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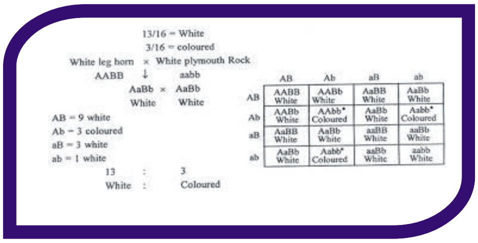

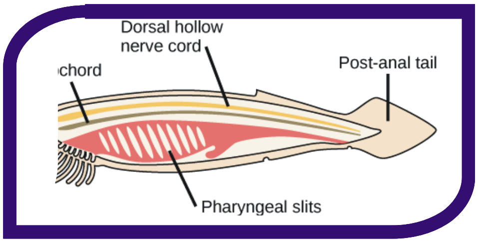

Biology of Chordates, Genetics & Microbiology

MZO-06

Vardhman Mahaveer Open University, Kota

Biology of Chordates, Genetics & Microbiology

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Preface

The present book entitled "**Biology of Chordates,Genetics and Microbiology**" has been designed so as to cover the unit-wise syllabus of MZO-06 course for M.Sc. Zoology (Final) students of Vardhman Mahaveer Open University, Kota. The basic principles and theory have been explained in simple, concise and lucid manner. Adequate examples, diagrammes , photographs and self-learning exercises have also been included to enable the students to grasp the subject easily.List of books suggested for further study will be a great help the students. The unit writers have consulted various standard books and internet as their reference on the subject and they are thankful to the authors of these reference books. Suggestions for the further improvement of the book will be thankfully acknowleged and incorporated in further editions.

Unit - 1

Lower Chordates – I

Structure of the Unit

1.1 Objectives

This unit describes the origin, classification and characteristics of phylum Protochordates. It gives detailed information on the characteristic features of the class and orders in the phylum protochordates. It describe the interrelationship between different classes of phylum protochordata. At the end of this unit you should be able to differentiate the major classes and orders of protochordates and their relationshios with other deuterostomes.

1.2 Introduction

The lower primitive chordates are collectively known as the protochordates or protochordata (Gr., protos, first; chorde. cord). They comprise three major subdivisions ranked as subphyla: Hemichordata, Urochordata and Cephalochordata. This division is chiefly based on the notochord found in the three subphyla. In Hemichordata, the notochord is of doubtful existence. It is repesented by a short stomodeal diverticulum or stomochord to the proboscis. For this reason, the Hemichordata is now a days treated as an independent invertebrate phylum. The Urochordata have notochord only in the tail of the tadpole larva. Only Cephalochordata have a persistent notochord extending along the whole length of their body throughout life.

The protochordates are of little economic importance. But they have great phylogenetic significance to the zoologists. They show great affinities and perhaps common origin with the living vertebrates. They retain the three basic chordate features throughout life. As such they are considered closer to the ancestor which probably gave rise to the final and the largest group of chordates the vertebrates.

Phylum Chordata is the largest of the deuterostome phyla. It is the highest and the most important phylum comprising a vast variety of living and extinct animals including man himself. Most of the living chordates are the well known familiar vertebrates animals such as the fishes, amphibians, reptiles, birds and mammals. Besides they include a number of marine forms such as the tunicates and lancelets, that are less well known. Cambrian to recent. Chordates about 50000 species.

Three fundamental Chordate characters

Chordates are sharply distinguished from nonchordates by three fundamental diagnostic characteristics:

1) **Notochord** : it is a supporting axial endoskeletal rod, forming from embryonic mesendoderm and extending in mid dorsal axis between nerve cord and alimentary canal. It consists of a series of large, vacuolated cells enclosed in a single or double layered sheath of connective tissue. It persists throughout life in some primitive chordates but in majority it occurs only during embryonic stages, being replaced in adult by a mesodermal, cartilaginous or bony vertebral column.

2) **Dorsal tubular central nervous system** : some higher nonchordates (Annelids and Arthropods) possess a double and solid, midventral nerve cord beneath alimentary canal. Chordates on the contrary always possess a single and hollow, tube like nerve cord, extending along middorsal line above alimentary canal and notochord. It develops from embryonic ectoderm. Its cavity is called neurocoel. In most chordates, its anterior part enlarges to form a brain, while the remaining part forms the spinal cord both together form the central nervous system(CNS).

3) **Pharyngeal gill clefts** : Paired slits, called gill clefts form in lateral walls of embryonic pharynx in all chordates primarily for respiration. These form in a peculiar manner finger like hollow pouches grow out from pharyngeal

wall and meet with corresponding inpockrtings of body wall; later the intervening walls dissolve forming the clefts which obviously connect pharyngeal cavity with the exterior. In lower aquatic chordates gill clefts persist for respiration throughout life; in higher, terrestrial chordates they close during later embryonic development.

Main differences between Chordata and Nonchordata

Chordata	**Nonchordata**
1. Notochord present throughout life or only in embryo.	1. Notochord absent.
2. Central nervous system hollow and middorsal.	2. Central nervous system solid and mid ventral.
3. Pharyngeal gill clefts present throughout life or in embryo.	3. Pharyngeal gill clefts absent.
4. Solid and muscular postanal tail in embryo; usually persists in adult.	4. Tail part is never a true postanal tail.
5. Heart ventral.	5. Heart dorsal.
6. Blood vascular system more developed. Blood flows in dorsal vessel from before backwards.	6. Blood vascular system less developed. Blood flows in dorsal vessel from behind forwards.
7. Hepatic portal system present.	7. Hepatic portal system absent.
8. Haemoglobin in red blood corpuscles.	8. Haemoglobin if present dissolved in plasma.

1.3 Origin of protochordates

Animals now living are unfortunately so modified and so far removed from significantly remote common ancestral structure that no conclusive picture has up to the present time, been gleaned from them of the pattern of evolution of the protochordates and vertebrates. Some evidences can be found supporting a variety of views, but the weighing of one theory as final is patently unjustified. Were fossils of ancient forms of prechordates more generally preserved, much uncertainty could be eliminated. Soft bodied as they seemingly were, they were rarely fossilized, since ordinarily only hard parts, notably bone, are preserved.

The perpetual argument about chordate phylogeny by serious students who continually sift and extract more possible bits of evidence from the structure and habits of living species has, however, resulted in the past 50 years in a marked restriction of the limits of acceptable inference. For one, serological tests have proved a close relationship of echinoderms, chordates, and hemichordates. Biochemical tests have shown a remarkable similarity of the phosphagens, essential for muscle contraction, between these groups. Creatin and arginin are amino acids found in the very labile phosphates, the phosphagens, essential for muscle contraction. Phosphocreatin and phosphoarginin are the two forms occurring in animal muscle tissue. Curiously only creatin is present in the phosphagens of all vertebrates and cephalochordates and only arginin in all invertebrates except the hemichordates and echinoderms which have both creatin and arginin. The same phyla (chordates, hemichordates and echinoderms) are also similar in having :

1. A bilaterally symmetrical dipleurula larva in primitive types
2. A mesodermal skeleton,
3. A regulation egg with no precise pattern of distribution of cellular components in the undivided fertile egg, so that in subsequent division several complete individuals may form from the one egg,
4. Indeterminate cleavage whereby the first division of the fertilized egg may take place between a number of parts of the egg and need not occur precisely in one plane and only that plane,
5. The mesoderm formed by evagination from the primitive gut,
6. The paired trunk coeloms forming in the mesodermal outpouching as an enterocele, in primitive types,
7. Basically five coelomic cavities,

8. Gastrulation by invagination,
9. A nervous system formed from a diffuse sheet or nerve net and
10. Location of the blastopore at the posterior end of the body forming or lying close to the anus.

The hemichordates are obviously closely related to chordates through possession of pharyngeal clefts, a stomochord and scattered cavities in the dorsal nerve cord. All of these characters form the basis for separation of one of the three great divisions of multicellular animals, the Deuterostomia to which not only the echinoderms and chordates belong, but also the few small phyla of Hemichordata, Brachiata, Bryozoa, Chaetognatha, Brachiopoda and Phoronida.

A second subkingdom the Archeostomia, is characterized by having :

1. Little serological similarity to chordates and echinoderms,
2. Arginin only in muscle phosphagens,
3. A radially symmetrical trochophore larva,
4. An ectodermal skeleton,
5. A mosaic egg with a precise pattern of distribution of cellular components so that only one individual can result from subsequent division of the egg,
6. Determinate cleavage, each division occurring in specific planes,
7. The mesoderm formed by splitting from other tissues,
8. The paired trunk coeloms forming as a schizocoele, as the mesoderm splits to form a central cavity on each side,
9. A varied arrangement and number of coeloms,
10. Invasive gastrulation by an internal rearrangement the gastrular cavity secondarily opening to the exterior,
11. A nervous system consisting of ganglionated cords, and
12. Location of the blastopore at the anterior end of the body, forming or lying close to the mouth.

The most significant of all the differences between the Archeostomia and Deuterostomia involves orientation of the blastopore, which lies near or forms the mouth in the former, the anus in the latter. The Archeostomia includes all the phyla with separate mouth and anus, except for those in the Deuterostomia: Acanthocephala, Annelida, Arthropoda, Aschelminthes, Echiuroidea, Endoprocta, Mollusca, Nemathelminthes and Sipunculoidea.

A third subkingdom is the Proctostomia, in which a single opening of the alimentary tract serves as both the mouth and anus. The Cnidaria

(Coelenterates), Ctenophora, Platyhelminthes and Porifera belong to this group. The Protozoa and Mesozoa may be regarded as constituting a separate subkingdom, the Astomia. The Proctostomia and Archeostomia are collectively referred to as the Proterostomia.

The conclusion that the echinoderms and chordates had a common ancestry is inescapable. But what was the common ancestor like? Little agreement on this point has as yet been attained. It does seem likely however that the deuterostomian line of evolution began at an extremely remote time with animals having a more or less spherical body and a blind gut like coelenterates, with no anus. Certainly no sudden or even gradual shift in antero posterior orientation of an animal already with anus, mouth, and cephalic end would occur to produce the deuterostomian condition from an established archeostomian one. As soon as an animal so evolves that it possesses an elongate body, with one end directed forward to seek food and test its environment the evolutionary door may be considered closed to a reversal of axis orientation.

In departing from a coelenterate like stage, apparently neither archeostome nor deuterostome ancestors had established an anterior or posterior end. When the gut become a continuous tube, in each line of evolution, the axial orientation with concomitant cephalisation and elongation of body, was free to evolve in either direction. Solely upon that axial orientation presumably was the functional fate of the former mouth anus (embryonic blastopore) determined. In the Deuterostomia the orientation evolved so that the blastopore derivative was directed posteriorly, thus retaining only anal functions, as at the same time the new opening at the opposite end of the alimentary tract served as the mouth. Exactly the reverse occurred in the Archeostome line of evolution. There is no indication that either of these two groups was derived from the other, there is every reason to believe that they are of common origin, from some proctostome group (Fig.).

The Pterobranchia are thus regarded as the most primitive of all living direct prevertebrates because of

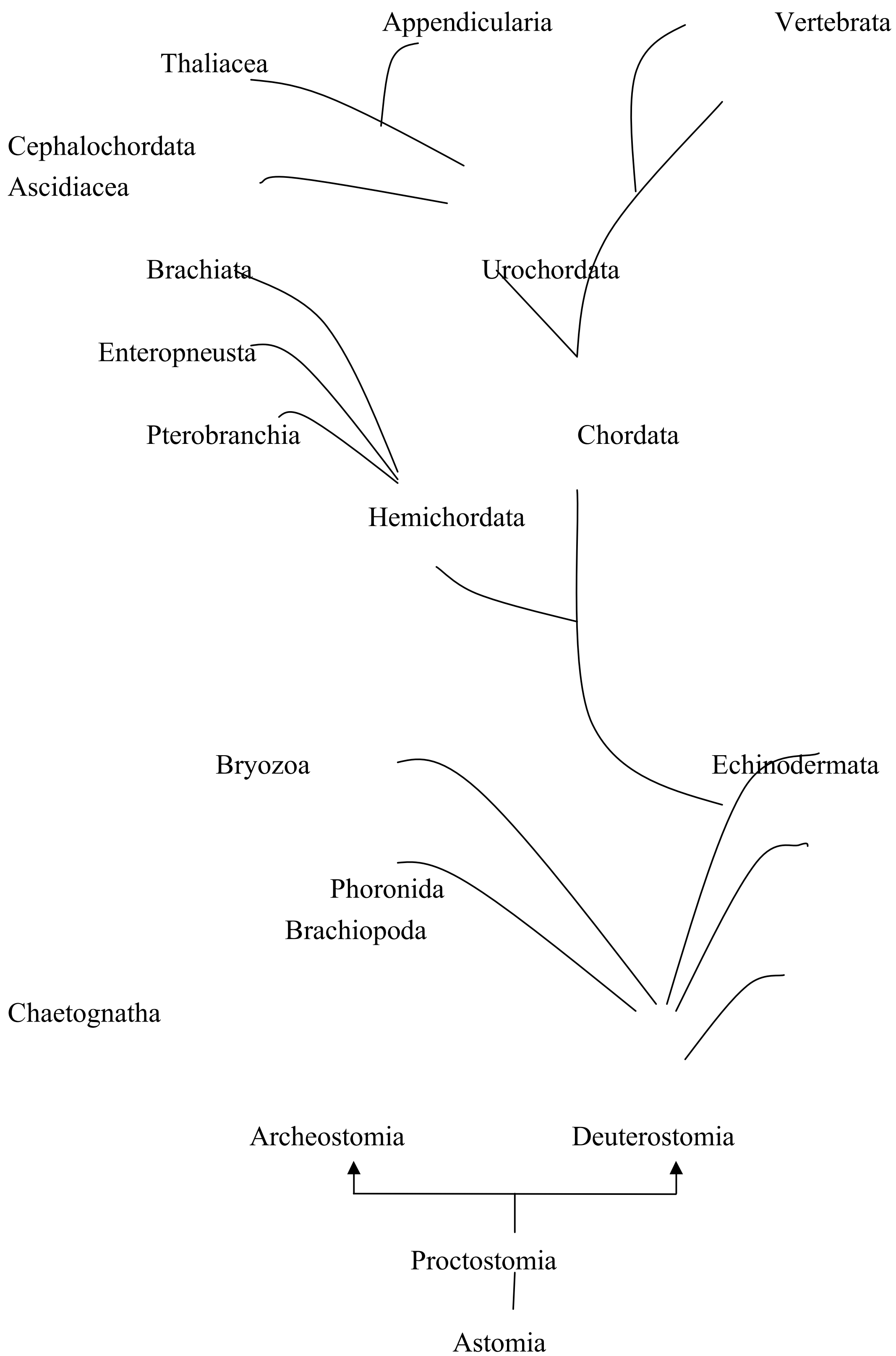
1. Their simple structure (few pharyngeal slits, no elaborate systems),
2. Their similarity to most other deuterostomians (save the echinoderms and most chordates) in having sessile habits and a lophophore like accessory feeding organ,

3. Possession of at least two of the most fundamental unique chordate characteristics or of a homolog there of (pharyngeal slits, notochord), and
4. Possession of a body form from which chordate animals may most easily have been differentiated.

Curiously enough fixation of common ancestry of archeostomes and deuterostomes does not establish whether the common ancestor

1. Was a sessile animal developing at first a U-shaped body axis which subsequently straightened as the animals became free swimming or
2. Was, from the first a free swimming animal. Either would be a reasonable possibility. Very likely both are true as either or both of the deuterostome and archeostome groups may have evolved from two or more proctostome groups (this is polyphyly).

Whatever the ancestral form of archeostomes so many protochordates and chordate like invertebrates (like the phoronida, Bryozoa, Pterobranchia, Ascidia) are sessile and with a U- shaped body axis that modern investigators are learning more and more to the view that from such forms at least the chordates evolved. The presence of the deuterostomian orientation even in radially symmetrical animals like the echinoderms can best be explained as a by product of at least a brief history as a sessile animal with a U- shaped body axis. That they evolved from free swimming, radially symmetrical animals remains a possibility. Further investigation of the problem will however be necessary before a definite conclusion is reached. At any rate present evidences makes wholly untenable all of the ingenious theories receiving much attention in the past that chordates have evolved from ammelids, arthropods or arachnids.


Appendicularia
Vertebrata
Thaliacea
Cephalochordata
Ascidiacea
Brachiata
Urochordata
Enteropneusta
Pterobranchia
Chordata
Hemichordata
Bryozoa
Echinodermata
Phoronida
Brachiopoda
Chaetognatha
Archeostomia
Deuterostomia
Proctostomia
Astomia

1.4 Outline classification of the protochordates

Phylum Chordata can be divided into two groups:

a) Acrania (Protochordata) and
b) Craniata (Euchordata)

Acrania (Gr. a, absent; kranion, head) or Protochordata (Gr. protos, first; chorde, cord).

All marine , small, primitive or lower chordates. Lacking a head, a skull or cranium, a vertebral column, jaws and brain. About 2000 species. The Acrania is divided into three subphyla : Hemichordata, Urochordata and Cephalochordata, chiefly on the position of notochord.

Phylum Chordata

- Group Acraniata or Protochordata
  - Subphylum Hemichordata
    - Classses
      - Enteropneusta
      - Pterobranchia
      - Planctosphaeroidea
      - Graptolite
  - Subphylum Urochordata
    - Classses
      - Ascidiacea
      - Thaliacea
      - Larvacea or Appendicularia
  - Subphylum Cephalochordata
    - Classses
      - Leptocardii
- Group Craniata or Euchordata
  - Subphylum Vertebrata
    - Classses
      - Pisces
      - Amphibia
      - Reptilia
      - Aves
      - Mammalia

1.5 Subphylum Hemichordata

A. General characters

(Gr. hemi, half; chorde, cord)

1. Exclusively marine, solitary or colonial, mostly tubicolous.
2. Body soft, fragile, vermiform, unsegmented, bilaterally symmetrical and triploblastic.
3. Body typically divided into 3 distinct regions- proboscis, collar and trunk.
4. Body wall of a single layered, epidermis with mucous gland. No dermis.
5. Coelom enterocoelous usually divided into protocoel, mesocoel and metacoel, corresponding to three body regions.
6. Digestive tube complete, straight or U- shaped.
7. Foregut gives out a hollow buccal diverticulum into proboscis, earlier considered as notochord.
8. Dorso lateral pharyngeal gill slits, when present one to several pairs. Ciliary filters feeders.
9. Circulatory system simple and open, including a dorsal heart and two longitudinal vessels, one dorsal and one ventral.
10. Excretion by a single proboscis gland or glomerulus connected to blood vessels.
11. Nervous system primitive consisting mainly of a subepidermal nerve plexus. Dorsal collar nerve cord hollow.
12. Reproduction mainly sexual. Sexes usually separate. Gonads one to several pairs.
13. Fertilization external, in sea water. Development direct or indirect with a free swimming tornaria larva.

Hemichordate includes about 80 known species which are generally grouped under two classes: Enteropneusta and Pterobranchia.

I. Class Enteropneusta

(Gr. enteron, gut + pneustos, breathed)

1. Solitary, free- swimming or burrowing animals, commonly called the ‘acorn’ or ‘tongue worms’.
2. Body elongated, vermiform, with no stalk.
3. Proboscis cylindrical and tapering.
4. Collar without ciliated arms (lophophore).

5. Alimentary canal straight. Mouth and anus at opposite ends. Filter feeding.
6. Several pairs of U- shaped gill slits.
7. Sexes separate. Gonads numerous, sac like.
8. Development includes tornaria larva in some. Asexual reproduction lacking.

   Examples : Balanoglossus, Saccoglossus, Speengelia.

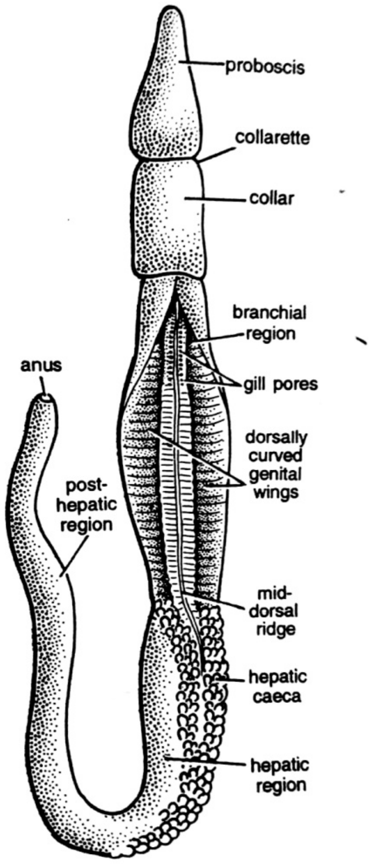


Fig. Balanoglossus

II. Class Pterobranchia

(Gr. pteron, feather + branchion, gill)

1. Solitary or colonial, sessile and tubicolous animals living inside chitinous tubes.
2. Body short, compact, with stalk for attachment.
3. Proboscis shield like.
4. Collar bearing ciliated arms.
5. Alimentary canal U- shaped. Anus dorsal lying near mouth. Ciliary feeding.

6. Gill silts one pair or absent, never U- shaped.
7. Sexes separate or united. Gonads 1 or 1 pair.
8. Development direct or with a larval stage. Asexual reproduction by budding in some.

a) Order Rhabdopleurida

1. Colonial, zooids connected by a stolon.
2. Collar with two tentaculated arms.
3. Gill slits absent.
4. Gonad single.

Example : single genus Rhabdopleura.

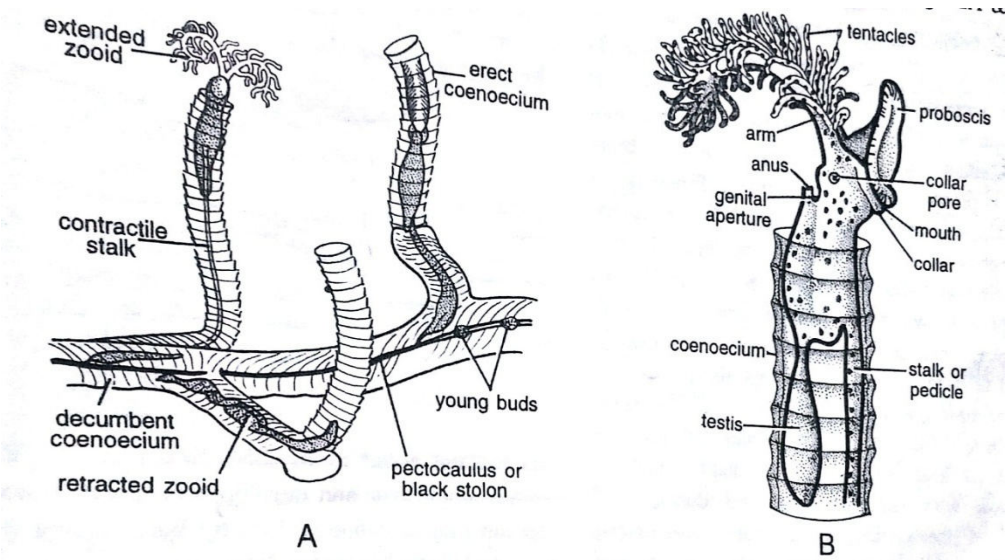


Fig. Rhabdopleura. A- Portion of colony. B- An individual zooid.

b) Order Cephalodiscida

1. Solitary or several zooids living unconnected in a common gelatinous case.
2. Collar with several tentaculated arms.
3. Gill slits single pair.
4. Gonads single pair.

Example : Cephalodiscus, Atubaria.

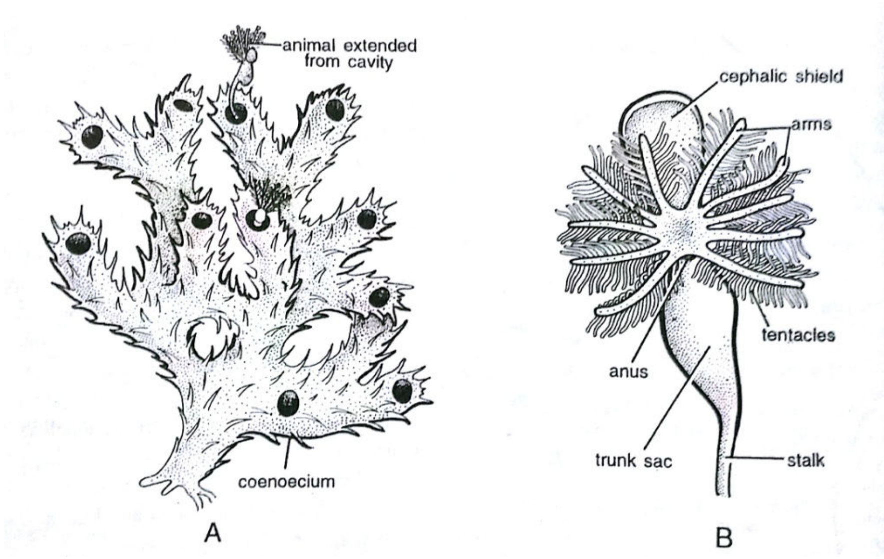


Fig. Cephalodiscus. A- A part of colony. B- An individual zooid.

III. Class Planctosphaeroidea

This class is represented by a few small, rounded, transparent and pelagic larvae, supposed to be specialized tornaria of some unknown hemichordate termed Plantosphaera pelagic. The larval body is covered by extensively branched ciliary bands and its alimentary canal is L-shaped.

IV. Class Graptolita

The fossil graptolites (e.g. Dendroglossus) were abundant in Ordovician and Silurian periods and often placed as an extinct calss under Hemichordata. Their tubular chitinous skeleton and colonial habits show an affinity with Rhabdopleura.

B. Affinities and systematic position of Hemichordata

Bateson proposed the name Hemichordata in place of Enteropneusta. Since then, due to their peculiar anatomical organisation and embryology, the Hemichordata have been considered closer to the Chordata as well as most non-chordate phyla by different workers from time to time. Some of these views regarding the phylogenetic relationship and taxonomic position of the Hemichordates are as follows:

I. Affinities with Chordata

Some earlier workers, such as William Bateson (1885), proposed closer affinities between Hemichordata and Chordata. Their resemblance was based on the presence of the three fundamental Chordate characteristics in Hemichordata, that is a notochord, a dorsal hollow nerve cord and the pharyngeal gill slits .

Affinities with Urochordata

Hemichordata are nearest to Urochordata, as they exhibit many close resemblance with them. The structure and function of pharynx and branchial apparatus in hemichordates are similar to those of urochordates. Also, the development of the central part of nervous system in quite similar in both.

Affinities with Cephalochordata

Besides similarities in the structure and function of the branchial apparatus the hemichordates also show similarity with cephalochordates in the arrangement of coelomic sacs and in development.

Due to these similarities Hemichordata had been considered as a subphylum of the phylum Chordata till recently, representing its lowest group, and probably having a common ancestry.

Objection

However the hemichordates are no longer included under chordates because they do not possess chordate characters in a typical condition. The main objections are:

1. A true notochord does not occur in hemichordates. Unlike that of the chordates, the so called notochord is very short confined to proboscis and without any supporting function. It is ventral to the main blood vessel and not covered by sheaths. Instead of being solid and made of vacuolated cells, it is hollow and lined by epithelial cells. It does not originate from the roof of larval archenteron but as a forward hollow projection of the foregut. Instead of being called notochord (Bateson, 1885) it is now termed the stomochord (Willy, 1899). Hyman prefers to name it as buccal diverticulum.
2. The nervous system is distinctly of the invertebrate type being intra epidermal in position and having a ventral nerve cord and a circumenteric nerve ring which are absent in chordates. In Balanoglossus, the dorsal tubular nerve cord is confined to the collar region only.
3. Gill slits of Balanoglossus are numerous and dorsal in position, whereas they are 5 to 7 and lateral in higher chordates.

Other differences

1. Lacking metameric segmentation, cephalisation, paired appendages, postanal tail, exoskeletom, living endoskeleton, dermis, liver, haemoglobin, red blood corpuscles, etc.
2. Having peculiar division of body and coelom (into proboscis, collar and trunk), single layered ciliated epidermis, hepatic caeca, dorsal heart, open neurocoel, colourless blood, numerous gonads, etc.

II. Affinities with Rhynchocephalia (Nemertinea)

Feeding and burrowing habits are similar in Balanoglossus and Nemertinea. Body in both is elongated, vermiform without external metamerism, with terminal anus, with smooth skin containing unicellular glands and ectodermal nerve plexus and having metamerically arranged simple gonads. But Nemertinea differ in lacking a dorsal nerve cord and in having lateral nerve cords and a protrusible proboscis.

III. Affinities with Phoronida

Some zoologist like A. T. Masterman (1897) advocated relationship of Balanoglossus with Phoronis on the following grounds:

1. Similar nature of epidermal nervous system.
2. The paired gastric diverticula of Phoronis, like the buccal diverticulum of Balanoglossus, forming so called notochord.
3. Actinotroch larva of Phoronis has several enteropneust features of tornaria such as similar disposition of coelom, anus surrounded by a ciliary ring, presence of a proboscis pore and a sensory apical plate with cilia and eye spots.
4. Both have great power of regeneration.

Objections

But the chordate features of Balanoglossus like pharyngeal gills are absent in Phoronis which also differs in having paired metanephridia.

IV. Affinities with Pogonophora

Marcus (1958) tried to relate Hemichordata with Pogonophora due to following similarities:

1. Enterocoelous formation of coelom.
2. Body and coelom divided into three regions.
3. Mesosome and metasome separated by a septum.
4. Nervous system intra epidermal .
5. Pericardial sac in some pognophores.
6. Gonads found in trunk.

Objection

But pogonophores differ in having protocoelic nephridial coelomoducts and lacking an alimentary canal. Moreover,nervous system is concentrated in protosome in Pogonophora, but in mesosome in Hemichordata.

V. Affinities with Annelida

Spengel (1893) first suggested affinities of Annelida and Hemichordata as follows:

1. Body vermiform and coelomate.
2. Burrowing habit, tubicolous life and ingesting mud which is passed out as castings through anus.
3. Collar of Balanoglossus similar to clitellum of earthworm.
4. Proboscis and prostomium similar and preoral.
5. Similar arrangement of blood vessel with blood flowing anteriorly in dorsal vessel and posteriorly in ventral vessel.
6. Dorsal position of heart.
7. Tornaria larva of Balanoglossus shows several structural resemblances with the trochophore larva of Annelida in being pelagic, ciliated, with apical plate, eye spots, sensory cilia well developed alimentary canal with similar parts.

Objections

However the two groups show striking differences as follows:

1. Annelids do not have pharyngeal gill slits, stomochord or buccal diverticulum and dorsal tubular nerve cord found in Balanoglossus.
2. Balanoglossus does not have double and solid ventral nerve cords and nephridia found in annelids.
3. In tornaria larva of Balanoglossus preoral or proboscis coelom is present, nephridia are absent and blastopore becomes anus of the adult (Deuterostomia). In trochophore larva of annelids preoral coelom is absent, nephridia present and the blastopore becomes the mouth (Proterostomia).

Thus compared to their great fundamental differences the similarities of the two group are only superficial and quite insignificant indicating probably a convergent evolution due to similar habits and habitat.

VI. Affinities with Echinodermata

Adult resemblance

Adult hemichordates and echinoderms are structurally quite different and it is difficult to suspect any phylogenetic relationship between them. They show few resemblances such as:

1. Enterocoelic origin of coelom and its division into three successive parts filled with sea water to serve a hydraulic mechanism.
2. Heart vesicle and glomerulus of enteropneusts are considered homologous to the dorsal sac and axial gland of echinoderms. Both the structures are related and combine vascular and excretory functions.
3. Nervous system is poorly developed and forms epidermal nerve plexus.
4. Proteins and phosphagens present in hemichordates closely resemble those of echinoderms.
5. Common habits and ecological niches and remarkable power of regeneration.

Larval resemblances

The two groups show a strong affinity on embryological ground as the tornaria larva of Balanoglossus has a striking structural similarity with an echinoderm larva, in particular the bipinnaria larva of asteroids. In fact the tornaria was regareded an echinoderm larva for a long time by Johannes Muller (1850), Krohn (1854), Agassiz (1864), etc., till Metschnikoff (1870) proved it to be an enteropneust larva. The larvae of the two groups possess the following common features:

1. Small, pelagic, transparent and oval.
2. Identical ciliated bands taking up a similar twisted course.
3. Enterocoelic origin and similar development of coelom.
4. Proboscis coelom opening to outside by proboscis pore of tornaria comparable to hydrocoel of echinoderm dipleurula.
5. Blastopore becomes the anus and digestive tract is complete with mouth, anus and same parts.

Objection

However the tornaria larva shows presence of apical plate with sensory hairs and eye spots and telotroch which are absent in echinoderm larvae. The protocoel is single in tornaria but paired in echinoderm larva. This raises doubts about the echinoderm affinities of hemichordates. Fell (1963) and others believe that their larval similarities are only because of convergent evolution due to same mode of habits and habitat.

1.6 Subphylum Urochordata

A. General characters

(Gr. uros, tail; chorda, cord)

1. Exclusively marine and cosmopolitan, found in all seas and at all depths.
2. Mostly sedentary (fixed), some pelagic or free-swimming.
3. Simple (solitary), aggregated in groups or composite (colonial).
4. Size (0.25 to 250 mm), shape and colour variable.
5. About body degenerate, sac-like, unsegmented, without paired appendages and usually without tail.
6. Body covered by a protective tunic or test composed largely of tunicine , $(C_6H_{10}O_5)_{n,}$ similar to cellulose, hence the name *Tunicata.*
7. A terminal branchial aperture and a dorsal atrial aperture usually present.
8. Coelom absent. Instead, an ectoderm-lined atrial cavity present which opens to outside through atrial aperture.
9. Notochord present only in larval tail, hence the name *Urochordata.*
10. Alimentary canal complete. Pharynx (branchial sac) large, with endostyle and two to several pairs of gill-slits. Ciliary feeders.
11. Respiration through test and gill-slits.
12. Blood-vascular system open. Heart simple, tubular and ventral. Flow of blood periodically reversed. Special vanadocytes in blood extract vanadium from sea water.
13. Excretion by neural gland, pyloric gland and nephrocytes.
14. Dorsal tubular nerve cord only in larval stage, reduced to a single dorsal nerve ganglion in adult.
15. Mostly hermaphrodite. Fertilization cross and external.
16. Development indirect including a free-swimming tailed larva with basic chordates characters. Metamorphosis retrogressive.
17. Asexual reproduction by budding common.

Classification

Subphlum Urochordata or Tunicata includes about 2,000 fixed and nearly 100 pelagic species exhibiting high degree of diversity. These have been variously classified by Herdman (1891), Lahille, Garstang (1895), Perrier (1898), Hartmeyer (1909-11) and S.M. Das (1957). But these have some drawbacks and are at times too complicated for students. The classification given below has been adopted from Storer and Usinger as given in their book General Zoology of 1965 edition. As usual, the subphylum Urochordata is divided into 3 classes.

I. Class Ascidiacea

1. Solidary, colonial or compound. Bottom living.
2. Body form and size variable.
3. Test permanent, well developed and thick.
4. Atrium opens dorsally by atriopore.
5. Pharynx large with many persistent gill-slits.
6. Sexes united. Larva free-swimming and highly developed.
7. Adults usually sessile after retrogressive metamorphosis when larval notochord, nerve cord and tail are lost and brain reduced to a solid dorsal ganglion.
8. Stolon simple or none.

a) Order Enterogona

1. Body sometimes divided into thorax and abdomen.
2. Neutral gland usually ventral to ganglion.
3. Gonad 1, lying in or behind intestinal loop.
4. Larva with 2 sense organs (ocelli and otolith).

i. Suborder Phlebobranchia

1. Pharynx with internal longitudinal vessels.
2. Budding rare.

Examples : *Ascidia, Ciona, Phallusia*.

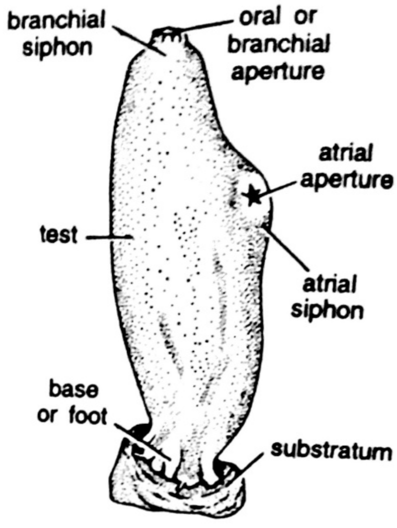


Fig. Ascidia

ii. Suborder Aplousobranchia

1. Pharynx without longitudinal vessels.
2. Budding common.

Examples : Clavelina.

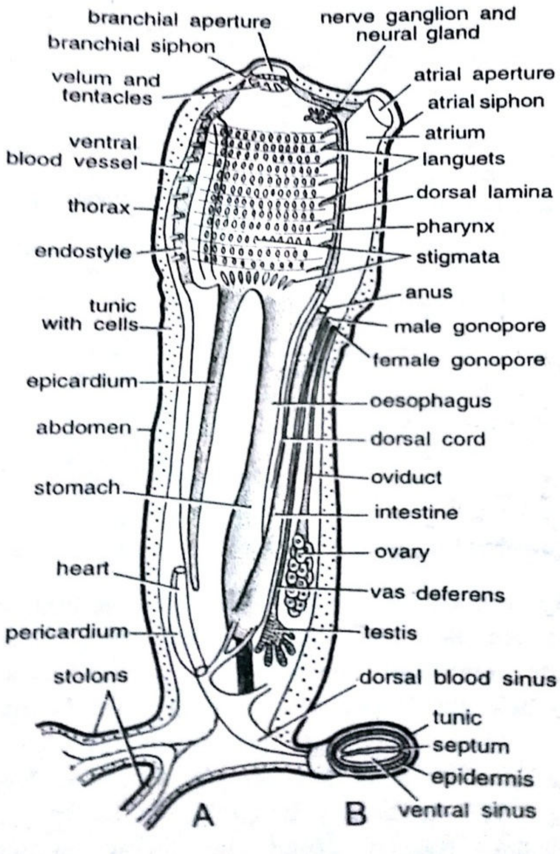


Fig. Clavellina. A – Single zooid. B – Stolon in T.S.

b) Order Pleurogona

1. Body compact, undivided.
2. Neutral gland dorsal or lateral to ganglion.
3. Gonads 2 or more embedded in mantle wall.
4. Larva with otolith. Separate eye absent.

Examples : *Herdmania, Botryllus, Molgula, Styela*.

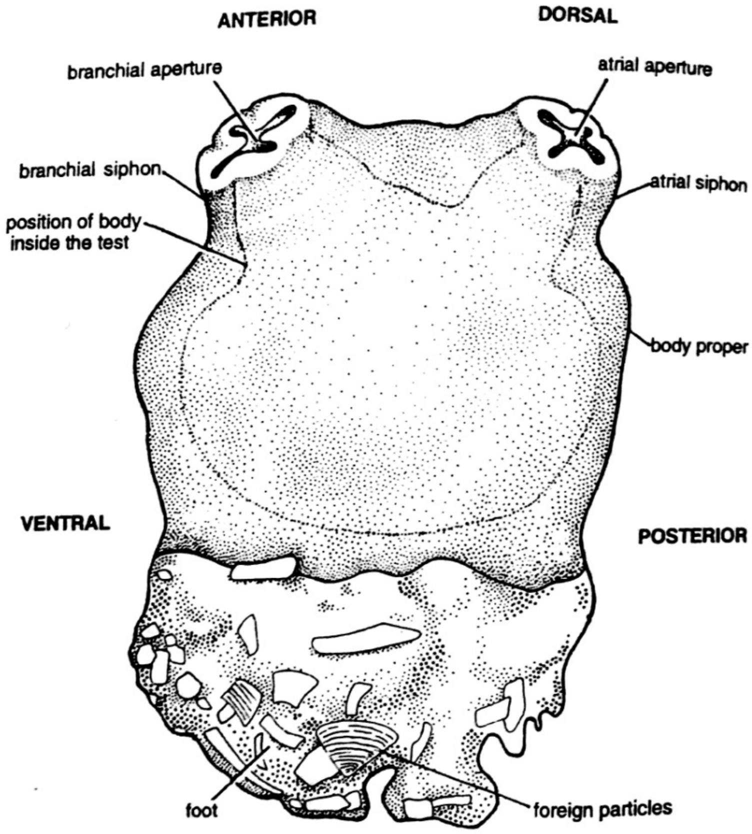


Fig. Herdmania

II. Class Thaliacea

1. Adults free living, pelagic, in warm and temperate seas. Solidary or colonial.
2. Body shape and size variable.
3. Tunic permanent, thin and transparent, with circular muscle bands.
4. Atriopore located posteriorly.
5. Pharynx with 2 large or many small gill-slits.
6. Sexes united. Larva formed or absent.
7. Adult without notochord, nerve cord and tall.
8. Asexual budding from a complex stolon.
9. Life history with an alternation of generations.

a) Order Pyrosomida

1. Colony compact, tubular, closed at one end and phosphorescent throughout the life, due to the invasion of its egg by a symbiotic luminescent bacteria.
2. Zooids embedded in a common test.
3. Muscle bands confined to body ends.
4. Gill-slits tall, numerous, upto 50.
5. No free-swimming larval stage.
6. Reproduces by budding.

Examples : *Single genus, Pyrosoma*.

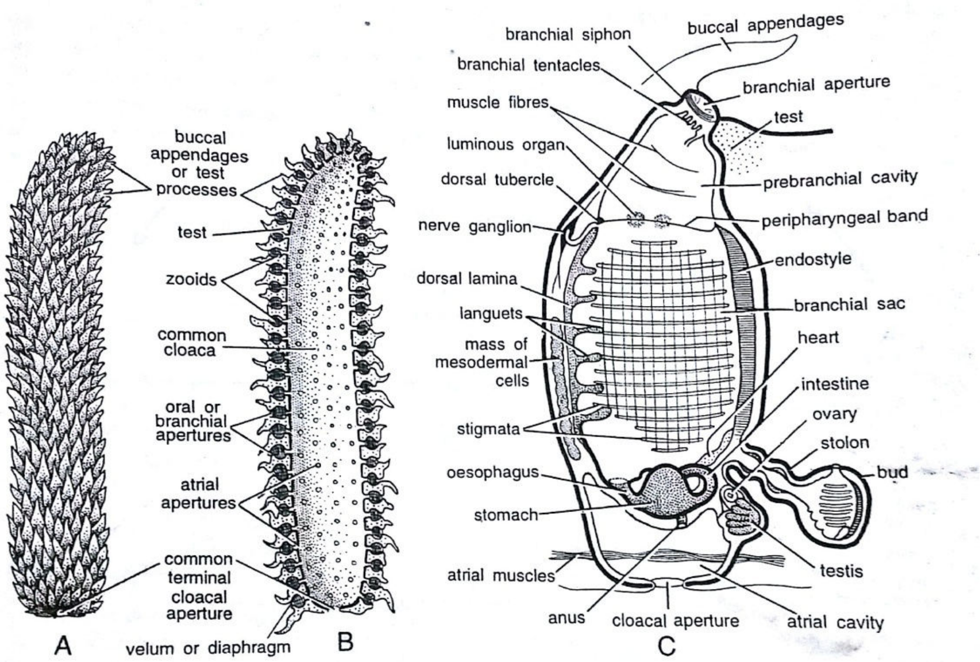


Fig. Pyrosoma. A – Entire colony. B – Colony in L.S. C- Single zooid.

b) Order Doliolida (=Cyclomyaria)

1. Body characteristically barrel-shaped.
2. Muscle bands form 8 complete rings.
3. Gill-slits small, few to many.
4. A tailed larva with notochord present.

Examples : *Doliolum, Doliopsis.*

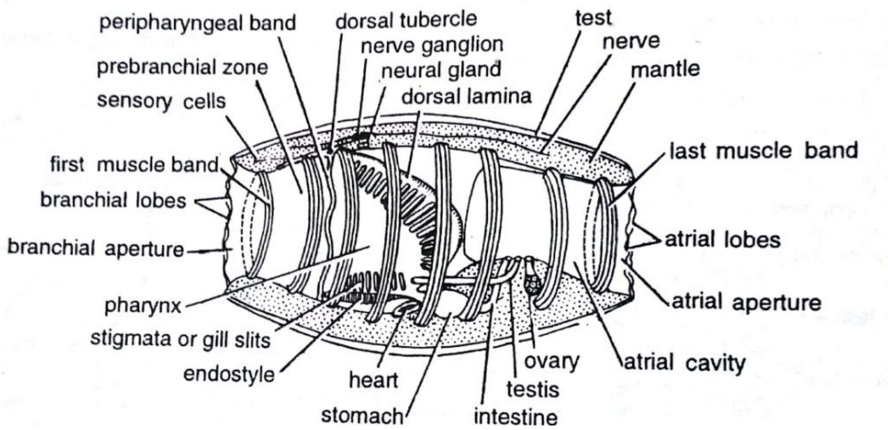


Fig. Doliolum

c) **Order Salpida (=Desmomyaria)**

1. Body cylindrical or prism-shaped.
2. Muscle bands incomplete ventrally.
3. Pharynx communicates freely with atrium through a large gill-slit.
4. Tailed larva absent.

Examples : *Salpa, Scyclosalpa*.

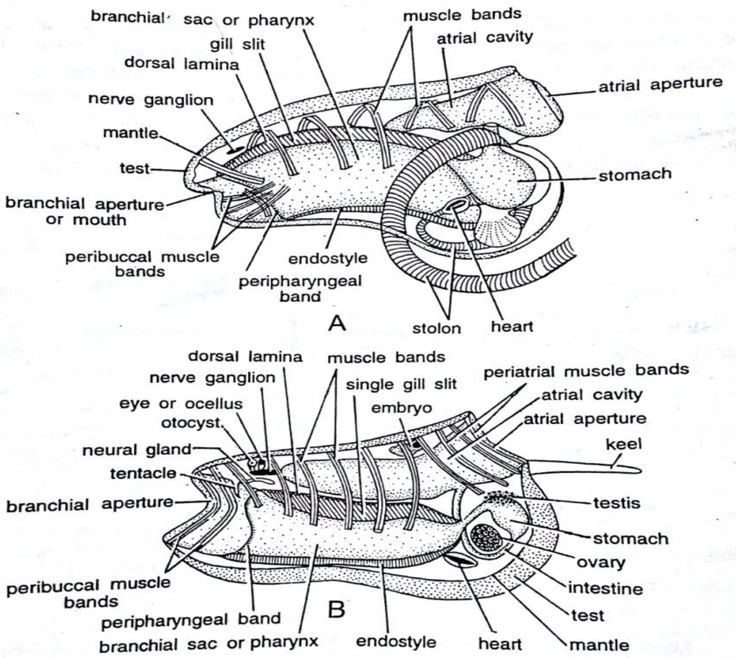


Fig. Salpa. A – Oozooid in left lateral view. B – Blastozooid in left lateral view.

III. Class Larvacea (=Appendicularia)

1. Small (5 mm long), solidary, free-swimming, pelagic, neotenic, larva-like forms with persistent tail, notochord, nerve cord and brain.
2. Test forming a temporary house, renewed periodically.
3. Atrium and atrial aperture absent.
4. Gill-slits 2, opening directly to outside.
5. Sexes united. No metamorphosis.

a) Order Endostylophora

1. House bilaterally symmetrical, with separate inhalant and exhalent apertures.
2. Pharynx with endostyle.

   Examples : *Oikopleura, Appendicularia*.

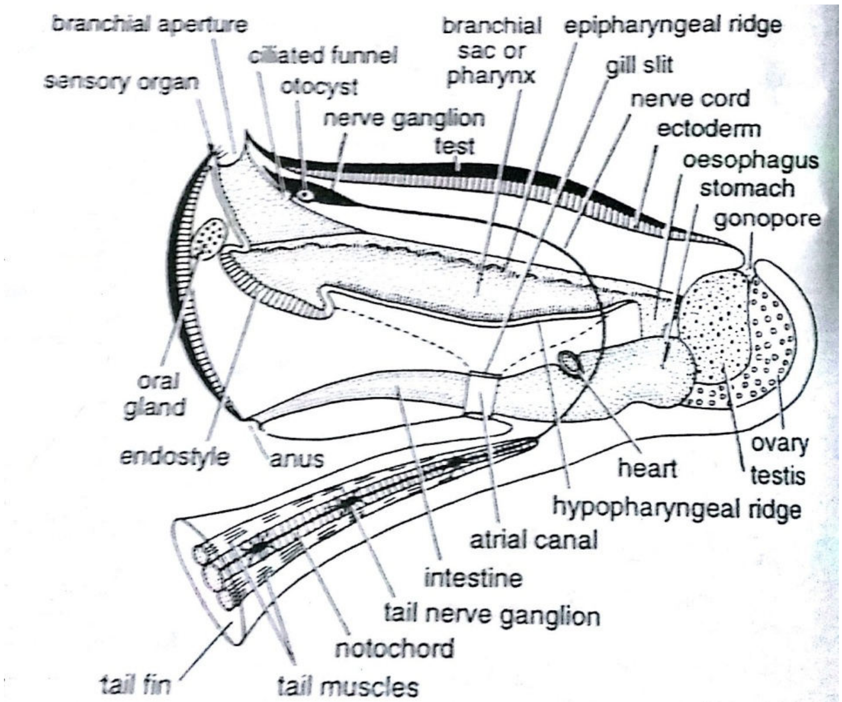


Fig. Oikopleura- animal in sagittal section.

b) Order Polystylophora

1. House biradically symmetrical, with single aperture.
2. Pharynx without endostyle.

   Examples : *Kowalevskia.*

B. Affinities and systematic position of Urochordata

I. Affinities with nonchordates

In some features, urochordates show some similarities with certain nonchordate groups.

1. Porifera, Coelenterata and Urochordates are sessile in nature.
2. Mechanism of filter feeding and respiration through a water current is parallel with that of sponges, molluscs and lophophores.
3. Budding chain of new zooids is common with coelenterates.
4. Larval eyes and otocysts are found in many invertebrates.
5. Colonial mode of life of simple and composite fixed ascidians is observed in a number of invertebrates.
6. Presence of typhlosole in intestine.

None of the above features establishes any nonchordate relationship. These similarities are only due to similar mode of life and parallel evolution.

II. Affinities with Chordates

As already mentioned the chordate affinities of urochordates are beyond any doubt. The ascidian tadpole larva possesses all the basic chordate characters such as : (i) rod like norochord forming axial skeleton of tail, (ii) dorsal tubular nerve cord and (iii) gill slits in the pharyngeal wall.

This is probably because both urochordates and the other chordates have originated from a common ancestor.

a) Affinities with Hemichordata

The assumption that nearest relatives of urochordates are the existing hemichordates is based on the following similarities :

1. Same structural plan of pharynx perforated by gill slits and having similar accessories.
2. Similar development of central part of nervous system.
3. Occurrence of restricted notochord.

Objections

However the two groups have important differences.

1. Balanoglossus lives in burrows but urochordates may be fixed, inert or pelagic.

2. Hemichordate body is divisible into proboscis, collar and trunk which is not found in urochordates.
3. A true notochord is present in the tail of ascidian larva whereas the buccal diverticulum of Balanoglossus is no longer considered a notochord.

   Thus, even inclusion of hemichordates as true chordates has become doubtful so that they are considered nowadays as an independent nonchordate phylum. The similarities of both the groups are probably because of their remote phylogenetic relationship with the ancestral common stock.

b) Affinities with Cephalochordata

The adult urochordates bears the following structural similarities with Branchiostoma :

1. Similar ciliary filter feeding or food concentration mechanism and respiratory mechanism.
2. Large pharynx with similar accessories.
3. Branchial tentacles are similar to velar tentacles.
4. Similar endostyle and associated parts.
5. Similar atrial complex.

Besides the fundamental three chordate characteristics the ascidian tadpole larva and Branchiostoma also share the following similarities :

1. Identical early stages of development.
2. Tail with median vertical fins without fin rays.
3. Median sensory organs (otocyst, ocellus, statocyst).
4. Pharynx with endostyle.
5. Atrial complex similar.

Objections

The important differences between the two groups are as follows:

Characters	Urochordates	Cephalochordates
Adult	Sedentary	Free swimming
Segmentation	Absent	Present
Notochord	Absent in adult	Present

Nerve cord	Absent in adult	Present
Coelom	Absent	Present
Test	Present	Absent
Liver	Present	Midgut diverticulum
Heart	Present	Absent
Nephridia	Absent	present
Sex	Bisexual	Unisexual
Metamorphosis	Retrogressive	Progressive

While their resemblances point out to a probable remote common ancestry their differences demand their taxonomic arrangement in two separate subphyla under the phylum Chordata.

c) Affinities with Vertebrata

The ascidian tadpole larva can be compared with a larval fish. It resembles higher chordates in having

1. Dorsal tubular nerve cord,
2. Axial skeletal notochord,
3. Pharyngeal gill slits,
4. Postanal tail covered by vertical caudal fin and
5. Type of cleavage and gastrulation.

Besides in the adult ascidian :

1. Neural gland is homologous with vertebrate pituitary,
2. Endostyle with vertebrate thyroid ,and
3. Typhlosole comparable to intestinal spiral valve of elasmobranch fishes.

Conclusion

From above discussion it is obvious that urochordates are primitive and degenerate descendants of ancestral chordates. The tadpole larva represents the relic of a free swimming ancestral chordate. Degeneration is a secondary modification due to sedentary and sessile mode of adult life.

1.7 Subphylum Cephalochordata

A. General Characters

(Gr., kephal, head; chorde, cord)

1. Marine, widely distributed in shallow waters.
2. Mostly sedentary and buried with only anterior body end, projecting above bottom sand.
3. Body small, 5 to 8 cm long, slender, fish-like, metameric and transparent.
4. Head lacking. Body has trunk and tail.
5. Paired appendages lacking. Medium fins present.
6. Exoskeleton absent. Epidermis single-layered.
7. Muscles dorso-lateral, segmented into myotomes.
8. Ceolom enterocoelous, reduced in the pharyngeal region by development of atrial cavity.
9. Notochord rod-like, persistent, extending from rostrum to tail, hence the name Cephalochordata.
10. Digestive tract complete. Pharynx large, perforated by numerous persistent gill-slits opening into atrium. Filter feeders.
11. Respiration through general surface. No special organs for respiration present.
12. Circulatory system well developed, closed and without heart and respiratory pigment. Hepatic portal system developed.
13. Excretion by protonephridia with solenocytes.
14. Nerve cord dorsal, tubular, without ganglia and brain. Dorsal and ventral nerve roots separate.
15. Sexes separate, gonads numerous and metamerically repeated. Gonoducts lacking. No asexual reproduction.
16. Fertilization external in sea water.
17. Development indirect, including a free-swimming larva.
18. The Cephalochordata comprise about 30 species mostly of the genus *Branchiostoma* and all put in the class Leptocardii.

B. Primitive, Degenerate & Specialized Characters of *Branchiostoma* (Cephalochordata)

I. Primitive characters

Branchistoma is regarded to be a primitive chordate. It differs from vertebrates because it retains several primitive chordate characters in most typical or unmodified form, as relics from its ancestors. The most prominent of these primitive characters are as enumerated below :

1. Asymmetrical body as in echinoderms, which are regarded to have common ancestry with chordates.
2. Absence of a specialized head or cephalization.
3. Absence of paired limbs or fins.
4. Epidermis one-cell thick. Dermis absent.
5. Coelom enterocoelous, arising as lateral pouches of larval archenteron.
6. Metamerically arranged muscles or myotomes.
7. Notochord persistent throughout life. Vertebral column or any other endoskeleton not developed.
8. Jaws absent. Alimentary canal straight.
9. Pharynx large, perforated by persistent gill-slits and specialized for ciliary mode of feeding by drawing a water-food current. Endostyle present without modification.
10. Liver represented by a midgut diverticulum.
11. Blood vascular system is simple, without a heart and any distinction between ateries and veins. Hepatic portal system is primitive.
12. No special respiratory organs and respiratory pigment.
13. Excretion by segmentally arranged protonephridia which are not coelomoducts.
14. Neutral tube hollow and lying dorsally above notochord. Specialized brain lacking. Dorsal and ventral roots of spinal nerves separate. Dorsal roots without ganglia so that impulses pass directly from skin to neutral tube.
15. Sensory organs simple and paired.
16. Gonads several pairs, alike, segmentally arranged and without gonoducts.
17. Eggs are small, almost yolkless. Blastula is spherical, hollow and one-layered. Gastrulation embolic.

II. Degenerate characters :

Workers like Gregory consider that Branchiostoma was once more developed but underwent specialization and degeneration during later course of evolution due to semisedentary mode of life. The degenerated characters include :

1. Poorly developed brain (cerebral vesicle) and simple sensory organs.
2. Lack of any cartilaginous or bony endoskeleton.
3. Lack of gonoducts.

III. Specialized, peculiar or secondary characters :

These changes developed perhaps due to their special mode of life and also probably prevented their further evolution. Because of their degenerate and special characters, they are not considered in direct line of evolution of chordates but as a side offshoot.

1. Peculiar asymmetry of adult and early stages of development.
2. Anterior projection of notochord into rostrum making it stronger for burrowing. Over-development of notochord may be responsible for the lack of brain.
3. Mouth surrounded by oral hood, with sensory oral cirri, meant for filtering and concentrating food particles from water.
4. Elaborate velum with sensory tentacles to permit only small food particles to enter pharynx.
5. Large, spacious and elaborate pharynx, with ciliated gill clefts which are more more numerous than actual body segments, to enable sufficient food collection.
6. Wheel organ and Hatschek's groove and pit developed to help in ciliary feeding.
7. Delicate pharynx surrounded by a protective atrial cavity opening to outside through atriopore.
8. Coelom displaced and reduced due to development of atrium.

C. Classification

Subphylum Cephalochordata includes a single class Leptocardii, a single family Branchiostomidae and only two genera, Branchiostoma with 8 species and Asymmetron with 7 spieces. Asymmetron differs from Branchiostoma in having unpaired gonads on the right side of body and asymmetrical metapleural folds.

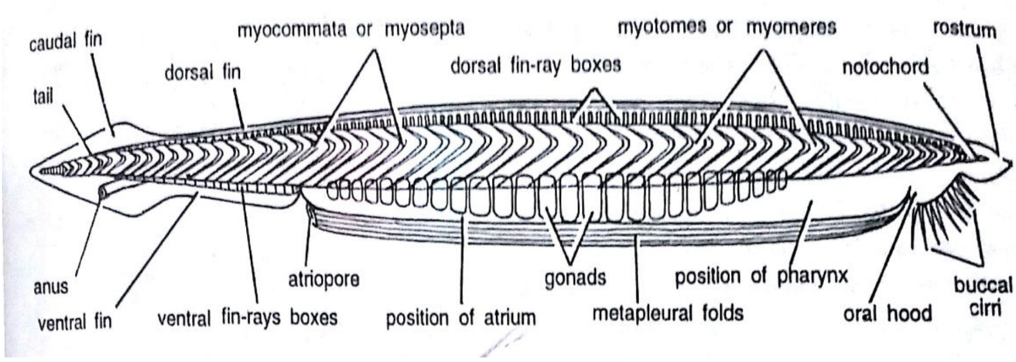


Fig. Branchiostoma

D. Affinities and systemic position of Cephalochordata

Cephalochordates (Branchiostoma) are uniqe in showing affinities with chordates as well as non chordates.

I. Non chordate affinities

Cephalochordates have been regarded to be phylogenetically related to several non chordate groups to one time or other. Only those with more important groups are being summarized below. But these can be overlooked in favour of the more chordate like characteristics of Cephalochordates.

Affinities with Annelida

Some of the common features are :

1. Body bilaterally symmetrical and metamerically segmented.
2. Metamerically arranged protonephridia with solenocytes (as in some polychaetes).
3. Well developed coelom.
4. Closed and similarly disposed blood vascular system and
5. Filter feeding method in some polychaetes.

Objections

In Cephalochordates unlike annelids metamerism is restricted only to myotomes and gonads. Coelom is enterocoelic and not schizocoelic as in annelids. The flow of blood in main blood vessels is in opposite directions in the two groups. Above all the three basic chordate characters of Cephalochordates are not present in Annelida.

Affinities with Mollusca

It was Pallas (1778) who first described and named amphioxus as Limex lanceolatus considering it to be a slug. But the ciliary mode of feeding and respiratory mechanism through water current which are common features of the two groups may be due to similar mode of life. Their anatomy is completely different. Moreover molluscs are unsegmented and their locomotory podium is also unknown in cephalochordates.

Affinities with Echinodermata

Echinoderms have asymmetrical body, enterocoelic coelom and similarly formed mesoderm. Perforations in the calyx of some fossil echinoderms look similar to gill slits of amphioxus. As in Branchiostoma, ophiuroids have similar phophagens (creatine phosphate). But all these similar features nay be because of a very remote common ancestry of the two groups.

II. Chordate affinities

Cephalochordates shows the three basic chordate features in the most typical manner and there is no doubt about its chordate nature. However it shows relationships with all the major groups of phylum Chordata and its real status in the phylum remains uncertain.

Affinities with Hemichordata

Hemichordate and Cephalochordata resemble in having similar

a) Pharyngeal apparatus with numerous gill slits and gill bars,
b) Filter feeding mechanism,
c) Respiratory mechanism,
d) Enterocoelic coelom, and
e) Numerous gonads without gonoducts.

Objections

But muscles in Hemichordata are unsegmented, nervous system distinctly of nonchordate type, gill slits dorsal in position instead of lateral, and a postanal tail is lacking. Moreover inclusion of Hemichordata under Chordata is also uncertain because of doubtful nature of notochord. As such Hemichordata without question are more primitive than Cephalochordata.

Affinities with Urochordata

Branchiostoma (Cephalochordata) and Herdmania (Urochordata) are regarded to be very closely related because of

a) Primitive ciliary feeding and respiratory mechanisms,
b) Large pharynx bearing numerous lateral gill slits, epipharyngeal groove, endostyle and peripharyngeal bands,
c) An ectoderm lined atrial cavity opening to outside through atriopore (atrial siphon),
d) Identical early stages (holoblastic cleavage, gastrulation by invagination) of development, and
e) The ascidian larva having a continuous notochord above it a dorsal hollow nerve cord and a post anal tail with median caudal fin without fin rays.

Objections

But the adult urochordates are extremely degenerate and sedentary animals having several features unrepresented in cephalochordates, such as

a. Body unsegmented,
b. Covered by a test made of ccellulose,
c. With enterocoelic coelom,
d. Without notochord and hollow nerve cord,
e. With a liver,
f. A well developed muscular heart covered by peritoneum,
g. Without nephridia,
h. Sexes united with hermaphrodite gonads and

a) Larva undergoing retrogressive metamorphosis to become the adult.

These differences shows that inspite of close similarities reflecting upon a probable common ancestry, the Cephalochordates are better evolved than the urochordates.

Affinities with Cyclostomata

The Ammocoete larva of lamprey (Cyclostomata) and Branchiostoma show a striking similarity in many characters, such as :

1. Elongated, slender fish like body,
2. Continuous dorsal median fin ,
3. Mouth surrounded by an oral hood and
4. Guarded by a velum and
5. Pharynx having endostyle and gill slits.

Besides these fundamental chordate characters their adults show metameric myotomes, persistent gill slits , velum and a postanal tail.

Affinities with Vertebrata

Besides Cyclostomes, Branchiostoma also resembles other vertebrates in several ways such as

1. Metamerically arranged myotomes,
2. Ture coelom lined by mesodermal epithelium,
3. Postanal tail,
4. Midgut diverticulum comparable with liver,
5. Well formed hepatic portal system and
6. Similar arrangement of main longitudinal vessels with forward flow of blood in ventral and backward flow in dorsal blood vessel.

Objections

Cephalochordates differ from cyclostomes and other vertebrates in most of their primitive features already described, such as (i) lack of head, paired limbs, skull, vertebral column, muscular heart, red blood corpuscles, brain, specialized sense organs, gonoducts, etc., and (ii) in possessing nephridia, atrium, numerous gonads, asymmetry etc.

1.8 Summary

- Chordates are sharply distinguished from nonchordates by three fundamental diagnostic characteristics i.e. a longitudinal supporting rod like notochord, a dorsal hollow or tubular nerve cord and a series of pharyngeal gill slits.
- Phylum Chordata can be divided into two groups:
  Acrania (Protochordata) and
  Craniata (Euchordata)
- The Acrania or Protochordata is divided into three subphyla : Hemichordata, Urochordata and Cephalochordata, chiefly on the position of notochord.
- Hemichordate includes about 80 known species which are generally grouped under two classes: Enteropneusta and Pterobranchia.
- Subphlum Urochordata or Tunicata includes about 2,000 fixed and nearly 100 pelagic species exhibiting high degree of diversity. Urochordata divided into 3 classes : Ascidiacea, Thaliacea and Larvacea.
- Subphylum Cephalochordata includes a single class Leptocardii, a single family Branchiostomidae and only two genera, Branchiostoma with 8

species and Asymmetron with 7 spieces. Asymmetron differs from Branchiostoma in having unpaired gonads on the right side of body and asymmetrical metapleural folds.

1.9 Self Assessment Questions

Section -A (Very Short Answer Type):

1. Which are three fundamental chordate characters?
2. Write down the name of classes of subphylum Urochordata?
3. Write down any two example of class Enteropneusta?

Section -B (Short Answer Type):

1. Describe outline classification of subphylum Urochordata?
2. Write short notes on :
   a) General characters of subphylum Hemichordata
   b) Affinities of Urochordates with other chordate phylum
3. Write short notes on :
   a) Enteropneusta
   b) Ascidiacea

Section -C (Long Answer Type)

1. Give an account on the origin of protochordates?
2. Describe the classification of protochordates upto order with suitable examples?
3. Write a detail account on general characters and classification of subphylum Hemichordata and its affinities with other chordates?
4. Write short notes on:
   a) Affinities of Cephalochordates with other non chordates and chordate phylum
   b) Classification of subphylum Urochordata

1.10 References

- Evolution of chordate structure – an introduction to comparative anatomy by Hobart M. Smith
- Modern text book of Zoology Vertebrates by R.L. Kotpal

Unit - 2

Lower Chordates – II

Structure of the Unit:

2.1 Objectives

By the end of the chapter, the student would acquaint himself with the General characters of Protochordates, salient features of Subphylum – Hemichordata, Subphylum – Urochordata, Subphylum – Cephalochordata, Structure, function and life histories of sessile and pelagic tunicates, *Doliolum, Oikopleura* and

Branchiostoma. Neoteny, its examples, various factors influencing neoteny, Evolution and phylogeny, Evolution, affinities and phylogeny of Protochordates- Hemichordata, Urochordata, Cephalochordata

2.2 Introduction

Distinguishingfeatures of Protochordates: Following are the important characters of group Protochordates:

- They have a small rod shaped notochord on the dorsal side of their body.
- They haveprimitive type of dorsal tubular nervous system.
- They have the primitive type of gill slits
- Protochordates may have the notochord in the different places like in tail region or in head region and in one half region of the body.

On the basis of the position of the notochord the group protochordata is again divided into following subphylum:

(1) **Subphylum -** Hemichordata (Half-chordates)in which the notochord is present in the anterior one half of the body. The representative animal is*Balanoglossus*.

(2) **Subphylum** – Urochordata(Tail-cord) in which the notochord is present in the tail region. The representative animal is *Herdmania*.

(3) **Subphylum** – Cephalochordata(Head-cord) in which the notochord is present in the head region. The representative animal is *Amphioxus*.

All these three subphyla of the group protochordata exhibits primitive characters of phylum chordata but cannot be included under vertebrata because they do not have well developed vertebral column. They have only the initial form of vertebral column in form of notochord in the dorsal side of the body. Protochordates are marine, small & primitive chordates, they lives singly & feed on microbes or particulate matter. There is absence of head, jaw, skull & cranium. Complete body is covered by single transparent layer called Tunic. The body cavity is enterocoelic. Pharynx with pharyngeal gill slits. Circulatory system includes blood without erythrocytes, heart, vessels and sinuses. Body bears protonepheric kidney. Nervous system is primitive. Sexes are separated or hermaphrodite. Development is indirect with larval stage. These are also called as Acraniata because cranium is absent

Hemichordata

These are solitary or colonial, mostly marine, tubicolous, soft and fragile animal. Theirbody is divided in to 3 parts - proboscis, collar and trunk. These are vermiform, triploblastic, bilaterally symmetrical, unsegmented animal. Body wall is made up of single layered epidermis and the lower layer dermis is absent. Animals have true coelom which is divided into 3 parts i.e. protocoel, mesocoel and metacoel. Alimentary canal is straight with terminal anus or 'U' shaped with anus nearer to mouth. Gill slits are present or absent, if present then number is variable. Circulatory system is of closed type. Blood is colourless without blood cell. Excretory system consist of the glomerulus and a proboscis gland lies in proboscis. Nervous system is of primitive type with intra-epidermal nerve plexus. Reproduction is sexual: Animal may be unisexual or bisexual. Gonads numbers may be one or many pairs. Fertilization is external. Development may be direct or indirect with a Tornaria larva. Their food are micro-organism and debris captured by cilary mechanism. There are about 70 species. Examles- *Balanoglossus, Cephalodiscus, Saccoglossus* etc.

Urochordata

All urochordates are exclusively marine and are found in all the seas at level of 5 km depths from surface water. They lives either solitary or colonial life. Majority of animals are sedentary, few are free swimming and pelagic. Body is covered with cuticular tunic or test in adult. Distinct head is absent and body varies in size, form, colours. Notochord present in tail of larva and not in adult. Body divided in to trunk and tail without appendages. Coelom is absent. Alimentary canal is complete with large pharynx perforated by two or numerous gill silts for respiration. Circulatory system is of open type with ventral heart. Excretion is carried out by nephrocytes, pyloric or neural gland. They are bisexual,fertilization is outside the body. Asexual reproduction by budding is common. Development include a small, free swimming tadpole larva with tail with dorsal nerve cord notochord restricted to tail region only. Approximately 2,200 Urochordate species are known Ex. *Herdmania*, *Doliolum*, *Ascidia* etc.

Cephalochordata

Cephalochordates are exclusively marine and show a burrowing life in shallow seas. Body is fish like adapted for burrowing and swimming. Head is absent but tail is present. There is no paired appendages but median fins i.e. dorsal caudal and ventral fins are present. Notochord covers the length of body and extended beyond the nervous system to the tip of the snout. A enterocoelomic true coelom is present. Alimentary canal is complete. It is cilliary feeder. There are no specialized organs for respiration, it occurs by diffusion through general body surface. Circulatory system is of closed type. Hepatic portal system is developed. Protonephredia consisted of specialized cells solanocytes. There is deficiency of sense organs. Dorsotubular nerve cord is without brain and ganglia. Two cerebral and several pairs of spinal nerves are present. Sexes are separated and gonads without gonoduct. Fertilization is external. Development is indirect including free swimming ciliated larva Ex.- *Amphioxus*

2.3 tunicates

Tunicate name derives from their unique outer covering or "tunic" which is formed from proteins and carbohydrates and acts as an exoskeleton. In some species, it is thin, translucent, and gelatinous, while in others it is thick, tough, and stiff.The earliest species of tunicate is supposed to be evolved in the early Cambrian period as per the fossil record. These animals are very close to the vertebrates shown by mobile larval stage, they possess a notochord or stiffening rod and resemble a tadpole. Some tunicates live as solitary individuals, but others in colonies, each unit being known as a zooid. They are marine filter feeders with a water-filled, sac-like body structure and two tubular openings, known as siphons, through which they draw in and expel water. During their respiration and feeding, they take in unfiltered water through the incurrent or inhalant siphon and expel the filtered water through the excurrent or exhalant siphon.

2.4 Structure, function and life history of sessile and pelagic tunicates, *Doliolum*

Doliolum

Phylum:	Chordata
Subphylum:	Tunicata
Class:	Thaliacea
Order:	Doliolida
Family:	Doliolidae
Genus:	*Doliolum*

Doliolum is cosmopolitan marine animal in distribution being observed from both cold and warm oceans of the world. It is free swimming, and pelagic in nature. It is typical, tunicate animal because it exhibits an dimorphic alternation of generation between the two morphologically distinct phases in its life history. The two phases of *Doliolum* life cycle are asexually reproducing solitary phase; and a sexually reproducing gregaria phase.

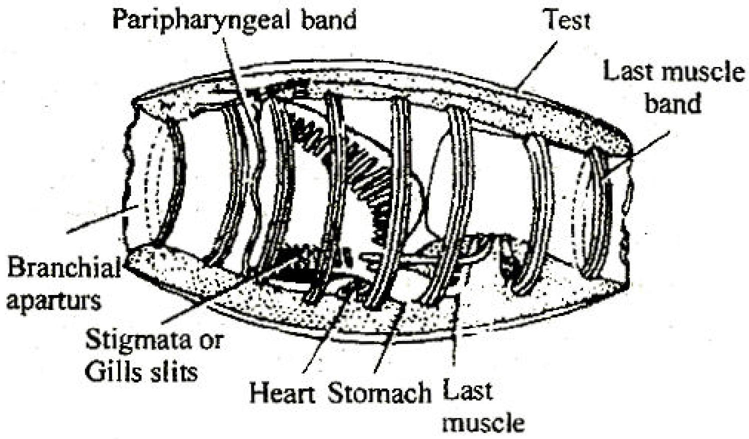


Figure- *Doliolum* Structure

Structure

The solitary phase of *Doliolum*is represented by a solitary gonozooid. It is one to two centimeters long approximately. It is barrel shaped animal with branchial and atrial apertures situated at the opposite ends. The branchial as well as atrial apertures are decorated with 10-12 sensory small lobes border. The gelatinous test is thin, tough, and transparent and contains no cilia. The body wall contains 8 & 9 annular muscular bands, which completely encircle the body like the loop. The 1st and last of these muscular bands serve as terminal sphincter muscles for their respective orifices. In *Doliolum* the tentacles are absent. There is large pharynx with peribranchial or atrial cavity in the posterior end. The postero-lateral walls of the pharynx are pierced by a large number of small stigmata. Thus there is a free straight passage for water, along the long axis of the body from branchial to atrial aperture through the pharynx, stigma and atrial cavity.

During swimming the animal rejecting water out forcefully through the atrial aperture with the help of annular muscular bands. In a few, however, stigmata occur on the lateral walls of the branchial sacs and in these cases the atrial cavity has corresponding anteriorly directed diverticulae. As usual a ciliated peripharyngeal band divides the pharynx into peribranchial zone and the branchial sac proper.

Endostyle is present ventrally in the branchial sac, but on dorsal side the lamina is absent. The remaining part of the alimentary canal is wide, a short oesophagus, a small subspherical stomach and a curved intestine lies ventrally and posteriorly to branchial sac. The heart is mid-ventrally present just posterior to endostyle. The neural complex and dorsal tubercle are at about the mid dorsal side of the body.

Sexual Reproduction

Doliolum is a bisexual animal, the ovary and testes both are present on the left of the middle line of the body. The alimentary canal and their ducts open in the atrial cavity. The ovary is nearly spherical in shape, while the testis is different in shapes in different species. In *D.mullom*testis areshort and narrow, while in *D. varnum*, *D. intermedium* elongated and cylindrical or rectangular, in *D. derticulatum* the testis are elongated anteriorly up to second muscular bandregion.

Development

The eggs of *Doliolum*are small and transparent and are layed at the time of fertilization. The cleavage is holoblastic and gastrula is formed by the invagination within five hours after fertilization. The gastrula extends and modified into cigar shape. At this time or stage it consists of an anterior rostral extension, a trunk region and a posterior tail.

A large perivitelline space is present surrounding the vitelline membrane in tailed embryo. The large median vesicular region of larva and the perivitelline envelope act as float during development. The temporary rostrum get modified into the stalk. Such a rostral outgrowth is commonly observed during development of many ascidians. After gastrulation the tail contains approximately 40 caudal cells. Along each side of the notochord about 40 fibrillated muscle cells form a band. The structure of notochord, number of its cells and the cell forming the muscle bands are just like the most of ascidians tadpoles. The tail is not functional and is retained within peri- vitelline membrane. Therefore it can be concluded that *Doliolum* have a partly relationship with ascidians.

Each tadpole larva of *Doliolum*during metamorphosis into a free swimming adult zooid which belongs to the sexually reproducing gregarine phase of *Doliolum*. This zooid is called as an oozooid or nurses and is characterized by the possession of nine muscular bands, a fewer stigmata confined to posterior wall of branchial sac and an oocyst on the left side of body. Later the oozooid develops in small ventrally placed complex stolon from near its heart and a larger lobe like posterio-dorsal out growth called as cadophore.On the ventral stolon, a number of minute projections or buds develop and become constricted from it. Each constricted bud is a little group of cells arranged in seven strings and covered with an ectodermal outlay. These ectodermal cells multiplies by division and get attached to the cadophore, some in lateral rows and some in median rows. As a result colony is formed in which oldest buds are at the tip of cadophore.

These buds now develop into three type of zooids-

1. **Trophozooid or Gastrozooid**

   Trophozooids are dorsoventral elongated structures with a large funnel shaped branchial aperture and their musculature is vestigial. These zooids are developed from the lateral buds of the cadophore and remain permanently attached to it. These are sterile structures with a vegetative function, aids the entire colony as well as the parents oozooids in respiration and nutrition. The development of gastrozooid is relatively simplified and restricted.

2. **Phorozooids**

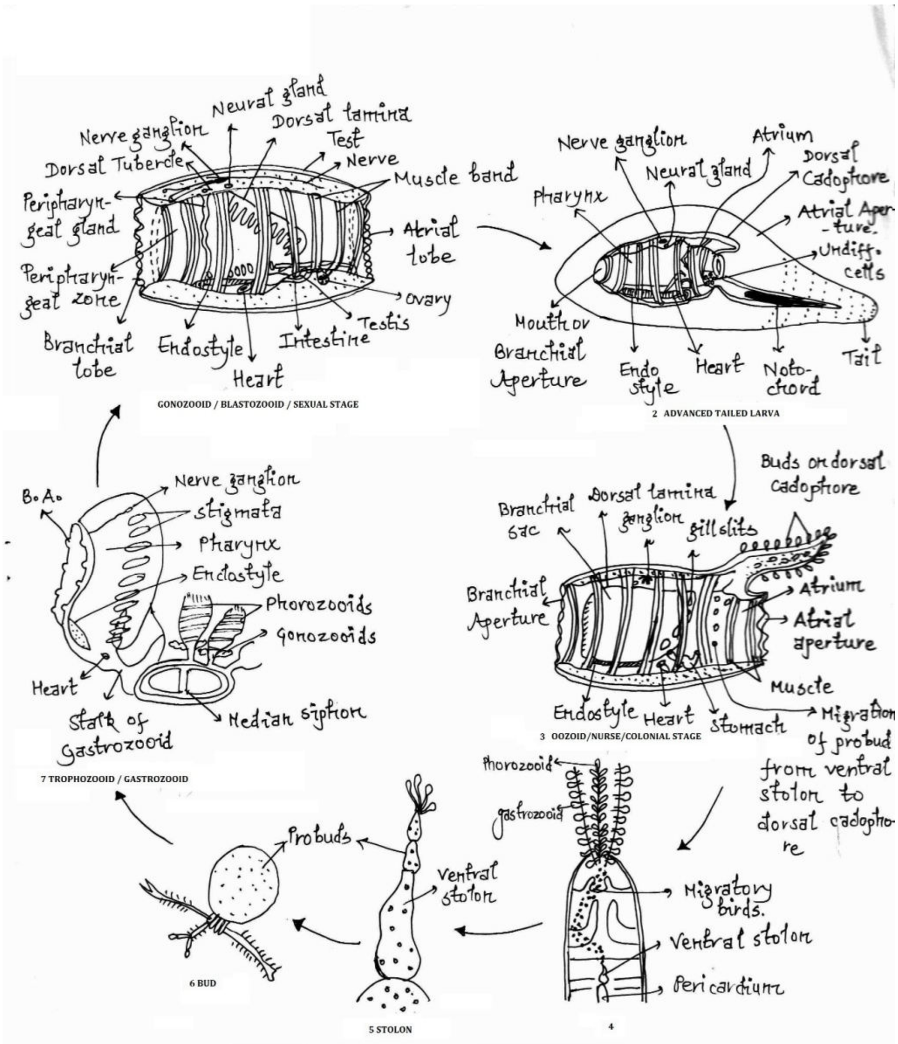
   Phorozooids are developed from some of the median buds of cadophore attached by means of a short ventral stalk. Similar to the gastrozooid these zooids are also sterile but are not permanent sessile on the cadophore. These zooids detach from the cadophore of parent zooid and swim freely in the form of cork shaped bodies having *Doliolum* appearance with 8 muscular bands. The main function of these zooids is to transport the third type of zooids, gonozooids.

3. **Gonozooid**

   As the name indicates, the gonozooids are sexual zooids which becomes matured on the stalks of the free swimming phorozooids. The structure of the gonozooids resemble with the parent but lack ventral stalk. After maturing the gonozooids get detached from the stalk of the parental posterior form and swim freely about and represent the solitary phase.

   The parent zooid present on nurse becomes modified, its branchial aperture, endostyle and alimentary canal, where as its muscular bands increase in thickness and nervous system becomes adapted to serve the function of a swimming organ of the entire colony.

The life cycle of *Doliolum* thus represents the regular alternation of generation between the sexually reproducing solitary phase and an asexually reproducing gregarious phase, the latter giving rise to temporary trimorphic colony, one type of which then develops into solitary individuals.



LIFE CYCLE OF DOLIOLUM

2.5 Structure, function and life history of sessile and pelagic tunicates*Oikopleura*

Oikopleura

- Systematic position
- Habit & habitat
- Shape & size
- Morphology

- House
- Body
- Reproduction

Systematic position

Phylum:	Chordata
Sub phylum:	Urochordata(Tunicata)
Class:	Larvacea (Appendicularia)
Order:	Endostylophora (Copelata)
Genus:	*Oikopleura*

Habit & habitat

Oikopleura is a neotenous, free swimming pelagic, tiny & solitary animal. *Oikopleura* is found in plankton of tropical & temperate zone. It resemble with ascidian tadpole in general characters. It is distributed in all seas except the Polar Regions.The oikopleurids are distributed in the tropical waters of all oceans and seas of the globe, having been reported widely in the Caribbean Sea and the western coasts of the Atlantic Ocean.

Shape & size

Oikopleura is a minute animal & it look like the larva. The animal possessed a rounded body & a long laterally compressed tail, loosely attached to the posterior end of the body. Its special feature is that it secretes the gelatinous test or home or house into which it lives for sometimes.

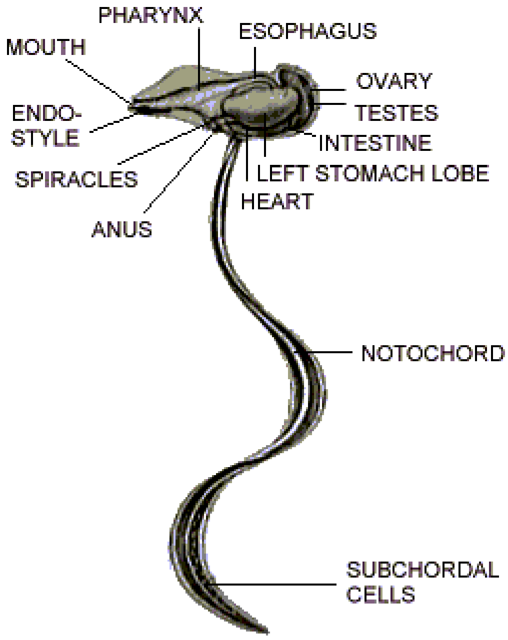


Figure – *Oikopleura* Structure

Morphology

Oikopleura like other appendicularians has a discrete body and tail as an adult and keeps its notochord throughout its life. It resembles a tadpole in appearance and has a transparent body with length of 5mmand a tail about four times that length.Its body is ovoid and the tail slender. It is differentiated into a strong trunk (only 1mm long) and long cylindrical laterally compressed tail.The tail is bend forward under the trunk. It is narrow at the base and contains an axial notochord, ganglionated nerve cord & segmented muscles.

House / Test

The whole body*ofOikopleura*is covered by a covering called as house. The animal secrets a temporary gelatinous house & many times larger than body size.This house is secreted from a special part of skin that is called as oikoplasts epithelium, generally house is made up of tunicin.House consists of inhalant & exhalent openings. Inhalant opening is consisted of sieve strainer which keep out sand & coarse particles. Within house water flows through complicated passages & perforated fold which also provide food filtering devices.House acts as an efficient food filtering apparatus It is protective hydrostatic & respiratory in function.Inhalant opening is called as a Branchial opening while exhalent opening is known as atrial opening. The tail movement produces a continuous current of water. The water flow brings food inside body which is caught by most elaborate filter arrangement in the house.Test can be casted off readily & new test can be secreted.

Body

Animal body has oval & compact trunk and vibratile tail attached to the ventral surface of the trunk and is bent downward and forward thus pointing anteriorly.Tail is laterally compressed and twisted to a less spiral through an angle of 90 degree so that the dorsal edge lies to the left side. Vibration of tail bring locomotion of the body during swimming.

Anatomy

There are two sub-chordal cells outside the central core of muscle in the tail, which are easily observable some half to two thirds of the way down the length of the tail. The mouth has a small lower lip and the buccal glands are small and globular. The endostyle is large, extending nearly as far as the anus. The right lobe of the stomach forms a sac behind the entrance to the intestine. In this species the sexes are separate, unlike in all other known appendicularians, and the ovary or testes are at the rear of the body.

Alimentary canal

The Alimentary canal is 'U' shaped. Mouth or Branchial aperture is anteriorly placed, this large aperture leads into large simple pharynx. Alimentary canal consisting of the spacious pharynx, narrow oesophagus, sac like stomach & tubular intestine.Pharynx is large simple, it bears mid-dorsally epipharyngeal ciliated band, Mid-ventrally hypopharyngeal ciliated band.No dorsal lamina present in pharynx. Endostyle is short tube like & closed from anterior and posterior sides.Their is a single pair of ciliated stigmata placed ventro-laterally each leading into a short funnel shape tube opening to the exterior through a wide atrial pore.Atrial pore is situated slightly behind the anus Anus open out directly on the ventral surface of the body.

Notochord

It is present in tail. It is homogenous rod surrounded with sheath containing nuclei.Muscular bands are present in tail. They are perfectly divided into ten segments on each side.

Nerve Cord

Nerve Cord of *Oikopeura* is dorsal & tubular. It extend from between the mouth and tail end. In the tail it shows ganglionic swelling from which paired nerves are given out. Anteriorly it form a cerebral ganglion (Brain) which is connected with an otocyst and a short richly ciliated tubular process called ciliated funnel. Otolish or statocyst, a minute vesicle is embedded on the left side of cerebral ganglion. The funnel opens into the branchial sac and represented the dorsal tubercles of other tunicates.

Heart

Heart is situated ventrally in front of the stomach & is surrounded by delicate pericardial membrane.There is absence of blood vessels. The blood circulate through blood lacuni in tissues & organs.There is a simple pair of gill slits. They are ciliated and open directly to the exterior by tubular passages.

Reproduction

All *Oikopleura* are bisexual animals and usually protoandrous. The gonads lie posteriorly and open posterior-dorsally by their short duct.Ovary is single and testis may be one or a pair in a body. Ovary releases ova by rupturing of body wall.Fertilization takes place externally in sea water.Cleavage is complete, holoblastic & determine. Cleavage is similar to that of *Herdmania*& result into larval forms resembling tadpole larva ascidian. This larva does not undergo metamorphosis but simply it twist its tail and develop gonad to attain maturity. It undergo no other changes.

Tadpole larva

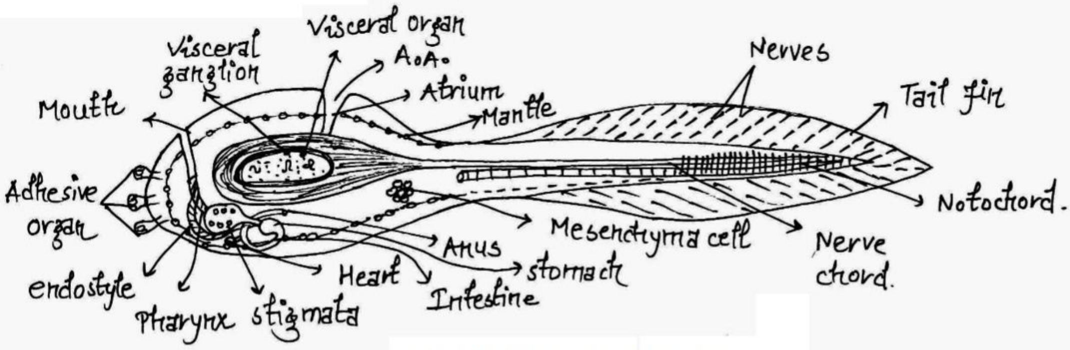
After fertilization within 8-24 hours a typical free swimming larva the ascidian tadpole is hatched out. Its length is of about 1-2 mm and swim actively with the help of its long tail vibrations. The shape of larva superficially resembles with a minute tadpole consisting of an oval trunk and a long laterally compressed tail. The tail is fringed with caudal fin.

The larva represents a full chordate characters. The trunk or the main body of the larva is cylindrical & bears prominent adhesive papillae at its anterior end. The nervous system of the tadpole is dorsal & consist of a long nerve cord extending from behind of mouth to tip of tail.The tip of nerve cord consist of a solid mass of nervous cells called as cerebral cone. The cerebral cone is followed by a pouch like sensory vesicle which contains photo receptor & a balancing organ following the sensory vesicle.

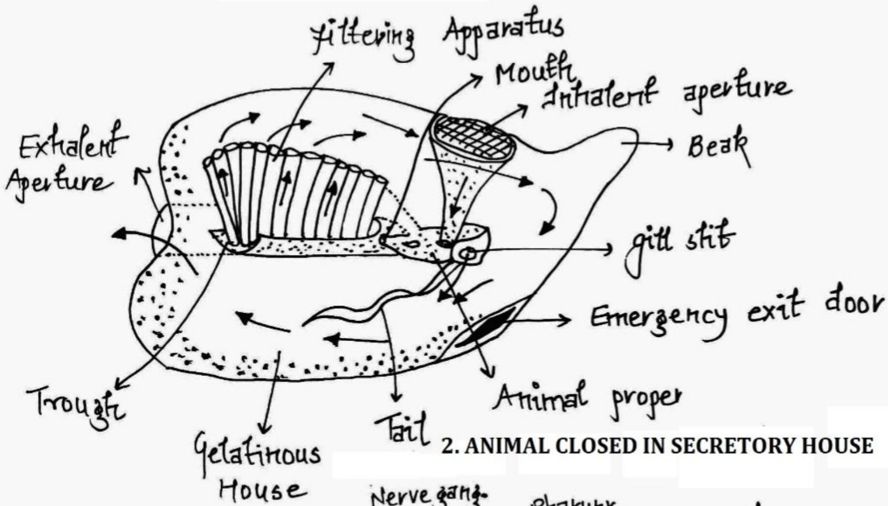
The digestive system is well developed & mouth is on dorsal side and lead into a sac like pharynx having three pairs of stigmata in its wall & a mid-ventral endostyle.The notochord is placed below the nerve cord & it extend from the posterior part of the trunk upto the tip of tail.The long posterior extension of body form a typical vibration tail having dorsal & ventral fin fold.Since there is no arrangement for feeding the life of larva very short.

During its larval phase it is geonegative and photopositive. Later on it becomes photonegative & geopositive, sinks to bottom & attach itself by anterior adhesive papillae. It now undergoes retrogressive metamorphosis during which some already formed organ are last & some undergoes differentiation growth.

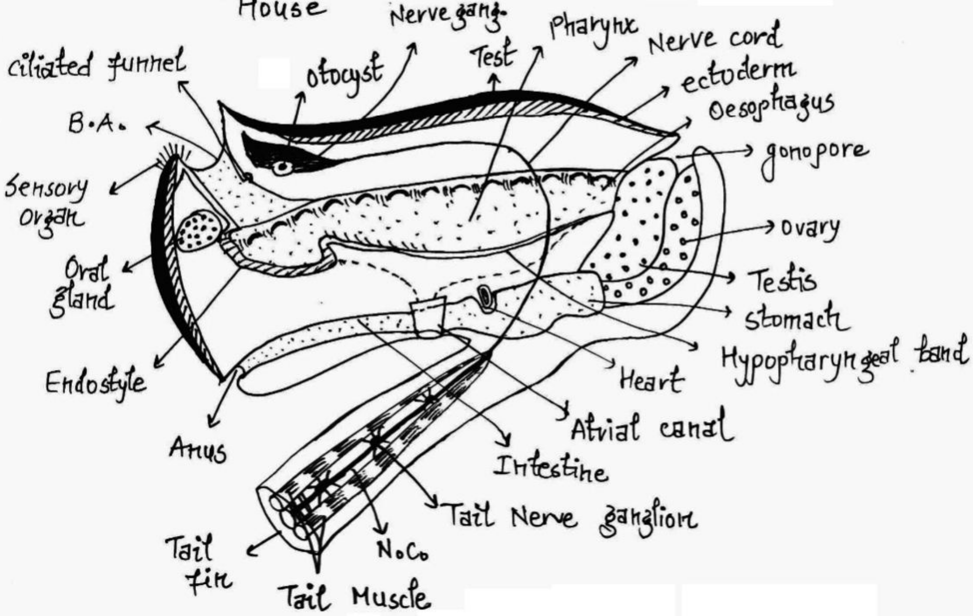
Finally sedentary adult ascidian is developed from a tail free swimming larva. The significant point about the ascidian metamorphosis is that the larva losses all its important chordate character in the process.



1. TADPOLE LARVA OF OIKOPLEURA



2. ANIMAL CLOSED IN SECRETORY HOUSE



3. SAGGITTAL SECTION OF OIKOPLEURA

Figure – Life cycle of *Oikopleura*

Significance

Oikopleura is used as a model organism, a role for which it has several features to recommend it. It has the typical chordate body plan, it is simple to keep and breed in the laboratory, it produces large numbers of eggs and the generation time is only four days at 20 °C (68 °F). The molecular base of a number of aspects of vertebrate development is identical in these simple chordates to those in higher vertebrates. As an example, the brachyury gene and the homolog of the PAX2 gene both play a similar role in the development of tunicates as they do in vertebrates. Complex aspects of vertebral development such as the differentiation of the central nervous system can thus be studied in the laboratory. The genome has been sequenced and contains about 15,000 genes, approximately half the number occurring in vertebrates. Comparison of the genome with that of other chordates will help identify the genes which appeared early in the vertebrate lineage.

2.6 Branchiostoma (Amphioxus)

Phylum: Chordata

Subphylum: Cephalochordata

Class: Leptocardii

Order: Amphioxiformes

Family: Branchiostomidae

Genus: *Branchiostoma*

Species: *lanceolatum*

Branchiostoma is commonly known as ‘lancelet’ or ‘lancet’ because of the sharp, pointed, lance-like ends of the body., It is a marine invertebrate found in soft substrates in shallow seas. It was first discovered by P.S.Pallas in 1778 who took it to be a slug (Mollusca) and naturally gave the name *Limax lancoelatus*. It was in 1834 that O.G.Costa recognized its true position in the animal kingdom and named it *Branchiostoma lanceolatus.* Yarrel in 1836 named it *Amphioxus* which is more famous and familiar to zoologists is retained as the common name for the cephalochordates in general.

Amphioxus is of great interest,it is used as a model organism to study the development of vertebrates.It is the most studied type of subphylum Cephalochordata. In the constitution of its digestive, respiratory, vascular, excretory, skeletal, nervous and muscular systems, and in its manner of development from the egg, it exhibits what appears to be a primordial condition of vertebrate organization, a condition which is in fact, partly recapitulated in the course of the embryonic development of craniate vertebrates. The absence of paired organs of special sense, olfactory, optic and auditory, which are characteristic of higher vertebrates suggest that the animal is degenerate rather than primitive. This is a puzzle that surrounds *Amphioxus*. It is true, however, that a definite gap separates this animal from the lowest fishes in many ways.

Distribution

Branchiostoma lanceolatum is found in shallow seas in the north-east Atlantic Ocean, from Norway, Scotland as well as further south to the Mediterranean Sea and the Black Sea. Its range has expanded through the Suez Canal to the northerly parts of the Indian Ocean and the coasts of East Africa. *Branchiostoma* is cosmopolitian in distribution but more common in warm seas like the Mediterranean. It is abundant near the coast of China and Japan where it serves as food. About 9 species are known of which *B. indicum, B. pelagieum* and *B. ceribbaeum* are found in Indian sea.

Habit and habitat

Branchiostoma burrows in soft substrates such as sand, gravel and shell fragments and is quite particular as to the size of the particles. It occurs from the low tide mark down to about 40 metres. It is found in the shore water and on the sandy beaches of the temperate subtropical and tropical parts of the world. It favours littoral habitat and rarely descends below 50 fathom line.

Branchiostoma is a burrowing animal. During burrowing it put its head first in the sand by vibratory body movement rapidly, but it rests with head protruding out and mouth wide open. Its swimming direction is vertical by means of lateral strokes of the posterior body part. When animal is disturbed by any reason it jumps out of burrow and swims for a short distance, dives into the sand with head downwards, takes a U-turn so that the anterior end protrudes above the sand.

Branchiostoma is a ciliary feeder. A respiratory-cum-food current enters the mouth and passes out through the antriopore. The food consists of planktonic microorganisms. The animal leaves the burrow particularly at night and during breeding season. Sexes are separate. Fertilization is external. Development is indirect.

External morphology

Branchiostoma lanceolatus is about 2-3 inches long, fish-like creature, has an elongated body, flattened laterally and pointed at both ends.The posterior end is more pointed than the anterior end. A stiffening rod of tightly packed cells, the notochord, extends the whole length of the body. Above it is a nerve cord with a single frontal eye. The mouth is on the underside of the body and is surrounded by a tuft of 20 or 30 cirri or slender sensory appendages. The gut runs just below the notochord from the mouth to the anus, in front of the tail. There is a flap-like, vertical fin surrounding the pointed tail. Gas exchange takes place as water passes through gill slits in the mid region, and segmented gonads lie just behind these. The animal is pearly white and semi-transparent which enables the internal organs to be seen from outside. Its appearance is similar to a "primitive fish".

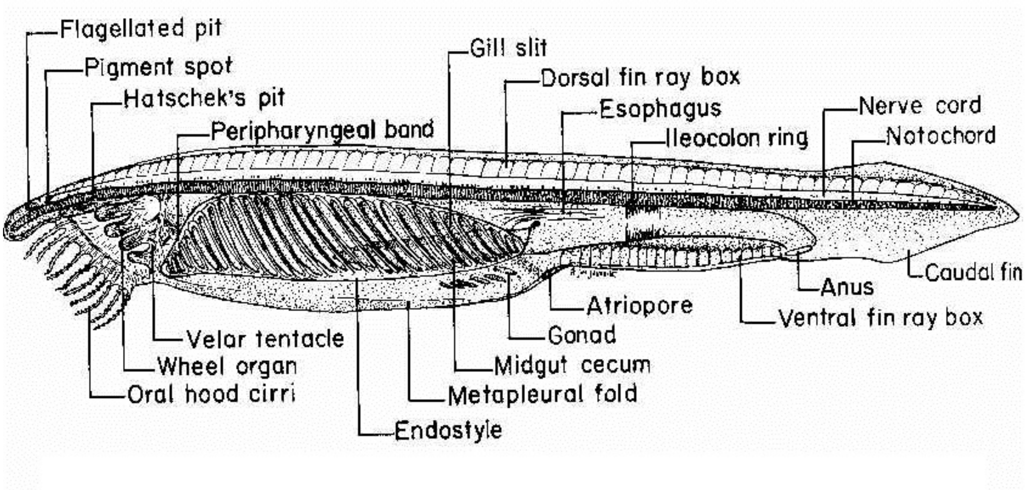


Figure – Lateral view of *Brachiostoma lanceolatus*

Body Divisions

Branchiostoma lanceolatus body can be divided into four regions: cephalic, atrial, abdominal and caudal.

1. **Cephalic region**

   The rostrum or preoral lobe and the mouth part forms the cephalic region. There is an absence of true head. The rostrum is the anterior pointed end of the body. The notochord covers the body portion from the mouth upto the tip of the rostrum. The mouth consists of two portions, an outer vestibule and an inner oral aperture which is surrounded by a sphincter with tentacles called the velum. There is a circlet of twelve velar tentacles which hang backwards into the pharynx. The vestibule is bounded by a lid-like oral hood fringed with tentacle-like sensory processes called buccal cirri. Wheel organ is present at the underside of the oral hood bears a complicated series of ciliated grooves and ridges. Its main function is to set up whirling currents of water.

2. **Atrial region**

   The Atrial region spreads from the mouth over about two-third of the length of the body, ending at a large median aperture, the atriopore. Atrioporemainly works as an aperture for the exit of the respiratory current of water and release of reproductive products therefore it is also termed as branchio-genital region. The ventral side of the body in the atrial region is extended and convex so that the body have a triangular appearance.

3. **Abdominal region**

   The small region between the atriopore and the anus of the body is called as an abdominal region and is characterized by the presence of a ventral fin. The atrial and abdominal region collectively forms the trunk part of the body.

4. **Caudal region**

   The posterior most, postanal region of the body surrounded by a caudal fin is called as caudal region. On the left side of the fin there is an anal opening present.

Fins

The fins of *Branchiotoma* are different from that of fishes. Three unpaired fins are present – the dorsal, ventral and caudal. The dorsal fin is spreaded mid-dorsally along the entire length of the body and posteriorly it is continuous with the much broader caudal fin surrounding the tail. The ventral fin covers the region mid-ventrally between the atriopore and anus. Its lower keel-like portion is continued behind the anus as the expanded caudal fin. The cavity of the dorsal and the ventral fins is divided into a number of fin chambers or compartments, into each of which projects a stout column-like fin ray made up of stiff connective tissue. The caudal fin has no fin chambers and fin rays.

Folds

Two metapleural folds are present longitudinally along the ventro-lateral edge of the atrial region of the body.These are hollow folds, which terminate symmetrically shortly behind the atriopore. Both metapleural folds are supposed to help in burrowing rapidly in sand due to turgidity caused by flow of lymph.

Myotomes and gonads

Myotomes or myomeres are a series of V-shaped muscle bands which are visible through the transparent body wall on each lateral side of the body. The muscles are arranged in 50-75 V-shaped segmental bundles called **myomeres**. Successive myomeres are separated from each other by connective tissue partitions called **myosepta**. The segmental organization of animals is referred to as **metamerism** and the vertebrates, as well as several other phyla of animals, such as annelids and arthropods, are said to be **metameric**. Myomeres on opposite sides of the body are asymmetric and out of register with each other.

A series of gonads are visible between the mouth and the atripore on either side below the myotomes. **There** are about 26 pairs of segmental **gonads which are s**egmentally arranged, paired, rectangular swellings along the ventral margins of the myomeres.*Branchiostoma* is gonochoric. The gametes are shed into the atrium and are carried to the exterior by the water passing through it and out the atriopore.

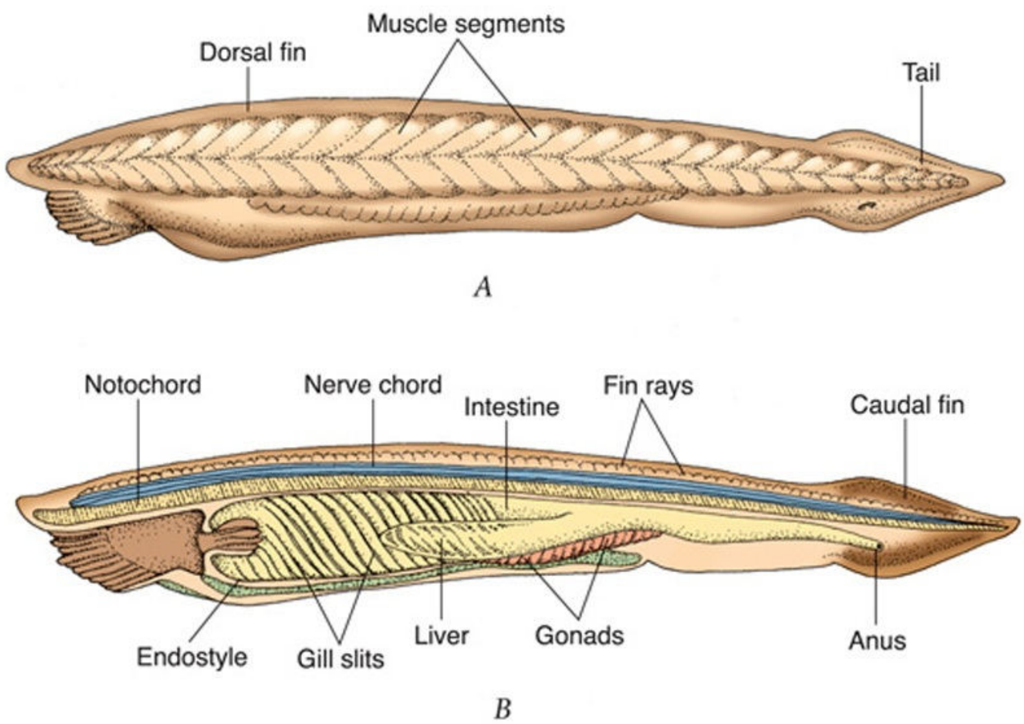


Figure – Morphology (A) Anatomy (B) View of *Branchiostoma*

Integument

The different layers of the body wall are epidermis, musculature and peritoneum.

1. **Epidermis**

   The outermost thin layer covering of body is called as epidermis which differs from that of the higher chordates in structure. In case of higher chordates epidermis is made up of a single layer of cuboidal epithelium while in this case initially cells are ciliated but later on after maturation the outer border becomes slightly cuticularized. The cuticle layer is thin, non-pigmented and perforated at places. The epidermis rests on a thin basement membrane. Below the epidermis lies a tough connective tissue layer distinguished into cutis and subcutis. The cutis layer is thin, compact and fibrous whereas the subcutis is thick, spongy and made up of a gelatinous matrix containing nerve fibres, blood cells and blood vessels. Cutis and the subcutis layers are secreted by few scattered nomadic cells. Gland cells and chromatophores are absent.
2. **Musculature**

   The musculature part is the larger part of the body wall. It consists of sixty segmentally arranged muscle segments or blocks called myotomes arranged on either side of the body along its whole length. The myotomes of the two sides alternate with each other. Each muscle block is V-shaped, the vertices of 'V' being directed anter iorly. Each myotome is enclosed in a complete connective tissue covering called as mycommata or myosepta which separates adjacent myotomes. In the myotomes the muscles are striped and arranged longitudinally. They help in lateral undulating movements of the body. They help in driving water out of the atrial cavity.
3. **Peritoneum :** The last layer of bodywall is thin parietal layer of peritoneum.

Locomotion

Branchiostoma is an inactive animal but when disturbed it swims in water by the contraction of the longitudinal muscle fibres of the myotomes. There is a rhythmic movement of muscle fibres in antero-posterior direction. The notochord prevents shortening of the body during contraction but its elasticity makes contraction more efficient. The animal moves in the forward direction by the propulsive movement of the myotomes. The fins are not very well developed and do not help in locomotion. The caudal fin may sometimes shows transverse movements.

Atrium

The entire branchial apparatus of the adult *Branchiostoma* is surrounded by a large water-filled chamber called the atrium. The atrium is a peripharyngeal cavity of secondary origin and opens to the exterior by a small aperture called the atriopore situated on the ventral side of the body just in front of the ventral fin. The atrium is lined by ectoderm. It arises as long ectodermal folds of the body wall called the metapleural folds which are united ventrally by a transverse shelf, the epipleur. The atrial cavity surrounds the branchial apparatus and the anterior part of the intestine. The gill slits open into the atrium and not directly to the outside as in other chordates. The water that comes through the pharyngeal gill clefts passes out through the atriopore. At the level of the posterior end of the pharynx the atrium gives rise to two forwardly directed pockets, one on each side, pushing into the dorsal coelomic canal with which it is reported to communicate. These pockets are called the brown funnels and are of unknown function. The atrium is protective in function, it protects the delicate pharynx while the animal is in its sandy burrow and helps to maintain an uninterrupted current of water.

Skeleton

There is absence of exoskeleton in *Branchiostoma.*

Notochord

The elongated, narrow, cylindrical structure, notochord is the main axial endoskeleton. It lies mid-dorsally below the nerve cord and gut. The notochord extends from the tip of the snout up to the end of the tail, projecting beyond the level of myotomes. This is probably related with the burrowing habit of the animal. The structure of notochord is complex. It is surrounded by a thick fibrous connective tissue sheath, which is covered by a thin elastic membrane. Initially the chord consists of large vacuolated cells filled with a fluid. In adults it consists of a series of flattened plates arranged with their flat surfaces in the transverse plane of the body. The two types alternatively arranged plates are present – fibrous and homogenous. The notochord does not support the myotomes and other visceral structures like the vertebral column. Its elasticity allows easy body movement and its rigidity prevents shortening of the body during locomotion.

Oral ring

The oral hood part of body is sustained by a thin oral ring made up of separate rodlike pieces. From each piece, rod like prolongations extend into each oral cirrus to support it. The oral skeleton is made up of gelatinous fibrous connective tissue resembling soft cartilage.

Gill rods

The gill rods are gelatinous skeletal rods supporting the gill bars of the gill slits. The gill rods are of two types: the primary gill rods with forked ends and the secondary gill rods with unforked ends. Dorsally the two rods are united.

Fin ray boxes

The fin ray boxes are made up of gelatinous connective tissue. The dorsal fin has a single row while the ventral fin has double row of fin ray boxes.They support the fins.

Food and feeding mechanism

Branchiostoma food consists of pelagic microscopic organisms like diatoms, protozoans, etc. *Branchiostoma* is a lazy animal. It does not move about in search of food. The water continously enters through the mouth and passes into the atrium through the gill slits and atlast it is exited through the aperture atriopore. This animal is ciliary feeder because it feeds upon the food brought by water currentby the ciliary movement. The food particles are filtered from the current of water.The rotatory movement of the cilia of wheel organ causes a whirling current of water into the mouth. The buccal cirri by folding forms a sieve for filtering the larger particles. When water current passes through the enterostome the velar tentacles further filter water, so that only fine food particles enter the pharynx. The chemoreceptors present on the buccal cirri and velar tentacles test the quality of food and water. The food particles are concentrated by mucous secreted by the Hatschek's groove and are pushed through the enterostome into the pharynx. The beating movements of the lateral and atrial cilia of the gill bars drive water out of the pharynx through the gill slits into the atrium and finally out through the atriopore.

The endostyle secrete mucous to the side walls of the pharynx. The food particles entrapped by the mucous move dorsalwards to the epipharyngeal groove by beating of the endostylar cilia and frontal cilia of gill bars. The food particles from the prebranchial region of the pharynx are swept up by the cilia of the peripharyngeal band to the epipharyngeal groove. From the epipharyngeal groove the mucous laden food is carried back to the opening of the oesophagus in the form of a food cord.

Digestive system

The digestive system of *Branchiostoma* includes the alimentary canal and the digestive glands.

The alimentary canal is a straight tube with ciliated epithelium lining. It begins at the mouth and terminates at the anus.The mouth is a wide oval aperture situated antero-ventrally below the rostrum. Mouth is guarded by a frilly membranous structure, the oral hood. The oral hood is a lidlike outgrowth around the mouth. It is fringed with 10-11 pairs of slender, stiff, ciliated, tentacle-like sensory structures called buccal or oral cirri. The oral hood and its cirri are supported by a solid skeletal axis. They have a special nerve supply and musculature by which they can either spread out or bend inwards. The cirri act as a sieve for filtering large particles with the water current.

The mouth opens into thefunnel-shaped buccal cavity or vestibule enclosed by the oral hood. The underside of the oral hood bears 6-8 pairs of finger like processes which form a series of ciliated grooves and ridges collectively known as the wheel organ or rotator organ or Muller's organ. It makes whirling water currents to move food organisms into the mouth. The mid-dorsal groove in the roof of the buccal cavity towards the right hand side of the notochord is the largest and is called the Hatchek's groove or pit. It is ciliated, glandular and secretes mucous. It is also considered sensory in nature. Its function is not known.

The vestibule is closed posteriorly by a circular vertical membrane called velum. It bears an aperture in the centre, called enterostome which opens into the pharynx. The velum has a sphincter to open and close the enterostome. It is also provided with a circlet of 10-12 slender, ciliated and sensory velar tentacles which hang backwards into the pharynx. It acts as a sieve and strains the water entering the pharynx.

The pharynx is a wide, laterally compressed chamber surrounded on all sides by the atrial cavity, except the dorsal. The lateral walls of the pharynx possesses about 200 oblique vertical slits called branchial apertures or gill slits or gill clefts through which the pharyngeal cavity are connected with the atrial cavity. The pharyngeal wall between two adjacent gill cleft is called gill bar. The gill bar is covered by ciliated epithelium. It encloses a mesodermal core containing fibrous connective tissue, blood vessel and a supporting gelatinous skeletal rod.

The cilia on the inner or pharyngeal side are quite long and few and are called frontal cilia, those on the outer or atrial side are short and sparsely distributed and are called atrial cilia and those on the anterior and posterior surfaces are long powered and dense and are called lateral cilia.

The endostyle of *Branchiostoma* is present mid-ventrally along the floor of the pharynx. It consists of five ciliated longitudinal grooves alternating with four longitudinal tracts of mucus secreting gland cells. The cilia of the median longitudinal groove are longer than that of lateral longitudinal grooves. The endostyle is supported by a pair of gelatinous endostylar plates. The endostyle is supposed to be similar structure like thyroid gland of vertebrates.

On the dorsal surface in the roof the pharynx there is a median ciliated epipharyngeal. It helps in food catching. The peripharyngeal bands are a pair of narrow ciliated tracts arising from the anterior end of the endostyle running upwards along the lateral sides of the pharynx and uniting with the epipharyngeal groove dorsally.

The pharynx opens into a short, narrow, tubular and ciliated oesophagus which leads into the gut or intestine. The intestine or gut is long, straight and tubular. It is suspended in the atrial cavity by a dorsal mesentry. Internally the intestine has many ciliated tracts. The intestine has three distinct regions. The anteriormost part is the midgut which is wider than remaining part of the intestine. It modifies posteriorly and gives rise to a narrow, large blind pouch called midgut diverticulum or liver, which lies to the right side of the pharynx. The midgut diverticulum or liver is the only digestive gland in *Branchiostoma*. It contains numerous gland cells which secrets digestive enzymes. The hind end of the midgut is marked by a specially ciliated region called the iliocolic ring whose cilia rotate the food tube, the hind-gut. The terminal part of the hindgut is the rectum which opens to the outside by anus.The anus is a small circular aperture present at the base of the caudal fin. It is controlled by a sphincter.

Physiology of digestion

The digestive enzymes are secreted by the epithelial lining of gut and the midgut diverticulum. The main digestive enzymes of*Branchiostoma* are amylase, lipase and protease. All the regions of the gut behind the oesophagus produce their own secretions and those of the midgut diverticulum are driven out into the gut by ciliary action. The food passing through the gut mixes with these secretions and process of digestion starts in the midgut and continues till the gut contents pass out. In the iliocolic region the food undergoes rotation for breaking up into smaller pieces and missing of the digestive enzymes with the food. Both extracellular and intracellular types of digestion are shown by this animal. Intracellular type of digestion takes place in the hind gut region. Some of the digested food is absorbed by the mid gut while mainly absorption takes place in the hind gut.

Circulatory system

The circulatory system of *Branchiostoma*is not well developed because it has no heart and the blood is without any respiratory pigment. It have closed type of circulatory system and resembles like the circulation of other chordates. The circulation is brought about by slow waves of contraction that pass along the major arterial vessels in such a way that the blood is driven forwards in the ventral vessel and backwards in the dorsal vessel. The blood vessels are muscular and contractile in nature. There is no structural difference between the arteries and veins but they are named so due to their similarities with blood vessels of higher chordates.

The circulatory system of *Branchiostoma* mainly consists of following components:

1. **Sinus venosus** : It is a large, thin-walled sac lying just below the posterior end of the pharynx. It receives blood from all parts of the body.
2. **Ventral aorta** : From the sinus venosus arises a large branchial artery, the ventral aorta or subendostylar aorta or truncus arteriosis, which runs forwards in the ventral wall of the pharynx below the endostyle in the subendostylar coelom. It is contractile and drives blood forwards. From the ventral aorta arise several fine vessels, the afferent branchial arteries, on each side. Each branchial artery has a small contractile dilation at the base called bulbillus or bulbule, presumably acting as a branchial heart. The afferent vessels of the primary gill lamella communicate with similar vessels in the secondary gill lamella by transverse vessels running through the synapticulae. While traversing the vessels the blood is exposed to the aerating influence of the respiratory current. The afferent vessels leave the branchial lamellae dorsally as efferent branchial arteries which open on each side into longitudinal vessels, the right and left lateral dorsal aortae. Before opening into the dorsal aorta each efferent vessel splits up into a capillary network called nephric glomerular sinus or glomus in the nephridium.

3. **Dorsal aorta** : The dorsal aorta is a pair of longitudinal vessels, the right and left lateral dorsal aortae lying one on either dorso-lateral side of the pharynx. Anteriorly both the aortae are continued forward as internal carotid arteries to supply the snout region. The right artery is relatively delicate. Posteriorly both the aortae unite behind the pharynx to form a single median dorsal aorta which runs between the notochord and intestine and continues in tail region as the caudal artery. The blood flows backwards in the dorsal aorta. The median dorsal aorta send a number of intestinal arteries to the intestine which form a capillary network or plexus in the intestinal wall. From the lateral and median dorsal aortae blood is carried by a system of lacunae to supply myotomes, body wall, lymph space, etc. There are no true capillaries.

   From the lacunae the blood is collected into veins of which the important ones are:
4. **Subintestinal vein:** The blood from the tail region is collected by a mid-ventral caudal vein and from the intestine by several intestinal veins which form a plexus on the gut. The caudal vein joins the intestinal veins to form a large median subintestinal vein in which blood flows anteriorly.
5. **Hepatic portal vein:** The subintestinal vein continues forward as the hepatic portal vein which extends along the ventral side of the midgut diverticulum and breaks up inot capillaries in that organ. From the liver blood makes its way into a hepatic vein which extends along the dorsal side of the digestive gland, runs downwards and forwards and joins the sinus venosus. All the blood from the intestine has to pass through the liver before reaching the sinus venosus, hence it is said to constitute the hepatic portal system.
6. **Cardinal veins:** There are two pairs of cardinal veins, the anterior and posterior cardinal veins which collect blood from ventro-lateral region of the body. The two unite just behind the pharynx to form a pair of vessels, the ductus Cuvieri or common cardinal vein which runs ventrally across the atrium to join the sinus venosus.
7. **Parietal veins:** There is a pair of parietal veins one on either side, collecting blood from the dorsal body wall. For some distance they run above the intestine and then turn ventrally to join the sinus venosus.

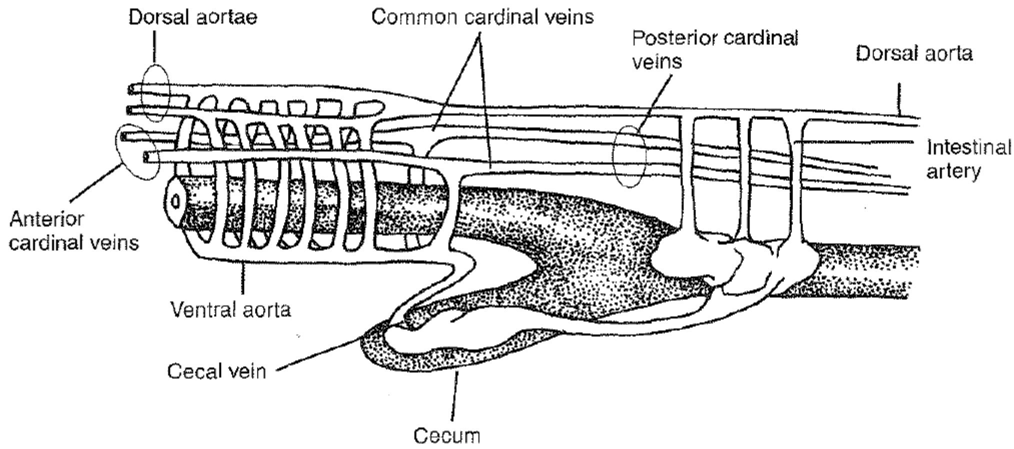


Figure- Circulatory system of *Branchiostoma*

Respiratory system

There is a deficiency of any specialized respiratory organs in *Branchiostoma*. Pharynx is supposed to be respiratory in function because it have richly vascular walls. Some gaseous exchange between blood and sea water takes place through the pharynx. Some researchers suggested that the exchange of gases takes place through the whole surface of the body, particularly the wall of the atrium, fins and metapleural folds containing lymph spaces.

Excretory system

The excretory system of *Branchiostoma* consists of protonephridia, different from chordates. It is similar to the protonephridia of many invertebrates like platyhelminths, annelids, branchiopods, etc. Protonephridia is ectodermal in origin whereas the vertebrates' kidneys are mesodermal in origin.

1. **Protonephridia**

   In *Branchiostoma*90-100 pairs of protonephridia are arranged segmentally in the dorso-lateral wall of the pharynx, one above each gill slit. It is small, simple, ciliated, closed, thin-walled, bent tube of ectodermal origin. It consists of a small upper horizontal limb and a long lower vertical limb. The horizontal limb is situated in the dorsal coelomic canal and opens into the atrium by a small ciliated excretory pore or nephridiopore situated on the dorsal edge of the gill slit. The vertical limb also lies in the coelomic canal of the primary gill lamella and terminates blindly. The anterior and dorsal surfaces of each nephridium bear numerous small branches, each covered with numerous elongated flame cells called as solenocytes. A simple nephridium may bear upto 500 solenocytes. Each solenocyte is an elongated cell, tiny circular, nucleated cell body and a long stalk or tubule that opens into the nephridial lumen by a separate aperture. A long vibratile flagellum, arising from a basal body, runs through the tubule and works freely into the lumen of the nepridium. It is resembles like a flickering flame. The nephridium is richly supplied with blood vessels. The tuft of solenocytes project into the dorsal coelomic canal and become directly bathed in the coelomic fluid, this facilitates extraction of nitrogenous wastes. The nitrogenous wastes extracted by the solenocytes is discharged into the atrium by the nephridiopore and passed out through the atriopore with the outgoing water.

Some other cells and organs are also supposed to be excretory in function.

2. **Nephridium of hatschek**

   Hatschek is ectodermal in origin and resembles the paired nephridia in structure. It is present in the roof of the oral hood, slightly to the left and ventral to the notochord. It is a straight narrow tube which opens at its posterior end into the pharynx just behind the velum and ends blindly anteriorly just in front of Hatschek pit. Its entire surface is covered by several solenocytes which extract the body nitrogenous wastes.

3. **Brown funnels**

   Brown funnels are sac-like brown funnel shaped structures. A pair is present dorsally near the posterior end of the pharynx. The narrow anterior end of the funnel opens into the dorsal coelomic canal of its side, while the broad posterior end opens into the atrium. According to some workers they are considered as receptor organs.

4. **Renal papillae**

   These papillae groups are present in the floor of the atrium and are supposed to be excretory in function.

Nervous system

*Amphioxus*have simple nervous system, consisting of a central nervous system which includes the hollow, tubular nerve cord dorsal to the notochord.The peripheral nervous system consists of nerves arising from the nerve cord and the autonomic nervous system have the nerve plexes.

Nerve cord

The nerve cord resembles like vertebrates, but the anterior end terminates bluntly in the rostrum just behind the anterior end of the notochord, and is slightly enlarged to form the cerebral vesicles or brain. The posterior part is called the spinal cord which reduces posteriorly just before the posterior end of the notochord. A central canal in the nerve cord is called as neurocoel which is filled with cerebro-spinal fluid. The nerve cord consists of inner grey matter and outer white matter.

Nerves

The peripheral nervous system consists of two sets of nerves cephalic and spinal nerves. The first two pair of nerves arises from the cephalic vesicle are called cephalic nerves. They innervate the snout, oral hood and buccal cirri. They are purely sensory in nature. The paired nerves which arise from the nerve cord behind the cephalic vesicle are called spinal nerves. One pair arises in each segment. Spinal nerve has a dorsal root and a ventral root, but the roots do not join to form a single mixed spinal nerve as in vertebrates. The ventral roots lie opposite to the myotomes, to which they carry motor fibres and these. The sensory fibres are like neuro-secretory cells of the invertebrates. The cell bodies are not collected into spinal ganglia, but lie either close below the epidermis or scattered along the course.

Autonomic nervous system

This system consists of two nerve plexes situated in the gut wall. The plexes are connected to the nerve cord by visceral nerves arising from dorsal root in each segment. The main function of this system is to control the involuntary muscles of the gut wall.

Reproductive system

Amphioxus is unisexual but there is no sexual dimorphism.

Gonads

The gonads are present ventro-laterally from the middle of the pharyngeal region up to the atriopore. These hollow sacs structures are arranged metamerically, one pair in each of the segments 25 to 51, thus are 26-27 pairs of gonad in adult *Amphioxus*. They are clearly visible through the transparent body wall. They are mesodermal in origin. They are covered on the outer side by the body wall and on the inner side by the atrial epithelium. Each gonad has an inner primary gonadial cavity which is completely surrounded by an outer secondary or perigonadial cavity. The germ cells are produced from the wall of the primary gonadial cavity.

Gonoduct

In *Amphioxus*, gonoducts are absent in the gonads. When gonads becomes matured the overlying tissue ruptures and the sex cells are released in the atrium to move for atriopore along with the outgoing water current. After the release of the sex cells the aperture in the tissue closes.

Development

Development is indirect, free swimming larval stage is present during development. The early stages of development resembles with invertebrates (Echinodermata) and vertebrates also.

Fertilization

Fertilization is external in surrounding water. The egg is 0.1 mm in diameter. It contains very small quantity of yolk. The first polar body separates and comes to lie just outside the vitelline membrane by the time the egg reaches the sea. The cytoplasm of the egg is divided in two layers : a peripheral layer, free from yolk, lying just below the vitelline membrane ; and a mass of yolky cytoplasm which lies mainly towards the vegetative pole of the egg. A major part of the animal pole is occupied by the egg nucleus (germinal vesicle).The sperm of *Amphioxus* is the smallest among the chordates. It is about 18 ulong. It enters the egg near the vegetal pole and this stimulates maturation of the egg. The second polar body is is released and comes to lie near the animal pole just within the vitelline membrane.

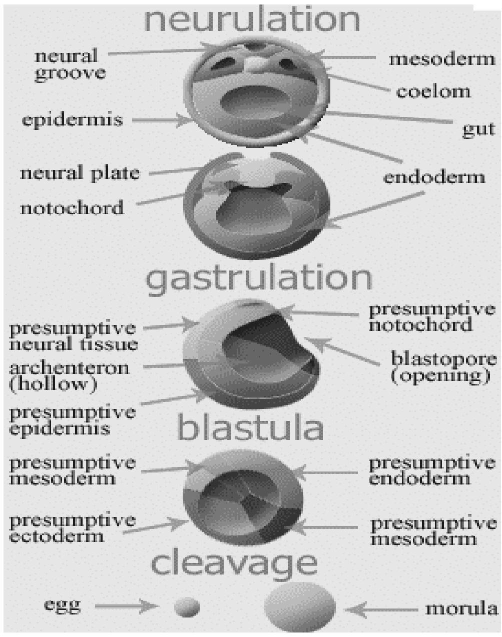


Figure – Development of *Branchiostoma*

Cleavage

The cleavage is holoblastic, the egg is completely divided into blastomeres. cleavage is a rapid process and begins soon after fertilization. The first cleavage is vertical and divides the egg into two equal halves. The second cleavage is also vertical, at right angles to the first. The third cleavage is horizontal passing just above the equator dividing the four blastomeres into an upper tier of four small cells, the micromeres and a lower tier of four large cells, the macromeres. At the sixth cleavage a ball of 64 cells called morula is formed. After sixth cleavage, process becomes irregular and the cells tend to flatten out against each other. The cleavage continues till somewhat spherical blastula is formed. It is somewhat pear-shaped, the pointed end being posterior. In the next stage the blastula flattens on one side and becomes hemispherical. Its wall show areas of histologically distinct cells. The bulk of the ventral half of the blastula (flat surface) is occupied by columnar cells form a plate-like area in the dorsal part of the blastula. The cells of the mesodermal crescent become smaller and granular and lie on the postero-lateral sides of the blastula. A small area of slightly small notochord forming chorda cells lie in the antero-dorsal part just behind the endodermal plate.

Gastrulation

Further changes convert the single layered blastula into double layered gastrula and the process is termed gastrulation. The blastocoels is gradually reduced in size and a secondary cavity called archenteron is formed, its opening is called blastopore (which later becomes anus) and its rim the lips of blastopore. At this stage the embryo is a cup-shaped, double- layered gastrula. The embryo now elongates and its dorsal side flattens. The dorsal lip grows backwards as a result of which the blastopore shifts from a nearly dorsal to posterior position, becomes reduced in size and persists as the anus.

Organogenesis

Formation of first definite organ begin now. This is known as organogenesis. The ectoderm will give rise to epidermis, nervous system and receptors; the endoderm will give rise to the alimentary canal and midgut diverticulum and the mesoderm will form muscles, connective tissue and germ cells.Formation of neural tube and notochord (notogenesis). Soon after the formation of mesodermal sacs, the embryo grows in length rapidly and is called neurula. It has an elongated fish-like body. The neurula hatches by rupturing the vitelline membrane into a minute free swimming larva which is incapable of feeding as the mouth and anus are not yet formed.

Larval development

The larva keeps on swimming for sometimewithout feeding. Then mouth develops and the anus is formed on the left side of the mid-ventral line near the posterior end of the body. The development now becomes asymmetrical due to spiral movement and method of feeding. The first two coelomic pouches also grow asymmetrically, the right one grows large and becomes the preoral body cavity or the cavity of the head and the left becomes the preoral pit or Hatchek's pit.

Simultaneously with these changes the pharynx becomes enlarged and gill clefts also appear but not in a regular sequence from front to rear. The first cleft arises before mouth opens, as a diverticulum from the anterior end of the pharynx on the right side. The wall of the pharyngeal floor become modified to form a V-shaped ridge of ciliated cells, this is the endostyle and its cells become glandular later on. The excretory organs are also formed during larval life. The genital organs also developes in form of small buds of cells which sprout from the anterior angles of the myotomes. They soon become cut off from the myocoel and project into the gonocoel.

Metamorphosis

First of all the larva settles down to the bottom, starts burrowing in the mud and gradually developes into adult. The changes which occur during metamorphosis are : the first gill slit, ectodermal cilia and the club-shaped gland disappear, and the mouth becomes anterior. Oral hood and cirri, wheel organ and velum develop, the gill slits increase in number because the primary gill slits are subdivided by tongue bars. The liver begins to develop as a little pouch of the gut just behind the pharynx. Nephridia develop. The tail region elongates and the notochord extends into the rostrum. The larva of *Amphioxus* is of special interest, especially its asymmetry. The strange sequence of gill formation, the large left-sided larval mouth and the club-shaped gland are some asymmetrical larva of *Amphioxus* becomes metamorphosed into an adult which is nearly bilaterally symmetrical. The larva *Branchiostoma* is so extraordinary because the initial bilaterally symmetrical embryo should pass through an asymmetrical pelagic larval stage in order to attain the form of a practically symmetrical burrowing adult.

The primitive features which *Amphioxus* seems to share with the prechordate ancestor include

1. Absence of jaws for biting.
2. Absence of paired fins.
3. Simple one-layered epidermis.
4. Simple straight tubular intestine with a simple liver diverticulum and the ciliary method of feeding.
5. Naked notochord from end to end.
6. Complete myotomic segmentation from end to end.
7. Simple circulatory system without a specialized heart.
8. Segmental nephridia which are not coelomoduct.
9. Segmental gonads with no ducts.
10. Separate dorsal and ventral roots of spinal nerves.
11. Small almost yolkless egg, hollow spherical blastula, embolic gastrulation and formation of anterior coelomic pouches.

Specialized features

1. The peculiar pharynx, with very large number of gill slits is one such important feature. The cilliary feeder relies on it for the maintenance of water current from which food is shifted. The food shifting is made possible by the presence of elaborate velum and oral hood equipment.
2. The atrium and atriopore is another such specialized feature.

The degenerate characters are:

1. The reduced brain and sense organs probably in response to its sedentary life.
2. Then extension of notochord up to the anteriormost tip of the body.

Amphioxus has been regarded as a generalized chordate. Its structure throws much light on the form of ancestral chordate, but unfortunately gives no hint of the way in which it might have evolved from any known type of non-chordate animal.

2.7 Neoteny

Introduction

- The term **neoteny** is derived from Latin *neotenia*; *neo* + Greek *teinein* = to extend, meaning when larval life is extended.
- Neoteny term was coined by Kollwann.

- **Neoteny** also called **juvenilization**, is the retention, by adults in a species, of traits previously seen only in juveniles.

Neoteny examples

- Larvaceans (or appendicularians) are small, transparent and solitary and **neotenous** urochordates, they achieve sexual maturity and reproduce without losing the larval form.
- Retention of embryonic cartilaginous skeleton in adult in chondrichthyes and the larval gills in some adult salamanders.

Effect of environmental factors

- Environmental factors affect metamorphosis in several ways.
- Abundance of food, cold temperature or insufficient iodine (component of thyroxin hormone that induces metamorphosis) may cause failure of metamorphosis and retention of larval features.
- When axolotls are experimentally treated with thyroxine or TSH, these axolotl lose their gills, assume lungs and become adult air breathing.

Extrinsic Factors

- Abundance of food
- Deep water
- Saline water
- Low temperature

These factors cause retention of larval features, inhibits thyroxin secretion, arrest of metamorphosis.

Intrinsic Factors

- Research shows that neoteny occurs because the hypothalamus of the brain fails to produce the hormone that causes the pituitary to stimulate the thyroid gland to produce growth hormones that trigger metamorphosis.
- The neurosecretory apparatus of the hypothalamus produces a substance called thyrotropin releasing factor (TRF) which stimulates the anterior lobe of pituitary gland to produce Thyroid Stimulating Harmone (TSH), which inturn enhances the rate of thyroid secretion

Significance Of Neoteny

- Weismann thought neoteny to be a case of retarded evolution or atavism, that is reversion to ancestral conditions.
- At present this is now regarded to be of secondary specialization of amphibians.
- A physiological adaptation of advantage to amphibians.

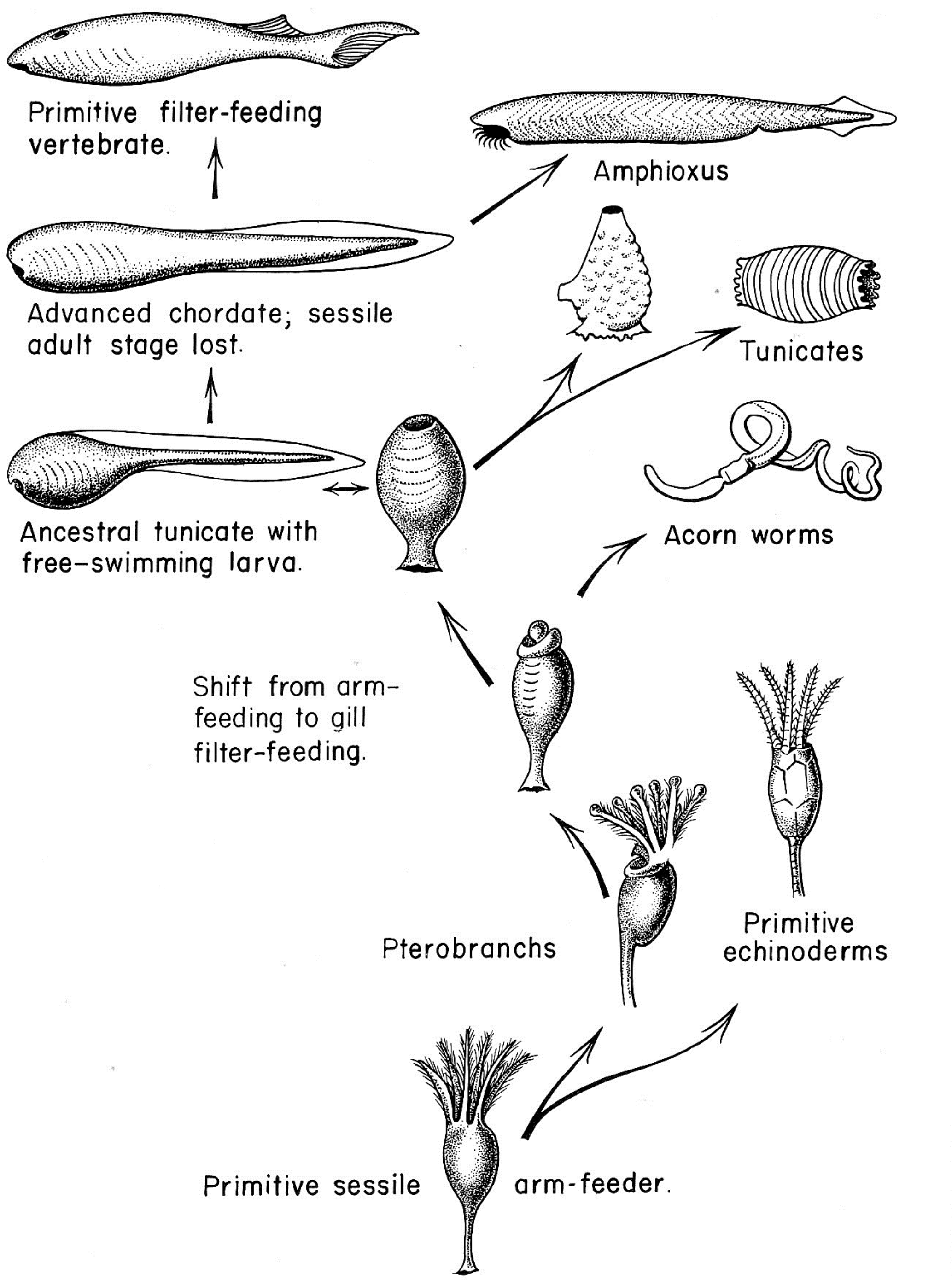


Figure : The tunicate origin of vertebrates by neoteny and further traces the chordates back to a sessile, lophophorate creature that was the common ancestor of living pterobranchs and echinoderms. (From Romer, A. S. 1964. *The Vertebrate Body.* W. B. Saunders. Philadelphia.)

2.8 Evolution and phylogeny

Chordates evolved sometime during Cambrian period, 500 million years ago during Cambrian explosion, almost at the same time when invertebrates were beginning to evolve. They may have evolved from some freshwater forms as Chamberlain (1900) pointed out that all modern chordates possess glomerular kidneys that are designed to remove excess water from body. However, early fossils of chordates have all been recovered from marine sediments and even modern protochordates are all marine forms. Also glomerular kidneys are also found in some marine forms such as myxinoids and sharks. That makes the marine origin of chordates more plausible. Chordates evolved from some deuterostome ancestor (echinoderms, hemichordates, pogonophorans etc.) as they have similarities in embryonic development, type of coelom and larval stages. Fossils of the earliest vertebrates are known from the Silurian-Devonian period, about 400 million years ago. The following theories have been given to explain the origin of chordates:

1. Echinoderm Origin. The theory was given by Johannes Muller (1860) and is based on the comparative studies of larval stages of echinoderms and hemichordates. Tornaria larva of hemichordates resembles echinoderm larvae such as Bipinnaria, Auricularia, Dipleurula and Doliolaria, which all possess ciliary bands and apical tuft of cilia. Johannes Muller, W. Garstang and DeBeers proposed that echinoderm larvae gave rise to chordates by neoteny. Also like chordates, echinoderms are also deuterostomes and possess mesodermal skeletal elements.

The discovery of fossil echinoderms called **Calcichordata** from Ordovician period (450 mya) further confirms echinoderm ancestry of chordates. Calcichordates were asymmetrical animals which demonstrate affinities with both echinoderms and chordates but their skeleton is made of $CaCO_3$ whereas in vertebrates the bones are made of hydrated Ca and phosphate. They had large pharynx with a series of gill slits, each covered with flaps for filter feeding, a small segmented body and a postanal tail. A perforated pharynx for filter feeding appears to have evolved in diverse groups of animals during Cambrian-Orodovician periods when planktons were abundant in water.

2. Hemichordate Origin. Romer (1959) suggested that ancestral deuterostomes were sedentary tentacle feeders whose mucous-laden ciliated tentacles served to trap planktons as they were waved in water as do the modern lophophorates and pterobranch hemichordates, *Cephalodiscus* and

Rhabdopleura. By some mutation pharyngeal gill slits evolved in these ancestors, which made the pharynx sieve-like to trap planktons as the water current passed through it. Extant pterobranchs possess both ciliated arms and pharyngeal gill slits. Tornaria larva of hemichordates shows phylogenetic relationship with echinoderm larvae and hemichordates also show affinities with chordates.

3. Urochordate Origin. W. Garstang (1928) and N.J. Berrill (1955) gave importance to the tadpole-like larva of urochordates which carries typical chordate characters, namely, a notochord in tail along with segmented myotomes, dorsal hollow nerve cord, sense organs and pharyngeal gill slits. Garstang (1928) suggested that chordates evolved from some sessile filter feeding urochordate by the larval stage evolving into adult by neoteny and by losing the sedentary adult stage.

4. Cephalochordate Origin. Chamberlain (1900) studied the primitive and advanced characters of cephalochordates and proposed that while extant cephalochordates possess all chordate characters in typical state, they also show some primitive features of non-chordates, such as, absence of heart, head, sense organs, respiratory pigment, filter-feeding mode of food capture and excretion by solenocytes. Fossils of 60 specimens from mid-Cambrian of the earliest chordate, *Pikaia gracilens* have been discovered from Burgess Shale in British Columbia, Canada. The Amphioxus-like fossils show streamlined, ribbon-shaped, 5 cm long body having notochord in the posterior two-third of body and myomeres. It has a small head with two tentacles and gill slits in the neck region. Other chordate-like fossils are: *Cathaymyrus* from early Cambrian sediments in China and *Palaeobranchiostomata* from early Permian from South Africa that appears to be more similar to Amphioxus.

5. Combined theory. E.J.W. Barrington (1965) combined all the above theories and proposed that the common ancestor of echinoderms and chordates was a sessile ciliary arm feeder that lived in the plankton-rich environment of the Cambrian. Modern Crinoidea (Echinodermata), Pogonophora and Pterobranch hemichordates evolved from a similar ancestor by retaining the original mode of feeding, perhaps because they continued to inhabit the same environment as occurred in ancestral days. However, pharyngotremy (perforation of pharynx with gill slits) must have evolved in a large number of groups at that time, which must have been much more superior method of food gathering by filtering water through pharynx as compared to ciliated arm feeding. Hence, the sedentary Protoascidians of that time lost ciliated arm feeding and adopted pharyngeal filter feeding as the only method of food gathering. Sometime later, when the plankton population in water declined, free-swimming tailed larva of these urochordates did not metamorphose and became a neotenic adult, since free-swimming mode was superior in food searching at a time of food scarcity. Cephalochordate-like ancestors evolved by perfection and expansion of chordate characters that were already present in the ascidian tadpole larva. We already have fossils of such primitive chordates, e.g. *Pikaia gracilens* from mid-Cambrian.

2.8 Affinities of Protochordata (Hemichordata, Urochordata, Cephalochordata)

- The term Protochordates means 'the first chordates' showing primitive features.
- The protochordates differ primarily from the vertebrates in not having a backbone.
- These animals of phylum chordata, resembling in possessing gill slits, notochord, and dorsal hollow nerve cord, or at least traces of these.
- The notochord is modified into the vertebral column in vertebrate chordates.
- Paired pharyngeal gill slits connect the lumen of the pharynx with the exterior and originally functioned in suspension feeding with respiration being added later.
- The protochordates shows various affinities to various other groups under following categories:-

Hemichordata

The group *Enteropneusta*, was established by Gegenbaur in 1870 which was replaced by term Hemichordata by Bateson (1885).After this due to their peculiar anatomical organization and embryology, these are known as Hemichordates.

[I] Affinities with Chordata

William Bateson (1885), proposed the closer affinities between Hemichordata and Chordata. Their resemblance was based on the presence of the three fundamental chordate characteristics in Hemichordata, that is, (i) a notochord, (ii) a dorsal hollow nerve cord and (iii) the pharyngeal gill-slits (pharyngotremy).

Affinities with Urochordata Hemichordates are nearest to Urochordata, as they resemble with them in various aspects. The structure and function of pharynx and branchial apparatus in hemichordates are similar to those of Urochordates. The development of the central part of nervous system is also similar in both.

Affinities with Cephalochordata The hemichordates shows similarity with cephalochordatesin the structure and function of the branchial apparatus, arrangementand development of coelomic sacs. Due to these similarities Hemichordata had been considered as a subphylum of the phylum Chordata.

Objection-The main objections are:

(1) The true notochord does not occur in hemichordates. It is ventral to the main blood vessel and without sheath covering. It is very short,hollow, restricted to proboscis and without any supporting function and lined many epithelial cells. It does not originate from the roof of larval archenteron but as a forward hollow projection of the foregut. It is also termed as buccal *diverticulum.*

(2) The nervous system is of the invertebrate type being intra-epidermal in position and having a central nerve cord and a circumcenteric nerve ring which are absent in chordates. In *Hemichordata* are, the dorsal tubular nerve cord is confined to the collar region only.

(3) Numerous gill-slits are present dorsally in*Hemichordata* whereas they are only 5 to 7 and lateral in higher chordates.

Other differences. The hemichordates further differ from the chordates in :

(1) Metamerism, cephalization, paired appendages, postanal tail, exoskeleton, living endoskeleton, dermis, liver, haemoglobin, red blood corpuscles, etc are absent.

(2) Body is divided into proboscis, collar and trunk, single-layered ciliated epidermis, hepatic caeca, dorsal heart, open neurocoel, colourless blood, numerous gonads, etc.

[II] Affinities with Rhynchocephalia (Nemertinea)

Feeding and fossorial habits are similar in *Hemichordata* and Nemertinea. Body is elongated, vermiform, without external metamerism, with terminal anus, with smooth skin containing unicellular glands and ectodermal nerve plexus, and having metamerically arranged simple gonads. Nemertinea lacks a dorsal nerve cord and in having lateral nerve cords and a protrusible proboscis.

[III] Affinities with Phoronida

Some zoologists shows the resemblance between*Hemichordata* with *Phoronis* on the following grounds :

(1) Both animals have regenerative power.

(2) Similar nature of epidermal nervous system.

(3) The paired gastric diverticula of *Phoronis,* like the *buccal diverticulum* of *Hemichordata,* forming notochord.

(4) Actinotroch larva of tornaria such as similar disposition of coelom, anus surrounded by a ciliary ring, presence of a proboscis pore and a sensory apical plate with cilia and eye spots.

Objection. But, the chordate features of *Hemichordata* like Pharyngeal gills, are absent in *Phoronis* which also differs in having paired metanephridia.

[IV]Affinities with Pogonophora

Hemichordata weith Pogonphora shows the following similarities :

(1) Enterocoelomic coelom.

(2) Same body divisions.

(3) Mesosome and metasome separated by a septum.

(4) Nervous system intra-epidermal.

(5) Pericardial sac in some pogonophores.

(6) Gonads found in trunk.

Objection.Pogonophores have protocoelic nephridial coelomoducts and lacks alimentary canal. The nervous system is at protosomic type in pogonophora, whereas in mesosomic in Hemichordata.

[V] Affinities with Annelida

Resembling characters of Annelida and Hemichordata are as follows:

1. Both are wormlike and coelomate.
2. These have burrowing habit, tubiculus life and ingesting mud which is passed out as castings through anus.
3. Collar of *Balanoglossus* similar to clitellumof earthworm.

4. Anterior part of body like proboscis and prostomium are similar and preoral.
5. Blood vessels are similarly arranged with blood flowing anteriorly in dorsal vessel and posteriorly in ventral vessel.
6. Dorsal position of heart.
7. Tornaria larva of *Balanoglossus* shows several structural resemblances with the trouchphore larva of Annelida in various aspects.

Objection. However, the two animals groups are different in following :

1) In Annelids pharyngeal gill-slits are absent while stomochord or buccal diverticulum and dorsal tubular nerve cord are present in *Balanoglossus.*
2) *Balanoglossus* does not have double and solid ventral nerve cords and nephridia found in annelids.

3) In tornaria larva of *Balanoglossus*, preoral or proboscis coelom is present, nephridia are absent and blastopore becomes anus of the adult .In trochophore larva of annelids, preoral coelom is absent, nephridia present and the blastopore becomes the mouth.

[VI] Affinities with Echinodermata

Adult Stage - Adult hemichordates and echinoderms shows few similarities such as:

1) They have common habits and ecological niches
2) Enterocoelom is divided into three successive parts filled with sea water to serve a hydraulic mechanism.
3) Heart vesicle and glomerulus of are considered homologous to the dorsal sac and axial gland of echinoderms.
4) Nervous system is poorly developed and forms epidermal nerve plexus.
5) Proteins and phosphagens of chordates are similar to those of echinoderms.
6) Both have a remarkable power of regeneration.

Larval stage –Both groups shows a resemblance on embryological ground as the tornaria larva of *balanoglossus* has a structural similarity with an echinoderm larva, in particular the bipinnaria larva of asteroids. The larvae of the two groups posses the following features:

1. Small, pelagic, transparent and oval.
2. Identical ciliated bands taking up a similar twisted course.
3. Enterocoelom.
4. Proboscis coelom opening to outside by proboscis pore of tornaria comparable to hydrocoel of echinoderm dipleurula.
5. Bastopore becomes the anus and digestive tract is complete with mouth, anus and same parts.

Urochordata

Urochordates are primitive and degenerate descendants of ancestral chordates. The tadpole larva represents the relic of a free-swimming ancestral chordate. Degeneration is a secondary modification due to sedentary and sessile mode of adult life.

[I] Affinities with nonchordates

Urochordates show some resemblances with certain nonchordate groupsdue to similar mode of life and parallel evolution.

(1) Porifera, Coelenterata and urochordates are sessile in nature.

(2) Mechanism of filter feeding and respiration through a water current is parallel with that of sponges, mollusks (oysters) and lophophorates.

(3) Budding process of new zooids is common with coelenterates and annelids.

(4) Larval eyes and otocysts are found in many invertebrates.

(5) Simple mode of colonial life and composite fixed ascidians is observed in a number of invertebrates.

[II]Affinities with chordates

The ascidian tadpole larva possesses all the basic chordate characters such as : rodlike notochord forming axial skeleton of tail, dorsal tubular nerve cord, and pharyngeal gill-slits.

Affinities with Hemichordata

The urochordates and hemichordates, shows following similarities :

Same structural plan of pharynx perforated by gill-slits and having samemechanisms, same development process of central part of nervous system, notochord is restricted.

Objections.

Above two groups have some differences also

Balanoglossus lives in burrows, but urochordates may be fixed, inert or pelagic, Hemichordate body is divisible into proboscis, collar and trunk which is not found in urochordates. A true notochord is present in the tail of ascidian larva, whereas the buccal diverticulum of *Balanoglossus* is no longer considered a notochord. Thus, even inclusion of hemichordates as true chordates has become doubtful so that they are considered nowadays as an independent nonchordates phylum. The similarities of both the groups are probably because of their remote phylogenetic relationship with the ancestral common stock.

ffinities with Cephalochordata.

The adult urochordate have following structural similarities with Hemichordata:

Similar ciliary filter feeding ,similar endostyle and associated parts,respiratory mechanism, large pharynx with similar accessories, branchial tentacles are similar to velar tentacles, similar atrial complex etc.

The ascidian tadpole larva and *Branchiostoma* also share the following similarities like Identical embryonic development, Tail consisting of median vertical fins without fin rays, Sensory organs are median, pharynx with endostyle, same atrial complex.

Affinities with Vertebrata.

The ascidian tadpole larva resembles with higher chordates in having axial skeletal notochord, pharyngeal gill-slits,dorsal tubular nerve cord, postanal tail covered by vertical fin and type of cleavage and gastrulation.Besides, in the adult ascidian, neural gland is homologous with vertebrate pituitary, endostyle with vertebrate thyroid, and typhlosole comparable to intestinal spiral valve of elasmobranch fishes.

Cephalochordata

Cephalochordatesshows affinities with chordates as well as non-chordates. It is considered to be a generalized and primitive type of chordate very close to the vertebrates. But, because of some variations, it is not included in the vertebrates. It occupies an independent subphylum Cephalochordata.

[I]Non-chordate affinities

Cephalochordates phylogenetically shows resembling features with several non-chordate groups.

Affinities with Annelida

Body have bilateral symmetry and metamerism is present. Protonephridia with solenocytes are arranged segmentally. Coelom is well developed. Closed and similarly disposed blood vascular system. Filter feeding mechanism is shown by some polychaetes.

Objections. In cephalochordates, unlike annelids, metamerismis restricted only to myotomes and gonads. Coelom is enterocoelic and not schizocoelic as in annelids. The flow of blood in main blood vessels is in opposite directions in the two groups. Above all, the three basic chordate characters of Cephalochordata are not present in Annelida.

Affinities with Mollusca

Pallas (1778) firstly described the *Amphioxus* as *Limax lanceolatus*, a slug. The ciliary mode of feeding and respiratory mechanism through water current are common features of the two groups. Their anatomy is completely different. Moreover, mollusks are unsegmented and their locomotory podium is also unknown in cephalochordates.

Affinities with Echinodermata.

Echinoderms have asymmetrical body, enterocoelic coelom. Perforations in the calyx of some fossil echinoderms look similar to gill-slts of amphioxus. As in *Branchiostoma,* ophiuroids have similar creatine phosphate. But all these features may be because of a very remote common ancestry of the two groups.

[II] Chordate affinities

The three basic chordate features, viz. the notochord, dorsal tubular nerve cord and pharyngeal gill-slits are present in cephalochordates. It shows relationships with all the major groups of phylum Chordata

Affinities with Hemichordata

Hemichordata and Cephalochordata resemble in having similar filter feeding, pharyngeal apparatus with numerous gill slits and gill bars, respiratory mechanism, entercoelic coelom, and numerous gonads in which gonoducts are absent.

Objections.

Hemichordata musculature is unsegmented, nervous system clearly of nonchordate type, gill-slits dorsal in position instead of lateral, and a postanal tail is absent. Hemichordata are more primitive than Cephalochordata.

Affinities with Urochordata.

Cephalochordates and Urochordates are regarded to be very closely related because of epipharyngeal groove, endostyle and peripharyngeal bands, large pharynx bearing numerous lateral gill-slits,primitive ciliary feeding, respiratory mechanisms, an ectoderm-lined atrial cavity opening to outside through atrial siphon,identical development, and the ascidian larva having a continuous notochord, dorsal hollow nerve cord, and a post-anal tail with median caudal fin without fin rays.

Objections.

The adult urochordates varies with cephalochordates in several features, such as body is unsegmented, covered by test, with entercoelic coelom, without notochord, and hollow nerve cord,with a liver, a well-developed muscular heart covered by peritoneum, without nephridia, bisexual, retrogressive metamorphosis. These differences shows that the cephalochordates are better evolved than the urochordates.

Affinities with Cyclostomata

The *Ammocoete larva* of Cyclostomat) and *Branchiostoma* show various similarities like elongated, streamlined fish-like body,pharynx having endostyle and gill slits, mouth surrounded by an oral hood and guarded by a velum andcontinuous dorsal median fin. Besides these other resembling chordate characters in the adults are metameric myotomes, persistent gill slits, velum and a postanal tail.

Affinities with other vertebrates.

Besides cyclostomes, *Branchiostoma* also resembles other vertebrates in several features like true coelom, midgut diverticulum comparable with liver, metamerically arranged myotomes, postanal tail, well-formed hepatic portal system and similar arrangement of main longitudinal vessels with forward flow of blood in ventral and backward flow in dorsal blood vessel.

Objections.

Cephalochordates differ from cyclostomes and other vertebrates in various features like head, paired limbs, skull, vertebral column, muscular heart, red blood corpuscles, brain, specialized sense organs, gonoducts, etc. are absent and in having nephridia, atrium, numerous gonads, asymmetry etc.

2.9 Summary

Protochordates exhibits primitive characters of phylum chordata but cannot be included under vertebrata because they do not have well developed vertebral column. They have only the initial form of vertebral column in form of notochord in the dorsal side of the body. Protochordates are marine, small & primitive chordates, they lives singly & feed on microbes or particulate matter. There is absence of head, jaw, skull & cranium. Complete body is covered by single transparent layer called Tunic. On the basis of the position of the notochord the group protochordata is again divided into following subphyla- Hemichordata (Half-chordates), Urochordata(Tail-cord), Cephalochordata(Head-cord).

Doliolum is cosmopolitan marine animal in distribution being observed from both cold and warm oceans of the world. It is free swimming, and pelagic in nature. It is typical, tunicate animal because it exhibits an dimorphic alternation of generation between the two morphologically distinct phases in its life history. The two phases of *Doliolum* life cycle are asexually reproducing solitary phase; and a sexually reproducing gregaria phase.

Oikopleura is a neotenous, free swimming pelagic, tiny & solitary animal. *Oikopleura* is found in plankton of tropical & temperate zone. It resemble with ascidian tadpole in general characters. It is distributed in all seas except the Polar Regions. The whole body *ofOikopleura* is covered by a covering called as house. The animal secrets a temporary gelatinous house & many times larger than body size. This house is secreted from a special part of skin that is called as oikoplasts epithelium, generally house is made up of tunicin.

Branchiostoma is commonly known as ‘lancelet’ or ‘lancet’ because of the sharp, pointed, lance-like ends of the body. , It is a marine invertebrate found in soft substrates in shallow seas. *Amphioxus* is of great interest, it is used as a model organism to study the development of vertebrates. It is the most studied type of subphylum Cephalochordata. In the constitution of its digestive, respiratory, vascular, excretory, skeletal, nervous and muscular systems, and in

its manner of development from the egg, it exhibits what appears to be a primordial condition of vertebrate organization, a condition which is in fact, partly recapitulated in the course of the embryonic development of craniate vertebrates. The absence of paired organs of special sense, olfactory, optic and auditory, which are characteristic of higher vertebrates suggest that the animal is degenerate rather than primitive. This is a puzzle that surrounds *Amphioxus*. It is true, however, that a definite gap separates this animal from the lowest fishes in many ways.

2.10 Self Assessment Questions

1. Describe the distinguishing characters of Protochordates.
2. Describe the salient features with examples of various subphyla of protochordates.
3. What do you mean by tunicates?
4. Explain the Structure, function and life history of sessile and pelagic tunicates, *Doliolum*
5. Draw a diagram of *Doliolum* life history.
6. Explain the various types of zooids of *Doliolum*.
7. Describe the Structure, function and life history of sessile and pelagic tunicates *Oikopleura* .
8. Write about significance of *Oikopleura.*
9. Draw a diagram of *Oikopleura*.
10. Describe the morphology of *Branchiostoma.*
11. Explain the digestive system of *Branchiostoma* with help of diagram.
12. Explain the circulatory system of *Branchiostoma*.
13. Give a short note on development of *Branchiostoma*.
14. Write an essay on neoteny.
15. Describe the various affinities of Hemichordata, Urochordata& Cephalochordata in details

2.11 Reference Books

- Modern Text Book of Zoology: Vertebrates by R.L.Kotpal
- Text Book of Vertebrate Zoology by *J. S. Kingsley*
- Hyman's Comparative Vertebrate Anatomy by Marvalee H. Wake
- Textbook of Vertebrate Zoology by Prasad S

Unit - 3

Vertebrates

Structure of the Unit:

3.1 Objectives

This unit describes the origin, evolution and adaptive radiation of phylum vertebrates. It gives detailed information on the characteristic features of the Agnatha and Gnathostomes. It describe origin and evolution of group Agnatha. At the end of this unit you should be able describe to general characters of ostracoderms, cyclostomes and placoderms.

3.2 Introduction

Vertebrates belong to the subphylum Vertebrata of the phylum Chordata. Their names is derived from the presence of serially arranged 'vertebrae' (L., vertebrates, jointed), which comprise a major part of their axial exoskeleton, the vertebral column or the backbone. Another feature, that all vertebrates share as a common diagnostic character, is the elaboration of anterior skeletal elements into a 'cranium' or 'skull' which houses various sense organs and a complex brain. This gives another name the ' Craniata', which is sometimes used for the group. There is reason to believe that the distinctive vertebrate cranium and brain evolved even before the vertebral column and are therefore more fundamentally characteristics of vertebrates than the backbone.

A vertebrate may be defined as a special kind of chordate animal that has a cartilaginous or bony endoskeleton consisting of a cranium, housing a brain and a vertebral column thorugh which the nerve cord passes. No other group of animals possesses these two fundamental and related characters which have existed in vertebrates since the late Cambrian and Ordovician.

3.3 Origin and evolution of vertebrates

Phylogeny or evolutionary history of vertebrates

The evolutionary history of group organisms is termed phylogeny. It is derived from Greek roots which mean "to beget a race": (Gr. phylon, race or tribe + gennao, to bring forth). The concept of phylogeny is used to place animal groups in proper evolutionary sequence. Thus in phylogenetic order fishes came before reptiles and reptiles before birds and mammals.

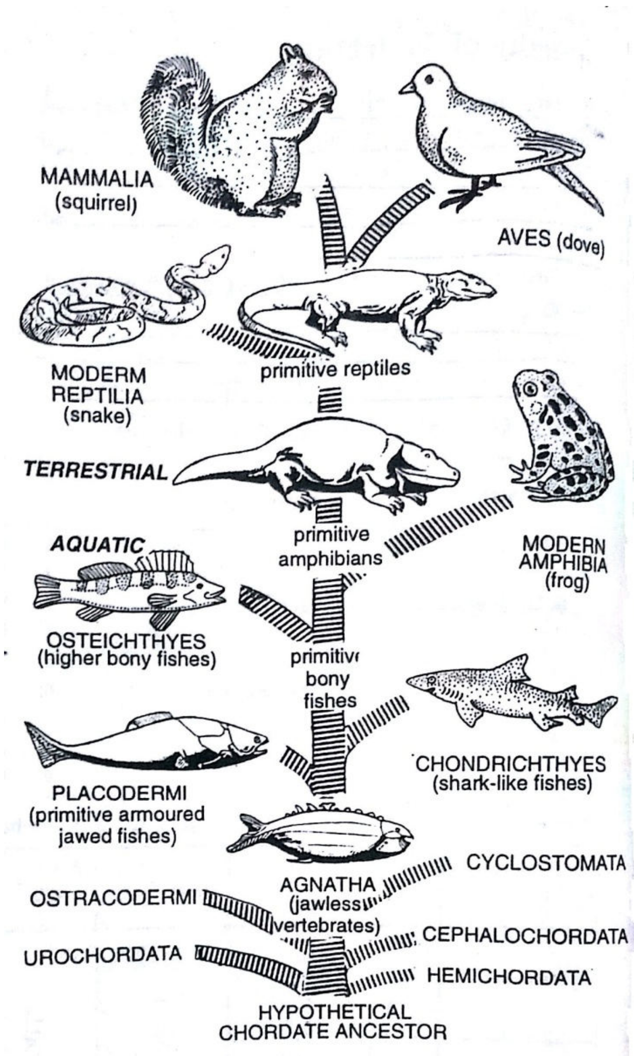


Fig. A simple phylogenetic tree of vertebrates showing classes.

A phylogenetic tree makes a good model to record the evolutionary history of vertebrates. The vertical dimension or height of the tree represent geological time. The diversification into group is represented by branches. The width of each branch indicates the relative abundance of each group in time. Various diagrams representing a phylogenetic tree have been presented reflecting different ideas of interrelationship of animal groups.

Cambrian and Ordovician

The first fossils of vertebrates were found in the rocks of the Ordovician period in the form of the Ostracoderms. These were small jawless, bony, fish like forms related to the cyclostomes, the lived some 480 million years ago. This shows that their chordate ancestors must have existed much before, in the late Cambrian. The scarcity of early vertebrates fossils is probably due to the fact that they evolved mainly in freshwater and did not have as much chance to become fossilized as marine forms did. The Ostracodermi became extinct but some Cyclostomata (modern lampreys and hagfishes) are still with us.

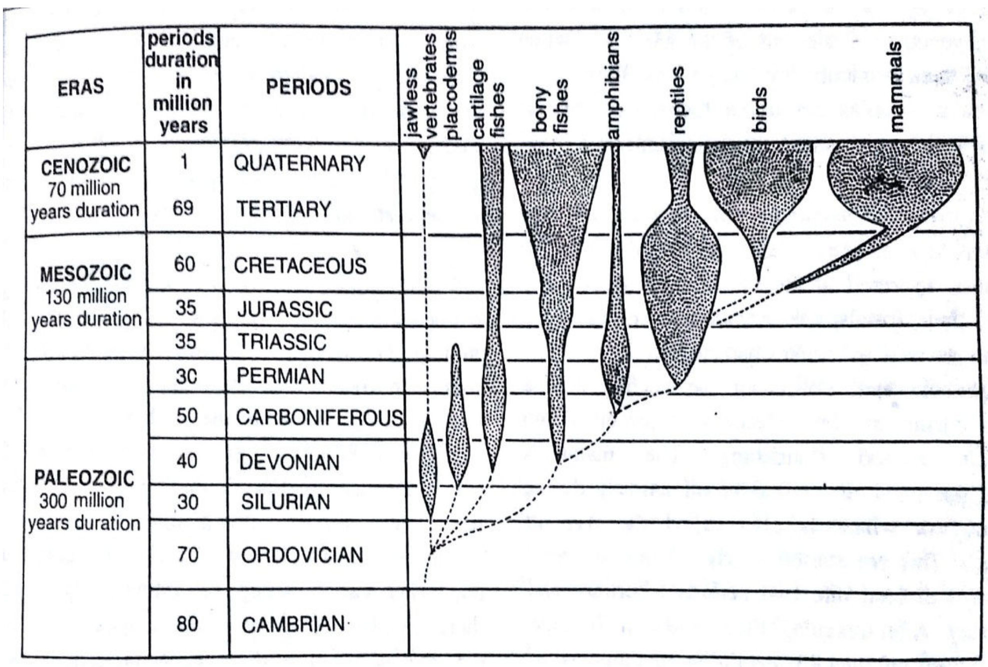


Fig. Geological chart with a simple family tree of vertebrate classes showing their relative abundance through time.

Silurian and Devonian periods

Some fossil fish are found in the Silurian period. But far more are present in the succeeding Devonnian period which is known as the Age of Fishes. Ostracodeerms were jawless fish but during Devonian the first jawed fish the placoderms arose. The Placodermi became extinct without leaving living representatives. It is likely that early placoderms wwere ancestors of cartilaginous and bony fishes both of which have true jaws but we do not have any direct evidence. Contrary to the former belief cartilaginous fishes (Chondrichthyes) did not give rise to bony fishes (Ostrichthyes).

Carboniferous period

In late Devonian or early Carboniferous period the lobe finned bony fishes (Crossopterygii) gave rise to labyrinthodonts or primitive stem Amphibia. They Amphibia became abundant and mutated in many directions during Carboniferous usually known as the Age of Amphibians.

Mesozoic era

In the early Carboniferous the primitive amphibians also gave rise to the primitive reptiles. They reached their peak and great abundance during Mesozoic era which is aptly known as the Age of Reptiles. They included among others the famed dinosaurs, the ichthyosaurs and the pterodactyls or flying reptiles. They dominated the world for nearly 130 million years, until the end of the Mesozoic when most of them suddenly became extinct. While only 4 orders of reptiles are living today as many as 10 orders are represented by their numerous fossil remains.

The ancestral mammals were derived from the primitive reptiles during Triassic period. The first birds also appeared in the late Jurassic period and one of their fossils the Archaeopteryx had both reptilian as well as avian characteristics.

Coenozoic era

Following the decline of the reptiles during the late Mesozoic, both birds and mammals started flourishing. The mammals became the most diversified of all animals during Cenozoic era which is also called the Age of Mammals. This era started nearly 70 million years ago and is divided into two periods : Tertiary and Quaternary. After reaching their peaks in Tertiary the mammals are steadily declining in numbers of species even though man a member of this group is considered to be the dominant form of life on earth today.

Origin and Ancestry of Vertebrates (Chordates)

Man being a vertebrate himself, it is not surprising that the problem of searching into the origin and ancestry of vertebrates has long been of particular interest to zoologists. But, like that of most of the animal phyla, the origin of vertebrates also remains obscure. Over the years, several hypotheses have been proposed to explain the origin of vertebrates, but none could stand the test of close scrutiny.

Time of Origin

The earliest known truly vertebrate animals were freshwater forms, abundant during the late Silurian and middle Devonian periods. Their fossils are collectively known as *ostracoderms.* They are placed with living cyclostomes (lampreys, hagfishes), in the jawless group called *Agnatha*. As their name refers to, their body was covered by a dermal bony armour forming an elaborate rounded solid shield on the head. Like cyclostomes, they had presumably a persistent notochord and no vertebrae. The mouth was anterior, ventral and lacked jaws and teeth. They had no paired appendages homologous with those of vertebrates. Paired eyes, median nostrial and pineal eye were present. A variable number of pharyngeal gill pouches opened by lateral common or separate gill openings. They were adapted for filter feeding.

The oldest fragmentary fossils belonging to ostracoderms occur in the late Cambrian and middle Ordovician. Absence of any vertebrate fossils in rocks older than the Cambrian, permits only speculation about the earlier history of the vertebrates. Which group was ancestral to the first true vertebrates (ostracoderms)? In fact there have been no fossils intermediate between the ostracoderms, which are already vertebrates, and any other earlier group of animals. As a result, there has been a great deal of speculation about the time of origin and the early progenitors of the vertebrates (chordates). Probably the vertebrate organisation had been evolving for several millions of years before the appearance of the first, late Cambrian fossils.

Place of Origin.

The American geologist Chamberlain gave the idea of freshwater origin of vertebrates, in 1900. It was also supported by Romer and Homer Smith. They argued that dilute body fluids, compared to sea water, and the glomerular kidney to get rid of excess water evolved as adaptations to freshwater conditions.

However, evidence for a marine origin of vertebrates is also overwhelming. The protochordates and dueterostome invertebrate phyla are exclusively marine forms. All known Cambrian and Ordovician vertebrates also occur as marine fossils. Further, a glomerular kidneyis found in hagfishes (Myxiniformes), which are exclusively marine and have body fluids similar to sea water in salt concentration. As professor James Robertson argues, the primary function of a glomerular kidney is excretory and not osmoregulatory, and it is valuable to an active and mobile vertebrate irrespective of whether it is adapted to sea, to freshwater, or to life on land.

Hypothetical vertebrate ancestor (prevertebrate)

Whatever this ancestral vertebrate or prevertebrate may have been, there are no fossil records to show. There is reason to believe that it was soft bodied, without any hard exo- or endoskeleton, which could be fossilized.

The simplest chordates living today are the invertebrate chordates or protochordates belonging to the subphyla Hemichordata, Urochordata and Cephalochordata. They possess the notochord, dorsal nerve cord, pharyngeal gill-slits and postanal tail, fundamentally associated with the vertebrate body plan. They lack the vertebrae and some other features of the earliest as well as the living vertebrates, but they show closest affinities and certainly a common origin with the vertebrates. Therefore, it seems most reasonable and logical to draw inferences about the imaginary, generalized or ancestral vertebrate among them. The ancestor of vertebrates (chordates) can be reconstructed from our present knowledge of existing protochordates.

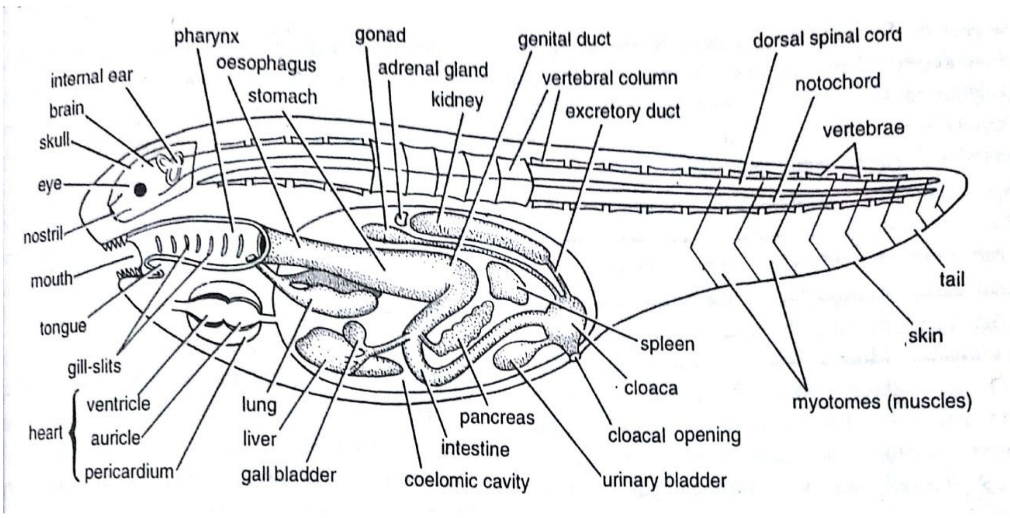


Fig. Hypothetical body plan of ancestral vertebrate.

The American geologist Chamberlain, who proposed the theory of freshwater origin of vertebrates in 1900, also gave the plan of a hypothetical protovertebrate. It was an aquatic, motile, actively swimming, fish-like animal having a bilaterally symmetrical body with definite head and tail ends. As in higher invertebrates and chordates, the basic internal organization would be some sort of modified tube-within-a-tube arrangement with the major internal organs present inside a large body cavity or coelom. It would also possess all the diagnostic chordate-vertebrate features. It had an internal skeleton in the form of a mid-dorsal longitudinal flexible rod, the notochord, surrounded by the vertebrae. There were internal supports in fins, bony plates in the skin and a rigid cranium or skull housing the tripartite brain and associated sense organs. Muscles were V-shaped and segmentally arranged along the sides of the body, particularly the tail, forming myotomes used in locomotion. Mouth was a simple anteroventral opening without jaws. It probably fed on microorganisms, filtered from the water or from bottom detritus. The water taken in through the mouth passed outwards through paired, lateral pharyngeal gill-slits, bathing the internal gills for aquatic respiration. A liver and primitive kidney was present. Whether the prevertebrate possessed a circulatory system with a single differentiated heart, is uncertain. The nervous system included a tripartite brain and a hollow nerve cord, dorsal to notochord. Sense organs were well developed including the lateral-line organs. The endocrine system was probably already well developed in the prevertebrate. Finally, the gonads were paired. The female laid eggs and the fertilization was external.

It was from such beginnings that the whole array of vertebrates evolved.

Origin of Chordata (vertebrates)

We shall now consider the origin of the earlier chordate ancestors of vertebrates. That the chordates have originated from the invertebrates is not doubted by most zoologists now-a-days. Since the earlier chordate ancestors were all soft bodied forms, they left no fossil remains to give us clues as to their origin. Therefore, the only basis for judging the origin of the group comes from the resemblances between the lower chordates (protochordates and the invertebrates). Some structural features shared by them, such as bilateral symmetry, anteroposterior body axis, triploblastic coelomate condition, matameric segmentation, etc., may be because of their common ancestry.

Theories ofInvertebrate ancestry of chordates

Several theories have been advanced to explain the origin of chordates either directly from some invertebrate group or through the invertebration of some protochordate. Almost every invertebrate phylum - Coelenterata, Nemertean, Phoronida, Annelida, Arthropoda and Echinodermata - has been suggested. But these theories are far from being satisfactory and convincing and have only a historical value. Only the echinoderm theory has received some acceptance and shall be considered and evaluated under deuterostome line of chordate ancestry.

Division of Bilateria

The greatest group of metazoan phyla, the Bilateria, is divided into two major divisions - Protostomia and Dueterostomia. The basis of division is the basic difference in embryonic and larval developments. The divisions probably represent two main lines of evolution within the Animal Kingdom.

Basic difference between Protostomia and Deuterostomia:

Characters	Protostomia	Deuterostomia
Cleavage	Spiral and determinate	Mostly radial and indeterminate
Blastopore	Forms mouth	Forms anus
Mesoderm formation	By cell cleavage between ectoderm and endoderm	By outpocketing from dorsolateral, endodermal wall of archenteron
Coelom formation	Schizocoelous, by a split of mesoderm	Enterocoelous, by fusion of gut pouches (except vertebrates)
Type of larva	Trochophore	Tormaria or Bipinnaria (except vertebrates)
Phosphogen	Arginine	Creatine
Major phyla included	Annelida, Mollusca and Arthropoda	Echinodermata, Pogonophora, Hemichordata and Chordata

Deuterostome line of chordate evolution

A mere glance at the Table, showing common features of all Deuterostomia, suggests strong evidence of embryological and biochemical nature of a closer evolutionary relationship between the three principal deuterostome phyla - Echinodermata, Hemichordata and Chordata. For example :

(1) Early cleavages of zygote are intermediate, i.e. in, each early blastomere is capable of developing into a whole adult if separated.
(2) Blastomere of gastrula forms the anus, while mouth is formed as a secondary opening.
(3) Pockets or folds arise from the endoderm of developing archenteron of the embryo. The fusion of spaces in the pockets forms the coelom (enterocoelous, except in vertebrates) and their walls become the mesoderm.
(4) The pelagic larvae of echinoderms and hemichordates bear a close structural resemblance. The vertebrates, however, having been lost in the course of evolution.
(5) Biochemically, all deuterostomes use an identical phosphagen, the creatine, in the energy cycle of their muscular contraction. The phosphagen of invertebrates is arginine. However, certain hemichordates as well as echinoids use both arginine phosphate as well as creatine phosphate. These facts are interpreted to show that the hemichordates are connecting link between chordates and nonchordates.
(6) Serological tests demonstrate that the proteins of the three deuterostome phyla are more closely related to one another than to those of any other phyla.

The precise relationship of the three deuterostome phyla remains unknown, but there is little doubt that they share a common evolutionary history. Several workers have attempted to explain the deuterostome line of chordate evolution. Some of the proposals are as follows:

1. Echinoderm ancestry

On the basis of anatomical, embryological, palaeontological, biochemical and serological evidences, various workers had tried to establish that the chordates probably had originated directly from some primitive echinoderm larva. The hemichordata larva (tornaria) is strikingly similar to the larva (bipinnaria or diplerula) of echinoderms. It was, in fact, mistaken for an echinoderm when first discovered. Both are small, transparent, free swimming and bilaterally symmetrical. Both have similar ciliated bands in loops, a dorsal pore, sensory cilia at the anterior end and a complete digestive system of ventral mouth and posterior anus. This striking larval resemblance led Johannes Muller and Bateson to suggest a common ancestry for the achinoderms and the hemichordates. But presence of apical plate with eyespots in tornaria larva raises doubts about the common ancestry of achinodermates and hemochordates. Garstang and de Beer proposed the Neotenous Larva theory suggesting that probably the auricularia larva of echinoderms became sexually mature and later this neotenic larva gave rise to the chordates.

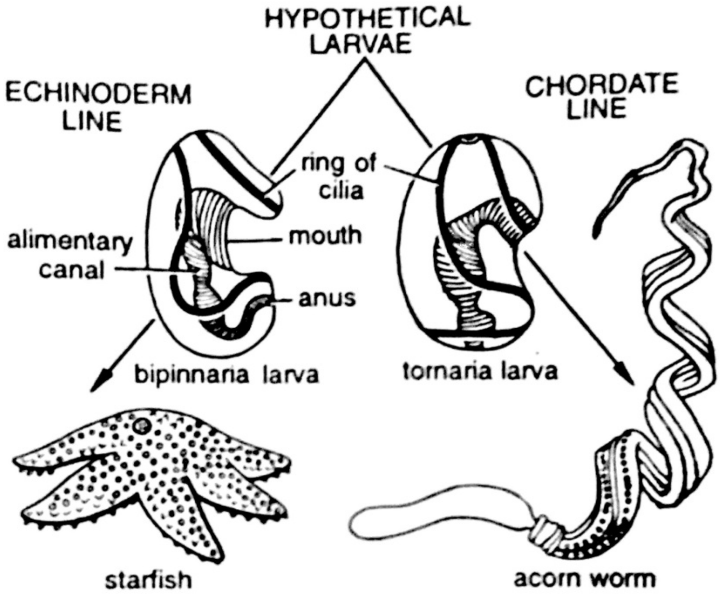


Fig. Similarity of larval forms of echinoderms and hemichordates has lent support to the idea that both came from a common ancestor.

Garstrong (1894) imagined that if ciliated bands together with underlying nervous tissue of auricularia larva of achinodermates, concentrates to form ridges leaving a groove between them and if lips of the groove fuses subsequently, it will give rise tube. It will resamble with the nervous system of chordates.

Cambrian and Ordovician fossil records of Carpoid aechinoderms lead Torsten and Gislen to assume that Carapoid echinoderms might have evolved from tornaria like creatures which have begun to settle down to lead sedentary life. The water vascular system might have developed out of ciliated grooves of these creatures. Besides this, it was also claimed that in the lower Silurian period, one carapoid echinoderm had the calyx perforated by a series of 16 small apertures. These apertures can be compared with the gill-slits of Branchiostoma.

Moreover, some isolated biochemical studies (Needham, 1932 and Wihelmi, 1942) also put some weight on the concept of diversion of chordates from echinodermates. Most of the nonchordates use arginine phosphate for the transfer of energy but ophiuroides, cephalochordates, ascidians and vertebrates use creatine phosphate. On the other hand hemochordates and echinoderms use both arginine and creatine phosphates as phosphate carrier.

The descent of chordate from the Echinodermata by the direct transformation of any echinoderm or its neotenous larva into a chordate is no longer accepted now-a-days. The view most widely accepted at present is that the living echinoderms and the living chordates had in common an immediate ancestor.

2. Hemichordate ancestry

There is a strong suggestive evidence that the early evolutionary stage of Deuterostomia group was sessile or sedentary. The pharynx perforated by gill-slits, a characteristic feature of chordates, is also a likely adaptation to sedentary habit. No doubt, hemichordates are sedentary and have pharyngeal gill-slits and a hollow dorsal nerve cord. But the presence of a true notochord is doubtful and their adult body plan is quite different from vertebrates. Therefore, the prospects of some hemichordate as a likely ancestor of vertebrates seems to be impossible so that they are put under a separate phylum of their own.

3. Urochordate ancestry

The urochordate or ascidian theory of vertebrate origin was advocated by W. Garstang in 1928 and later elaborated by N.J. Berrill (1955) in his book, "Origin of Vertebrates", Romer (1959) and others. The adult tunicates or ascidians reflect the primitive sessile marine and filter feeding condition of the ancestral chordates. But their body plans are so divergent that it is impossible to imagine a direct evolutionary transformation of an adult ascidian into a vertebrate. On the other hand, the ascidian larvae are tadpole-like, elongated, bilaterally symmetrical and free-swimming creatures with pharyngeal gill-slits, notochord, dorsal hollow nerve tube, and a muscular postanal tail. They represent only slightly modified living caricature of the ancestral chordate that gave rise to the vertebrate line of evolution. According to this theory, certain of these larvae failed to metamorphose into adults, but became neotenous, that is, sexually mature by developing gonads precociously, and later evolved into the cephachordates and vertebrates. The sessile nature of life of the primitive chordate ancestry, pterobranch hemichordates and primitive echinoderms by the workers is considered resulting from common ancestry.

However, the ascidian theory of chordate origin does not seem to be perfect. The principal drawback is that the theory considers sessile urochordates to be ancestral to chordates. Whereas, they are highly specialized because sessility is a specialized condition wherever it occurs in the Animal Kingdom.

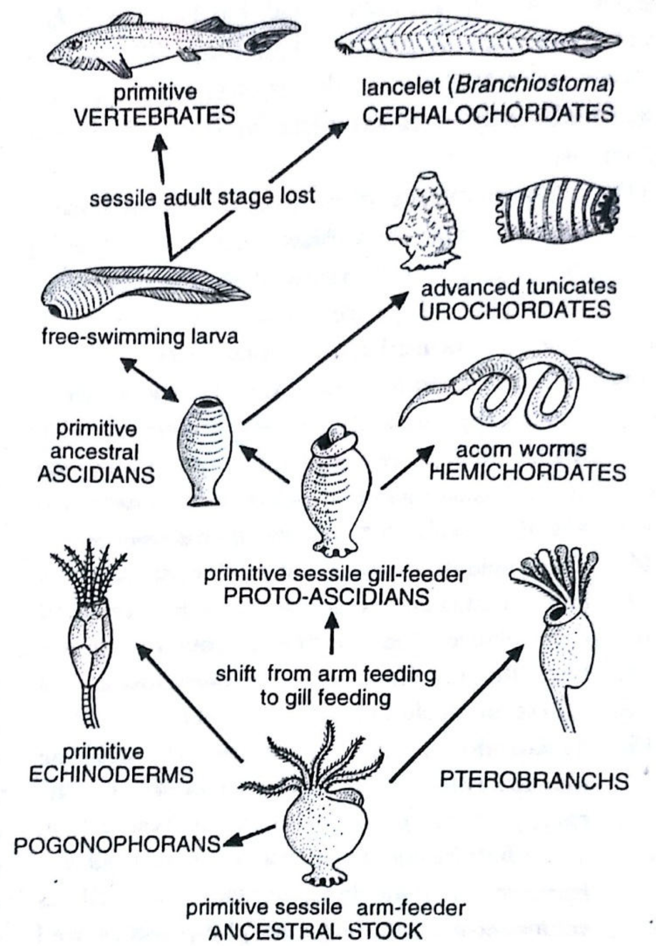


Fig. Diagrammatic representation of chordate evolution based on the hypotheses of Berrill and Barrington.

4. Cephalochordate ancestry

The cephalochordates, particularly the lancelets (Branchiostoma lanceolatum) are an interesting group of animals. They possess the three basic chordate features in diagrammatic form. According to Colbert, the living Amphioxus (=Branchiostoma) answers the logical structure of a model prevertebrate. Homer Smith's reconstruction of the hypothetical protovertebrate in his book, *FromFish to Philosofer* (1953), also greatly resembles Amphioxus. But, the excretory system of cephalochordates consisting of flame cells called solenocytes, is altogether different from that of vertebrates. The solenocytes are ectodermal and therefore not homologous with the mesodermal vertebrate kidneys. Further, lack of strong cephalization and sense organs, and the unique forward extention of notochord indicate that the cephalochordates may hint about the likely ancestral body plan of vertebrates, but they are not themselves ancestral. Body may represent divergent paths of evolution from a common remote ancestor.

5. Barrington's hypothesis

The most plausible hypothesis by E.J.W. Barrington (1965) is based on the deuterostome line of chordate evolution. The common echinoderm¬¬chordate ancestor was in all probability a small, sessile or semisessile, lophophorate or arm feeding creature. It fed by waving tentacles. From this ancestral stalk were derived early stalked echinoderms and pogonophores. The next logical step was the derivation of a sessile filter feeder or stem chordate. The cumbersome external gill-slits and a mucus-secreting endostyle. Cephalodiscus, a living pterobranch hemichordate, shows the transitional stage between the two modes of feeding because it has a single pair of gill-slits besides the crown of tentacles. Pharyngotremy, that is, perforated pharynx with internal food-trapping mechanism, resulted in the evolution of free-living hemichordates on one hand and the sessile ancestral urochordates (tunicates) on the other. Some ancestral tunicates, instead of producing ciliated larvae common to the earliest groups, formed tadpole larvae with all the typical somatic features of the chordates. According to Garstang, the larva became elongated and increased in size, the longitudinal ciliary bands shifted mid-dorsally and changed to the hollow nerve cord, the adoral cilia developed into the endostyle, and muscle fibres evolved in the tail. This typical chordate larva by paedogenesis suppressed the sessile adult stage, developed gonads precociously and became the ancestor of cephalochordates (Branchiostoma), vertebrates and the larvaceans probably presenting three cases of parallel evolution.

3.4 Adaptive radiation in vertebrates

In evolutionary biology, adaptive radiation is a process in which organisms diversify rapidly into a multitude of new forms, particularly when a change in the environment makes new resources available, creates new challenges, or opens new environmental niches. Starting with a recent single ancestor, this process results in the speciation and phenotypic adaptation of an array of species exhibiting different morphological and physiological traits with which they can exploit a range of divergent environments.

Adaptive radiation, a characteristic example of cladogenesis, can be graphically illustrated as a "bush", or clade, of coexisting species (on the tree of life). Caribbean anoline lizards are a particularly interesting example of an adaptive radiation. The Hawaiian islands are very isolated and contribute numerous examples of adaptive radiation. An exceptional example of adaptive radiation would be the avian species of the Hawaiian honeycreepers. Via natural selection, these birds adapted rapidly and converged based on the different environments of the Hawaiian islands

Identification

Four features can be used to identify an adaptive radiation:

1. A common ancestry of component species: specifically a *recent* ancestry. Note that this is not the same as a monophyly in which *all* descendants of a common ancestor are included.
2. A phenotype-environment correlation: a *significant* association between environments and the morphological and physiological traits used to exploit those environments.
3. Trait utility: the performance or fitness advantages of trait values in their corresponding environments.
4. Rapid speciation: presence of one or more *bursts* in the emergence of new species around the time that ecological and phenotypic divergence is underway.

Causes

Innovation

The evolution of a novel feature may permit a clade to diversify by making new areas of morphospace accessible. A classic example is the evolution of a fourth cusp in the mammalian tooth. This trait permits a vast increase in the range of foodstuffs which can be fed on. Evolution of this character has thus increased the number of ecological niches available to mammals. The trait arose a number of times in different groups during the Cenozoic, and in each instance was immediately followed by an adaptive radiation. Birds find other ways to provide for each other, i.e. the evolution of flight opened new avenues for evolution to explore, initiating an adaptive radiation. Other examples include placental gestation (for eutherian mammals), or bipedal locomotion (in hominins).

Opportunity

Adaptive radiations often occur as a result of an organism arising in an environment with unoccupied niches, such as a newly formed lake or isolated island chain. The colonizing population may diversify rapidly taking advantage of all possible niches.

In Lake Victoria, an isolated lake which formed recently in the African rift valley, over 300 species of cichlid fish adaptively radiated from one parent species in just 15,000 years.

Adaptive radiations commonly follow mass extinctions: following an extinction, many niches are left vacant. A classic example of this is the replacement of the non-avian dinosaurs with mammals at the end of the Cretaceous, and of brachiopods by bivalves at thePermo-Triassic boundary.

Examples

Classic examples of vertebrate adaptive radiation, includingNorth American postglacial fishes, African cichlids,coral reef-dwelling parrotfish, Caribbean anoline lizardsand Darwin's finches, have captivated evolutionary biologistsfor decades. These examples provide tangibleevidence of phenotypic divergence and the operation ofnatural and sexual selection. Recent progress in molecularphylogenetics and population genetics has yielded insightinto the cladogenic pattern and genetic architecture ofevolutionary radiation.

A. Darwin's finche

One famous example where adaptive radiation is seen is with Darwin's finches. It has been observed by many evolutionary biologists that fragmented landscapes oftentimes are a prime location for adaptive radiation to occur. The differences in geography throughout disjointed landscapes such as islands are believed to promote such diversification. Darwin's finches occupy the fragmented landscape of the Galápagos Islands and are diversified into many different species which differ in ecology, song, and morphology, specifically the size and shapes of their beaks. The first obvious explanation for these differences is allopatric speciation, speciation that occurs when populations of the same species become isolated geographically and evolve separately. Because the finches are divided amongst the islands, the birds have been evolving separately for several million years. However, this does not account for the fact that many of the species occur in sympatry, with seven or more species inhabiting the same island. This raises the question as to why these species split when living in the same environment with all the same resources.

Petren, Grant, Grant, and Keller proposed that the speciation of the finches occurred in two parts: an initial, easily observable allopatric event followed by a less clear sympatric event. This sympatric event which occurred second was adaptive radiation. This occurred largely to promote specialization upon each island. One major morphological difference among species sharing one island is beak size and shape. Adaptive radiation led to the evolution of different beaks which could access different food and resources. Those with short beaks are better adapted to eating seeds on the ground, those with thin, sharp beaks eat insects, and those with long beaks use their beaks to probe for food inside cacti. With these specializations, seven or more species of finches are able to inhabit the same environments without competition or lack of resources killing several off. In other words, these morphological differences in beak size and shape brought about by adaptive radiation allow the island diversification to persist.

B. Cichlid fish

Another famous example is the cichlid fishes in lakes of the East African Rift. The lakes in this area are believed to support and sustain about 2,000 different species of these fish, each with different ecological and morphological characteristics such as body size. Like the Galápagos Islands, these lakes form a fragmented landscape that isolates the cichlid fish from one another, allowing them, and many of the organisms they live with, to evolve separately. The diversity of the lakes is in fact quite extraordinary because the adaptive radiations here are sometimes so young. One thing that has interested scientists about the cichlid fish case is the possibility of convergent evolution, or the evolution of analogous structures independently, driven by similar environmental selection pressures. However, quantitative studies on the convergent evolution of the cichlid fish are limited.

C. Hawaiian honeycreepers

Another example of an adaptive radiation would be an endemic species of the Hawaiian Islands. The Hawaiian honeycreepers are a large, highly diverse species which have been part of a vast adaptive radiation, that began as the Hawaiian Islands started to form. The honeycreeper species was shaped by island formation and natural selection. The mechanism by which this adaptive radiation occurred can be described as allopatric speciation via the peripheral isolate model. Each time a new island formed, a dispersal event would occur which would result in new community structures on each island. New selection pressures forced the adaptive radiation of the Hawaiian honeycreepers, as they needed to exploit new resources from the different environments of each island. It has been determined that many of the similar morphologies and behaviors of the Hawaiian Honeycreepers, located on distant islands, are due to convergence of analogous traits caused by similar environments.

D. Hawaiian silverswords

Though the most famously recognized cases of adaptive radiation have occurred in animals such as Darwin's finches or the cichlid fish, adaptive radiation certainly occurs in plant species as well. The most famous example of adaptive radiation in plants is quite possibly the Hawaiian silverswords. The Hawaiian silversword alliance consists of twenty-eight species of Hawaiian plants which range from trees to shrubs to vines. This is exceptional diversification as can be seen through the significant morphological differences between each species of the Hawaiian silverswords. With some species, it is virtually impossible to distinguish visually that they were ever part of one species to begin with. These radiations occurred millions of years ago, but through studies over the past few decades, it has been suggested that the rate of speciation and diversification was extremely high. These high rates, as well as the fragmented landscape of the Hawaiian Islands, are key characteristics which point directly to adaptive radiation.

E. Anolis lizards

Anolis lizards have been radiating widely in many different environments, including Central and South America, as well as the West Indies and experience great diversity of species just as the finches, cichlid fish, and silverswords. Studies have been done to determine whether radiations occur similarly for these lizards on the mainland as they do on the Caribbean islands or if differences can be observed in how they speciated. It has been observed that in fact, the radiations are very different, and ecological and morphological characteristics that these lizards developed as part of their speciation on the islands and on the mainland are unique. They have clearly evolved differently to the environments they inhabit. The environmental pressures on the Anolis lizards are not the same on the mainland as they are on the islands. There are significantly morepredators preying on the Anolis lizards on the mainland. This is but one environmental difference. Other factors play a role in what sort of adaptive radiation will develop. Among the Caribbean islands, a larger perch diameter correlates with longer forelimbs, larger body mass, longer tails, and longer hind limbs. However, on the mainland, a larger perch diameter correlates with shorter tails. This shows that these lizards adapted differently to their environment depending on whether they were located on the mainland or the islands. These differing characteristics reconfirm that most of the adaptive radiation between the mainland and the islands occurred independently. On the islands specifically, species have adapted to certain "microhabitats" in which they require different morphological traits to survive. Irschick (1997) divides these microhabitats into six groups: "trunk–ground, trunk–crown, grass–bush, crown–giant, twig, and trunk." Different groups of lizards would acquire traits for one of these particular areas that made them more specialized for survival in this microhabitat and not so much in others. Adaptive radiation allows species to acquire the traits they need to survive in these microhabitats and reduce competition to allow the survival of a greater number of organisms as seen in many of the examples before.

3.5 Origin, evolution and general characters of Agnatha (ostracoderms and cyclostomes)

Agnatha (Greek, "no jaws") is a superclass of jawless fish in the phylum Chordata, subphylum Vertebrata, consisting of both present (cyclostomes) and extinct (conodonts and ostracoderms) species. The group excludes all vertebrates with jaws, known as gnathostomes. They are descendants of extinct armored agnathans that were once widespread and were the precursors also of the "jawed vertebrates," which includes the bony fish, tetrapods, and humans.

The oldest fossil agnathans appeared in the Cambrian, and two groups still survive today: the lampreys and the hagfish, comprising about 120 species in total. Hagfish are considered members of the subphylum Vertebrata, because they secondarily lost vertebrae; before this event was inferred from molecular and developmental data, the group Craniata was created by Linnaeus (and is still sometimes used as a strictly morphological descriptor) to reference hagfish plus vertebrates. In addition to the absence of jaws, modern agnathans are characterised by absence of paired fins; the presence of a notochord both in larvae and adults; and seven or more paired gill pouches. Lampreys have a light sensitive pineal eye (homologous to the pineal gland in mammals). All living and most extinct Agnatha do not have an identifiable stomach or any appendages. Fertilization and development are both external. There is no parental care in the Agnatha class. The Agnatha are ectothermic or cold blooded, with a cartilaginous skeleton, and the heart contains 2 chambers.

General Characters

1. **Metabolism**

   Agnathans are ectothermic, meaning they do not regulate their own body temperature. Agnathan metabolism is slow in cold water, and therefore they do not have to eat very much. They have no distinct stomach, but rather a long gut, more or less homogenous throughout its length. Lampreys feed on other fish and mammals. They rely on a row of sharp teeth to shred their host. Anticoagulant fluids preventing blood clotting are injected into the host, causing the host to yield more blood. Hagfish are scavengers, eating mostly dead animals. They also use a sharp set of teeth to break down the animal. The fact that Agnathan teeth are unable to move up and down limits their possible food types.

2. **Body covering**

   In modern agnathans, the body is covered in skin, with neither dermal or epidermal scales. The skin of hagfish has copious slime glands, the slime constituting their defense mechanism. The slime can sometimes clog up enemy fishes' gills, causing them to die. In direct contrast, many extinct agnathans sported extensive exoskeletons composed of either massive, heavy dermal armour or small mineralized scales.

3. **Appendages**

   Almost all agnathans, including all extant agnathans, have no paired appendages, although most do have a dorsal or a caudal fin. Some fossil agnathans, such as osteostracansand pituriaspids, did have paired fins, a trait inherited in their jawed descendants.

4. **Reproduction**

   Fertilization in lampreys is external. Mode of fertilization in hagfishes is not known. Development in both groups probably is external. There is no known parental care. Not much is known about the hagfish reproductive process. It is believed that hagfish only have 30 eggs over a lifetime. Most species are hermaphrodites. There is very little of the larval stage that characterizes the lamprey. Lamprey are only able to reproduce once. After external fertilization, the lamprey's cloacas remain open, allowing a fungus to enter their intestines, killing them. Lampreys reproduce in freshwater riverbeds, working in pairs to build a nest and burying their eggs about an inch beneath the sediment. The resulting hatchlings go through four years of larval development before becoming adults. They also have a certain unusual form of reproduction.

Evolution

Although a minor element of modern marine fauna, agnathans were prominent among the early fish in the early Paleozoic. Two types of Early Cambrian animal apparently having fins, vertebrate musculature, and gills are known from the early CambrianMaotianshan shales of China: *Haikouichthys* and *Myllokunmingia*. They have been tentatively assigned to Agnatha by Janvier. A third possible agnathid from the same region is *Haikouella*. A possible agnathid that has not been formally described was reported by Simonetti from the Middle Cambrian Burgess Shale of British Columbia.

Many Ordovician, Silurian, and Devonian agnathans were armored with heavy bony-spiky plates. The first armored agnathans—theOstracoderms, precursors to the bony fish and hence to the tetrapods (including humans)—are known from the middle Ordovician, and by the Late Silurian the agnathans had reached the high point of their evolution. Most of the ostracoderms, such as thelodonts,osteostracans, and galeaspids, were more closely related to the gnathostomes than to the surviving agnathans, known as cyclostomes. Cyclostomes apparently split from other agnathans before the evolution of dentine and bone, which are present in many fossil agnathans, including conodonts. Agnathans declined in the Devonian and never recovered.

Classification of Agnatha

It is divided into two classes : Ostracodermi (extinct) and Cyclostomata.

A. Class - Ostracodermi (extinct)

(Gr., ostrakon, shell + derma, skin)

The earliest known vertebrate to appear in fossil record were jawless primitive fishlike animals collectively known as the ostracoderms, and placed under the class Ostracodermi. They resembled the present day cyclostomes (lampreys and hagfishes) in many respects and togedher with them, constitute a special group of jawless vertebrates, the Agnatha.

Important features

These primitive vertebrates were small to medium sized. Their body form was fishlike, usually flattened with a huge head and gill region, a tapering but muscular trunk and some sort of tail fin. They had no jaws and no pectoral or pelvic fins but had only median fins. Remarkably these earliest vertebrates were very bony and heavily armoured. The head was encased in a solid shield made of broad bony dermal plates, while the rest of the body surrounded by a series of smaller plates often called dermal scales. This has led to their names 'ostracoderms', 'armoured fishes' or 'bony skin' (Gr., ostrakon, shell + derma, skin). Why were they so heavily armoured? It has been suggested that the heavy exoskeleton served as a protection against the giant scorpion-like arthropods, the eurypterids, which were dominant predators of Cambrian, Ordovician and Silurian periods. Later, when these enemies disappeared, the jawed descendants of ostracoderms also lost their heavy armour which only hindered rapid progress.

The ostracoderm head was rather unusual. Most kinds had a pair of large lateral eyes and a median pineal eye on top of head. A single median nostril was located anterior to pineal eye. Very little is known about ostracoderm internal anatomy. They had no axial endoskeleton or vertebrae. Mouth was anteroventral small and without jaws or teeth. Sensory fields on head were probably a part of the lateral line system. Judging by their flattened body and feeble fins, they were probably sluggish bottom dwellers and filter feeders, like most of the present day lower chordates. An internal ear with 2 semicircular canals was present

Classification

All ostracoderm fossils have been generally put under a single class Ostracodermi. It is divided into 2 sub-classes and 5 orders, as follows:

I. Subclass Monorhina

A single, large, slit-like median nostril on top of head between eyes.

a) Order - Euphanerida

Silurian. Represented by a single genus and species, Jamoytius kerwoodi, previously placed under order Anaspida by Stensio and Ritche (1960). Newth regarded it as ammocoet larva of ostracoderms, discovered in the Silurian strata of England. About 18 cm long, fusiform, blunt-head and naked. Indications of two lateral and a median dorsal fin folds, notochord, and muscle myotomes like those of Branchiostoma. No gill-slits. Regarded as an ancestor of ostracoderms.

b) Order - Anaspida

Silurian-Devonian. Body small about 15 cm long. Head naked or covered by a complex of small plates. Arrangement of scales was complicated. In the trunk region, scales were oriented in longitudinal rows. Mouth was terminal. Eyes laterally placed. Nostril, single pair of pectoral spines. A series of spines was found in the mid-dorsal line of the trunk. An anal spine might have also been present. About 8 gill-slits were present in front of the pectoral spine in slanting fashion. Tail hypocercal (lower lobe larger). They were active swimmers. Birkenia, Lasanius, Pteroleps, Rhyncholepis and Pharyngolepis.

c) Order - Osteostraci (Cephalaspida)

Silulrian-Devonian. A single piece head shield or carapace without sutures. Tail heterocercal (upper lobe larger). A pair of palps behind the gills served like pectoral fins. Several pairs of gill slits. Sensory fields on head. Lateral eyes close together. Lampreys (Petromyzontiformes) probably were derived from Osteostraci. Tail, heterocercal. *Cephalaspis, Hemicyclaspis, Tremataspis, Kiaeraspis, Didymaspis*, *Benneviaspis* etc.

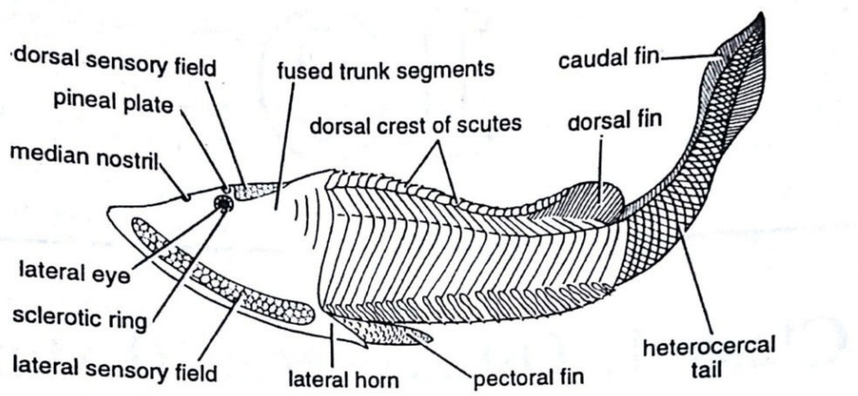


Fig. Restoration of *Cephalaspis.*

II. Subclass Diplorhina

Two separate nasal openings, where known.

a) Order - Heterostraci (Pteraspida)

Ordovician-Devonian, Body large, head encased in a shield. Tail was laterally compressed, covered with small scales hypocercal.

Mouth slit like aperture. Anterior portion of the snout is prolonged into rostrum. Lateral eyes widely separated.

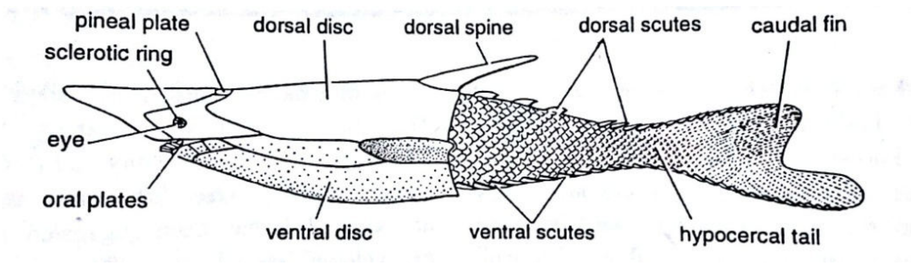


Fig. Restoration of *Pteraspis rostrata*.

A single large gill-slit on each side. No signs of sensory organs. Lateral line was distinct. A backwardly directed dorsal spine projected upwards from the posterior part of cephalic shield. Entire trunk was found covered with rectangular scales. Hagfishes (Myxiniformes) were probably derived from Heterostraci. *Pteraspis, Drepanaspis*

b) Order Coelolepida

Ordovician-Devonian Body small (10 to 12 cm long) and fusiform or torpedo-like, Surface covered by numerous minute placoid-scale like denticles. Tail hypocercal or heterocercal. Lateral eyes widely apart. Broad flanges project antero-laterally from body. *Phlebolepis, Lanarkia, Coelolepis.*

Biological Significance of Ostracoderms

(1) Ostracoderms are especially interesting because they represent the oldest known vertebrate fossils in the late Cambrian and Ordovician rocks dating back to 500 million years. They are the remote ancestors of all the vertebrates including man.

(2) Microscopic examination of their fossilized bony tissues reveals a great complexity of structure, thereby implying that these vertebrates were far advanced and had undergone a considerable period of evolution before becoming fossilized.

(3) Lack of earlier vertebrate fossils shows that they had perhaps evolved in a habitat (freshwater?) which was inimical to fossilization. It is also likely that the earliest ancestors lacked hard skeletal materials, i.e., bone.

(4) They developed heavy bony armours perhaps for survival against the attacks of contemporary giant arachnid predators, the eurypterids. As these enemies disappeared, the descendants of ostracodermis, the cyclostomes, also lost the unwanted heavy armour which was a hinderance in rapid progression.

(5) The cartilage of cyclostomes and sharks and skates (Chondrichthyes) was previously considered a precursor to bone and more primitive. Since the ostracoderms had bony skeletons, the bone is now considered more primitive and the cartilage is interpreted as a degenerate condition.

Origin of Agnatha

The oldest known group of vertebrates is regarded to be Agnatha which includes the extinct ostracoderms and the living cyclostomes. The living cyclostomes are regarded to be highly specialized relics of the ostracoderms line. Stensio (1927) believed that cyclostomes had descended from some group of ostraderms by the evolution of a sucking mouth, and development of cartilage. Their structural organization has been considered higher than that of Branchiostoma (Cephalochordata) but lower than that of Gnathostomata (jawed vertebrates).

There is increasing evidence to believe that there were two major and not so much related groups of ostracoderms : (i) the cephalaspides having several pairs of gill slits, pairs fin-like appendages, heterocercal tail and a single median nostril; and (ii) the pteraspids having a single pair of gill-slits, no paired appendages, hypocercal tail and two separate nostrils where known. According to Stensio (1927), the lampreys (order Petromyzontiformes) represent derivatives of cephalaspid line and the hagfishes and slime eels (order Myxiniformes) are probably derived from pteraspid stock. This view holds that existing cyclostomes are diphyletic in origin.

In 1968, E. Jarvick in his article “Aspects of Vertebrate Phylogeny” in the book entitled “Current Problems of Lower Vertebrate Phylogeny”, put forth a new idea that cyclostomes and gnathostomes are sister groups, and that gnathostomes betray several features even more primitive than the earliest recorded cyclostomes.

To conclude, the phylogenetic status or systematic position of cyclostomes remains doubtful. They are a dead line now. The gnathostomes had their origin much earlier than cyclostomes in the ostracoderm line.

1. Salient features of Cyclostomata

(Gr., cyklos, circular; stoma, mouth)

General Characters

1. Body elongated, eel-like.
2. Median fins with cartilaginous fin rays, but no paired appendages. Tail diphycercal.
3. Skin soft, smooth, containing unicellular mucous glands but no scales.
4. Trunk and tail muscles segmented into myotomes separated by myocommata.
5. Endoskeleton fibrous and cartilaginous. Notochord persists throughout life. Imperfect neutral arches (arcualia) over notochord represent rudimentary vertebrae.
6. Jaws absent (group Agnatha).
7. Mouth ventral, suctorial and circular, hence the class name Cyclostomata (Gr. cyklos, circular + stoma mouth).
8. Digestive system lacks a stomach. Intestine with a fold, typhlosole.
9. Gills 5 to 16 pairs in lateral sac-like pouches of pharynx, hence another name of class, Marsipobranchii, Gill-slits 1 to 16 pairs.
10. Heart 2-chambered with 1 auricle and 1 ventricle, with a conus arteriosus anteriorly. Many aortic arches in gill region. No renal portal system. Hepatic portal system present. Blood with leucocytes and nucleated circular erythrocytes. Body temperature variable (poikilothermous).
11. Two mesonephric kidneys with ducts to urinogenital papilla.
12. Dorsal nerve cord with differentiated brain. 8 to 10 pairs of cranial nerves.
13. Single median olfactory sac and single median nostril. Auditory organ with 1 or 2 semicircular canals.
14. Sexes separate or united. Gonad single, large, without gonoduct.
15. Fertilization external. Development direct or with a prolonged larval stage.

Classification

About 50 species of the living jawless fishes are recognized. They belong to two major divisions (Petromyzontiformes and Myxiniformes) termed variously as subclasses, orders or families. Because they possess a round jawless mouth, they are combined in the class Cyclostomata. The similarity of these two groups is probably the result of convergent evolution. However, they show important and basic morphological differences which can be attributed to their long phylogenetic separation and different habits and habitats. Their differences have been enumerated in the Table 1.

Order 1. Petromyzontiformes

(Gr., pertos, stone + myzon, suck)

Members of this order are called lampreys or lamper eels or lamperns or sand pride etc.

1. Mouth ventral, within a suctorial buccal funnel beset with many horny teeth.
2. Nostril dorsal. Nasohypophyseal sac closed behind, not connected to pharynx.
3. Gill pouches and gill slits 7 pairs each, opening in a separate respiratory pharynx.
4. Dorsal fin well developed.
5. Branchial basket complete.
6. Dorsal and ventral roots of spinal nerves remain separate.
7. Ear with 2 semicicular canals.
8. Eggs numerous, small. Development indirect with a long larval stage and metamorphosis.
9. Both marine and freshwater forms.

   Examples : Lampreys. Over 30 species. *Petromyzon, Lampetra, Entospherus,Ichthyomyzon.*

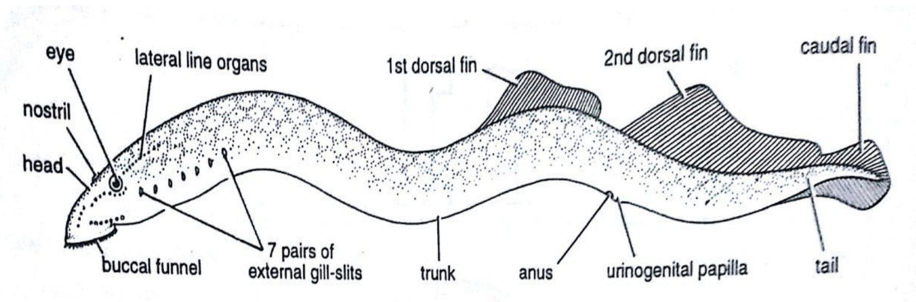


Fig. Petromyzon

Order 2. Myxiniformes

(Gr., *myxa*, slime + *oidea*, type of)

Representatives of this class are called hagfishes. They are exclusively marine.

1. Mouth terminal with 4 pairs of tentacles and few teeth. No buccal funnel.
2. Nostril terminal. Nasohypophyseal duct opens behind into pharynx.
3. Gill pouches 6 to 15 pairs. Gill slits 1 to 15 pairs.
4. Dorsal fin feeble or absent.
5. Branchial basket poorly developed.
6. Dorsal and ventral roots of spinal nerves united.
7. Ear with only 1 semicircular duct.
8. Eggs few, large. Development direct.
9. Hagfishes are all marine 15 species.

Examples : *Myxine, Eptatretus (=Bdellostoma), Paramyxie.*

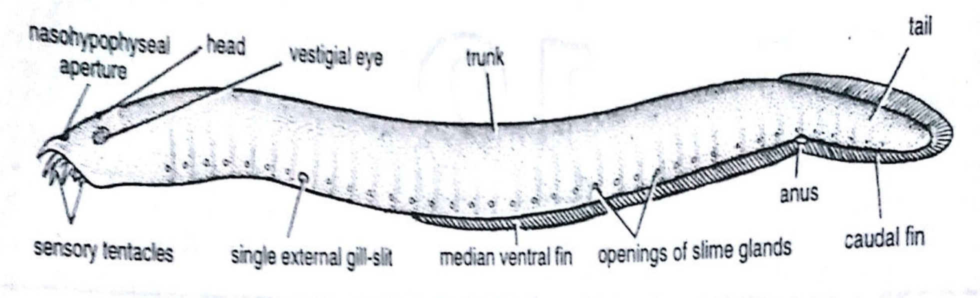


Fig. Myxine

Affinities of Cyclostomata

The chordate characteristic of cyclostomes are clear-cut. They represent the most primitive members among living vertebrates. The similarity of several features of ammocoete larva of lamprey with Cephalochordata (Branchiostoma) indicates primitive relationship. But the adult cyclostomes have certain specialized as well as degenerate features as adaptations to a parasitic habit. Thus, the affinities of cyclostomes may be best described by discussing their (i) primitive, (ii) advanced, (iii) specialized and (iv) degenerate characters, as under.

Primitive characters

1. Resemblances with Cephalochordate (Branchiostoma)

   The adult cyclostomes and Branchiostoma have many characters in common, such as:

   a) Lack of jaws, exoskeleton, paired fins and gonoducts.
   b) Persistent and continuous notochord.
   c) Segmental muscle blocks or myotomes.
   d) Numerous gill slits.

e) Straight and simple alimentary canal.
f) Dorsal and ventral roots of spinal nerves separate in lamprey.

Besides these, the ammocoete larva of lampreys further resembles Branchiostoma in:

a) Fish-shaped body.
b) Vestibule (oral hood) anterior to mouth,
c) Continuous median dorsal and caudal fins,
d) Ciliated gut,
e) Microphagus filter feeder,
f) Endostyle functions in feeding.

2. Differences from fishes (vertebrates)

Both cyclostomes and fishes are aquatic vertebrates, but cyclostomes present many primitive characters in which they differ from fishes, such as :

a) Absence of biting jaws, scales, true teeth, paired appendages, true fin rays , girdles, ribs, stomach, spleen and gonoducts,
b) Diphycercal caudal fin,
c) Continuous median dorsal fin,
d) Single median nostril rather than paired,
e) Incomplete or poorly developed cranium, vertebral column, intestinal spiral valve, pancreas, brain, sympathetic nervous system and lateral line organs,
f) Heart S-shaped tube,
g) 9^{th} and 10^{th} cranial nerves not enclosed by cranium,
h) Non-myleinated nerves,
i) 1 to 2 semicircular canals in ear instead of 3 of advanced vertebrates,
j) Poorly developed lateral line sense organs.

3. Affinities with ostracoderms

The oldest fossils of vertebrates are fragments of ostracoderms belonging to Ordovician. They become abundant in Silurian but died out in Devonian. Palaeontologists believe that they were the forerunners of higher fish. The fossil ostracoderms and living cyclostomes are grouped together under Agnatha because of the following structural similarities:

a) Absence of biting jaws.
b) Single nasal opening.
c) Pineal eye.
d) no paired limbs.
e) Pouch-like branchial sacs.
f) Internal ear with 2 semicircular canals.
g) Lateral line system.

Advanced or vertebrate characters

Cyclostomes are undoubtedly vertebrates as they have many advanced though simple of fishes and higher vertebrates. These are:

(i) Formation of a distinct head bearing paired eyes and internal ears.
(ii) Differentiated brain like embryonic vertebrates with several pairs of cranial nerves.
(iii) Cranium for housing brain.
(iv) Beginning of segmental vertebrae.
(v)Stratified or multilayered epidermis.
(vi) Dorsal root ganglia on spinal nerves.
(vii) Sympathetic nervous system.
(viii) Lateral line organs.
(ix) Gills primarily used for respiration and not for food collection as in Branchiostoma.
(x)Water enters into pharynx by muscular activity and not by ciliary activity as in Branchiostoma.
(xi) E-shaped myotomes as in fishes.
(xii) Presence of liver, gall bladder, bile duct, pineal and parietal eyes, pancreatic cells in midgut wall and thyroid and pituitary glands.
(xiii) Well developed circulatory system with a muscular, contractile heart.
(xiv) Blood with erythrocytes and leucocytes.
(xv) Hepatic portal system.
(xvi) Mesonephric kidneys.

Specialized characters

Adult cyclostomes are too specialized or too degenerative in many respect. It is probable that many adult characteristics are adaptations for parasitic mode of feeding. Some of their specialized features are:

(i) Suctorial mouth and buccal funnel with armature of horny spikes in lampreys for attachment to host body.
(ii) Powerful, muscular tongue, heavily armed with sharp horny teeth serves as a rasping organ while feeding.
(iii) Production of anticoagulants in saliva to feed on blood and body fluids of prey.
(iv) Peculiar sac-like gill pouches located far behind head.
(v)Posterior position of gill openings, probably an adaptation to burrowing.
(vi) Complete separation of ventral sac-like respiratory pharynx from dorsal oesophagus.
(vii) Respiratory water entering gill pouches as well as leaving them through mouth which mostly remains attached to rocks or fishes for feeding.
(viii) Large mucous glands secreting enormous quantities of mucus inn hagfishes.
(ix) Dorsal position of single nosril high on head in lampreys.

Degenerate characters

The degenerate characters of cyclostomes include :

(i) Simple, cylindrical eel-like body form compared to broad fish-like shape of ostracoderms.
(ii) Lack of bony armour or exoskeleton.
(iii) Lack of bony endoskeleton which is cartilaginous.
(iv) Absence of paired fins and girdles.
(v)Vestigial eyes covered by thick skin and muscle in hagfishes.
(vi) Reduced liver and disappearance of gall bladder and bile duct in adult lamprey.

3.6 The early Gnathostomes (Placoderms)

(Gr., gnathos, jaw; stoma, mouth)

The First Jawed Vertebrates

In the mid-Silurian, some 450 million years ago, as the ostracoderms were disappearing, a host of more efficient and jawed fishes appeared. Earlier grouped in a single category, they are now placed into two separate classes;

Placodermi and Acanthodii. Taxonomists place them lower than Chondrichthytes in the evolutionary scale.

Aconthodians were little shark-like fishes covered by diamond-shaped scales. A common fossil genus, Climatius, was about 8 cm long. Between larger pectoral and pelvic fins, they had extra smaller paired fins some of which in the form of stout spines, hence the name, 'spiny sharks' (Gr., acantha). Some of them features true teeth, especially on the lower jaw. The acanthodians survived into early Permian before they became extinct.

Occurrence

Placodermi were earliest jawed vertebrates of fossil record. They appeared in Silurian, flourished in Devonian and Carboniferous and became extinct in Permian. They probably lived both in fresh water as well as seas. Some primitive agnath ostracoderms were probably the ancestors of placoderms. But their fossil record does not show any connecting link between the jawless and the jawed fishes. Like ostracoderms before them, the jawed fishes also appear fully formed without intermediates. Fossil evidence for the ancestry of Placodermi simply does not exist.

Important Features

The placoderms combined the heavy external bony armour of the ostracoderms with powerful jaws and sufficient fins.

1. Bony armour. In their heyday the placoderms were highly diversified, ranging in length from a few centimetres to 3 metres or more. The giant predator, Dunkleosteus, grew to 10 metres. Despite the differences among them, all were characterized by the presence of a bony skeleton. Some in particular (Dunkleosteus) featured a heavy armour of bony plates over the heads and anterior part of trunk, while the rest of the body was nearly naked. The name Placodermi means 'armoured fish' or 'plaate-skinned' (Gr., plakos, plates + derma, skin). Their dermal armour links them genetically with their predecessors, the ostracoderms.
2. Jaws. All placoderms possess jaws which are supposed to have originated from th first pair of gill bars (mandibular arch) in front of the first gill slit. Hyoid gill arch persisted as a result of which spiracles failed to arise. Hyoid arch remained unmodified and extended support to the jaw. This type of primitive jaw suspensorium is called aphetohyoidean type. With paired fins and bony jaws they became

more active, predaceous and specialized than the jawless ostracoderms. Jaws enabled them to spread into new habitats, make use of a wider variety of food and evolve immeasurably. Jaws also provided protection or defense and the ability to manipulate objects. With a biting mouth, heavy external armour probably became unnecessary and tended to disappear with the result that descendants could grow still larger and swim faster. No wonder the success of jawed fishes probably contributed to the extinction of the ostracoderms and to the limitation of cyclostomes.

No placoderm ever developed teeth of a modern type as did the acanthodians. Also their jaws were immovably bound to cranium or rest of the head. These handicaps may have been involved in their eventual extinction.

3. Paired fins. Most placoderms possess paired fins. In an aquatic environment, development of strong mobile fins was coincident with the evolution of jaws, for swimming faster. The lateral fins served, like the elevators and ailerons of an aircraft, to produce turning movements in any direction (right, left, up or down) and to prevent roll, pitch, and yaw when swimming in a straight path. placoderms survived for a short period only and are often considered as 'unsuccessful ancient experiment', in the evolution of gnathostomes.

Classification

Various schemes of classification have been forwarded by workers. Berg (1940) and Romer (1959) forwarded detailed classification of fishes, which have been generally accepted. Bertin and Armbourg (1958) and Greenwood et. al.,(1989) suggested altogether new scheme of classification, often called modern classification of fishes.

All the extinct earliest jawed fishes were once believed to be generally related and therefore placed in a group or class, the Placodermi. But the spiny sharks are now placed in a separate class Acanthodii. Two groups, subclasses or orders of class Placodermi are generally recognized as follows:

I. Order Arthrodiriformes

Earliest placoderms. Resembled ostracoderms in appearance and habitat. Heavy bony armour shields of head and trunk meeting in a movable joint (Gr., arthros, joint). Powerful gaping jaws with sharp shearing blades. Violently predaceous. Devonian. Coccosteus, Dinichthyes.

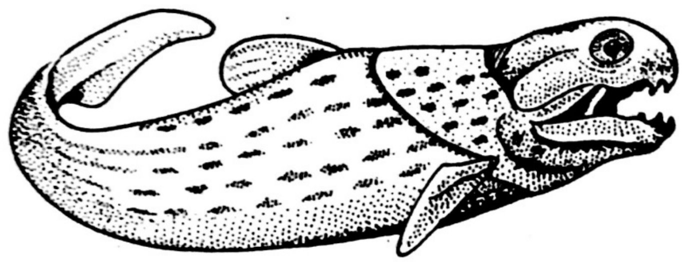


Fig. Dinichthyes

II. Order Antiarchiformes

Bottom-dwellers and mud-feeders in fresh water. Ecologically similar to and competing with flat agnaths. Head and fore part of trunk covered by a depressed bony shield. These cephalic plates had a different arrangement and the mode of attachment of head with body shield was also different. Pectrol fins long. Devonian. Bothriolepis, Pterichthyodes (=Pterichthys)

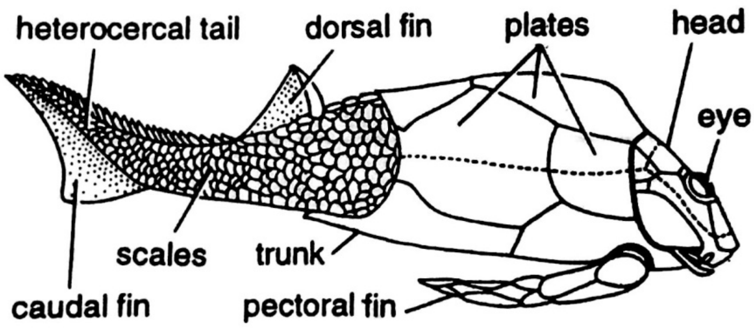


Fig. Pterichthyodes

Boilogical Significance

Placoderms were the earliest of the known fossil vertebrates with jaws, mostly creatures of the Devonian. Their bony armour links them genetically with their predecessors, the ostracoderms. With bony jaws and paired fins, they were more specialized than ostracoderms. A new era in the history of fishes opened with the advent of jaws. As Prof. A.S. Romer recalls in his book, The Vertebrate Body, that, "Perhaps the greatest of all advances in vertebrate history was the development of jaws and the consequent revolution in the mode of life of early fishes". There is no fossil evidence for the ancestry of placoderms. Some primitive ostracoderms were probably their ancestors. According to one view, the placoderms all became extinct by the end of the Palaeozoic era, without giving rise to any surviving forms. According to another view, the cartilaginous fishes or Chondrichthyes and the bony fishes or

Osteichthyes, both arose in early Devonian from some primitive group of cartilaginous fishes did not give rise to bony fishes. Thus bone, rather than cartilage, is to be considered a primitive characteristic.

3.7 Summary

- Vertebrates belong to the subphylum Vertebrata of the phylum Chordata. Their names is derived from the presence of serially arranged 'vertebrae' (L., vertebrates, jointed), which comprise a major part of their axial exoskeleton, the vertebral column or the backbone.
- The evolutionary history of group organisms is termed phylogeny. It is derived from Greek roots which mean "to beget a race": (Gr. phylon, race or tribe + gennao, to bring forth). The concept of phylogeny is used to place animal groups in proper evolutionary sequence. Thus in phylogenetic order fishes came before reptiles and reptiles before birds and mammals.
- In evolutionary biology, adaptive radiation is a process in which organisms diversify rapidly into a multitude of new forms, particularly when a change in the environment makes new resources available, creates new challenges, or opens new environmental niches.
- Agnatha (Greek, "no jaws") is a superclass of jawless fish in the phylum Chordata, subphylum Vertebrata, consisting of both present (cyclostomes) and extinct (conodonts and ostracoderms) species.
- Placodermi were earliest jawed vertebrates of fossil record. They appeared in Silurian, flourished in Devonian and Carboniferous and became extinct in Permian. They probably lived both in fresh water as well as seas. Some primitive agnath ostracoderms were probably the ancestors of placoderms.

3.8 Self Assessment Questions

Section -A (Very Short Answer Type)

1. Give two examples of group Agnatha?
2. Which is first jawed vertebrate?
3. Define Agnatha?
4. What is adaptive radiation?

Section -B (Short Answer Type)

1. Describe difference between protostomia and deuterostomia?
2. Write short notes on :

a) Ostracoderms
b) Placodermii

3. Describe general characters of cyclostomata?

Section -C (Long Answer Type)

1. Write an essay on the origin and ancestry of vertebrates.
2. Describe in detail adaptive radiation in vertebrates.
3. Write short notes on :
   a) The early Gnathostomes
   b) Cyclostomata
4. Write an essay on origin, evolution and general characters of Agnatha.

3.9 Reference

- Modern text book of Zoology Vertebrates by R.L. Kotpal
- https://en.wikipedia.org/wiki/Evolutionary_radiation
- https://books.google.co.in/books?id=tEhO-

Unit-4

Pisces

Structure of the Unit

4.0 Objectives

This unit is designed to understand the diversity, physiology, morphology and behavior of class Pisces. Object in this Unit is, therefore, to help the reader/student to learn as much as possible about Fish life:

- The diversity and various degrees of structural difference in the fishes to divide and subdivide them in a systematic classification
- The Origin and Evolution of representative fish forms which are very unlike in structure and origin
- The harsh environment have led deep sea fishes to fascinating adaptations of deep sea life for sensing, feeding, reproducing, moving and avoiding being eaten by predators
- Various functions and ways in which the boy fishes have become specially modified for different ecological conditions
- The practice of parental care in marine and many more freshwater forms in different modes, from simple to complex
- Offense and defense; the ways of influencing relationships of fishes with each other that is what one need while avoiding being what else needs

- The vital role played by the sense organs in the fish in smooth surviving
- How the Air Bladder works in fish as Hydrostatic Organ, also to learn about its position, function and types
- Distribution, Position and sense perception of Lateral Line System.

4.1 Introduction

Fish are an important resource for humans worldwide, especially as food. Commercial and subsistence fishers hunt fish in wild fisheries or farm them in ponds or in cages in the ocean. They are also caught by recreational fishers, kept as pets, raised by fishkeepers, and exhibited in public aquaria. Fish have had a role in culture through the ages, serving as deities and religious symbols also. Besides all these the fish exhibit great species diversity than any other group of vertebrates.Fish are abundant in most water bodies.

Fish are limbless aquatic vertebrate animal with fins and internal gills. There are three living classes of fish: the primitive jawless fishes, or Agnatha; the cartilaginous (sharklike) fishes, or Chondrichthyes; and the bony fishes or Osteichthyes. These groups, although quite different from one another anatomically, have certain common features related to their common evolutionary origins or to their aquatic way of life. Fish were the earliest vertebrates and presumably evolved from a group of aquatic lower chordates; the terrestrial vertebrates evolved from fishes.

The earliest organisms that can be classified as fish were soft-bodied chordates that first appeared during the Cambrian period. Although they lacked a true spine, they possessed notochords which allowed them to be more agile than their invertebrate counterparts. Fish would continue to evolve through the Paleozoic era, diversifying into a wide variety of forms. Many fish of the Paleozoic developed external armor that protected them from predators. The first fish with jaws appeared in the Silurian period, after which many (such as sharks) became formidable marine predators rather than just the prey of arthropods.

Fish have great ability to adapt a diverse environment of light, pressure, currents, temperature, oxygen, nutrients, predators, food, mates, competitors or symbionts. Some marine forms and many more freshwater forms practice parental care and influence relationships with each other that are what one need while avoiding being what else needs.

The sense organs, olfactory sacs, eyes, and auditory organs, are present. The olfactory sacs are a pair of simple depressions or invaginations of the epidermis open in front of the mouth. The eyes have a flatter cornea and a more spherical lens than those of terrestrial vertebrates.The auditory organs are membranous sacs entirely enclosed in the auditory capsules of the skull, The skin possess the sense of touch all over its surface as a result of the presence of terminations of sensory nerves, without any special organs, but there are also definite sense-organs in the epidermis composed of groups of sensory cells united into spindle-shaped bodies called end-buds; these are irregularly distributed over the body, on the fins and lips, and also in the epithelium of the mouth and pharynx. It will be noted that the series of lateral-line organs radiate from a focus formed by the auditory organ which is really to be regarded as a special and enlarged sense-organ of the same system. Go through the unit and learn all these in detail.

4.2 Classification

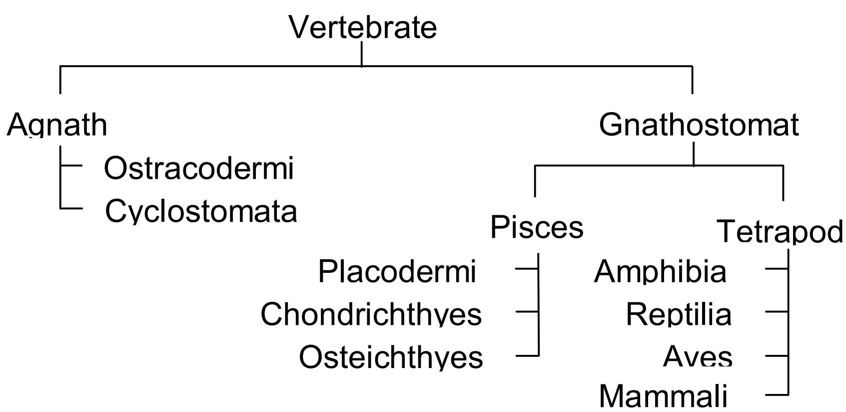
4.2.1. Gnathostomata

4.2.2. Super-class Pisces

4.2.2.1. Division Elasmobranchii
4.2.2.2. Division Acanthodi
4.2.2.3. Division Holocephali
4.2.2.4. Division Teleostomi
4.2.2.5. Sub-class Dipnoi

Fishes are vertebrates with usually scaly skins, permanent gills, and paired fins. The heart is two or three-chambered. The skeletons may be cartilaginous or bony. Gill-apertures are four to seven pairs. Dorsal and ventral spinal nerves join to form mixed trunks. The liver has at least two lobes.

In accordance with the various degrees of structural difference the fishes can be divided and subdivided in a systematic classification.



Some of the oldest and least satisfactorily known Palaeozoic fishes are gathered under Class Ostracodermi. So aberrant are they in structure that their very right to be placed among the Pisces at all has been disputed. Other Class cyclostomata are without visceral arches transformed into jaws, and without paired limbs. Nasal tube unpaired median. Liver is a compact gland. Pronephros persistent, gonomeres fused. With bag-shaped gill-pouches or gill-slits and mouth is suctorial. Lampreys and hagfishes are members of the class. The true fishes are grouped as under.

4.2.1 Gnathostomata

1. With visceral arches
2. One pair of which is modified into jaws
3. With an anterior and a posterior pair of limbs.
4. Super classes are (i) Pisces and (ii) Tetrapoda.

4.2.2 Super-class Pisces

1. Respiring by gills only
2. Heart entirely venous
3. Nasal blind-sacs not communicating with the mouth.
4. Classes are (i) Placodermi (ii) Chondrichthyes and (iii) Osteichthyes.

4.2.2.1 Division Elasmobranchii

1. With seven, six, or mostly five, pairs of branchial arches, enclosing as many gill-clefts
2. The gill-bearing clefts are separated by complete septa, which are continuous with the outer skin
3. Vertebrae with chorda-centra, unless still acentrous
4. Without membrane bones
5. Skin with numerous small enamelled denticles
6. Males with a pair of mixopterygia
7. Orders (i) Proselachii and (ii) Plagiostomi.

Order Proselachii

1. Mouth subterminal, without a rostrum
2. With archipterygium biseriale
3. Vertebrae still acentrous
4. *Pleuracanthus* (*Xonacanthus*) Decheni Lower Permian and Carboniferous. *Cladodus*. Lower Carboniferous. Fins with almost preserial archipterygium.

Order Plagiostomi

1. Mouth transverse and ventral, with a rostrum
2. With Ichthyopterygium preseriale
3. Pectoral fins separated from the head
4. i.e.*Pristis*, *Zygaena*, *Torpedo etc*.

4.2.2.2 Division Acanthodi

1. With five Elasmobranch gill-clefts, but each with an external fringe-flap
2. Mouth sub-terminal
3. Vertebrae acentrous without mixipterygia
4. Paired and median fins with a strong dermal spine
5. Large dermal bones on the jaws; dermal armature of trunk

   and cranium consisting of small granules, i.e. *Acanthodes* from Devonian to Permian.

4.2.2.3 Division Holocephali

1. Palato-quadrate bar fused with the cranium
2. Vertebrae acentrous; numerous calcified rings in the notochordal sheath
3. Agreeing with Elasmobranchi : males with mixipterygia absence of membrane bones; conus arteriosus with three series of valves.
4. Agreeing with Tectobranchi : four gill-clefts, with one large operculum; the gill-septa are thin and incomplete, no longer reaching the surface; with a few large teeth resembling those of Dipnoi; mouth subterminal.
5. i.e. *Ptyctodus*, *Squaloraia*, *Chimaeropsis*, *Myriacanthus*, *Ischyodus*, *Callorhynchus antarcticus*, *Chimaera monstrosa*, *Onchus*, *Homacanthus*, *Ctenacanthus*.

4.2.2.4 Division Teleostomi

1. Vertebrae acentrous or arcocentrous
2. Without mixipterygia
3. Tectobranchi, i.e. gills with one large operculum
4. With membrane bones
5. Mouth terminal or subterminal
6. Ova numerous and small
7. Orders (i) Crossopterygii and (ii) Actinopterygii

Order Crossopterygii

1. Paired fins lobate, with a thick axis and biserial fin-rays
2. With a pair of jugular plates

3. i.e. *Osteolepis*, *Diplopterus*, *Holoptychius*, *Glyptolepis*, *Megalichthys*, Coelacanthidae, *Macropoma*, *Polypterus*.

Order Actinopterygii

1. Paired fins, with multibasal, uni-preserial ichthyopterygium
2. Sub-orders
   (i) Chondeostei i.e. *Cheirolepia*,*Platysomus*,*Chondrosteus*, *Acepenser*, *Polyodon*
   (ii) Holostei i.e.*Lepidotu*, *Eurycormus*, *Callopterus*, *Osteorhachis*, *Amia*, *Aspidorhynchus*, *Lepidosteus* and
   (iii) Teleostei i.e. *Scopelus*, *Cyprinus*, *Salmo*, *Clupea*, *Xiphias*. *Cyclopterus*, *Channa*, *Anabas*, *Pleuronectes*, *Tetrodon*, *Diodon*, *Hippocampus*.

4.2.2.5 Sub-class Dipnoi

1. With gills and lungs. Heart trilocular, with mixed blood
2. Nasal ducts lead into the mouth-cavity
3. With archipterygium, no mixipterygium
4. Tectobranch, conus with numerous series of valves
5. Membrane bones, teeth and the acentrous but potentially chordocentrous vertebral column much resembling Holocephalous conditions; the same applies to the holostylic arrangement -spiral valve.
6. Orders (i) Arthrodira and (ii) Sirenoidei

Order Arthrodira

1. Strong dermal armour, also on the ventral side
2. Paired fins vestigial or absent
3. With maxilla and premaxilla, which are toothless
4. Teeth on mandible and palatal region
5. i.e. *Coccosteus*, *Dinichthys*, *Titamchthys*.

Order Sirenoidei

1. Dermal plates restricted to the trunk with imbricating scales, or naked
2. Paired fins present, archipterygial

i.e. *Dipterus*, *Ctenodus*, *Ceratodus*, *Protopterus*, *Lepidosiren*.

4.3 Origin and Evolution

4.3.1 Characteristics of ostracoderms

4.3.2 Origin of Phyla

4.3.2.1 Cyclostomata

4.3.2.2 Elasmobranchii and Holocephali

4.3.2.3 Arthrodira, Teleostomii and Dipnoi

The full recognition of the ostracoderms as the common ancestor and it forms the starting point for all vertebrates. The representative fishes forms elasmobranchii, holocephali, teleostomi and cyclostomes are very unlike in structure and origin.

4.3.1 Characteristics of ostracoderms

Ostracoderms are consist of a comparatively small number of metameres, and are provided with a highly developed dermal armor, cephalic locomotor appendages, paired jaws, a large atrial or peribranchial chamber, and with eyes and olfactory organs located near the middle of the aboral surface of the forehead. The form and general appearance suggest that of a trilobite or merostome, or an amphibian tadpole, rather than that of a true fish. But the notochord is diminutive in them. They are without definite constrictions or thickenings of the sheath to form centra, and without recognizable neural or haemal arches, pectoral or pelvic appendages, or teeth.

Probably not later than the Silurian period, three or four well defined phyla grew out of the ostracoderms. The main line of ascent probably leads from the typical ostracoderms, through the antiarcha and arthrodires, to the crossopterygians, dipnoi and amphibia. Evolution along this line leads to the first air-breathing land vertebrates, the culminating metamorphosis depending on remote antecedent changes in the dermal skeleton, appendages, air bladder, and heart. The remaining phyla stand quite apart from this main stem. They are characterized by the breaking up of the dermal armor into minute plates (elasmobranches) or by their absence altogether (the later holocephali); by the absence of an air bladder, branchial chamber, and leg-like fins. The cyclostomes end in lampreys; the holocephali in chimeras, and the elasmobranches in sharks and rays. We may characterize the several phyla arising from the ostracoderms.

4.3.2 Origin of Phyla

4.3.2.1 Cyclostomata

The cyclostomes may be regarded as one of the off-shoots of the ostracoderms. We may consider their chief characteristics under three heads, namely those derived from the ostracoderms, those gained, and those lost since their separation from them.

1. The cyclostomes retain the following organs derived from the ostracoderms: A median, practically unpaired, olfactory organ, merged with a persistent hypophysis that opens on the dorsal surface of the head. An uncommonly large and well developed parietal eye.

   There are, in the adults of some genera, three pairs of oral arches provided with rudimentary appendages. The arches are comparable with the three pairs in amphibian embryos, i.e., premaxillae, maxillae, and mandibles, and with the three dental-plate arches of adult ostracoderms and arthrodires. The true mouth is not always circular, but may be a narrow, longitudinal slit. The oral region may be surrounded by a wide papillae fold of ectoderm that forms a shallow, circumoral antechamber, or pre-oral hood, comparable with the membranous folds on the free anterior edges of the dorsal and ventral shields of Bothriolepis.

2. The cyclostomes have lost, probably at a very early period, their ancestral dermal armor, including the jaw plates; also the cephalic swimming appendages and lateral folds; and the tadpole-like form does not appear in any phase of their development.

3. The cyclostomes have failed to develop many important organs that have appeared in the other descendants of the ostracoderms. There are no paired pectoral or pelvic appendages; the notochord persists in a practically unmodified condition; no traces of ring-like calcifications of its sheath appear, and only the most diminutive neural and haemal spines are developed. The air bladder and teeth of the vertebrate type are absent. The actual progress made by the cyclostomes since their separation from the ostracoderms is therefore insignificant, the only noteworthy gain being in the increased number of body segments, giving additional freedom and facility of locomotion, and an imperfect adaptation to a parasitic mode of life. The cyclostomes may therefore be regarded as very ancient animals, deriving their underlying primitive characters from the ostracoderms, and owing the present simplicity of their organization.

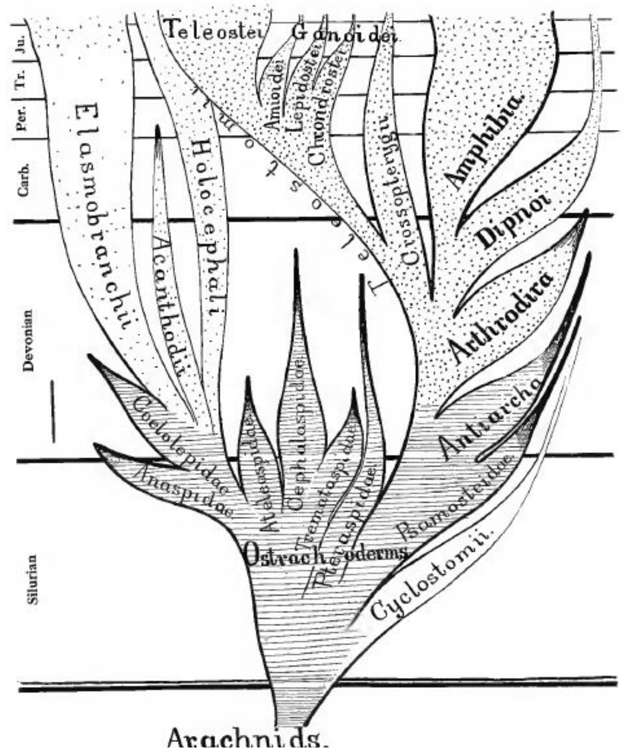


Fig.: Diagram to illustrate the probable phytogeny of the vertebrate stock

4.3.2.2 Elasmobranchii and Holocephali

Elasmobranchii : The advent of the elasmobranchs is clearly foreshadowed in the Silurian period by the appearance of the coelolepidae with their fish-like form and shagreen-like armor. The successive steps in the fragmentation and final disappearance of the dermal armor are well shown in this branch of the ostracoderms. The process begins in the cephalaspidas with the formation of wellmarked, but immovable polygonal areas, followed by the appearance of the small free plates of Ateleaspis and Lasanius, and by the isolated dermal denticles of Thelodusand the elasmobranchs, which finally disappear altogether in their naked-skinned, modern representatives. The primitive elasmobranchs quickly acquired a fish-like form, losing the extensive ancestral peribranchial chamber, and at no phase of their development showing, so far as known, any trace of a tadpole stage.

The notochord, at an early period, is invested and largely replaced by well developed cartilaginous centra, and an elaborate system of more or less calcified cartilaginous gill bars, and neural and haemal arches are developed.

The lateral fold is generally retained for a longer or shorter period, giving rise by local enlargement to well defined pectoral and pelvic fins. The parietal eye is not conspicuously developed; neither are the three pairs of embryonic oral arches, nor the corresponding appendages. True teeth appear on the upper and lower margins of the mouth, but they are not preceded by any recognizable dental plates. An air bladder is absent.

The ova are very large, fertilized within the body, and the males are provided with highly specialized intermittent organs or claspers.

The elasmobranches, owing largely to their well developed sensory and locomotor organs, and to their formidable jaws and teeth, developed rapidly in efficiency during the late palaeozoic period, but they pass the climax of their evolution without producing a noticeably higher type of organization. While the internal skeleton may be highly developed and more or less calcified, it never develops into true bone.

The elasmobranchs are pelagic or deep water fishes rather than frequenters of the shallow brackish waters of the shore.The absence of an air-bladder excluded the possibility of their becoming air breathers, and the pectoral and pelvic fins show no signs of developing into elongated, digitate appendages suitable for locomotion on land.

Holocephali probably arose from the coelolepid branch of the ostracoderms, developing along somewhat similar lines as the elasmobranchs, but retaining certain features of the parent stock not seen in the latter. The notochord is persistent, but enveloped by ring-like calcifications of its sheath more numerous than the cartilagenous neural and haemal arches. They retain the large head and small trunk, or tadpole form, of the ostracoderms; a peribranchial chamber, and a short body cavity, with the viscera and anus placed well forward. Three pairs of dental plates are present, consisting of vascular dentine and growing from persistent pulps. They probably represent the premaxillary, maxillary, and mandibular plates of the ostracoderms and arthrodires, and belong to the three pairs of primitive oral arches seen in amphibian embryos. The general shape of the mouth and the form of the jaws resemble those of the ostracoderms and amphibian tadpoles rather than those of an elasmobranch. No true vertebrate teeth are developed.

The primitive characters above mentioned are those of the ostracoderms and are sufficient to distinguish the chimseras from all other adult vertebrates. But the cartilaginous internal skeleton, the large-sized ova, the internal fertilization, the anal claspers of the males, and the absence of an air bladder, indicate an affinity, although probably a remote one, with the elasmobranchs.

4.3.2.3 Arthrodira, Teleostomii, and Dipnoi

The antiarcha, at some time probably not later than the early Devonian, gave rise to the arthrodira. From the latter sprang the teleostomii and dipnoi, and from them, near the beginning of the carboniferous, the first air-breathing vertebrates, or amphibians from which, at some subsequent period, all the higher vertebrates had their origin, directly or indirectly.

These animals, the most vigorous and varied offspring of the ostracoderms, form a homogeneous stock that stands distinctly apart from all other primitive vertebrates. In practically all of them the ancestral dermal armor has a prolonged and flourishing existence, and is rarely, or never, entirely suppressed. In the more primitive groups it survives in the form of large superficial plates ornamented with low rounded tubercles, or with sinuous, beaded ridges. On the trunk and tail they are usually smaller, forming irregular, polygonal, or rounded scales which tend to sink deeper into the skin and eventually to disappear, leaving even in such primitive forms as Bothriolepis and some coccosteans a practically naked skin behind. But in the adults of all branches of the phylum a considerable number of the ancient, large-sized cranial plates are retained in the head region, forming a characteristic covering for the roof and sides of the head, jaws, gill chamber, and pectoral arches. With this prolonged survival of the primitive dermal armor, there is an early and vigorous development of an endoskeleton, consisting of true bone, which here makes its appearance for the first time in the history of the animal kingdom.

A bony floor and sides to the endocranium are formed, as well as complete bony vertebrae consisting of centra intimately united with neural and haemal arches and transverse processes.

A large peribranchial chamber is always present, but the rigid, armored walls of this chamber, so characteristic of the ostracoderms, are greatly shortened in the arthrodires, and in the teleostomes, dipnoi, and amphibia, give place to membranous folds that may or may not be strengthened by movable opercular plates.

Three pairs of oral arches are usually conspicuous in the embryonic stages, and the branchial arches may retain remnants of arachnid appendages, in the form of external gills, hyoidian "balancers," oral arch papillae, tentacles, or adhesive discs.

The parietal eye is generally well-developed, and so far as known, a large, lung-like air bladder occurs in all the main subdivisions of the phylum. The primitive pectoral and pelvic appendages may be narrow and elongated, with a bony internal skeleton that in the earliest fish-like descendants of the arthrodires shows, for the first time in the evolution of the vertebrates, distinct traces of the radiate terminal digits, and the jointed axis characteristic of all primitive land vertebrates.

The ova are of moderate size, frequently covered with an adhesive, gelatinous substance, and are generally fertilized externally. The antiarcha, coccosteans, dipnoi, and primitive teleostomes were preeminently shallow water, shoreloving forms, as are their survivors to-day. Their highly developed air bladder, leg-like fins, specialized breeding habits, and the general structure and mode of life characteristic of the higher members of this phyla, afford an easy anatomical and physiological transition to the amphibia, and hence to the higher air-breathing land vertebrates.

On the other hand, the young of many amphibia, dipnoi and, teleostomes pass through a larval, or tadpole, stage generally characterized by a large head, by the presence of rudimentary appendages on the oral and branchial arches, by a small mouth with a feeble lower jaw, short body cavity and slender tail, and by the absence of postbranchial paired appendages. This tadpole larva is clearly the recurrence of the ostracoderm stage in their phylogeny.

The arthrodires closely resemble the ostracoderms in the structure of their jaws and in the arrangement of their cranial plates, and without doubt are directly descended from them. While it is not possible to identify in detail all the various structures involved in the general resemblance that runs tjirough them all, we can trace a progressive series of structures and events that lead steadily upward from the ostracoderms, through the arthrodires, to the vertebrates.

Without entering into a detailed discussion of the arthrodires, an examination of Coccosteus, a fairly well-known and typical representative, will show us the more important respects in which they approach the vertebrates.

In *Coccosteus*, the central aggregate of procephalic sense organs seen in the ostracoderms has separated. The lateral eyes have increased greatly in size and have taken up an antero-lateral position, losing apparently some of their dermal armor and their power to rise and fall in the orbits; the parietal eye is lodged in a small median plate, located in about the same position as before, while the olfactory organs have moved forward and lateraly, occupying a more nearly terminal position.

Three pairs of jaws are present. The premaxillae are relatively smaller than in the ostracoderms, and although still distinctly paired, are less freely movable in a transverse direction. The rudiments of toothed maxillae are present, for the first time, as small free plates that probably represent the small ventrolateral plate of Bothriolepis. The mandibles have increased greatly in size and may be provided with prominent tooth-like spikes on their anterior and median borders, being in this respect more like arthropod jaws than those of Bothriolepis, the only ostracoderm whose mandibles are known. Like the ostracoderm mandibles, they were capable of very complex movements. Both ends were free; that is, they were not firmly articulated to any cartilage or bone, and could be either rotated, or moved in a transverse and longitudinal direction. Their exposed median ends were probably held in place by the integument, while their lateral ends were buried in the tissues of the head, and served for the attachment of the sinews and powerful muscles that controlled their movements.

A, single hyoid arch, covered with dermal bone, extended across the throat behind the mandibles, in place of the two arches of similar structure seen in *Othriolepis*.

The cranial bones are more numerous than in the antiarcha, owing probably to the breaking up of the large orbital plate by the lateral migration of the eyes. The same movement has probably opened the way for the formation, for the first time, of a supra-orbital line of cutaneous sense organs.

The branchial shield retains nearly the same number and arrangement of plates as in the ostracoderms, but it is greatly reduced in size, and deeply incised on the flanks, thus opening up the peribranchial chamber. It thereby takes on more distinctly the character of a true operculum, and apparently serves solely for the protection of the gills and heart, the digestive and urogenital organs taking up a position farther back, wholly posterior to the respiratory region, as indicated by the large size of the postbranchial section of the trunk, and by the probable location of the cloacal opening.

In probably all arthrodires, the notochord persisted throughout life with little or no hange. Although there are no indications of vertebral centra, we see for the first time a well-developed series of neural and haemal arches, the forerunners of a true vertebral column.

The cephalic appendages, a, that were so characteristic of the ostracoderms, are here rudimentary and probably functionless. With the relative decrease in the size of the head and the increase in the size of the trunk, the whole body is better balanced and more suitable for an active, free swimming existence. Locomotion was probably effected largely by the flexible trunk and tail, although immediately behind the branchial region there are traces of supports for small pectoral fins, the first appearance in this phylum of paired appendages of the vertebrate type. Pelvic fins were apparently absent.

The arthrodires clearly represent a higher type of animals than the ostracoderms. They have successfully emerged from the precarious period of profound metamorphosis in which the ostracoderms were engaged. While it lasted, an active life was inhibited by the changes going on in the old organs, and by the imperfect adjustment of the new.

With the arthrodires that period is past. They have increased notably in size; the eyes are fully adjusted to their new location within the neural tube; the mouth has become capacious, and the jaws large and powerful, with formidable cutting, or toothed margins, well suited for capturing and devouring animal food; the respiratory region is set apart from the digestive and urogenital regions, and the body is better balanced and better adapted for an active, free swimming life. The arthrodires, therefore, quickly developed from the sluggish, plant-eating stage of the ostracoderms, into the most active, rapacious, and formidable animals of their time. But as a class they were short-lived, for the structural conditions within had as yet attained only a temporary equilibrium, and the new mode of life was rapidly producing new creative forces. Out of these conditions arose the first true vertebrates of this phylum, the dipnoi and the teleostomes.

The anatomical changes involved in the creation of the new types of animals were comparatively insignificant. The head became relatively smaller and more compact, the trunk larger, and the whole body assumed a more fish-like appearance. The lateral eyes grew still larger and took up a position on the sides of the head, well behind the olfactory pits, which have also greatly increased in size. The premaxillae fused, forming the fronto-nasal process, and together with the maxillae became permanently fixed to the floor of the cranium. The distal ends of the mandibles united in the median line, and their proximal ends articulated with a cranial cartilage, to which the hyoid arch was also attached; they then lose their rotary and transverse movements, and swing forward and backward against the maxillary arch in typical vertebrate fashion.

With the fusion in the median line of the three pairs of oral arches, their dermal armor becomes the three pairs of fixed dental plates characteristic of the dipnoi and from which the isolated socketed teeth of the higher vertebrates arose.

The plates in the dorso-lateral walls of the primitive branchial shield now form movable opercula, and those in the ventral wall become attached to the mandibular and hyoid arches to form the gular plates.

The dermal plates on the dorsal surface of the head (mesocephalon) increase in number, and approach the typical arrangement seen in the primitive air-breathing vertebrates. The base of the endocranium becomes ossified; bony centra appear in the sheath of the notochord and, uniting with the neural and haemal arches, form true vertebrae.

The pectoral fins enlarge and the girdle extends dorsally, uniting with the occipital portion of the cranium.Within the pectoral fins, for the first time in the phylogeny of the vertebrates, appears an axial skeleton that approaches, in the arrangement of its elements, the characteristic structure of the appendages of the land vertebrates, i.e., Eusthenopteron. Pelvic fins appear in the anal region.

Thus the broad foundations for the evolution of the first air-breathing land vertebrates is laid in the dipnoi and ganoids, the armored fishes of the upper Devonian; reaching down through them to the arthrodires and to the ostracoderms of the Silurian, and then again beyond them to the merostomes, trilobites, and primitive phyllopods of the Ordovician, Cambrian, and Proterozoic.

In this vigorous phylum, evolution follows a logical and consistent course, each important event being the direct, or indirect result of the preceding ones, and they themselves creating the conditions that bring about those that follow. The various independent sets of organs, such as the brain, sense organs, appendages, jaws, internal and external skeleton, in their own peculiar ways move steadily onward toward the same end, in a manner that could hardly be possible except in a real, not an imaginary, line of evolution. The perfection with which these immensely varied and complicated facts and details fit together to form a definite, intelligible picture, carries with it the overwhelming conviction that that picture is an image of the truth. The precision with which each event creates again and yet again, new conditions, new organs, and new readjustments, shows that the primary forces that sustain and direct the main lines of evolution are self-creative, and lie within, not without, the organism.

Classes ostracodermi, cyclostomata, placodermi, chondrichthyes and osteichthyes are aquatic and are fishes. Out of these five classes two ostracodermi, placodermi are extinct whereas cyclostomata, chondrichthyes and osteichthyes are living. Three classes placodermi, chondrichthyes and osteichthyes are put in super class Pisces.

4.4 Deep seaAdaptations

Deep Sea Fishes face harsh environment of light, pressure, currents, temperature, oxygen, nutrients, predators, food, mates, competitors or symbionts. All these factors have led to fascinating adaptations of deep sea life for sensing, feeding, reproducing, moving and avoiding being eaten by predators. These can be studies as follow:

4.4.1 Light (Bioluminescence)

4.4.2 Swimming and Locomotion

4.4.3 Pressure

4.4.4 Temperature

4.4.5 Oxygen

4.4.6 Food

4.4.7 Body Color

4.4.8 Reproduction

4.4.9 Gigantism

4.4.10 Long Lives

4.4.1 Light (Bioluminescence)

The deepest ocean waters below 1,000m are as black as night as far as sunlight is concerned. The light present is because of bioluminescence. In many species the light is due to organs containing luminous bacteria, whose appearance may be controlled by the movement of a fold of skin, or of the whole organ, or of chromatophores. This light is low, so animals need special sensory adaptations. Many deep-sea fish such as the stout blacksmelt (*Pseudobathylagus milleri*) have very large eyes to capture what little light exists. Other animals such as tripod fishes (*Bathypterois grallator*) are essentially blind and instead rely on other, enhanced senses including smell, touch and vibration.

Functions of bioluminescence:

1. As the forward-facing light organs

2. Unique light patterns for attracting mates
3. Lures to attract curious prey
4. Counter illumination, fish produce faint light making fish invisible to predators
5. Confusing predators
6. Serve to startle attackers and in a few cases to illuminate the prey

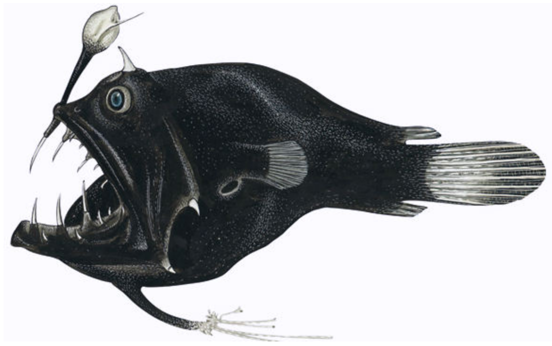


Fig.: Bioluminescence: Deep-sea Anglerfish (*Lophius*)

Fig.: Bioluminescence: Deep-sea Lanternfish (*Symbolophorus barnardi*)

Most bioluminescence is blue, or blue-green, because those are the colors that travel farthest in water. As a result, most animals have lost the ability to see red light, since that is the color of sunlight that disappears first with depth.

4.4.2 Swimming and Locomotion

Bathypelagic fishes, living in deep waters, below the thermocline at about 75m, encounter special problems. Here currents and turbulence are low, but since the water is cold it is very viscous, making swimming difficult but sinking slow. Many deep-sea fishes have elaborate lures, often phosphorescent. They may be described as 'floating fish traps'. They often have no swim-bladder and achieve almost neutral buoyancy by great reductions of the skeleton and muscles (e.g. *Ceratias*). The only parts to be well ossified are the jaws. On the other hand, deep-sea fishes that retain the swim-bladder have a well-developed skeleton and powerful muscles.

4.4.3 Pressure

The hydrostatic pressure is one of the most important factors affecting deep sea life. Life appears to cope with pressure effects on biomolecules in two ways. First, their membranes and proteins have pressure-resistant structures that work by mechanisms not yet fully understood, but which also mean their biomolecules do not work well under low pressure in shallow waters. Second, some organisms may use "piezolytes". These are small organic molecules that somehow prevent pressure from distorting large biomolecules.

Animals brought from great depth to the surface in nets and submersible sample boxes generally die; in the case of some deep-sea fishes, their gas-filled swim bladder (adapted to resist high pressure) expands to a deadly size.

4.4.4 Temperature

Difference in temperature between the euphotic or sunlit zone near the surface and the deep sea is dramatic because of thermoclines or the separation of water layers of differing temperatures. In most parts of the deep sea, the water temperature is more uniform and constant. With the exception of hydrothermal vent communities where hot water is emitted into the cold waters, the deep sea temperature remains between about -1 to about +4°C. However, water never freezes in the deep sea. This is by having "loose" flexible proteins and unsaturated membranes which do not stiffen up in the cold. However, as with pressure, there is a trade off: loose membranes and proteins of cold-adapted organisms readily fall apart at higher temperatures.

4.4.5 Oxygen

The dark, cold waters of much of the deep sea have adequate oxygen. This is because cold water can dissolve more oxygen than warm water and the deepest waters generally originate from shallow polar seas. In certain places in the northern and southern seas, oxygen-rich waters cool off so much that they become dense enough to sink to the bottom of the sea. These so-called thermohaline currents can travel at depth around the globe and oxygen remains sufficient for life because there is not enough biomass to use it all up. However, there are also oxygen-poor environments in intermediate zones, wherever there is no oxygen made by photosynthesis and there are no thermohaline currents. These areas, called oxygen minimum zones, usually lie at depths between 500 - 1,000m in temperate and tropical regions. Here, animals as well as bacteria that feed on decaying food particles descending through the water column use oxygen, which can consequently drop to near zero in some areas. Biologists are still investigating how animals survive under such conditions.

Although most of the deep seafloor has oxygen, there are exceptions in isolated basins with no circulation. Some of these basins that have no oxygen are found at the bottom of the Mediterranean Sea. How they can survive these conditions is not yet known.

4.4.6 Food

Deep sea creatures have evolved some fascinating feeding mechanisms because food is scarce in these zones. In the absence of photosynthesis, most food consists of detritus; the decaying remains of microbes, algae, plants and animals from the upper zones of the ocean and other organisms in the deep. Scavengers on the seafloor that eat this "rain" of detritus include sea cucumbers (the most common benthic animal of the deep), brittle stars and grenadier or rattail fish (*Coryphaenoides*). The corpses of large animals such as whales that sink to the bottom provide infrequent but enormous feasts for deep sea animals and are consumed by a variety of species. This includes jawless fish such as hagfish (*Myxini*), which burrow into carcasses, quickly consuming them from the inside out; scavenger sharks an crabs grow root-like structures into the bone marrow.

Deep-sea pelagic fish such as gulper eels (*Eurypharynx pelecanoides*) have very large mouths, huge hinged jaws and large and expandable stomachs to engulf and process large quantities of scarce food. Many deep-sea pelagic fish have extremely long fang-like teeth that point inward. This ensures that any prey captured has little chance of escape. Some species, such as the deep sea anglerfish (*Ceratias*) and the viperfish(*Chauliodus*), are also equipped with a long, thin modified dorsal fin on their heads tipped with a photophore lit with bioluminescence used to lure prey. Many of these fish don't spend much energy swimming in search of food; rather they remain in one place and ambush their prey using clever adaptations such as these lures. Others, such as rattails or grenadiers cruise slowly over the seafloor listening and smelling for food sources failing from above, which they engulf with their large mouths.

4.4.7 Body Color

This is often used by animals everywhere for camouflage and protection from predators. In the deep sea, animals, bodies are often transparent (such as many jellies and squids), black (such as blacksmelt fish (*Pseudobathylagus milleri*), or even red (such as many shrimp and other squids). The absence of red light at these depths keeps them concealed from both predators and prey. Some mesopelagic fish such as hatchetfish (*Sternoptyx obscura*) have silvery sides that reflect the faint sunlight, making them hard to see.

4.4.8 Reproduction

How hard it must be to find a mate in the vast dark depths? Deep-sea anglerfish (*Ceratias*) may use such light patterns as well as scents to find mates, but they also have another interesting reproductive adaptation. Males are tiny in comparison to females and attach themselves to their mate using hooked teeth, establishing a parasitic-like relationship for life. The blood vessels of the male merges with the female's so that he receives nourishment from her. In exchange, the female is provided with a very reliable sperm source, avoiding the problem of having to locate a new mate every breeding cycle.

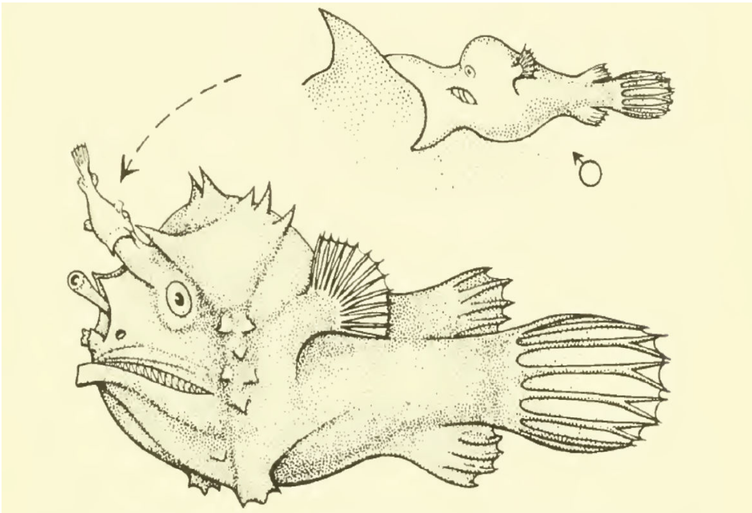


Fig.:Deep-sea fish *Photocorynus* with male attached

In some deep-sea fishes the male is much smaller than the female, to which it remains permanently attached. Breeding is often preceded by a migration of the fishes to suitable situations and the association into large shoals.

4.4.9 Gigantism

Deep-sea gigantism is the tendency for certain types of animals to become truly enormous in size. A well-known example is the giant squid, but there are many others such as the colossal squid, the giant isopod, the king-of-herrings oarfish (*Regalecus glesne*). While the giant tubeworms of hydrothermal vents grow well due to abundant energy supplies, the other gigantic animals live in food-poor habitats and it is not known how they achieve such growth. It may simply be a result of the feature of long lives.

4.4.10 Long Lives

Many deep-sea organisms, including gigantic but also many smaller ones, have been found to live for decades or even centuries. Long-lived fishes include rattails or grenadiers and the orange roughy, which are of special concern as they are targets of deep-sea fisheries. These species reproduce and grow to maturity very slowly, such that populations may take decades to recover after being over fished. This has happened repeatedly to the orange roughy, a deep-sea fish easily found congregating around seamounts in the southern oceans. Once fisheries have wiped out one seamount population, they move on to another seamount.

4.5 Adaptive Radiation in Bony Fishes

The variety of Actinopterygii is so great that it would be impossible to try to give a complete idea of it and the best that we can do is to consider various functions in more detail and specify some of the ways in which the animals have become specially modified.

4.5.1 Swimming and Locomotion
4.5.2 Various Body Forms and Swimming Habits
4.5.3 Structure of Mouth and Feeding-habits
4.5.4 Protective Mechanisms
4.5.5 Scales and other surface Armour
4.5.6 Spines and Poison Glands
4.5.7 Electric Organs
4.5.8 Luminous Organs
4.5.9 Colours of Fishes
4.5.10 Colour change in Teleosts
4.5.11 Aerial Respiration and Air-bladder
4.5.12 Special Reproductive Mechanisms in Teleosts

4.5.1 Swimming and Locomotion

Teleosts are adapted for swimmingby propagation of waves.The situation is different from elasmobranchs that they have air-bladder. The stabilization of the animal duringlocomotion is different problem, and thefins are correspondingly changed. In sharks the pectoral fins serveto steer the animal upwards or downwardsin the water.

A fish with an air-bladderneeds only very little fin movement to maintain it at a constant depthor to change its depth. The elaborate mechanismof pectoral "aerofoils" and a lifting heterocercal tail is no longer neededfor the maintenance of a constant horizontal cruising plane. Concomitantwith the loss of the heterocercal tail in evolution occurs arapid and tremendous adaptive radiation of the pectoral fin in formand function. Paired fins produce oscillating movements during hovering,and this is still found in *Amia* and *Lepisosteus*, fishes that remainrelatively slow and clumsy.

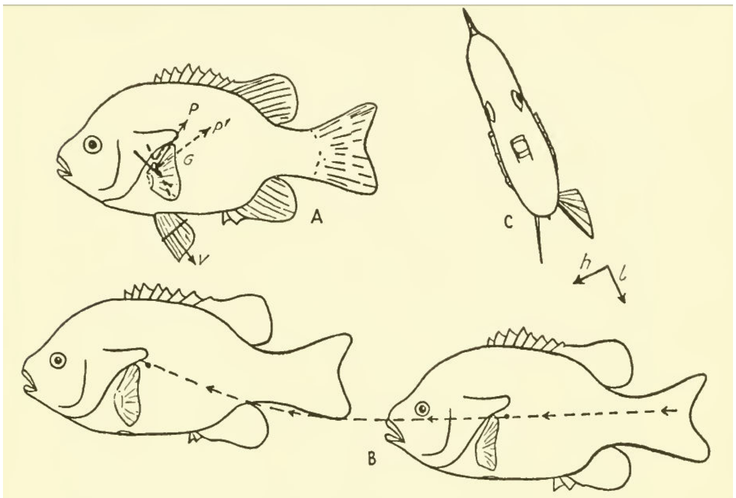


Fig.: Use of the paired fins for braking

The caudalfin of bony fishes has, of course, adopted its symmetrical shape and is used to increasethe efficiency in turning. After its amputation a fish is not able to turnin its own length as normally.The locomotion of each type of fish is adapted to its habits. Mostfreshwater fishes are 'sprinters' but there are varying degrees ofstaying power. Thus we may distinguish (1) typical sprinters (pikeand perch), (2) sneakers (eel) with some staying power, (3) crawlers(rudd, bream), with considerable staying powers for escape, (4)stayers, either for migration (salmon) or for feeding (carp). In the fishwith staying powers there is a lateral strip of narrow red muscle fibresin addition to the characteristic broad white fibres of fish muscles.

Bathypelagic fishes, living in deep waters, below the thermoclineat about 75 m, encounter special problems. Here currents and turbulenceare low, but since the water is cold it is very viscous, makingswimming difficult but sinking slow. Many deep-sea fishes haveelaborate lures, often phosphorescent. They may be described as'floating fish traps'. They often have no swim-bladder and achievean almost neutral buoyancy by great reductions of the skeleton andmuscles (e.g. *Ceratias*). The only parts to be well ossified are thejaws.On the other hand, deep-sea fishes that retain the swim-bladderhave a well-developed skeleton and powerful muscles.

4.5.2 Various Body Forms and Swimming Habits

Various body forms and swimming habits in teleosts departure from the streamlined body form typical of pelagic fisheshave been very numerous; in nearly every case they are associatedwith a reduction in the efficiency of swimming as such and thedevelopment of some compensating protective mechanism.

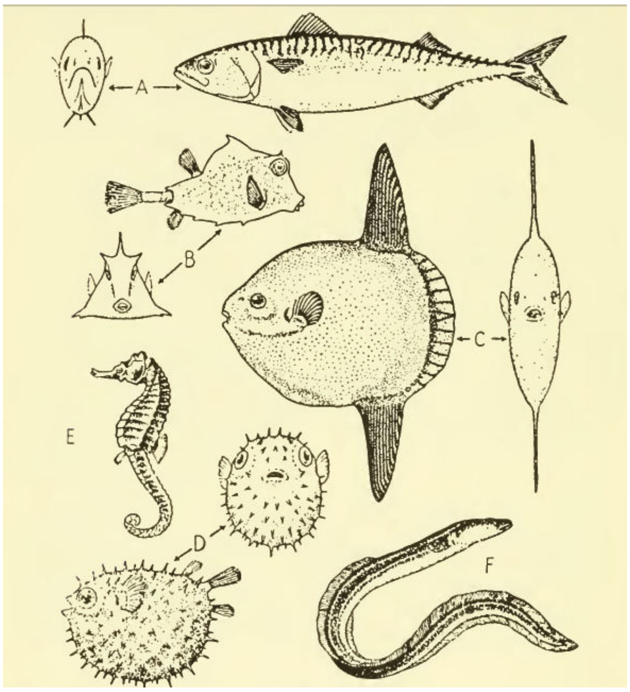


Fig.: Differences in form of fishes

(A) mackerel (*Scomber*); (B) trunk-fish (*Ostracion*); (C) sun-fish (*Mola*); (D) globe-fish

(*Chilomycterus*); (E) sea-horse (*Hippocampus*); (f) eel (*Anguilla*)

Lateral flattening, which is already a feature of all teleostean organization,is carried to extremes in many types. Thus the angel-fish,*Pterophyllum*, often seen in aquaria, is provided with long filamentsand a brilliant coloration, which, in its natural habitat, give it a protective resemblance to plants, amongwhich it slowly moves. They feed on molluscs andother invertebrates on the sea bottom and lie always on one side.The upper side becomes darker and protectively coloured, the lowerside white. In order to have the use of both eyes the whole head istwisted during the post-larval period. These forms are mostly poorswimmers, but their coloration gives them a remarkable protectiveresemblance to the background.

Flattening in the dorso-ventral plane is less common among teleoststhan selachians. The flattened forms are mostly angler-fishes, of which there are several different sorts; *Lophius piscatorins* is common in British waters. It is much flattened, with a huge headand mouth and short tail. It 'angles' by means of a dorsal fin, modifiedto form a long filament with a lump at the end, which hangs over themouth. Swimming, though vigorous, is slow, and protection (both forattack and defense) is obtained by sharp spines, protective coloration,and flaps of skin down the sides of the body, which break up theoutline. There is even a special fold of pigmented skin over the lowerjaw, serving to cover the white inside of the mouth.

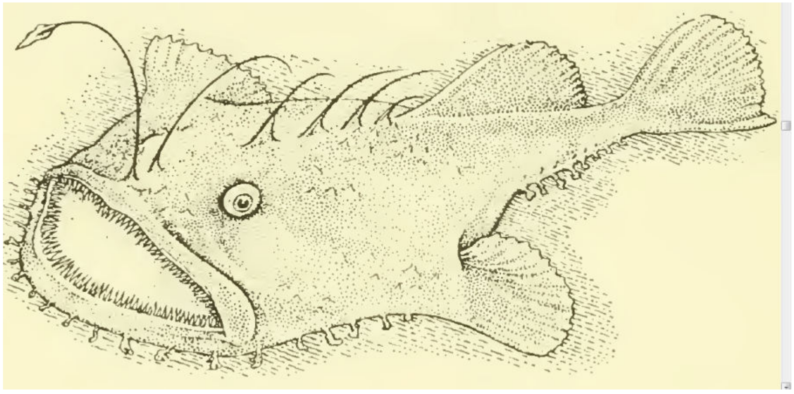


Fig.: The angler-fish, *Lophius*

Other anglers are the star-gazers, *Uranoscopiis* of the Mediterraneanand *Astroscopes* from the Western Atlantic seaboard. Theirlure is a red process attached to the floor of the mouth and they lie inwait buried in the sand, with the mouth opening upwards and onlythe eyes showing. The colour is protective, there are poison spines,and in *Astroscopus* there are electrical organs located near the mostvulnerable spot, the eyes, and formed from modified eye-muscles.

Other fishes abandon the swift-moving habit for the protectionafforded by the development of heavy armour, such as that of thetrunk-fish (*Ostracion*) and the globe-fish (*Chilomycterus*).Special spinous dorsal rays, such as those of the sword-fish (*Xiphias*)may be developed, without loss of the swift-moving habit; indeedthese fish are among the fastest swimmers. There are many groups inwhich an elongated body form like that of the eel has been developed.In *Anguilla* itself this is associated with the habit of moving over land.The Syngnathidae, sea-horses and pipe-fishes, no longer swim withthe typical fish motion but by passing waves along the dorsal fins.The long and often grotesquely cut-up body form gives a strongprotective resemblance to the weeds among which they live and onwhich they feed. The tail of the sea-horses has lost its caudal fin andis used as a prehensile organ, being wrapped around the stems ofsea-weeds for attachment.

Evidently the mastery that the Actinopterygii have acquired in thewater has depended to a large extent on the freedom given by the useof the air-bladder as a hydrostatic organ. The bladder was first a respiratory diverticulum of the pharynx. Only the active swimmers, continually venturing into new waters, would beable to make full use of the new organ.

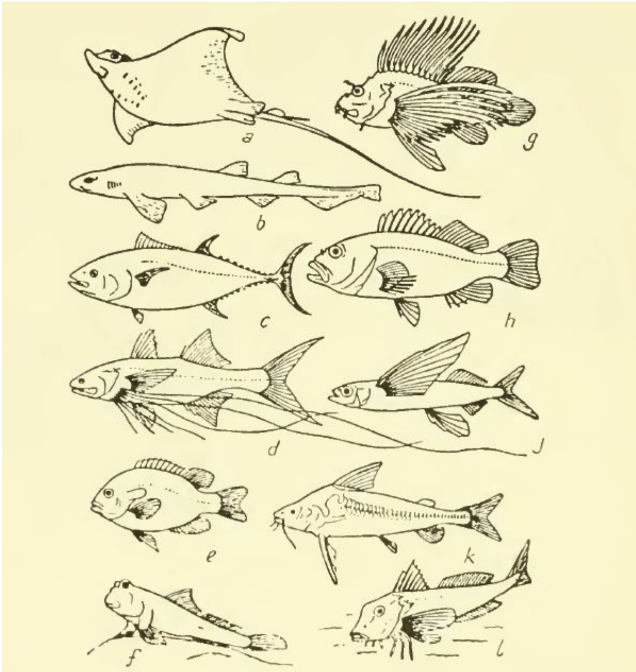


Fig.: Fishes showing special conditions of the pectoral fins

(A) eagle-ray (*Myliobatis*); (B) dog-fish (*Scyliorhimis*); (C) tunny (*Thynmis*); (D) thread fin

(*Polynemus*) ; (E) sun-fish (*Lepomis*); (F) mud-skipper (*Periophtlialmus*); (G) scorpion fish

(*Pterois*); (H) cirrhitid fish (*Paracirrhites*); (J) flying-fish (*Exocoetus*); (K) catfish (*Doras*);

(L) gurnard (*Trigla*)

Many different fishes are able to jump out of the water, presumablyto escape enemies. Salmon and tarpon can jump to 8 or 9 ft abovethe water. The flying-fishes have special structures to assist in suchjumps. In *Exocoetus* the enlarged pectoral fins serve forgliding for distances up to 400 metres, but in the flying gurnards(*Dactylopterus*) they are actually fluttered up and down, though theflight is feeble.

Several types of fish have the pectoral fin modified to allow 'walking'.The gurnards (*Trigla*) move in this way over the sea bottom, and the mud-skipper (*Periophthalmus*) chases about catching Crustacea and insects on land, using the pectoral fins as levers,provided with special anterior and posterior muscles.

Fishes that live in situations from which they are likely to be carriedaway develop suckers. Thus in the gobies, found between tide-marks,the pelvic fins form a sucker. The cling-fishes (*Lepadogaster*) are anothergroup with the same habit. The remoras have developed a suckingplatefrom the first dorsal fin and by means of this they attach themselvesto sharks and other large fish. In order to catch their food theyleave the transporting host, though they also feed on its ectoparasites.

4.5.3 Structure of Mouth and Feeding-habits

Majority of fishes are carnivorous. The species with other methods of feeding also available. The more activepredators have strong jaws and sharp teeth, such as those of the pike(*Esox*), cod (*Gadus*), and many others. The teeth on the edge ofthe jaw serve to bite and catch the prey, those on the walls of thepharynx to prevent its escape if, as is often the case, it is swallowedwhole. The teeth can often be first lowered to allow entrance of theprey and then raised to prevent its exit (e.g. in *Lophius*). In connexionwith this habit the walls of the oesophagus and even stomach areoften composed of striped muscle, capable of quick and powerfulcontraction.

Many carnivorous fishes are very fierce. For instance, the blue fish(*Pomatomas*) of the Atlantic move in shoals, cutting up every fishthey meet, making a trail of blood in the sea. The barracuda (*Sphyraena*)of tropical waters may attack man. They are said to chaseshoals of fish into shallow waters and to keep them there to serve forfood as required.

Other fishes feed on invertebrates and are then usually bottomfeeders.Thus the plaice (*Pleuronectes*) has developed chisel-like teethon the jaws and flattened crushing teeth in the pharynx; it feedslargely on molluscs. The Labridae (wrasses) also have blunt teethand eat molluscs and crabs. The sole (*Soled*) has a weaker dentitionand eats mostly small Crustacea and worms. Fish such as the herring (*Clupea*) that live on the minute organisms of the plankton have small teeth and weak mouth, but are provided with a filtering system ofbranched gill-rakers, making a gauze-like net, comparable with thefiltering system found in basking sharks, paddle-fish,and whale-bone whales.

Herbivorous and coral-eating fishes have crushing teeth similar tothose of the mollusc-eaters; indeed, many forms with such dentitionwill take either form of food. The parrot-fishes (Scaridae) have a beakand a grinding mill of flattened plates in the pharynx. With this theybreak up the corals, rejecting the inorganic part from the anus as acalcareous cloud. The Cyprinidae, including many of our commonestfreshwater fishes (goldfish, carp, perch, and minnow), have no teethon the edge of the jaw, hence the name 'leather-mouths'. There are,however, teeth on the pharyngeal floor, biting against a horny padon the floor of the skull. These fishes are mainly vegetarians, but manytake mouthfuls of mud and extract nourishment from the plants andinvertebrates it contains.

4.5.4 Protective Mechanisms

In general teleosts depend for protection against their enemies onswift swimming, powerful jaws, good receptors, and brain. Themajority of them have thus been able to abandon the heavy armourof their Palaeozoic ancestors. In many cases, however, subsidiaryprotective mechanisms have been developed, and are especially prominentin fishes that have given up the fast-swimming habit and takeneither to moving slowly among weeds or to life on the bottom. Thesedevelopments are a striking example of the way in which, followingadoption of a particular mode of life, appropriate subsidiary modificationstake place, presumably by selection of those varieties of structurethat are suited to the actions of the animal.

These protective devices may be classified as follows:

1. Protective armour of the surface of the body
2. Sharp spines and poison glands
3. Electric organs
4. Luminous organs
5. Coloration

4.5.5 Scales and other surface Armour

A covering of thin overlapping bony plates, providing somemeasure of protection, but not interfering with movement is found in teleosts. Thehinder edges of the scales are sometimes provided with rows of spines,and are then said to be ctenoid. In many fishes the scales bearupstanding spines and possess a pulp cavity, which recalls that ofdenticles. In the tropical globe-fishes (or puffers) and porcupinefishes(*Diodon*) these spines are very long and sharp and the puffersare able to inflate themselves and cause the spines to project outwards,a very effective protective device. In a few fishes the scales havebecome developed to form a bony armour even more complete thanthat of the Palaeozoic fishes. Thus in the trunk- or the coffer-fishes(*Ostracion*) the scales are enlarged and thickened into a rigid box,from which only the pectoral fins and tail emerge as movable structures,the former apparently assisting the respiration, the latter theswimming. These fishes live on the bottom of coral pools and havea narrow beak with which they browse on the polyps.

4.5.6 Spines and Poison Glands

Sharp protective spines are often found in teleosts, especially on theoperculum and dorsal fins. These may be provided with modifieddermal glands that inject poison into the wound. Thus the Europeanweever (*Trachinus*) lives buried in the sand and has poison spines onthe operculum and the dorsal fins. It is suggested that the darkcolour of the fins serves as a warning. Some catfishes, scorpion fishes,and toad-fishes also have poison spines. Spines may be effective evenif not poisonous; the stargazer, *Uranoscopus*, of the Mediterraneanand tropical waters has powerful spines on the operculum, whichinflict a most unpleasant wound if the animal is disturbed by handor foot while lying in the sand angling for its prey. *Lophius*,the angler, is also armed with dangerous spines. Several species ofcatfish have large spines, sometimes serrated. In the trigger-fishes(Balistidae and related families) of the tropics one or more of the finsis modified to make a spine that can be raised and locked in that position.These fishes have very brilliant coloration, but since some ofthem live in the highly coloured surroundings of coral reefs it cannotbe considered certain that the colours serve as a warning.

4.5.7 Electric Organs

The power to produce electric discharges has been developedindependently in four distinct families of teleosts, as well as intorpedoes and rays. The electric organs arise bilaterally from modifiedmuscle fibres, the cells of which are plate-like and arranged in rows,the electroplaques. Each plate is innervated on only one surface bymotor neurons whose activity is controlled from the forebrain, insome fish there is a controlling nucleus located in the medulla. Thephysiological properties of transmission at the nerve endings with theelectroplaques are similar to those of motor end plates. Unlike otherelectrogenic tissues such as muscle or nerve, electric organs candevelop appreciable voltages in the surrounding fluid, up to 550 voltsin *Electrophorus*, the electric eel of the Amazon. These voltages areachieved by series summation of the electromotive forces generatedby the individual cells.

The columnar array of several hundreds or thousands of electroplaquesin series in the strongly electric fish, *Electrophorus*, *Malapterurus*and *Torpedo* are paralleled so that the electric organs of these fish cangenerate considerable current at high voltage. A maximum peak powerof up to 600 watts has been observed in *T. nobiliana*. The electricorgans form the major part of the body of the strongly electric fish.

The discharges are used for offence and defense. The weaklyelectric fish (Gymnotidae, Mormyridae) have only a few columnsof series arrays, and relatively few electroplaques in each column.However, many of the species emit pulses of low voltages moreor less continuously and regularly (60-400/sec). These pulses probablyserve as the power components in an electrical guidancesystem. All species of the continuously emitting fish are sensitive tochanges in the conductance of the water. Presumably the fish sensethe altered electric field of their discharges; although the receptorshave not yet been identified specialized lateral line organs (mormyromasts)are often present.

4.5.8 Luminous Organs

Fishes of many different families live at great depths and 95 percent of individuals caught below 100 fathoms are luminescent. Thedevelopment of luminescent organs is therefore a further example ofparallel evolution. The organs usually show as rows of shining beadsof various colours on the sides and ventral surface of the fish.

In many species the light is due to organs containing luminousbacteria, whose appearance may be controlled by the movement of afold of skin, or of the whole organ, or of chromatophores. Someteleosts, however, have self-luminous photophores and these are alsofound in *Spinax* and a few other Squalidae. They are formed frommodified mucous glands, and may be provided with reflectors andeven lenses. They can be flashed on and off, probably by sympatheticstimulation.

The luminous organs probably often serve for recognition of thesexes and often show distinctive patterns. They may serve to startleattackers and in a few cases to illuminate the prey. In the deep-sea

anglers (*Ceratias*) the luminous tip of the fin is used as a lure.

4.5.9 Colours of Fishes

The bony fishes show perhaps the most brilliant and varied colorationof any animals, rivaling even the Lepidoptera and Cephalopodain this respect. The enormous range of colour and pattern providesan excellent example of the detailed adjustment of the structure andpowers of animals to enable them to survive. A great difficulty isintroduced into the study of animal coloration by the fact that we areusually ignorant of the capacity for visual discrimination possessedby the animals likely to act as predators. Moreover, it is very difficultfor us to obtain this information. When we examine any two objectswe are able to say not merely that they are different but that one isred and the other green. A person or animal that is colour-blind mayalso be able to detect a difference, but yet remain unaware of anydistinction of colour; the objects appear to him only as differing inbrightness. In order to decide whether animals are able to distinguishbetween light of two wavelengths we must present them with objectsof different colour but the same brightness.

We are therefore faced with the possibility that some of the coloursthat appear to us so brilliant are to other animals merely differences oftone, and animals to us conspicuous because coloured, when seen inmonochrome, may be protected. Some of the colours of fishes maybe only a means of producing a pattern of protective greys, as seenthrough the eyes of an attacker. However, there is no doubt that somefishes are able to discriminate between illuminated bodies whichthough of different wavelength reflect light of equal brightness. Inthe subsequent description of fish coloration we shall not be able toconsider predators further, but shall describe the colours as theyappear to the eye of a normal man.

The colour of fishes is produced by cells in the dermis, (a) thechromatophores and (b) the reflecting cells or iridocytes.The chromatophores are branched cells containing pigment, whichmay be either black (melanin) or red, orange, or yellow (carotenoidsor flavines). The iridocytes contain crystals of guanin, making themopaque and able to reflect light so as to produce, where no chromatophoresare present, either a white or a silvery appearance. Thismaterial is used in the manufacture of artificial pearls, the scales of thecyprinoid *Alburnus lucidus* (the bleak) being used for the purpose.The iridocytes may be either outside the scales, when they producean iridescent appearance, or inside them, giving a layer, the argenteum,that produces a dead white or silvery colour. By a combination of thechromatophores, and of these with the iridocytes to produce interferenceeffects, a wide range of colour is produced.

Thus by mixingyellow and black either brown or green is produced. Blue is usuallyan interference colour.

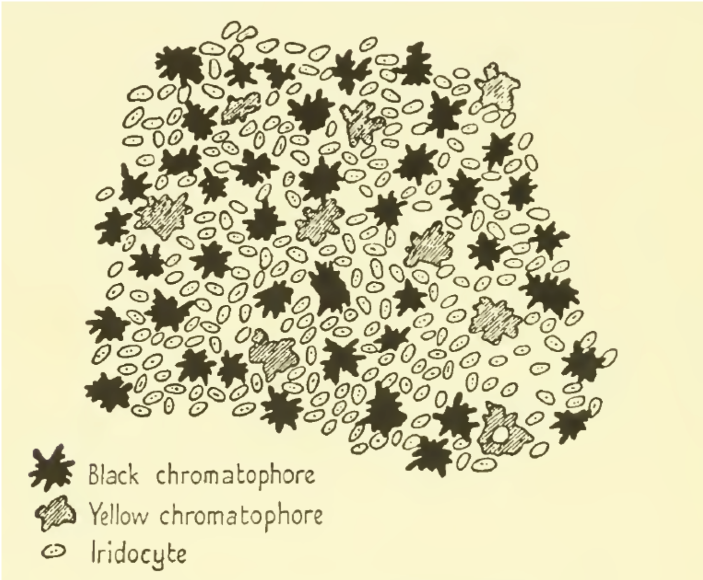


Fig.: Coloration elements in the skin of the upper side of a flounder (*Platichthys*)

The use of the colour by the fish may be classified, according to thescheme introduced by Poulton, as cryptic or concealing, sematic orwarning patterns, and epigamic or sex coloration. Cryptic colorationmay be achieved in various ways and may be subdivided into twomain types: (1) assimilation with the background, (2) breaking up theoutline of the fish. Assimilation is common, but is often associatedwith some degree of disruption of outline. The absence of all pigmentationin pelagic fishes, for instance the Leptocephalus larvae ofeels, is an example of assimilation. Fishes living among weeds, suchas the sea-horses and pipe-fishes, or *Lophius* the angler, often resemblethe weeds in colour, and in addition develop 'leaf-like' processes.The colour of many familiar fishes, such as the green of the tench,may be said to resemble that of the surroundings by assimilation.When we consider the much more numerous examples of patternsinvolving several colours the distinction between assimilation and disruptionis more difficult to draw. Many free-swimming pelagic fisheshave the upper side dark and striped with green or blue, whereas theunder-side is white, the beautiful pattern that is seen in the mackerel(*Scomber*). This gives them protection from above and below, thestriping probably making the animal less conspicuous in disturbedwater than it would be if of uniform colour. The white under-side alsoserves to lessen any shadows, an important factor for animals that livein shallow water; similar shading is used by land animals.

Devices of spots and stripes are found on fishes that live against avariegated background. The beautiful red and brown markingsof a trout are a good example. Flat fishes, living on sandy orgravelly bottoms, adopt a spotted pattern, which gives them a highdegree of protection, and we shall see later that they are able to changecolour to suit the ground on which they rest. The brilliant coloursof many tropical fishes probably serve mainly to break up the outline,though no doubt the surroundings in which they live are also brilliant.Great variety of colours may be found on a single fish, especially inthc trunk-fishes (*Ostracion*), one species of which is described ashaving a green body, yellow belly, and orange tail, while across thebody are bands of brilliant blue, edged with chocolate-brown. Moreover,the female has another colour scheme and was for long consideredas a different species!

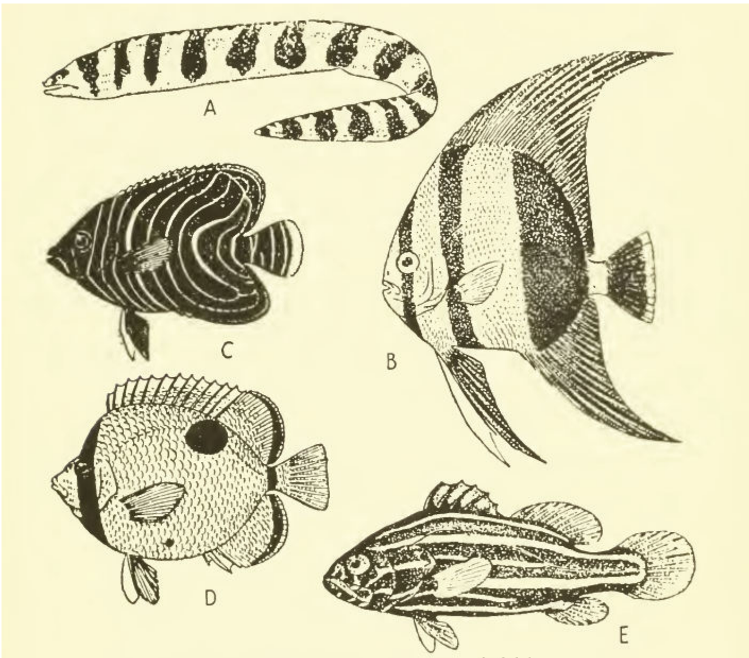


Fig.: Colour patterns of various tropical fishes

(A) Muraena (*Gymnothorax*);(B) bat-fish (*Platax*);(C) butterfly fish (*Holacanthus*);

(D) butterflyfish (*Cliaetodon*); (E) perch (*Grammistes*)

Colour differences between the sexes are frequent in fishes, the malebeing usually the brighter. Thus in the little millions fish, *Lebistes*,there are numerous 'races' of males with distinctive colours, but thefemales are all of a single drab coloration. The genetic factors thatproduce the various types of male are carried in the Y chromosome.Presumably the colour of the males acts as an aphrodisiac as a partof the mating display, but the significance of the different races is notknown.

Sematic or warning coloration involves the adoption of some strikingpattern that does not conceal but reveals the animal. This typeof colouring is found in animals that have some special defense orunpleasant taste (such as the sting of the wasp), and its use implies thatanimals likely to attack are able to remember the pattern and theunpleasant effects previously associated with it. It is not easy to becertain when colours are used in this way, but it is possible that theconspicuous spots on the electric *Torpedo* ocellata have this function.Among teleosts there is the black fin of the weevers (*Trachinus*), possiblya warning of their poison spines, and the spiny trigger-fishesand globe-fishes also have conspicuous colours.

4.5.10 Colour change in Teleosts

In spite of the reputation of the chameleon the teleosts are thevertebrates that change their colour most quickly and completely.The melanophores are provided with nerve-fibres, andthese cause contraction of the pigment and hence a paling of the skincolour. The processes of the cells themselves are not withdrawn; thecolour change is produced by a movement of pigment within them.

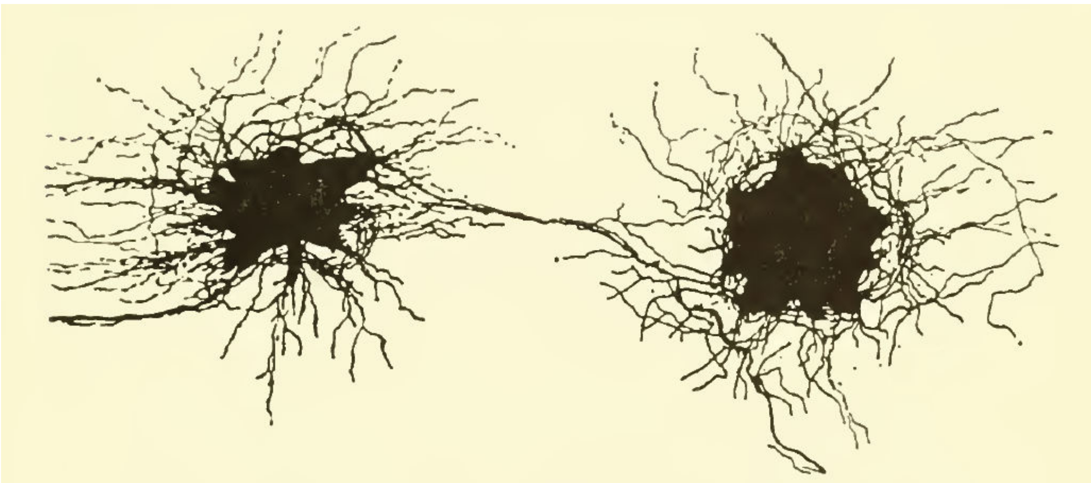


Fig.: Nerves of the melanophores of a perch

The nerve-fibres in question are post-ganglionic sympathetic fibres,leaving the ganglia in the grey rami communicantes to allthe cranial and spinal nerves. The pre-ganglionic fibres that operatethem, however, emerge only in a few segments in the middle of thebody, so that severance of a few spinal roots will affect thecolour of the whole body. When a nerve to any part of the skin is cutthe chromatophores in that region at first expand, making a dark area. After a few days, however, the skin involved graduallybecomes lighter, the process, it is alleged, beginning at the edges andmoving inwards. Parker, who has made a careful study of thesephenomena, believes that they indicate the presence of melanophoreexpandingnerve-fibres (said to be of 'parasympathetic' nature). Followingthe cut these fibres are supposed to be stimulated to repetitivedischarge and hence to make the dark band. Later the band palesbecause the stimulating substances (neurohumors) produced at thenerve-endings of the melanophore-contracting fibres in the neighbouringareas diffuse in gradually.

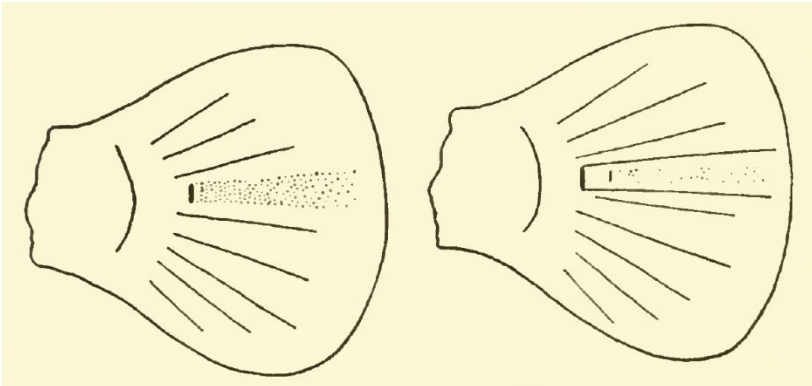


Fig.: Diagrams showing, *left*, a cut in the tail of the fish *Funduhis* producing

a band of dark melanophores; *right*, when the dark band has faded

a second cutmakes the melanophores again dark

This hypothesis involves two physiological propositions which areso novel that they would require detailed evidence for acceptance.Firstly the act of cutting is presumed to set up a discharge of impulseslasting for several days, which is unlikely. Moreover, the discharge ispresumed to be only in the melanophore-expanding fibres and not inthose that produce contraction. Secondly, electrical stimulation ofnerves in teleosts always produces paling and never darkening (exceptafter the use of ergotamine). We must assume, therefore, that thisform of stimulation has the opposite effect to section and only stimulatesthe melanophore-contracting nerve-fibres. Since neither of thesepropositions is adequately demonstrated, we must reject the hypothesisand say that there is not sufficient evidence of melanophoreexpandingnerve-fibres.

There is, however, another agent that causes expansion of melanophoresin a wide variety of vertebrates, namely the posterior lobe ofthe pituitaryand there is evidence that this works also inteleosts. Hypophysectomized specimens of the Atlantic minnow*Fundulus* are nearly always lighter than normal individuals, especiallywhen on a dark background. Injection of posterior pituitary extracts,or placing of isolated scales in the extract, causes expansion of thechromatophores of any teleost. We may conclude that colour changeis produced by the nerve-fibres tending to make the animals pale andsecretion of the posterior pituitary to make them dark. Adrenalineinduces contraction of chromatophores, and is presumably similar tothe sympathetic transmitter.

It is more difficult to decide how external influences are linked withthis internal mechanism. Fishes mostly become pale in colour on alight background and vice versa, and the effect is produced predominantlythrough the eyes. There may also be a slight direct effectof light on the chromatophores. The change in colour begins rapidly,but its completion may take many days. Analysis of the rates ofchange in normal fishes and in those with anterior and posteriorpituitary removal has led to the suggestion that the anterior lobeproduces a substance tending to make the fish lighter in colour, atleast in the eel. A similar hypothesis has been fully worked out byHogben and his colleagues in amphibia. The colour is alsoinfluenced by the pseudobranch, a secretary tissue in the first gillarch. After removal of this a fish becomes dark and the choroid glandof the eye, which receives blood from the pseudobranch, degenerates.It is suggested that the pseudo branch produces a hormone,whose entry into the circulation is controlled by the choroid gland.

The value of the colour change in bringing the animals to the sametint as their surroundings is considerable. Fishes kept on a light backgroundare very conspicuous for the first few minutes when transferredto a dark one. The fisherman acknowledges this by paintingthe inside of his minnow-can white, to make the bait conspicuous. Inthe flat fishes, living on the sand, the protection assured by the colourchange is of special importance. It has been suggested that it is possible for the fish to assume a pattern similar to that of the groundon which it lies, but it is probable that the degree of expansion of thechromatophores is adjusted to suit the amount of light reflected fromthe ground; by increasing or decreasing the areas of dark skin, effectsapproximately appropriate to various backgrounds are produced.

4.5.11 Aerial Respiration and the Air-bladder

Many fishes are able to live outside the water. The excursions on tothe land vary from the wriggling of the eel through damp grass to thelife of the Indian climbing perch (*Anabas*) spent almost entirely onland. In the eel there is no special apparatus for breathing air (thoughoxygen may be taken in through the skin). The climbing perch is providedwith special air chambers above the gills and evenwhen in water it comes to the surface to gulp air and will 'drown' ifprevented from doing so, even though it is placed in well-oxygenatedwater.

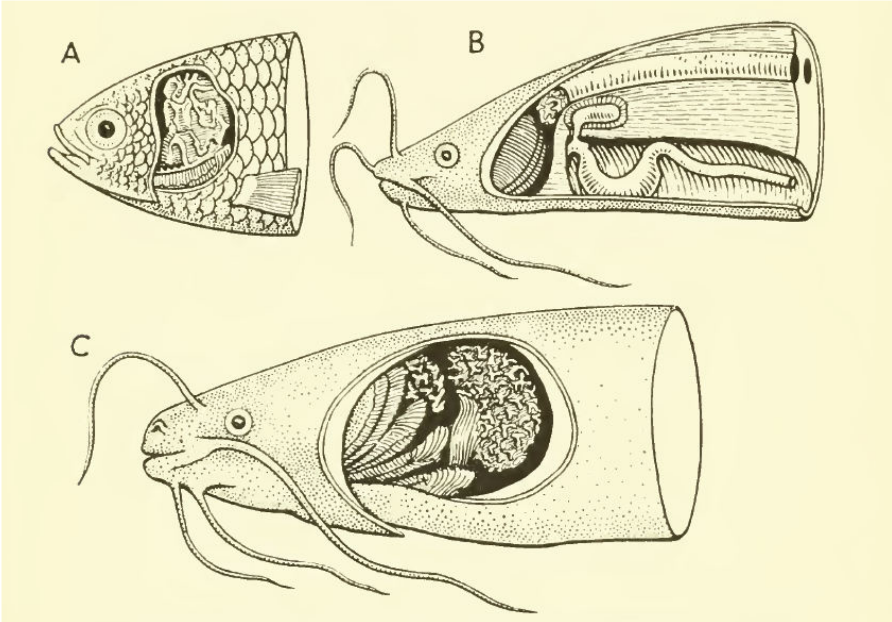


Fig.:Special respiratory apparatus

(A) in climbing perch (*Anabas*); (B) Indian catfish (*Saccobranchus*); (C) African catfish (*Clarias*)

Many other fishes gulp air, especially those living in shallow tropicalwaters, which readily become deoxygenated. There may be otherspecial mechanisms for gaseous interchange. In the Indian catfish *Saccobranchns* there are large air sacs growing a long way down thebody from the gill chambers.

The air-bladder, which has contributed so largely to the success ofthe later teleosts, may have arisen as an accessory respiratory organ, used in the same way as those described above. In all the moreprimitive teleosts (Isospondyli) the air-bladder preserves in the adult its opening to the pharynx (physostomatous), whereas in higherforms it becomes completely separated ('physoclistous'). Survivals ofstill earlier Actinopterygii have the opening especially well developed,though it varies from group to group. Thus in the sturgeonsthere is a wide opening into the dorsal side of the pharynx.In *Amia* and *Lepisosteus* the opening is also dorsal and the walls ofthe sac are much folded and used for respiration. In *Polypterus*the opening is ventral and the bladder has the form of a pair of lobesbelow the gut. This arrangement recalls that of the tetrapod lungs and is also found in the modern lung- fishes and presumably in theirDevonian ancestors,from which we may suppose that the tetrapods arose. This ventral position of the air-bladder was one of thefeatures that for a long time led zoologists to suppose that *Polypterus* was a member of the crossopterygian line of fishes. It is probable,however, that the affinity is only that which persists between allprimitive members of both Actinopterygii and Crossopterygii andis to be taken as an indication that the air-bladder was originally awidely open respiratory sac, or perhaps pair of sacs. Once the powerto produce a pharyngeal diverticulum had been developed it is easyto imagine that the actual position of the opening might shift eitherdorsally, as in the later Actinopterygii, or ventrally, as in the tetrapods.

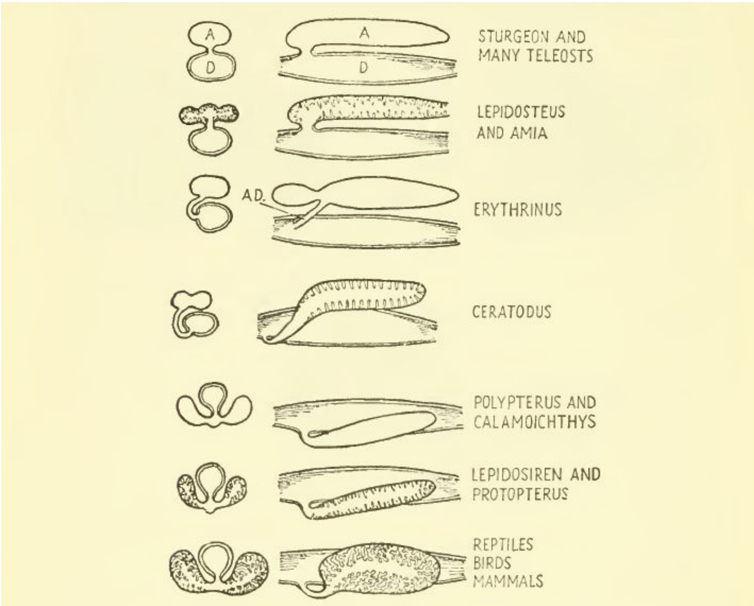


Fig.:Air-bladder of various fishes, seen from in front and from the left side

A: air- or swim-bladder; AD: air-duct; D: digestive tract

The blood-supply of the air-bladder should provide some indicationsboth of its origin and function. In *Polypterus* and Dipnoi thereare pulmonary arteries springing from the last (sixth) branchial archnandpresumably containing venous blood. Blood returnsto the heart by pulmonary veins. Essentially the same arrangementis found in *Amia*, but in all other Actinopterygii oxygenated blood issupplied to the bladder from the dorsal aorta (or sometimes from thecoeliac artery). Probably, then, the original function of the air-bladderwas respiratory, and this may still be its main function not only in*Amia* and *Lepisosteus* but also in some of the physostomatous Teleostei.However, in the majority of teleosts its dorsal position, closedduct, and arterial blood-supply show that it has some other functionand it has long been supposed that this is concerned in some way withflotation. The air-bladder is absent from bottom-living forms, suchas flat-fishes, *Lophius* and *Uranoscopus*, though it may be present intheir pelagic larvae.

The bladder is provided with special glands by which it is filledand the gas they secrete is mostly oxygen; only in some physostomatousforms is the bladder filled by gulping air. Nitrogen and carbondioxide are also present and the former even constitutes the main gasin some freshwater fishes at great depths. In the more primitive formsgas is secreted all over the surface of the bladder, but later theredevelop special anterior oxygen-secreting and posterior oxygenabsorbingregions. The former, known as the red gland, has a specialapparatus of blood-vessels, the rete mirabile, and the latter, or 'oval',which may be developed from the closed end of the pneumatic duct,has a special sphincter by means of which it can be closed off.

The pressure of the gases in the swim-bladder is adjusted to makethe fish neutrally buoyant, which is achieved when the bladderoccupies 7-10 per cent of the total volume in fresh-water and 5 percent in marine fishes. This may involve partial pressures of oxygen,carbon dioxide, and nitrogen many times greater than those in theblood. The mechanism by which the gases are secreted against adiffusion gradient of several atmospheres has been much discussed.Carbonic anhydrase is present in the gas gland and the oxyhemoglobinof fish blood is especially sensitive to carbon dioxide, giving upits oxygen even at high oxygen concentration.

If weights or floats are attached to a fish it maintains its positionin mid-water by swimming while gas is secreted or absorbed. Thereceptors concerned are therefore not activated by the tension in thebladder but perhaps by the movements that are necessary when thefish is not in equilibrium. The bladder is innervated by the vagus andsympathetic nerves and after severing the former gas secretion ceases.

The various diverticula connecting the bladder with the ear (and the Weberian ossicles) may be associated with pressure receptorsthat assist in the control of the bladder. Loaches are famous asfish barometers, whose behaviour can be used to predict weatherchanges.

4.5.12 Special Reproductive Mechanisms in Teleosts

The teleosts show great variation in breeding habits, the eggs beingsometimes left to develop entirely by themselves, in other cases lookedafter by one or both parents, while in a few species they developviviparously within the mother. Hermaphrodite individuals are notuncommon and in some species of Sparidae and Serranidae areinvariably monoecius and self-fertilizing. The method of associationof the sexes is correspondingly varied and there are numerous devicesfor bringing sexes together, such as colour differences, sound production,and the liberation of stimulating substances into the water. Insome deep-sea fishes the male is much smaller than the female, towhich it remains permanently attached. Breeding is oftenpreceded by a migration of the fishes to suitable situations and theassociation into large shoals.

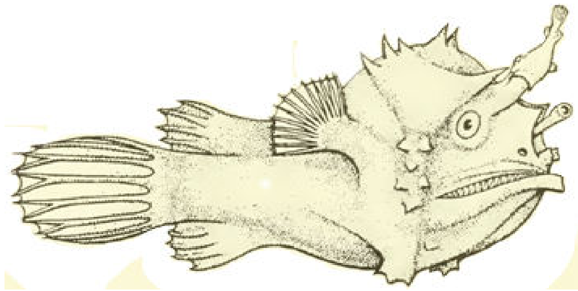


Fig.:Deep-sea fish *Photocorynus* with parasite male attached

The eggs may be classified as either pelagic, if they float, or demersal,if they sink to the bottom. In the former case they are sometimes providedwith an oil globule and are exceedingly numerous. Thus asingle female turbot has been calculated to contain nearly 10 million eggs, a cod 7 million, and a ling 28 million, whereas the herring,whose eggs sink to the bottom, probably does not lay more than 50,000 eggs. The large numbers laid by the pelagic species are presumablyinsurance against failure of fertilization and especiallyagainst random elimination of the eggs and young. The greater thecare devoted to the young by the parents the smaller the number ofeggs produced.

Demersal eggs, especially of freshwater animals, are usually laidwith some special sticky covering, by means of which they areattached to each other and to the bottom or to stones, weeds.Thus the eggs of many cyprinids (carp) are attached to weeds.The eggs of salmon and trout, however, though demersal, are notsticky. From depositing eggs on weeds it is only a short step to thebuilding of a nest and guarding of the eggs by one or both parents.Thus the sand goby (*Gobius minutus*) lays its eggs in some protectedspot, where they are guarded by the male, who aerates them by hismovements. Quite elaborate nests may be built, as by the sticklebacks(*Gasterosteus*), where pairs remain together throughout the breedingseason.A still further development is the retention of the youngwithin the body. In some catfishes they develop within the mouth ofeither parent. In pipe-fishes and sea-horses the males are providedwith special pouches for the young.

Although external fertilization is usual, various teleosts showinternal fertilization and the young then develop within the ovary(*Zoarces, Gambnsia,* *Lebistes*). The mechanisms by which mating andthe nutrition of the embryos are assured in these cases show someinteresting parallels with the conditions in mammals, including theformation of placentae or nutritive material. In *Lebistes* the femaleadopts a special position of readiness for copulation, and this has beenshown to depend partly on an internal factor in the female and partlyon a substance secreted into the water by the male. The embryos arenot attached to the wall of the ovary but develop free in the sac, feedingupon an 'embryotrophic' material, apparently produced by thedischarged ovarian follicles, which become highly vascular and remainthroughout the several months of 'pregnancy'.

4.6 Parental Care

Some marine forms and many more freshwater forms retain their eggs after they are laid, that is, they practice parental care. Parental care takes on a host of different modes, from simple to highly complex.

4.6.2 Forms of Parental Care

4.6.1.1 Male parental care: sea catfishes (Ariidae), sticklebacks (Gasterosteidae), pipefishes (Syngnathidae), and greenlings (Hexagrammidae)

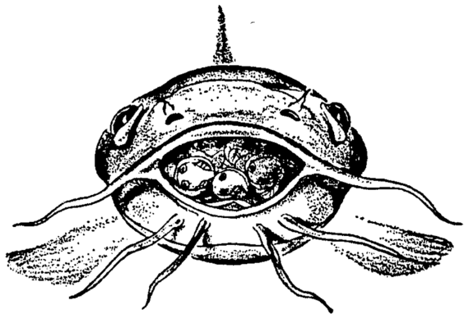
4.6.1.2 Female parental care:

1. Oviparity with post-spawning care: the cichlidaegenus *Oreochromis*
2. Ovoviviparitywithout post-spawning care: rockfishes, genus *Sebastes*
3. Viviparitywithout post-spawning care: Elasmobranchs, livebearers (e.g., genus *Poecilia*), surfperches(Embiotocidae)

4.6.1.3 Biparentalcare: bullheads (Ictaluridae), several cichlid genera (e.g., *Cichlasoma*and *Symphysodon*)

4.6.1.4 Juvenile helpers: some African cichlids (e.g., genus *Lamprologus*)

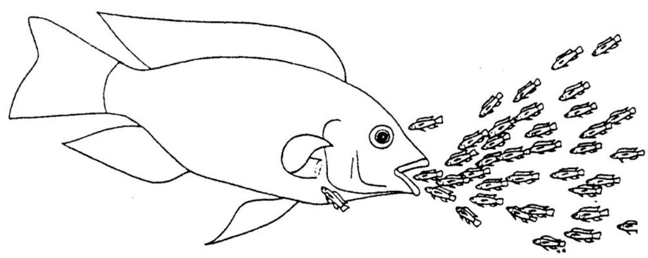
Within the first of these categories are many of the oral incubatorsor mouth brooders. In the marine catfish*Bagremarinus*, as many as 55, large (about 20 mm in diameter) eggs are laid.



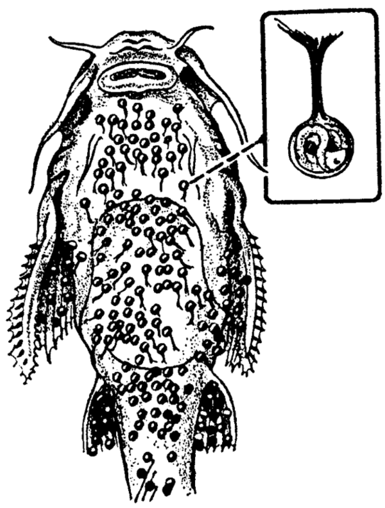
♀ Mouthbreeding catfish

After fertilization, the maleretrieves the eggs in his mouth, keeping them all well oxygenatedand very well protected from predation. After about a month the young emerge but remain near the male, retreating to the shelter of his mouth whenever there is a nearby disturbance. The male continues to protect the young for another two weeks or so.

In freshwater fishes, members of the family Cichlidaeare the best known oral incubators. In this group, the females do the incubating as much as the males.

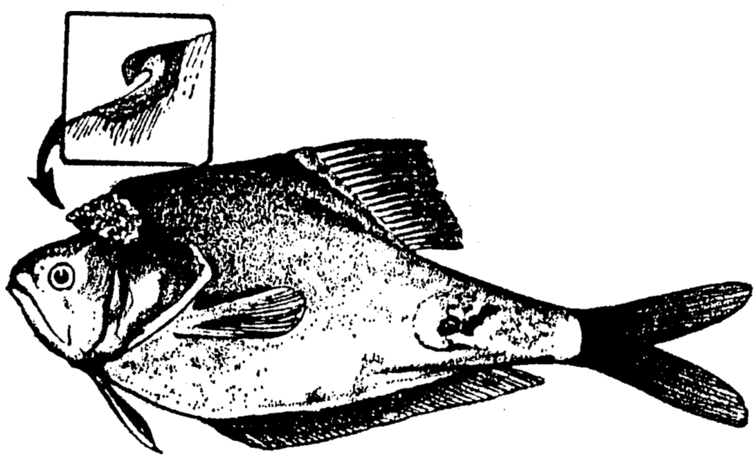


Bunocephalidcatfisheshave evolved a strange system in which the female lies down on the eggs after they have been laid and fertilized. The eggs sink into the soft, spongy skin of her underside, under the head, thorax, and abdomen. The eggs stick there and eventually each egg is carried on a stalked cup that grows from the skin.



♀ Obstetrical catfish

The males of the nurseryfishes, genus *Kurtus*, which lives in brackish-water habitats of the Indo-Australian region, are equipped with a bony hookon their forehead. The hook is used to carry the eggs, which are in two bundles connected by a fibrous thread.



♂ Forehead brooder

4.7 Offensive and Defensive Mechanism

4.7.2 Offensive Behaviour

4.7.1.1 Stickleback

The social behaviour of many species includes the development of special 'releasers', shapes, colours, or postures that are displayed by one individual and elicit specific reactions in another.

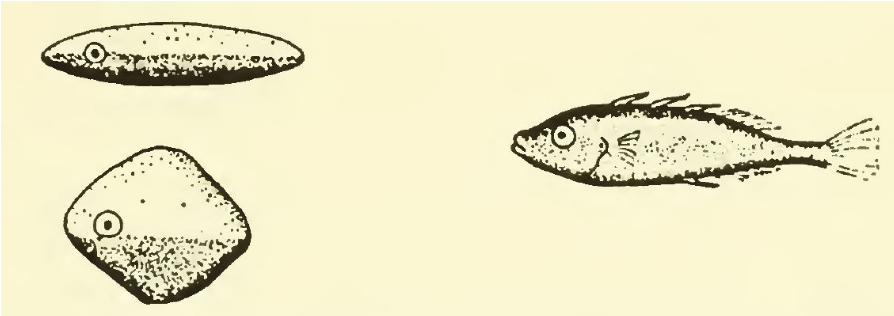


Fig.: The red belly of the stickleback (*Gasterosteus)* releases attacking

behaviour in other malesand following by females. Of the above

models only the two on the left acted as releasers

There is no doubt that fishes possess great powers of learning. They can form conditioned reflexes involving discrimination of tones, also second-order conditioned reflexes, in which after the animal has learnt to give a certain behaviour in response to a visual stimulus it is many tales of carp coming to be fed at the ringing of a bell, and similar powers of association must play a part in the life of fishes in more natural situations. Bull has shown that fishes can be trained to discriminate between very small differences of water flow, temperature, salinity or pH, and no doubt it is by means of such powers that they normally find a suitable habitat then taught to associate the latter with an olfactory stimulus. There are many other examples of such powers, but unfortunately we have as yet little information as to the way in which they are brought about by the brain. Nor have the naturalists provided us with very clear examples of the use of these powers by fishes in nature. There are many tales of carp coming to be fed at the ringing of a bell, and similar powers of association must play a part in the life of fishes in more natural situations. Bull has shown that fishes can be trained to discriminate between very small differences of water flow, temperature, salinity or pH, and no doubt it is by means of such powers that they normally find a suitable habitat.

4.7.1.2 Lion fishes

Lionfishes (*Pterois*) make up any of several species of showy Indo-Pacific fishes of the scorpion fish family, Scorpaenidae (order Scorpaeniformes). They are noted for their venomous fin spines, which are capable of producing painful, though rarely fatal, puncture wounds. The fishes have enlarged pectoral fins and elongated dorsal fin spines, and each species bears a particular pattern of bold, zebra-like stripes. When disturbed, the fish spread and display their fins and, if further pressed, will present and attack with the dorsal spines.

4.7.1.3 Candiru

Candiru, (*Vandellia cirrhosa*), is a scaleless, parasitic catfish of the family Trichomycteridae found in the Amazon River region. It is translucent and eel like. The candiru feeds on blood and is commonly found in the gill cavities of other fishes. It sometimes also attacks humans and has been known to enter the urethras of bathers and swimming animals. Once in the passage, it erects the short spines on its gill covers and may thereby cause inflammation, hemorrhage, and even death to the victim.

4.7.1.4 White shark

In the areas where they are most common, white sharks are responsible for numerous unprovoked, and sometimes fatal, attacks on swimmers, divers, surfers, kayakers, and even small boats. A white shark (*Carcharodon charcharias*) tends to inflict a single bite on its human victim and then retreat. In many instances, however, the shark rarely returns for a second bite. If the victim suffers a moderate bite, he or she may have time to seek safety. In situations where a large bite occurs, however, serious tissue and organ damage may result in the death of the victim.

4.7.1.5 Electric eel

Electric eel (*Electrophorus electricus*) is an elongated South American fish that produces a powerful electric shock to stun its prey, usually other fish. The electric eel's penchant for shocking its prey may have evolved to protect its sensitive mouth from injury from struggling, often spiny, fish. The shocked prey is stunned long enough to be sucked through the mouth directly to the stomach. Sometimes the electric eel does not bother to stun prey but simply gulps faster than the prey can react. The eel's electrical discharges may be used to keep prey from escaping or induce a twitching response in hidden prey that causes the prey to reveal its position.

4.7.2 Defensive Behaviour

4.7.2.1 Defending Eggs and Fry

When parents defend their young from attack by members of their own species is aggression. Male stickleback fish (*Gasterosteus)* defend eggs and fry against cannibalistic attack. Biology of gamete production also has an influence on aggressive behaviour because a female's eggs are larger, are fewer in number, and require more energy to produce than a male's sperm, competition among males over females is usually more frequent and intense than competition among females over males. As a result, the most spectacular fights among salmon occur between males over fertile females.

4.7.2.1 Defending Territory

Aggression may be focused on a specific area, such as a defended territory from which rivals are vigorously excluded. A notable example is shown by mudskippers (*Periophthalnus*), intertidal fish that defend small territories where they browse on microscopic plants. The fish build mud walls around the borders of their territories, and at low tide water is retained within the walls. Territorial behaviour is also shown by rag worms and fiddler crabs when they defend their burrows, by male dragonflies and sticklebacks defending breeding grounds, by male tree frogs, sage grouse, and Uganda kob defending high-quality sites for courting and mating, and by spiders, reef fish, and hyenas when they defend feeding areas.

4.7.2.1 Schooling Behavior

With few exceptions, the important behavioral characteristics of clupeiforms are schooling and diurnal vertical movements. Schools are formed with larvae or young juveniles. When the schools do persist after nightfall, the lateral line system may also play a significant role in preventing one fish from straying.

The primary advantage of the schooling habit seems to lie in the safety of the individual fish. Sardines react to attacks by predators by swimming closer together and milling around in tight, compact balls; herring (*Clupia*) form a close school with any approach of danger. The reaction of anchovies to predators is even more intense; a school that may be spread over several hundred meters contracts at the approach of a predator to a moving, writhing sphere of thousands of fishes only a few meters across. In such a situation the predator cannot concentrate on a single individual and may be frustrated in its attempt to catch any fish.

The adaptive value of schooling behaviour is poorly understood, but several logical explanations have been advanced. Schooling evidently provides a better chance for small fish to survive many environmental hazards than if they live solitarily. The instinctive tendency of the tiny larvae to associate, even though hatched from scattered eggs, ensures the formation of the school—with its protection from predation. Certain hydrodynamic interactions between members of the school are thought to facilitate feeding movements, and the aggregation of so many fish simplifies the finding of mates.

4.7.2.1 Defending Behavior in other Fishes

Hagfish

Hagfish (*Myxini*) are eel-shaped marine animals with the incredibly useful ability to slime their enemies. When threatened, the hagfish emit a slime from their pores that, when mixed with water, expands into a gelatinous goo that can either trap predators or suffocate them by clogging their gills. Each predator took one bite before immediately spitting the hagfish out and swimming away, gagging. "The hagfish, uninjured and oblivious, just carried on feeding. Its defense is so effective that it can totally ignore the fact that a shark just tried to bite it.

Lantern fishes

The flashing sequence of these organs is dictated by its hormonal control since photophores are connected to its nervous system. Some species of lantern fishes (*Symbolophorus*) consist of light organs on their tails to entice potential predators. During the night they migrate to the surface to feed and descend back to the depths during the day. By doing this, they also save themselves by the risk of predation from the larger species.

4.8 Sensory, Hydrostatic and Lateral Line System

4.8.1 Sensory Organs

4.8.2 Hydrostatic System

4.8.3 Lateral-line System

4.8.1 Sensory Organs

The three chief pairs of sense organs, olfactory sacs, eyes, and auditory organs, are present. The olfactory sacs are a pair of simple depressions or invaginations of the epidermis opening in front of the mouth; the division of the opening by the dermal bones of the upper jaw.

The eyes have a flatter cornea and a more spherical lens than those of terrestrial vertebrates; in Teleosteans there is a special layer of reflecting tissue, composed of a chemical compound called guanin, behind the retina.

The auditory organs are membranous sacs entirely enclosed in the auditory capsules of the skull, each is divided into an upper part provided with the usual three semicircular canals and a lower part called the sacculus; opening into the latter is a narrow tube which is the vestige of the epidermic invagination by which the auditory sac is formed in development; for the organ of the sense of hearing like that of the sense of smell is nothing but a portion of the external skin, which in the development of the embryo becomes pushed in to form a hollow bladder; the bladder or sac becomes complicated, and the tube by which it originally opened to the surface becomes much narrowed or entirely closed. In Elasmobranchs this tube has a small opening on the surface of the skull throughout life, but in other fishes it is closed in the adult. There is no middle or external ear in fishes, for the middle ear or tympanum is represented by the spiracle and no external ear is formed. The auditory sac contains calcareous matter secreted by its wall and known as otoliths; the function of these is to convert the sound vibrations into movements of solid matter which have more effect in stimulating the terminations of the auditory nerve. In the dog-fish the calcareous particles are small and separate, in the bony fish they are consolidated into large concretions which increase in size during growth by the addition of layers to the exterior; the deposits of successive years can often be distinguished, and in this way the age of the fish when it was killed can be ascertained.

The skin may be supposed to possess the sense of touch all over its surface as a result of the presence of terminations of sensory nerves, without any special organs, but there are also definite sense-organs in the epidermis composed of groups of sensory cells united into spindle-shaped bodies called end-buds; these are irregularly distributed over the body, on the fins and lips, and also in the epithelium of the mouth and pharynx. In this latter position they form the organs of the sense of taste. But the most peculiar and special sense-organs of fishes are the sense-organs of the lateral line. These are somewhat similar in structure to those already mentioned, but they are usually sunk beneath the surface and contained in tubes, grooves, or closed canals; in all cases they are developed on the surface of the epidermis and afterwards enclosed in invaginations. The most constant part of this system is the lateral line, which is a tube in the skin extending from the auditory region along each side of the body to the root of the tail. In the dog-fish, as in most Teleosts, this tube is closed and communicates with the surface of the epidermis by pores at regular intervals; it has thus a structure very similar to that of a tube-railway, the pores corresponding to the stations at which the tubular tunnel communicates by vertical shafts with the surface of the ground.

The sense-organs are in the epithelium lining the tube, on its inner surface, alternating with the pores; in Teleosts the tube perforates a series of modified scales which make a conspicuous line on the side of the fish. The sense-organs are innervated by a branch of the tenth cranial nerve called the vagus, this branch running parallel to the tube and sending a small branch to each sense-organ. On the head the structures of this system are not so constant; in Teleosteans they form tubes like that of the lateral line, one running above the eye, another below the eye and along the upper jaw, and a third along the front of the operculum and the lower jaw; these tubes are all supplied by branches of the seventh cranial nerve. In the dog-fish, on the other hand, and in most Elasmobranchs, the tubes on the head are represented by separate tubes each opening by a pore and running obliquely beneath the surface of the skin; these sensory tubes are situated in the same regions as the continuous tubes of Teleosts, and are supplied by the same nerves. It will be noted that the series of lateral-line organs radiate from a focus formed by the auditory organ which is really to be regarded as a special and enlarged sense-organ of the same system.

4.8.2 Hydrostatic System

Hydrostatic organ, the swim bladder, may be used as a supplementary and emergency respiratory organ in fishes. It is generally assumed that the swim bladder is the homolog of the lung, although there are differences. The lung arises primitively from paired anlagen from the ventral wall of the pharynx, while the teleost swim bladder arises from an unpaired dorsal or dorsolateral diverticulum of the esophagus. The blood supply of the lung is from the sixth aortic arch, while that of the swim bladder is usually from the dorsal aorta.

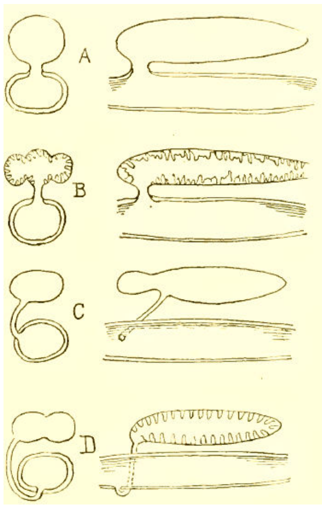


Fig. Diagrams of air bladder in fishes;

A. Physostomous fishes, B. *Lepidosteus*

and *Amia,* C. *Erythrinus,* D. *Ceratodus*

Dipnoan

In two of the lung fishes, *Protopterus* and *Lepidosiren*, paired sac-like lungs extend the length of the body cavity. The stem of these lungs attaches to the ventral aspect of the esophagus just behind the expanded branchial chamber. There is a slit-like glottis opening into the pharynx or esophagus. The lungs in these forms serve not only a respiratory function but also a hydrostatic one. In *Protopterus* the lungs lie retroperitoneally above the level of the kidney and the gonad, and they extend to the extreme posterior end of the body cavity.

Teleost

The swim bladder of the teleost is a thin-walled, midline sac in the dorsal mesentery. It lies below the kidneys: the gonads lie lateral to it and the gut is suspended below it. The swim bladder may be connected with the esophagus dorsally, or this connection may be lacking in the adult. The salmon is an example of the first, physostomous condition, while the perch is an example of the second, physoclystous condition. This bladder generally has a hydrostatic function, but in some physostomes it is an air-respiratory organ.

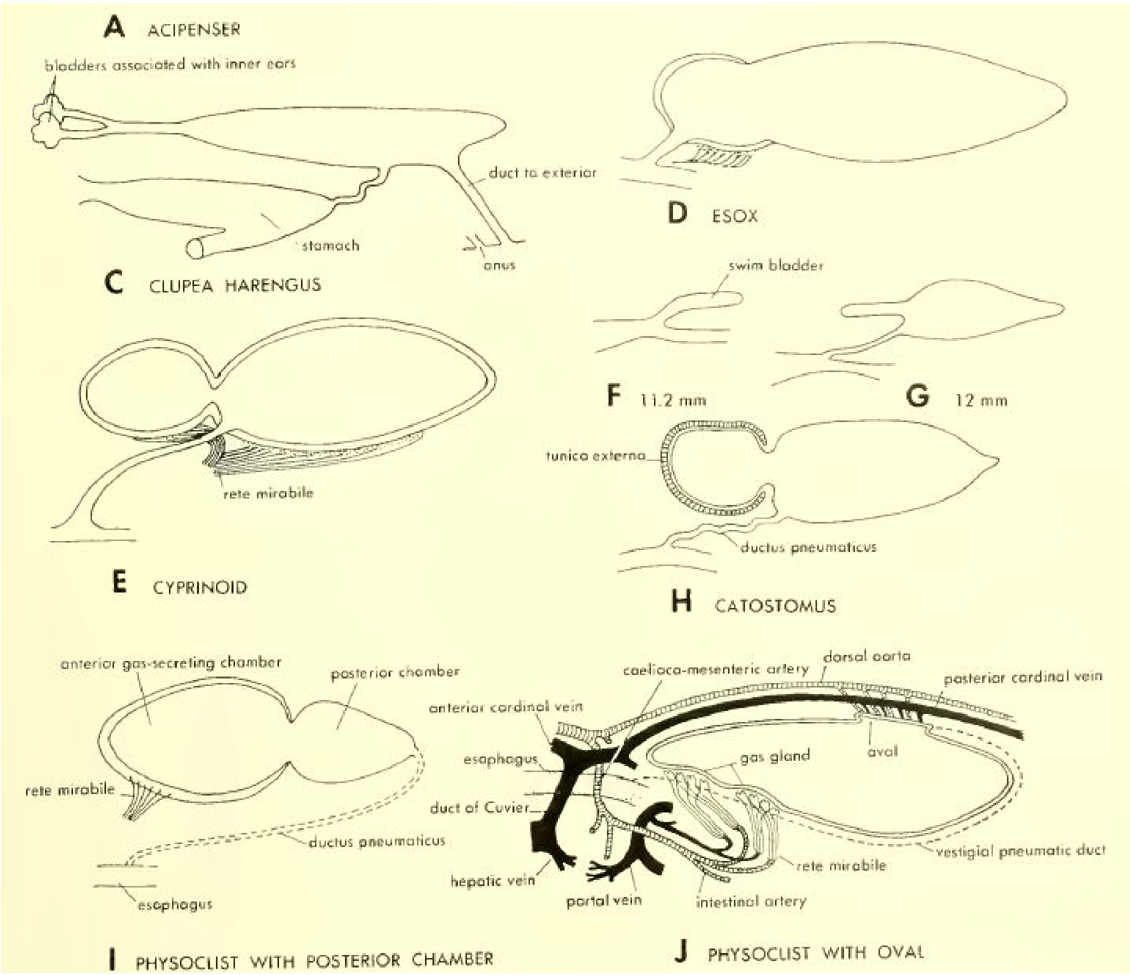


Fig.: Variations in the structure and relationships of the swim bladder

In both physostomes and physoclists the swim bladder arises as a dorsal diverticulum from the esophagus. In the cod the swim bladder wall contains peptic (stomach) glands. In the physoclist the swim bladder undergoes modification so as to function as a hydrostatic, sensory, or sound-producing organ. There is an anteroventral secretary region, the gas gland, which contains a retia mirabilis (wonderful net) of blood capillaries, and a posterodorsal absorbing region, the oval, which develops from the degenerating pneumatic duct. These two parts may lie in nearly separated anterior and posterior chambers. The eel (*Anguilla*) is an intermediate type, retaining the pneumatic duct while having the oval and gas glands.

In physostomes, blood is supplied to the swim bladder through a branch of the coeliacomesenteric artery and is returned to the heart through a vein joining the hepatic portal system or the postcardinal vein (*Anguilla*). In physoclists the coeliacomesenteric artery supplies the retia mirabilis of the gas gland, the oval area, and the bladder wall. These structures also receive blood from the dorsal aorta. The blood returns from the gas gland through the hepatic portal stem, and the rest of the bladder is mainly drained through the posterior cardinal veins. Right and left branches of the vagus (Xth cranial) nerve innervate the structure, particularly the gas gland; the oval is innervated by sympathetic branches which parallel the vagus nerves.

Amia, Lepidosteus, Acipenser

In *Amia* and *Lepidosteus* the swim bladder extends nearly the length of the body cavity. Unlike the teleost, the walls of the bladder are sacculated laterally as in *Neoceratodus*. The pneumatic duct opens through a dorsal, slit-like glottis in the region of the esophageal constriction posterior to the pharynx.

In *Amia* arterial blood comes from right and left pulmonary arteries, while in *Lepidosteus* branches of the dorsal aorta descend at intervals along the length of the bladder. Venous drainage is into the left duct of Cuvier in *Amia*, into the right postcardinal of *Lepidosteus*. The teleost *Gymnarchus* is intermediate; the efferent branchial arteries of the third and fourth gill arches unite to form a root for the coeliacomesenteric and pulmonary arteries.

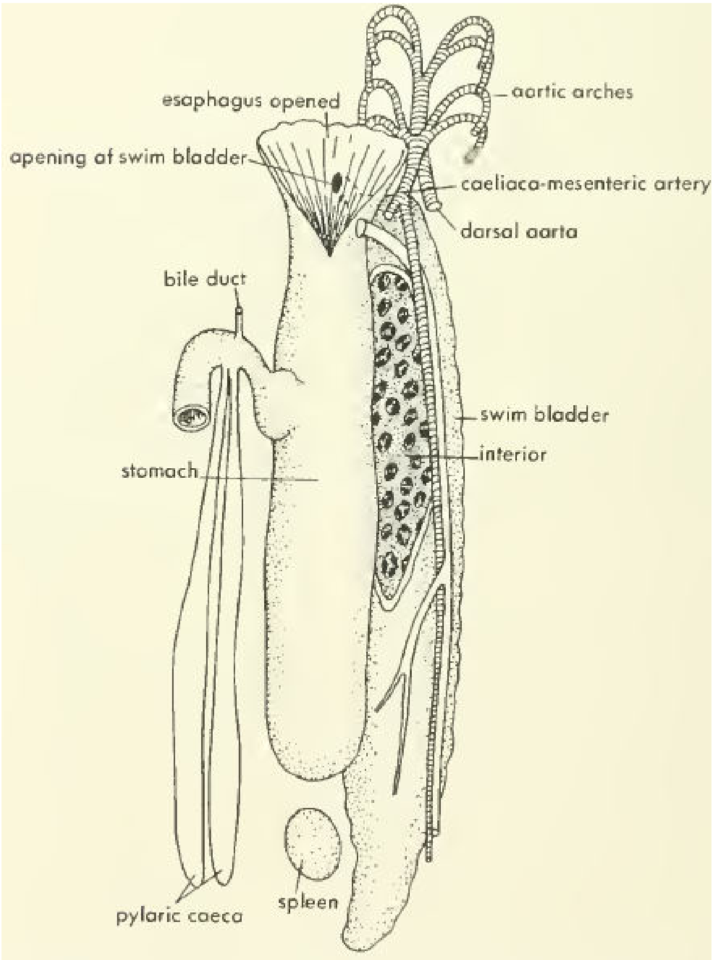


Fig.: Swim bladder and its relationships in *Gymnorchus*

In *Acipenser* the swim bladder is short and ovoid. The pneumatic duct enters the bladder ventrally, well behind the anterior end, and it joins the esophagus, or stomach, well back from the pharynx. The point of entrance into the esophagus is closed by simple constriction of the duct, and there is no glottis. Internally the bladder is smooth walled. The walls are fairly thick and fibrous brittle in preserved specimens. Isinglass, a very pure gelatin, is produced from the swim bladders of sturgeons. The arterial and venous connections are with the dorsal body channels.

Polypterus

Polypterus has bilateral air sacs joined anteriorly and opening through a ventral glottis to the right of the median line. It is assumed that these sacs arise as bilateral diverticula from the underside of the esophagus, just behind the pharynx. The left glottis is reduced to an epithelial strand or lost with the fusion of the anterior ends of the sacs. In cross sections it is seen to be broadly attached dorsally between the kidneys with the gut suspended below it.

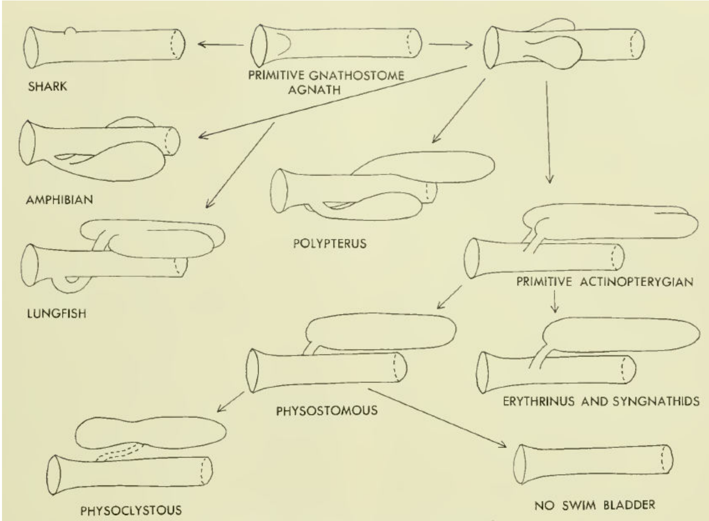


Fig.: Possible phylogeny of the swim bladder

The sacs of *Polypterus* contrast strongly with those of other actinopterygians. It is difficult to assume that the chondrosteans, holosteans, and teleosts were derived from a form with bilateral ventral sacs of which all trace has been lost except in *Polypterus*. It is equally or more difficult to assume that *Polypterus* is a modified choanate. The paired sacs of *Polypterus* give foundation to the theory of the separation of a brachiopterygian group from the actinopterygian.

Other gnathostome fishes

Miklucho-Maclay (1867) found a rudimentary dorsal diverteculum from the esophagus in embryos of *Galeus, Mustelus*, and *Squalus.* These rudiments were not generally observed by other workers, although Mayer (1894) found one in the adult of *Mustelus laevus*. Pits in the esophageal wall are observed in *Scytlium, Squatina, Carchartus, Pnsliiirus, Heptanchiis*, and rays. Wassnezow (1928, 1932) found one to three pairs of pits, dorso- and ventrolaterally behind the fifth pouch in *Trygon, Torpedo,* and *Pristiurus*. The most posterior of these in *Trygon* forms a dorsomedial pit comparable to that of *Muslelus*. In most adult sharks there is no evidence of a swim bladder and in the development of the shark, there is only this hint of a rudimentary swim bladder.

In the fossil antiarch *Bothnolefiis canadensis*, casts of what appear to be lungs have been described. This interpretation has been objected to on the grounds that these structures lie too far forward and are thus casts of the gill chambers.

4.8.3 Lateral-line System

Lateral-line organs are a specialized type of cutaneous sense organs limited to fishes and water-dwelling amphibians. It is believed that they respond to currents of water and to sudden changes in pressure. The lateral line in most fishes consists of a series of modified scales, each one provided with a mucous tube extending along the side of the body from the head to the caudal fin. The canal which pierces each scale is simple at its base, but its free edge is often branched or ramified. In most spiny-rayed fishes it runs parallel with the outline of the back. In most soft-rayed fishes it follows rather the outline of the belly. It is subject to many variations. In. some large groups its surface structures are entirely wanting. In scaleless fishes the mucous tube lies in the skin itself. In some groups the lateral line has a peculiar position, as in the flyingfishes, where it forms a raised ridge bounding the belly. In many cases the lateral line has branches of one sort or another. It is often double or triple, and in some cases the whole back and sides of the fish are covered with lateral lines and their ramifications. Sometimes peculiar sense-organs and occasionally eye-like luminous spots are developed in connection with the lateral line, enabling the fish to see in the black depths of the sea.

It is an integral part of the acusticolateralis system which includes the ear. It consists of:

1. Sensory lines radiating over the head and body,
2. Pit organs and
3. Ampullae of Lorenzini.

The basic lines are as follows:

1. One above the eye, the supraorbital
2. Another behind and below the eye, the infraorbital
3. One paralleling the mandibular arch and having jugal and oral parts
4. One passing down over the hyoid arch and on to the lower jaw and having preopercular and mandibular segments
5. A temporal division, which continues posteriorly as the main lateral line and
6. A supraoccipital connective joining the temporal lines.

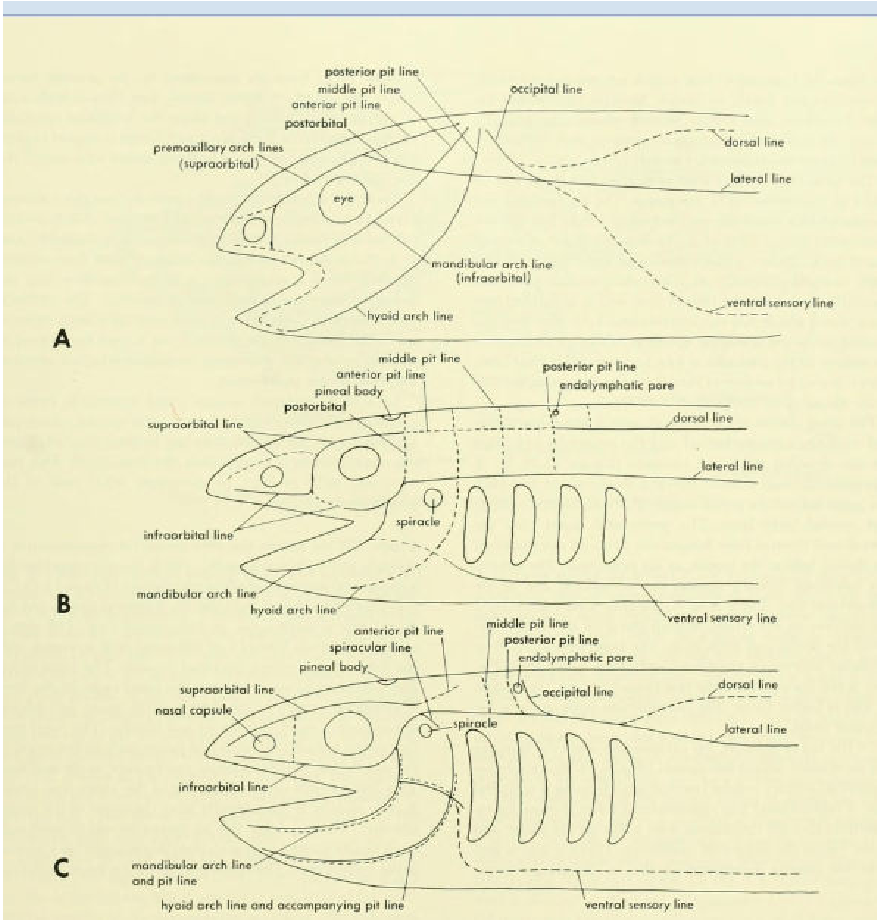


Fig.: A. Arthrodire; B. Primitive vertebrate; C. Chondrichthyes, and Osteichthyes

The sensory lines of living forms have organs called neuromasts or maculae formed of clumps of cells. The cells of a neuromast have hair-like projections enclosed in a cupola of gelatinous material. Movement of the cupola, acting through the hairs, stimulates the cells.

This system functions in the detection and location of disturbances in the water. Such a

1. sense aids in finding food
2. in social behavior
3. in avoiding enemies
4. in echo location and
5. as accessory rheotactic sense (orientation in flowing water)

This system in fossil agnaths appears to involve a "mucous-canal" reticulum as well as sensory canals. The system is assumed to be one of epidermal canals forming a reticular pattern over the head and trunk.

From the agnaths one could conclude that the ancestral vertebrate lacked a sensory-canal system or that it had an extensive reticular system in which certain channels came to be the primary sensory lines. The reticular and sensory-line systems may have originally been separate, the former functioning in mucous production, protecting the body both from abrasion and from water penetration.

In a well-developed sensory canal system, in terms of primary lines distinct from the reticular system, three primary pairs of longitudinal lines can be distinguished. These are connected by a single canal, the postorbital. This pattern can only in part be compared with that of the gnathostome groups.

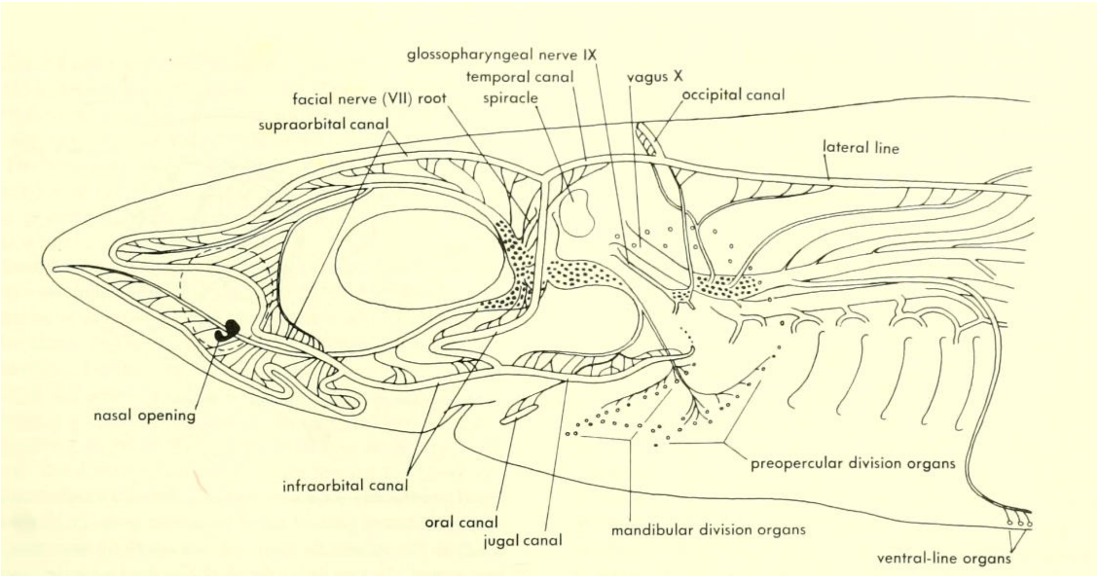


Fig.: Sensory-line system of *Squalus acanthias*

Sharks the most primitive representative is thought to be *Chlamydoselachus,* which closely resembles the hypothetical ancestral gnathostome pattern. Here the main lateral line joins the temporal canal, and the dorsal line is interrupted on the cranial roof. The supra-orbital line loops forward on the snout and connects with the infraorbital behind the nasal capsule. The infraorbital line continues forward beneath the nasal capsule; the lines of either side touch below the tip of the snout and are re-flexed back a short distance to end blindly. The jugal portion of the mandibular arch line passes back to the angle of the mouth, where it turns down and forward as the oral line. This line extends nearly to the tip of the lower jaw, where it ends blindly or occasionally joins the canal of the opposite side. The jugal line has an extension which continues back nearly to the hyoid arch line; this is probably a section of the ventral line.

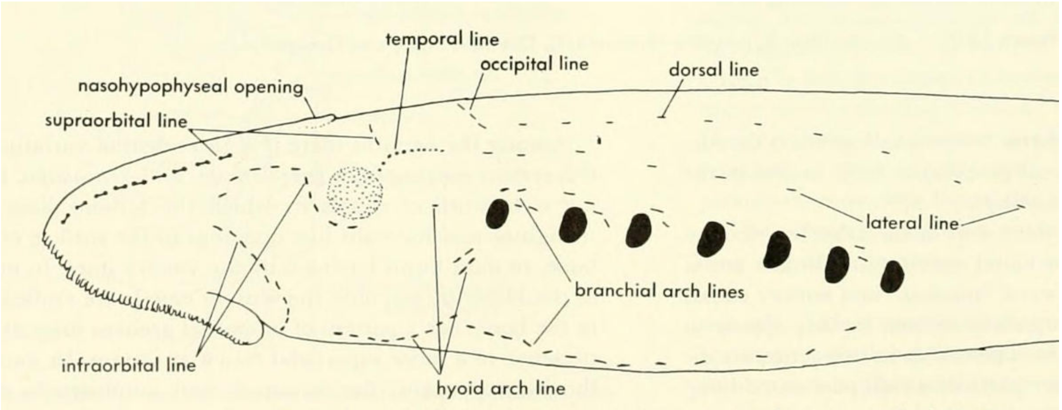


Fig.: Distribution of sensory-line organs and grooves in the lamprey

The preopercular division ends behind and below the spiracle without connecting with the temporal portion of the main lateral line. The posterior pit line, single or double, extends toward but not to the mid-line in front of the endolymphatic pores. Some of the lines are open grooves; these include the entire hyoid arch line, the oral portion of the mandibular arch line, the posterior tip of the jugal extension, and the lateral line posterior to the spiracle. The others are closed canals opening to the surface through irregularly spaced pores.There is a pit line from the spiracle down toward the junction of the jugal and oral lines. The significance of this pit line is not known.

In most selachians, such as the dogfish, the canal pattern is suggestive of that observed in *Chlamydoselachus;* however, the mouth is far back below a strongly developed rostrum. The infraorbital canal ends below the tip of the snout. In *Laemargus* the supraorbital, infra-orbital, and lateral lines come together at a point high on the head.

In most sharks the hyoid line is represented only by pit organs; the oral canal may be much reduced and without direct connection with the jugal canal, which has a strong posterior extension. The occipital canal lies behind the endolymphatic pores and the canals of either side connect. This canal is also called the supratemporal, aural, or commissural canal. A short branch of the occipital canal may pass in front of the endolymphatic pore; this is the posterior pit-line canal. The sensory lines are canals in most sharks although the posterior end of the lateral line may be a groove as in *Squalus.*

*Holocephalan,*the pattern observed in *Hydrolagus* is distinct from that of the shark, but suggestive of it. The hyoid and mandibular arch lines join the infra-orbital below the eye. The first is the better developed. The infraorbital line has a break in it and passes above (behind) the nasal capsule. Assuming that this lower line represents the infraorbital, the upper could correspond to the reflexedend of the supraorbital line of the shark.

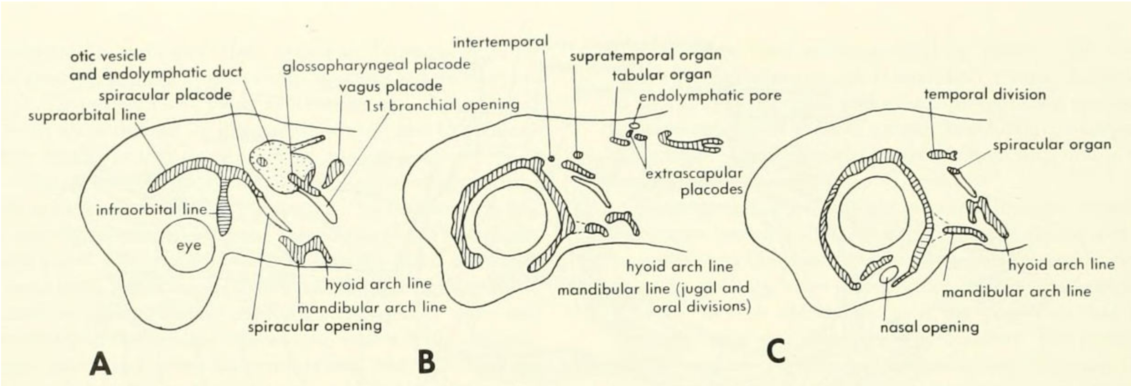


Fig.: Three stages in the development of the sensory-line system of *Squalus acanthias*

The infraorbital is then peculiar because of its relationship to the olfactory capsule and also its forking (only in *Hydrolagus);* both parts connect across the midline, and the upper also connects along the midline with the supraorbital connective.

Fossil gnathostome fishes In the arthrodires and acanthodians, the same general canal pattern is seen. In the acanthodian the canals lie in the scales covering the head. In one genus, *Poracanthodes*, there is a reticular canal system embedded in the scales. There are distinct mandibular and hyoid arch lines which join at the angle of the mouth, and there is a pit line of the cheek which joins the jugal section with the preopercular section. The preopercular section lies far forward of the spiracle and does not connect with the temporal line. The position of this line indicates that these fishes did not have a complete mandibular cleft (the canal could not have been displaced forward on the cheek if such a cleft were present). In some acanthodians a dorsal hyoid section extends from the spiracle to the temporal canal.

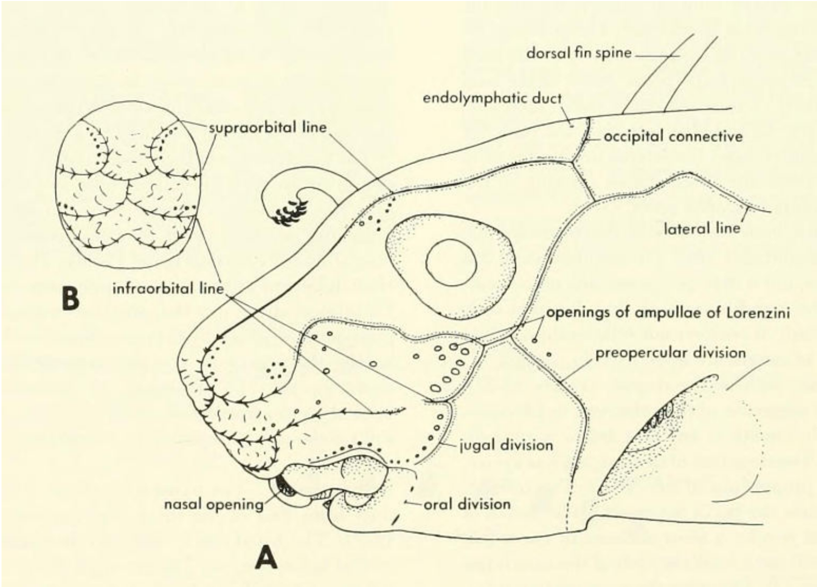


Fig.: Sensory-line grooves on the head of *Hydrolagus*.

A, lateral view; B, interconnections on snout

In the arthrodires the canal system is indicated by grooves in the dermal bones; this system is peculiar because of the radiating nature of the supraorbital, infraorbital, and lateral lines. This is also observed in the shark *Laemargus.* Unlike any other gnathstomes, there are also middle and posterior pit lines in this radiation. The posterior pit line is a canal in this group; the occipital canal, passing behind the endolymphatic pore, is lacking or poorly developed. The temporal line loops down toward the cheek and the preopercular canal is indicated only dorsally. A jugal connection between mandibular and hyoid arch lines is perhaps present in *Homosteus.* Like the shark the supraorbital canal extends down behind the nasal capsule, but it does not join the temporal line posteriorly. It is unlikely that the infraorbital canal passed below the nasal capsule.

The sensory-canal system of the **actinopterygian** fishes is difficult to compare with the hypothetical pattern or the patterns seen in other groups. As in the chondrichthyes, most acanthodians, and arthrodires, there is no evidence of a reticular canal system in any of the living or extinct forms. The supraorbital canal may or may not connect with the temporal division of the lateral-line canal.

The infraorbital canal passes forward below the nasal capsule; anterior connection between the supraorbital and infraorbital canals is lacking with the exception of *Polypterus.* The hyoid arch canal attaches to the temporal canal behind the spiracle or ends on a line passing behind the spiracle. The mandibular arch line is poorly developed. There is an occipital canal, and anterior, middle, and posterior pit lines on the top of the head. The anterior pit line is a continuation of the supraorbital canal behind the point of connection with the temporal canal. This suggests that it is a part of the dorsal line as seen in the agnath fish or the basic model.

Not all actinopterygians agree in detail. In the palaconiscoids, *Pteronisculus* or *Boreosomus* as examples, the supraorbital canal does not join the temporal canal. This state is observed in all lines of palaeoniscoid fishes. The living chondrosteans, holosteans, and teleosts in contrast have these lines joined. Although the preopercular line invariably extends up behind the spiracle, *Polyodon* is the exception. In this chondrostean it connects with the infraorbital line below the spiracle. In the haplolepid palaeoniscoids, dorsal and ventral body lines as well as a main lateral line are observed.

In the actinopterygian the sensory lines are usually embedded in the dermal bones with pores to the surface. The dermal bones may be reduced to tubes enclosing the canal. In some the canals lie superficial to the bone or are lost.

The development of this system in *Amia* has been reported. Here again are seen supraorbital, infraorbital, joined mandibular and hyoid, and temporal placodes.

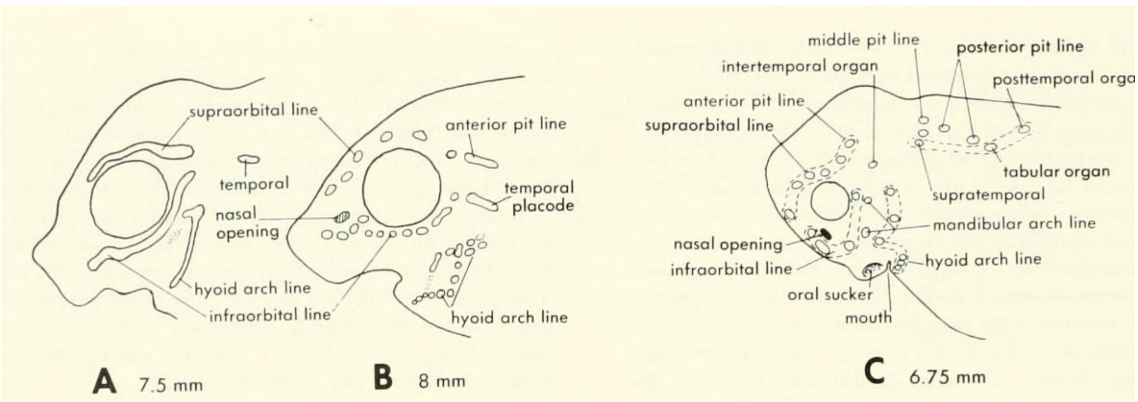


Fig.: Development of the sensory-canal system of the head in actinopterygians.

A and B, two stages in *Amia*; C, an early stage of *Polypterus*

Choanates

In the early **crossopterygians** and **dipnoans** both reticular and sensory-line canals were present and enclosed in the dermal bones *(Porolepis,* *Osteolepis).* In the later forms *(Holoptychius, Eusthenopteron)* the reticular system is lacking.

The primitive **dipnoan** had an embedded sensory line similar to that of the crossopterygian and, in the case of *Dipterus,* a reticular system, which was lost early in this group. The dipnoan differs in that the infraorbital line did not passforward below the opening of the nasal capsule, or between the internal and external nares, as in the amphibian.

In living dipnoans a pattern similar to that of larval amphibians is observed. The sensory organs are located primarily in grooves in *Protopterus* but in canals in *Neoceratodus;* these lie in the skin, not in the bones. *Protopterus* differs from *Neoceratodus* in having a connecting line between the mandibular and hyoid arch lines. This groove could represent the jugal extension, turned down rather than up as in the crossopterygian. Whereas the infraorbital line of *Neoceratodus* is reflexed upward above the nasal capsule, that of *Protopterus* is bent down and back as if to enter the mouth to pass between the flares. *Protopterus* has a ventral line on the body as well as a lateral line.

The development of this system has been described for *Neoceratodus*. The pattern appears to be the same in the other lungfishes and is much like that observed in the shark or *Amia.*

4.9 Summary

The detailed discussions in the unit is of great importance for the students to lean diversity, physiology, morphology and behavior of class Pisces. The diversity and various degrees of structural difference in the fishes to divide and subdivide them in a systematic classification which clear the specific position in the living world. Fish are limbless aquatic vertebrate. There are three living classes of fish: the primitive jawless fishes, or Agnatha; the cartilaginous fishes, and the bony fishes. These groups, although quite different from one another anatomically, have certain common features related to their common evolutionary origins or to their aquatic way of life. Fish were the earliest vertebrates and presumably evolved from a group of aquatic lower chordates; the terrestrial vertebrates evolved from fishes.

Fish first appeared during the Cambrian period. They possessed notochords. Fish would continue to evolve through the Paleozoic era, diversifying into a wide variety of forms. The first fish with jaws appeared in the Silurian period.

In order to provide the conditions necessary for the maintenance ofsuch a watery system, in very different environments, many auxiliaryactivities have been developed to compete light, pressure, currents, temperature, oxygen, nutrients, predators, food, mates, competitors or symbionts. The sense organs, olfactory sacs, eyes, auditory, hydrostatic and lateral line organs helps fish to compete with the diverged environment. The harsh environment have led deep sea fishes to fascinating adaptations of deep sea life for sensing, feeding, reproducing, moving and avoiding being eaten by predators.

Fish is specially modified for different ecological conditions; show parental care and exhibit offense and defense mechanism are additive in their survival. The fish is the most successful animal in water.

4.10 Glossary

- **Adaptation:** the process of change by which an organism or species becomes better suited to its environment
- **Adaptive radiation**: diversification of a group of organisms into forms filling different ecological niches
- **Bathypelagic**: the open ocean or pelagic zone that extends from a depth of 1000 to 4000 meters below the ocean surface
- **Benthic zone**: the ecological region at the lowest level of a body of water such as an ocean or a lake, including the sediment surface and some sub-surface layers.
- **Benthos**: aquatic organisms which live on or in the seabed, also known as the benthic zone. Included are both mobile animals, such as crabs and abalone, and non mobile animals, such as corals and sponges
- **Bony fish**: fish that have a bony skeleton and belong to the class osteichthyes
- **Dead zone**: an area in an ocean or large lake where oxygen levels are extremely low, often due to eutrophication
- **Deep ocean currents**: currents in the deep ocean, also known as thermohaline circulation or the "conveyor belt", are driven by density and temperature gradients
- **Detritus:** dead organic matter and the decomposers that live on it; when broken up by decomposers
- **Elasmobranch**: cartilaginous fish that includes sharks, skates and rays

- **Eutrophication**: an increase in chemical nutrients typically compounds containing nitrogen or phosphorus in an ecosystem. Eutrophication in water often results in an increase in algae growth and decay, which can lead to decreased levels of oxygen and fish populations
- **Fish**: a true fish is a vertebrate with gills that lives in water. However, in the context of fisheries, the term "fish" is generally used more broadly to include any harvestable animal living in water, including molluscs, crustaceans and echinoderms
- **Genus**: a rank in classification below family and above species
- **Gills:** the respiratory organ found in fish and other aquatic animals
- **Habitat**: the place where an organism lives
- **Hydrostatic :** relating to or denoting the equilibrium of liquids and the pressure exerted by liquid at rest
- **Hypoxia**: occurs in aquatic environments when dissolved oxygen becomes depletion to a level which is harmful to aquatic organisms
- **Isobath**: a contour line linking regions of the same depth
- **Lateral line:** system of sense organs found in aquatic vertebrates used to detect movement and vibration in the surrounding water
- **Littoral**: the shallow water region around lake or sea shores where significant light penetrates to the bottom
- **Parental care**: form of altruism since this type of behaviour involves increasing the fitness of the offspring at the expense of the parents
- **Phylum:** a taxonomic rank between kingdom and class (the plural is phyla)
- **Species:** used in the classification of living organisms, referring to related organisms capable of interbreeding

4.11 Self-Learning Exercise

Section -A (Very Short Answer Type):

1. Most fish do not sink in water because of the presence of......................
2. Hydrostatic isdenoting the ....of liquids.
3. Dipnoi are with gills and...................... for respiration.

4. The electric organs arise from modified muscle fibers, the cells of which are plate-like andarranged in rows, the arrangement is known as ....
5. Elasmobranches have bony skeleton. T/F
6. Fish were the second earliest vertebrates. T/F
7. The heart fish is two or three-chambered. T/F
8. The poison gland on the operculum and dorsal fins of fish, that inject poison into the woundis modified dermal glands. T/F
9. What is isobath?
10. Agnath means?
11. In which period Ostracoderms became extinct?
12. What is source of light in bioluminescence?

Section -B (Short Answer Type):

1. What are the two main classes into which fish are divided?
2. Which features give chondrichthyes and osteichthyes their names?
3. What is the evolutionary origin of jaws in fish?
4. Identify at least 3 characteristics that are essential to deep sea fish and their survival.
5. Name at least 2 constraints that deep-sea fish must deal with on a daily basis.
6. What are the advantages and disadvantages of a cartilaginous skeleton?
7. How do fish care for their young?
8. What is the role of slimes Hagfish?
9. Imagine that all species had the same traits and characteristics. What problems might this create in the natural world?
10. How does the swim bladder of fish allow them to control their depth under water?
11. What role do the spines play in spiny-rayed fishes?
12. What are the lateral lines of fish?
13. In what habitats would you expect to find a fish with as strongly depressed body?
14. Why do omnivorous fishes tend to have longer gut lengths relative to their size than carnivorous fishes?
15. How does-sea fish not get crushed by the tremendous pressure of the ocean at the sea floor?

Section -C (Long Answer Type):

1. Give a detailed classification of fish with suitable examples.
2. Write a note on origin and evolution of fish.
3. What are traits or adaptations found in deep sea fish that allow them to live in the deep sea?
4. What are adaptive radiations? Discuss adaptive radiations in bony fishes.
5. How fish care their eggs and young ones? Explain.
6. Explain offensive and defensive mechanism in fishes.
7. Explain how sensory system helps fishes in their survival?
8. What is lateral line system? Discuss lateral line system in fishes.

Answer Key of Section-A:

1. swim bladder/air bladder
2. equilibrium
3. lungs
4. electroplaques
5. F
6. F
7. T
8. T
9. contour line linking regions of the same depth
10. Jaw less
11. Devonian period
12. luminous bacteria

4.12 References

- Life of Vertebrates by J.Z. Young
- The Class Pisces by Baron Cuvier
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Unit - 5

Amphibia

Structure of the Unit

5.1 Objectives

This unit is designed to understand the diversity, physiology, morphology and behavior of class Amphibia. Object in this Unit is, therefore, to help the reader/student to learn as much as possible about Amphibian life:

- The diversity and various degrees of structural difference in the fishes to divide and subdivide them in a systematic classification
- The Origin of representative Amphibian forms which are very unlike in structure and origin
- The varied environment have led Amphibia to fascinating adaptations for different ecological conditions
- The biology and ecology for extinction of large amphibians
- The mechanisms to protect their eggs and developing young ones by Amphibians

5.1 Introduction

The word amphibian comes from the Greek *amphibios* meaning "both lives". This is an apt description because most adult amphibians are better adapted to life on land than in water, while their larval phases are entirely aquatic.

Amphibians are ectothermic, tetrapod vertebrates of the class Amphibia. Modern amphibians are all Lissamphibia. They inhabit a wide variety of habitats with most species living within terrestrial, fossorial, arboreal or freshwater aquatic ecosystems. Amphibians typically start out as larvae living in water. The young generally undergo metamorphosis from larva with gills to an adult air-breathing form with lungs. Amphibians use their skin as a secondary respiratory surface and some small terrestrial salamanders and frogs lack lungs and rely entirely on their skin. They are superficially similar to reptiles but, along with mammals and birds, reptiles are amniotes and do not require water bodies in which to breed. With their complex reproductive needs and permeable skins, amphibians are often ecological indicators.

Amphibians have other defining trademarks that make them who they are. First, they are cold-blooded creatures. If you enjoy warm and fuzzy pets, an amphibian may not be the one for you. Amphibians are at the mercy of the environment around them. They have to regulate their temperature by physically moving to or away from the heat source. If it's an extremely hot day, you'll probably find them lounging in the pond.

Amphibians absorb their water right through their skin. Unlike their reptile cousins that have thick scales covering their bodies, the skin of an amphibian is extremely thin and fragile. This allows water to penetrate but also puts them at high risk of dehydration. And so, their favorite hangouts are watery destinations such as ponds, lakes, and swamps.

An amphibian's skin has another crucial job, which is gas exchange. Frogs and their relatives actually breathe through their skin as adults. Although adult amphibians do have lungs, they function rather poorly. Oxygen gas is absorbed, and carbon dioxide is released, right through the very thin covering of these creatures. Thus, it is even more important for them to keep their skin moist and protected.

The earliest amphibians evolved in the Devonian period from sarcopterygian fish with lungs and bony-limbed fins, features that were helpful in adapting to dry land. They diversified and became dominant during the Carboniferous and Permian periods, but were later displaced by reptiles and other vertebrates. Over time, amphibians shrank in size and decreased in diversity, leaving only the modern subclass of Amphibia is Lissamphibia is composed of only 3 orders. Anura, which refers to tailless amphibians, includes frogs and toads. This is the largest order. Caudata refers to amphibians with tails salamanders, newts, and sirens. The Gymnophiona order is made up of caecilians, legless, tailless amphibians that spend most of their time burrowing.

Amphibians evolved in specialized situations during Devonian period, just after the Devonian mass extinction when climate became warm and humid, and huge swamps and marshlands appeared on land. By Carboniferous period there were dense gymnosperm forests all over the land areas. Advancement of forests on land attracted insects, worms and molluscs that had already achieved the capability to inhabit terrestrial forests and which were part of fish and amphibian diet.

Swamps of Devonian period were inhabited by highly specialized lobe-finned fishes that were already equipped with lungs to obtain oxygen from air as the marshland water was low in oxygen content. These fishes also had lobed fins with strong bony elements to support the body in muddy water or on submerged vegetation. Perfection of these two anatomical features, that is, lungs and lobed fins transforming into tetrapod limbs must have taken place in marshy conditions, which later proved to be important adaptations to conquer land, as seen in labyrinthodonts. As forests started invading land, followed by invertebrates, these primitive labyrinthodonts also tried to venture on land in pursuit of abundant prey or to escape predators. They soon mastered terrestrial living and became the dominant predators on land in Carboniferous and Permian periods. Large amphibians were wiped out by the Permian mass extinction.

As amphibian include anurans, urodelans and apodans. In all these groups of amphibians show a great deal of parental care. Amphibians show several mechanisms to protect their eggs and developing young ones because they lay few eggs.

The smallest amphibian (and vertebrate) in the world is a frog from New Guinea (*Paedophryne amauensis*). The largest living amphibian is Chinese giant salamander (*Andrias davidianus*), The study of amphibians is called batrachology, while the study of both reptiles and amphibians is called herpetology.

5.3 Classification

5.2 Class: Amphibia

5.2.1 sub-class: Stegocephali

5.2.1.1 Order: Labyrinthodonti/Stereospondyli

5.2.1.2 Order: Temnospondyli

5.2.1.3 Order: Lepospondyli

5.2.2.1 sub-class: Lissamphibia

5.2.2.1 Order: Urodela (Caudata)

5.2.2.2 Order: Anura (Salientia)

5.2.2.3 Order: Apoda (Gymnophiona)

5.2 Class: Amphibia

This Class, next to Cyclostomata, is the smallest of the vertebrate classes. Living amphibia counts some 2500 species and 234 living genera of frogs, toads, and salamanders. There are 19 genera and 55 species of the snakelike caecilians (*F. Nieden*). The Class breaks into two sub-classes (i) Stegocephali (extinct) and (ii) Lissamphibia (living).

5.2.1 sub-class: Stegocephali

1. Cranial roof with dermal bones
2. Two supraoccipitalia, two postorbitalia, two supratemporalia
3. With interparietal foramen
4. With three bony pectoro-jugal plates
5. Tailed
6. Extinct (Carboniferous, Permian, and Triassic epochs)
7. Orders (i) Labyrinthodonti (ii) Temnospondyli and (iii) Lepospondyli.

5.2.1.1 Order: Labyrinthodonti/Stereospondyli

1. Oldest known tetrapod,like crossopterygian
2. Examples are *Trematosaurus, Capitosaurus, Mastodonsaurus, Labyrinthodon*

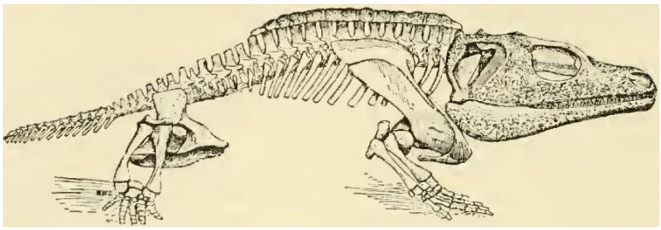


Fig.: *Cacops,* a small labyrinthodont amphibian

5.2.1.2 Order: Temnospondyli

1. Component units of the vertebrae remain in a separate, unfused state
2. Examples are *Chelydosaurus, Sphenosaurus, Trimerorhachis, Archegosaurus, Actinodon, Euchirosaurus*.

5.2.1.3 Order: Lepospondyli

1. Pseudocentrous
2. Sub-order: Branchiosauri (with gills, *e.g. Branchiosaurus);* Microsauri (without gills, *e.g.Urocordylus*); Aistopodes (without limbs and without pectoral girdles, *e.g.Dolichosoma*)

5.2.2.1 sub-class: Lissamphibia

1. Without bony dermal armour
2. Without supratemporalia
3. Modern amphians.
4. Orders (i) Urodela (Caudata), (ii) Anura (Salientia) and (iii) Apoda (Gymnophiona).

5.2.2.1 Order: Urodela (Caudata)

1. These are tailed amphibians with elongated body.
2. Adult retains larval tail.
3. Fore and hindlegs are of nearly equal size.
4. Larval gills and gill clefts may or may not be retained by adult.
5. Living mainly in the northern hemisphere; most abundant in the more temperate parts of it.
6. The common examples are *Necturus, Proteus, Siren (lacertina), Cryptobranchus, Amphiuma, Ambystoma*.

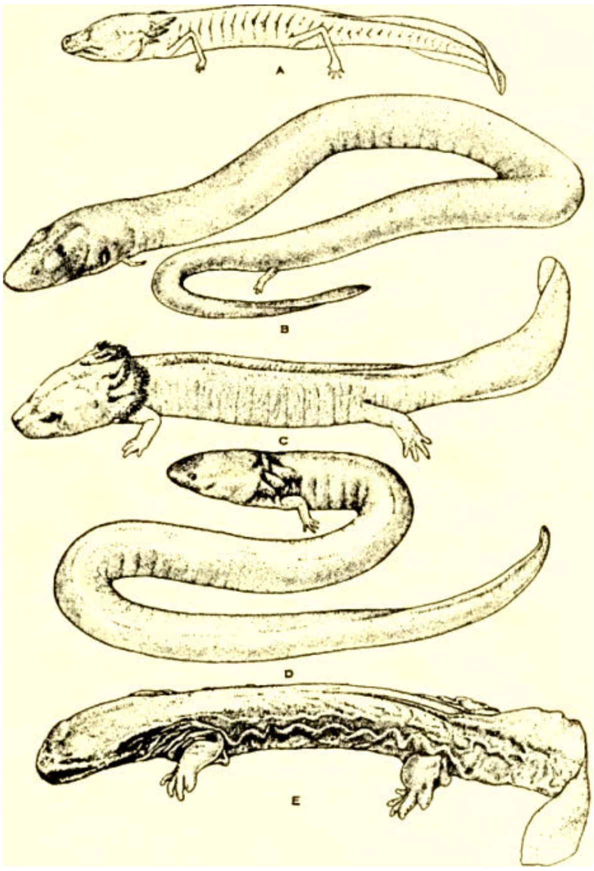


Fig.: Salamanders (A) *Typhlomolge ralhbuni* (B) *Amphiuma means* (C) *Necturus maculosus (D) Siren lacerlina (E) Cryptobranchus*

5.2.2.2 Order: Anura (Salientia)

1. Frogs and Toads
2. Body short and broad
3. Larval tail is not retained by adult
4. No gills or gill-clefts in adult
5. Vertebrae, only nine, sometimes fewer, within the region of the trunk
6. First vertebra articulates with the skull and ninth is strongly joined to the pelvic girdle
7. Behind the ninth, sacral vertebra is urostyle. Urostyle is a caudal structure, but in a strict sense it is not caudal and the adult anuran has no tail.
8. Centra of Anura are usually procoelous (concave only on the anterior face) or opisthocoelous (concave only on the posterior face) or may have sonic modification of these forms.
9. Forelegs short; hind legs much elongated and adapted to leaping locomotion.

10. Ear in Anura possesses an accessory mechanism not found in other modern amphibians or in fishes i.e. a cavity which internally opens into the pharynx.

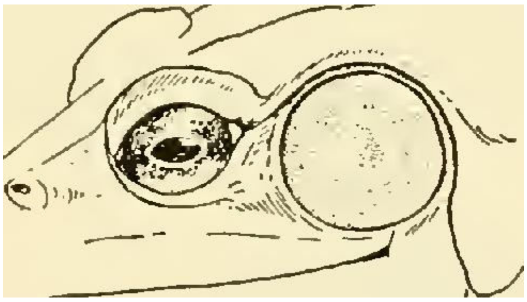


Fig.: Head of bullfrog, *Rana catesbeiana* showing tympanic membrane just behind eye

11. A slender rod of bone, the columella, extends across this cavity (tympanic cavity). Vibrations of the external medium impinge upon the tympanic membrane and are transmitted by the columella to the internal ear.

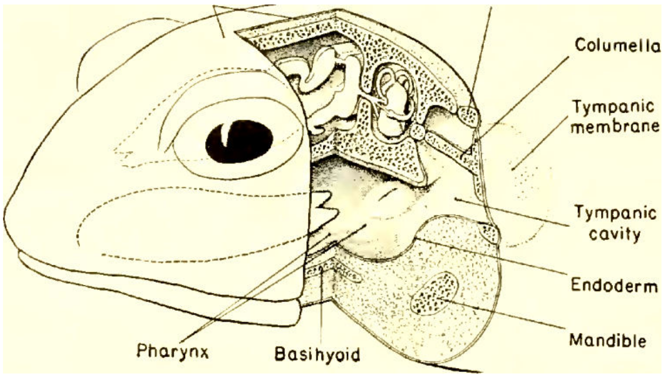


Fig.: Auditory apparatus of an Anura

12. The Anura are much more specialized than the Urodela. It is easy to distinguish the common toad from certain equally common frogs.

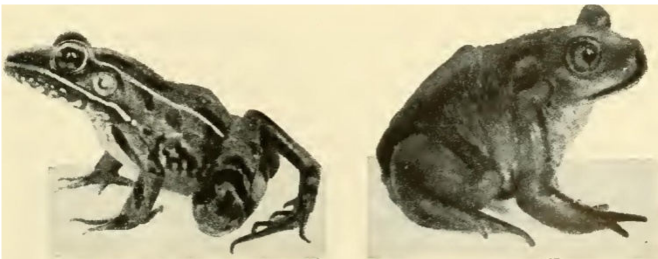


Fig.: Frog, *Ranasphenoeephala (Left)*
Toad, *Seaphiopus holbrookii (Right)*

13. Most of them are amphibious. Some are wholly aquatic, others wholly terrestrial.
14. The terrestrial anurans may live on the surface, or burrow, or spend most of the time in trees. In Java and Borneo there are flying frogs.
15. Examples are *Bufo americanus*, *Rana clamitans*, *R. pipiens*, tree frogs (or tree toads)*, Hy*la *versicolor*.

5.2.2.3 Order: Apoda (Gymnophiona)

1. This smallest of amphibian orders includes animals which might easily, at first glance, be mistaken for small snakes or for earthworms.

2. 
3. Fig.: *Siphonops,* one of the Apoda

4. Their amphibian nature is revealed by the fact that most of them pass through the typical larval stage of amphibians.
5. A metamorphosis takes place, during which the three pairs of external gills are absorbed.
6. No trace of legs appears in either adult or larva.
7. No accessory auditory structures (tympanic cavity and membrane) are developed.
8. The lungs are well developed. Left lung is very much smaller. This is an adaptation. In reptilian snakes there is a similar reduction of the left lung.
9. The skeleton is well ossified; the vertebrae of which there may be 250 or more, are amphicoelous.

10. The skin is typically amphibian in being thin and highly mucous.
11. Most of the apoda are burrowing animals. One genus is aquatic.
12. In the burrowing species the eyes are very small, poorly developed. In compensation for poor sight most of these animals have a pair of sensory facial tentacles.
13. Apoda, otherwise known as caecilians or blindworms, are widely distributed in the tropic regions of both Eastern and Western Hemispheres,
14. The best known genus is Ichthyophis, meaning fish-snake.

5.4 Origin

5.3 Origin of Ambhibia

That the amphibia arose from fish and their ancestor must have been one of the primitive Osteichthyes, related to the stock which also gave rise to Osteolepis and Dipterus.

5.3.1 Resemblances between Osteolepis and Stegocephalian

The resemblances between Osteolepis and one of the earliest Stegocephalian amphibia such as *Loxomma* can be extended to the following features:

1. Skull is a complete bony box.
2. Amphibia also had lateral-line canals which occupied grooves in the bones.
3. The bones of the palate are similar, and both had nostrils which lead into the cavity of mouth.
4. Amphibian Eogyrinus had a shoulder girdle the dermal bones of which were attached to the post-temporal bone of the skull by the supra-cleithrum, as in the fish.

5. Early amphibia had no sacrum, for the ilium was not attached to the ribs. The walls of the teeth were folded, in the Labyrinthodont pattern.
6. Amphibia are autostylic, as are the Dipnoi including Dipterus.
7. Otic process in Osteolepis did not reach the auditory capsule. So, whether the common ancestor of Osteolepids, Dipnoi, and Tetrapods was autostylic or not.
8. Lung was almost certainly present in Osteolepis.

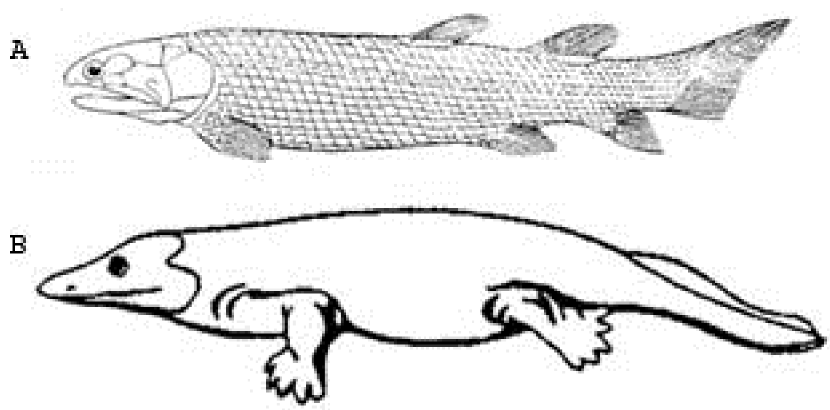


Fig.: A. Osteolepis (Crossopterygian), B. Labyrinthodonts

5.3.2 Transition and modification of organs

The transition from water to air necessitated a development of the olfactory organs to greater sensitiveness, for the concentration of substances in water is very much greater than that which can be obtained in air.

1. Increase in development of the olfactory organs and of the corresponding centers in the forebrain. The latter development accompanied and perhaps assisted the formation of the cerebral hemispheres, which are regarded as connected with an adaptation to the poor oxygen-content of the water in which the amphibia and their ancestors evolved.
2. In connexion with respiration, it will be remembered that the amphibia breathe by means of respiratory movements performed by the visceral muscles in the floor of the mouth, in a manner very similar to that of the fish. The difference is that the fish take in water and pass it back and out through the gill-slits, the amphibia take in air and pass it back and into the lungs. The mechanism is the

same, and it is obvious that the transition from water to air involved no functional rearrangement of importance as regards respiration.

3. The same holds true with regard to locomotion. The amphibia were beasts with bodies disproportionately large in comparison with their limbs. The limbs were used as oars to row the animal along on land, and the same muscles and nervous connexions came into play as in the aquatic ancestor.

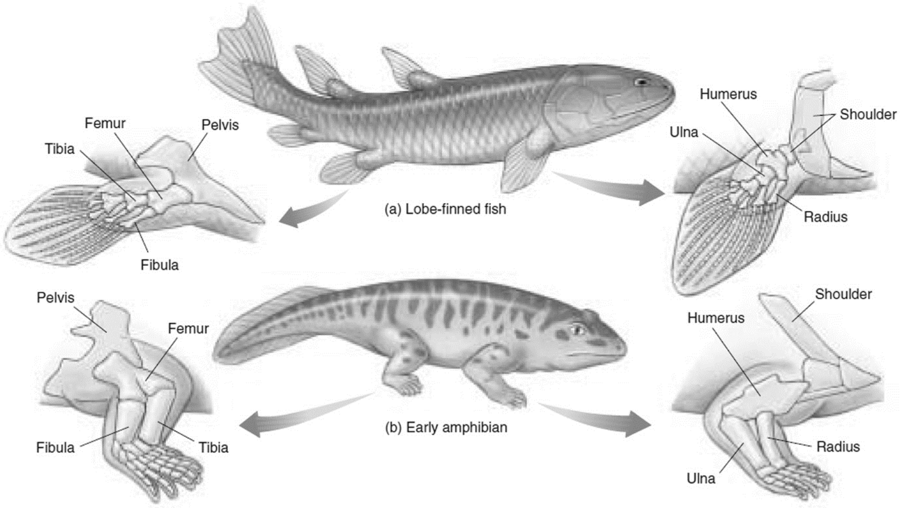


Fig.: Comparison between the limbs of a lobe-finned fish and those of a primitive amphibian

1. In the very earliest amphibia, the sacrum was absent (*e.g.Eogyrinus*). In the others it was present, and by anchoring the pelvic girdle on to the vertebral column, it strengthened the hind limbs.
2. The hyomandibula being no longer required to suspend the quadrate from the auditory capsule, its function became converted into that of conveying vibrations from the skin covering the spiracular cleft to the auditory capsule. In this way the hyomandibula became the columella auris; the covering of the spiracular cleft became the tympanic membrane, and the cavity of the spiracular cleft became the middle-ear and Eustachian tube; all quite simply and without involving any great rearrangement. So the ear became an organ for the delicate appreciation of sound as well as balance.
3. The early amphibia had a covering of dermal bones more or less all over the body. The vertebral column of the earliest forms or Embolomeri is remarkable in that each vertebra possessed two centra. There was an anterior hypocentrum and a posterior

pleurocentrum. The later amphibia preserved the hypocentrum at the expense of the pleurocentrum, which disappeared. It will be seen that in the reptiles the opposite occurred.

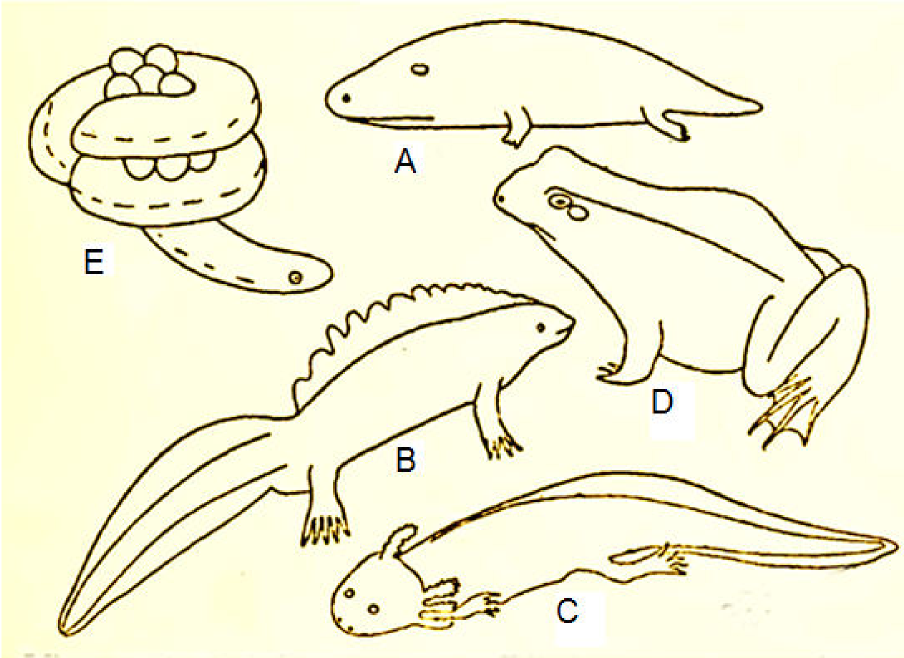


Fig.: Different types of Amphibian; A. Stegocephalian, B. Newt (Urodele), C. Ambystoma, D. Frog (Anuran), E. Ichthyophis (Gymnophiona)

Collectively, the early amphibia are known as the Stegocephalia or Labyrinthodonts, the former term referring to the complete bony covering of the skull. They flourished in the Carboniferous, and persisted until the Triassic period, when they were extinguished by the competition of their more successful descendants the reptiles, leaving only the frogs (Anura), newts (Urodela) and Gymnophiona alive to-day.

Based on the lack of agreement in the dermal bones of the snout, specifically the presence of a frontal and nasal as opposed to a series of nasals, and also on the autostylic palatoquadrate of the amphibian and the possible loss of the hyomandibula. Save-Soderbergh has pointed out the unlikelihood of the origin of amphibians directly from crossopterygians.

Holmgren has assumed a dipnoan origin for the urodeles in spite of the obvious specializations of the Dipnoi when first encountered in the fossil record. Even though Jarvik's analysis of the nasal openings of dipnoans is discounted, the loss of maxilla and premaxila and the form of the pterygoid and prearticular dental plates remain as salient features. Offering some support to the dipnoan origin are the autostylic jaw suspension, reduction of the hyomandibula, and connection of the pharyngosuprahyal to the otic capsule.

To complicate the discussion of the origin of amphibians, Save-Soderbergh, Holmgren, and Jarvik have asserted that the Amphibia arose from two choanate-fish sources. Save- Soderbergh and Holmgren derive the Urodela from a source near the Dipnoi and the other amphibians indirectly from the osteolepid crossopterygians. Jarvik derives the urodeles from a porolepiform and the anurans and other tetrapods from an osteolepiform source.

5.5 Adaptations in Amphibia

5.4.1 Metamorphosis

5.4.2 Axolotls

5.4.3 Protective and warning coloration

5.4.4 Adaptations for Locomotion

5.4.5 Adhesive discs of Tree frogs

5.4.6 The spade of Pelobates

5.4.7 Respiratory adaptations

5.4.8 Allantoic gills in embryos

5.4.9 Adaptations in the male for pairing

5.4.10 Adaptations for Protecting the Young

5.4.11 Convergent evolution in tree frogs

5.4.1 Metamorphosis

In the species, such as newts, frogs and toads, the young animal is hatched in the water as a larva, known as a tadpole. At the time of hatching the tail is still short, the mouth is indicated by a rhomboidal depression, but it has no opening, the anus is formed, there are branchial or gill-arches visible as transverse ridges, but no gill clefts. On the first and second branchial arches on each side are small branched external gills. On the ventral surface of the head behind the mouth is a transverse crescentic groove bounded by ridges; this is the adhesive organ commonly but erroneously called the sucker; it has no muscles of suctorial action, but consists of cutaneous or skin glands, producing a sticky secretion which enables the larva to attach itself to the surface of leaves or other objects. After hatching it divides into two separate parts and at an early stage of larval life disappears altogether. When the mouth is formed it is not provided with true teeth, but has prominent lips which are marked with transverse grooves and the ridges between these grooves are developed into papillae; on the outer margin and more internally into several transverse series of minute horny teeth formed by the external horny layer of the epidermis. The jaws also within the lips are covered by a horny beak composed of similar teeth closely joined together.

Four gill-clefts are formed and a third pair of external gills grow out on the third pair of gill-arches; the tail grows long and develops a median vertical fin-membrane. The paired limbs are entirely wanting. This structure characterizes what may be called the first stage of larval life. After about a week in the frog and its allies the second stage begins in which the external gills disappear and a transverse flap of membrane grows backwards from the hyoid arch and covers up the gill-clefts. This membrane is known as the "operculum" or "opercular fold". In the frog the posterior edges of the opercular folds unite with the skin of the body behind the gill-clefts leaving only a small opening on the left side. This aperture is sometimes called the spiracle, but it does not of course correspond to the spiracle of the shark-tribe which is represented in the frog by the tympanic chamber or ear-cavity; the latter communicates with the throat by the Eustachian aperture but is closed externally by the tympanic membrane. In the tadpoles of the Aglossa, *Pipa* and *Dactyletkra*, there are two "opercular apertures" one on each side, and in the Discoglossidae the two lateral apertures unite into one which is ventral and median. In the tadpoles of the Urodelathe operculum is always small and rudimentary, it extends only a short distance from the hyoid arch, and does not cover the external gills; the latter are more dorsal in position than in the Anura and the opercular fold is sometimes continuous with the base of the first gill. The two opercular folds meet below the throat and often persist in the adult as a transverse ridge of skin known as the gular fold. Considering the structure of the gills, or branchial organs, in Dipnoi (lungfish) and Crossopterygian (fringe-finned) fishes from which the Amphibia must have been originally descended, the rudimentary operculum in Urodela cannot be regarded as a primitive feature, but we must conclude that in the course of evolution the ancestral operculum has undergone divergent modifications in the Urodela and the Anura, in the former having been reduced, in the latter forming a closed branchial chamber with the exception of the small apertures above described.

Considerable difference exists between the Anura (frog tribe) and Urodela (newts and salamanders) in the metamorphosis with respect to the limbs and tail. In the Anura the fore-limbs are at first concealed beneath the operculum, that on the left protruding through the branchial aperture, that of the right side actually bursting through the operculum. The hind limbs grow much faster than the fore-limbs; although they begin to develop at the same time the hind pair are at first alone visible because the anterior pair are concealed by the operculum. The limbs develop directly, first as cylindrical outgrowths and then becoming segmented into the parts of the terrestrial limb; they show no trace in their earlier stages of the structure of the fin of a fish. In the frog-tribe, when the hind limbs are well developed but small, the tail begins to grow smaller, but is not entirely absorbed when the frog leaves the water and becomes terrestrial. In the Urodela the only change which takes place in the tail is the partial or entire disappearance of the fin-membrane. Ih newts, which are species of the genus *Triton*, the fin-membrane is enlarged every season when the animals resume aquatic habits, and is reduced when they leave the water and lead a terrestrial existence. In Triton the fore-limbs appear much earlier than the hind. In the larvae of *Triton vulgaris* and other Salamandridae there are a pair of tentacle-like organs situated just above the angle of the jaw on each side. They are little rods developed from the skin and have thickened ends; they are movable and are used chiefly to prevent the head from sinking into the soft mud at the bottom of the water; they are known as balancers. These organs occur also in Ambystoma, and in the larvae of the African clawed toad, *Xenopus* they are long, tapering and conspicuous. It is possible that the retractile tentacles of the Apoda correspond to these balancers.

The lungs develop as a ventral "out-pocketing" of the throat behind the gill-clefts, dividing posteriorly into two sacs. Each receives an artery from the dorsal part of the fourth arch, and as the gill-clefts close up, the first arch forms the carotid arch sending blood to the head and brain, the second on each side remains connected with the dorsal aorta, the main artery of the trunk, while the third arch disappears altogether. In some cases and under abnormal conditions the transformation is postponed and the animal retains its gills and continues its aquatic respiration throughout life. The most remarkable instance of this is the celebrated axolotl. This creature is abundant in the lakes near the city of Mexico, it was found there by the Spanish conquerors of the country and was then and is still habitually used as food.

5.4.2 Axolotls

The axolotl has three pairs of large external gills and four pairs of gill-clefts, the two pairs of limbs are well developed but rather slender, with five toes on the hind-foot, four on the fore-foot.

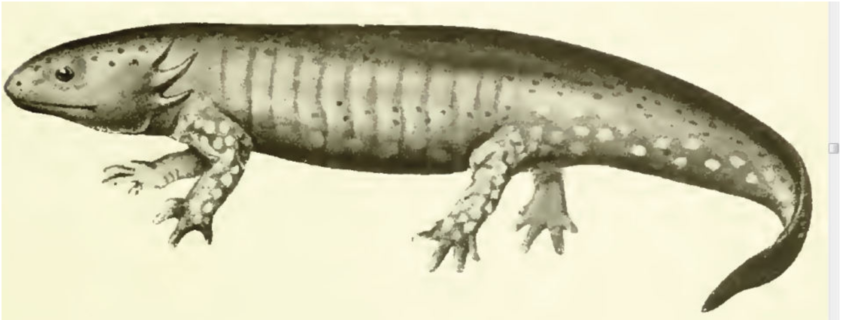


Fig.: Intermediate stage in metamorphosis of Axolotal

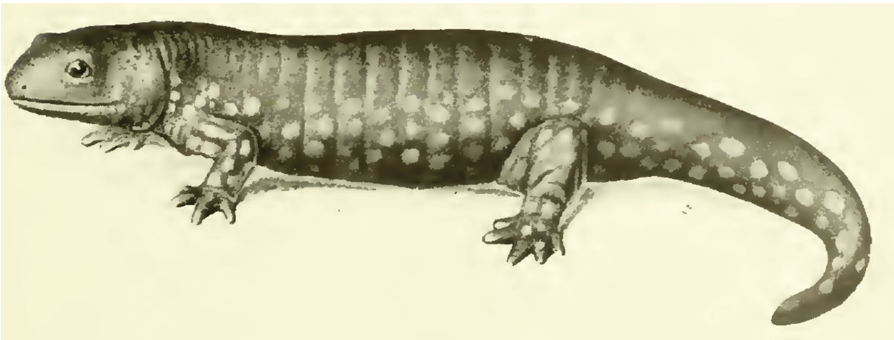


Fig.: Fully developed *Amblystoma tigrimnn*

The head is depressed with a somewhat pointed snout; the tail is compressed from side to side and has a broad fin-membrane which extends dorsally along the body almost to the neck. These axolotls reach a length of eight or nine inches or even sometimes as much as a foot; they pass their whole lives in the water and reproduce in the water-breathing condition. Originally therefore they were regarded as "Perennibranchiata" and under the name *Siredon pisciforme* were placed in the same division as *Proteus* and *Necturus*. Axolotl are sexually mature in the gill-breathing condition, but several undergo metamorphosis and became entirely terrestrial. It was thus shown that the axolotl was capable of changing into an air breathing form which would naturally belong to the family Salamandridag. It was therefore a probable conclusion that this terrestrial form was the species from which the axolotl was descended and that the latter was a larval form which for some reason had become permanent and sexually mature, but still retained the power of undergoing metamorphosis under suitable conditions. Actually this gill less creature axolotls were specimens of a well-known Salamandrine species named *Amblystoma tigrimnn*.

Neoteny occurs in a large number of species and he distinguished between cases in which the metamorphosis is only temporarily postponed, the tadpoles passing through the winter in the larval condition and passing through their metamorphosis in the following summer, and cases in which the animal becomes sexually mature in the gill breathing condition. It is a curious fact that examples of the first kind occur in Anura and of the latter only in Urodela. The following are the species in which neoteny has hitherto been observed: I. Partial Neoteny. *Pelobates fuscus, Bombinator pachypus, Pelodytes punctatus, Alytes obstetricans, Hyla arborea, Rana esculenta, Rana temporaria, Bnfo vulgaris and B. viridis.* II. Total Neoteny: *Triton vulgaris, T. alpestris, T. cristatus, T. boscai, T. waltlii, and Amblystoma tigrinum.*

5.4.3 Protective and warning coloration

The elements of coloration in the skin of Amphibia, especially of Anura are similar to those of the skin of fishes. They are of three kinds, black chromatophores, coloured chromatophores and bodies composed of reflecting substance called iridocytes. Diffuse pigment also occurs and is usually yellow; the coloured pigment ranges in tint from yellow to red; as in fishes there is no green pigment, although tree frogs, like many fishes, exhibit a distinct green colour. Amphibia, like fishes, have the power of changing their colour in accordance with their surroundings, a power which has been lost almost entirely by reptiles, birds and mammals; it is, however, retained by some reptiles and is highly developed in the chamaeleons. The change of colour with change of surroundings is conspicuously seen in tree-frogs; when they sit on green leaves they are green, when they sit on brown bark they become brown. When *Hyla arborea* in a box with moss; were unpacked they were of a dull greenish-grey, when placed in an enclosure with freshly cut branches of a lime tree, and if they were sitting on the dark brown branches, had turned to a light brown colour mottled with darker patches; this is an instance of variable protective resemblance.

Fig.: *Hyla arborea*, A Tree-frog:

An example of Protective Coloration

The colour elements in this frog are a mosaic of polygonal iridocytes or interference cells and black branched chromatophores. When the black chromatophores are expanded, the frog is of some shade of brown, when they are contracted the light passes through the yellow colour and then, being reflected from the white reflecting substance, by interference becomes green. In frogs the chromatic function depends chiefly on sensory impressions received by the skin, while that of fishes depends on the eye.

Few species show warning coloration, that is conspicuous colours and markings associated with some poisonous or dangerous quality, and bold, fearless habits. One of the best examples of these characteristics is afforded by the spotted or fire salamander which is black with large irregular patches on the back and limbs of bright yellow or orange, the colours we see in a wasp; the lower surface is bluish-black. The animal is poisonous, producing a poisonous secretion from the glands in a thickened patch of skin behind the tympanic membrane on each side; there are also glands along each side of the back and on the flanks. The secretion is a milky white liquid which if it accidentally touches the human eye causes burning pain and inflammation; a few drops of the poison in the stomach or injected into the blood of a small animal are fatal. The parotid glandular patch is present in most of the Anura but the development and the activity of the poison vary greatly. The toad is much more poisonous than the frog, the latter being eaten by many other animals, the former being usually avoided, and if a dog bites a toad the poison causes great pain from its effect on the mucous membrane of the mouth. Among the Anura *Bombinator zgneus*, called the fire-bellied toad, has the same association of warning colours and poisonous qualities as the spotted salamander. In *Bombinator* the upper side is dark grey or nearly black and the warning colour is on the lower side which is black with large red or orange-red patches.

Fig.: *Bombinator zgneus*, The Fire-bellied toad,

Showing Warning Coloration and Warning Attitude

It has often been stated that these toads when disturbed on land turn over on their backs, but this is not the case, the animal remains in its normal position but turns its limbs over its back so as to show as much as possible of their lower surface and of the red markings of the belly. *Dendrobates* in South America offers an example of a tree-frog which instead of being protectively coloured like the Hylidae is warningly coloured and poisonous: some are black and white, others black and yellow or black and red, one species is red with small dark marks on the back, and black legs. A very curious use is made in Brazil of the poison secreted by the skin of *Dendrobates tinctorius*: the feathers of the head and neck or other parts of green Amazon parrots are plucked out and the skin is then rubbed with the poison or simply with the skin of the living frog and as a consequence the new feathers which grow are yellow instead of green, so that artificially coloured parrots are produced, which are valued as curiosities. In Colombia the Indians are said to use the same poison for poisoning the tips of their arrows, which are employed especially for shooting monkeys. The poison acts on the heart and central nervous system.

5.4.4 Adaptations for Locomotion

The contrast between the lizard-like shape of the newts and their modes of locomotion and the structure of the frog or toad, adapted for leaping and destitute of tail, is very conspicuous. As in the original Amphibian the limbs were evolved from the fins of fishes by adaptation to terrestrial conditions, so in the Anura the elongation of the hind limbs for leaping and the loss of the tail could scarcely have been due to the requirements of locomotion in the water. At the same time both the Urodela and the Anura usually pass part of their lives in the water, and their different types of structure are adapted to both aquatic and terrestrial movement. In the Urodela when the animals are either temporarily or permanently aquatic the tail is furnished with a membranous fin; in the common British newts this fin diminishes greatly when the animals leave the water after the breeding season; in the salamanders which are almost entirely terrestrial, the fin is absent, as it is also in the genus *Amblystoma*. In some species there is a membrane along the dorsal edge of the body as well as on the tail; this is known as the crest and is confined to the males as in *Triton cristatus* and *T. vulgaris*. This crest occurs in those species which perform a lengthy courtship in the water, while in forms that practice an amplexus, such as *Triton waltlii*, *T. asper*, etc., there is no crest. On the hypothesis of the inheritance of acquired characters this difference is explicable, the crest of the male being attributed to the effect of the pressure of the water in the active vibratory movements which the male continues for hours or days in presence of the female.

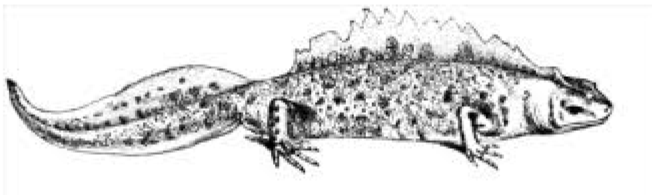


Fig.: *Triton cristatus*

In the Anura the hind limbs are elongated in adaptation for jumping as in many other animals, such as the kangaroo, the grasshopper, and the flea. The segments of the legs are all elongated, including the tarsus (ankle) and the attachment of the pelvic girdle to the vertebral column is shifted far forward so that the number of presacral vertebrae, the vertebrae in front of the sacrum is reduced to nine or less. The effect of this is to place the application of the force of propulsion near to the middle of the body and so give greater efficiency. The hind toes are usually united by a web or membrane for the purpose of swimming in the water, the frog swimming with its hind feet, while the newt swims with its tail. In some cases the toes of the fore-foot are also webbed, but this seems not to be an adaptation for swimming, as in the most completely aquatic species such as the Aglossa *Pipa* and *Xenopus* the front toes are free, and the webbed condition of these toes occurs in arboreal species. In *Nectophryne*, a genus of the Bufonidae occurring in Africa, India and the East Indies, the toes of both fore and hind limbs are webbed, and the species of this genus are believed to be arboreal, while in the allied species *Nectes subasper* of

Java the fore toes are free and the animal is aquatic. Perhaps the webbing of the fore toes is always adapted for supporting the animal in the air during its leaps in the trees, as in *RhacopJiorus pardalis* and other species of the same genus. *R.pardalis* was first described by A. R. Wallace in his *Malay archipelago*. It was brought to him by a Chinese workman who said he saw it come down in a slanting direction from a high tree as if it flew. The feet are not only fully webbed, but much enlarged, so as to present a greater surface; this species lives in the forests of Borneo and the Philippine Islands; its total length is two and a half inches.

5.4.5 Adhesive discs of Tree frogs

In the arboreal Anura the ends of the toes are expanded into adhesive discs by which the animal is able to attach itself firmly to the surfaces of leaves. It is a well-known fact that if a specimen of the common tree-frog, *Hyla arborea*, is thrown against a pane of glass it sticks to the glass almost like a piece of putty, adhering by these discs. In the majority of cases, as in the Hylidae and in *Ceratophyla* among the Cystignathidae, the terminal phalanges which bear the discs are claw-shaped and bent upwards, their bases being enlarged.

Fig.: Tree frog: adhesive discs

Between the terminal and the penultimate phalanx is a cartilaginous disc which projects ventrally and helps in the formation of the pad, which however is chiefly carried by the terminal phalanx. When not in use, as for example when the frog is sitting on a rough stone, the pad is round and turned upwards, and the phalanx is seen as a slight ridge on its upper surface; when the pad is adhering it is flattened and the phalanx is sunk into it. The pad contains unstriated muscular fibres the contraction of which produces one or more longitudinal grooves on the lower side. The adhesion of the discs is due not to sucker-like action, but merely to the attraction of two surfaces in close contact assisted by the viscosity of the secretion of the glands present in the skin of the discs. In *Rhacophorus* among the subfamily Raninae the terminal phalanges which bear the adhesive discs instead of being claw-like are bifurcated, and in the subfamily Dendrobatinae, which are also Ranidse modified for arboreal habits in South America and Madagascar, the terminal phalanges are T-shaped. Within the limits of South American Cystignathidag we find similar differences in the shape of the terminal phalanges which carry the adhesive discs; in *Centrolene* they are bifurcated, in *Hylodes*, *Plectromantis* and others T-shaped. Possibly these differences may be due to the different positions in which the toes are habitually held in the different forms; it would seem that the discs in Hylidae are developed at the base of the terminal phalanges, where the bones are bifurcated on the under sides and where the bones are T-shaped on the tips of the toes.

5.4.6 The spade of Pelobates

An interesting adaptation to the habit of digging occurs in the spade-footed toads of Europe, *Pelobates* and *Scaphiopus*. The inner tarsal (ankle) tubercle which occurs in Anura generally is developed into a curved oblique ridge covered with a hard horny sheath with a sharp edge; this organ is used as a spade or shovel with which the animal digs its way into soft soil, especially into sandy ground. The loose sand falls on the toad as it shovels away that which is underneath and so it sinks backwards into the ground quite rapidly and remains buried in the daytime, emerging at night in search of food. This is one of the cases which to a Lamarckian seem inexplicable except on the theory of the inheritance of the effects of external stimulations. We can scarcely doubt that if the feet were used for digging as they actually are used, the friction would cause a thickening and cornification of the epidermis at the part most affected, and if such effects were inherited the organ as it exists would be produced. The opposing view is that although in the individual the epidermis would be thickened and hardened, yet the evolution of the structure was due to the selection of spontaneous variations which were independent of the friction due to the act of digging.

5.4.7 Respiratory adaptations

The most surprising fact concerning the respiratory organs of Amphibia is the absence of lungs as well as gills in certain species of Urodela. These species all belong to the family Salamandridae, in which the gill and gill-clefts are normally lost in the adult condition. The lungs may be entirely absent or reduced to a vestigial condition of no functional value. All the Desmognathinse and Plethodontinae, so far as known, are lung less and also *Amblystoma opacum* and *Salamandrina perspicillata*. With the disappearance of the lungs are correlated certain changes in the circulatory organs: the pulmonary veins are naturally absent and the left auricle is much reduced in size and not completely separated from the right, so that the heart has practically only two chambers, one auricle and one ventricle, as in a fish. In all Amphibia the skin is an important organ of respiration and we know that in the hibernating condition the animals depend on the skin alone for the little oxygen which is required. According to one investigator the skin is the respiratory organ in the lung-less salamanders, but others maintain that the action of the skin is unimportant and that the function is performed by the buccal cavity and pharynx. In *Autodax lugubris*, a lung less species which is common in California and which never enters the water, the throat vibrates rapidly, from 120 to 180 times per minute, drawing in and sending out air; since a frog breathes by forcing air into its lungs, raising the floor of the mouth while the external nostrils are closed, it is easy to understand that the same action carried on with the nostrils open would only drive air in and out of the mouth. In this case the lungs would no longer be used and would therefore in the course of evolution diminish and at last disappear or become vestigial and functionless. We see then that the loss of the lungs may have been caused by a very slight change in the respiratory movement. In *Spelerpes porphyriticus*, which lives in the Alleghany mountains of North America, that the respiration by movements of the throat was very limited, most of the oxygen required being absorbed through the skin which is very moist and slimy. This species is found only in wet places and is very sensitive to drought; when the skin becomes dry the animals show signs of suffocation and great discomfort.

5.4.8 Allantoic gills in embryos

The respiratory adaptations in the larvae especially in those whose development takes place within the egg, not in the free state, are remarkably varied and interesting. It is curious to compare the various modes in which the larval stage is modified in Amphibia with the embryonic adaptations of reptiles, birds and mammals. In all these three classes the embryo is always provided with oxygen by means of the allantois, which is an outgrowth of the hind-gut. The Amphibia, on the other hand, although they possess in the urinary bladder which opens into the cloaca an organ answering to the allantois of the embryos of higher vertebrates, make no use of this structure as a respiratory organ. In the Californian newt, *Autodax lugubris*, in which the whole of the metamorphosis takes place within the egg, the external gills, instead of being branched or fringed, form on each side a broad three-lobed membrane of which the outer surface is applied to the inner surface of the egg capsule, in this respect resembling the allantois of the higher classes. In *Plethodon cinereus*, on the other hand, in which also the gilled stage is passed within the gelatinous capsule of the egg, the gills are long and branched. In the viviparous *Salamandra atra* the long gills of the embryo not only serve for respiration but also in the later stages of development absorb nourishment from the walls of the uterus, thus performing another

function of the allantois which in the Mammalia is performed by the allantois. Somewhat similar allantoic gills, as they have been called, occur in the embryos of some species of *Nototrema*, in which the whole development is passed through in the egg. In this case the gills envelope the embryo and are only connected to the gill-arches by narrow stalks. In other cases again, as in *Hylodes martinicensis*, in which also the development takes place within the egg, the gills and clefts have been lost altogether, and the organ of respiration of the embryo is the large and vascular tail. It may be pointed out here that when the tadpole is free and active in the water it is properly called a larva, but when the tadpole develops in the egg with more or less modification it becomes an embryo. All these different embryonic adaptations in Amphibia may be regarded as partially successful experiments as compared with the one method which, originally adopted by one group, has given rise to the reptiles, and has persisted with modifications in the birds and mammals which arose from them. This method consisted in the development of a large yolk, foreshadowed in some of the Amphibia, the formation of a tough usually calcareous egg-shell and the development of the amnion and allantois. The amnion is a growth of the outer membrane covering the yolk-sac, a fold of the membrane being formed which grows up and encloses the embryo dorsally. It is perhaps possible that the large yolk was the original cause in the development of both allantois and amnion, for the increase in the size of the yolk-mass necessarily involves a distension of the ventral region of the embryo, and an extension of the body cavity ventrally: into this enlarged body-cavity the allantois is able to grow, and it may have been the growth of the allantois which originally pushed the amniotic fold before it.

5.4.9 Adaptations in the male for pairing

The Amphibia present some interesting cases of adaptation in relation to the fertilization of the eggs and the protection of the young, that is to say, in structures related to the sexual and parental instincts. An amplexus of the sexes takes place the male is distinguished by modifications of the parts which are used in holding the female. A typical example of this occurs in the common frog, the male having a swollen tubercle on the inner side of the fore-foot covered with black skin, while the muscles of the fore-leg and the leg generally are larger and stronger than those of the female.

Fig.: Common frog: amplexus

(male modified for holding female)

The tubercle is pressed into the flanks of the female and the greater strength of the limb muscles enables the male to hold the female with sufficient power. The sexual instinct in male frogs is so great that occasionally they fix on to fresh-water fishes and blind them by pressing the tubercles into their eyes. These secondary sexual characters develop to a maximum in the breeding season and diminish very much afterwards; they are therefore sometimes termed nuptial organs. It has been recently shown that their development in the male and absence in the female depends on a chemical secretion produced by the testis and present in the blood. In all Anura which practice an embrace similar to that of the common frog there are similar modifications of the anterior limbs. In Urodela various modes of amplexus occur with corresponding modifications of structure. In *Diemyctylus viridescens*, an American newt, the male clasps the female with his hind legs either just before or just behind her fore-legs, and the hind legs in the male are larger than in the female, and in the breeding season hard rough black warts are developed on the inner surfaces of these legs. In the Pyrenean newt, *Triton asper*, the male holds the female by twisting his tail round the hinder part of her body and the part of the tail used in this way by the male is larger and more muscular than in the female.

5.4.10 Adaptations for Protecting the Young

The most extraordinary of the adaptations for protecting the young are the dorsal pouch in *Nototrema* and the united and enlarged vocal sacs in the little

Rhinoderma darwini. In the former case the pouch is present only in the female and the eggs are probably introduced by the male. In *Rhinoderma* the brood sac is confined to the male and it is difficult to explain the origin of such a condition except as the result of the distension of the vocal sacs by the eggs. The male apparently developed the habit of taking the eggs into the mouth and thence they passed into the vocal sacs. The sacs are united, but they still open by two apertures into the mouth as in other Anura. When empty the sac only extends back to the pectoral region, but when filled with eggs it reaches to the groins and upwards to the dorsal surface, a truly extraordinary modification, sacs belonging to the mouth cavity acting as a brood-pouch and extending beneath the skin over the greater part of the body. The vocal sacs themselves in ordinary cases are at the same time an adaptation and a secondary sexual character; they serve as resonators to the voice which is produced by the larynx and in the breeding season the males are continually expressing their sexual excitement by their loud croaking, though whether this music has any effect in promoting the satisfaction of the sexual instincts may be doubted.

5.4.11 Convergent evolution in tree frogs

Animals of different lines of descent may become adapted to similar conditions of life, and thus, although descended from different ancestors, come to resemble one another to a greater or less degree. Where the structure was originally fundamentally different the resemblances are only superficial and the essential differences are easily distinguished, as for example, when we compare a bat and a bird, or a whale and a fish. When on the other hand, the forms in question are originally somewhat closely related, and belong to the same group, adaptive convergence may produce a much greater similarity of structure, so that it is often difficult to distinguish such similarity from true affinity or community of descent. This is well illustrated by the Anura adapted to arboreal life, commonly known as tree-frogs. The arboreal species all agree in the possession of adhesive discs on the toes, which are necessary to enable them to cling to the foliage, but when we examine the internal anatomy we find that they often differ greatly from one another, and show affinities to different groups and to other species which may be aquatic, terrestrial, or burrowing. Although the discs have a similar structure and act in the same way, the structure of the terminal claws which support the discs differs considerably, as we have already seen, and the internal anatomy, such as the structure of the pectoral girdle, may be very different in forms equally arboreal. Thus the Hylidae belong to the Arcifera and are therefore more closely related to the toads than to the true frogs, *Hylodes* belongs to another group, the Cystignathidae, and the Dendrobatinae are arboreal modifications of the true frogs or Ranidae. Tree-frogs are therefore not all related together, but those of each region are most closely related to the other Anura of that region.

5.6 Extinct Amphibians

5.5.1 Early Amphibians

The earliest of the amphibians belonged to the ichthyostegid group from the uppermost Devonian of Greenland. This kind of animal is imagined as looking much like a salamander.

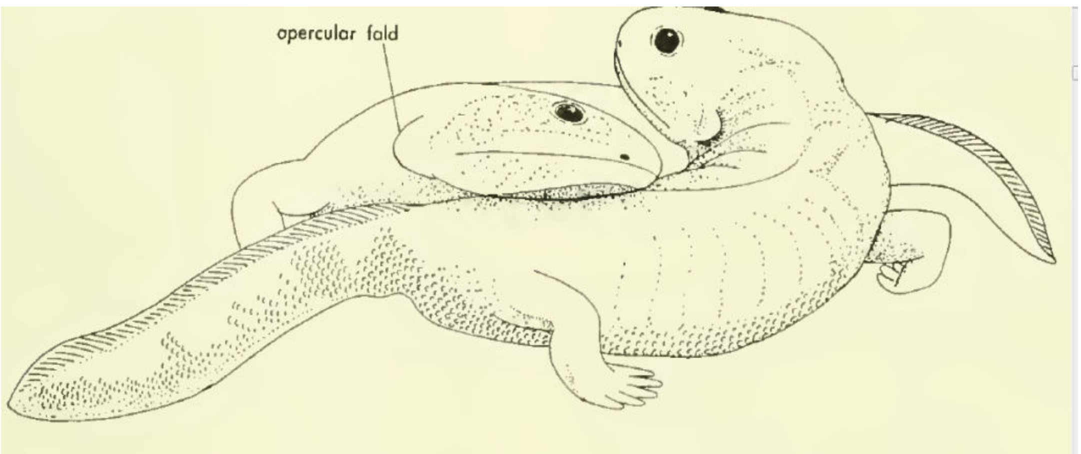


Fig.:Devonian Amphibian Icthyostega

Salamander-like organisms are known from the Upper Pennsylvanian; these are the branchiosaures. The branchiosaurs are small in size. They went through a period with internal and external gills followed by a period with only external gills. As adults they were lung breathers. These small animals have been viewed as the larvae of larger forms, but, if not adults themselves, they approximate the adult form of the ancestral amphibian better than the larger forms. At least some of the genera of this group agree with the living forms in having four digits on the forelimb; these are *Amphibamus* and *Branchiosaurus*.

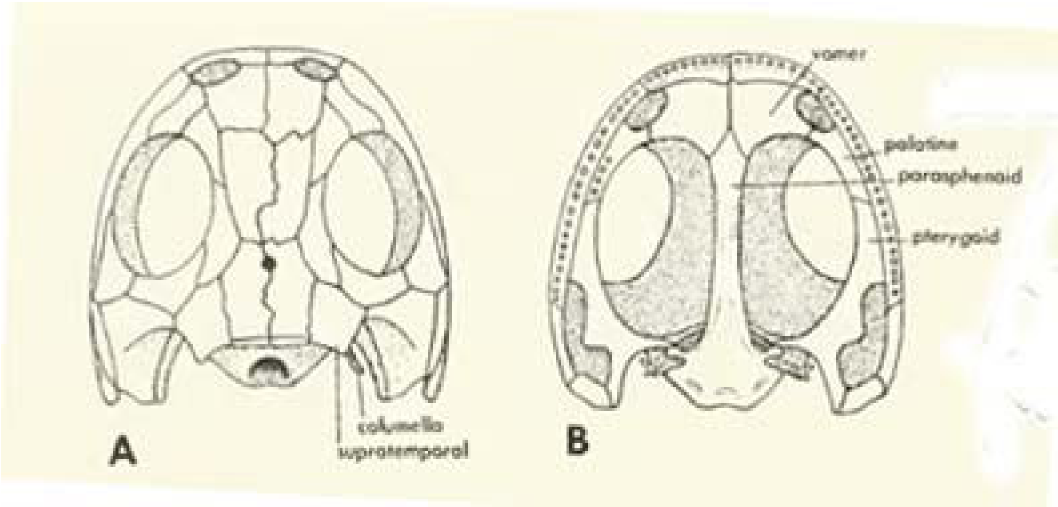


Fig.:Fossil Amphibian Branchiosours and *Eogyrinus*

5.5.2 Extinct Amphibians

Some of the later labyrinthodonts were very large with proportionally larger heads and shorter tails. They are assumed to be modified in structure as compared with the primitive fish-like form.

The earliest known terrestrial vertebrates provided with two pairs of five-toed, obviously terrestrial limbs, are the animals known from the structure of the teeth in some of the most typical forms as Labyrinthodonts, from the bony covering of the head as Stegocephali. In studying the remains of the skeletons of these extinct vertebrates the question arises whether they can be distinguished from reptiles, especially from the reptiles of the same or slightly later periods. It is impossible to avoid the conclusion that the existing Amphibia as well as the earliest reptiles were derived from the Stegocephali: the latter are distinguished by the possession of two occipital condyles or none at all, by the structure of the vertebrae, and by the primitive characters of the skeleton to which that of the Apoda and Urodela is evidently allied. It is usually stated that the whole of the dorsal surface of the skull is covered or roofed over by dermal bones, which means that in these primitive Amphibia the flat or membrane-bones of the skull were still in the condition of dermal scutes, as in the primitive bony fishes such as the African fish *Polypterus* and the lung-fishes. Dermal bones, or scales, were also present in many types of the group on the body, either on all parts or only on the lower surface, but some were destitute of such structures. The fossil skeletons naturally do not throw much light on the condition of the gills or lungs, but in the Branchiosauri, one of the sub-orders, representatives of which are found in the Carboniferous and Permian formations, the young specimens show very distinct gill-arches with numerous nodules and denticles on them; there can be little doubt that these arches carried functional gills like those of the aquatic larvae of existing Amphibia. *Branchiosaurus salamandroides* is found abundantly in the Permian beds of Europe and specimens are found of almost every size and stage of development from larvae of three-quarters of an inch to adults of two and three-quarter inches. Gill-arches and gills seem to be absent in the adult so that in all probability this form at least went through the characteristic Amphibian metamorphosis. In the Carboniferous strata are found skeletons of another sub-order in which the body was long and snake-like, without any limbs; in these gill-arches have been recognized behind the head and these carry skeletal rods supposed to have been the supports of external gills which were retained throughout life; it has been suggested that these were the ancestors of the existing Apoda, but no connecting forms from intermediate periods are known. In the Carboniferous are also found *Keraterpeton* and *Urocordylus*, animals shaped like a newt, with a ventral armour, and about a foot in length; these had terrestrial limbs but traces of gills or gill arches have not been found in them.

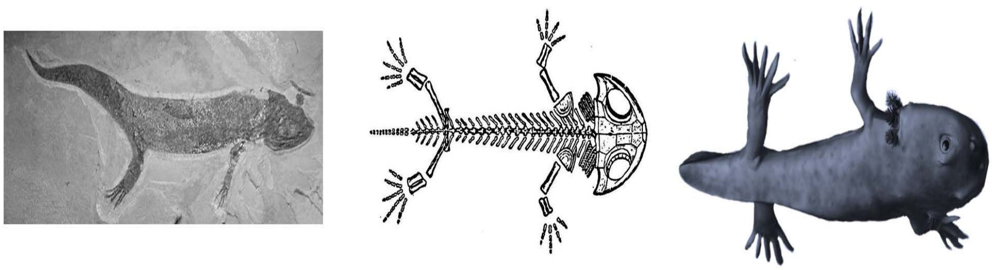


Fig.:*Branchiosaurus salamandroides* (Fossil, Skeleton diagram and Restoration)

In another Order of Stegocephali we have *Archegosaurus*, represented by many well-preserved skeletons which reach a length of four or five feet. These have well-developed terrestrial limbs with four toes in front and five behind; the enamel of the teeth is much folded in the Labyrinthodont fashion; young specimens show traces of gill-arches and the surface of the bones of the skull is marked with grooves which probably contained dermal sense-organs like those of fishes. *Archegosanrus* occurs in the lower Permian of Germany. The third Order of the sub-class, called Stereospondyli, contains the most highly developed Stegocephali; *Labytinthodon* itself is one of the latest genera; it occurs in the Upper Triassic of Warwickshire. Some of these creatures were of gigantic size; the largest called Mastodonsaurus, from the Triassic of England and Germany, had a skull which was nearly a yard in length. No evidence of young or larvae with gills has been obtained in connection with these later Labyrinthodonts.

5.5.3 Systematic Position of extinct Amphibia

The class Amphibiaconsists of subclasses Stegocephali (extinct) and Lissamphibia (living). Stegocephali had cranial roof with dermal bones, two supraoccipitalia, two postorbitalia, two supratemporalia with interparietal foramen. They were tailed and extinct in Carboniferous, Permian, and Triassic epochs. The subclass Stegocephali further divided into orders Labyrinthodonti, Temnospondyli and, Lepospondyli.

5.7 Parental Care

The different modes of protection will be made clear by the following synoptic arrangement:

5.6.1 Protection by means of nests or nurseries

- 5.6.1 .1 In enclosures in the water
- 5.6.1 .2 In holes near the water
- 5.6.1 .3 In nests, on trees or rocks, overhanging the water
- 5.6.1 .4 In a transparent gelatinous bag in the water
- 5.6.1 .5 On trees or in moss away from the water

5.6.2 Direct nursing by the parents

- 5.6.2.1 Tadpoles transported from one place to the other by the male
- 5.6.2.2 Eggs protected by the male, who covers them with his body
- 5.6.2.3 Eggs carried by the parent
- 5.6.2.4 Eggs retained in the uterus (viviparous species)

5.6.1 Protection by means of nests or nurseries

5.6.1 .1 In enclosures in the water

A large tree-frog (*Hyla faber*) known in Brazil as the "Ferreiro" (= smith) from its peculiar voice, sounding like a mallet slowly and regularly beating upon a metal plate, protects its progeny by building basin-shaped nurseries in the shallow water of the borders of ponds. The mud is scooped out by the female to a depth of three or four inches, and with the material thus removed a circular wall or parapet is built which emerges above the surface of the water. The eggs and early larvae, which do not depart from the normal type described above, are thus protected from the attacks of many aquatic insects, fishes, or other batrachian larva;, at least for a time, for it is not unusual for heavy rains to destroy the walls of the enclosure and thus to prematurely release the larvae.

5.6.1 .2 In holes near the water

A still better mode of protecting the offspring during the early stages of development has been adopted by a Japanese tree-frog of the family Ranidae (*Rhacophorus schlegelii*). The male and female in embrace bury themselves in the damp earth on the edge of a ditch or flooded rice-field, and make a hole or chamber, a few inches above water-level; after polishing the walls, oviposition begins. The female first produces from the vent a secretion which, by rapid movements of the feet, is beaten up into froth and afterwards, in the midst of this agglomeration of air-bubbles, she deposits the eggs; the male, who has all along been clinging to her back without taking part in the operation, at once impregnates them. This being accomplished, the pair separate and proceed to make their way out of the chamber by boring a gallery of exit; instead of returning the way they came, the tunnel which they now bore is in the side of the bank and directed obliquely downwards towards the water; and this tunnel will be utilized later by the larvae to gain access to the ditch or pond, in which they will complete their metamorphosis.

The eggs of this *Rhacophorus* are about one millimetre in diameter and absolutely devoid of pigment; the segmentation, though holoblastic, is of a type approaching the meroblastic, as in other Batrachian eggs containing much yolk; the embryo is at first quite distinct from the yolk. Eggs taken from the nest invariably die if put into water. The tadpoles, when leaving the nest, are said to resemble those of ordinary frogs.

5.6.1 .3 In nests, on trees or rocks, overhanging the water

Some tree-frogs, *Phyllomedusa* in South America, *Rhacophorus malabaricas* in India, and Chiromantis in tropical Africa, deposit their spawn on trees, in nests of froth attached to a leaf or to several leaves stuck together, and overhanging a pool. The larvae move with considerable freedom in the frothy mass, and after a few days, having lost the external gills, drop into the water, where they complete their metamorphosis in the ordinary way. As in the preceding forms, the eggs contain much yolk, and are comparatively few in number, *viz*, not over 200.

Nests very similar to the above have been observed in Japan, and ascribed to *Rhacophorus schlegelii*, but this determination requires confirmation. Other species of *Rhacophorus* inhabiting India and Ceylon, produce masses of green frothy spawn which have been found sticking to the walls of wells, per pendicular rocks in quarries, or trunks of trees, in such a position as to allow the larvae to readily drop into the water when strong enough to swim about and procure their food, this being but a simplified variante of the nests which some of their congeners are known to produce.

5.6.1.4 In a transparent gelatinous bag in the water

Another form of nest is that offered by a small Engystomatid from New Guinea: *Phrynixalus biroi*. The large, impregnated eggs, measuring seven millimetres in diameter, and only twelve to eighteen in number, are enclosed in a sausage-shaped transparent common membrane, secreted by the female, which is abandoned in mountain streams. As in *Hylodes*, the whole development takes place within the egg, which the little frog leaves in the perfect condition. No gills have been observed and the large tail serves as a breathing organ whilst the young is in the egg.

5.6.1.5 On trees or in moss away from the water

In several species of the tropical American genus *Hylodes*, small tree-frogs related to *Leptodactylas*, of which the West Indian "Cogni," *H. martinicensis*, is the best known, the eggs are deposited in damp places, under stones or moss or on the leaves of plants, and are of large size. The metamorphosis is hurried through within the egg, and after subsisting on the large yolk-bag, the young frog hops out as an air-breather, with a mere vestige of the tail which was fully developed and so richly supplied with blood that it no doubt functioned as a breathing organ, no gills or gill-slits having been detected.

Another small tree-frog of the family Hylidse, *Hylella platycephala*, from Mexico, is said to lay its eggs in the axils of the leaves of *Tillandsia*, where it undergoes the whole of its metamorphosis.

A large frog inhabiting the Solomon Islands, *Rana opisthodon*, morphologically very similar to our European species of the same genus, also undergoes the whole of its development in the egg and away from water. The eggs, which measure six to ten millimetres in diameter, have been found in the moist crevices of rocks, with the small frog coiled up, in an advanced stage of development, without even a vestige of a tail and differing from the perfect frog only in the presence of several folds of skin on the sides of the belly, the function of which is probably that of breathing organs, like the tail of *Hylodes*, and of a small, hard, conical protuberance at the end of the snout, which, like the well-known egg-tooth of manyhigher vertebrates, is used to perforate the rather tough envelope of the egg.

A curious frog from the Malay Peninsula, *Megalophrys longipes*, of the family Pelobatidze, measuring only sixty millimeters from snout to vent, is believed to deposit its ova in clusters of about a dozen under damp moss or tree trunks, the young emerging in the perfect condition. These eggs are most remarkable as being the largest on record among frogs, their diameter being thirteen millimetres.

5.6.2 Direct nursing by the parents

5.6.2.1 Tadpoles transported from one place to the other by the male

Small South American frogs of the genera *Dendrobates* and *Phyllobates*, not very far remote from the typical Ranids, have been repeatedly observed carrying well-developed

tadpoles on their back. These tadpoles are essentially similar in form and anatomical structure to those of our European frogs, and they adhere to the back of their parent by their sucker-like lips and flattened abdomen. It has been observed in the case of *Dendrobates trivittatus* that the frog spawns in water, and that the free-swimming tadpoles attach themselves to the parent—which, in *Phyllobates trinitatis*, has been ascertained to be the male. It is inferred that the young are thus transported from one pool to another, an excellent plan to adopt in districts where the water may dry off in two or three days.

The frog in which this mode of nursing had been first observed in the Guianas had been determined as *Hylodes lineatus*, but the figure which accompanies its description shows it to have been probably a *Phyllobates* or *Dendrobates*. The true *Hylodes lineatus* has since been observed, in Peru, to lay its eggs under grass far from water, and the young before hatching are perfect little frogs, without even the remains of a tail.

A somewhat similar little frog in the Seychelles, *Sooglossus sechellensis*, also carries its tadpoles. It is found in forests at about 5000 feet altitude, where there is no still water. The eggs are concealed under dead leaves, and the tadpoles, as soon as hatched, place themselves, with the aid of their tails, on the male's back, to which they stick partly by suction, partly by a viscous secretion produced by the parent. The tadpoles are not attached to the parent for transportation to water, but undergo in that position the greater part of their development; no gills .have been found in them, only the usual rudiments of lungs.

5.6.2.2 Eggs protected by the male, who covers them with his body

The eggs of a Papuan frog allied to the *Phiynixalus* mentioned above, *Mantophryne robusta*, are strung together by an elastic gelatinous envelope similar to that known in the European Midwife Toad, and, seventeen in number, form a clump over which the male sits, holding it with both hands. The diameter of these eggs is six or seven millimetres, and on the occasion on which they were observed each contained an embryo with well-developed legs, no gills, and a large tail, the membranous lobes of which are rich with capillary vessels and act no doubt as a breathing organ.

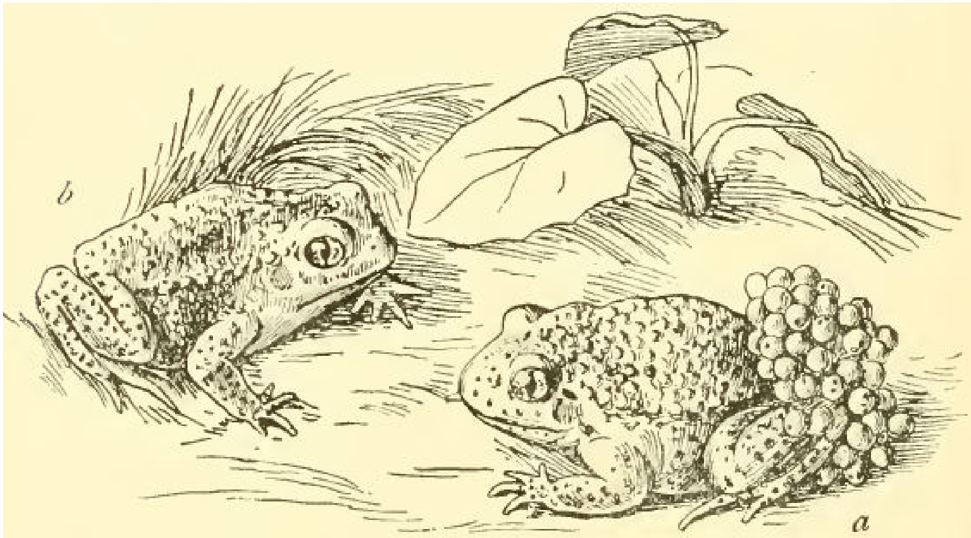


Fig.: Midwife Toad (*Alytes obstetricans*): a. male with eggs; b. female

Here again, as in *Phrynixalus*, the mode of development is essentially the same as in *Hylodes*; like the latter, and unlike the former, it takes place out of the water.

5.6.2.3 Eggs carried by the parent

a. Round the legs by the male

We now come to the well-known example of the Midwife Toad, *Alytes obstetricans*, discovered in France in the middle of the eighteenth century, in the very act which has rendered it famous. For over a century it, with the equally famous Surinam Toad, remained the only known examples of parental solicitude among the batrachians.

Pairing and oviposition take place on land. The male first seizes the female round the waist, and when, after having submitted to a process of lubrification of the cloacal region by rapid movements of his toes, she stretches out her hind limbs, he places his between them, bent at angles at the knees, the tarsi erect and pressed close together, thus forming a receptacle in which the eggs are suddenly extruded. The yellow eggs, as if threaded together by elastic filaments continuous with the gelatinous capsules, form a large mass, two to four layers of about ten eggs, in this receptacle. The very moment the eggs are produced, the male unclasps the waist of the female and shifts his hold to the base of the head; the body then stretched out, but the legs remaining in the same position as before, fecundation commences; it takes place in two or three emissions at short intervals. After a few minutes' rest, the male proceeds to attach the strings of eggs to his legs, by passing the latter into the egg-mass and then folding them against his body. There they remain until hatched. Thus laden, and yet so little impaired in his movements as to occasionally resort again to hymen during the nursing period, and successfully add on a second burden, the male retires to his usual retreat, in a hole in the ground, or between the stones of some old wall, but going about at night in order to feed himself and to keep up the moisture of the eggs, even resorting to a short immersion in the water during exceptionally dry nights. The development in the egg takes about three weeks. At the expiration of this period, he enters the water with his burden; the larvae, in the full tadpole condition, and limbless, measuring fourteen to seventeen millimetres, bite their way through the tough envelope or egg-string, which is not abandoned by the father until all the young are liberated. The rest of the development does not differ from that of other frogs.

The rosary-like string -contains about twenty to fifty eggs, which, considering the small size of the frog (forty to fifty millimeters from snout to vent) are remarkably large, measuring three and a half to five millimetres in diameter. When first laid the eggs are nearly spherical, but they soon acquire a more oval shape. Through the transparent capsules the whole development can be easily followed. An enormously large vitelline sac is present, and the embryo is provided with uncommonly long, unpigmented, branched external gills, one only on each side, which are absorbed and replaced by internal ones before the larvae are hatched,

b. On the back of the female

(i) Exposed

In a Brazilian tree-frog, *Hyla goeldii*, it is the female which takes charge of the eggs, carrying them on the back. How they get there is still unknown, but we may surmise that they are placed by the male. The whole surface of the back is occupied by one layer of twenty-six large yellow eggs, four millimetres in diameter, on which, in the specimen described, the embryos, coiled round the enormous vitelline sphere, can be distinguished with the naked eye. The skin of the back is extended into a narrow fold which borders and supports the egg-mass on the sides, thus suggesting an incipient stage of the dorsal pouch to be described hereafter in the allied genus *Nototrema*.

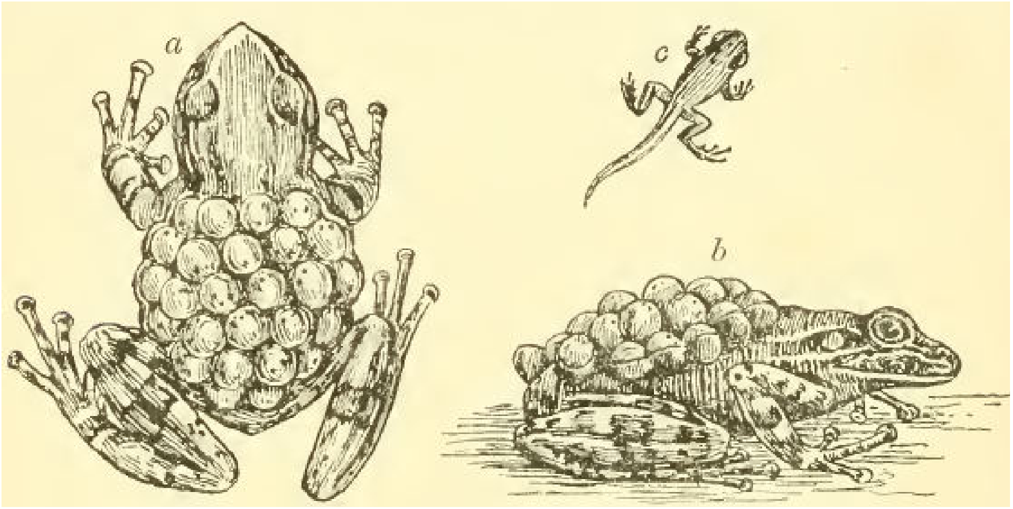


Fig.: *Hyla goeldii*, a tree-frog carrying eggs on its back: a. from above; b. from the side; c. young when hatched

The embryos are much elongate in shape, colourless, with a large flat head, in which the eyes are distinguishable as two black points; no traces of gills are to be seen. The young leaves the egg in the perfect state, but still provided with a longish tail. An allied, but larger, species from British Guiana, *Hyla evansii*, has adopted the same mode of nursing. The eggs, twenty-two in number, measure eight or nine millimetres in diameter.

A frog of the family Hemiphractidae, *Ceratohyla bubalus*, an inhabitant of the Andes of Ecuador, Bolivia, and Peru, also carries its eggs on the back. A female specimen, measuring sixty-three millimetres from snout to vent, has been obtained in Peru, carrying nine large spherical eggs, ten millimetres in diameter, each containing a little frog distinctly visible through the transparent membrane which at this stage constitutes the egg-capsule.

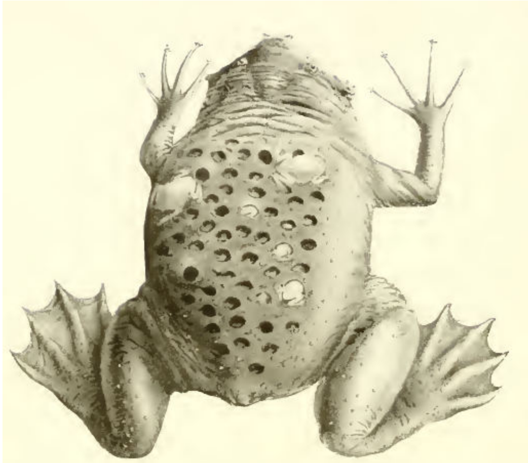


Fig.: Surinam Toad, female

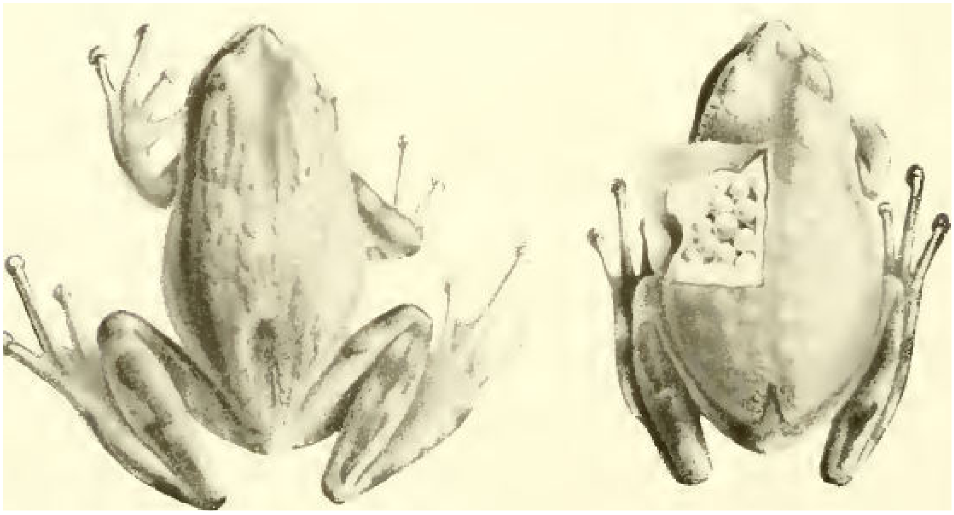


Fig.: *Notorema marsupiatum* with dorsal pouch

half developed same with pouch full of eggs

The little one, with the abdomen, tumid with yolk, turned towards the back of the mother, and the limbs folded against the belly, is connected v/ith the membrane by two string-like cords on each side, proceeding from the throat, such as we shall describe presently in some species of *Nototvema*, and these cords serve to convey the blood, for the purpose of respiration, to the vascular, allantois-like membrane.

(ii) In cell-like pouches

In the preceding examples, the eggs are simply adherent to the back, leaving shallow hexagonal impressions in the skin. In the well-known Surinam toad, *Pipa americana*, an inhabitant of the Guianas and Northern Brazil, the eggs are likewise carried on the back of the mother, but the skin thickens and grows round the eggs, until each is enclosed in a dermal cell, which is finally covered by a lid, believed to be formed by a secretion from the glands of the skin. The eggs, which may number about 100, and measure five to seven millimetres in diameter, develop entirely within these pouches, and the young leap out in the perfect condition, without even a vestige of a tail. External gills exist but are lost at a very early period, and a long tail is present in the embryo.

The Pipa is a thoroughly aquatic Batrachian, and pairing of course takes place in the water. The male clasps the female round the waist. The way in which the eggs reach the back of the female has been observed in specimens kept in the London Zoological Gardens. During oviposition the cloaca projects from the vent as a bladder-like pouch, which is directed forwards, between the back of the female and the breast of the male, and by means of this ovipositor the eggs are evenly distributed over the whole back. How the eggs are fertilized has not been ascertained.

(iii) In a common pouch

Whilst in the *Pipa* each egg is enclosed in an outgrowth of the skin of the female's back, in the South American tree-frogs of the genus *Nototrema* the whole of the brood is sheltered in a common pouch, a structure which, as we have seen, is foreshadowed by the lateral everted fold of *Hyla goeldii*. This dorsal pouch develops only at the approach of the breeding season, and is evolved by the skin of the back forming a horse-shoe-shaped fold on the sacral region, which gradually deepens; the inner wall of the pouch is therefore part of the outer layer of the skin turned inwards. How the eggs are introduced into the pouch is still unknown. In most species of this genus, the opening to this invaginated dorsal fold is small and situated on the posterior part of the back, often so small, when the pouch is distended by the ova, as to simulate a second anal opening. In one species, however, *Nototrema pygmaeum*, from Venezuela, the pouch is formed by two lateral folds, meeting on the middle line of the back, a mere slit separating them when the back is filled with eggs.

In some species, *Nototrema marsupiatum* and *N. plumbeum*, the eggs are large and numerous (about 100), and, as n *Alytes*, only a part of the metamorphosis is undergone within them, the young escaping from the pouch as ordinary tadpoles, with a powerful tail, internal gills, and a beak-like mouth surrounded by a large circular lip. In others, *N. oviferum, N. testudineum, N. fissipes, N. cornutum,* and *N. pygmceum*, the eggs are enormous and few in number (four to sixteen) and in these species the whole development takes place within the pouch, which the young frog leaves in the perfect condition, differing only in size from its parents. Within the pouch, the breathing organs of the larvae consist of a pair of bell-shaped membranes, veined with a capillary network, each connected with the second and third branchial arches by a pair of stringlike filaments. These gills, the form of which can only be seen by floating them in water, form a sort of envelope to the embryo, like an allantois, and may be designated by the term allantoic gills, which has been proposed for the lobate gills of the Tailed Batrachian *Autodax.*

Amphignathodon guentheri, the sole representative of the family Amphignathodontidae, which inhabits the Andes of Ecuador, is provided with a dorsal pouch similar to that of *Nototrema marsupiatum*, but the eggs are still unknown.

c. On the belly, exposed, by the female

The female of the Ceylonese *Rhacophorus reticularis* carries its eggs on the belly, which bears shallow impression when the eggs are removed. These, in the single specimen observed, measuring forty-seven millimetres from snout to vent, were spherical and unpigmented, four to five millimetres in diameter and about twenty in number. Nothing further is known of the breeding habits and development.

d. In the mouth, or in a gular pouch

(i) By the male

One of the most remarkable modes of nursing is that of the Chilian *Rhinoderma darwini*, a small frog of the family Engystomatidse It was first believed to be viviparous, but a more careful examination revealed the fact that the young, about ten to fifteen in number, are sheltered and develop in the gular pouch (the modified vocal sac with which the males of many Tailless Batrachians are provided) of the father, which pouch extends over the entire ventral side.

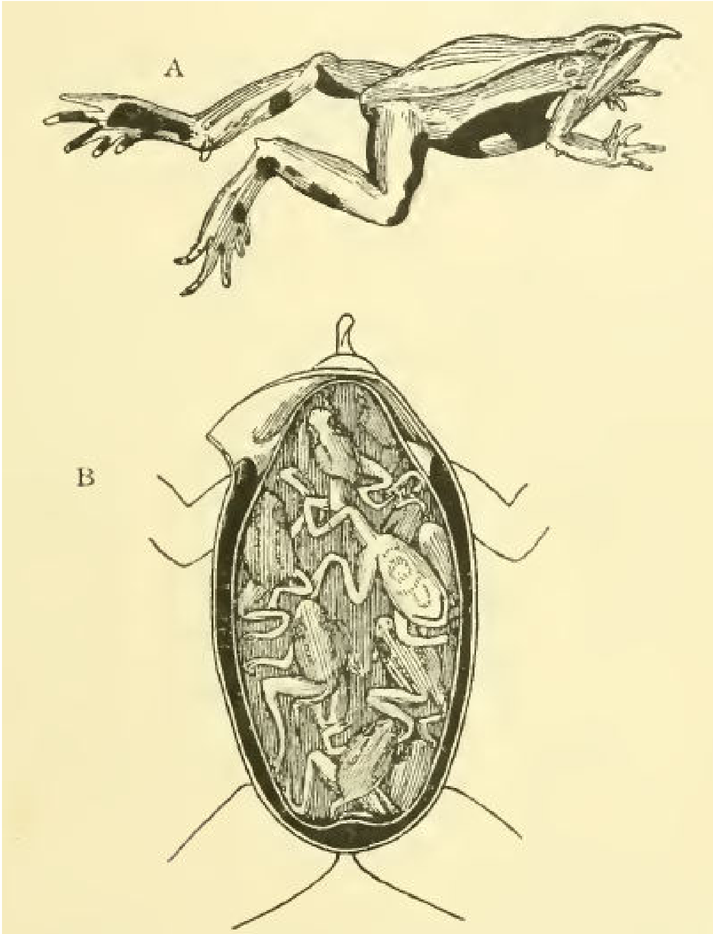


Fig.: A. *Rhinoderma darwini* external appearance; B. A specimen of the same with the gular sac cut open, showing contained embryos.

No gills or other breathing organs have been observed, and the tail, which is never large, is absorbed before the young leaves the paternal pouch. We do not know how the young get into the pouch, but it is highly probable that the male takes the eggs into his mouth as soon as they are deposited and, with the aid of his hands, forces them into the subgular vocal sac which, as usual, communicates with the floor of the mouth by a long slit on each side of the tongue.

(ii) By the female

The female of a West African tree-frog, *Hylambates breviceps*, carries the eggs in her mouth, as do some Silurid and Cichlid fishes. These eggs are large (four millimeters in diameter) and few in number.

5.6.2.4 Eggs retained in the uterus (viviparous species)

Two small East African toads, one referred by Tornier to *Pseudophryne* (*P. vivipara*), the other by me to *Nectophryne* (*N. tornieri*) are known to be viviparous, but no observations have yet been made on them beyond the fact that larvae are found in the uteri.

5.8 Summary

The discussions in the unit are of great value for the students to learn diversity, physiology, morphology and behavior of class Amphibia. The Cold-blooded, amphibians are ectotherms, as they are unable to regulate their own body temperature independently of the temperature of their surroundings. Amphibians are generally small with thin skin permeable to air and water. In general, amphibian reproduction strategy consists of egg-laying and external fertilization of a large number of eggs in a moist or fully aquatic environment. Fertilized eggs develop into amphibian larvae that live part of their lives dependent on an aquatic environment requiring gills and specialized feeding habits. Following a pattern of development unique to amphibians. Amphibian larvae undergo marked changes and metamorphose into a terrestrial form that lives on land. Typically, this metamorphosis is demonstrated by loss of gills, changes in overall appearance, and changes in diet. Amphibians live in diverse habitats, often in large numbers, and play several important ecological roles. Amphibians skin types limit distribution to warm, moist climates.

The systematic classification which clear the specific position of Amphibians in the living world. Amphibians are vertebrates of the taxonomic class Amphibia which is represented by subclasses Labrinthodontia (extinct) and Lissamphibia (living). Modern (living) amphibians appeared in Triassic. Three primary orders of Amphibia within the Subclass Lissamphibia are Caudata (Urodela) - Salamanders; Anura (Salientia) - frogs and toads; Apoda (Gymnophiona) – Caecilians.

Amphibia arose from two choanate-fish sources. Save- Soderbergh and Holmgren derive the Urodela from a source near the Dipnoi and the other amphibians indirectly from the osteolepid crossopterygians. Jarvik derives the urodeles from a porolepiform and the anurans and other tetrapods from an osteolepiform source.

Being tetrapods that facilitate moving about on land. These limbs evolved from the pectoral and pelvic fins. Skin is thin, soft, glandular and magid (lack scales except in the caecilians). Skin of caecilians with scales similar to those of fish. They are both gill and lung breathers, usually gills in the larval stage, replaced by lungs in the adult; cutaneous respiration in many. Three-chambered heart with two atria and one ventricle. Fertilization may be internal or external; egg-layers (oviparous) but may have modifications associated with development. Eggs are anamniotic, does not have a shell but covered with a series of gelatinous layers hatchling in aquatic larval form which breath by gills. Most larvae herbivorous, some omnivorous to carnivorous. Amphibia is specially modified for different ecological conditions which are additive in their survival. With few exceptions, amphibians do not actively care for their young.

Labyrinthodonts ventured on land in pursuit of abundant prey or to escape predators. They soon mastered terrestrial living and became the dominant predators on land in Carboniferous and Permian periods. Large amphibians were wiped out by the Permian mass extinction.

As amphibian include anurans, urodelans and apodans. In all these groups of amphibians show a great deal of parental care. Amphibians show several mechanisms to protect their eggs and developing young ones because they lay few eggs.

5.9 Glossary

- **Advertisement Calls**: Calls used by frogs to define their territories and to attract female frogs during breeding season
- **Aestivation**: A summer version of hibernation. Especially prevalent in very hot dry regions
- **Aggressive Calls**: Males usually make these calls, when other males are trying to occupy their territory or are very close to their territory
- **Amphibian:** A vertebrate that usually has two stages in its life. Spends part of its life in water and part on land. Usually has moist skin
- **Amplexus**: Sexual embrace of amphibians; the grasping of the female's body by the male's forelimbs from a dorsal position. The grip is strong, and is not released until oviposition is completed.
- **Anuran**: Amphibians without tails (i.e. frogs and toads)
- **Archosaurs**: A group of animals including crocodiles, birds, dinosaurs and pterosaurs
- **Barbels**: A fleshy, tubular extension of the skin, usually on the head or the neck, which appear to be sensory, probable tactile in function
- **Brachiosaurs**: A group of sauropod dinosaurs

- **Caecilians**: Caecilians are tropical amphibians that look like large worms or slick snakes. They have no arms or legs, and sometimes it's hard to tell which end is the head and which is the tail
- **Call**: The noise, cry, whistle or song of an anuran, usually confined to males. In a very few species, the female has a voice
- **Camouflage:** An animal's ability to use its color or shape to hide within its environment
- **Choana**: Internal opening of the nasal passage in the roof of the mouth (plural: choanae)
- **Cloaca**: The common chamber into which the intestinal, urinary and reproductive duct discharge their contents, opening to the outside through the anus
- **Courtship Calls**: Calls that are given by both males and females just before amplexus
- **Defensive calls**: The adults of both sexes and juveniles make these calls when startled or attacked by the potential predator
- **Ectotherm**: A cold-blooded animal; that is, an animal whose body temperature varies with the temperature of its surroundings. Ectotherms cannot produce their own body heat
- **Extinct:** No longer in existence; lost or having died out
- **Extinction:** The loss of an entire species
- **Fossil:** What is left of an animal that lived long ago, usually molded or embedded in rock
- **Frogs:** The frog is an amphibian in the order Anura. Characterized by long hind legs, short body, webbed digits, protruding eyes and the absence of a tail
- **Guttural Resonator**: Referring to the sound coming out of throat; harsh sound
- **Habitat**: The environment to which an organism is adapted
- **Hibernation**: Period of time spent dormant (over the winter)
- **Ichthyosaur**: A group of aquatic reptiles that lived from the Triassic Period to the Cretaceous

- **Interorbital**: The measurement between the eyes, usually taken across the top of the head
- **Labyrinthodont**: A species of primitive amphibian that lived from the Devonian to the Cretaceous Period
- **Larva**: An embryo which becomes self-sustaining and independent before it has acquired the characteristic features of its parents
- **Lissamphibians**: A subclass of amphibians that includes all the living amphibians in the order Anura, Urodela and Apoda
- **Metamorphosis**: The process of changing from one form to another during development. In amphibians it is usually associated with a switch from an aquatic larval stage to a terrestrial adult phase
- **Newts**: Small, usually bright-colored, semi aquatic salamanders
- **Paratype**: Specimen other than the holotype that the author uses when describing the species
- **Periosteum**: The connective tissue covering a bone
- **Salamanders**: Common name for a group of approximately 500 species of amphibians typically characterized by slender bodies, short legs, and long tails
- **Sexual Dimorphism**: Referring to the differences in males and females in addition to sex organs, such as size or color
- **Snout-vent length**: The body length measured as the distance between the tip of the snout and the end of the anus (vent)
- **Spawn**: Amphibian eggs
- **Toadlet**:Young toad that has just completed its metamorphosis from a tadpole and left the pond
- **Toadpole**: Sometimes used to describe toad tadpoles
- **Toads**: Toads are frogs. The family Bufonidae, one of the frog families, has over three hundred toad species. Members of this family have drier skins and many have short hind legs for walking instead of doing much hopping
- **Trogloxene**: Any organism that spends some of its time in dark caves
- **Tympanum**: The membrane covering the external opening of the middle ear chamber or vestibule

- **Vocal Cords**: These are pair of elastic fibers stretched across the larynx in amphibians, reptiles and birds. The cords produce sounds as air is expelled
- **Vocal sac**: Pouch(es) of skin under an amphibians chin; these are inflated in order to make noises to attract a mate
- **Warm-blooded**: An animal that can produce its own body heat and maintain its body temperature at a fairly constant level

5.10 Self-Learning Exercise

Section -A (Very Short Answer Type):

1. Flying frog is ……………………..
2. Order …………………….. is tail-less amphibians that includes frogs and toads.
3. A frog with body temperature of 20 ^{o}C is transferred to an area with 30 ^{o}C temperature. The body temperature of frog in the new environment will be ……………………..
4. Maternal care take place in …………………….. and …………………….. species.
5. Adult frogs excrete nitrogen waste as ammonia to conserve water. T/F
6. Stegocephali (extinct) and Lissamphibia (living) are two subclasses of class Amphibia. T/F
7. In anurans species maternal care take place? T/F
8. Gymnophiona is the smallest of amphibian orders includes animals which might easily, at first glance, be mistaken for small snakes or for earthworms.T/F
9. Define parental care.
10. Which chordate class is considered evidence of the transition of vertebrates from an aquatic environment to dry land?
11. What is critical temperature for salamanders and anurans?
12. What is nictitating membrane?

Section -B (Short Answer Type):

1. Write note on Anurans.
2. Write a brief note on adaptation in skin of amphibians.
3. What is paedomorphosis?
4. Why are species of amphibians reducing?

5. What features of amphibians make them dependent on water to survive?
6. How do amphibians protect themselves from predators?
7. Give a brief account of extinct amphibian.
8. Give a brief classification of extinct amphibian with examples.
9. How Brazilian tree-frog, *Hyla goeldii*, protect their eggs?
10. How Chilian *Rhinoderma darwini*, nurses small frogs? Explain.

Section -C (Long Answer Type):

1. Give a detailed classification of amphibian with suitable examples.
2. Discuss origin of three orders of amphibians.
3. What are adaptations? Discuss adaptations in amphibia.
4. Discuss parental care in amphibians.
5. Why do some species survive while others go extinct?

Answer Key of Section-A:

1. *Rhacophorus*
2. Anura (Salientia)
3. 30 ^{o}C
4. Salamanders and Caecilians
5. F
6. T
7. T
8. T
9. looking after by parents to ensure development and survival
10. Amphibians
11. Salamanders (2-27°C) and anurans (3-41 °C)
12. Transparent part of eyelid

5.11 References

- Life of Vertebrates by J.Z. Young
- Reptiles, Amphibia, Fishes and Lower Chordata by Cunningham, J.T.
- Chordate Morphology by Malcolm Jollie
- Vertebrate by Kotpal

Unit - 6

Reptilia

Structure of the Unit:

6.1 Objectives

By the end of the chapter, the student would acquaint himself with the General characters & classification of Reptilia, Origin & evolution of Reptiles, a brief idea about Seymouria, Snake Bite, Biting mechanism, various components of the biting apparatus of snake, how to identify snake bite, Signs and symptoms of snakebites, first Aid measures for Snake Bite, various precautions kept during snake bite accident, How to prevent snakebite, Snake venom- Production, Chemical Composition, Types of Snake Venom, Symptoms and Treatment, production of anti-sera, Types of Antisera.

6.2 Introduction

The term Reptilia refers to the mode of movement(L., reptum, to creep or crawl). The reptiles are the first vertebrates adapted for life on dry land. In the carboniferous period of palaeozoic era "Reptiles" arose from Labyrinthodont Amphibians. They flourished and attained maximum development during the Mesozoic era. In the Mesozoic era "Reptiles" dominated the entire world and that era is called 'Golden age of reptiles". Cotylosauria' is considered as "stem - reptiles". They closely resemble the Labyrinthodont amphibians. In the Mesozoic era giant dinosaurs dominated the earth. In the late cretaceous period of Mesozoic era they became extinct. In the present day world nearly 5,000 known species of reptiles are living. Reptiles are (cold blooded)poikilothermic vertebrates. They are first true land vertebrate's. They are the first amniotic group of vertebrate animals. They have radiated towards all kinds of habitats, that is, land, water and air. It is now definitely known that the reptiles arose from the amphibians and in turn gave rise to birds and mammals. The characters of reptiles are, in fact, a combination of characters found in fishes and amphibia on one hand and in birds and mammals on the other hand. The reptiles breathe by lungs throughout their life and have body covered with scales. In winter they become dormant and hibernate in crevices. The study of reptiles is known as Herpetology. Some examples of reptiles are sphenodon, lizards, snakes, turtles, crocodiles etc.

6.3 General Characters

1) Reptiles are cold blooded, terrestrial or aquatic, creeping or burrowing animals.
2) The body of Reptiles is covered with an exoskeleton of scales, horny scales shields, plates or scutes.
3) In Reptiles Skin is dry& glands are absent.
4) Reptiles are tetrapods.
5) They are pentadactyl having 5 fingerswith clawed digits.
6) Limbs absent in snakes and some lizards.
7) Body is provided with Body is bilaterally symmetrical and divided into four regions : head, neck, trunk and tail.

1) Endoskeleton is of bony type.
2) Skull articulates with the vertebral column by a single medianoccipital condyle. (monocondylic).

3) Mandibles made up of many small pieces and articulate with the cranium by the quadrate bone.
4) In Reptiles vertebrae are gastrocentrous.
5) In Reptiles Ribs from a true sternum.
6) A characterstic T-shaped interclavicle is present.
7) Reptiles are carnivorous.
8) Mouth terminal, simple, conical teeth present on the jaws. In turtles teeth are replaced by horny beaks.
9) Alimentary canal terminates in a cloacal aperature.
10) Respiration is carried on by the lungs.
11) Heart with two auricles and incompletely divided ventricle, exceptional case is four chambered in crocodiles.
12) Right & left aortic arches are completely formed.
13) Red blood corpuscles are oval and nucleated in Reptiles.
14) Brain is more developed than that of amphibians. Twelve pairs of cranial nerves present. Lateral line system absent. Jacobson's organ present in the roof of the mouth.
15) Excretion is of uricotelic type.
16) In Reptiles Kidneys are metanephric. Each kidney is provided with separate ureter.
17) Unisexual animals.
18) Males having a pair of copulatory sacs or hemipenis.
19) Fertilization is inside the body. Eggs are laid on land. Development direct. Young ones resemble the adults.
20) In Reptiles extra embryonic membranes(amnion, chorion, allantios and yolk sac) are present during the development of the embryo.
21) No parental care.

Classification

On the basis of presence or absence of certain openings in the temporal region of the reptilian skull the class Reptiles can be divided into five subclasses. It is represented by about 7000 living species placed in 4 out of the 16 orders that flourished during the Mesozoic era. Remaining orders have become extinct.

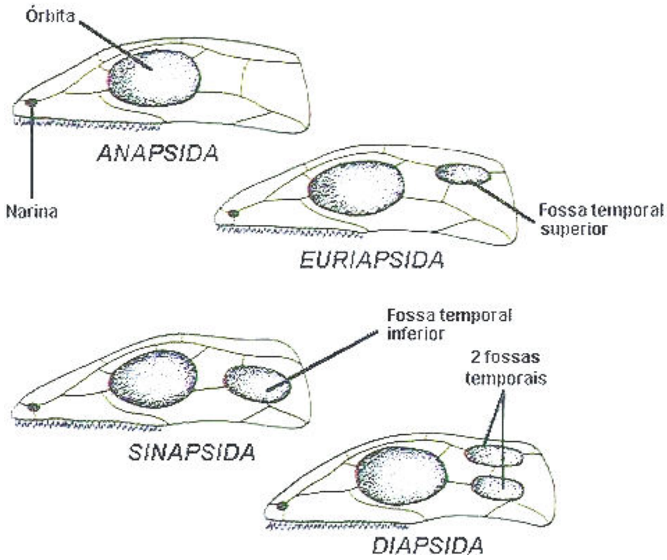


Figure – Different types of reptilian skulls

Subclass I: ANAPSIDA

Anapsid reptiles are those in which the dermal bones form a complete roof over the skull, Skull roof issolid, temporal openings are absent. Two main orders possess anapsid skull, the extinct Cotylosauriaand the existing Chelonia.

Order 1. Cotylosauria

1. This is extinct order. Their existence is supposed to be from Carboniferous to Triassic.
2. They resembleswith Labyrinthodont amphibians.
3. Limbs are feeble and project laterally.
4. Jaws and teeth poorly developed.
   Examples:*Seymouria, Camptorhinus*.

Order 2. Chelonia (Gr., chelone, turtle) or Testudinata (L., testudo, turtle)

1. These animals are existing from Permian to present.
2. Body short, broad and oval, encased in a shell of rounded dorsal carapace and flat ventral plastron.
3. Limbs pentadactyle with clawed and/or webbed digits, or paddle – like.
4. Thoracic vertebrae and ribs usually fused with the carapace.
5. Skull solid, quadrate immovable, sternum absent.
6. Jaws without teeth, covered with horny sheath.
7. Cloacal aperture is longitudinal.
8. Copulatory organ is single.
9. Marine forms are turtles, freshwater forms terrapins and lands forms tortoises.

Examples : *Chelone, Chrysemys, Trionyx, Testudo, Dermachelys.*

Subclass II: EURYAPSIDA(Extinct)

1. Extinct.
2. Skull with single dorso-lateral temporal opening on either side behind the eye, bounded below by supratemporal and postfrontal and bones and above by the parietal bone.
3. They lived during Triassic period.

Subclass-III PARAPSIDA (Extinct)

1. Skull with a single dorso-lateral temporal opening on either side.
2. These openings are bounded below by the supratemporal and postfrontal bones.
3. They lived during Mesozoic to cretaceous period.
4. Ex: - Ichthyosaurus, mixosaurus etc.

Sub-class – IV – SYNAPSIDA (Extinct)

1. Extinct. From Carbobiferous to Permian.
2. Skull with a single lateral temporal opening on either side.
3. These openings are bounded above by the postorbital and squamosal bonesand by the jugal part of the squamosal bones below.
4. Skullwith a single lateral opening on either side behind the eye, bounded by the post orbital squamosal bones above

Order 1. Pelycosauria (=Theromorpha)

Examples : *Varanosaurus, Dimetrodon.*

Order 2. Mesosauria

Example :*Mesosaurus.*

Sub-class – V – DIAPSIDA

(i) Skull with two temporal openings on either side separated by the bar of postorbital and squamosal bones.

This subclass includes 4 orders.

Order 1. Eosuchia

Extinct. Permian to Eocene.

Examples : *Youngina, Prolacerta*.

Order 2. Rhyncocephalia (L., rhynchos, snout + Gr., kephale, head).

1. Body small, elongated, lizard –like burrowing reptiles with elongated body.
2. Limbs pentadactyle, clawed and burrowing.
3. Skin covered by granular scales and a mid-dorsal row of spines.
4. Vertebrae amphicoelous or biconcave.
5. Numerous abdominal ribs present.
6. Ribs single headed and with uncinate process.
7. Parietal foramen with vestigial pineal eye present.
8. Quadrate bone immovable.
9. Teeth acrodont.
10. Cloacal aperture transverse slit
11. No copulatory organs in male.

Example – Represented by a single living species, **Sphenodon punctatum** or tuatara

Order 3. Squamata (L., squama, scale)

1. Includes lizards and snakes.
2. Terrestrial, burrowing or arboreal.
3. Body covered with horny epidermal scales and shields.
4. Limbs clawed, absent in snakes and some lizards.
5. Limbs and girdles usually well developed.
6. Quadrate movable. Vertebrae procoelous. Ribs single-headed.
7. Skull with single occipital condyle. Jugal bone present.
8. Sternum present.
9. Eyelid movable. Nictitating membrane, ear openings and tympanum present.
10. Maxillae, palatines and pterygoids fixed. The two rami of mandible fused in front. Premaxillae bear teeth.
11. Teeth acrodont or pleurodont.
12. Shows caudal autotomy with regeneration.
13. Urinary bladder present.
14. Cloacal aperature transverse.
15. Copulatory organ paired and eversible.

Examples: *Hemidactylus, Chaemeleon, Uromastix, Draco, Phrynosoma, Varanus, Heloderma, Gecko, Mabouia, Iguana.*

This order includes two sub-orders.

Sub order – 1 Lacertilia

Sub order – 2 Ophidia

Suborder 1. Lacertilia or Sauria

1. Commonly known as lizards.
2. Body small to medium, elongated and advanced.
3. Body covered by horny epidermal scales, shields and spines.
4. Limbs, clawed, absent in snakes and few lizards.
5. Limbs and girdles usually well-developed.
6. Eyelids movable. Nictitating membranes present.
7. Ear openings and tympanum present.
8. Maxillae, palatines and pterygoids fixed.
9. Mouth is non-expansible.
10. Vertebrae procoelous.
11. Ribs single headed.
12. Teeth acrodent or pleurodent.
13. Sternum, episternum and urinary bladder usually present.
14. Tongue rarely notched or extensile.
15. Cloacal aperture is transversely present
16. Male with eversible double copulatory organs (hemipenis)

Example – *Hemidactylus, Calotes, Uromastix Varanus, Chamaeleon, Draco*.

Suborder 2. Ophidia or Serpentina

1. Commonly known as snakes.
2. They are existing from Cretaceous to present.
3. Body elongated, slender and narrow.
4. Limbs and griddles absent, vestigial hindlimbs and pelvic girdle in boa, python etc.
5. Terrestrial or aquatic, burrowing or arboreal.
6. Skull with triple occipital condyle. Jugal bone absent.
7. Eye lids fixed or immovable.
8. Nictitating membrane, ear opening and tympanium absent.
9. Manilae, palatines and pterygoids movable helping in bifing mechanism.
10. Mouth can be widely separated.
11. Sternum, episternum and urinary bladder usually absent.
12. Tongue slender, bifid and extensible / protrusible.
13. Maxillae, palatines and pterygoid freely movable and help in biting mechanism.

14. The two rami of the mandible joined by an elastic ligament and can be widely stretched to swallow a large prey. Premaxillae without teeth.
15. Do not show caudal autotomy.
16. Urinary bladder absent.

Ex - *Python, Typhlops, Eryx, Hydrophis, Crotalus, Bungarus, Boa, Naja, Bungarus*, Viper etc.

Order 4. Crocodilian (Gr., krokodeilos, crocodile).

1. Body large-sized, carnivorous and aquatic reptileswith a long laterally compressed tail having denticulated dorsal crest.
2. Limbs short but powerful, clawed and webbed, forelimbs with five and hindlimbs with four clawed and webbed digits, modified for swimming.
3. Tail long, strong and laterally compressed.
4. Body surface covered by horny epidermal scales, bony plates and scutes.
5. Quadrate fixed, parietal foramen absent. Maxillae, palatines and pterygoid united along the midde to form a pseudopalate.
6. Teeth thecodont, numerous.
7. Vertebrae procoelous.
8. Sternum present.
9. Abdominal ribs present.
10. Ribs two-headed.
11. Thoracic ribs with uncinate process.
12. Heart is completely divided into four chambers (2 auricles and 2 ventricles).
13. Lungs well developed and spongy.
14. Cloacal aperture longitudinal.
15. Male with single median, erectile and grooved penis.

    Ex – *Crocodylus, Alligators, Gavialis.* etc.

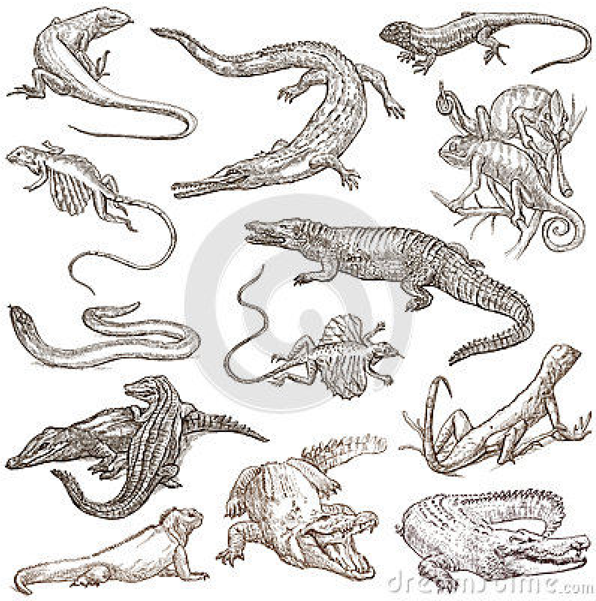


Figure – Different Reptile species

6.4 Origin of Reptiles

Reptiles are supposed to be evolved about 250 million years ago from amphibians of Carboniferous period of the Palaeozoic era, which were depended on water bodies for completion of life cycle i.e. laying eggs and development of larval stages and hence could not survive themselves in arid habitats far away from water bodies. First time a large yolk-laden shelled egg was also found in reptiles that could be laid on land which contains an amniotic sac contained fluid in which embryo get nourishment during development to an advanced stage, capable of fending for itself when hatched.

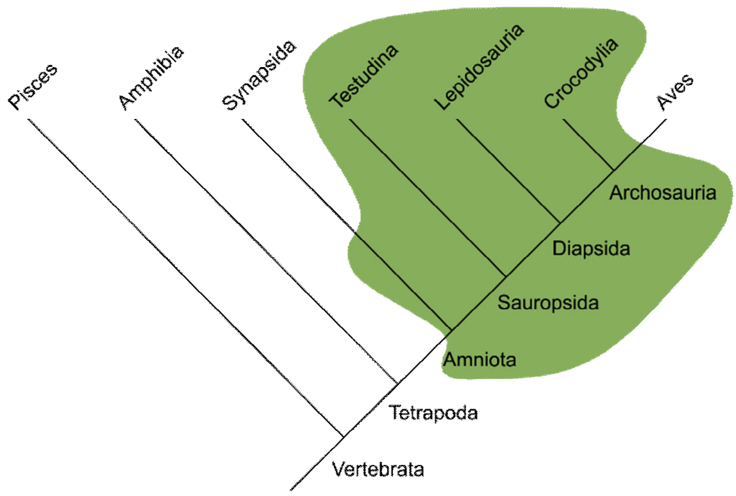


Figure –Flow chart of origin of Reptiles

Origin from amphibian

The primitive reptiles most probably originated from some primitive Labyrinthodont Amphibia which flourished through the Carboniferous and Permian periods and finally became extinct in the Triassic. It is presumed that the reptiles had a polyphyletic origin, that is, they arose along a number of independent lines.

The following anatomical changes were observed in the ancestral amphibians into land adapted reptiles:

- Reptiles are a group of cold blooded creeping animals successfully adapted for life on land. Although amphibians are considered as the first land vertebrates, but the reptiles are the first successful land vertebrates completely independent of water.
- The amphibians were first vertebrates to emerge on land but they have to depend on water for completion of their lifecycle. They frequently visited water bodies to keep their skin moist. They laid eggs in water and their early life, that is, the tadpole larva, was aquatic and had gills for breathing.
- In reptiles the body is covered with epidermal scales to prevent loss of body moisture, and skin glands are absent.
- Skull is monocondylic for better movement and flexibility. Atlas and axis vertebrae combinedly permits skull movement in all directions.
- Limb bones and girdles were stronger but limbs were attached on the sides of body, and belly touched the ground during creeping mode of locomotion.
- Sacral region is supported by two strong and fused vertebrae.
- Well-developed pentadactyle limbs with claws are present to help in climbing on rocks and trees easily.
- Lung respiration became more efficient.
- For more water conservation, metanephric type of kidneys are present, which excrets uric acid with less loss of water.
- Reptiles are ectothermal animals because ventricle was not completely partitioned by a septum and blood mixed in heart.
- Internal fertilization is present in order to more survivality chances to a large cleioid shelled egg was on land.

- Embryonic membranes amnion, allantois and yolk sac are presnt in order to enable embryonic develpment in arid conditions.

The reptiles are successful on land was because:

1. They had dry skin covered with an exoskeleton of scales or bony plates to prevent loss of water.
2. Evolution of shelled or amniotic eggs which are usually laid on land.
3. Development of extra-embryonic membrane, the amnion around the developing embryo. The amnion encloses amniotic cavity filled with amniotic fluid in which the embryo develops.

Stem reptiles (cotylosauria)

It is now sure that the reptiles have originate in the Palaeozoic era from Lavyrinthodont Amphibia, but there is no agreement with regard to the Protoreptilia. The extinct progenitors of the reptiles belong to the order Cotylosauria of the subclass Anapsida. They have been referred to as the stem reptiles because they have primitive and unspecialized characters. They have no space in the temporal region. The first traces of these were found in the carboniferous period in deposited formed in the great coal swamp, when the amphibians were at the peak of their development. Some cotylosaurs regarded as the probable ancestors of reptiles.

6.5 Adaptive Radiation and Evolution

The evolution of a single group of animal in different directions invading different habitats with different types of adaptations is called adaptive radiation or divergent evolution. It generally results from competition for food and living space. Among vertebrate groups, the reptiles have shown greatest adaptive radiation during the course of their evolution.

The Cotylosaurians are regarded as the first or most primitive reptiles. They are also called as stem reptiles because they possess primitive characters and have given rise to all other reptilian orders. The skull of Cotylosauria had complete roofing, that is, there was no vacuity or fossa in the temporal region of the skull, hence they were placed in the sub-class Anapsida.

Temporal fossae and arcade-

The region of skull behind the orbit is known as temporal region. In the skull of reptiles the temporal region contains one or two cavities called temporal fossae. The fossae are bordered by a set of bones which form an arch or bar called arcade. The fossae provide surface for attachment to temporal muscles which help in the movement of lower jaw. The fossae play an important role in the classification of reptiles.

Evolution of reptiles

The reptiles have evolved along several lines from the stem reptiles but the five main lines based on skull roofing are:

1. Anapsid line
2. Synapsid line
3. Euryapsid line
4. Parapsid line
5. Diapsid line.

1. **Anapsid line:** The anapsid line means a skull resembling the stem reptiles. There is absence of fossa in the temporal region of the skull. Among the living reptiles, the chelonians (turtles and tortoises) have retained anapsid skull. They arose as an early offshoot of stem reptiles in the Triassic period and have remain underchanged since 160 millions years.
2. **Synapsid line:** The synapsid line represents the mammal-like reptiles or Synapsida. This type of skull is not found in any living reptile. The early Pelycosauria or Theromorpha were similar to the cotylosaurs. The later Therapsida were more mammal-like. Here the temporal region of the skull had a single vacuity or fossa called infratermporal fossa, on each side behind the orbit. The fossa was bounded above by the post-orbital and squamosal and below by the quadratojugal and jugal. The lower bones together form the infratemporal arcade. These reptiles became extinct in the Jurassic period after giving rise to mammals.
3. **Euryapsid line:** The euryapsid reptiles also had a single fossa on each side of the skull, its position was slightly above therefore it is called as supratemporal fossa. Its lower border is called supratemporal arcade and is formed by post-orbital and squamosal. The euryapsid line is represented by Plesiosaurs, large marine, turtle – like, heavy bodied creatures with long neck. The became extinct by the end of Cretaceous period.
4. **Parapsid line:** The parapsid line is represented by marine fish-like creatures, the Icthyosaurs. The skull had a single fossa on each side which was far high so that post orbital and squamosal meet far below and have no connection with the fossa, and post frontal and

supratemporal lie immediately ventral to the fossa. They became extinct by the end of Mesozoic era:

5. **Diapsid line:** The diapsid reptiles have two fossae on each side of the skull. The post-orbital and squamosal are present between them. The fossa present above is called supratemporal fossa and that present below is called infratemporal fossa. The squamosal and post-orbital situated below the supratemporal fossa from the supratemporal arcade and the jugal and quadratojugal present below the infratemporal fossa form the infratemporal arcade.
6. The early diapsids divided into two lines: Lepidosauria and Archosauria. The Lepidosauria gave rise to modern Squamata (lizards and snakes) and Rhynchocepalia (Sphenodon). The Archosauria were the ruling reptiles dominating the Mesozoic era. They gave rise to Pterosauria, dinosaurs, Crocodilia and birds.

The conquest of land; *Seymouria* and related forms.

Seymouria was a lizard like reptile tetrapod from the early Permian of North America and Europe, supposed to be existed approximately 280 to 270 million years ago. It was small, only 2 feet long. *Seymouria* was well adapted to life on land, with many reptilian features, in fact, that it was first thought to be a primitive reptile. According to the most accepted view *Seymouria* belonging to the order Cotylossauria is the ideal stem reptile.

Seymouria was about 2 feet long, aquatic, lizard-like animal with a thick body. The head was small and pointed and the nostrils were dorsally placed. The limbs were short and pentadactyle and the tail was short. The dry climate of the Permian suited reptiles better than amphibians, but *Seymouria* had many reptilian features that helped it in this harsh environment. It had long and muscular legs, and may have had dry skin and the ability to conserve water. It may have been able to excrete excess salt from its blood through a gland in its nose, like modern reptiles.

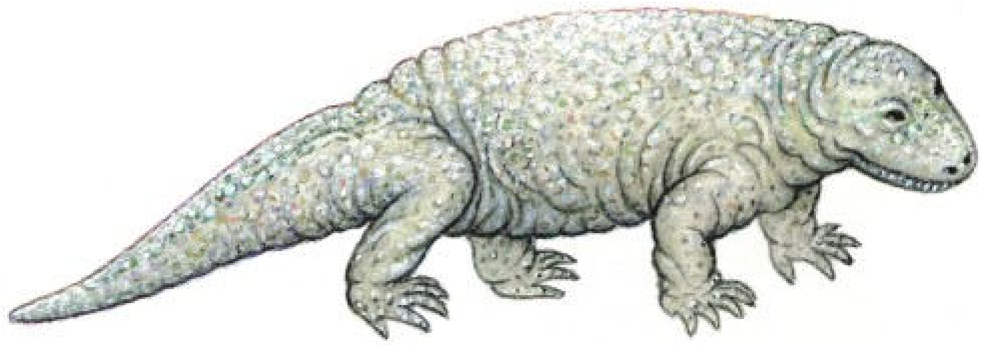


Figure - *Seymouria*

All of this meant that *Seymouria*, unlike amphibians and other early tetrapods, might have lived for extended periods of time away from water. If so, this would have allowed it to move about the landscape in search of insects, small amphibians, and other possible prey, such as the eggs of reptiles and synapsids. Male *Seymouria* had thick skulls that may have been used to batter rivals in mating contests. After mating, the females would have had to return to water to lay their eggs. As in amphibians, the larvae would develop in water, hunting for worms and insects until they were strong enough to live on land. While no larvae are known from *Seymouria* itself, fossil larvae of its relative *Discosauriscus* and other species in the order *Seymouria*morpha have been found, with impressions of external gill structures as in modern amphibians.

Characteristics

Seymoria was a lizard-like animal, with pentadactyle limbs and a short tail. It had homodont labyrinthine teeth on the jaw bones as well as on vomer and palatine bones. Presence of lateral line indicates its amphibious habits. Skull was monocondylic for better movement of head. It possessed characters both of reptile and Amphibia. Romer (1962) considered *Seymouria* as a reptile- like amphibian and Colbert (1969) considered it a connecting link between Amphibia and primitive reptile.

Amphibian characters

1. Skull flat with little ossification.
2. A prominent otic notch for accommodation of ear drum present.
3. Single occipital contyle in the skull.
4. Intertemporal bone present behind the eye.
5. Palate primitive and incomplete.
6. Teeth folded (labyrinthine); also present on vomers and palatines.
7. Head region with traces of lateral line canal.
8. Vertebrae not properly differentiated.

9. Neck short so pectoral girdle lies behind the head.
10. Only one pair of sacral ribs.

Reptilian characters

1. Skull anapsid type and moncondylic.
2. Interclavicles of pectoral girdle long.
3. Neural arches of vertebrae were broad.
4. Ilium of pelvic girdle expanded for attachment of muscles used for walking.
5. Pelvic girdle attached to vertebral column by sacral vertebrae.
6. Limbs pentadactyle, muscular and arise mid ventrally. The arrangement of toe bones similar to early reptiles and the phalangal formula was reptile-like 2:3:4:5:3or 4.

When *Seymouria* was living the reptiles were already present for about 50 million years. So it cannot be ancestor of reptile. Romer treated *Seymouria* as a reptile and placed it under the order *Seymouria*morpha in Amphibia. However, now it is regarded as a connecting link between Labyrinthodontia (Amphibia) and Cotylosauria (Reptila).

Contemporary to *Seymouria* are Didectomorphs and Captorhinomorphs which are very primitive reptiles but had their own marked specialization. The didectomorphs were more tortoise like and were on the road to chelonians while the captorhinomorphs were more lizard like and were on the road to typical reptiles and mammal-like reptiles.

The main phase of evolution for the tetrapods occurred during the Carboniferous. A wide range of early tetrapods are known, such as the aquatic *Diadectes* and the bizzare *Diplocaulus* with its boomerang shaped head. Large terrestrial amphibians such as *Eryops* also evolved. Reptiles evolved from amphibians when some forms such as *Seymouria* 'split' from the amphibian lineage. This 'splitting' pattern explains how amphibians and reptiles were able to evolve seperately along different 'branches', and is the reason why reptiles and amphibians exist together today, even though one evolved from the other.

Hence *Seymouria* possessed characters that were intermediate between amphibians and reptiles. A major evolutionary step occurred with the development of the amniotes, animals able to lay shelled eggs, as this allowed animals to reproduce out of water for the first time. The evolutionary tree below shows the relationships of the early tetrapods.

6.6 Snake Bite

Introduction

Snakes are found on every continent except Antarctica. Snakes are remarkable animals, successful on land, in the sea, in forests, in grasslands, in lakes, and in deserts. Most snakes do not act aggressive toward humans without provocation. Snakes are meat eaters and they catch prey that includes insects, birds, small mammals, and other reptiles, sometimes including other snakes. Only about 400 of 3,000 snake species worldwide are poisonous. Number of species diminishes progressively through the Polar Regions.

In India, there are 330 species only and among them only 69 species are poisonous. Many snakes kill their prey by constriction. In constriction, a snake suffocates its prey by tightening its hold around the chest, preventing breathing or causing direct cardiac arrest. Snakes do not kill by crushing prey. Some snakes grab prey with their teeth and then swallow it whole. Snakes are cold-blooded. Thus, they are unable to increase their body temperature and stay active when it is cold outside. It is very difficult to determine the number of snakes bites, but as per the record of the W.H.O., nearly 30000 to 40000 persons die of snake bite in the world every year. Snakebites are more common in tropical regions and in areas that are primarily agricultural. In these areas, large numbers of people coexist with numerous snakes.

Poisonous snakes inject venom using modified salivary glands. Envenomation means the snake bite that injects venom or poison into prey. During envenomation the venom passes from the venom gland through a duct into the snake's fangs, and finally into its prey. Not all bites lead to envenomation. Snakes can regulate whether to release venom and how much to release. Dry Bite is a bite where no venom is injected by snakes, this type of bite occurs in between 25%-50% of snake bites. This variation is species specific with approximately 25% of pit-viper bites being "dry" and up to 50% of coral snake bites. Snake venom is a combination of numerous substances with varying effects.

Biting mechanism

The poison apparatus of snakes consists of a pair of poison glands, their ducts and a pair of fangs. In poisonous snakes the poison glands are situated one on either side of the upper jaw. The poison glands are modified salivary glands, possibly the superior labial glands or parotid glands. Each poison gland is sac-like and provided with a narrow duct at its anterior end. The duct passes forward along the side of the upper jaw and loops over itself just in front of the fang and opens either at the base of the fang or at the base of the tunnel on the fang. These glands are one on either side of the head surrounded by muscle which, when contracted, forces the clear or yellowish venom along the venom ducts and down through the fangs, squirting out under pressure as if from a pair of hypodermic syringes. Venom may be injected with each of a possible series of consecutive bites. Interestingly however, venom is not always injected. The poison gland is held in position by ligaments. An anterior ligament attaches the anterior end of the gland to the maxilla. A posterior ligament extends between the gland and the quadrate. Fan-shaped ligaments are situated between the side walls and **squamoso-quadrate** junction. The fangs are sharply pointed and are enlarged maxillary teeth. The fangs regenerate when lost.

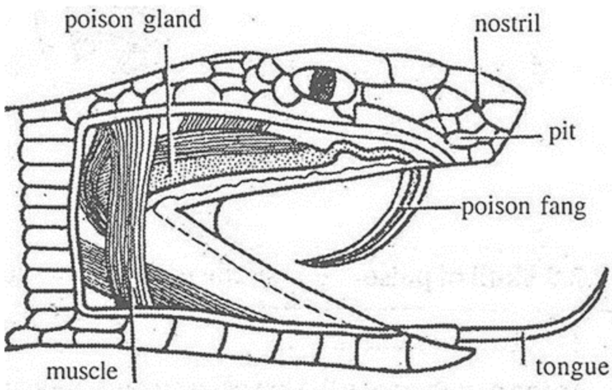


Figure- Snake biting apparatus

The biting apparatus taking part in the biting process are —

1. Poison glands
2. Poison ducts
3. Poison teeth or Fangs

Poison Glands

There is one pair of poison glands each one is situated on either side of the upper jaw. The poison glands are actually the parotid glands. Each poison gland is sac like in appearance. They are held in position by some ligaments. With the help of anterior ligament, the gland is attached with the maxilla. The posterior ligament is present between the gland and the quadrate. In addition to these, fan-shaped ligaments are also situated between the side walls and squamoso-quadrate junction**.**

Poison Duct

Each poison gland is provided with a narrow duct at its anterior portion which passes along the side of the upper jaw, loops over itself and finally opens at the base of the fang.

Poison Fangs

- *There is one pair of fangs in the upper jaw. They are enlarged maxillary teeth which are very sharp and pointed. There is great power of regeneration (when lost for some reason). On the basis of structure and position, the fangs are of the following types :*

    1) *Proteroglyphous type: The fangs are comparatively small and they are present in front of the maxillae. The fang has a groove all along its anterior face. Examples : Cobra, Krait, Sea snakes and Coral snakes.*

    2) *Stenoglyphous type: The fangs are movable and turned inside. Poison canal runs through the fang and opens at the tip. Examples: Vipers and Rattle snakes.*

    3) *Opisthoglyphous type. The fangs are small and lie at the back portion of maxillae. The fang has a groove along its posterior face. Examples : Some colubrid snake (African tree snakes)*

    4) *Aglyphous type: Aglyphous dentition is present in the non-poisonous snakes.*

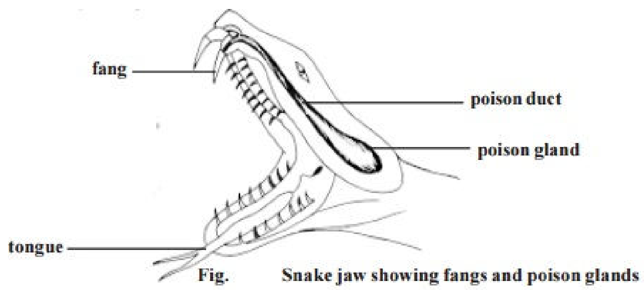


Fig. Snake jaw showing fangs and poison glands

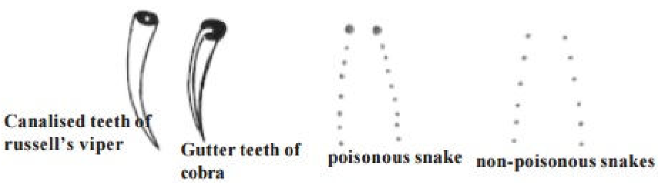


Fig. Fangs of snakes and marks left by the bite of poisonous and non-poisonous snakes

6.7 Associated Bones and Muscles

There are some important bones and muscles which are directly or indirectly associated with the mechanism of biting.In the skull, maxillae, quadrate, pterygoid, squamosals, ectopterygoids and palatines are movably articulated. Premaxillae are very much reduced. The joint of quadrate and lower jaws acts as fulcrum. Quadrates are also loosely articulated with the cranium, pterygoid and lower jaw. The important muscles are Digastric muscle, Anterior and Posterior temporalis muscles and Protractor-Pterygoid or Sphenopterygoid muscle. In addition to these, there are two more muscles associated with the poison glands. These are masseter muscle and Mandibular constrictor muscle.

Snake Biting Mechanism

The mechanism of biting is a complicated process and it can be described in the following three steps.

i. Opening of the mouth:

By the contraction of digastric muscles the mouth is opened.

ii. Rotation of maxilla:

As the mouth opens the lower jaw moves forward and a rotation of the squamosal, quadrate and mandible in relation to each other occurs. Now the sphenopterygoid muscles contract. Thiscontraction results in the forward movement of pterygoid and up-pushing of the ectopterygoid. The upward movement of the ectopterygoid brings about a rotation of maxilla on its own axis round the lacrymal and as a result the fang is raised and becomes directed forward. The fang is nearly horizontal in position when the mouth remains closed. But during opening of the mouth to bite, the fang assumes almost vertical position.

iii. Closing of mouth:

The closing of the mouth is brought about by the contraction of the temporalis and sphenopterygoid muscles. The point of fang is directed back ward while the mouth is closed. It takes longer time to open the mouth than to close it.

Identification of snake bite

Diagnosis of snakebite or identification or description of the snake can be helpful in developing a treatment plan as not all snakes are venomous, and different kinds of antivenom exist for different species of snakes that are venomous. The doctor also looks for evidence of fang marks or local trauma in the area of the bite. Pain and swelling accompany many snakebites, venomous or not. The doctor treats breathing problems, shock, or immediately life-threatening injuries even before a full workup is complete. The wound will be examined and cleaned.

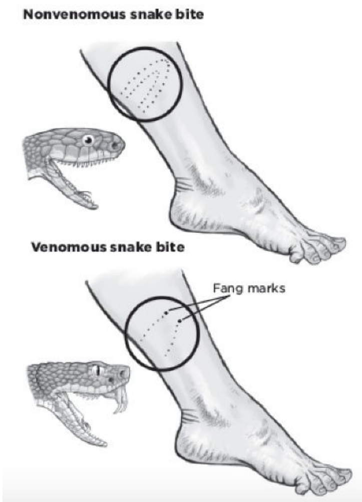


Figure –Non- venomous and venomous snake bite marks

If a patient has symptoms, the doctor will likely send blood and urine samples to the laboratory to look for evidence of bleeding, problems in the blood clotting system, kidney problems, or muscle death. These problems may not be initially apparent, but can have dire consequences if missed. The patient is monitored to look for worsening symptoms at the wound site, or worsening systemic symptoms in the breathing or cardiovascular systems. A rare complication in very swollen limbs is compartment syndrome. Limbs are divided into compartments of muscles, blood vessels, and nerves. Severe swelling can cut off the blood circulation to a compartment. When the circulation is cut off, the patient usually has severe pain and numbness. Later, the limb may get white and cold. If not treated in time, the limb may need to be amputated. The most common symptom of all snakebites is overwhelming fear, which contributes to other symptoms, including nausea and vomiting, diarrhea, vertigo, fainting, tachycardia, and cold, clammy skin. Television, literature, and folklore are in part responsible for the hype surrounding snakebites, and people may have unwarranted thoughts of imminent death.

Most snakebites, whether by a venomous snake or not, will have some type of local effect. There is minor pain and redness in over 90 percent of cases, although this varies depending on the site. Bites by vipers and some cobras may be extremely painful, with the local tissue sometimes becoming tender and severely swollen within five minutes. This area may also bleed and blister and can eventually lead to tissue necrosis. Other common initial symptoms of pit viper and viper bites include lethargy, bleeding, weakness, nausea, and vomiting. Symptoms may become more life-threatening over time, developing into hypotension, tachypnea, severe tachycardia, severe internal bleeding, altered sensorium, kidney failure, and respiratory failure.

Venom emitted from some types of cobras, almost all vipers and some sea snakes causes necrosis of muscle tissue. Muscle tissue will begin to die throughout the body, a condition known as **rhabdomyolysis**. Rhabdomyolysis can result in damage to the kidneys as a result of myoglobin accumulation in the renal tubules. This, coupled with hypotension, can lead to acute renal failure, and, if left untreated, eventually death

Signs and symptoms of snakebites can be broken into a few major categories:

Bites by venomous snakes result in a wide range of effects. They range from simple puncture wounds to life-threatening illness and death. The findings following a venomous snakebite can be misleading. A victim can have no initial significant symptoms, and then suddenly develop breathing difficulty and go into shock.

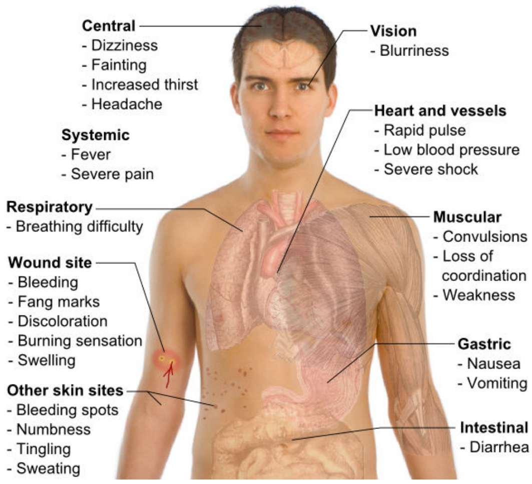


Figure –General Symptoms of snake bite

- **Local effects:** These are the effects on the local skin and tissue surrounding the bite area. Bites by vipers and some cobras (*Naja* and other genera) are painful and tender. They can be severely swollen and can bleed and blister. Some cobra venoms can also kill the tissue around the site of the bite.
- **Bleeding:** Bites by vipers and some Australian elapids can cause changes in the victim's hematologic system causing bleeding. This bleeding can be

localized or diffuse. Internal organs can be involved. A victim may bleed from the bite site or bleed spontaneously from the mouth or old wounds. Unchecked bleeding can cause shock or even death.

- **Nervous system effects:** The effect on the nervous system can be experienced locally close to the bite area or affect the nervous system directly. Venom from elapids and sea snakes can affect the nervous system directly. Cobra (*Naja* and other genera) and mamba (*Dendroaspis*) venom can act particularly quickly by stopping the breathing muscles, resulting in death without treatment. Initially, victims may have vision problems, speaking and breathing trouble, and numbness close to or distant to the bite site.
- **Muscle death:** Venom from Russell's vipers, sea snakes, and some Australian elapids can directly cause muscle death in multiple areas of the body. There can be local effect of muscle death or necrosis, or distant muscle involvement (rhabdomyolysis). The debris from dead muscle cells can clog the kidneys, which try to filter out the proteins. This can lead to kidney failure.
- **Eyes:** Spitting cobras and ringhals can actually eject their venom quite accurately into the eyes of their victims, resulting in direct eye pain and damage.

6.8 Snakebite Treatment

First Aid for Non-Poisonous Snake Bite: Getting bitten by a non-poisonous snake might cause pain or skin infections. A non-poisonous snake bite may lead to death just because of the shock. So follow these steps:

- Help the person to come out of the shock and calm down. Assure the victim, that it was a non-poisonous snake bite, so there's nothing to worry about.
- Also, tell him/her that all poisonous snakes are not fully charged with venom and even the fully charged ones don't necessarily inject the poisonous dose. This will help reduce blood pressure, sweating, tremors, shivering or palpitation.
- Though we have identified that it was a non-poisonous snake, take the victim to a doctor as soon as possible.

First Aid for Poisonous Snake Bite: A lot of promptness and instant action is required in this case. Call the emergency medical service before we start the first aid process. Keep in mind, we have to save the victim. Catching the snake is not important, as we pose a risk of getting a snake bite again. Following are the first aid tips:

- Keep the victim calm and assure him that he will be alright. Help him come out of the shock as an increase in the heart rate or tremors may lead to more complications.
- Remove the clothes and jewellery around the affected body part.
- Immobilize the affected area without pressurizing it.
- Wash the wound immediately using warm water and soap.
- Do not elevate the casualty and keep the bite below the heart level.
- Observe the patient keenly.
- Never try to suck the venom with our mouth as we usually do when someone is hurt and the wound bleeds.
- We should not try to remove the venom by cutting into the wound or by applying suction
- Do not apply any ice on the wound.
- Do not give alcohol or hot beverages to the patient.
- Take the patient to the hospital as soon as possible, for further treatment.
- Inform the doctor about any signs that we noticed after the snake bite. Conditions like drowsiness, bleeding from gums or dropping-eyelids should be reported to the doctor without fail.

Figure – Venom sucking pump

Precautions

- **Do not cut and suck -** Cutting into the bite site can damage underlying organs, increase the risk of infection, and sucking on the bite site does not result in venom removal.
- **Do not use ice -** Ice does not deactivate the venom and can cause frostbite.
- **Do not use electric shocks -** The shocks are not effective and could cause burns or electrical problems to the heart.
- **Do not use alcohol -** Alcohol may deaden the pain, but it also makes the local blood vessels bigger, which can increase venom absorption.
- **Do not use tourniquets or constriction bands -** These have not been proven effective, may cause increased tissue damage, and could cost the victim a limb.

Snakebite Prevention

The snake is almost always more scared of the human, than the human is of the snake. However, most snakes will try to bite if cornered or frightened.

- Do not attempt to handle, capture, or tease venomous snakes or snakes of unknown identity. 40% of snakebites occur when the victim tries to capture a snake or handles a snake carelessly.
- Snakebites are often associated with alcohol use. Alcohol intake can weaken a person's inhibitions, making it more likely that they might attempt to pick up a snake.
- Individuals can help prevent significant bites by wearing boots while hiking or working where snakes may live. Long pants can reduce the severity of a bite. and never walk barefooted.
- Providing information regarding the type of snake, type of venom, and the procurement and use of antivenom can help the medical staff treat the victim.

6.9 Snake venom

Venomous snakes are found throughout the world. According to one study over 3 million bites happens worldwide every year. They causes a significant health problem both in mortality and morbidity, causing deaths and renal failure. Approximately over 100,000 deaths a year are caused due to snake bites. The major death causing snakes are vipers, elapids, colubrids and sea snakes. Of these, elapids such as cobras and kraits have developed the most potent toxins.

Snake venom is a highly developed form of saliva, injected by the snake into its victim through hollow, modified fangs. Snake venoms contain a multitude of biologically active toxins that work together for the capture of prey. Their effects include pro- and anti-blood coagulation, neurotoxicity, mycotoxicity, nephrotoxicity, cardiotoxicity and necrotoxicity. Amongst these, the neurotoxins play a key role in immobilising prey through paralysis, disorientation and depressed respiration.

Venom Production in snake

Generally the venom is produced in to the glands which are located in the head of the snakes, it is never produced in the fangs of the snake. The glands are of two types - False venom Glands and True venom Glands.

False venom glands produces venom but during prey capturing the snake needs to hold the animal continuously to keep the venom spray intact whereas in case of true venom glands, the snake doesn't require continuous holding of the animal instead they can be used once for a short time only.

Chemical Composition of venom

There are approximately 20 types of toxic enzymes found in snake poisons throughout the world. Each of these enzymes has its own special function. Some aid in the digestive process, while others specialize in paralysing the prey. This is a list of various toxins that have been identified in snake venom:

Proteolytic enzymes

- Phosphomonoesterase
- Arginine ester hydrolase
- Phosphodiesterase
- Thrombin-like enzyme
- Acetylcholinesterase
- Collagenase
- RNase

- Hyaluronidase
- DNase
- Phospholipase A2 (A)
- 5'-Nucleotidase
- Phospholipase B
- L-Amino acid oxidase
- Phospholipase C
- Lactate dehydrogenase
- Adenosine triphosphatase

Enzymes

Enzymes are biological proteins catalysts. A catalyst is a compound that increases the rate of the reaction but does not affect the yield or equilibrium of the reaction. The enzymes in the snake venom can speed up chemical reaction going on in an organism so much, that they can kill the organism. Enzymes bind temporarily to one or more of the reactants of the reaction they catalyse. In doing so, they lower the amount of activation energy needed and thus speed up the reaction.

The function of some of the key enzymes have been summarised below -

- **Cholinesterase:** attacks on the nervous system, relaxing muscles to the point where the victim has very little control.
- **Amino acid oxidase:** plays a part in digestion and the triggering of other enzymes, and is also responsible for venom's characteristic light yellowish colouring.
- **Adenosine triphosphates:** believed to be present in most of the snakes venom and is one of the central agents resulting in the shock of the victim and immobilizing smaller prey.
- **Peptide bradykinin potentiators** :- responsible for dilation and increased permeability of blood vessels, stimulation of pain receptors, and contraction of some smooth muscles, thereby enhancing diffusion of

venom in the bloodstream, increasing bleeding, and stopping the ability to flee. Bothrops, Rattlesnakes contain this venom.

- **Polypeptide toxins:** Directly disrupt nerve-impulse transmission, usually causing heart or respiratory failure Taxon with the compound in its venom: Mambas and colures: Cobra (*Naja*)(cobratoxin), Cobra (*Naja*) (cardiotoxin), Mojave Rattlesnake (*Crotalus scutulatus*) (Mojavetoxin), Vipera (viperptoxin).
- **Proteolytic enzymes:** Catalyse the breakdown of structural components of tissues. All venomous species contain this venom.
- **Hyluronidases**: Catalyse reactions that break mucopolysaccharide links in connective tissues, thereby enhancing diffusion of venom. Several types of snake contain this venom.
- **Proteases:** Catalyse reactions that disrupt protein peptide bonds in tissues, causing blood-vessel wall damage and haemorrhaging and muscle-fibre deterioration. Vipers, pit vipers contain this venom.
- **Phospholipases:** Catalyses reactions that harm musculature and nerves. Almost all venomous species (e. g., phospholipase A, in Copperheads, cottonmouths, Fer-de-lances, Rattlesnakes, Cobras, Vipera)
- **Thrombin like enzymes:** This inhibits blood clotting. Vipers, pit vipers, a few elapids (but rare) contain this venom.
- **Nerve growth factor** (an enzyme): NGF stimulates the growth of nerve cells. Copperheads and Cottonmouths, Rattlesnakes contain this venom. This component disrupt normal cellular function, causing death of the affected cells. Vipers and elapids (occurrences vary) contain this venom.
- **Glycoproteins**: The main function of this is to suppress normal immune response of tissues through anticomplementary reactions. Some vipers contain this venom.
- **Biogenic amines**: This disrupts normal transmission of nerve impulses and other types of signalling between cells. Cottonmouths, Copperheads, Rattlesnakes, Tree vipers, contain this venom.
- **Other enzymes**: ribonucleases, deoxyribonucleases, nucleotidases, lactate dehydrogenases, acidic and basic phosphatases.

Types of Snake Venom, Symptoms And Treatment

1. **Cytotoxic venom (cell destroying)**

Symptoms

• This venom destroys blood vessels, cells and tissue.

• The symptoms develop slowly, 10 – 15 minutes after the bite.

• The bite is very localized and painful, with severe swelling, bleeding, redness and blistering often occurring.

• Nausea and vomiting may also occur.

After the entry of venom inside the body, the body sends plasma (white blood cells) to the site of the bite to try and dilute the venom. Because of effect of venom excess amounts of plasma cause the body tissues to swell up to the degree and the veins are compressed very tightly up against the skin, resulting in loss of blood circulation. The area often turns to a blackish-blue color and there is limited blood circulation. If not treated promptly, within 4 hours, amputations may necessary.

Treatment

• Keep the victim calm and advise not to move, either sitting or lying down.

• Cover the site of the bite with a clean dressing and apply a pressure bandage from the start of the limb, then wrapping towards the bite area.

• It is very important to take the pressure bandage off every 10 – 15 minutes then re-applying it once again as severe swelling may cause circulation to be cut off by the bandage.

•Splint and elevate the limb if possible.

• If a suction syringe is available, then use on the site of the bite within three minutes, any time after that would be prove useless.

Don't use mouth to suck the venom out as we may have a cut or mouth ulcer where the venom can enter through.

•Don't cut the sight of the bite as this may lead to infection of the wound.

•Keep the proper ventilation and monitor respiration and heart rate.

•Identify the snake if possible but don't waste time doing this.

Don't try catching the snake if it is still alive as this may result in another snake-bite.

- Transport the victim to hospital promptly.

2. **Haemotoxic venom (poisoning of blood)**

Symptoms

• Often no pain or symptoms occur for 1 -3 hours and as long as 8 hours.
• Casualty feels lethargic with headaches.
• Nausea and vomiting may occur.
• Constant bleeding from the bite wound.
• Bruising as well as blood spots may occur under the skin.
• Blood oozing from other bodily openings.

This venom affects the blood clotting mechanism, resulting in headaches, and loss of blood through the bodily openings and if left untreated internal bleeding of the organs will occur.

Treatment

• Keep the victim calm and advise not to move. Make him/her to sit or lie down.
• Cover the site of the bite with a clean dressing and apply a pressure bandage from the start of the limb, then wrapping towards the bite area.
• Splint and elevate the limb if possible.
• If a suction syringe is available, then use on the site of the bite within three minutes, any time after that would be prove useless. Don't use mouth to suck the venom out as we may have a cut or mouth ulcer where the venom can enter through.
• Don't cut the sight of the bite as this may lead to infection of the wound.
• Keep the airways open and monitor respiration and heart rate.
• Try to identify the snake if possible but don't waste time doing this. Don't try catching the snake if it is still alive as this may result in another snake-bite.
• Transport casualty to hospital promptly.

3. **Myotoxic venom (muscular necrosis)**

Symptoms

• Casualty feels a sensation of the tongue thickening.
• Dryness of the throat with a sense of thirst.
• Stiffness of the jaw, neck, trunk and limbs muscles.
• Severe pain when moving muscles.
• Muscular spasms and convulsions.

• Eyelids may start drooping.
• Difficulty breathing.
• Urine turns to a blackish-brown color after a few hours.

Myotoxic venom contains peptides that destroy the protein in the muscle fibers, that have a high oxidative capacity, resulting in myocrosis (muscle destruction).

The broken down protein from the muscle fibers then enters the blood stream through to the kidneys. The kidneys overwork trying to filter out all the excess body tissue often resulting in kidney failure and the urine turning very dark in color.

Treatment

• Keep casualty calm and advise not to move. Either sitting or lying down.
• Cover the site of the bite with a clean dressing and apply a pressure bandage
from the start of the limb then wrapping towards the bite area.
• Splint and elevate the limb if possible.
• If a suction syringe is available, then use on the site of the bite within three minutes, any time after that would be prove useless. Don't use mouth to suck the venom out as we may have a cut or mouth ulcer where the venom can enter through.
• Don't cut the sight of the bite as this may lead to infection of the wound.
• Keep the airways open and monitor respiration and heart rate.
• Try to identify the snake if possible but don't waste time doing this. Don't try catching the snake if it is still alive as this may result in another snake-bite.
• Transport casualty to hospital promptly.
• With certain species of snakes such as sea snakes the venom is extremely powerful, so a tourniquet may be used but should be released and put back on every 10 – 15 minutes to allow for blood circulation.

4. **Neurotoxic venom (Nerve destroying)**

Venoms often contain different neurotoxins that work synergistically to cripple the nervous system. Neurotoxins can be classified according to their site of action: pre-synaptic neurotoxins block neurotransmission by affecting acetylcholine transmitter release; post-synaptic neurotoxins are antagonists of the acetylcholine receptor. Together these neurotoxins effectively block skeletal neuromuscular transmission by crippling receptors, while at the same time acting to destroy any neurotransmitter that might compete with the toxin for receptor binding. Venoms often contain several post-synaptic neurotoxins, each with a high affinity for a nicotinic receptor subtype - in this way the venom can cripple as many receptors as possible. The post-synaptic neurotoxins are found only in elapids and sea snakes (*Hydrophiidae*) and a pre-synaptic & post-synaptic toxin are bungarotoxin.

Symptoms

• The casualty has speech and swallowing difficulties often accompanied by drooling.

• Difficulty breathing.

• Respiratory arrest.

• Dizziness.

• Tunnel vision or blurred vision.

• Visible increase in sweating.

• Convulsions.

• Unconsciousness.

This venom attacks the central nervous system by causing a high loss of the synaptic vesicles, resulting in the blockage of nerve impulses from the brain too the muscles.

Treatment

• Keep casualty calm and advise not to move. Either sitting or lying down.

• Cover the site of the bite with a clean dressing and apply a pressure bandage from the start of the limb then wrapping towards the bite area.

• Splint and elevate the limb if possible.

• If a suction syringe is available, then use on the site of the bite within three minutes, any time after that would be prove useless. Don’t use mouth to suck the venom out as we may have a cut or mouth ulcer where the venom can enter through.

• Don't cut the sight of the bite as this may lead to infection of the wound.
• Keep the airways open and monitor respiration and heart rate.
• Mouth to mouth respiration may be required to keep the casualty alive.
• Try to identify the snake if possible but don't waste time doing this. Don't try catching the snake if it is still alive as this may result in another snake-bite.
• Transport casualty to hospital promptly.

• Neurotoxic venom is extremely powerful, so a tourniquet may be used if the casualty is far from any hospital. The tourniquet should be released and put back on every 10 – 15 minutes to allow for blood circulation.

Snake venom is not dangerous if medical treatment is speedily provided. Where it is not possible to get medical aid quickly, it is always advisable to be aware of the first aid measures. First aid measures for snake bites should be teach in schools. People should live with the snakes, by simply being aware and taking care. They try to live in harmony, where no man kills the snake and the snakes return the favor.

Anti-Sera (Antivenin / Antivenom) and their Production

Introduction

The term "antivenin" is derived from the French word "venin," meaning venom. Antivenin was the resultant product of several researches carried out simultaneously in different parts of the world. Though this usage used to be common, the World Health Organization decided in 1981 that "venom" and "antivenom" would be the preferred terms in English.

The successfully scientific immunization of an animal by repeated injections of animal venom was first reported by Fornara in 1877. He successfully protected a dog after several inoculations of small amounts of toad skin secretions. Later in Michigan, Sewall described a similar experiment in 1887 on pigeon protection. After treating them with gradually increasing doses of venom. Discovery of antivenin was claimed the same day (February 10, 1984) by the team of Phisalix and that of Calmette (Brygoo, 1985; Calmette, 1884; Phisalix and Bertrand, 1884). Calmette in Paris, Fraser in Edinburgh, Brazil in *São* Paulo, and McFarland in Philadelphia began preparation of antivenin against various species of venomous snakes.

Snake *antisera* are a man-made biological product use in treatment of venomous snake bites. Anivenin serum treatments are based on the vaccine process developed by Louis Pasteur, the famous French microbiologist & chemist. Today, antisera is very important from the epidemiological, biochemical, immunological, commercial and economic points of view. Serotherapy is currently the only specific treatment method of snake envenomation. Although antivenin availability is difficult and expensive in relation to the way of life.

6.10 Effect of Venom on body

Venom is made up of a combination of many different protein molecules that change the way our cells behave. The huge variety of toxins lead to many different effects on our body, depending on what kind of cells they target. On the basis of action venoms are divided into following categories of toxins:-

i) Neurotoxins affect the cells in our brain and nervous system.

ii) Hemotoxins mostly target cells in our bloodstream, though they have impacts on other tissues as well. They can kill red blood cells, which deliver oxygen to the rest of our body, as well as disrupt normal blood clotting and cause organ failure.

iii) Cytotoxins are responsible for spontaneous cell death in which a cell explodes and releases its fluid into the body. The tissue swells up and causes extraordinary pain.

Effect of Venom on body

Antisera is produced when a small amount of snake venom is injected into an animal, usually a horse, goat, sheep, or rabbit. The animal has an immune response to the venom and produces specialized antibodies which are small proteins designed to counteract the effects of the toxic proteins in venom. Scientists can collect the antibodies from the animals and use them to treat people who have been bitten by a Snake. Because each type of venom produces a slightly different immune response and therefore a slightly different antibody, at present we only have a handful of antisera available for use on humans.

Antisera production

Since the discovery of antisera by Calmette in 1894, the method for preparation has changed little. The basic principle is to immunize an animal with venom for production of specific antibodies that will be able to neutralize this venom. Today for antivenin production modern techniques are in use.

A. Sources of Venom

First step in production of antisera is venom is milked from the snake by mechanical pressure on the venom gland or by electrical stimulation of striated muscles surrounding the gland. A direct electric current of 6-12 V is applied to the mucous membrane of the mouth. The venom is collected and then dried or lyophilized. The quantity of venom obtained by both methods is similar, but the later process is much safe for both snakes and human. Previously the snakes were killed before milking; however, at present this procedure is no longer used, and snakes are kept alive for several years. The yield of venom from an individual snake decreases after the first milking, if they are too frequent. There should be a four-week interval between milking to maintain the average yield of venom from individual snakes. The problem is quality, specificity and efficacy of antivenin.

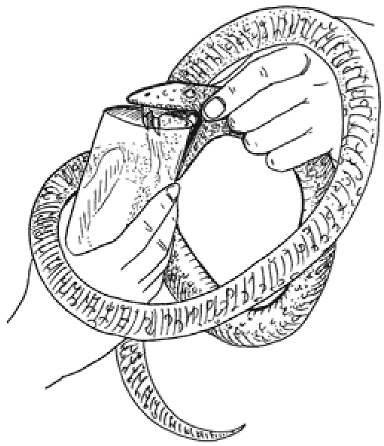


Figure - Mechanical method of venom extraction

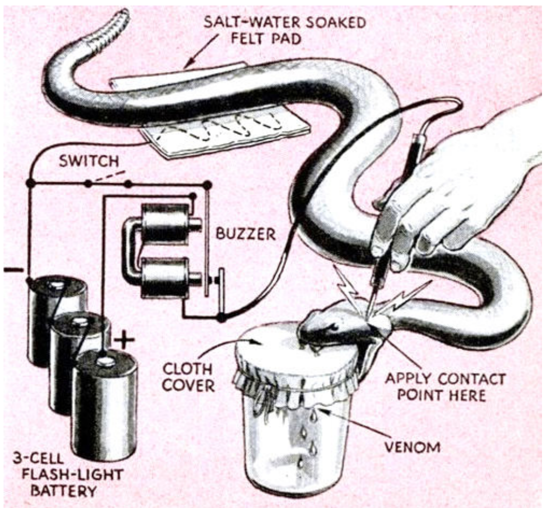


Figure - Electrical stimulation method of venom extraction

B. Immunization

Animals Used

Usually the **horse** is the preferred animal for antisera production. The amount of serum obtained in one bleeding is larger than that from any other easy-to-breed and to handle animal. Donkies, mules, cows, or oxen, can also be used but least preferred by producers. Sometimes goats, sheep, or rabbits can also be used.

These animals are injected with small, non-lethal doses of venom over a period of time, with the dosage slowly increased. Their immune systems respond by creating antibodies to attack the venom. Once the animal is immune to the venom, the antibodies are then extracted to make anti-venom. Horse serum often causes serum sickness which is when the immune system reacts against the foreign proteins in the serum, the reaction can cause kidney failure and even death.

Sheep serum is made by a purified fraction of the plasma, specifically refined antibody fragments and is a lot safer to use. Sheep serum is used for the treatment of the American rattlesnake bites.

C. Inoculation

Direct inoculation of pure venom is not recommended usually but numerous methods of venom preparations have been proposed to reduce toxicity and preserve antigenic properties. Detoxification has been obtained with various processes: heat-treatment, hypochlorite, soap, mixtures with lipoids, hydrogen peroxide, bile, and others. Monovalent antivenin is prepared with venom from an unique species, or pooled venoms from very close species belonging to the same genus. Polyvalent antivenin is produced with venom from various species, genera, or families.

D. Purification

The purpose of purification is to concentrate and purify the antibody activity of the immunized animal blood. First, cells must be removed from the plasma by centrifugation. Then nonimmunological proteins are removed from the plasma especially albumin . The hazards of adverse reaction to antivenin are reduced.

E. Immunological Control

The immunological composition of an antivenin can be checked by immunoelectrophoresis against the venom used for immunization or against another venom. Venom is submitted to an electrophoresis that separates its components, which then diffused against the antivenom through a gel.

Adverse Reactions

One of the main problems of serotherapy is adverse reactions to horse serum in previously sensitized persons. This allergic reaction can be rapid. In this case, it can be very severe with urticaria, edema, asthma, urticaria, edema, asthma, or shock.

Indian Snake Antivenin Producers Organizations

In a recent world 50 snake antivenin producers are reported throughout the world. In our country, India following are main producers:

1. Central Research Institute, Kasauli, 173205 (HP) India.
2. Haffkine Biopharmaceutical Corp. Ltd, Acharya Donde Marg,Parel,Bombay 400012 India
3. Serum Institute of India Ltd,212/2, Hadapsar, Pune - 411028 India

Why doesn't venom kill the venomous animal?

There are a couple reasons for this. First, venom is only effective if it makes it into the bloodstream. If a snake, for example, eats its own venom, the toxic proteins will be broken up in the stomach before they have a chance to do damage in the bloodstream. The snakes and other animals can make their own antivenomous proteins that circulate in their blood and neutralize any venom that makes its way through.

6.11 Types of Antisera

1. Monovalent anti-sera – this is made by injecting the venom of a specific species of snake into an animal to develop the anti-venom. This is effective against one specific snake species.
2. Polyvalent anti-sera – this is made by injecting the venom from a variety of different snake species into an animal to develop the anti-venom. This is effective against a broad range of snake species.

   For instance, there is not a specific Antivenom developed for an Australian Copperhead bite strike so emergency medical providers are advised to use either Tiger Snake Antivenom or a polyvalent one. The first snake Antivenom was discovered in 1895 by Albert Calmette against the deadly Indian *Naja* Snake, better known as the Cobra. Since that time and through the beginning of the 19th century antivenins were also formulated for many venomous arachnids (spiders), scorpions and the Amphibia class of animals which includes poisonous frogs and toads.

Difficulties

The main difficulties observed during antiserum preparations are as follows-

1) Preservation of antivenin is an important problem.
2) Inactivation of venom before inoculation of the animal
3) Purification of antivenin
4) Evaluation of antivenin potency
5) Adverse reactions to antivenin

Summarized process

1. First, snake venom is injected into a horse or other animal and the weekly dosage is gradually increased until the horse/animal is able to produce its own natural antivenin. To ensure that the antisera has the required strength, blood is drawn from the animal and tested at intervals.
2. Second, when the antisera meets the required strength, some blood is drawn from the horse (without causing any harm to the animal) and preserved in a clean, safe container. Then the antivenin is purified thanks to sedimentation and sifting processes so that non-antibody-related proteins which tend to cause allergy when administered to patients are extracted from the compound that is the most efficient against snake venom.
3. Third, the serum is bottled and dehydrated to make it easy to transport and store for long periods (up to 5 years).
4. Snake antiserum is now ready but to ensure the serum's effectiveness and safety many quality control tests are also conducted on animals under laboratory conditions in compliance standards to be sure that antivenom is proper for human use and 100% safe for patients.

- Since **1894** little progress has been made in antivenin manufacture. In severe envenomation, large amounts of antivenin must be injected and adverse reaction risks are increased. Preservation of antivenin in tropical countries remains problematic. The cost of antivenin limits the supply where such a therapy is strongly useful. One solution could be to improve antivenin manufacturing to better concentrate the specific antibody activity and remove any unsuitable proteins. It would be necessary to identify the toxic components in venoms and to discard venom antigens that are not involved in the envenomation.

6.12 Summary

Reptiles are first true land vertebrate's. They are the first amniotic group of vertebrate animals. They have radiated towards all kinds of habitats, that is, land, water and air. It is now definitely known that the reptiles arose from the amphibians and in turn gave rise to birds and mammals. The characters of reptiles are, in fact, a combination of characters found in fishes and amphibia on one hand and in birds and mammals on the other hand.Reptiles are supposed to be evolved about 250 million years ago from amphibians of Carboniferous period of the Palaeozoic era, which were depended on water bodies for completion of life cycle i.e. laying eggs and development of larval stages and hence could not survive themselves in arid habitats far away from water bodies.

Seymouria was a lizard like reptile tetrapod from the early Permian of North America and Europe, supposed to be existed approximately 280 to 270 million years ago. It was small, only 2 feet long. *Seymouria* was well adapted to life on land, with many reptilian features, in fact, that it was first thought to be a primitive reptile.

In India, there are 330 species only and among them only 69 species are poisonous. Many snakes kill their prey by constriction. It is very difficult to determine the number of snakes bites, but as per the record of the W.H.O., nearly 30000 to 40000 persons die of snake bite in the world every year. Snakebites are more common in tropical regions and in areas that are primarily agricultural. In these areas, large numbers of people coexist with numerous snakes. The poison apparatus of snakes consists of a pair of poison glands, their ducts and a pair of fangs.Diagnosis of snakebite or identification or description of the snake can be helpful in developing a treatment plan as not all snakes are venomous, and different kinds of antivenom exist for different species of snakes that are venomous. The doctor also looks for evidence of fang marks or local trauma in the area of the bite.The snake is almost always more scared of the human, than the human is of the snake. However, most snakes will try to bite if cornered or frightened.

Snake venom is a highly developed form of saliva, injected by the snake into its victim through hollow, modified fangs. Snake venoms contain a multitude of biologically active toxins that work together for the capture of prey. Their effects include pro- and anti-blood coagulation, neurotoxicity, mycotoxicity, nephrotoxicity, cardiotoxicity and necrotoxicity. Amongst these, the neurotoxins play a key role in immobilising prey through paralysis, disorientation and depressed respiration.

Snake *antisera* are a man-made biological product use in treatment of venomous snake bites. Anivenin serum treatments are based on the vaccine process developed by Louis Pasteur, the famous French microbiologist & chemist. Today, antisera is very important from the epidemiological, biochemical, immunological, commercial and economic points of view.

6.13 Self Assessment Questions

1. Describe the distinguishing characters of Reptilia.
2. Describe the salient features with examples of various subclasses of Reptilia.
3. What do you mean by stem Reptiles?
4. Explain the Structure, function and life history of sessile and pelagic tunicates, *Doliolum*
5. Draw a diagram of poison apparatus of snakes.
6. Explain the origin of reptiles.
7. Describe the structure, mechanism of snake biting .
8. Write about first aid measures of snake bite.
9. Describe the precautions during the snake bite.
10. Write an essay on snake venom.
11. Explain the production of anitsera.
12. Give a short note on signs and symptoms of snake bite.
13. Write a short note on *Seymouria*
14. Describe the various components of snake venom.

6.14 Reference Books

- Modern Text Book of Zoology: Vertebrates by R.L.Kotpal
- Text Book of Vertebrate Zoology by *J. S. Kingsley*
- Hyman's Comparative Vertebrate Anatomy by Marvalee H. Wake
- Textbook of Vertebrate Zoology by S. Prasad

Unit - 7

Aves

Structure of Unit:

7.1 Objectives

By the end of the chapter, the student would acquaint himself with the General characters & classification of Aves, Origin & evolution of Aves, a brief idea about origin and evolution of birds from reptiles, Migration of birds, its types and causes. Types of palate in birds- Dromaeognathous or palaeognathous, Schizognathous, Desmognathous, Aegithognathous, Aquatic and flight adaptations in birds, Characteristic features of flightless birds, Flightless birds (Ratitae/ Palaeognathae), Salient Features *of Archaeopteryx,* Different types of modifications of beaks and feet in birds

7.2 Classification of Aves (up to orders)

Introduction

Birds are a specialized group of vertebrates. Young (1958) stated that birds are "the masters of air". Birds are found all over the entire Earth. There are approximately 9700 species of birds living today, and most species are particularly well known. Of the 9700 species, some 5000 species belong to the order Passeriformes, the perching birds or songbirds. Modern Birds are warm bodied creatures belongs to a class of vertebrates characterized by being feathered and bipedal; and by having very high metabolic rates and a forelimb modified into a wing which, together with a long tail, forms part of a flight mechanism.

Birds have originated from reptiles in the Mesozoic era. Birds are supposed to be evolved from some group of reptiles within the larger group of ancient diapsid reptiles known as archosaurs. Externally reptiles and birds are quite different but closer examination reveals that both of them are basically similar. Birds are supposed to be descended from reptiles, as reptiles and birds are very similar Huxley placed these two classes of vertebrates in a single superclass Sauropsida. Birds are better evolved in their organisation but have many reptilian features so Huxley stated that "birds are glorified reptiles."

The once-toothed jaws of birds are modified into the lighter beak in which the upper and lower jaws are covered by a horny rhamphotheca, which may vary in different birds species serving different functions as per their feeding habits.

Feathers are unique to birds. The feathers of birds are filamentous, lightweight modifications of the outer skin that have remarkable aerodynamic qualities. Feathers serves as flight structures by generating lift and thrust and also acts as insulation body cover to maintain high body temperatures. The tail feathers of modern birds are attached to a specialized bone known as the pygostyle, which is formed by a number of fused tail vertebrae. The pygostyle also accommodates the uropygial gland, or oil gland, an essential part of the anatomy of birds that provides a rich waterproofing oil for preening the feathers. In addition to all these functions, feathers can produce different colour patterns and structural forms that allow for species recognition and courtship displays.

Birds have lightweight hollow bones, a well-developed air-sac system and flow-through lungs, a wishbone or furcula made up of fused clavicles, and a hand reduced to three digits. Birds lay eggs which they incubate until hatching or, in some cases, get some other bird to do the incubating. In some species such as our songbirds, the young hatch naked and helpless and must be fed and cared for by a parent bird. They are still dependent for protection upon adult birds.

Birds range in size from thumb-sized hummingbirds to ostriches larger than a big man. Birds walk, run, hop, swim, perch, cling, fly and even dig. They live in woodlands, open areas, cities, farms, lakes, swamps even the open ocean. They lay their eggs and raise their young in holes in the ground, in nests of varying complexity in vegetation or on the ground, in holes in trees, in human-constructed nest boxes, and in or on various parts of buildings.

In birds different modifications of feet are also present. Birds have well developed senses of vision and hearing. The sense of smell (olfaction) is not particularly well developed, although in some birds there is a good sense of smell. Birds have well developed respiratory system with a flow-through lung and an extensive air-sac system. Birds have a well-developed sternum with a keel, or carina, for the attachment of the large flight musculature.

Class Aves: General Characters and Classification

The birds are best known and most- easily recognized animal. They have branched off from the reptiles. Both reptiles and birds resemble each other in many respects, so Huxley placed the two classes of vertebrates in a single superclass, Sauropsida. Although birds have many reptilian features but they are highly specialized than reptiles. The specialized features of birds are:

1. The body is covered with feathers, spindle-shaped and consists of four regions; head, neck, trunk and tail.
2. These creatures are warm-blooded, air breathing, oviparous, bipedal flying vertebrates.
3. The jaws are modified into a toothless beak or bill.
4. The birds have two pairs of limbs. The forelimbs are modified into wings for flying. The hind limbs are adapted for walking, running, perching, swimming, etc. They bear four clawed digits of which the first one or hallux is directed backwards and the remaining three in front.
5. The body is covered with an epidermal exoskeleton in the form of feathers covering the body, scales on legs, claws on toes and horny sheath on beak.
6. The skin is dry and skin glands are absent, except oil or preen gland at the root of tail.
7. The endoskeleton is made up of pneumatic bones which are hollow and without bone marrow. As a result body is light weighted.
8. The skull is smooth, with indistinct sutures, diapsid type and monocondylic, bearing single occipital condyle.

9. The lower jaw or mandible consists of five or six bones and articulate with the quadrate.
10. The vertebrae have heterocoelous centrum. Neck long, so cervical vertebrae numerous.
11. A bony structure synsacrum is present in the vertebral column which is formed by the fusion of posterior thoracic, lumber, sacral and anterior caudal vertebrae. The last 3 or 4 caudal vertebrae are fused to form a "ploughshare bone", the pygostyle.
12. The sternum is large and bears a keel for the attachment of flight muscles.
13. The ribs are double headed or bicephalous and each bears a backwardly directed uncinate process.
14. The pectoral girdle has a stout coracoids and a sabre-shaped scapula. The clavicles of the two sides fuse to form a V-shaped bone called furcula or wish bone or merry thought bone.
15. There is a large, strong pelvic girdle which is fused with the synsacrum. The acetabulum is perforated.
16. The distal carpals are made up with fusion of three metacarpals called as carpometacarpus. The proximal carpals are free.
17. The proximal tarsals fuse with tibia to form tibio-tarsus. The distal tarsals fuse with II, III and IV metatarsals to form tarso-metatarsus. First metatarsal remains free. The ankle joint is inter-tarsal.
18. The heart is four chambered.
19. Sinus venosus and truncus arteriosus are absent. The right systemic arch only persists, the left one disappears. The red blood celld are nucleated.
20. The lungs are compact, spongy and non-distensible and provided with thin walled air sacs.
21. Larynx is without vocal cords. A sound box or syrinx is present.
22. The kidneys are metanephric and three-lobed, urinary bladder is absent.
23. Birds are uricotelic.
24. Brain with greatly developed cerebrum, cerebellum and optic lobes.
25. There are twelve pairs of cranial nerves.
26. The eyes are surrounded by sclerotic plates and contain vascular pectin.
27. The olfactory organs are less developed.
28. The birds have three chambered cloaca.

29. Birds are unisexual and sexual dimorphism is present, female has single ovary and oviduct. Male without copulatory organ except in ratitae, ducks, etc.
30. Fertilization is internal.
31. Birds are oviparous.
32. Body development is direct.
33. The eggs are cleidoic and amniotic and require external incubation for development.
34. Cleavage is discoidal and meroblastic.
35. The embryo develops four extra-embryonic membranes performing specific functions.

7.3 Classification

The classification of birds involves grouping of birds into categories according to physiological similarities, and more recently, by consideration of their genetic make-up. The system of classification was created by Carl Linnaeus. There are about 9000 living species of birds.

The class Aves is divided into two subclasses: Archaeornithes and Neornithes.

Subclass I: ARCHAEORNITHES (Gr. Archios, ancient + Ornithos, bird).

1. These are ancient and extinct birds that lived about 155 million years ago in the Mesozoic era.
2. Tail is long tapering and lizard-like, with more than 30 vertebrae, without pygostyle.
3. Wings are of primitive type, with poor power of flight.
4. Forelimbs with three clawed digits.
5. Jaws are facilitated by teeth.
6. Vertebrae is of amphicoelous type.
7. Sternum without a keel.
8. Ribs without uncinate process, abdominal ribs present.
9. Carpals and metacarpals free.
10. Cerebellum small.

Examples: Archaeopteryx and *Archaeornis.*

Subclass II: NEORNITHES (Gr. Neos, modern, ornithos bird)

1. This subclass is of modern as well as extinct birds.
2. Wings usually well developed, with some exceptions.
3. Vertebrae is of heterocoelous type.

4. The digits of forelimbs without claws.
5. The metacarpals are fused with distal carpals to form carpometacarpus.
6. The sternum has a keel, ribs bear uncinate process.
7. Abdominal ribs are absent.
8. Jaws without teeth except in some fossil birds.
9. Cerebellum large.
10. Tail is short, with 13 or less caudal vertebrae, pygostyle present.

Superorder 1. ODONTOGNATHE (Gr. Odontos, teeth).

1. These all are extinct Cretaceous birds.
2. Toothed beak is present.

Order 1. Hesperornithiformes

1. These are marine, flightless, large-sized birds.
2. Teeth are of pleurodont type(present in grooves)
3. Vertebrae is of amphicoelous type.
4. Pectoral girdle is reduced.
5. Sternum without a keel.
   Examples: *Hesperornis, Enaliornis.*

Order 2. Ichthyornithiformes

1. Teeth are present or absent is not define.
2. Sternum with a keel.
3. Pectoral girdle is well developed.
4. Neck vertebrae amphicoelous.
   Examples: *Icthyornis, Apatornis*.

Superorder 2. PALAEOGNATHAE or RATITAE

1. These birds distribution is restricted. Not represented in India.
2. This superorder is commonly called as flightless, terrestrial birds or ratites.
3. Big body and are good runners.
4. Vestigial or rudimentary wings are present.
5. Feathers without interlocking mechanism.
6. Rectrices absent or irregularly arranged.
7. Oil glands absent except in kiwi and tinamous.
8. Palate dromaeognathous or palaeognathours.
9. Teeth are absent.
10. Sternum without keel, ribs without uncinate process.

11. Bones are not pneumatic.
12. Syrinx absent.
13. Pectoral muscles are poorly developed.
14. Tail vertebrae free, pygostyle small or absent.
15. Male has a copulatory organ.
16. Male incubates the eggs.
17. Young ones are precocious.

Order 1. Struthioniformes

1. Head, neck and legs sparsely feathered.
2. Head comparatively small, neck long and flexible, beak short and broad.
3. Feathers without after shaft.
4. Legs strongly developed, only two toes (third and fourth) present on each foot.
5. Sternum without keel.
6. Pubic symphysis present.
7. Pygostyle absent.

   Examples: *Struthio camelus* (ostrich) found in Africa and Arabia.

Order 2. Rheiformes

1. Swift running birds.
2. Head, neck and thigh feathered.
3. Feathers without aftershaft.
4. Legs bear 3 toes with claws.
5. Sternum without keel.
6. Ischial symphysis present.

   Example: *Rhea americana* (rhea) of America.

Order 3. Casuariformes

1. Head sparsely feathered, bears a comb-like structure, neck and body densely feathered.
2. Feathers with aftershaft nearly equal to shaft.
3. Wings small or rudimentary.
4. Legs with three clawed toes.
5. Sternum without keel.

   Examples: *Casuarius* (cassowaries) of Australia and New Guinea, *Dromaius* (Emu) of New Zealand.

Order 4. Apterygiformes

1. Feathers hair-like, without aftershaft.
2. Wings rudimentary or degenerate.
3. Beak long and slender with nostrils at the tip.
4. Neck and legs comparatively short. Legs with four toes.
   Example: *Apteryx* (kiwi) of New Zealand.

Order 5. Dinornithiformes

1. Giant, extinct birds, became extinct about 700 years ago.
2. Feathers with large aftershaft.
3. Wings almost absent.
4. Beak short.
5. Legs massive bearing four toes.
6. Sternum reduced and without keel.
7. Pectoral girdle completely absent.
   Example: *Dinornis* (moas) of New Zealand.

Order 6. Aepyornithiformes

1. Recently extinct birds.
2. Wings vestigeal or tiny.
3. Legs powerful, with four toes.
4. Sternum short, broad and without keel.
5. Coracoids, scapula and wing bones reduced or absent.
   Examples: *Aepyornis, Mulleornis* (giant elephant birds) of Africa and Madagascar.

Order 7. Tinamiformes

1. They are great runners (cursorial)
2. Sternum with keel, pygostyle reduced.
3. Egg shell glossy.
   Examples: *Tinamus (tinamous), Eudromia*.

Superorder 3. IMPENNAE

1. Include aquatic flightless birds.
2. Wings paddle-like
3. Feet webbed.
4. Small scale-like feathers cover the whole body.
5. Come ashore to breed.
   Example: Aptenodytes (penguins) of Southern Hemisphere.

Superorder 4. NEOGNATHAE or CARINATAE

1. These are modern flying birds.
2. Wings well developed, feathers with interlocking mechanism.
3. Rectrices arranged regular.
4. Pterylae regular.
5. Oil gland present.
6. Skull neognathus, that is, vomer is short allowing the palatines to meet. Skull sutures disappear. Quadrate double-headed.
7. Sternum with well-developed keel.
8. Ribs with uncinate process.
9. Pygostyle present.
10. Scapula and coracoids meet at a right angle. Clavicle well developed.
11. Ilium and ischium united posteriorly.
12. Copulatory organ absent in male.
13. Young are altricious.
14. Distributed throughout the world.

The superorder Neognathae includes several orders. Only a few interesting orders are mentioned below with known examples. For convenience sake these orders have been grouped in six ecological group on the basis of the habits.

GROUP A. ARBOREAL BIRDS

This group includes those birds which live in and around tree and shrubs. Majority of birds belong to this group.

Order 1. Passeriformes

1. Largest order including half of the known species.
2. Toes three in front and one behind, adapted for perching.
3. Beaks adapted for cutting.

   Examples: *Passer domesticus* (common house sparrow), *Corvus splendens* (common house crow), *Corvus macrorhynchos* (Indian jungle crow), *Acridotheres tristis* (common myna), *Saxicoloides fulicata* (Indian robin), *Oriolus oriolus (*golden oriole), *Molpestes* (Bulbul), Swallows, Sturnus (starlings), *Alauda* (Larks), Loxio (crossbill), *Fringilla (*finches).

Order 2. Piciformes

1. Includes highly specialized wood-boring birds like woodpeckers, toucans, sap-suckers, etc.
2. Bill is hard and powerful.
3. Tongue is long and protrusible.

4. Insectivorous.
   Examples: *Dendrocopos mahrattensis* (Yellow front-pied wood pecker), *Dinopium benaghalensis* (golden-backed woodpecker).

Order 3. Columbiformes

1. Includes doves and pigeons.
2. Beak usually soft and slender.
3. Crop large, producing pigeon milk to feed young ones.
4. Fruit and grain-eating birds.
5. Mostly good fliers.
   Examples: *Columba livia* (blue-rock pigeon), *Crocopus* (green pigeon) *Goura crustata* (crowned pigeon), *Streptopelia risoria* (ring dove), *Streptopelia chinensis* (spotted dove).

Order 4. Psittaciformes

1. The main members of this order are parrots, parakeets, cockatoos, macaws, etc.
2. Beak stout, narrow, sharp-edged and hooked at the tip.
3. Upper jaw movably articulated with the skull.
4. Feet zygodactylus, that is, two toes in front and two toes behind. Outer toe not reversible and have considerable memory.
5. Feathers green, blue, yellow or red.
   Examples: *Psittacula eupatria* (large Indian parakeet), *Psittacula lameri* (green parrot).

GROUP B. TERRESTRIAL BIRDS

Order 5. Galliformes

1. These are game birds with short powerful flight.
2. They have feet usually modified for scratching and running.
3. Beak short and feathers are with aftershaft.
4. Graminivorous (grain-eating) and palatable.
   Examples: *Gallus* (jungle fowl), *Pavo cristatus* (peacock), *Phasianus* (pheasant), *Francolinus* (partridge), *Cotrunix* (quil), *Alectoris grecca* (chukor).

Order 6. Cuculiformes

1. The main members of this order are different species of cuckoos.
2. The female usually lays eggs in the nests of other birds, only some species make their own nests.

3. Two toes in front and two behind, outer one reversible and tail is long and beak moderate.
   Examples: *Cuculus canorus* (cuckoo), *Eudynamis scolopaceous* (koel).

GROUP C. SWIMMING AND DIVING BIRDS

Order 7. Anseriformes

1. Includes aquatic birds like ducks, geese and swans.
2. Beak broad and flattened, covered with soft cornified epidermis, margins of beak bear many transverse horny ridges or lamellae. Tongue fleshly.
3. Tail usually short, with many feathers.
4. Legs short and feet webbed.
   Examples: *Anas* (wild duck or mallard), *Cygnus* (swan), *Anser indica* (bar-headed goose).

Order 8. Coraciformes

1. Includes kingfishers.
2. Beak strong.
3. Third and fourth toe fused at the base.
   Examples: *Halcyon smyrnensis* (white-breasted kingfisher), *Ceryle rudis* (pied kingfisher), *Dichoceros bicornis* (grey hornbill), *Upupa epops* (hoopoe).

Order 9. Gaviiformes

1. Legs short, toes webbed.
2. Tail consists of 18-20 short stiff feathers.
   Example: *Gavia,* represented by four species.

Order 10. Podicipediformes or Colymbiformes

1. Freshwater divers with legs placed for back on the body, feet lobed.
2. Tail with a tuft of downfeathers.
   Example: *Podicipes* (grebes).

Order 11. Procellariformes

1. Includes seabirds like albatrossuc, petrels and shearwaters.
2. Nostrils tubular.
3. Wings long and narrow.
4. Feathers compact and oily in texture.
5. Nest usually on islands.

Examples: Diomedea (albatross), *Procellaria* (petrel) and Puffins (shearwaters).

Order 12. Pelecaniformes

1. Fish-eating and colonial. Nest in colonies on rocks.
2. Beak long, with wide gap for catching and swallowing the prey.
3. Nostril vestigeal or absent.
4. Feet four-toed and webbed.
5. Gular pouch present on the throat.
   Examples: *Pelecanus* (pelican), *Phalacrocorax* (little cormorant), *Anhinga* (darter).

GROUP D. SHORE BIRDS AND WADING BIRDS

This group includes aquatic birds. They live near the shore and rarely swim or dive.

Order 13. Charadriformes

1. The legs are long and slender, toes are webbed.
2. Beak mud-probing.
3. Feathers dense and firm.
   Examples: *Lobivanellus indicus* (red-wattled lapwing), *Hydrophasianus chirugus* (pheasant-tailed jacana), *Tringa glariola* (sandpiper), *Capella* (snipe), *Larus* (gull).

Order 14. Ciconiformes

1. Wading birds are with long-legged living in a marshy areas.
2. Neck long and snake-like and beak javelin or pincer-like for piercing aquatic prey (mainly fishes)
3. Feathers decorative and strong fliers.
4. Toes without web, young ones are borne naked.
5. Some are migratory.
   Examples: *Bubulcus ibis* (cattle egret), Ardea (great blue heron), *Phonicopterus* (flamingo), *Ciconia* (stork).

Order 15. Gruiformes

1. This order consists of small to large-sized wading birds with long legs and partially webbed feet.
2. Beak heavy and omnivorous diet.
   Examples: *Fulica atra* (common coot), *Antegone antegone* (sarus crane), *Choriotis* (bustard).

GROUP E. PREYING BIRDS

Order 16. Falconiformes

1. Diurnal and strong fliers.
2. Beak short and curved at the tip.
3. Feet with sharp curved claws adapted for grasping and holding the prey.
   Examples: *Milvus migrans* (common kite), *Sarcogyps calvus* (king vulture), *Astur badius* (hawk), falcon.

Order 17. Strigiformes

1. Nocturnal birds with large rounded head.
2. Eyes large and directed in front. Each located in a disc of radial feathers.
3. Beal short.
4. Feet feathered upto the toes. Claws sharp, adapted for grasping prey.
   Examples: *Bubo bubo* (great horned owl), *Ketupa ceylonensis* (brown fish owl).

GROUP F. AERIAL BIRDS

Aerial birds are the birds with well-developed wings and poorly developed feet

Order 18. Micropodiformes or Apodiformes

1. Small insectivorous birds are present in this order.
2. Beak small and weak, with long tubular tongue.
3. Wings long and pointed.
   Examples: *Micropodus* (Indian swift), *Trochus* (humming bird).

Order 19 Caprimulgiformes.

1. These birds are shy in nature, nocturnal and insectivorous.
2. Feathers are soft.
3. Beak is small and delicate.
4. Mouth wide, margined with long bristle-like sensory feathers.
   Examples: *Caprimulgus* (goat suckers), *Chordeiles* (night hawk).

7.4 Origin and evolution of birds

The birds are supposed to be evolved from the reptiles, birds of today are more closely related to reptiles than to mammals. Long ago the anatomists predicted that birds emerged out from basal reptilian stock and according to Goodrich, the Sauropsid line. The birds originated about 165 million years ago in the Jurrasic period.

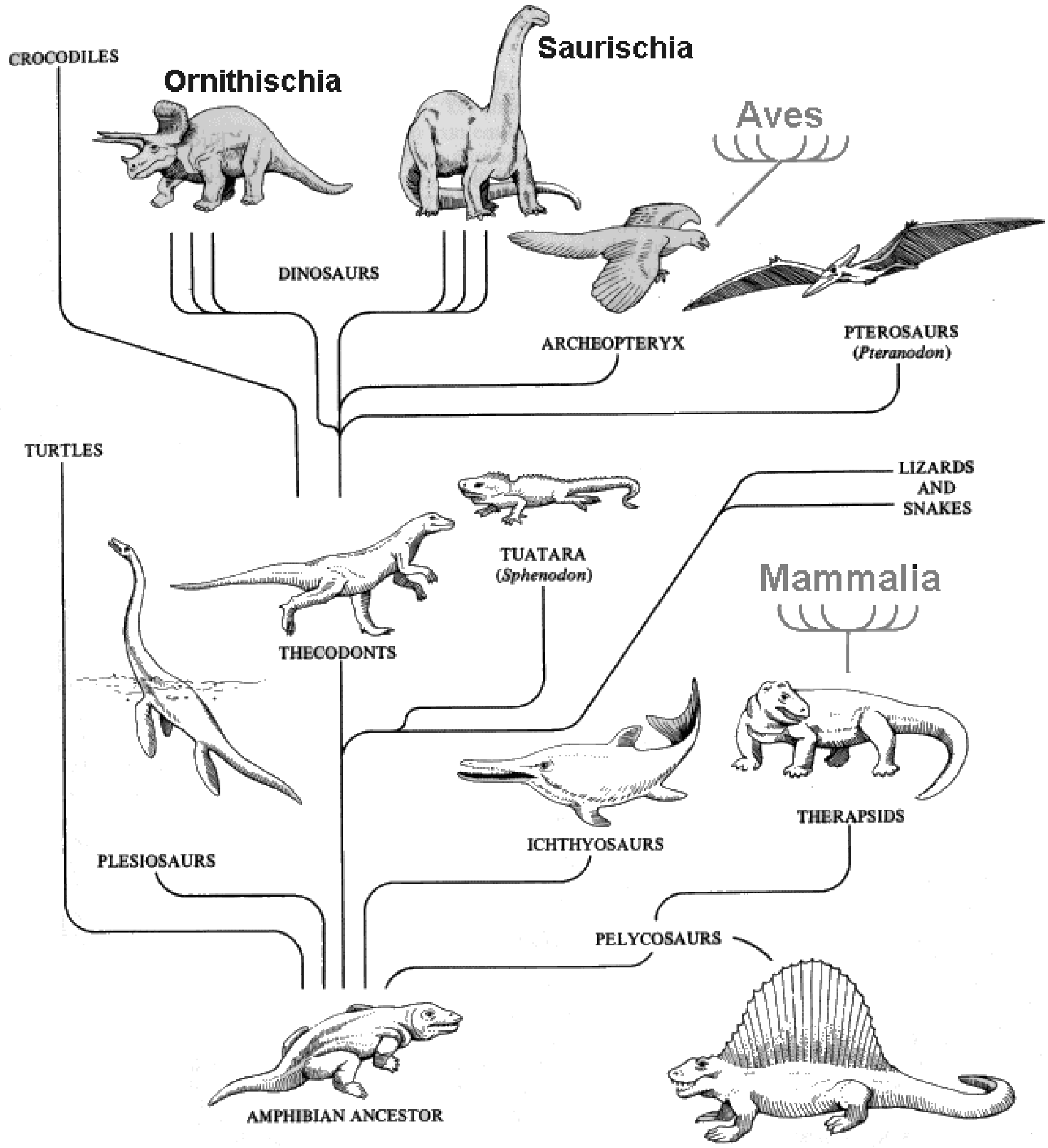


Figure – Evolution of Birds from reptiles

The fossil record of birds is very incomplete and fragmentary. The transitory stages in bird evolution are missing. This led to various speculations regarding the immediate ancestors of bird. Some derived it from Pterodactyles and some from dinosaurs. It now seems likely that the Jurrasic bird *Archaeopteryx* is a form intermediate between reptiles and modern birds. It shows avian as well as reptilian features, hence it is a connecting link between the two groups. The newly discovered fossil bird Protoavis is nearer to the ancestors of birds than *Archaeopteryx.* Protoavis was nearly as big as a crow. The bones of forelimbs and hand had a number of nodes to which feathers were attached. The hindlimbs were strong and it could run like dinosaur.

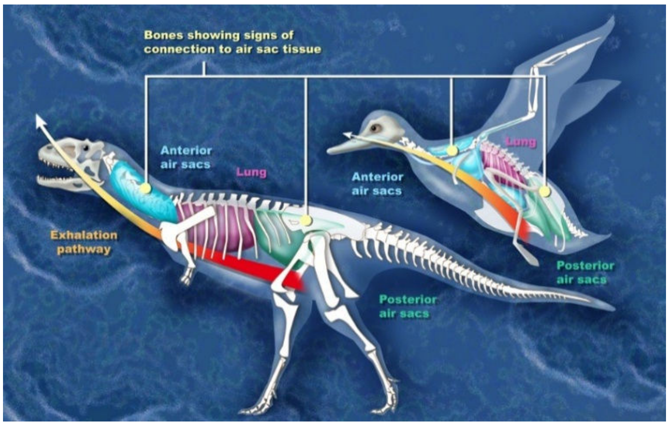


Figure – Relation between reptiles and birds

The different evidences for reptilian origin of birds can be furnished by their comparative anatomy, embryology and palaeontology.

7.5 Anotomical Evidences

Exoskeleton

1. The body of reptiles is covered by epidermal scales which persists in birds on the legs and feet.
2. Birds have feathers which are homologous to the scales of reptiles. Both are epidermal derivatives.
3. The beak of turtles and tortoises is like the beak of birds. The horny covering (rhamphotheca) of the beak of bird is comparable to the reptilian scales. In some birds like puffin the outer covering of beak is shed off every year. This process is similar to the moulting of scales in reptiles. Moulting is also seen in many birds which cast off and renew their feathers periodically.
4. The horny claws present on the toes have the same basic structure in reptiles, birds and mammals.

Endoskeleton

1. The skull in birds and reptiles is well ossified, diapsid type and with single occipital condyle (monocondylic). The jaw suspensorium is autostylic. The basisphenoid is well developed and parasphenoid is poorly developed. An interorbital septum is present (absent in snakes and legless lizards).
2. In both in birds and reptiles the lower jaw is complex and is made up of five or six bones in each half. It articulates with the skull by the quadrate bone of upper jaw.

3. The vertebrae are without epiphyses (present in mammals). The neck vertebrae variable in number.
4. In class both cervical and thoracic ribs present. In young birds the cervical ribs are free but in adult they fuse with the centra of cervical vertebrae. The thoracic ribs form a complete basket and meet the sternum in the mid-ventral line. Ribs are provided with uncinate processes in birds, crocodiles and Sphenodon.
5. The pectoral and pelvic girdles are similar in structure in both the classes. There is a well-developed single coracoids in birds but in reptiles it is divided into precoracoid and coracoids proper.

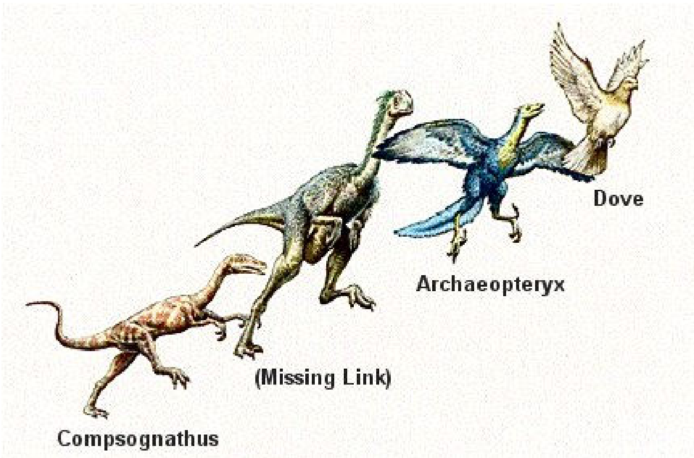


Figure - Evolution of birds from reptiles or dinosaurs

Digestive system - In birds and reptiles the alimentary canal has a gizzard, they habitually swallow stones to help in digestion. In both reptiles and birds the alimentary canal ends in a tripartite cloaca (the three chambers are coprodaeum, urodaeum, and proctodaeum). The division in birds is not very distinct. A caecum is present.

Respiratory system -The lungs are the organs of respiration in both reptiles and birds. The air sacs characteristic of birds occur only in Charmaeleon among lizards. The trachea had complete tracheal rings.

Circulatory system -The heart is completely four chambered in birds and in crocodiles among reptiles. The blood in both groups is similar and the red blood cells are nucleated. In birds the right aortic arch forms the systemic aorta and the left aortic arch disappears. In crocodiles the left aortic arch is much smaller than that of the right side.

Nervous system -In both the groups the cerebellum is divided into a median vermis and lateral floculi and the mid-brain contains two optic lobes called corpora bigemina. Both have 12 pairs of cranial nerves except snakes.

Sense organs -In birds, crocodiles and dinosaurs, the front part of the eye has a ring of minute bony plates, the sclerotic ossicles. The eyes of birds and crocodiles contain a comb-like pectin which projects into the vitreous chamber from the region of the blind spot. The external ear or pinna is absent in both the groups.

Urinogenital system- In both birds and reptiles the kidneys are of metanephric type. Both are uricitelic, they excrete semisolid urine in the form of uric acid. The cloaca reabsorbs water. The rectum and genital ducts open into the cloaca.

Embryological Evidence

On the basis of embryogeny both reptiles and birds show following similarity in embryology.

1. Copulation occurs in both.
2. Fertilization is internal.
3. Both are oviparous and the eggs are large and telolecithal and laid on land.
4. The eggs are cleidoic, i.e., surrounded by a thick hard calcareous shell.
5. The ovum is surrounded by albumen, an egg membrane and a shell, all of which are secreted by special glands present in the wall of the oviduct.
6. Sperms are similar in size and structure.
7. Development requires incubation.
8. Cleavage is meroblastic and discoidal.
9. Extraembryonic membranes – amnion, chorion, allantois and yolk sac are present to protect the embryo.
10. The mechanism of gastrulation and mesoderm formation is similar.
11. The blastoderm has two regions: area pellucid and area opaca.
12. Primitive streak appears during gastrulation.
13. Both groups develop gill slits in the embryonic stages which disappear in the adults.
14. In reptiles an interclavicles is present in the pectoral girdle. A similar interclavicle develops in the embryo of birds.
15. Jacobson’s organ is present in reptiles and in birds it is present in the embryonic stage.

7.6 Migration of birds

Bird Migration is one of the most spectacular event in the animal kingdom. It is the seasonal movement of a number of birds as a habit to obtain the advantage of favourable conditions in other regions. Bird migration is the regular seasonal movement, often north and south along a flyway, between breeding and wintering grounds. Many species of bird migrate. Migration is different from unidirectional movement and those in which animals are helplessly carried by some other agency. It is a two way journey between their summer and winter homes. It is not compulsory activity for all birds. Those bird which remain in one country throughout the year are called resident birds and those which migrate are called migratory birds. Migration carries high costs in predation and mortality, including from hunting by humans, and is driven primarily by availability of food. It occurs mainly in the northern hemisphere, where birds are funnelled on to specific routes by natural barriers such as the Mediterranean Sea or the Caribbean Sea.

Birds migrate to obtain better surroundings for food, nesting and breeding and for getting favourable climatic conditions. Bird migration is an inborn custom or an instinct. There are several factors which act as stimulus in initiating migratory instinct. These are scarcity of food, shortening of day length, fall in temperature and ripening of gonads.

With regard to periodic seasonal movements, or migration, all birds can be classified as belonging to one of four groups:

- **Permanent residents**, or just "residents," are non-migrating birds such as House Sparrows who remain in their home area all year round.
- **Summer residents** are migratory birds such as Purple Martins who arrive in our Northern backyards in the spring, nest during the summer, and return south to wintering grounds in the fall.
- **Winter residents** are migratory birds who have "come south" for the winter *to* our backyards. White-throated Sparrows, who are summer residents in much of Canada, are winter residents in much of the U.S.
- **Transients** are migratory species who nest farther north than our neighbourhoods, but who winter farther south; thus we see them only during migration, when they are "just passing through.

7.7 Types of migration

Birds migrate in a different ways. Following kinds of migration has been observed in birds:

1. Latitudinal migration: This type of migration is the most common type of bird migration. The bird moves from north to south and south to north. Most birds migrates towards the north temperate and subarctic region where there are facilities for feeding and nesting during warmer months and then leave to South during winter when the north is covered with ice and snow. Many birds of North America and Eurasia cross the equator and spend winter in warmer parts of South America and Africa. Siberian birds migrates to spend their winter in the plains of Himalayas in India. Another bird American golden plover migrates for the nine months of winter, 8000 miles south in the plains of Argentina thus enjoying two summers in a year. In the Southern hemisphere of earth, lesser migration occurs when the seasons change.
2. Altitudinal or vertical migration: Almost everyone knows that birds fly south for the winter. But few are familiar with the concept of vertical migration, the seasonal movement of birds and mammals in mountainous areas. Rather than changing latitudes, many mountain residents adapt to the seasons by altering the elevation of their residence. Heavy snows and severe winter weather can impair the survival of mountain dwellers and they have thus developed the instinct to move on to lower terrain as the days shorten. Some birds migrate upper area and downward area on the mountain slopes as weather changes.

   For example in India many species of birds migrate from the lowlands to the slopes of Himalayas several thousand feet above sea level during summer and return to valleys as winter begins. Vertical migration also occurs in grebes and coots of Andes in Argentina and violet green swallows of Great Britain. Few other examples are Townsend's solitaires, red-breasted nuthatches, dark-eyed juncos (gray-headed race), Cassin's finches, pine siskins, mountain chickadees, red crossbills, golden-crowned kinglets and yellow-rumped warblers.
3. Longitudinal migration: Some birds migrates as per longitudinal path i.e. from east to west and vice versa to avoid winters. For example, the starling of Eastern Europe passes through Atlantic coast to avoid

continental water, sterlings move from East Europe or Arid (breeding place) towards Atlantic coast (feeding place).

4. Partial migration: Many species of birds of temperate region show partial migration. Some new individuals are added to the resident population of the same species for a short period. All the birds of a group of migratory birds do not leave the home land. They stay at the breeding place. Such a migration is known as partial migration For example, barn owls, blue birds and blue jays of Canada and northern United States move southwards and mix with the resident population of that region.
5. Irregular or erratic or vagrant migration: Some birds migrates in irregular patterns such as heron, thrushes and warblers, after breeding move away from their home with the young ones and disperse in all directions over a few hundred miles in search of food and safety from enemies.
6. Seasonal migration: By seasonal migration we means that some birds migrate regularly at definite seasons year after year. For example, swifts, swallows, cuckoos and nightingales migrate from south to north during summer. These birds are called summer visitors. Certain birds like fieldfare, snow bunting and redwig migrate from north to south during winter. They are called winter visitors. Some birds like snipes and sandpipers are called birds of passage as they are seen for a short time twice in a year on their way to north or south.

7. Daily migration: Some birds fly from their nest early in the morning and return to the same nest in the evening or vice versa. Thus daily migration is of two types:
   a) Diurnal migration: This migration takes place during daytime for example some birds like crows, swallows, robins, hawks, blue birds, jays, cranes, ducks, geese, swan, pelicans, etc. fly during the day. They are called diurnal migrants. They usually travel in flocks which may be well organized (ducks, geese, swans) or loose (swallows).
   b) Nocturnal migration: when migration takes place during night time it is called as nocturnal migration. For example some small sized birds like sparrows, warblers, thrashes, etc. are nocturnal migrants because they fly at night and rest during the day. They thus escape their enemies.

Speed of migration -The speed or velocity of flight in migration depends upon birds species type. The birds usually fly with tremendous speed ranging from 30 miles per hour in case of small birds like golden plover to 200 miles per hour for large birds like swift, etc. They can travel non-stop at this speed in a day or a night, usually they travel for 5-6 hours and then stop for food or drink. The body fat serves as an efficient fuel during flight. The longest non-stop flight has been recorded for golden plover which covers a distance of 2,400 miles from Hudson Bay and Alaska to South America.

Range of migration - Birds travel can be range even to thousands of miles to reach their destination. The distance covered depends on species and environmental conditions. The bird which covers the longest distance is the arctic tern which travels 11,000 miles from ice free coasts of Labrador inside the Arctic Circle to Antarctica in winters and returns again through the same route in summers. White storks cover a distance of 8,000 miles and golden plover 2,400 miles. Himalayan snow partridges come down only a few feet while the cicadas may descend nearly 8,000 feet.

Regularity of migration - Bird show striking regularity and accuracy in migration. They are very punctual in their time of arrival. The purple martins are known to return on the same day every season.

Routes of migration -The most remarkable and astonishing feature of bird migration is that the birds come and go by the same route year after year. For example, when the swallows return from their winter abode in South Africa to Europe they visit the same village and also the same tree or building for nesting.

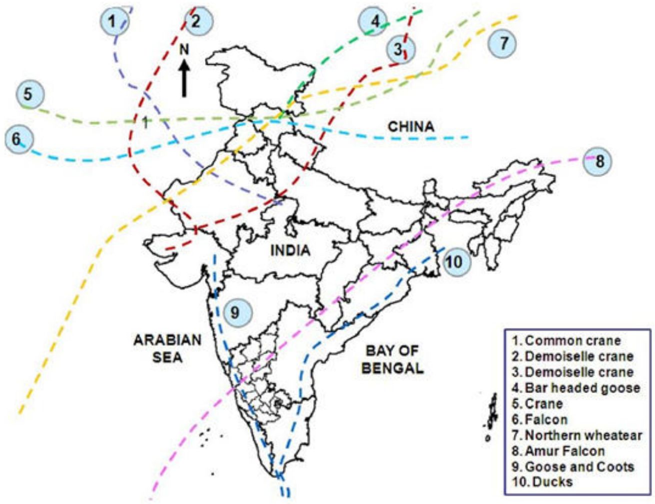


Figure: The different migratory birds route through India

7.8 Bird navigation

Even today there is a big mystery that how do birds navigates or find their way has been the greatest mystery among the ornithologists. There are a few explanations or theories-

1. Visual landmarks: Some migratory birds detects their path by identifying various types of landmarks or topographical features such as rivers, river valleys, coastal lines, oceans, seas, oceanic islands, deserts, mountain ranges, etc. These landmarks enable them to recognize and remember the routes and entrances to the country. The birds migrating at night, however, cannot easily make use of these landmarks.
2. Magnetic fields: Some naturalists like Van Middendroff and H.L. Yeaglev believes that birds are directed by the earth's magnetic field. Their inner ear reacts with the mechanical Coriolis Effect produced by the rotation of the earth. But there is no evidence to support it.
3. Celestial bodies: Sauer (1957) demonstrated that celestial bodies like stars serve as a guiding force for nocturnal birds. This is true for warblers. Gustava Kramer (1949) Mathews emphasized that the diurnal migratory birds are guided by the position of the sun. Experimenting with sterlings cages, they showed that birds possess an internal time clock or biological clock with the help of which they make necessary adjustments in their course according to the position of the sun.

4. Experience: Some believes that birds learn to find their way by experience. This is possible when old members become leaders and guide the younger generation. However, most birds do not learn from their elders as they do not fly in flocks and in many cases, young birds make their first journey independently. Individual memory may play some part in migration.
5. Air currents: Some ornithologists says that soaring birds like vultures migrate with the help of air currents.
6. Homing instinct: Some ornithologists believe that homing instinct plays a major role in navigation. But experiments with carrier pigeons have proved that vision helps in navigation. In short, how birds find their way is still unknown.

7.9 Causes of Migration

Though the desire to migrate is a hereditary impulse, however, it is influenced by some external and internal causes.

The external causes include —

1. The scarcity of food in the original locality where the birds reside permanently.
2. Shortening of day length
3. Increase of cold and sudden changes in the weather conditions continued for a longer time

The internal stimulus is the maturation of gonads and secretion of certain concerned hormones. It has been suggested by Rowen (1925) that the birds migrate when the gonads are in changing condition. The secretion of interstitial cells in males might be providing stimulus for migration. Increase or decrease in day light affects the maturation of gonads, and thus, migration is affected.

The pituitary hormones are also concerned with the migration of birds as they stimulate the activities of the gonads in both the male and female individuals. Fat deposition in the migratory birds has also been considered as the controlling factor by some workers. Increasing day length affects the activities of the hypothalamus, Pituitary and gonads, which are functionally linked. Thus, the physiological changes in the body induce readiness to migrate. The thyroid hormones are also said to be directly or indirectly concerned with the migratory activities of the birds.

Figure- Migratory Bird (Demoiselle cranes) at Khichan Village, Jodhpur, Rajasthan

Advantages of migration

The following are the main points of advantages daring migration period.

1. The birds avoid harsh environmental condition by migrating in winter.
2. They get long day light hours for searching food when they migrate in winter and thus avoid scarcity of food due to snowfall and freezing and birds get an abundance of food supply and Short day light is avoided which is not sufficient and convenient for food collection.
3. They get suitable and uncongested dwelling places.
4. In summer when they return to the north they get suitable nesting grounds and abundant food.
5. Birds avoid excessive cold and stormy weather for their safety.

The phenomenon of migration is very much essential for the birds. If the suitable breeding area and feeding area are not available to birds at the proper time, several bird species will be subjected to natural elimination. Suitable climatic conditions for safety and comfortable breeding are necessary for them.

7.10 Types of palate in birds

Palate is a structure formed from the assemblage of bones situated in the roof of buccal cavity of vertebrates. It separates the oral cavity from nasal passage. It helps the nasal passage to open far behind into the buccal cavity. The palate helps the animal to chew and breathe at the same time. Palate first appeared in some reptiles and is well developed in birds and mammals.

Mainly there are four types of palate in birds.

1. Dromaeognathous or palaeognathous

2. Schizognathous
3. Desmognathous
4. Aegithognathous.

1. Dromaeognathous or Palaeognathous - Dromaeognathous palate is found in flightless birds or Ratitae. Vomer is large and broad and is connected posteriorly with the palatines. Palatines do not articulate with the parasphenoidal rostrum because the vomer intervenes between the two. Basipterygoid processes of the basisphenoid are large and articulate with the posterior part of pterygoid. Maxillo-palatine processes are short and do not unite with one another or with the vomer. Pterygoid is unmovably fixed to vomer.
2. Schizognathous - Schizognathous palate is found in birds like pigeon, fowl, gull, crane, woodpecker, penguin, etc. Vomer is small and pointed or absent. Palatines and pterygoids articulate with the parasphenoidal rostrum where they meet each other. Maxillo-palatine processes are small and do not unite with each other or with the vomer. Basipterygoid processes are small or absent. Pterygoids movably articulated.
3. Desmognathous - Desmognathous palate is found in duck, goose, stork, parrot, heron, birds of prey,etc. Vomer, when present, is slender and tapering anteriorly or it may be absent. Palatines and pterygoids articulate with the parasphenoidal rostrum. Maxillo-palatine processes are large and spongy and unite with each other in the middle to form a flat spongy palate. Basipterygoid processes absent.
4. Aegithognathous - Aegithognathous palate occurs in crow, swift, bulbul, etc. It is similar to schizognathous type of palate. Vomer is short and broad and truncated in front instead of being pointed. Porteriorly it is deeply cleft embracing the parasphenoidal rostrum. In parrot specialized form of desmognathous palate called sliding palate is found. The nasals and the ascending process of the premaxilla are very thin and elastic where they join the skull, thus there is large amount of movement in the vertical plane. A cranio-facial joint or hings occurs between the upper beak and the skull.

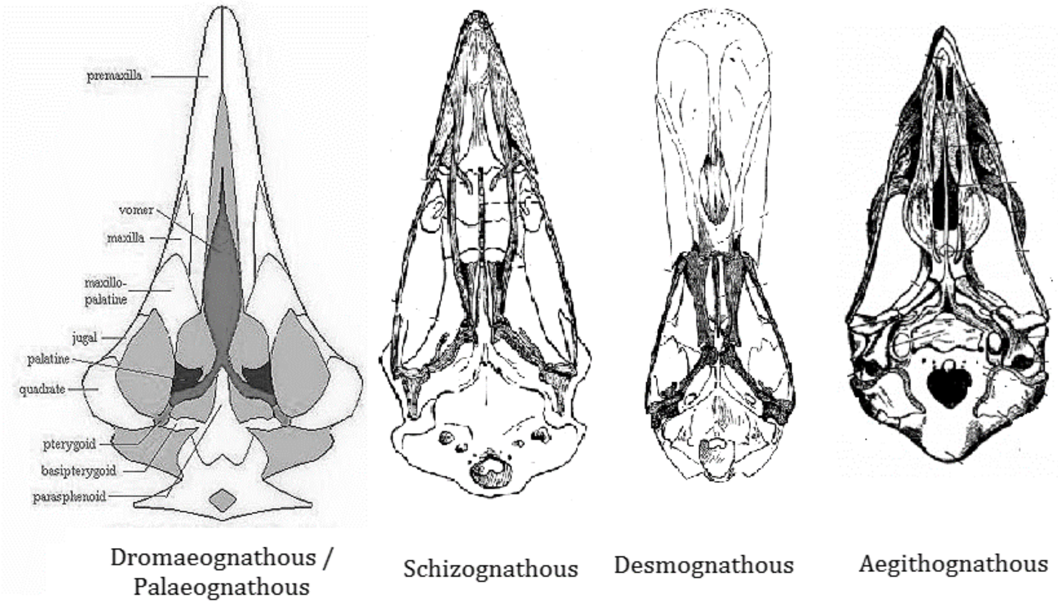


Figure – Different types of Palates

Importance of palate

The palate of birds plays an important role in the classification of birds. Modern birds are classified into two super-orders-Palaeognathae and Neognathae.

Palaeognathae includes all flightless birds (Ratitae). They have palaeognathous palate in which the vomer is large and the two palatines do not meet each other and with the rostrum. Neognathae includes all flying birds. They have neognathous palate which is divided into three subtypes – schizognathous, desmognathous and aegithognathous. In these types the vomer is small or absent and the palatines meet the rostrum.

Aquatic and flight adaptations in birds

Adaptation is the ability of organisms to adjust in the existing environment. It is the adjustment of organisms in the surrounding environment for their existence by changing the body structure accordingly.

Aquatic adaptations

Aquatic environment provide critical habitat to a wide variety of bird species**.** Some aquatic birds divide their time between aquatic and terrestrial environments, while others spend most of their lives in water, returning to land only to breed. Many familiar bird groups are aquatic, including gulls and penguins as well as recreationally important species such as ducks and geese.

Wading birds are most commonly associated with wet lands, streams, and other aquatic habitats. Most wad ing birds possess long legs and toes, and long and sometimes curved bills adaptations enabling them to live and feed in shallow-water habitats. This leaflet addresses birds in the orders Ciconiformes (herons and their allies) and Gruiformes (cranes and their allies).

Wading birds occupy shallow-water habitatsin both fresh-water and saltwater environments. They have long, thin legs that allow them to walk through water easily while keeping the rest of their bodies dry. Some wading species find food by stirring the water with their feet; others use their beaks to filter food. Larger species with long legs and great height also possess long flexible necks that allow them to reach food below the water surface. Well-known wading species include flamingos, cranes, herons, storks, and egrets. Smaller birds include sandpipers and plovers.

Diving birds describe a broad group of species that occupy waters deeper than wading species. These birds dive, plunge, or swim after fish. The American widgeon is a common marsh duck which spends much of its time in deep water. Well-known diving birds include ducks, geese, swans, pelicans, and penguins. These highly aquatic birds have evolved special adaptations to their habitat, such as webbed feet for swimming and waterproof feathers. Salt-water species also possess special salt glands that help excrete the excess salt that results from drinking sea water.

Diving birds associated with marine habitats also are called seabirds. Many seabirds spend large portions of time far from land, and all obtain food from the sea. However, all species return to land to lay eggs. Large breeding colonies of colorful puffins, as well as murres, stormpetrels, and other species attract numerous tourists in the spring.

Some aquatic birds examples

Penguins

Penguins are the most aquatic of all diving birds. In some species, individuals return to land only to breed and molt. Like marine mammals, penguins that dive underwater must return to the water surface to breathe. Many penguins feed on krill which is a small, shrimp-like crustacean, though some species hunt larger prey such as squid and fish also.

Penguins possess a streamlined body and are well-adapted to underwater swimming, achieving speeds as great as 15 kilometers (9 miles) per hour. A specialized fat layer and dense, waterproof feathers help them maintain appropriate body temperatures in frigid Antarctic waters. Penguin wings have been modified through evolution to form paddle-like flippers. Penguins are often described as "flying through water" because their wing motions during swimming resemble those of flying birds.

Sandpipers & Phalaropes

The sandpipers are a large and diverse group of shorebirds. They are widely distributed throughout the world, and are common even in the High Arctic. Sandpipers live in a variety of different habitats- terrestrial, marine and freshwater- and generally spend time in all three over the course of the year. They are typically found in open areas, often, but not always, near water.

Sandpipers are wading birds that tend to have cryptically patterned plumage that enables them to blend into their surroundings. Their plumage ranges from white and pale buff to grey, chestnut brown or black, often with streaks or spots. Both sexes share similar markings. They can be distinguished from other bird families by their long, slender bill, bare upper legs, and long toes. The long legs and toes of sandpipers are an adaptation for running along shores and wading in shallow water. Their long bills allow them to probe through the water to the mud below in search of invertebrates; species that forage in deeper water tend to have proportionally longer bills and legs. Most sandpipers migrate and tend to be gregarious, associating in flocks both with members of their own species and with other species.

Phalaropes spend the majority of their time swimming, rather than walking or wading along the shoreline. Their feet are well adapted to help them swim, having both lobes on their toes to push water, and webs between their toes. They also have very dense plumage on their underside to keep their bodies warm in cold water. This plumage traps pockets of warm air against the bird's body, adding warmth as well as helping it to float. Phalaropes feed on small aquatic crustaceans and insect larvae by spinning on the spot in the water to stir up organisms and then pecking with their long, thin beaks. The vortex created in the water by the spinning bird sucks organisms and particles upwards towards the surface, where they are more easily reached. This behaviour- which is made possible partly by the phalarope's unusually high buoyancy- is unique among birds.

Although in most birds, such as the peacock, the female is much duller than the male, this situation is reversed in phalaropes. Females are much brighter, and slightly larger, than males, and do not participate in caring for the young in any way and therefore do not require camouflage. Instead, females defend territories and use their bright colours to compete for males, who are well camouflaged by their inconspicuous plumage as they build the nests and incubate the eggs. Females often have multiple mates and many nests at once.

Risks

Wading birds rely heavily on wetland habitats including inland and coastal emergent marshes and wooded swamps. Unfortunately at present many wetlands have been converted for agriculture, residential, commercial, and other land uses. Many adjacent native grassland and forest habitats have also undergone significant land use changes.

As a result, many species of wading birds that depend on these habitats have suffered significant population reductions, with some populations still in decline. Protecting and properly managing existing wetland communities can help maintain and enhance populations of wading birds and other wildlife species that live in similar habitats.

Aerial or flight adaptations

Birds are known as the masters of air. Birds are good fliers and the fastest of all animals. Over millions of years, birds have perfected the body structure needed for flight. In fact, a bird's entire being has adapted to a life of soaring through the air. Besides insects and bats, no other group of animals can truly fly. The wings of birds are uniquely adapted to their way of life, from the daily search for food to yearly migrations lasting thousands of miles. Birds inherited from their ancestors wing structures that allow them to escape from predators, take advantage of more food sources, and make life less stressful. The complete body of birds have been modified for flight. In fact, a bird is like a flying machine. It flies on the principle of an aeroplane, a heavier than air machine. The morphology, anatomy, physiology and embryology of birds are highly modified for flight. The birds shows following flight adaptations:

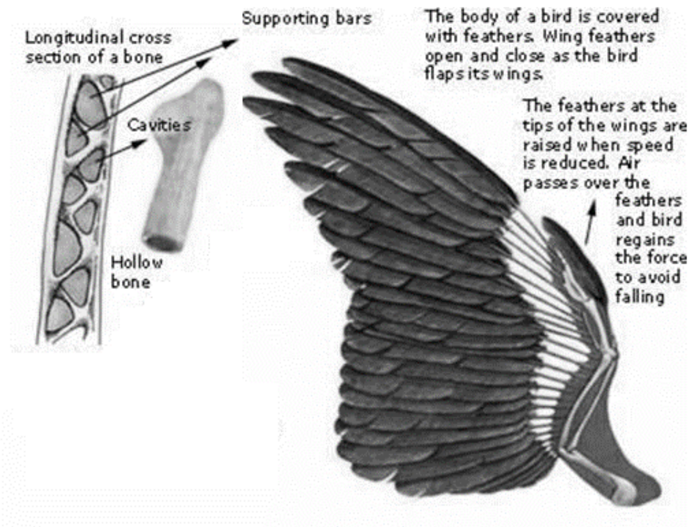
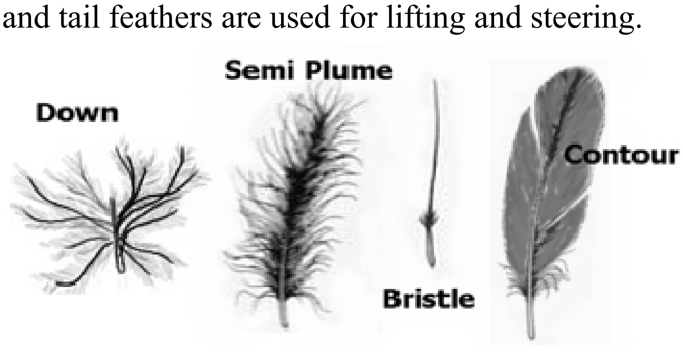


Figure –Different flight adaptations of birds

1. Adaptive body shape and structure - Birds have a stream-lined, boat-shaped or spindle-shaped body which offers minimum frictional force from the air during flying. The body is quite compact. It is light but strong dorsally and heavy ventrally, it helps in maintaining balance in the air. The internal vital organs are well centralized. The attachment of wings high up on the thorax, the high position of light organs like lungs and air sacs and low and central position of heavy structures like muscles, sternum and digestive organs beneath the line of attachment of the wings lowers the centre of gravity.
2. Feathers - The feathers are characteristic features of birds. They fully cover the body. The feathers covering the body reduces frictional force and they are light so they reduce weight. The feathers insulate the body and prevent loss of body heat. This enables the bird to tolerate extreme cold at height and maintain a constant body temperature. The body feathers helps in buouancy. The wing feathers help in striking the air and tail feathers are used for lifting and steering.



3. Figure –Different types of birds feathers

4. Wings - The forelimbs of birds are modified into wings which are used for propulsion through the air. The shape of the wings figure into a bird's flying ability. Short, rounded wings help birds take off rapidly. Long, pointed wings provide speed. Long, narrow wings allow for gliding. Broad wings with slots let birds both soar and glide.
5. Tail -In birds the tail is reduced to a degenerate skeletal stub. It is light and air resisting tail feathers are present. They act as a rudder for lifting, steering and stopping during flight. It also helps in balancing during perching.
6. Beak - The jaws are drawn out into a horny beak. The beak compensates for the modification of forelimbs into wings. It is used for picking up food, in nest building, for preening and for offence and defense.
7. Flexible neck and head - The head is small and light and the neck is long and flexible. It can be retracted during flight, thus shortening the body-axis. The flexible neck allows free movement of head which helps the beak in performing its function easily.
8. Skin – Body is covered with loose covering of skin is quite loose which allows free movement of flight muscles.
9. Flight muscles – Highly developed flight muscles are present forming nearly one-sixth of the total body weight. There are four set of flight muscles. They are:
    a. Pectoralis major
    b. Pectoralis minor
    c. Coracobrachialis
    d. Tensor muscles
10. The pectoralis major and coracobrachialis lower or depress the wings (down stroke) and the wing is raised or elevated (up stroke) by pectoralis minor. The tensor muscles keep the wing stretched while flying. The flight muscles are well aerated and provide the sustained power to maintain flight.
11. Perching -The hindlimbs and their muscles are well developed and help in perching. Thus a resting or sleeping bird is automatically clamped to the perch and does not fall down.
12. Air sacs - The air sacs are thin walled, non-muscular sacs connected to the lungs. There are four paired and one unpaired air-sacs. The paired air sacs are cervical, anterior thoracic, posterior thoracic and abdominal and unpaired interclavicular air sacs. They occupy the space between the

internal organs and extend into the cavities of bones. They serve as reservoirs of air. In birds blood is oxygenated twice during a single breathing. This is called double oxygenation or double ventilation. Thus the respiratory system of birds is highly adapted to supply more oxygen to flight muscles. The air sacs also help in internal perspiration which help in regulation of body temperature.

13. Warm bloodedness - Birds are warm blooded animals, therefore they have perfect aeration of blood and a great output of energy over a long period. The high body temperature helps the birds to take flight and keep them survive at high altitudes and in all seasons.
14. Effective circulatory system - High amount of energy and more oxygen is required for active flight. The circulatory system of birds is highly adapted for carrying more oxygen by one large four chambered heart, efficient double circulation of blood and presence of high proportion of haemoglobin.
15. Digestive system - Birds consumes large amount of food and there is need for rapid digestion because of high metabolic rate. Digestive system is compact but effective. Food eaten by birds is generally of high caloric value, easily digested and with minimum undigested waste. The rectum is short because of small amount of waste material which is excrete out at once.
16. Excretory system - The excretory system of birds is highly modified to reduce weight by absence of urinary bladder. Body excreta liquid is also reabsorbed in the kidney tubules and cloaca such that excretory waste is in the form of semisolid uric acid and urate particles which are emptied at once.
17. Vision -The birds optical senses are more developed in comparison to olfactory sense organs, therefore they depend on their sight rather than smell in contrast to reptiles and most mammals. The eyes are large and their ability to accommodate rapidly is also well developed. This enables them to see objects from very high altitudes. A comb-like structure called pectin is present in the eye of birds helping them in accommodation.
18. Endoskeleton - In the vertebral column about fourteen vertebrae are fused together to form a plate-like structure called synsacrum. The fusion of pelvis with the synsacrum provides firm attachment to the legs, supports body weight during locomotion on the ground and counteracts

the effect of shocks when the bird lands. The heterocoelous cervical vertebrae giving flexibility to the neck. The vertebrae are one thoracic (last), six lumbar, two sacral and five caudal. It acts as a support for the entire weight of the body and also provides a firm fulcrum during flight for wings. The shortening of caudal vertebrae and formation of pygostyle (fusion of last four caudals) provides stability in the air. The sternum bears a mid-ventral ridge called keel or carina to which the flight muscles are firmly attached.

19. The clavicles of the pectoral girdle are fused ventrally to form a V-shaped furcula or merry thought bone or wish bone. It works like a spring between the two wings.
20. The mid-ventral symphysis between the pubes and Ischia is absent. This results in a more posterior displacement of the viscera, shifting the centre of gravity towards the legs.
21. Loss of weight – Less body weight is achieved by the following modifications in body:
    (a) Bones are pneumatic, i.e., they are provided with air sacs.
    (b) The skeletal framework is light but strong.
    (c) Skull bones are thin and light and firmly fused together.
    (d) Thoracic ribs bear uncinate processes which provide compactness by concentrating the mass.
    (e) Teeth are absent
    (f) Ovary and oviduct of right side in female are absent.
    (g) No urinary bladder.
    (h) Production of solid excretory waste.
    (i) Presence of feathers.

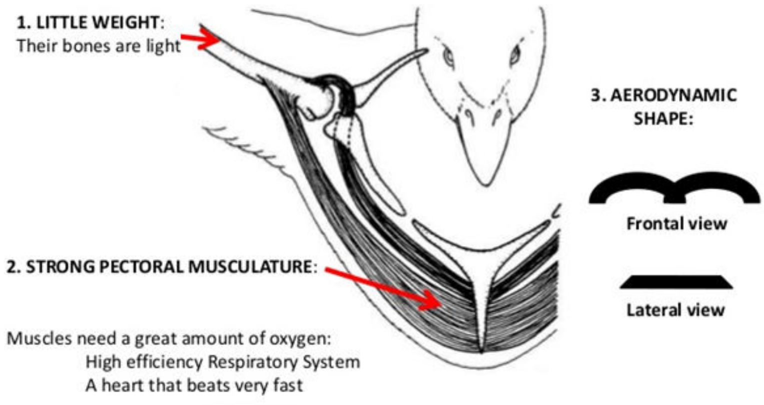


Figure – Flight adaptations of birds

Flightless birds (Ratitae/ Palaeognathae)

Flightless birds belongs to diverse group of super order Ratitae, these are large birds of Gondwanan origin, most of them now extinct. The flightless birds have no keel on their sternum, hence their name which comes from the Latin (*ratis*) for raft. The keel provides an anchor to which a bird's wing muscles attach, thereby providing adequate leverage for flight. Without keel, these birds are not able to anchor their wing muscles therefore they could not fly even if they have well developed suitable wings. The wings and flight muscles are very much reduced, so they are not capable of flight. They are all alike and ground dwellers. They are good runners because their hind limbs are strong and powerful. The flightless birds are generally large in size and some may reach a height of 4 meters.

The superoder Palaeognathae includes 7 orders of which only 4 orders have living representatives and 3 are extinct.

Living orders

1. Struthioniforms – Ostriches
2. Rheiformes – Rheas.
3. Casuariformes – Cassowaries and Emus.
4. Apterygiformes – Kiwis.

Extinct orders

1. Diornithiformes – Moas.
2. Aepyornithiformes – Giant elephant birds.
3. Tinamiformes – Tinamous.

Distribution

The flightless birds are distributed irregularly in different parts of the world. They are found in widely separated places of the world – ostriches in Africa and South-west Asia, rheas in South America, cassowaries and emus in Australia and kiwis in New Zealand. All living flightless birds occupy those parts of the world where there are no terrestrial competitors and enemies. Another interesting fact is that they are found in the southern parts of the world and are totally absent in the northern parts. On the basis of fossils of flightless birds discovered, it is supposed that have also been found in North America and Europe but because of carnivorous animals they disappeared.

1. The bones are solid and not pneumatic with air sacs.
2. There is an absence of anchoring bone keel in sternum.
3. The ribs have no uncinate process which normally supports the thorax.
4. The clavicles are small or absent.
5. The ilium and ishium of the pelvic girdle are unfused behind.
6. The palate is palaeognathous type (the palatines do not articulate with the rostrum, the vomer intervening).
7. The wings are reduced or vestigeal.
8. The feathers are soft and curly. They are not arranged in regular feather tracts. The barbs of the feathers are disconnected and the barbules are without hooks.
9. The pectoral muscles are poorly developed.
10. Syrinx is absent.
11. Oil glands are absent.
12. The newly hatched young one are precocious, i.e., they are not developed on their parents. Male has a copulatory organ, the penis.
13. Female have a clitoris.
14. Male incubates the eggs.

7.11 Origin of Ratitae

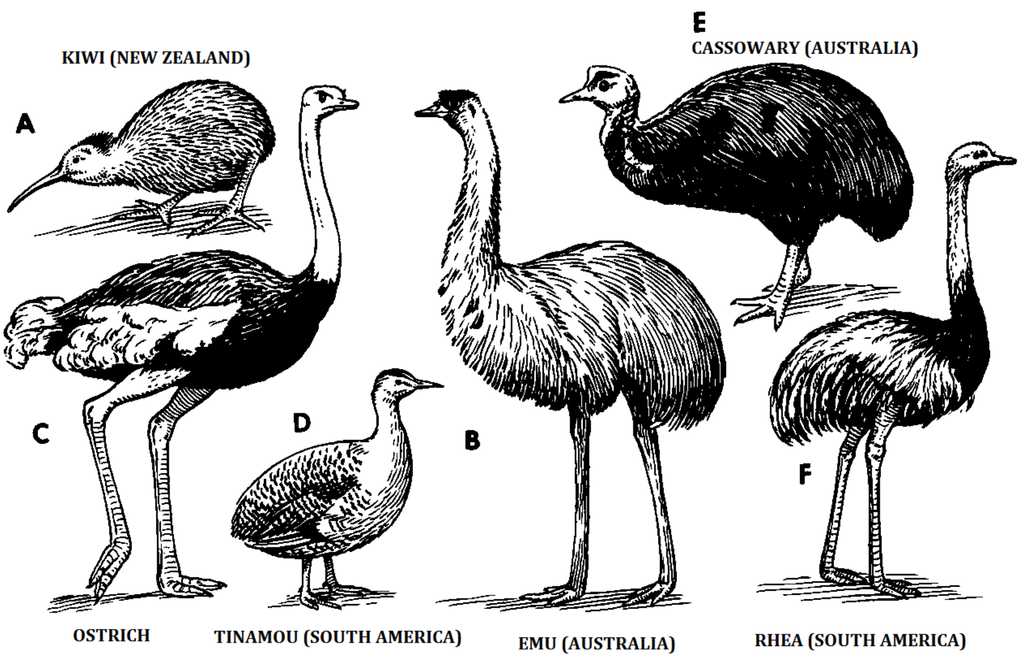
There are two major theories regarding the origin of Ratitae.

1. They have evolved from true flying ancestors.
2. They have evolved from ancestors who never developed the power of fight.

The most accepted view was proposed by Romer. According to him, the Ratitae have evolved from true flying ancestors and only secondarily readapted for a flightless mode of life because the flightless birds possess certain morphological characters showing adaptations to flight. He says that advancement of flight nature in birds is a tool to escape enemies on the ground. If there are no enemies then there is no need for flight. The Ratitae stopped flying and gradually became flightless because of the absence of predators enemies. If the distribution of present day Ratitae is studied and the ancestry of some Ratitae is observed this concept is more acceptable. In the early Coenozoic era a number of neognathous birds were ground dwelling. The mammals also evolved at the same time, so there was strong competition between the ground-dwelling birds and mammals to conquer the ground vacated by the large reptiles which had become extinct. The mammals being carnivorous succeeded, this might have gave rise to the modern flightless birds.

Other concept was given by young, according to him the ancestors of ratitae were not flying birds. The Ratitae never passed through a flying stage, they diverged early from the main stock of flying birds.

Common flightless birds



PALAEOGNATHOUS BIRDS (INCLUDING RATITES)

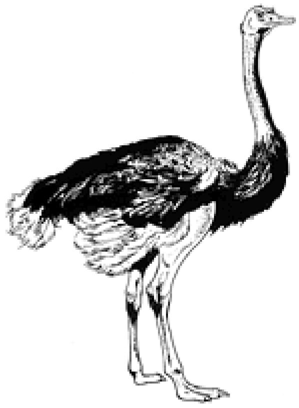
Struthio camelus **(African ostrich)**

- The **Ostrich**, *Struthio camelus*, is a large flightless bird native to Africa (and formerly the Middle East). It is the only living species of its family**, Struthionidae** and its genus, ***Struthio***. Ostriches share the order Struthioniformes with the Emu, kiwis, and other ratites. It is distinctive in its appearance, with a long neck and legs and the ability to run at maximum speeds of about 72km/hour. The Ostrich is the largest living species of bird and lays the largest egg of any bird.

- The diet of the Ostrich mainly consists of plant matter, though it also eats insects. It lives in nomadic groups which contain between five and fifty birds. When threatened, the Ostrich will either hide itself by lying flat against the ground, or will run away. If cornered, it can attack with a kick from its powerful legs. Mating patterns differ by geographical region, but territorial males fight for a harem of two to seven females.
- The Ostrich is farmed around the world, particularly for its feathers, which are decorative and are also used for feather dusters. Its skin is used for leather and its meat marketed commercially. Ostriches usually weigh from 63 to 130 kilograms, with exceptional male Ostriches weighing up to 155 kilograms. The feathers of adult males are mostly black, with white at the ends of the wings and in the tail. Females and young males are greyish-brown and white. The head and neck of both male and female Ostriches is nearly bare, with a thin layer of down.
- The long neck and legs keeps their head at 1.8 to 2.75 metres above the ground, and their large eyes which are said to be the largest of any land vertebrate help them detect predators at a great distance. The eyes are shaded from sun light falling from above.
- Their skin is variably coloured depending on the sub-species. The male tarsus has red horn plates, while the female's are black. The strong legs of the Ostrich, like other birds, are scaled and unfeathered. The bird has just two toes on each foot (most birds have four), with a nail on the larger, inner toe resembling a hoof. The outer toe lacks a nail. This is an adaptation unique to Ostriches that appears to aid in running. The wings reach a span of over about two meters and are used in mating displays and to shade chicks. The wing feathers lack the tiny hooks that lock together the smooth wing feathers of flying birds, and are soft and fluffy and serve as insulation. They have 50-60 tail feathers, and their wings have 16 primary, four alular and 20-23 secondary feathers. The Ostrich's sternum is flat, lacking the keel to which wing muscles attach in flying birds. The beak is flat and broad, with a rounded tip. Like all ratites, the Ostrich has no crop, and it also lacks a gallbladder. They have three

stomachs, and their caecae is 28 inches. Unlike all other living birds, the Ostrich secretes urine separately from feces. They also have unique pubic bones that are fused to hold their gut. The copulatory organ is retractable and 8 inches long. Their palate is different than other ratites, in that the sphenoid and palatal bones are unconnected.

- At sexual maturity (two to four years), male Ostriches can be between 1.8 and 2.8 metres in height, while female Ostriches range from 1.7 to 2 metres. During the first year of life, chicks grow about 25 centimetres per month. At one year of age, Ostriches weigh around 45 kilograms.



***Casuarius casuarius* (Cassowary)**

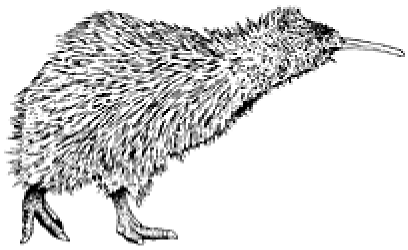
- *Casuarius casuarius* is found in New Guinea (Papua, formerly Irian Jaya, **Indonesia** and **Papua, New Guinea**), including the islands of Seram and Aru, and north-eastern **Australia**. It occurs throughout the lowlands of New Guinea. In Australia, there are 3 subpopulations in Queensland. The Australian population was estimated to number c. 2,500 birds in 2010, but it is declining
- It is a shy, Nocturnal bird. The head is small with compressed beak and a helmet-shaped bony knot on top. The young ones are brown while adults are black in colour. The skin of head and neck is brightly tinted. The quill feathers of the wing are like stiff long rods and hang down freely. The after shaft is as long as the main shaft. The rectrices or tail feathers are absent. There are three toes on each foot. The bird is quarrelsome and quite vicious, if disturbed, the male may even attack man. The native people sometimes domesticate young birds. The flesh of cassowary is palatable.
- It is a solitary and sedentary inhabitant of rainforest, occasionally using adjacent savannah forests, mangroves and fruit plantations. Its diet

largely comprises fallen fruit, although it is fairly undiscriminating. It ranges between 0 m and at least 500 m in Papua New Guinea, and has been recorded up to 1,400 m in Australia.

- In Australia, it was historically threatened by habitat loss and fragmentation. In Indonesia and Papua New Guinea, the species is heavily hunted, captured and traded close to populated areas, being of high cultural importance, and constituting a major food source for subsistence communities

***Apteryx* (Kiwi)**

- Kiwis are native to New Zealand and limited to just three regions in the country. The three main areas are northwest Nelson, Paparoa Range, and the Arthur's Pass-Hurunui district of the Southern Alps.
- Kiwis are stout, long-billed birds with a total length from 45 to 55 cm. They weight 2.4 to 3.3 kg and have bill lengths from 8.3 to 13.5 cm. Females are typically larger than males. Great spotted kiwis appear spotted because they have an arrangement of grey, brown, and off-white feathers. The legs are short and muscular and they have large 4-toed feet; three toes facing forward and one in the back. Kiwis are flightless birds so their wings and tails are reduced in size. Because they do not fly, their feathers are soft, much like the hair of mammals. Kiwis are nocturnal, so their eyes are underdeveloped and their olfactory organs are larger than those of diurnal birds. On their elongated bills, the nostrils are located closer to the tip of the bill. This nostril placement is unique to kiwis. The bills are white grey and take up to five years to develop to their adult size. In captivity the average life span of great spotted kiwis is 12 to 18 years. The maximum lifespan can range from 28 to 33 years. In the wild, kiwis can live 20 years.



Dromaius novaehollandiae **(Emu)**

- Emu is the second largest living bird. The emu is most commonly found in wooded areas but emus are common all over Australia. It avoids dense forests and largely populated areas. Although the emu does prefer to be in woodland or shrub land where there is plenty to eat as well as cover, they like to know exactly what is around them. In Australia there are enormous emu farms where the emu is bred for meat, oil and leather. Its body fat is used as a lubricant. Emu oil is said to hold medicinal healing properties when rubbed onto painful joints and is commonly used across the world mainly for sports injuries but also arthritis.

- Emus can grow to nearly 2 meters tall and have extremely soft feathers. Emus are flightless birds mainly due to their enormous size, which means that they are just too heavy to fly. Emus are nomadic animals which means that they rarely stay in the same place for long. This travelling lifestyle means that the emu can make the most of the food that is available and emus are known to travel long distances in order to find more food.

- Emus are omnivorous birds feeding mainly on fruits, seeds and insects. Emus are generally found close to water and are therefore not keen on more arid regions. However, the introduction of better water supplies to inland Australia has meant that despite the population decrease of the wild emu, their range has expanded. Emus have long necks and long legs in comparison to their body size. The long, flexible legs of the emu mean that the emu is able to run a high speeds, with emus generally running at around 25 mph. Emus however are able to reach a top speed 30 mph in short bursts should the emu need to get away quickly from a dangerous situation.

- Emus form breeding pairs during the Australian summer (December) and mating usually occurs when the climate becomes cooler a few months later. The female emu can lay up to 20 eggs, which hatch after a couple of months. The male emu eats very little throughout the breeding process and it is he that incubates the eggs. By the time the emu chicks hatch, the male emu has lost a considerable amount of body weight and lives of his fat reserves. Emus have few predators due to their large size and fast speed. Emus are most commonly preyed upon by wild dogs and crocodiles and are hunted by humans. Emu eggs are eaten by many animals including dogs, birds of prey and large reptiles.

- Emus tend to live for between 10 and 20 years in the wild, although it is not uncommon for an emu to be more than 30 years old, particularly when in captivity. Emus are known to be very versatile animals and can easily adapt to many different environments.

Rhea americana **(American ostrich)**

- The common rhea lives in south-eastern part of South America. Rheas live in pampas, campos, cerrado and open chaco woodland of South America. They avoid open grassland. Rheas live in areas with at least some tall vegetation. During the breeding season, they stay near rivers, lakes, or marshes. Largest South American bird, flightless, unique due to its great height, massive legs, and terrestrial habits. Their breeding season is from August to January, depending on the region. Males court two to twelve females. Once mating has occurred, the males build nests, which are shallow holes in the ground with a rim that is surrounded by twigs and vegetation. Each of the females lay one egg in the male's nest every other day for a period of seven to ten days. After the first two or three days of egg laying, the male stays with his nest and eggs and begins incubating them. A male usually incubates ten to sixty eggs. The chicks hatch within thirty-six hours of each other. The male takes care of the chicks by himself. Female rheas move from male to male throughout the breeding season.

- During the spring, male rheas are solitary, and females form into small groups. Yearlings form a flock until they are two years old, which is when they are ready to breed. At the end of the summer males, females, and chicks come together to form large flocks for the winter months. The flocks formed after the breeding season contain twenty to thirty and sometimes as many as one hundred rheas. Rheas are omnivorous, preferring broad-leaved plants and clover. However, they eat a variety of seeds, roots and fruits. They also eat insects, including grasshoppers; small vertebrates, such as lizards, frogs, small birds and snakes. Rheas continuously move as they feed.
- Rhea feathers are used to make feather dusters in South America. Their skins are used for leather, their meat and eggs are consumed by man and dogs. Rheas are a pest to farmers because they will eat almost any agricultural crop.
- The rhea population has declined considerably and is considered to be near threatened. In 1980, over 50,000 skins were traded; however a permit is now needed for their export and import. Rheas and their eggs are eaten by local people. They are also killed to be used as dog food. Rhea chicks are threatened by many predators. The rhea's habitat has become limited due to agricultural progress. Rheas are eliminated near agricultural areas, because they will eat almost any crop.
- Rheas cannot fly. However, they have unusually long wings for flightless birds. They use their wings like an airplane rudder, in order to help them dodge predators.

Extinct forms

- *Aepyornis*, the "elephant bird" of Madagascar, was the largest bird ever known. Although shorter than the tallest moa, a large *Aepyornis* could weigh over 450 kilograms and stand up to 3 metres tall.
- At least 11 species of moa lived in New Zealand before the arrival of humans, ranging from turkey-sized to the Giant Moa *Dinornis giganteus* with a height of 3.3 metres and weighing about 250 kilograms. They went extinct by A.D. 1500 due to hunting by Māori settlers, who arrived by A.D. 1300.
- In addition, eggshell fragments similar to those of *Aepyornis* were found on the Canary Islands. The fragments date to the Middle or Late

Miocene, and no satisfying theory has been proposed as to how they got there due to uncertainties about whether these islands were ever connected to the mainland.

Archaeoptery x Salient Features

The palaeontological studies of fossil birds give strongest evidence about evolution of birds from reptiles. One of the earliest-known fossil bird is *Archaeopteryx lithographica*. It is considered a connecting link between reptiles and birds. *Archaeopteryx lithographica* means "ancient wing from the printing stone". It is named after the limestone in which it was discovered. It was extracted from in and around the Solnhofen area of Germany which was formed in the Late Jurassic, about 150 million years ago. It was discovered in 1861 from Bavaria. Germany by Andres Wagner. A second skeleton was discovered in 1877 and a third in 1956 from the same locality. *Archaeopteryx* possesses both reptilian and avian features.

7.12 Archaeopteryx's avian features

1) Feathers- Feathers are the diagnostic feature of modern birds. This is one of the main criterion for classifying *Archaeopteryx* as a bird, as no other modern animal has feathers. The possession of feathers is a characteristic of birds, so strike one up for the birds. However, in late 1996, a discovery in China may change this view. A small theropod dinosaur *Sinosauropteryx* was found with what appear to be feathers preserved along the back. Forelimbs modified as wings bearing flight feathers (remiges). Tail bears two lateral rows of elongated tail feathers (rectrices).

2) Opposable hallux (big toe)- Each foot bears 4 clawed toes with 2,3,4 and 5 phalanges respectively. The first toe or hallux is directed backward and is opposable.This also is a character of birds and not of dinosaurs. Although opposable big toes are found in other groups, they are not found in dinosaurs. A reversed big toe is found in some dinosaurs however, and the condition is approached in some theropod dinosaurs.

3) Furcula (wishbone) formed of two clavicles fused together in the midline - A V-shaped furcula bone is present. It used to be thought that the possession of a furcula distinguished birds from dinosaurs. Indeed, up until recently even clavicles were few and far between in even theropod dinosaurs. However, it has been found that theropod dinosaurs did indeed have clavicles and they have been found in several species, e.g., *Segisaurus, Velociraptor, Euparkeria, Ornithosuchus, Saltoposuchus, Ticinosuchus.* nosaur. It has been found that the clavicles are often small and poorly ossified.

4) Pubis elongate and directed backward - This is a feature of birds, but it is also a feature of some theropod dinosaurs so is not diagnostic of birds - another neutral character. However, the pubic shafts of *Archaeopteryx* and dromaeosaurs (a group of theropod dinosaurs which are thought to be closely linked to birds) share a plate-like, slightly angled transverse cross-section which not found in any other archosaurs.

5) Skull large, bones intimately fused, brain case large and rounded and orbits large.

6) Jaws are modified into a beak.

7) Tibia and fibula are separate.

9) Eyes provided with sclerotic ossicles.

9) Bones of limbs and girdles are bird-like.

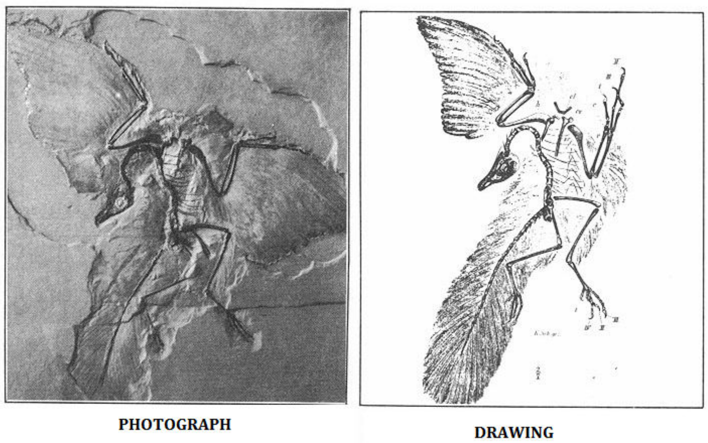


Figure - The berlin specimen of *Archaeopterys lithographica*

***Archaeopteryx's* reptile features**

1. Bones are not pneumatic.
2. Scales are present on body and limbs.
3. Jaws are provided with homodont teeth lodged in sockets.
4. Vertebrae are amphicoelous type as in Sphenodon.

5. Cervical vertebrae few (9 or 10).
6. Free cervical and abdominal ribs are present in addition to thoracic ribs as in Sphenodon and crocodiles. Ribs single-headed and without uncinate process.
7. The tail is long, lizard-like and with about 20 free caudal vertebrae. Pygostyle absent.
8. Eyes provided with sclerotic ossicles.
9. **Premaxilla and maxilla are not horn-covered -** The premaxilla does not have a keratinized covering, so *Archaeopteryx* has no bill. The bill is produced via the process of 'cornification' which involves the mucus layer of the epidermis and thus its formation is independant of jaw bone formation.
10. **Trunk region vertebra are free -** In birds the trunk vertebrae are always fused.
11. **Pubic shafts with a plate-like, and slightly angled transverse cross-section -** A Character shared with dromaeosaurs but not with other dinosaurs or birds
12. **Cerebral hemispheres elongate, slender and cerebellum is situated behind the mid-brain and doesn't overlap it from behind or press down on it -** This again is a reptilian feature. In birds the cerebral hemispheres are stout, cerebellum is so much enlarged that it spreads forwards over the mid-brain and compresses it downwards. Thus the shape of the brain is not like that of modern birds, but rather an intermediate stage between dinosaurs and birds
13. **Neck attaches to skull from the rear as in dinosaurs not from below as in modern birds -** The site of neck attachement (from below) is characteristic in birds, Archaeopteryx does not have this character, but is the same as theropod dinosaurs:
14. **Center of cervical vertebrae have simple concave articular facets -** This is the same as the archosaur pattern. In birds the vertebrae are different, they have a saddle-shaped surface.
15. **Long bony tail with many free vertebrae up to tip (no pygostyle) -** Birds have a short tail and the caudal vertebrae are fused to give the pygostyle.
16. **Premaxilla and maxilla bones bear teeth -** No modern bird possess teeth. Bird embryos form tooth buds, but do not actually produce teeth. Some birds subsequently produce ridges in the bill, but there is no

connection between them and the embryonic tooth buds, since the ridges also form in other areas of the bill where no tooth buds have previously formed.

17. **Ribs slender, without joints or uncinate processes and do not articulate with the sternum -** Birds have stout ribs with uncinate processes and articulate with the sternum.
18. **Pelvic girdle and femur joint is archosaurian rather than avian (except for the backward pointing pubis) -** The pelvic girdle as a whole is basically free and similar to archosaur girdles, the pubis points backward - a character shared with birds and some other bird-like theropod dinosaurs.
19. **The Sacrum (the vertebrae developed for the attachment of pelvic girdle) occupies 6 vertebra –** Like in reptiles and especially ornithipod dinosaurs. The bird sacrum covers between 11-23 vertebrae! So, while the variation seen in modern birds is large, it is nowhere near the number found in *Archaeopteryx*
20. **Metacarpals (hand) free (except 3rd metacarpal), wrist hand joint flexible** Metacarpals are free like in reptiles, in birds the metacarpals are fused together with the distal carpals in the carpo-metacarpus, wrist /hand fused. All modern birds have a carpo-metacarpus, all fossil birds have a carpo-metacarpus
21. **Nasal opening far forward, separated from the eye by a large preorbital fenestra (hole) -**This is typical of reptiles, but not of birds. Where a fenestra is present in birds, it is always greatly reduced, and is involved in prokinesis (movement of the beak)
22. **Deltoid ridge of the humerus faces anteriorly as do the radial and ulnar condyles.**
23. **Claws on 3 unfused digits -** No modern adult bird has 3 claws, nor do they have unfused digits. The juvenile hoatzin and Touracos do have 2 claws but loose them as they grow, the ostrich appears to retain its 2 claws into adulthood, due to the early termination of development. In fact almost all birds exhibit claws, but in the embryonic stage and they are lost by the time the bird leaves the egg. In the case of the few which do retain claws into the juvenile stage, this is merely the extension of the condition into the post-embryonic stage.
24. **The fibula is equal in length to the tibia in the leg -** This again is a typical character of reptiles. In birds the fibula is shortened and reduced.

25. **Metatarsals (foot bones) free -** In birds these are fused to form the tarsometatarsus. However, in modern bird embryos, the foot bones are initially separate as in the adult *Archaeopteryx* and is another character supporting a reptilian ancestry for birds.
26. **Gastralia present -** Gastralia are "ventral ribs," elements of dermal bone in the ventral wall of the abdomen. This is a typical structure present in reptiles but are absent in birds.

Archaeopteryx is a bird because it had feathers but it retained many dinosaurian characters which are not found in modern birds, whilst having certain characters found in birds but not in dinosaurs. This is why *Archaeopteryx* is a true transitional species, because it shares some characters which are diagnostic of one group whilst still retaining characters diagnostic of its ancestral group. It is a bird is nothing but a reptile clothed with feathers. Hence birds are described as glorified reptiles.

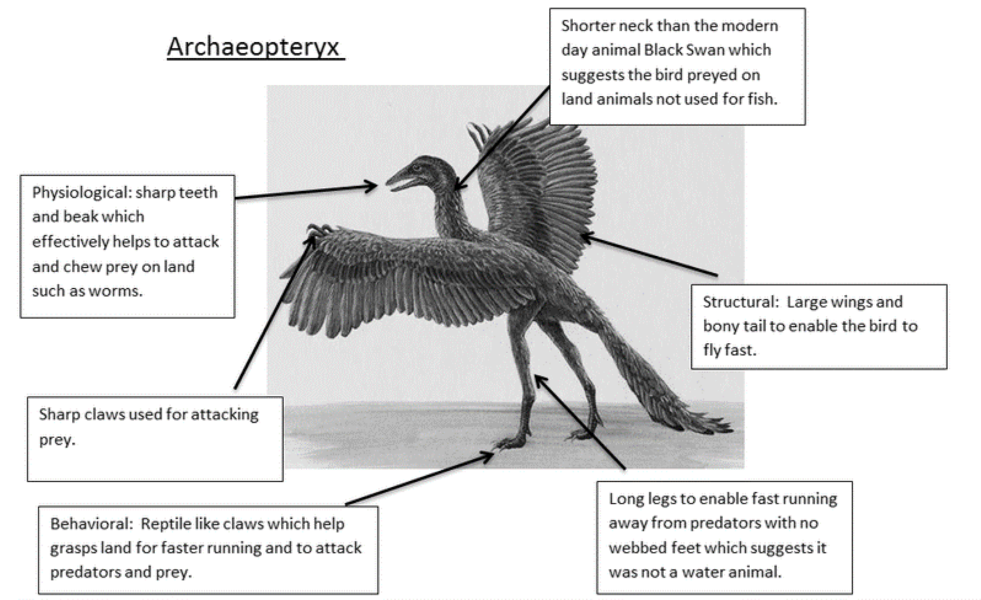


Figure - Archeopteryx

7.13 Modification of beak in birds

Bird's beaks are very important functioning structures. They help the bird gather food and sometimes they rip, tear, or crush food. They can act as an extra hand, such as when parrots use their beak for climbing. They are used for building nests, and in some species of birds they can even tie knots. They are also important for preening, which is making sure all of the bird's feathers are in their proper place. In birds teeth are absent.

The bones of the upper and lower jaws become elongated to form a beak or bill covered by a horny sheath called rhamphotheca. The beak serves both as mouth and hand. According to the type of food and feeding habit beak of birds show many variation in shape, size and structure.

Functions of beaks

1. **Feeding:** The primary function of bird for using his beak is simply to eat. There are, however, many different methods of feeding, depending on the type of bird and beak.
2. **As a Tool:** Since bird has no hands, his beak is used to do many of the things that our hands do.
3. **Communicating:** Just like we use our mouths, birds use their beaks to communicate. While some species of parrot can mimic human voices, most birds communicate using different noises and gestures, such as chirping, clicking, singing, or and many others.
4. **As a Weapon:** One of the most vital uses a bird has for its beak is as a weapon. Bird beaks are often very hard, strong, and sharp, and can inflict heavy damage. Bird may use its beak as a weapon for a variety of reasons, they may fight for control or dominance, may even try to bite out of fear or territoriality.

A few common types of beaks in birds are being described here.

1. Seed-eating beak: Some small birds like sparrow, finches and cardinals have short, peg-like, conical beaks which are used to eat grains or seeds. In some seed eating birds the beak is weak and is used to pierce small seeds, while some have strong beak which is used to crush hard-shelled seeds and fruit stones.
2. Cutting beak: Crows have long and slender beak with cutting edges. It is used to cut plants.
3. Fruit- eating beak: In parrot the beak is sharp, strong, massive, deeply hooked and well adapted for breaking and opening hard seeds and nuts. Hornbills have a large and heavy-looking beak but actually it is very light as its interior has a cellular structure. These cells act as a resonator, thus enabling the bird to produce loud cry.
4. Insectivorous beak: In robin, frog-mouth, swift and swallow the beak is small, delicate and wide to scoop living insect prey while on wing. In hoopoe the beak is long slender and slightly curved to open rolled leaves or probing into the soil for insect larvae and pupae.

5. Wood-chiselling beak: Woodpeckers have elongated strong, chisel-like beak for drilling into wood for insect larvae or for nest construction. They have strong neck muscles and shock-absorbent skull bones to tolerate the shocks while drilling.
6. Tearing and piercing beak: Flesh eating birds like eagle, hawk, kite, etc. and carrion feeding birds like vultures have short, pointed, sharp-edged and powerful hooked beak and strong mandibular muscles for tearing the flesh.
7. Mud-probing beak: This type of beak is found in snipe, sand-piper, stilt, yellow-leg, kiwi, lapwing, etc. The beak is extremely long, slender and used to probe deep into water and mud in search of worms and larvae.
8. Water and mud straining beak: In birds like ducks, geese and teals, the beak is broad and flat and the margins of the jaws are provided with transverse lamellae which act as a sieve or strainer allowing water and mud to pass out and retaining the food in the mouth. In flamingos the beak is distally curved downwards and provided with transverse lamellae. The two halves of the lower jaw are considerably enlarged so that the comparatively narrow upper jaw fits into a wide cavity.
9. Fish-catching beak: In storks, herons and kingfishers the beak is long, powerful and sharply pointed to capture fish, frog, tadpole and small aquatic animals. In cormorant the beak is long and narrow and the edges are provided with sharp backwardly directed teeth-like processes meant to capture fish.
10. Spatulate beak: Spoonbill has a long flattened beak which terminates in a broad spatula-like or spoon-like expansion meant for splashing water and mud in search of its prey which includes worms, insects, fish, molluscs, etc.
11. Pouched beak: In pelicans the beak is large and provided with a gular pouch which is extensile skin attached to the mandible. It serves as a fishing net.
12. Flower-probing beak: In humming birds the beak is extremely long, pointed and rapier-like and can dive down the corollas for sucking honey and insects.

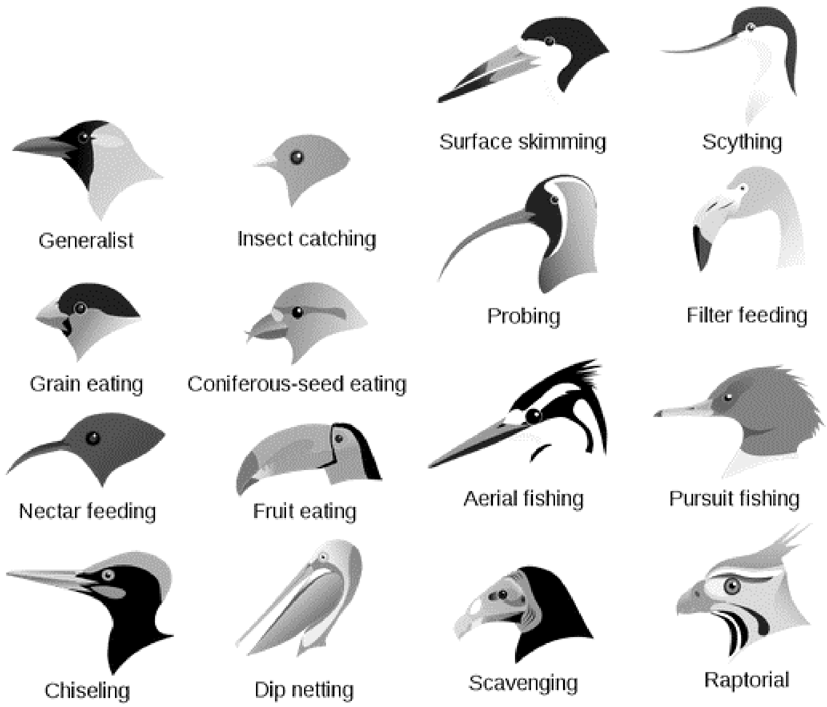
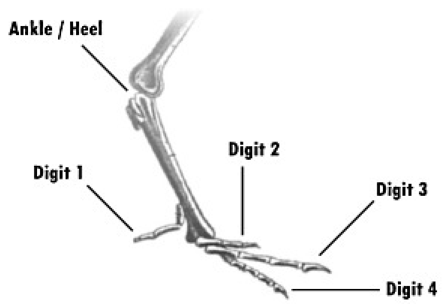


Figure – Different types of beaks of birds

7.14 Modification of feet in birds

Birds have many different shapes and sizes to their feet. Like the shape of the bill, the anatomy of birds' feet tells us much about the ecology of different species of birds. Below are several examples of birds feet and what each one can tell us about the group of birds who possess them.

Another name for a bird's toe is a digit. When comparing a bird's digit with our toes, the first digit would be our big toe. The second digit would be the next toe and so on. Bird's are missing the fifth digit, or our little toe. In fact, some birds have only three toes, and the Ostrich has only two.



Bird's have amazing feet that are much different than ours. Most people think that a bird's knee bends backwards, but in fact what we think looks like a knee is actually the bird's ankle / heel joint. The knee is hidden under feathers close to the bird's body. Since everything under the ankle is the foot, it is easy to see that a bird actually walks on its toes.

The types of feet of birds on the basis of modifications according to their habit and habitat.

1. Cursorial or running feet: In running birds the legs are powerful and number of toes is reduced. In bustards, flightless birds such as emu, rhea and cassowary, only 3 toes directed forward are present. In ostrich only 2 forward directed toes are present, the outer one is smaller and without a nail.
2. Perching feet: Most birds like finche, sparrow, crow, bulbul, mynah, robin etc. have perching feet. Three toes are slender and anterior and one toe or hallux is posterior, strongly built and opposable, so that they can securely hold a branch or a perch.
3. Scratching feet: Fowl, quail, pheasant, etc. have stout feet with strong claws, well adapted for running as well as scratching the earth. Feet of male are provided with a pointed bony spur for offence and defence.
4. Raptorial feet: Carnivorous birds such as eagle, kite, owl and vulture have strong feet for striking and grasping their prey. Toes have well developed, strong, sharp and curved claws. Large, fleshly bulbs called tylari are present on the under surface of the toes.
5. Wading feet: Wading marshly bird such as heron, jacana, snipe, lapwing, etc. have exceptionally long and slender legs and toes which help them to walk over aquatic vegetation or marshes. Web absent or feebly developed.
6. Swimming feet: In aquatic birds the toes are webbed partially or completely. In duck, teal, etc. only anterior three toes are enclosed in a web and in pelican and cormorant all the four toes are united in a web. In diving birds like coot and grebe the web is lobate and the toes are free.
7. Climbing feet: In parrot and woodpecker the second and third toes point in front and the first and fourth point backwards. Such feet are adapted for climbing vertical surface. They are also used as grasping organ.
8. Clinging feet: In martinet, swift, kingfisher and humming bird all four toes point forwards and help to cling to steep surfaces of cliffs, caves or houses.

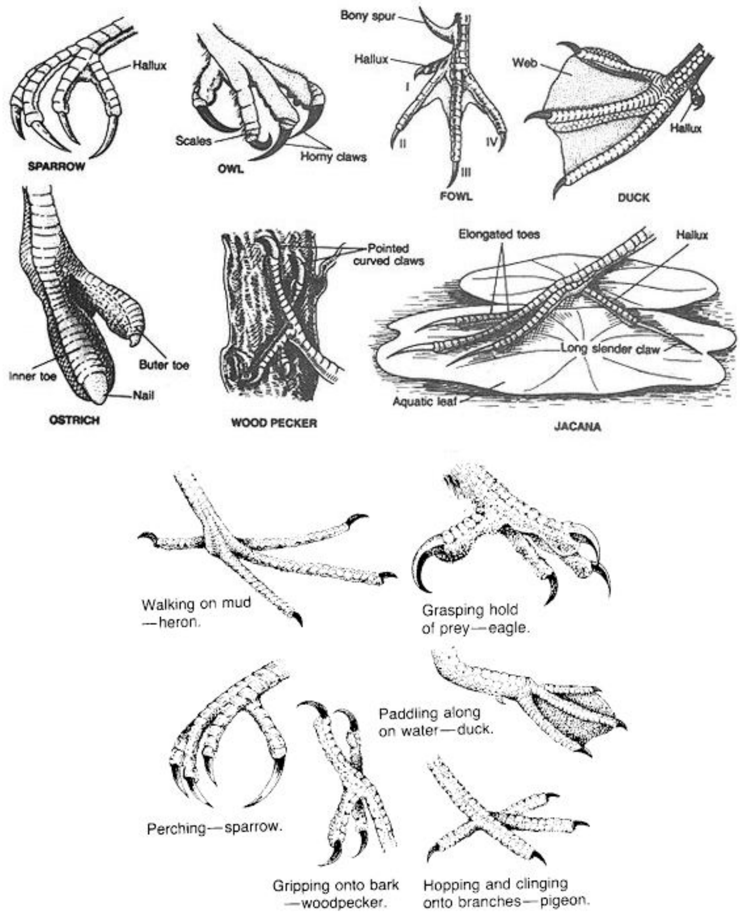


Figure – Different types of feet of birds as per their habit and habitat

The types of feet of birds on the basis of modifications according to their structure-

1. Zygotactyl feet are feet where the 2nd and 3rd digits face ahead, while the 1st and 4th digits point backwards (arboreal species such as woodpeckers).
2. Heterodactyl feet are similar to zygodactyl feet except 3 and 4 face ahead and 1 and 2 point backwards (found in trogons).
3. Anisodactyl feet have the 1st digit pointing backwards and the other three pointing ahead (most common arrangement in birds, seen in passerines).
4. Syndactyl feet are similar to anisodactyl feet but have the 3rd and 4th digit partially joined together (seen in Coraciformes such as kingfishers).
5. Pamprodactyl feet have all four digits pointing ahead though two can be rotated backwards (birds such as swifts).

6. Tridactyl feet have only three digits all pointing ahead (birds such as sanderlings).
7. Didactyl feet have only two toes both pointing ahead (ostriches).

Webbing on the feet is and degree of webbing is also used in identification. Palmate feet are feet where webbing connects only the three front digits. Totipalmate feet have webbing connecting all four digits (pelicans). Lobate feet have membranous lobes that increase the size of the digits and help the bird swim.

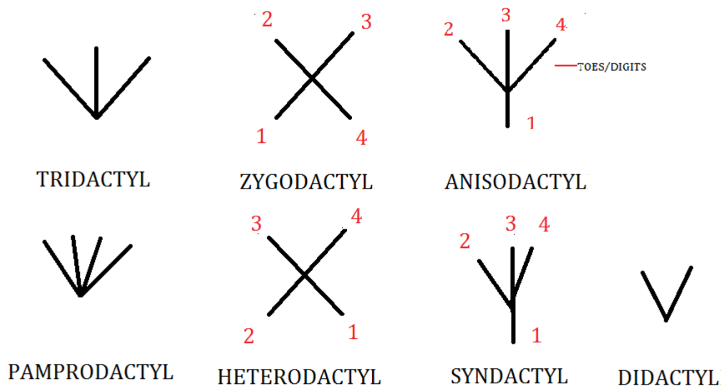


Figure – Different types of feet of birds as per their structure.

7.15 Summary

Birds are "the masters of air". Birds are found all over the entire Earth. There are approximately 9700 species of birds living today, and most species are particularly well known. Of the 9700 species, some 5000 species belong to the order Passeriformes, the perching birds or songbirds. Modern Birds are warm bodied creatures belongs to a class of vertebrates characterized by being feathered and bipedal; and by having very high metabolic rates and a forelimb modified into a wing which, together with a long tail, forms part of a flight mechanism.

The birds are supposed to be evolved from the reptiles, birds of today are more closely related to reptiles than to mammals. Long ago the anatomists predicted that birds emerged out from basal reptilian stock and according to Goodrich, the Sauropsid line. The birds originated about 165 million years ago in the Jurrasic period.

Bird Migration is one of the most spectacular event in the animal kingdom. It is the seasonal movement of a number of birds as a habit to obtain the advantage of favourable conditions in other regions. Bird migration is the regular seasonal movement, often north and south along a flyway, between breeding and wintering grounds. Many species of bird migrate. Migration is different from unidirectional movement and those in which animals are helplessly carried by some other agency. It is a two way journey between their summer and winter homes.

Palate is a structure formed from the assemblage of bones situated in the roof of buccal cavity of vertebrates. It separates the oral cavity from nasal passage. It helps the nasal passage to open far behind into the buccal cavity. The palate helps the animal to chew and breathe at the same time. Palate first appeared in some reptiles and is well developed in birds and mammals.

Adaptation is the ability of birds to adjust in the existing aquatic or aerial environment. It is the adjustment of organisms in the surrounding environment for their existence by changing the body structure accordingly.

Flightless birds belongs to diverse group of super order Ratitae, these are large birds of Gondwanan origin, most of them now extinct. The flightless birds have no keel on their sternum, hence their name which comes from the Latin (*ratis*) for raft. The keel provides an anchor to which a bird's wing muscles attach, thereby providing adequate leverage for flight.

Archaeopteryx lithographica is considered a connecting link between reptiles and birds. *Archaeopteryx lithographica* means "ancient wing from the printing stone". It is named after the limestone in which it was discovered. It was extracted from in and around the Solnhofen area of Germany which was formed in the Late Jurassic, about 150 million years ago.

Bird’s beaks are very important functioning structures. They help the bird gather food and sometimes they rip, tear, or crush food. They can act as an extra hand, such as when parrots use their beak for climbing. They are used for building nests, and in some species of birds they can even tie knots.

Birds have many different shapes and sizes to their feet. Like the shape of the bill, the anatomy of birds’ feet tells us much about the ecology of different species of birds. Below are several examples of birds feet and what each one can tell us about the group of birds who possess them.

7.16 Self Assessment Questions

1. Describe the distinguishing characters of Aves.
2. Describe the salient features of *Archaeopteryx*.
3. What do you mean by palates? Explain its types
4. Draw a diagram of different types of palates.
5. Explain the origin of birds.
6. Describe the classification of birds up to order in details .
7. Write about flightless birds.
8. Describe the Bird migration in details.
9. Write an essay on Flight adaptations of birds.
10. Explain the types of migration in birds.
11. Give a short note on different types of beaks of birds.
12. Write a short note on different types of feet of birds.
13. Describe the Aquatic and flight adaptations in birds

7.17 Reference Books

- Modern Text Book of Zoology: Vertebrates by R.L. Kotpal
- Text Book of Vertebrate Zoology by J. S. Kingsley
- Hyman's Comparative Vertebrate Anatomy by Marvalee H. Wake
- Textbook of Vertebrate Zoology by S. Prasad

Unit - 8

Mammals

Structure of the Unit:

8.1 Objectives

By the end of the chapter, the student would acquaint himself with the General characters & classification of Mammals, Origin & evolution of Mammals and different theories related to origin of mammals, mammals of the Mesozoic and coenozoic,different evolutionary modifications observed in mammals, characteristics and classification of prototheria (Primitive mammals), characteristics and classification of Metatheria (Marsupials), General characteristics of Eutherians, different types of adaptive radiations of Eutherians - Central or focal type, Cursorial type, Arboreal type, Scansorial type, Fossorial type, Aquatic type, Flight or Volant type, Fossorial type etc

8.2 Classification of Mammals (up to orders) Class

Introduction

The word "mammal" is coined by Carl Linnaeus in 1758, derived from the Latin word "mamma" (mammary glands). All female mammals nurse their young with milk, which is secreted from special glands, the mammary glands. The mammals are the final product of evolution. They are the highest group in the animal kingdom. The class Mammalia includes man, the master of the present age.Mammals are a clade of endothermic amniotes distinguished from reptiles and birds by the possession of a region of the brain - neocortex, hair, three middle ear bones and mammary glands.

Mammals include the largest animals on the planet, the great whales, as well as some of the most intelligent, such as elephants, primates and cetaceans. The basic body type is a terrestrial quadruped, but some mammals are adapted for life at sea, in the air, in trees, underground or on two legs. The largest group of mammals, the placentals, have a placenta, which enables the feeding of the fetus during gestation.

Mammals range in size from the 30–40 mm bumblebee bat to the 33-meter blue whale. With the exception of the five species of egg-laying mammals - monotreme, all modern mammals give birth to live young. Most mammals, including the six most species-rich orders, belong to the placental group. The three largest orders in number of species are Rodentia: mice, rats, porcupines, beavers, capybaras and other gnawing mammals; Chiroptera: bats; and Soricomorpha: shrews, moles and solenodons. The next three biggest orders, depending on the biological classification scheme used, are the Primates including the great apes and monkeys; the Cetartiodactyla including whales and even-toed ungulates; and the Carnivora which includes cats, dogs, weasels, bears and seals.

The early synapsid mammalian ancestors were sphenacodont pelycosaurs, a group that produced the non-mammalian *Dimetrodon*. At the end of the Carboniferous period, this group diverged from the sauropsid line that led to today's reptiles and birds. The line following the stem group Sphenacodontia split-off several diverse groups of non-mammalian synapsids—sometimes referred to as mammal-like reptiles—before giving rise to the proto-mammals (Therapsida) in the early Mesozoic era. The modern mammalian orders arose in the Paleogene and Neogene periods of the Cenozoic era, after the extinction of non-avian dinosaurs, and have been among the dominant terrestrial animal groups from 66 million years ago to the present.

8.3 Mammals: General Characters

The salient features of this class are:

1. Mammals live in all types of habitats from Polar Regions to the tropics and from the sea to the densest forests and driest.
2. The mammals are warm blooded animals characterized by special glands called as mammary glands by means of which the females suckle their young ones. In males, the mammary glands are undeveloped.
3. The body is usually covered with hairs which are moulted periodically.
4. The body is divided into head, neck, trunk and tail.
5. The limbs are pentadactyl and modified for different functions like walking, running, climbing, burrowing, swimming, flying etc.
6. The integument system consists of sweat, sebaceous, and sometimes scent glands.
7. There are dead exoskeletal structures like horny epidermal hairs, scales, claws, nails, hoofs, horns, etc.
8. Skull is dicondylic with large cranial cavity and the temporal region has a zygomatic arch.
9. Elongated nasal region is present in the body. A secondary palate isolates the nasal passage from the food passage.
10. The auditory capsule projects outwards as a swollen structure, the tympanic bulla.
11. The prefrontal, post-frontal, quadratojugal, supra-orbital and basipterygoids bones are absent in the skull.
12. Jaw suspensorium craniostylic.
13. Lower jaw is formed of a large dentary bone which articulates with the squamosal of the skull.
14. Vertebrae is with flat centrum and with terminal epiphysis.
15. Seven cervical vertebrae are present.
16. Coracoid and precoracoid bones of pectoral girdle are reduced or vestigial.

17. Ribs are bicephalous.
18. Teeth are thecodont, present in sockets, different types like incisors, canines, premolars and molars (heterodont) and two sets of teeth are developed during life time-milk teeth and permanent teeth called as diphyodont teeth.
19. A muscular diaphragm separates the thoracic and abdominal cavities.
20. Alimentary canal ends with anus, cloaca absent.
21. Respiration by lungs contained in pleural cavity. Larynx with vocal cords.
22. Heart four-chambered, only left aortic arch present and renal portal system absent.
23. Red blood cells are biconcave and non-nucleated cells.
24. Kidneys are of metanephric type and ureters open into a urinary bladder.
25. Excretory waste of body is urea which is excrete out as urine.
26. Brain of mammals is highly developed which shows greatest intelligence. Corpus callossum present and it connects the two cerebral hemispheres. Optic lobes four in number called corpora quadrigemina.
27. Fleshly and cartilaginous external ear or pinna present to collect sound waves and protect external ear opening.
28. Three ear ossicles – malleus, incus and stapes present in the middle ear.
29. Cochlea of inner ear highly coiled structure.
30. Mammals are unisexual with well-marked sexual dimorphism.
31. The testes are usually in scrotum, external to abdomen.
32. Fertilizationtakes place inside the body.
33. Eggs are usually minute, without protective shell and are retained in the uterus for development.
34. Development of the foetus takes place by attaching to the uterine wall by placenta through which it derives nutrition, respires and removes wastes.
35. All mammals except Prototheria, are viviparous, i.e., they give birth to young ones.
36. Mammals show parental care of high level.

Mammals - Classification (up to orders)

Approximately there are 5,000 living species in Class Mammalia, about 15,000 subspecies and many extinct forms. Class Mammalia is mainly categorized into three subclasses: Prototheria, Allotheria and Theria.

Subclass I. Prototheria(Gr; Protos, first + therios, beast)

- Primitive, reptile-like, egg laying mammals.
- Mammary glands unspecialized and without teats.

Order 1. Monotremata (Gr; monos, single + trema, opening).

- Ear external part pinna is absent.
- Cloaca present.
- Teeth in young, adults with horny beak.
- Confined to Australian region.
- Examples: *Ornithorhynchus* (duckbill platypus), *Tachyglossus* = *Echidna* (spiny anteater).

Subclass II. Allotheria

- This subclass consists of extinct mammals, only fossils of mandibles and teeth are available.
- Nothing is known about the soft parts but the skeleton resembles with Monotremata.
- Occur from Jurassic to Eocene.

Order 1. Multituberculata.

- Examples: *Microlestes,Plagiaulax*

Order 2. Triconodonta

- Example: *Triconodon*

Subclass III. Theria

- The characteristic animals of this subclass are Marsupials and placental mammals.

- External ear or pinna present.
- Mammary glands provided with teats.
- These are viviparous animals.

Infraclass A. Metatheria (marsupialia)

- These animals are intermediate between monotremes and placental mammals.
- Females with a pouch (marsupium) in the abdomen.
- With or without rudimentary yolk sac abdomen.
- Occur mostly in Australia and South America.

Order 1. Marsupialia

- Marsupials are characterized by presence of special pouch like structure called as marsupium in which the underdeveloped born young oneget shelter and feeded by the teats of mammary gland present inside.
- Uterus and vagina double.
- Usually 3 premolars and 4 molars on each side in each jaw.
- Examples: *Macropus* (kangaroo), *Phascolarctos*(koala), *Didelphis* (opossum),.

Infraclass B. Eutheria (Placentalia)

- These are higher advanced, placental mammals.
- Here the special pouch like structure marsupium is absent.
- Vagina single.
- Well-developed young one is born.
- There are several orders of extinct and living eutherians. Only living orders are being described.

Order 1. Insectivore

- Primitive mammals with a long pointed snout modified for eating insects.
- Body covered with fur or spines.
- Feet plantigrade or semiplantigrade, generally pentadactyl with clawed digits.
- Molars rooted, with pointed, peg-like cusps.
- Widely distributed, terrestrial and nocturnal.
- Testes never descend into scrotum.
- Examples: *Erinaceus* (hedgehog),*Talpa* (mole), *Sorex* (shrew).

Order 2. Dermoptera

- Members are called as flying lemurs which resemble flying squirrels.
- A hairy, muscular, fold of skin which is modified like parachute- pataguim extending from neck to the wrist and down the sides of the body to the ankle including the tail.
- Nocturnally active animals and usually feed on fruit and leaves.
- Four equal sized limbs.
- Can glide with the help of pataguim.
- Example: Only one living genus *Cynocephalus* (*Galeopithecus*)..

Order 3. Chiroptera

- These are flying mammals, commonlycalled as bats.
- Forelimbs modified as wings (patagium), second to fifth digits greatly elongated to support a broad web of skin extending back to the short hindlimb.
- Tail if present connected with the pataguim.
- Teeth are sharp, small and are of different types.
- Sternum with keel for attachment of flying muscles.

- Eyes are small with less sense of vision.
- Ear pinna are in size large.
- These are the only true flying mammals because of capability of true flight.

Suborder i. Megachiroptera

- Frugivorous, large size as the name indicates, which sleep by day on tree hanging head downwards, with wings folded, cloak-like around the body, in deserted areas.
- Live in groups.
- Eyes large, snout elongated and without foliaceous appendages, ear pinna large and without appendages, first and second digits clawed.
- Tail is completely absent.
- Molars with longitudinal grooves.
- Found in tropical and subtropical regions of Eastern hemisphere.
- Commonly known as flying foxes or fruit bats.
- Examples: *Cynopterus,Pteropus*

Suborder ii. Microchiroptera

- Insectivorous, as the name indicates, small sized animals which hang by hind claws head downwards during day at dark places
- Found in temperate and tropical regions of both hemispheres.
- Some gregarious, others solitary.
- Eyes are small, snout short, with foliaceous appendages.
- Ear pinna large with leaf-like appendages. Only first digit clawed.
- Tail is long, enclosed in inter-femoral membrane.
- Molars with sharp cusps.

- Examples: *Tadarida mexicana* (free tailed bat), *Desmodus* (blood-sucking vampire bat),*Myotis* (small brown bat), *Vespertilia,*.

Order 4. Edentata

- Animals without teeth or less developed structure, reduced to molars in anterior part of the mouth, enamel absent.
- Dentition is monophyodont.
- Toes with strong, curved claws for scratching.
- Testis are present on abdomen.
- Some species may be armoured.
- Solitary, nocturnal and arboreal.
- Examples: *Dasypus* (armadillo), *Myrmecophaga* (giant anteater), *Bradypus* (3-toed sloth).

Order 5. Pholidota

- Body is covered with overlapping epidermal scales which can be erected.
- Teeth are absent.
- Tongue is very long, sticky and protrusible and modified for capturing insects.
- Limbs short and have five digits.
- Terrestrial and burrowing animals.
- In case of danger it can roll and convert themselves into a ball like structure for protection.
- Example: Only one genus *Manis*or Pangolin or Scaly anteater.

Order 6. Tubulidentata

- These animals have long snout like a Pig.
- Teeth are 4 to 5 in each jaw, enamel, incisors and canines are absent.

- Insect feeding tongue is modified into a long, sticky and protrusible structure.
- Short and stout forelimbs with four toes, hindlimbs with five toes.
- Ears are long, erect and pointed.
- Example: Only one genus *Orycteropus* (Cape anteater) belongs to South Africa.

Order 7. Rodentia

- Small herbivorous,gnawing mammals.
- Canines are absent leaving a small gap called diastema between incisors and cheek teeth.
- Body enclosed with fine fur, in some animals it may be spinous.
- Nails performs claw-like functions.
- Examples: *Funambulus* (squirrel), *Castor* (beaver), *Bandicota* (bandicoot), *Rattus* (rat), *Mus* (mouse), *Hystrix* (porcupine), *Cavia* (guinea pig),.

Order 8. Lagomorpha

- These are small to moderate sized animals with a small broad tail.
- Incisors are chisel-like, which grows continously, upper incisors two pairs.
- Canines are absent with a large gap diastema.
- These mammals are gnawing animals, eat leaves, stem and bark.
- Example: *Ochotona* (pika), *Lepus* (hare), *Oryctolagus* (rabbit),.

Order 9. Carnivora

- As the name indicates this order includes beasts of prey.
- Body size may be from tiny weasel to large sized bear.
- Many of them are known for their speed.
- Powerful and strong jaw.
- Mammae are abdominal.

- Flesh-eating animals, show adaptations for hunting.
- The canines are large.

Suborder i. Fissipedia

- These are terrestrial Carnivorous mammals with large and strong projecting canines, poorly developed incisors.
- Limbs are ungulate and provided with strong claws.
- Majority of mammals of this category are flesh eaters.
- Examples: *Felis domesticus* (domestic cat), *Canis familiaris* (domestic dog), *C.lepus* (wolf), *Urus* (bear), *Panthera tigris* (tiger), *Acinonyx* (cheetah), *Hyaena* (hyaena), *Ailuropoda* (panda),*Herpestes mungo* (mongoose), *Panthera leo* (lion),.

Suborder ii. Pinnipedia

- These are marine carnivores mammals with limbs modified into flippers for swimming.
- Body is streamlined in shape, powerful swimmers, slow and clumsy on land where they come to rest and raise their young ones.
- Their food is fish and other marine animals.
- Examples: *Odobenus* (walrus), *Callorhinus* (fur seal), *Phoca* (seal), *Mirounga* (sea elephant).

Order 10. Cetacea (Whales and dolphins)

- These mammals have a fish-like body, naked and protected with a thick layer of subcutaneous blubber.
- Nasal apertures on top of the head for easy breathing in water.
- Forelimbs are modified into broad paddle-like flippers.
- Hind limbs are absent.
- Minute ear opening devoid of external ear or pinna.

- Live in schools or herds and swim with great speed.
- Largest animal that have ever lived.
- Carnivorous and predaceous, found in all seas.

Suborder i. Odontoceti

- These aresmaller size toothed whales.
- Upper surface of skull asymmetrical.
- The nipples are nearer to the anusin females.
- A single blow holeon top of the head is formed by the fusion of two nostrils.
- Sternum many jointed,
- Body oil is used in different cosmetic products, lubricants, etc.
- Examples: *Delphinus* (dolphin), *Phocaena* (porpoise), *Orcinus* (killer whale), *Monodon* (nar whale), *Platanista gangetica* (Ganges dolphin), *Physetes catodon* (sperm whale).

Suborder ii. Mysticeti (whalebone whales or baleen or toothless whales)

- This suborder members are larger in size.
- The two nostrils are separate not fused like previous suborder.
- Teeth are completely absent.
- Many horny triangular whalebone or baleen plates hang down from the upper jaw for filtering planktons on which they feed.
- Upper surface of skull symmetrical.
- Nasal passages directed up and anteriorly.
- Sternum consists of a single piece.
- Body oil is used in various eating products and medicines.
- Examples: *Balenoptera musculus* (blue whale), *Balaena* (right whale).

Order 11. Sirenia (sea-cows)

- These are aquatic mammals.
- They are short-necked, thick-skinned, naked or with very sparse hairy covering,
- Herbivorous mammals feeding on sea weeds and other aquatic plants.
- Forelimbs are modified into paddle-like structure, hindlimbs are absent.
- Body is streamlined, with rounded muzzle and a prominent cleft in the lip.
- Tail is horizontally modified into a caudal fin.
- Mammae are two in number.
- Testes are present on abdomen.
- Examples: *Dugong*, *Trichechus* (manatee).

Order 12. Hyracoidea

- Herbivorous, small rodent-like mammals.
- They have close set fur, small ears, split snout and reduced tail.
- Canines are absent and cheek teeth are of lophodont type.
- Diastema present.
- Testes are present on abdomen, mammae six pairs, four pairs inguinal and two pairs axillary.
- Example: *Hyrax* (conies) found in South Africa.

Order 13. Proboscida

- These are the mammals having a long proboscis.
- It is also a largest living land animal with an enormous head.
- Proboscis is a mobile, muscular expansion of the nose,long, structure having nostrils at the tip.
- Proboscis is prehensile and capable of movement in all directions.

- Incisors of great size and modified into tusks.
- Canines and premolars absent, molars are of lophodont type.
- These animals usually lives in herds of 10 to 100.
- Favourite food is grass and bamboos.
- Examples: Only two species, *Loxodonta africana* (African elephant) and *Elephas maximus* (the Indian elephant).

Order 14. Perissodactyla

- These are hoofed mammals with the odd-toed ungulates.
- Axis of the limbs passes through the middle of the third digit.
- These are herbivorous mammals.
- Incisors present, molars and premolars similar and form a continuous series.
- Stomach is of simple type.
- Horns are absent, body size is large, legs are long, actively used for locomotion.
- Examples: *Rhinoceros* (rhino),*Tapirus* (Malayan tapir), *Equus equus* (horse), *E.asinus* (wild ass), *E.zebra* (zebra).

Order 15. Artiodactyla

- These are ungulates like sheep, pig, hippopotamus, etc.
- Herbivorous, usually of large size.
- The axis of the limbs passes between 3 and 4 digits.
- Usually number of toes is even (2 or 4).
- Cheek teeth bunodont or solenodont.
- Incisors and canines or upper jaw usually lacking.
- Stomach is divided into 4chambers.
- Many with antler or horns on head.

- All but pigs ruminate or chew the cud.

Suborder i. Bunodontia

- Pigs and its associated animals comes in this category.
- Horns or antlers absent.
- Teeth number is from 38 to 44.
- Molars are bunodont and provided with blunt cusps.
- Canines are enlarged to form curved tusks which is used for defence.
- Examples: *Hippopotamus amphibious* (hippopotamus),*Sus* (pig), *Pecari tajacu*, New World pigs.

Suborder ii. Pecora

- Ruminantslike camel, deer, giraffe, antelope, cattle, etc comes under this suborder.
- The skull bears horns which are outgrowths from the frontal bones.
- Teeth are 32 in number, usually canines are small or absent, but upper incisors are absent.
- Stomach is divided into four chambers - rumen, reticulum, omasum and abomasums.
- Examples: Giraffe (giraffe), *Ovis* (sheep), *Antilope* (black buck), *Capra* (goat), *Bubalus bubalis* (buffalo), *Bos grunniens* (yak), Bison (bison),*Camelus* (camel), *Axis axis* (spotted deer), *Moschus* (musk deer).

Order 16. Primates

- This order is characterized by lemurs, monkeys, apes and man.
- Limbs are long, hand and feet enlarged, each with five digits with flattened nails.

- The toe and thumb or both usually opposable, used for holding and climbing.
- Eyes are of large size, vision is of binocular type.
- Hairs cover the entire body except palm, sole and face.
- Testes are enclosed in scrotal sac.
- Two pectoral mammae are present.
- Orbit usually directed forward and surrounded by bony ring.
- Well-developed nervous system with extraordinary intelligence in comparison to other animals.

Suborder i. Lemuroidea

- These are small, solitary, primitive type mammals.
- These are active during night time, omnivorous, arboreal
- Snout usually elongated.
- Eyes usually lateral and vision poor.
- Both pollex and hallux opposable.
- They have a long non-prehensile tail.
- Brain case low, cerebellum not covered by cerebrum, orbit confluent with temporal fossa and nasals large.
- Dental formula 2.1.3.3./2.1.3.3. = 36.
- Uterus is of bicornuate type, placenta is diffused and non-deciduate.
- Found in Madagascar, Africa and South-East Asia.
- Examples: *Lemures*, *Tupaia* (tree shrew).*Loris* (loris),

Suborder ii. Tarsioidea

- These are solitary, insectivorous mammals with long thin tail tufted at the end.
- By nature these are arboreal, nocturnal.

- Snout is of short size.
- Tarsus very long, fingers and toes ending in flattened discs, two toes with claws and three with nails.
- Optical and auditory sense organs are well developed.
- Orbit largely separated from temporal fossa.
- Dental formula 2.1.3.3./1.1.3.3. = 34.
- Upper incisors large, molars with numerous pointed cusps.
- Uterus bicornuate and placenta metadiscoidal and deciduate.
- Found in Indo-Malaya, Archipelago and Philippines.
- Example: Single genus *Tarsius spectrum* (tarsier).

Suborder iii. Anthropoidea

- These animals are world widely present.
- Most developed mammals including monkeys, apes and man.
- Cranial cavity is spacious, orbits rounded, separated from temporal fossa by a bony partition.
- The snout short and eyes large and forwardly directed, with binocular vision.
- Quadripedal and plantigrade gait.
- All digits carry nails.
- Tail is prehensile when present.
- Dental formula 2.1.2.3/2.1.2.3 = 32.
- Premolars and molars bunodont.
- Testes are present in scrotum.
- Uterus is of simple type, metadiscoidal.
- Examples: *Gorilla* (gorilla), Pan (anthropopithecus),*Hylobatis* (gibbon), *Satyrus* (orangutan), *Troglodytes* (chimpanzee), *Homosapiens* (man).

8.4 Origin and evolution of mammals

First Mammals originated in the upper Triassic period of Mesozoic era about 200 million ago, but for over 100 million years they remained an insignificantanimals group. In the Palaeocene to Pleistocene period of the Cenozoic era the mammals became dominant, as such this period is known as the 'age of mammals'. Within this period of about 55 million years many ancient mammals have disappeared leaving no descendants.

Mammals holds the top position in the evolutionary tree as they are the final product of evolution.Mammals are supposed to be originated from reptiles, the fossils show that the reptiles that gave rise to mammals were Synapsids. Synapsids diverged from the main reptilian stock almost at the base of phylogeny during Permian period. Cynognathus, a mammal like reptile, belonging to suborder Theriodontia, order Therapsida and subclass Synapsida is regarded as the probable mammalian ancestor. It lived about 230 million years ago in the early Triassic period.

Synapsids were heavy-bodied, large-headed, stumpy-legged creatures, adapted to various types of habitats and food. Some were rodent-like seed-eaters, some insectivores. Cynodonts were dog-sized carnivorous therapsids known from all over Gondwanaland and dominant predators. Sailbacks or Pelycosaurs from North America and Russia were primitive synapsids, without a false palate and heterodont dentition. The large sail on their back was perhaps a thermoregulatory device.

As per the fossil remains, the therapsids had developed many mammalian characteristics due to which they became dominant predators as well as herbivores of Permian period.Majority of therapsids died out in the Triassic mass extinction.

- Mammalian ancestors were tiny creatures not bigger than rats and mice.
- They were active during night time therefore there was no competition with large-sized diurnal reptiles.
- They were burrowing or arboreal in habit.
- Body was completely covered and protected with hairs.
- Brain was large and welldeveloped, so they all were intelligent.
- They have acute sense of smell, sight and hearing in early mammals.

- Skull was with two occipital condyles, with enlarged temporal fossa and secondary palate.
- Lower jaw with enlarged dentary and different types of teeth were present like incisors, canines, premolars and molars.
- Limbs are of mammal-like, fast running speed.
- Efficient and increased feeding and respiratory rate.
- Presence of diaphragm indicates high metabolism and endothermy in therapsids.
- These were insectivores, of small size like cat that flourished in hidden habitats away from the reach of the mighty dinosaurs and gradually evolved into archaic mammals.
- They carried their young ones in pouches for further development after birth.
- They were an advanced, small -sized, carnivorous animal showing both mammalian and reptilian characters.

8.5 Theories of origin of mammals

Three theories have been proposed to explain the origin of mammals.

- Amphibian ancestry -This concept was given by T.H.Huxley (1880) on the basis of presence of two occipital condyles in the skull of both Amphibia and Mammalia. Although it was, not accepted because the origin of condyles were from exoccipitals in Amphibia and basioccipitals in Mammalia.
- Reptilian ancestry–On the basis of various fossils of extinct reptiles and mammals, mammals are from a reptilian ancestry. The Cynognathus, a theriodont therapsid, belonging to subclass Synapsids (mammal-like reptile) are the probable ancestor of mammals. This theory is further supported by the fact that the monotremes of prototherian mammals resembles with living reptiles in many anatomical features.
- Polyphyletic theory–As per taxonomy the class Mammalia has been divided into two subclasses on the basis of their reproductive habit Prototheria or primitive egg-laying mammals and Theria or viviparous mammals. Therians aresubdivided into two infraclasses - Metatheria or

marsupials and Eutheria or higher placental mammals. It is supposed that mammal-like reptiles (Theriodontia, suborder Therapsida) are the probable ancestors of mammals, but it is difficult to conclude that the exact ancestors of mammals were theriodonts. Some theriodonts were more advanced than mammals in certain characters but comparatively primitive in others. Therefore, it was established that mammals had a polyphyletic origin, that is, early mammals arose from mammal – like reptiles whereas present mammals originated from at least two mammal – like reptiles. Prototheria probably originated from the decodonts, while Metatheria and Eutheria originated independently from pantotherians by the end of Cretaceous period.

8.6 Mammals of the Mesozoic

Mesozoic mammals were small rabbit-sized insectivores, herbivores or seed eaters, partially arboreal and active animals that were not allowed to proliferate due to the presence of ubiquitous dinosaurs. Cretaceous mass extinction eliminated most of them, leaving only small insectivorous pantotherians which found all ecological niches vacant after the extinction of dinosaurs. That led to the unusually rapid evolution in Palaeocene epoch when 7 distinct mammalian groups were differentiated, namely, insectivores, carnivores, primates, ungulates, rodents, edentates and cetaceans. By Pleistocene, mammals had become giant rulers of the earth, when another mass extinction eliminated the largest of them. Today mammals are on the decline. The mesozoic mammals were the following:

1. **Triconodonts.** They were the size of cat and had three sharp cusps on the grinding teeth. Ex. *Priacodon*.
2. **Symmetrodonts.** Cusps were three but were symmetrically placed in a triangle. Ex. *Spalacotherium*.
3. **Pantotheres.** Upper molars triangular with two cusps and lower molars with three cusps. Ex. *Amphitherium*.
4. **Docodonts.** Molars were nearly squarish having 4 cusps for grinding grasses. Ex. *Disacus*.
5. **Multituberculates.** Rodent-like herbivores with chisel-like incisors and broad molars with rows of grinding cusps. Diastema was present and canines were absent. Ex. *Plagiaulax*.

8.7 The Coenozoic Placentals

Early branching of marsupials, placentals and perhaps monotremes had been accomplished before the end of cretaceous period. During early coenozoic period at least 7 mammalian groups had already been differentiated, namely, insectivores, carnivores, primates, ungulates, rodents, edentates and whales.

1. **Insectivores.** Shrew-like insectivores lived in hidden situations. Ex. hedgehogs, shrews etc.
2. **Carnivores.** They probably evolved from insectivores and continued with their ancestral carnivorous habit. Creodonts were archaic carnivores that rapidly diverged from earlier insectivores. Ex. cats, dogs, tiger, lion
3. **Primates.** Their evolutionary process can be traced through tree shrews, lemurs, tarsiers, monkeys.
4. **Ungulates.** Condylarths were primitive ungulates of Palaeocene and Eocene epochs that gave rise to all modern ungulates. Ex. Perissodactyla such as horse and Artiodactyla such as cow, buffalow, deers, goat, sheep etc.
5. **Rodents.** Their fossils are known from the late Palaeocene strata that possessed chisel-like incisors.They are related to Lagomorpha. Ex. Rats, mice, guinea pigs etc.
6. **Edentates.** Evolutionary origin of armadillos and South American anteaters is not known.
7. **Whales.** Earliest known whales of the middle Eocene were completely aquatic with fore limbs modified as flippers but whales evolved from mesonychids in Palaeocene.
8. **Proboscideans.** They evolved from some marsh-dwelling ancestor that was forced to migrate to land due to arrival of dry conditions. Sirenia and Hyracoidea are related orders that evolved from a common ancestor. Ex. Elephants.

Evolutionary trends

During evolution from mammal-like reptiles to mammals, following body structure modifications occurred:

- Hair developed on the body.
- External ear called as pinna was developed.

- They became warm-blooded from cold blooded.
- Mammary glands for nursing young ones were developed.
- Diaphragm, a muscular partition between thoracic and abdominal cavities, developed.
- Dentition became of different shapes and in sockets (heterodont and thecodont).
- Heart became completely four-chambered.
- Females transformed from oviparous to viviparous condition.
- Male developed scrotal sacs.
- Skull became dicondylic.
- Lower jaw bones reduced to a single large dentary bone.
- Brain developed specialized part called as corpus callosum.

8.8 Prototheria(Primitive mammals)

The prototheria are described as primitive unfinished mammals by Romer. Prototherians are the only hairy which lay eggs. Presence of hair is a mammalian character and laying eggs is a reptilian character. The prototherians also feed their young ones on milk like higher mammals. Hence prototheria is regarded as a connecting link between reptiles and higher mammals.

Prototherians possess primitive and degenerative features. They are not in the main line of mammalian evolution. It is concluded that Prototheria originated as a side line from mammal-like reptile ancestor and continued to survive in isolation retaining those primitive characters through which higher mammals have passed. It is not the ancestor of higher mammals.They are regarded as a connecting link between reptiles and higher mammals.

8.9 Distinctive Features of Prototheria

- Habitat of prototherians are semi-aquatic (*Ornithorhynchus*) or terrestrial (*Tachyglossus*), burrowing, nocturnal and insectivorous mammals.

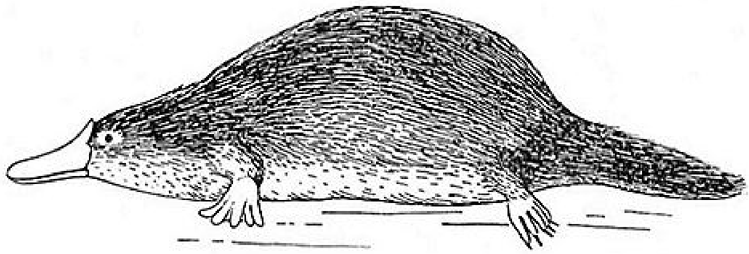
- The snout is pointed (*Tachyglossus*) or produced into a beak (*Ornithorhynchus*). Pinna absent. Limbs short, digits clawed and webbed (in aquatic form) and used for burrowing.
- Body is covered with hairs and spines with a short tail.
- Mammary glands are modified sweat glands and without teats.Males possess functional mammary glands. The feeding of young with milk by father and mother or parentsis known as gynaecomastism.
- For defense males have a poisonous tarsal spur on each hind leg. Skin glandular, sweat and sebaceous glands present.
- Body cavity is divided into two cavities, anterior thoracic and posterior abdominal, by a diaphragm.
- Skull has two occipital condyles, sutures between skull bones absent. Orbits continuous with temporal fossae. Tympanum ring-like. Ear ossicles three. Each half of lower jaw (mandible) composed of a single dentary bone.
- Vertebrae are without epiphyses. Cervical vertebrae are seven in number, ribs single-headed, with only capitulum.
- Tongue is long and sticky, teeth absent in adults, replaced by horny beak. Anus absent, rectum opens into a cloaca.
- Heart is divided into fourchambered. Right auriculo-ventricular valve is muscular and incomplete. Right valve tricuspid. Chordae tendinae is absent. Abdominal vein present. Only left systemic aorta present. RBC circular and non-nucleated. Temperature of the body low (25 ^{D} C to 28 ^{D} C)hence incompletely warm-blooded.
- Small brain without corpus callosum.
- Optic lobes four. Cochlea of internal ear are of simple structure.
- Kidneys are of metanephric type. Ureters connects and open into the cloaca on a urinogenital sinus.
- Testes are present in abdominal region, penis retractile and only passes out sperms. Oviducts open into cloaca independently. Vagina and uterus absent.

- Females lays cleidoic and megalecithal eggs, cleavage meroblastic, placenta absent. Immature young ones feed on mother's milk in a temporary abdominal pouch developed during the breeding season.
- Prototherians possess a number of reptilian characters in addition to the mammalian characters. So they form the bridge to fill the gap between reptiles and mammals, i.e they act as connecting link between Reptiles and Mammals.

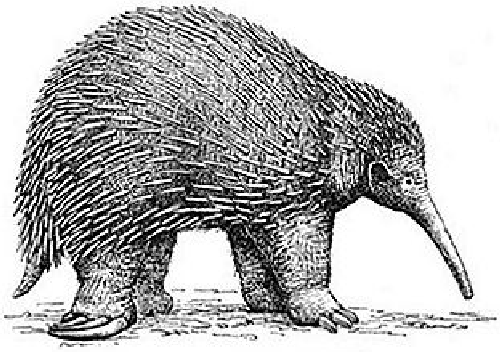
1) Monotremes

Prototheria is a subclass of class Mammalia. It includes only one living order monotremes. In monotreme mammals there are only two kinds in the world, echidnas and platypuses, and they both live only in Australia, Tasmania, or New Guinea. Monotremes are a very rare type of animal and different from other groups of mammals of placentals and marsupials. Monotremes are divided into triconodonts, docodonts, and multituberculates in the subclass Prototheria. Monotremes are primitive mammals known since the early Mesozoic in Australia from 200 million years, far longer than the therian mammals. There is one another monotreme lower jaw from the Cretaceous (*Steropodon*) known from Australia also. Australian platypuses are known since the Oligocene and echidna fossils are known in Australia since the Miocene.

Monotremes are warm-blooded, have fur, and produce milk to feed their young just like all mammals.The thing that is different about them is that they lay eggs instead of bearing live young. Some parts of their skeletons are more similar to a reptile than to a mammal.More primitive kinds of mammals such as monotremes would generally have been expected to be outcompeted when more advanced mammals like the placentals evolved. Australia contained no advanced mammals at that time, and its island isolation prevented advanced placental mammals from finding their way there. With no competition from other mammals, the monotremes survived. It is for this same reason that Australia contains so many marsupials which are also considered to be a more primitive kind of mammal.



ORNITHORHYNCHUS



TACHYGLOSSUS

Classification

Order Monotremata is divided into two families.

Family 1. Ornithorhynchidae : It have only one genus *Ornithorhynchus* commonly called duck-billed platypus. It is semi-aquatic, burrowing animal with clawed and webbed feet. Snout is produced into a beak like that of a duck. The body is covered with soft hairs.The platypus is the only animal that has a bill, webbed feet, fur, and feeds in water. They inhabit freshwater creeks, rivers, and lakes and can live in both hot and cold climates. They live in the eastern part of Australia and in Tasmania. Platypuses can stay under water for 2 to 8 minutes, and then it must surface to breathe through nostrils which are located at the front of its bill. Unlike female echidnas, female platypuses do not have a pouch.

Family 2. Tachyglossidae: It is also represented by single genus *Tachyglossus* (*Echidna*) commonly known as spiny ant eater.There are two species of echidnas. Echidnas have long snouts and spiny-like fur similar to a porcupine. The echidnas with the shorter snouts live in Australia and the lowlands of New Guinea and are called short-beaked echidnas. The ones with the longer snouts live in the highlands of New Guinea and are called long-beaked echidnas. The spines of an echidna do not look like fur, but they are. The spines themselves are stiff hairs. Normal fur grows between the spines and on the face, belly, and legs. Short-beaked echidnas feed almost exclusively on ants, although they will eat termites if no ants are available. Long-beaked echidnas eat ants and other small insects and earthworms.

Echidnas protect themselves from danger by curling up into a ball to protect the soft part of their bellies where they are most vulnerable. When confronted with danger, echidnas also use their powerful claws and quickly dig a hole and sink their soft bellies into the hole to protect them. Predators to echidnas are dingoes and goannas. Echidnas use their strong claws to dig into ant nests and termite mounds. When an echidna digs into an ant or termite nest, it smells out the ants or termites and feels for them with its sensitive snout. It sticks the snout in and out of the broken nest, flicking out its tongue which is covered in a special sticky saliva. When many ants or termites are stuck to the tongue, the echidna pulls it in, dirt and all and swallows. Echidnas often eat as much dirt as they eat ants or termites. Echidnas have no teeth. They crush the insects between the spiny base of their tongue and the roof of the mouth, and the grains of dirt help with the crushing. The crushed ants and dirt are then swallowed down into the stomach. Echidnas and other ant-eating mammals have a stomach with a special lining that can accommodate alot of dirt.The long snouts, long, sticky tongues, toothless mouths, and claws for digging are all adaptations that allow echidnas to successfully eat ants and termites. About 3 weeks after mating, a female echidna lays 1-2 eggs. Female monotremes have a pouch like marsupials. The female echidna lays her eggs directly into her pouch.

After about 10 days, the leathery eggs hatch. In marsupials like kangaroos and koalas, the newborn anchors itself in its mother's pouch by attaching itself to a teat. However, monotremes do not have teats, so baby echidnas and platypuses use their front legs to hold on to the hairs in the pouch. The mother secretes milk from pores in her skin. The newborns suck or lap up the milk from the skin and hairs. The baby stays in the pouch for about 2 months until it starts to grow spines. After that, the mother digs a burrow for the baby to shelter in. She continues to nurse the baby until it is about 5 months old.

Reptilian features of Monotremes

- A large coracoid with precoracoid and T-shaped interclavicle in pectoral girdle.
- Pelvic girdle is with epipubic bone.
- Vertebrae are without epiphyses. Ribs are single headed.

- Cloaca is present and testes are abdominal.
- Laying of large eggs with abundant yolk.
- Meroblastic cleavage is seen in the development.

Mammalian features of Monotremes

- Hair is present all over the body.
- Diaphragm is present.
- Only left aortic arch is present.
- Heart is 4 chambered.
- Non-nucleated red blood corpuscles are seen in blood.
- Mid brain shows four optic lobes.
- Skin possesses mammary, sweat and sebaceous glands.
- Three ear ossicles are present in the middle ear.
- A single bone (dentary) is present in each half of the lower jaw.
- A slightly coiled cochlea is present.

Metatheria (Marsupials)

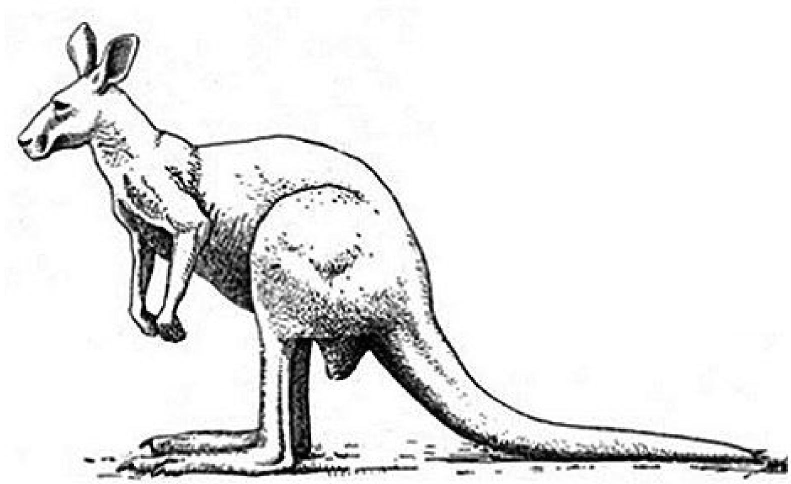
The marsupials occupy an intermediate position between the primitive mammals (monotremes) and the higher mammals (eutherians). Infraclass metatheria includes pouched mammals called marsupials. Marsupials are more advanced than the prototherians. They are all pouched mammals characterised by the presence of an integumentary brood pouch or *marsupium* in which the immature young ones are fed with the milk of the mother. The young ones are born in a much undeveloped state and are sheltered in a pouch called marsupium present in the abdomen of female. They are intermediate between monotremes and placental mammals.

Distinctive Features of Metatheria are:

- Metatherian mammals wereworldwidedistributed till the Miocene period, almost 90 million years ago, but today they are limitedto Australia and America only.
- By habitat point of view they are terrestrial, arboreal, burrowing, rarely aquatic, herbivorous or carnivorous animals.

- Marsupials exhibit discontinuous distribution. Some marsupials like *kangaroos* live in Australia and *oppossums* live in South America. Australia is described as the land of marsupials.
- Body covered with hairs with a well-developed, sometimesprehensile tail used for body balance during locomotion.
- External ear pinna is well developed.
- Mammary glands are modified sebaceous glands and have teats.
- Forelimbs are short whereas hindlimbs are long supported by narrow rod-like parafibula bone.
- Skin is glandular.
- The body temperature ranges from 36° C to 40° C.
- Female with a specialized pouch called as marsupium in the abdomen sustained by marsupial or epipubic bones and enclosing teats.
- Muscular diaphragm divides the body cavity into anterior thoracic and posterior abdominal cavities.
- Skull dicondylic and with distinct sutures. Mandible with a single dentary bone.
- Vertebrae with epiphyses. Seven Cervical vertebrae in the neck, atlas incomplete, thoracic vertebrae thirteen, lumber seven and caudal vertebrae with a chevron bone.
- Thoracic ribs are bicephalous.
- Pelvic girdle has epipubic or marsupial bones to support the marsupium.
- Dentition is heterodont, thecodont or monophyodont.
- Number of teeth is always more than 44. Number of incisors always not constant. Dental formula 5,1,3,4/4,1,3,4 =50.
- Anal and urinogenital apertures open individually into a shallow cloaca surrounded by a common sphincter.
- Stomach variable in shape and size.

- Circulatory, respiratory and excretory systems resembles like that of higher mammal.
- Small Brain without Corpus callosum.
- Olfactory lobes large and cerebral hemispheres small.
- Cerebellum small and exposed. Middle ear has 3 ossicles.
- Cochlea of internal ear spirally coiled.
- Testes lie in scrotrum in front of the penis which is bifurcated at the tip in some animals.
- In females number of vagina may be two or three, if three in number one is used for the birth of young one and the remaining two are used for copulation.
- Testes are situated in scrotal sacs. The penis is present behind the scrotum and it is bifid.
- The females have 2 oviducts, 2 uteri and 2 vaginae (didelphic condition) 2 clitoris are present which separately open into urinogenital sinus.
- Fertilization and a major part of development are internal,cleavage is holoblastic but the young ones are born in immature state (mammary foetus).
- Yolk sac placenta is present in marsupialia. True allantoic placenta is absent (except parameles). Yolk sac is large with villi.
- Gestation period short, 2 weeks in opossum and 5 weeks in kangaroo.
- Placenta develops for the first time in Metatheria, yolk sac placenta in all metatherians and allantoic placenta in Perameles.
- Young ones are immature at birth, about 2 cm long and 1 gm in weight.
- They are naked and blind and transferred to the marsupium.
- Youngones feeds and sleeps inside the pouch for about 190 days.



MACROPUS RUFUS

Metatherians are found in Australia and America. In Australia, due to absence of competition with higher mammals they inhabit all kinds of habitat and have developed different kinds of adaptations. Development of different kinds of habitat and have developed different kinds of adaptations. Development of different kinds of adaptations to live in different habitats by members of a single group of animals is called adaptive radition.

Classification

In this infraclass there is only one order Marsupialia represented by about 240 living genera. It is divided into two suborders on the basis of their dentition and structure of foot.

On the basis of dentition:

Suborder 1. Polyprotodontia: Each half of upper jaw has more than three incisors. It includes four families. Didelphidae, Dasyuridae, Notoryctidae and Peramelidae.

Suborder 2. Diprotodontia: Each half of upper jaw has less than three incisors. It includes four families. Caenolestidae, Phalangeridae, Phascolomidae and Macropedidae.

On the basis of structure of foot:

Suborder 1. Didactyla: Foot with all toes separate. Marsupium opens posteriorly. It includes families: Didelphidae, Dasyuridae, Notoryctidae Caenolestidae.

Suborder 2. Syndactyla : Second and third toes of hind foot united by a common sheath. Pouch opens forwards. It includes families Peramelidae, Phalangeridae, Phascolomidae Macropodidae.

Family 1. Didephidae: *Didelphys* (America opossum). *Chironectes* (water opossum).

Family 2. Dasyuridae: *Dasyurus* (Australian cat), *Thylacinus* (Tasmanian wolf). Myrmecobius (banded ant eater).

Family 3. Notoryctidae: *Notoryctes* (marsupial moles).

Family 4. Peramelidae: *Perameles* (bandicoots of Australia).

Family 5. Caenolestidae: *Caenolestes*, *Rhyncolestes*, (shrew-like marsupials).

Family 6. Phalangeridae: *Petaurus* (flying phalanger), *Phascolarctos* (Koala).

Family 7. Phascolomidae: *Phascolomys* (wombat).

Family 8. Macropodidae: *Macropus* (kangaroo), *Dendrolagus* (tree kangaroo), *Petrogale* (rock kangaroo).

Affinities

Metatheria possesses primitive as well as advanced characters, so it shows relationship with Prototheria and Eutheria.

8.10 Affinities with Prototheria

Similarities

- Clavicles present, epipubic or marsupial bones present, tympanic bone ring-like, tympanic bulla absent.
- Brain is well developed with large olfactory lobes and anterior commissure and without corpus callosum.
- True allantoic placenta is absent.
- Cloaca structure is present.

Differences

- Adults are with teeth.
- Vertebrae have epiphyses, ribs are bicephalous, interclavicle absent, coracoids reduced.

- Permanent marsupial pouch present.
- Mammary glands are with teats.
- External ears pinna is well developed.
- Cochlea of internal ear is not simple but spirally coiled.
- Testes are present in scrotal sacs, penis with bifid tip.
- Viviparous, underdeveloped young ones develop in uterus.

Affinities with Eutheria:

Similarities

- Mammary glands modified sebaceous glands, with teats.
- Dentition is of different shapes called as heterodont type.
- Four optic lobes (corpora quadrigemina) present in the brain.
- External ear part pinna is present.
- Cochlea of internal ear is of spirally coiled shape.
- Interclavicle absent, coracoids reduced and ribs bicephalous.
- Testes present in scrotal sacs, penis present.
- Uterus and vagina present.
- Viviparous, eggs microlecithal, cleavage holoblastic, development internal, placenta present.

Differences

- Cloaca is present.
- Specialized marsupial pouch is present.
- Small flat cranium, tympanic bulla absent, epipubic bone present, jugal extending back, palate with perforations, incisors more.
- Brain is without corpus callosum.

- Two vagina and uterus.
- Scrotal sac in front of the penis, penis with bifid tip.
- True allantoic placenta absent.
- Gestation period is short.

Metatherians mammals are more advanced than the primitive Prototherians and is close to the placental mammals but not close enough to belong to the same line of evolution. It is now believed that the Prototheria is a side line of evolution from mammal – like reptile and Metatheria and Eutheria have evolved independently from a common Pantotherian ancestor.

8.11 General account of Eutherians

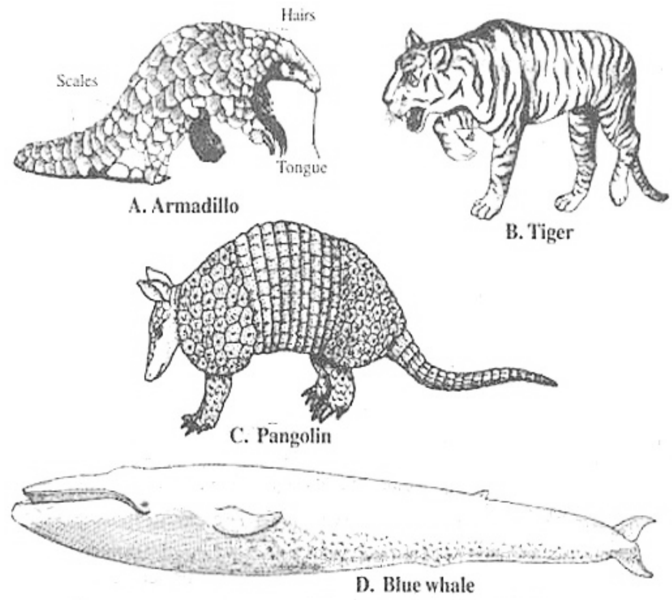
Eutherian mammals are a rather diverse group, with nearly 4000 described species, mostly rodents and bats. The eutherian mammals include such diverse forms as whales, elephants, shrews, and armadillos. They are also some of the most familiar organisms to us, including pets such as dogs and cats, as well as many farm and work animals, such as sheep, cattle, and horses and humans, of course, are also placental mammals.

Placental mammals all bear live young, which are nourished before birth in the mother's uterus through a specialized embryonic organ attached to the uterus wall, the placenta. The placenta is derived from the same membranes that surround the embryos in the amniote eggs of reptiles, birds, and monotreme mammals. The term "placental mammals" is somewhat of a misnomer because marsupials also have placentae. The difference is that the placenta of marsupials is very short-lived and does not make as much of a contribution to fetal nourishment as it does in eutherians, as "placental mammals" are known scientifically.

Eutheria ("true beasts") is a group of mammals which is distinguished from non-eutherians by various features of the feet, ankles, jaws and teeth. One of the major differences between placental and non-placental eutherians is that placentals lack the epipubic bones, which are present in all other fossil and living mammals. The absence of these bones is probably an adaptation that helps them be faster on the run than their relatives.

Distinctive Features of Eutheria are:

- These mammals have truly mastered all sorts of environments (Its distribution is world-wide). They range from the polar caps(polar bears) to the equator (gorilla) to the oceans(blue whale). Among other things Eutherians support vast orders that have the ability to completely modify their body temperature and heart beat. This helps them beat the odds in adverse climes like deserts and ice caps.
- These mammals are known to live in very harsh climates and have adapted to eat anything that they can, at an extremely fast pace. They are so diversified that there is no one behavior that can be truly called eutherian aside from giving birth to well defined and very active babies.
- Eutherians have adapted to be niche based eaters. They generally eat everything and each other when time and opportunity arises.
- The most numerous eutherians are rodents and humans. They seem determined to have a population competition and the rodents are losing. We humans are eating and despoiling the planet for all the rest of the animals and so are the biggest threat to ourselves.
- These are highly evolved mammals with advanced organisation.
- Marsupium and marsupial bones are entirely absent.
- Nourishment, respiration and excretion of young takes place through a complex *allantoic placenta.*
- The young ones are born in relatively advanced state (miniature adults). Complete development takes place in the uterus only. Gestation period is generally long.
- Testes are usually present in scrotal sacs. Penis is situated infront of the testes.
- Vagina is single. Urinogenital passage and anus are separate.
- Cerebral hemispheres in brain have more convolutions.
- Corpus callosum is well developed.
- Eutherians are viviparous.
- Common esamples: *Rats, bats, rabbits, monkeys, cats, dogs, whales, horses, deers, pigs, elephants, man etc.*



Adaptive Radiations of Eutherians

Starting with a recent single ancestor, this process results in the speciation and In evolutionary biology, adaptive radiation is a process in which organisms diversify rapidly from an ancestral species into a multitude of new forms, particularly when a change in the environment makes new resources available, creates new challenges, or opens new environmental nichesphenotypic adaptation of an array of species exhibiting different morphological and physiological traits with which they can exploit a range of divergent environments

The Osborn's law of adaptive radiation or divergent evolution states that "Each isolated region, if large and sufficiently varied in its topography, soil, climate and vegetation will give rise to a diversified fauna". There is a central or focal type from which animals diverge in different directions. The impelling causes of adaptive radiation are the need of food and safety which lead towards aquatic, amphibious, aerial, fossorial or arboreal habitat.

The earliest placental mammals arose during Cretaceous history as primitive insectivores and there was a wide range of adaptive radiation from this base through the Coenozoic era. With the disappearance of dinosaurs of the Cretaceous period, there was an "evolutionary explosion" of mammals to fill the ecological niches which were left by the dinosaurs. These mammals became adapted to many aspects of varied environment.

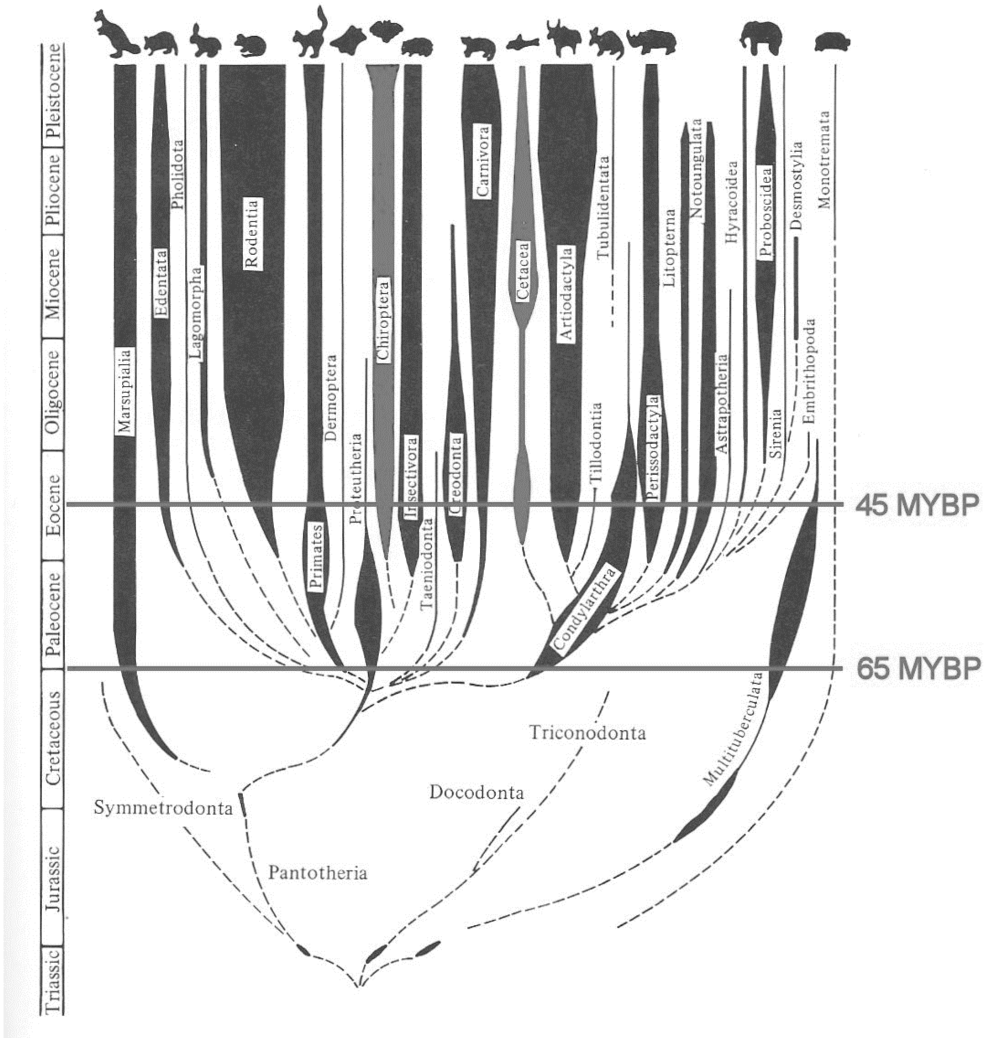


Figure – Adaptive radiations of mammals (MYBP - Million Years Before Present)

Central or focal type: It is represented by Insectivores like the Malayan insectivore Gymnura,modern elephant shrews of Africa, old world hedgehogs, shrews, moles and related forms and the golden moles. Most of the insectivores have been small and they have survived with little change from Cretaceous times to the present. They had flat plantigrade feet.

Cursorial types: The cursorial mammals includes the Eutherians like dogs, swift fox and other carnivores. The ungulates whichwalk on the tips of their toes usually have hoofs to protect their feet and take up the shock of running over hard ground. The ungulates are fast runners and include deer, the African and Mangolian antelope, horses, Rhinoceros, zebra, ass, tapir, etc.

All speedy animals show certain specific features :

- Body is modified externally to reduce resistance to the medium through which they pass.
- The hindlimbs are more efficient and better functioning than forelimbs which helps to get food as they are nearer to mouth and the extended hands pull the body forward in running.
- The primitive terrestrial foot is plantigrade but for cursorial adaptation the feet became elongated and there is reduction in the number of digits and hoof is formed.
- The length of neck also increases so that the animal may reach the ground for food and drink.

Arboreal type:

These are represented by the mammals which jumps and take long roaring leapsinto the air supported by folds of skin between the limbs and the sides of the body and thus have become semi-aerial. The different orders which have arboreal forms are :

- Rodentia which includes *Anomalurus* with six species found in Africa, *Pteromys*, *Sciuropterus* (flying squirrels) and *Eupetaurus* which is not perfectly arboreal but can climb rocks.
- Insectivora which includes *Galeopithecus* (flying lemur). It shows highest degree of aviation. The pataguim reaches its highest development as it extends from head between the fingers to the base of the claws, forming a web as in aquatic forms.
- Primates includes *Propithecus* of family Lemuroidae. It has powerful hindlimbs with which it takes long leaps. Modern lemurs are nocturnal and roam through the trees at night.
- Chiroptera includes bats which can fly by flapping of wings formed by patagium of skin fold supported by bones of the forelimbs. Their rate of metabolism is very high to provide them energy for flight. They are nocturnal and they have an echo-location system which helps them to fly during darkness of night.

Scansorial type: Some mammals take on the climbed habit for protection and for feeding. During the age of reptiles arboreal life was one of the means that protected the mammalian race and rendered its subsequent evolution possible.

The scansorial adaptations of mammals are as following-

- In gibbon (*Hylobates*) the arms are so long that the hands touch the ground when the animal stands erect.
- Feet are prehensile, with opposable digits to prevent the animal from slipping.
- Tail may be prehensile or non-prehensile. If non-prehensile spines and scales are present on the underside as in flying squirrel *Anomalurus*.
- The chest and ribs support the body while climbing. In sloth the ribs are numerous to support their viscera in inverted position.
- The shoulder girdle has strong clavicles and scapula to withstand the compression of powerful breast muscles.
- The hip bone (ilium) is broadened to support the viscera.
- The proximal limb elongates in direct ratio to the animals climbing powers.

The different orders which have scansorial forms are:

- Edentata – Tree Sloth, Anteater.
- Carnivora – Cat, Jaguar, Racoon, Brown Bear.
- Rodentia – Tree Porcupine, Flying Squirrel.
- Insectivora – Pen-Tailed Shrew, Flying Squirrel.
- Chiroptera – Bat.
- Primates – Baboon Monkey.

Fossorial type : There are a number of mammals which show fossorial (burrowing) adaptations. Some important subterranean forms are :

- Edentata – Armadillos (*Dasypus*).
- Insectivora – Common Mole (*Talpa*), Golden Mole (*Chrysochoris*), Shrew Mole (*Scalopus*), Water Shrew (*Crossopus*), Hedgehog (*Erinaceus*).

- Tubidentata – Aardvark (*Orycteropus*).
- Rodentia – Ground Squirrel (*Spermophilus*), Prairie Dog (*Cynomys*), Wood Chunck (*Aroctomys*), Mole Rat, Bamboo Rat.
- Lagomorpha – Hare (*Lepus*).
- Carnivora – Otter (*Lutra*), Skunk (*Mydaus*), American Badger.
- A Few Ungulates Dig For Food – Swine, Elephant And Mastodons.

Aquatic type : A number of mammals have become amphibious or secondarily aquatic. In transformation from terrestrial to aquatic forms they have undergone a number of modifications to suit aquatic mode of life. These specialized modifications will be described later. The different orders which have aquatic forms are :

- Insectivora – Aquatic Shrew.
- Rodentia – Musk Rat, Water Rat.
- Carnivora – Otter, Mink, Seal.
- Cetacea – Porpoise, Whale.
- Sirenia – Sea Cow.
- Artiodactyla – Hippopotamus.

8.12 Aquatic Adaptations in Mammals

Mammals are fundamentally terrestrial animals but because of competition on land for food and shelter some have developedaquatic adaptations, most probably. Aquatic mammals have originated from terrestrial ancestors. They are lung breathers and do not have gills. Their aquatic adaptations are secondary but they are very well adapted to lead aquatic life.

There are two categories of aquatic mammals :

1. Amphibious mammals : They are not completely aquatic. They live on land but go into water for food and shelter. They show incomplete aquatic adaptations. A few examples are Seal, Otter Walrus, Beavers, *Ornithorhynchus*, Hippopotamus, Etc.

2. Completely aquatic mammals: There are two groups of mammals which are completely aquatic. They are Cetacea and Sirenia. They do not come on land.

Aquatic adaptations

Completely aquatic mammals show many secondary aquatic adaptations which can be described as following-

Modification of structures

- Shape of body aquatic mammals is streamlined and boat-shaped like that of fish to reduce frictional force during movement in water.
- The body of aquatic mammals is usually large and heavy, but it does not create any problem for support in water due to buoyancy. Large size reduces skin friction and heat loss.
- The bones are light weighted and spongy and in Cetacea are filled with oil. In Sirenia the bones are heavy and solid, enabling the animal to stay under water for browsing on sea weeds. The cranium is shortened and produced into a pointed snout or rostrum which cuts the water efficiently. The neck is shortened and very much reduced. In whales the cervical vertebrae are fused. It helps the animal to pass through the water easily. The vertebrae are of simple type, the sacral region is not distinct due to reduction of hindlimbs.
- The forelimbs are modified into flattened and unjointed structures called paddles or flippers. They have no indication of fingers. They can move as a whole from the shoulder joint. They act as balancers and provide stability during swimming. Hyperdactly and hyperphalangy serve to increase the surface area of flippers and thus help in swimming.
- The nostrils are situated on top of the head so that the animal can breathe air easily without raising the head much out of water.
- The diaphragm is slanting making the thoracic cavity larger, dorsal and barrel-shaped, thus providing more space to lungs to expand.
- The lungs are like air sacs, large, highly elastic and extensible which can store large volumes of air. This helps the animal to remain under water for a considerable period of time. The dorsal lungs also act as hydrostatic organ.

- Aquatic mammals cannot use oxygen dissolved in water. They depend on atmospheric oxygen which is stored in various parts of the body.
- The circulatory system consists of a network of blood vessels, these networks are termed as*ritia mirabilea*. Their function is to store oxygen. The oxygen carrying capacity of haemoglobin is very high.
- Osmoregulation is maintained by the large and lobulated kidneysby excreting excess salt in the form of urine.
- These aquatic mammals feed their babiesunder water by mammary glands which storesa large amount of milk.
- The skin surface is without hair covering except for a few bristles near the mouth.
- Ear pinnae and hindlimbs are completely absent to avoid any type of hindrance in movement in water.
- All type of skin glands like sweat and sebaceous are absent as they are of no use in water.
- Eyes are without nictitating membrane and lacrimal glands.
- Testes are present in abdominal part not in scrotal sacs.
- The tail develops large, lateral or horizontal expansions called tail flukes. Their up and down strokes propel the animal forward and also enable them to return rapidly to the surface for breathing.
- The median dorsal fin of Cetacea serves as a rudder during swimming.
- A thick subcutaneous layer of fat called blubber is present below the skin. It acts as a heat insulator and a reservoir of food. It also reduces the specific gravity of the animal and thus help in maintaining buoyancy.
- Whalebone whale feeds on plankton.
- Teeth are absent. There are numerous triangular horny plates called baleen or whalebone which hang down from the upper jaw in two transverse rows like the leaves of a book. During feeding large quantities of water is allowed to pass through these plates which serve as an effective sieve for filtering plankton.

- Middle ear cavity is filled with a fine emulsion or foam consisting of fat, mucus and gas. It improves hearing under water.
- The specialized harderian glands produce an eye protective special fatty secretion.

Flight or Volant type

There are some mammals which can fly in the air. The adaptations for flight are called volant adaptations.

Flight is of two types :

1. Passive flight or gliding.

2. Active flight or true flight.

Gliding or passive flight: Gliding is a simply an aerial leap covering a distance of 10 – 20 meters. The animal glides from one tree to another with the help of its stretched patagium and limbs. Gliding is a passive flight therefore does not require much energy. Energy is utilized only during initiation, no energy is utilized when the animal is in the air. Some common example of gliding mammals are: flying lemur, *Cynocephalus* (order Dermoptera), wolly flying squirrel, *Eupetaurus* and brown flying squirrel, *Petaurista* (order Rodentia).

Gliding adaptations

- Body is elongated, flattened and spindle – like.
- Limbs are long and equal in size.
- Tail is long and tapering, helps in maintaining body balance
- A specialized structure Patagium is located on the side of the head, neck, limbs and tail, supported by ribs which acts like parachute during gliding.

Active or true flight: Wings are used for active flight. As the name shows large amount of energy is utilized during true flight. True flight is exhibited by bats (order Chiroptera). Examples-*Pteropus* (flying fox),*Cynopterus* (fruit eating bat), *Vampyrus* (American vampire), *Megaderma* (Indian vampire).

Flight adaptations

- The forelimbs are modified into wing-like structures called patagia which are modified forms of thin folds of skin. They extend on the sides of the body and are supported by skeleton elements of the forelimbs.

- The bones of forelimbs are elongated and its digits are much elongated to support the wing. In *Microchiroptera* the first finger or pollex is short and free and bears a well-developed curved claw. The remaining fingers are clawless. In *Megachiroptera* the second finger is also clawed. The forelimbs are also used for walking, climbing, holding food and killing prey.
- A fold of membrane called inter-femoral membrane extends between the hindlimbs and also encloses the tail. The hindlimbs are rotated outwards so that the knee is directed backwards instead of forward. This helps in maximum spread of wing membranes. The claws help the bat to hang from trees.
- Light weighted bones minimizes the body weight. The ribs are flattened and fused with the vertebrae. This gives large surface area for the attachment of flight muscles. A spur of bone called calcar projects from the tarsus of each foot. It supports the inter-femoral membrane.
- The flight muscles are well developed for flying.
- Tail may be small, large or scarcely visible which can act as a brake to fight, as a pouch to hold food and for the reception of a new born during flight.
- Teeth comes 2 times during life time and are of different shapes. Young ones can grip the teats of mammary glands even while the mother is flying with help of these structures.
- Bats are provided with an advanced echo location detecting apparatus like radar system or ultrasonic sound system. They fly with their mouth open and continuously produce ultrasonic sound waves. These waves strike on solid objects and are reflected back. These reflected waves and received by the ears. This helps the bats to locate the obstacles in their path. Bats are nocturnal animal. Due to this system they are able to fly easily and swiftly even in total darkness. The pinnae is also sensitive accessory lobe called targus which help to pick up echoes. The nose is provided with leaf-like lobe called nose leaf which probably acts as an antenna and perceive air vibrations.
- The wings are richly vascularized with blood vessels and nerve fibres. They probably can also act as tactile organs.

8.13 Fossorial type

The animal which lead subterranean life are called fossorial. Fossorial adaptations are adaptations for burrowing. Fossorial mammals are of two types :

1. Semifossorial: These are partially fossorial. They do not use burrows for living purpose only for searching their food or for retreat. Mammals like swines, elephants and mastodon dig the ground for food. They dig with the help pointed snout (swine) or long tusks (elephant and mastodon). Some mammals like rabbit, hare, otter, badgers and skunk dig burrows for retreat. Their limbs are modified for digging.
2. Completely fossorial: These animals lead a completely subterranean life. Some examples are: – Armadillo, Aardvark, Moles, Shrews, Hedgehog, Ground Squirrel, etc.

Fossorial adaptations

- Fusiform body have little resistance in entering and exiting out of burrows. The head tapers anteriorly forming a snout for digging. The shoulders are broad, strong and powerful for digging burrows.
- Tail is usually short (hedgehog) or vestigial (moles) to reduce hindrance during burrowing.
- The eyes are reduced or functionless as they are of no use in dark habitat.
- The external ears are absent to avoid any hindernace in burrowing.
- The shape of snout is conical which supports in digging. In mole a prenasal bone is well developed at the tip of the nose. Incisors and sometimes canines are directed forward for digging. Limbs are short and stout. The forelimbs are broad and powerful with strong claws for digging.
- Hindlimbs produces pushing force by which the body moves forward into the burrow. The sacral vertebrae are fused together to provide solid support to hindlimbs.
- Hibernation or winter sleep is an adaptive measure for fossorial animals in unfavourable conditions.

8.14 Summary

The mammals are the final product of evolution. They are the highest group in the animal kingdom. The class Mammalia includes man, the master of the present age.Mammals are a clade of endothermic amniotes distinguished from reptiles and birds by the possession of a region of the brain - neocortex, hair, three middle ear bones and mammary glands.

Mammals include the largest animals on the planet, the great whales, as well as some of the most intelligent, such as elephants, primates and cetaceans. The basic body type is a terrestrial quadruped, but some mammals are adapted for life at sea, in the air, in trees, underground or on two legs. The largest group of mammals, the placentals, have a placenta, which enables the feeding of the fetus during gestation.

First Mammals originated in the upper Triassic period of Mesozoic era about 200 million ago, but for over 100 million years they remained an insignificantanimals group. In the Palaeocene to Pleistocene period of the Cenozoic era the mammals became dominant, as such this period is known as the 'age of mammals'. Within this period of about 55 million years many ancient mammals have disappeared leaving no descendants.

Mammals holds the top position in the evolutionary tree as they are the final product of evolution.Mammals are supposed to be originated from reptiles, the fossils show that the reptiles that gave rise to mammals were Synapsids. Synapsids diverged from the main reptilian stock almost at the base of phylogeny during Permian period. Cynognathus, a mammal like reptile, belonging to suborder Theriodontia, order Therapsida and subclass Synapsida is regarded as the probable mammalian ancestor. It lived about 230 million years ago in the early Triassic period.

Prototherians possess primitive and degenerative features. They are not in the main line of mammalian evolution. It is concluded that Prototheria originated as a side line from mammal-like reptile ancestor and continued to survive in isolation retaining those primitive characters through which higher mammals have passed. It is not the ancestor of higher mammals.They are regarded as a connecting link between reptiles and higher mammals.

The marsupials occupy an intermediate position between the primitive mammals (monotremes) and the higher mammals (eutherians). Infraclass metatheria includes pouched mammals called marsupials. Marsupials are more advanced than the prototherians. They are all pouched mammals characterised by the presence of an integumentary brood pouch or *marsupium* in which the immature young ones are fed with the milk of the mother.

Eutherian mammals all bear live young, which are nourished before birth in the mother's uterus through a specialized embryonic organ attached to the uterus wall, the placenta. The placenta is derived from the same membranes that surround the embryos in the amniote eggs of reptiles, birds, and monotreme mammals.

Adaptation is the ability of birds to adjust in the existing aquatic or aerial environment. It is the adjustment of organisms in the surrounding environment for their existence by changing the body structure accordingly.

The earliest placental mammals arose during Cretaceous history as primitive insectivores and there was a wide range of adaptive radiation from this base through the Coenozoic era. With the disappearance of dinosaurs of the Cretaceous period, there was an "evolutionary explosion" of mammals to fill the ecological niches which were left by the dinosaurs. These mammals became adapted to many aspects of varied environment.

8.15 Self-Assessment Questions

1. Describe the distinguishing characters of Mammals.
2. Describe the salient features of prototheria.
3. What do you mean by Metatheria? Explain its classification
4. Explain various theories of origin of mammals
5. Explain the distinctive features of Marsupials.
6. Describe the classification of mammals up to order in details .
7. Write about mammals of the mesozoic.
8. Describe the affinities of metatheria details.
9. Write an essay on different types of adaptive radiations of eutherian mammals.
10. Explain the distinctive features of eutheria.
11. Give a short note on flight adaptations of mammals.
12. Write a short note on aquatic adaptations of mammals.
13. Describe the Scansorial and fossorial types of mammals.

8.16 Reference Books

- Modern Text Book of Zoology: Vertebrates by R.L. Kotpal
- Text Book of Vertebrate Zoology by J. S. Kingsley
- Hyman's Comparative Vertebrate Anatomy by Marvalee H. Wake
- Textbook of Vertebrate Zoology by S. Prasad

Unit - 9

Ancestory of man and horse

Structure of the Unit:

9.1 Objectives

In this chapter, the student would acquaint himself with the various aspects about human evolution, evolution Process, the place of human origin, details of different evolutionary stages of human- *Ramapithecus, Australopithecus, Homo habilis, Homo erectus,* Java men, Peking man, Heidelberg man, Neanderthal man, Cromagnon Man, Modern Man (*Homo sapiens*), changes in body, behaviour of human during evolution, various similarities and dissimilarities of of man with other primates, distinguishing features of primates with man and brief idea about different fossil records of man's ancestory

Various evolutionary Changes in horse, the place of Origin, Details of small Eocene Horses- *Hyracotherium***or** *Eohippus (Dawn horse), Orohippus, Epihippus, Mesohippus, Miohippus, Anchitherium, Hypohippus, Arshacohippus*(pigmy horses of Miocene), *Parahippus, Merychippus, Hipparion, Neohipparion* and *Nannippus,* True Equines /One-Toed Horses, *Calippus, Pliohippus, Astrohippus, Dinohippus, Equus,* Modern Equines, Different characteristics of the Living Modern Horses

9.2 Evolution of Man

Introduction

Human evolution is the long process of modifications by which people originated from apelike ancestors. Scientific evidences shows that the physical and behavioural traits shared by all people originated from ape like ancestors and evolved over a period of at least 5 million years.The biological name for "human" or "man" is *Homo*. The modern human species is called *Homo sapiens*. "*Sapiens*" means "thought". *Homo sapiens* means "the thinking man".

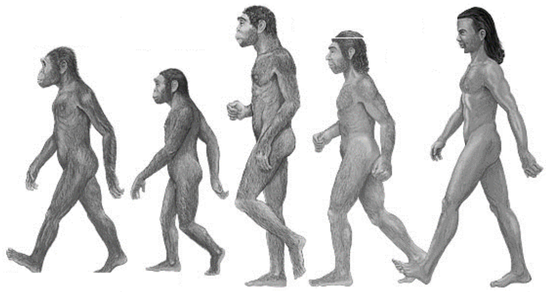
One of the earliest defining human traits, bipedalism - the ability to walk on two legs, evolved over 4 million years ago. Other main human characteristics are such as a large and complex brain, the ability to make and use tools, and the capacity for language are developed more recently. Many advanced traits like complex symbolic expression, art, and elaborate cultural diversity are emerged mainly during the past 100,000 years.

The scientific study of human evolution is called as **Paleoanthropology**. Paleoanthropology is a subfield of anthropology, the study of human culture, society, and biology. It involves an understanding of the similarities and differences between humans and other species in their genes, body form, physiology, and behavior. Paleoanthropologists search for the origins of human physical traits and behavior. Man always want to discover how evolution has shaped the potentials, tendencies, and limitations of all people. This scientific field investigates the origin, over millions of years, of the universal and defining traits of our species.

Human evolution is about the origin of human beings. All humans belong to the same species, which has spread from its birthplace in Africa to almost all parts of the world. Its origin in Africa is proved by the fossils which have been found there.

9.3 Human Evolution Process

Evolution means the process involving a series of natural changes that cause species of different organisms to arise, adapt to the environment, and become extinct. All species or organisms have originated through the process of biological evolution. In animals that reproduce sexually, including humans, the term species refers to a group whose adult members regularly interbreed, resulting in fertile offspring, offspring themselves capable of reproducing.



Whenever there is change in the genetic structureevolution happens. This genetic material inherited from the parents and especially in the proportions of different genes in a population. The hidden information inside genes can be changed by a process known as mutation. The way particular genes are expressed that is, how they influence the body,behaviour and other factors of an organism can also change. Genes affect how the body and behaviour of an organism develop during its life, and this is why genetically inherited characteristics can influence the likelihood of an organism's survival and reproduction. Evolution does not change any single individual but it changes the whole population by inherited means of growth and development. Parents inherits selected changes or adaptive genetic changes to their offspring, and ultimately these changes get regularize throughout a population. As a result, the offspring enhance the chances of their survival and ability to give birth, which may work well until the environment changes. Human evolution took place as new genetic variations in early ancestor populations developed new abilities to adapt to environmental change and so altered the human way of life.

Modern humans are termed as *Homo sapiens*.It is assumed that the modern man might have been evolved from some primitive Eutherian mammals .The first prosimion primates evolved from these ancestors in coenozoic era. These primates were appeared like the present day Lemur and tarsius.Most of the tarsius extinct in Eocine epoch and their descendants diverged in different directions and were the ancestors of modern anthropoids.The ancestors of modern anthropoids had reduced nose, grasping hands and feet with nails in fingers and toes. The divergent line of their evolution gave rise to the ancestors of modern old world monkeys new world monkeys, apes and man.

The fossil record suggest that Man and modern apes had a common ancestor known as homonids. It is presumed that homonids existed 24 millions years ago.Apes and modern man diverged out from the main line of homonids evolution at different periods. The main changes in the evolution of humans was the development of an upright posture, increase in intelligency and the increase in brain size.

9.4 Place of human origin

The oldest known human fossils are found in Asia, China and Java. The place of origin of man is supposed to be Central Asia as per evidences available. It was a central place for migration and place of oldest civilization. The climatic conditions in Asia in Miocene or Early Pliocene were favorable for ancestors to descend from the trees and it was necessary for the further human development.

9.5 Major Evolutionary stages of human

1. Ramapithecus

The earliest fossils bearing the traits of the hominid are those belonging to genus *Ramapithecus*. It is the most important hominid from Miocene period. In recent years *Ramapithecus* has been accepted as the first true hominid. There are at least two dozen fossils specimens that have been identified as belong to *Ramapithecus*. Most of these specimens consist of teeth and jaws and they principally come from two areas – the Siwalik Hills in India and Fort Ternan in Kenya.

The first discovery of *Ramapithecus* fossils was made by G.E. Lewis in 1932 in the Siwalik hills regions of India. He assigned one of the fossils, an upper jaw, to a new genus and species he named *Ramapithecus brevirostris*. Next *Ramapithecus* fossil was discovered by L.S.B. Leakey near Fort Ternan in south western Kenya in 1961. The specimen included parts of both sides of an upper jaw.

Characteristics

- Incisors and canine are vertical and not in slight procumbent position as in apes.
- Little or no canine diastema.
- The canines of the *Ramapithecus* are not projected and they possess narrow faces.
- The dental arcade is rounded.
- The palate of the *Ramapithecus* is arched as in man.

- Flattened and thick enameled premolars and molars that appear to be adapted for heavy chewing and processing of hard food stuffs.
- *Ramapithecus* has a canine fossa (*Kenyapithecus*).
- The molars possess the *Dryopithecus* Y-5 cusps pattern.
- Slightly divergent tooth rows. The tooth rows have been identified as parabolic by some and V-shape by some others.
- Reduction of size of third molar as compared to first and second molar.
- The ratio between the sizes of front tooth (incisors and canine) and those of cheek teeth (premolars and molars) is roughly the same which indicates the human position.
- Large inferior torus on mandible.
- Short maxilla that would indicate a placement of the chewing muscles that increase the chewing pressure brought to bear on the food being eaten.
- Facial profile is orthognathus.

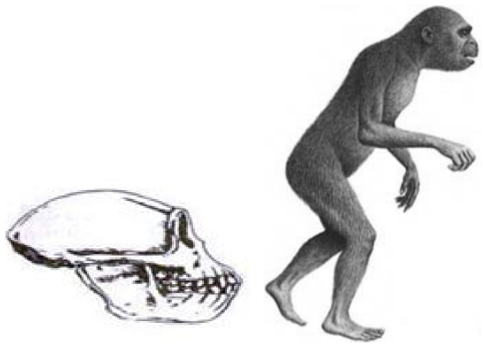


Figure -*Ramapithecus*

2. *Australopithecus*

It is the first human like form which existed about 5 million years ago in the dry land of Africa. It provides a lot of information about the evolution of man. *Australopithecus*was the connecting link between apes and man.In 1925, Prof. Raymond Dart discovered a skull consisting of the face embedded in limestone, the cast of brain and the jaw with teeth, from a limestone cave near Taungs, Bucchana land, South Africa. The skull was of an anthropoid ape and it was named *Australopithecus africanus* or southern ape. These forms are similar to primitive apes in certain characteristics and to man in many others. The most ancient of these near-men lived in the Early Pleistocene (about two million years ago) and some of them persisted until as recently as 900,000 years ago.

Characteristics

- The brain case has only a frontal bone which was not complete.
- The brain capacity of Australopithecus was 500 c.c., resembling to that of ape. In other features, it resembles to man.
- Its brain was more man-like in shape than that of gorilla or chimpanzee.
- The parietal lobe was much better developed than in the living anthropoids.
- Occipital condyles anterior.
- Nuchal crest reduced.
- Palate reduced but longer than in man.
- *Australopithecus* was able to stand and walk erectly as *Pithecanthropus* and was over four feet tall.
- They lived in caves and uses weapons of bones.
- The face of Australopithecus had better developed eyebrows and had no bony ridges above the eyes (dissimilarity from gorilla and chimpanzee).
- The front teeth of Australopithecus were like those of man, small in size and less forwardly projected.
- They had long sized canines and incisor.
- 20 milk teeth and four permanent teeth (molars).
- Pelvic broad and flattened.
- They ate both meat and vegetables and they manufactures, and used crude tools of stones.

There is a gap of about six to seven million years in *Ramapithecus* and *Australopithecus*. But recent discoveries from Omo valley in Ethiopia showed that the *Australopithecus* is much older (two to four million years' old) than it was thought previously. *Australopithecus*is supposed to be nearer to *Ramapithecus.*It is believed that *Australopithecus* is also nearly related to man and possibly very near to the human ancestor. They form a connecting link between man and apes.

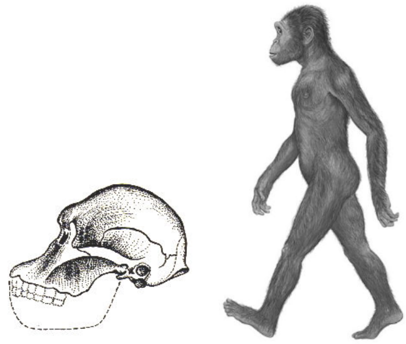


Figure -*Australopithecus*

Modern human ancestors

3. Homo habilis

In the early 1960s, at Olduvai Gorge, Tanzania, Louis Leakey, anatomist John Napier, and paleoanthropologist Philip Tobias described a newly discovered group of early human fossils that showed a cranial capacity of 590 to 690 cc. Based on this brain size, the scientists concluded this as a new species, *Homo habilis*, should be recognized.

H. habilis lived in eastern and possibly southern Africa between about 1.9 million and 1.6 million years ago, and maybe as early as 2.4 million years ago. Although the fossils of this species somewhat resemble those of *Australopithicus*, *H. habilis* had smaller and narrower molar teeth, premolar teeth, and jaws than did its predecessors and contemporary robust species.

Characteristics

- It was about 4.5 feet tall, walked erect and weighted about 40 to 50 kg.
- The cranial capacity was about 700 cc.
- It have longer arms relative to her legs in comparison to Lucy (*Australopithicusafarensis*).
- Apelike body proportions.
- H. habilis also had more modern-looking feet and hands capable of producing tools. They used earliest stone tools.
- They used tools for hunting and defence.
- *H. habilis* had a large amount of sexual dimorphism(female skeleton was dwarf in comparison with male)

- It existed about 2 million years ago, its fossils were recoverd from east Africa.
- They were omnivorous.

4. Homo erectus

The first discoveries of early human fossils in the 1800s were made in Europe, later discoveries made from Asia and included fossils from the Indonesian island of Java. The first finds from Java were in 1891 by Dutch physician Eugene Dubois. Dubois named this early human *Pithecanthropus erectus*, or "erect ape-man". Today paleoanthropologists refer to this species as *Homo erectus*. H. erectus was the first human species known to have spread in large numbers beyond the African continent.*H. erectus* was very successful, and was widelyspreaded in Africa and Asia. It appears to spread to Asia between 1.8 million and 1.5 million years ago. They are the youngest known fossils of this species, from the Solo River in Java, have been dated to about 50,000 years old.

Characteristics

- They existed about 1.7 million years ago.
- Two similar fossils were recovered from Java and Peking and are supposed to belong the same species *homo erectus.*
- The fossils recovered from Java was named as Java men and those recovered from Pecking was named and Pecking man.
- *H. erectus* had a low and rounded braincase that was elongated from front to back, a prominent brow ridge.
- An adult cranial capacity of 800 to 1,250 cc, an average twice that of the australopiths.
- Its bones, including the cranium, were thicker than those of earlier species.
- Prominent muscle markings and thick, reinforced areas on the bones of *H. erectus* indicate that its body could withstand powerful movements and stresses.
- Its body was well adapted for bipedal walking.
- Although its teeth were much reduced in size from *Australopithecus*, its lower jaw was still quite thick and rugged looking.

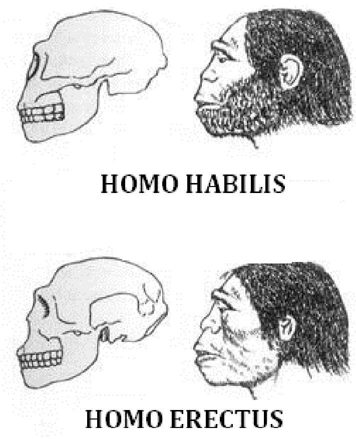


Figure – Comparison of *Homo habilis, Homo erectus*

5. *Pithecanthropus erectus (*Java men)

Java men was found at Trinil on the banks of the Solo river in Java by Dr. Eugene Du Bois in 1891. This species has been designated by Haeckal as "**the last link**" in human genealogy. It was the first known human fossil till now, dated from Lower Pliestocene epoch.

Characteristics

- It occupied an intermediate position between the *Australopithecus* apes and the most ancient ape man such as *Pithecanthropus*, the Peking man and the Heidelberg man.
- *Homo habilis* was an ape man who hunted large animals with the aid of self made stone implements.
- They might have used tools and also learned to light fire.
- It was more than 5 feet tall.
- Its brain capacity was 940 c.c., while the average brain capacity of a modern man is about 1400 c.c., and of gorilla between 450 to 550 c.c.
- The brain case was low, forehead flat.
- The skull was broad and has a rounded back.
- The skull cap is very human, but with very prominent eye brow ridges, like those of the famous *Neanderthal cranium*.
- They possessed essentially human articulate speech, however, rudimentary.
- The upper jaw is very large and protrudes forward.
- The lower jaw is also heavy and lacks a chin.

- Its have school lzm
- Teeth are very like those of man.
- The teeth are large, canines project slightly beyond the level of the other teeth.
- The femur also resemble with human, but also shows some resemblance to that of gibbons.
- Its size, however, indicates an animal which stood, when erect, not less than five feet six inches high.
- Prominent chin was absent but eyebrow ridges were present over the eyes.
- The eyebrows were heavy and protruding.
- The impressions left by the cerebral convolutions are also very human, more highly developed then in recent apes.

Figure - *Pithecanthropus erectus (*Javamen)

6. *Sinanthropus pekinensis* (Peking man)

The first fossil remains of early human types were discovered near Peking around 1922. They consist of several teeth, lower jaw and a number of fragmentary skulls. These fossils are frequently known as the Peking man or *Sinanthropus pekinensis*. They belonged to Lower middle Pleistocene.

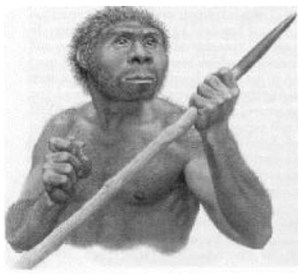


Figure - *Sinanthropus pekinensis* (Peking man)

Characteristics

- *Sinanthropus* and *Pithecanthropus* may prove variants of the same human type.
- Weidenreich thinks that *Sinanthropus* was the ancestors of the modern Mongoloids and was a contemporary of *Pithecanthropus*.
- The first known users of fire and of primitive stone tools.
- The cranial capacity varies from 850 c.c. to 1300 c.c.
- It had under developed chin but developed eyebrow ridges.
- The skull resembles to that of *Pithecanthropus* in having large supra-orbital ridges.
- The frontal region was better developed than in Pithecanthropus.
- The prominent ridges, running down the middle of the skull, is a characteristic, found today in Eskimos and Australians.
- The molar teeth were much more human-like than those of the Pithecanthropus.
- Mandible chinless with massive canine teeth.
- Skeleton unknown, but evidence suggests an erect posture.

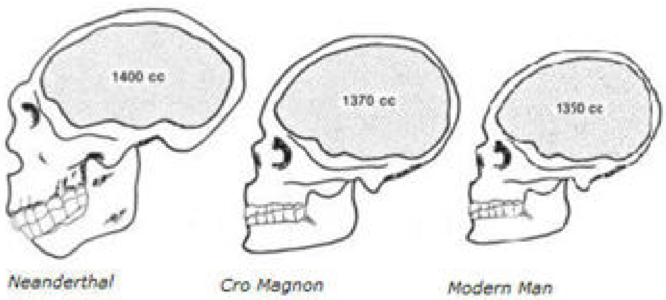


Figure – hominid cranial capacities

7. Heidelberg man

In 1907, the fossil remain of Heidelberg man was found from the river sand at Maver near Heidelberg in Germany.

Characteristics

- Its age appears to be Early Pleistocene.
- It consists of lower jaw with all the teeth.

- Its teeth are human-like, the massive jaw is ape-like.
- The chin slopes away as the Piltdown man, while the area for muscular attachment is extremely powerful.
- The teeth, relatively small for the jaw, form a continuous series and the canines do not rise above the level of the other teeth, both of which are human features in contrast with those of apes.
- Teeth have short divergent roots, a bulging crown and a relatively large pulp cavity, which Keith considers as an adaptation to a peculiar sort of diet.
- Its anatomical similarities suggest that Heidelberg man might have been the ancestor of Neanderthal man.

8. Neanderthal man

The first Neanderthal skull was found in 1848 in a cave in the rock of Gibraltar and it remained ignored for several years. The Neanderthal skull found by Prof. Schaffhausen in 1856 in a limestone cave in the Neanderthal near Dusseldorf (Germany) is the most notable. This skull represents the most primitive and least specialized of any skull type known to be distinctly human. It has therefore been recently named as a distinct species of man, *Homo Neanderthalensis*.

Characteristics

- They lived about 40000 years ago and flourished in Europe, Asia and North America.
- It resembled modern man but was relatively short.
- Short stature but powerfully built and walked with a bend at the knees.
- Average stature was about 5 feet
- They made tools for hunting and used animal skin for clothing.
- They made use of fire and buried his dead.
- They were capable of communication with each other and had primitive type of social life, division of labour, religion and culture.
- The brain case of Neanderthal man is large and its volume is surely human but proportions are again less like those of recent man than like anthropoids.

- Its cranial capacity was 1450 cc.
- The presence of large supra-orbital ridges (primitive).
- The head was thrust-forward.
- Chin was prominent.
- Limbs stout, short and thick.
- Chest is large and robust, shoulders broad and stooped forward.
- The knees bent forward, leg powerful with a short shin and clumsy foot, clearly not of cursorial adaptation.
- The hands are large and the fingers and thumb are devoid of much movement which is characteristic of modern man.
- The heavy head and enormously developed face.
- It is quite different from the modern man and so it is named *Homo neanderthalensis*. The man was hunter and used stone tools and weapons.

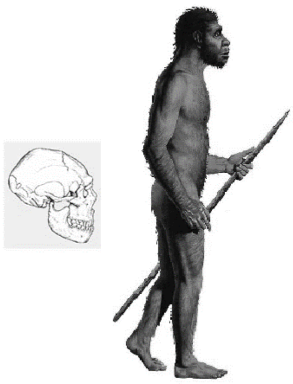


Figure – NeanderthalMan

9. Cromagnon Man

Its fossils were recovered from cromagnon rocks of France. The Cro-Magnon show their elaborate burial customs and their belief in life and death.

Characteristics

- The sum of the Cro-Magnon characters is certainly Asiatic rather than African.
- They resembled to the modern man in size and appearance.
- They were expert in making weapons and tools.

- They lived in the caves of Europe and left some beautiful pictures of animals.
- They were about 4 feet tall.
- The cranial capacity was 1600 cc.
- He had massive but narrow skull with a broad face.
- The forehead was broad and had raised narrow nose and prominent chin.
- Stature of old man was about 5 feet 11 inches. Average for males being 6 feet 1.5 inches and for women 5 feet 5 inches.
- Jaw thick and strong.
- Fore arms long as compared with his upper arms, in contrast with Neanderthal type. Hence Cro-Magnon were swift-footed, while Neanderthal were slow.
- The forehead was broad, and orbital ridges reduced.
- Cro-Magnon was progressive and was expert in making weapons and tools.
- He was a hunter and also an artist.
- He used fire and clothing.
- There was decline of Cro-Magnon in their artistic culture due to environmental causes

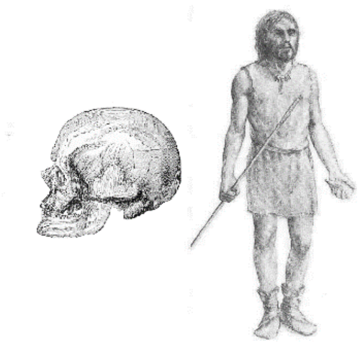


Figure – CromagnonMan

10. Modern Man (*Homo sapiens*)

Homo sapiens first begins to appear in fossil record about 3,00,000 years ago, i.e., at about the time of Java and Peking man, man of definite modern characters evolved in Europe. Another important finding of *Homo sapiens* was made in France in 1947. Africa has also produced an early *Homo sapiens*, which was as old as Swanscombe man.Hence, *Homo sapiens* includes all the existing men and also some extinct men. *Homosapiens sapiens* emerged from damp dark caves, built huts of animal skins into the open place and began sedentary communal life in fixed places. They spread all over the world and become a dominating species. They domesticated animals, learned to plant crops, store food and brought about cultural revolution.

Characteristics

- The entirely erect posture, with four reversed curves in the spine which act as a shock- absorbing device to protect the nicely poised skull.
- The limbs are straight, but the segmental proportions vary racially and individually.
- The skull also varies in size and relative proportions, such as length to breadth.
- The forehead is generally steep and the continuous brow ridge is absent.ive feature is the protruding chin prominence, the result of the reduction of the dental arch.

Figure – *Homo sapiens*

Changes during human evolution

Man's physical evolution has reached to an end point now, but if any change is being effected, it is largely retrogressive. Such retrogressive changes are :

(1) Reduction of hair and teeth, and of hand skill.
(2) Senses of sight, smell and hearing will become more efficient in response to the selective force of a high speed society.
(3) Bones of skull will generally become thinner because of the reduced stress from the jaw muscles.
(4) Face will become generally more refined and beautiful because of reduced physical stress and because of the action of sexual selection.

(5) Forehead will be larger and the eyes set more deeply. Nose will be more prominent and narrow. Mouth will be smaller, but the chin will be more prominent.

(6) Body will be slender. Breasts of the female will be smaller.

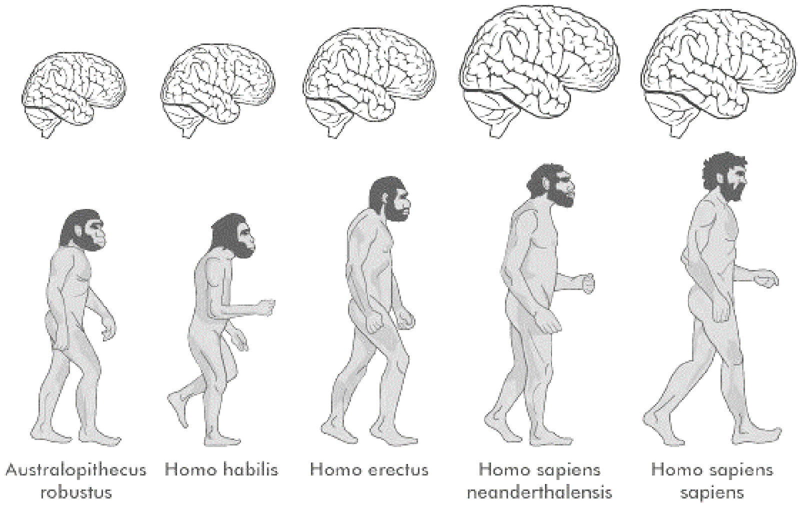


Figure – Brain size of different human evolutionary stages

9.6 Relationship of man with other primates

Human beings belong to the mammalian group known as Primates - the scientific category that contains over 230 species of chimpanzees, lemurs, lorises, tarsiers, monkeys of the Old and New World, apesand gorillas. The modern human species, *Homo sapiens*, has a very close relationship with primate. Modern humans, early humans, and other primate species all share many similarities and have some important differences. Knowledge of these similarities and differences helps scientists to understand the roots of many human traits and the significance of each development in human evolution. They share a common ancestor that lived between 5 and 8 million years ago. They shows various physical and genetic similarities

All primates, including humans, share at least part of a set of common characteristics that distinguish them from other mammals. Many of these characteristics evolved as adaptations for life in the trees, an environment in which the earliest primates evolved. These characteristics include more support of sight than smell; overlapping fields of vision, allowing stereoscopic - three-dimensional sight; limbs and hands adapted for clinging on, leaping from and swinging in the trees; the ability to grasp and manipulate small objects by using fingers with nails instead of claws; large brains in relation to body size; and complex social lives.They share all of the basic characteristics of primates, although their brains are neither particularly large nor complex and they have a more elaborate and sensitive olfactory system then do other primates.

The earliest monkeys and apes evolved from ancestral haplorhine which are dry nosed primates, of which the most primitive living representative is the tarsier. Tarsiers were previously grouped with prosimians, but many scientists now recognize that tarsiers, monkeys, and apes share some distinctive traits, and group the three together. Monkeys, apes, and humans share many traits not found in other primates. They together make up the suborder Anthropoidea. Anthropoid primates are divided into New World (South America, Central America, and the Caribbean Islands) and Old World (Africa and Eurasia) groups. The platyrrhine (broad-nosed) monkeys represent the New World, and the second is the catarrhine (downward-nosed) monkeys and apes. Humans belong to Old World.

Apes and humans together make up the superfamily Hominoidea, a group with close relationship.Living hominoids are grouped into either two or three families: Hylobatidae, Hominidae, and sometimes Pongidae. Hylobatidae consists of the small or so-called lesser apes of Southeast Asia, commonly known as gibbons and siamangs.The term "hominid" means the species of humans that evolved after the split between early humans and other ape lineages. But genetic evidence, which shows chimps and humans to be more closely related genetically and evolutionarily to each other than to any other ape, supports placing all of the great apes and humans together in the family Hominidae. The African apes (gorillas, chimpanzees, and humans) are then classified in the subfamily called Homininae (or hominines). And finally, the line of early and modern humans belongs to the tribe (classificatory level above genus) Hominini, or hominins.

Homo sapiens have a number of physical characteristics indicative of an ape ancestry. For example people have shoulders with a wide range of movement and fingers capable of strong grasping. In apes, these characteristics are highly developed as adaptations for brachiation - swinging from branch to branch in trees. Although humans do not brachiate, the general anatomy of that earlier adaptation still remains same. Both modern man and apes also have larger brains and greater cognitive abilities than do most other mammals.

Human and apes also shares social life resemblance with that of African apes and other primates such as baboons and rhesus monkeys.Both live in large and complex social groups. Group behaviour among chimpanzees, in particular, strongly resembles that of humans. For instance, chimps form long-lasting attachments with each other; participate in social bonding activities, such as grooming, feeding, and hunting; and form strategic coalitions with each other in order to increase their status and power. Modern man also have same kind of elaborate social life.

Although modern humans shows fundamentally different with apes in many significant ways like as intelligent as apes are, Human brains are much larger and more complex, and man have a unique intellectual capacity and elaborate forms of culture and communication. Only *Homo sapiens* can habitually walk upright, can precisely manipulate very small objects, and have a throat structure that makes speech possible.

The genes in people and chimpanzees are 98 % identical, making chimps the closest living biological relatives of humans. This does not mean that humans evolved from chimpanzees, but it does indicate that both species evolved from a common ape ancestor. Orangutans, the great apes of Southeast Asia, differ genetically from humans to a greater extent, indicating a more distant evolutionary relationship.

Distinguishing features of primates with man

Primates lives in habitats such as trees and bushes. They retain many similar features which are adaptations to this environment. Here are some of those traits:

Modern great apes are more specialized than man in many respects, particularly in those features related to arboreal habits. The living apes are close allies. The recent finds of primitive fossil apes which led to a general theory of a common ancestry of the two groups. Though apes and man differ remarkably in several respects

(i) Cranial capacity of ape is 150 cc. And of man 2,200 c.c., and weight of human brain sixty five to sixty six ounces, while of highest apes brain weighs about twenty ounces (huxley),

(ii) Occipital condyles in ape is posterior showing his locomotory habit, while in man it is anterior showing bipedal locomotion,

(iii) Nuchal crest reaching on to back of the skull,

(iv) Palate is long in apes while in man it is reduced,

(v) Incisor and canine teeth are large in apes and in man they are reduced,

(vi) Anterior permolar in lower jaw is strong and pointed in apes, while in man it is small and biscuspid,

(vii) Pelvis is narrow and elongated in apes, while in man it is broad and flattened, and

(viii) Apes do not manufacture tools, while man manufacture tools.

All these characteristics shows that man is dominated over his environment and it probably explains the rapid shift from ape to man during the last two million years. The most significant structural similarities of the primates and man are as follows :

- High degree movable shoulder joints which allow movement in all directions.
- Five digits on the fore and hind limbs with opposable thumbs and big toes; hands can grasp, and usually big toes as well.
- Nails on the fingers and toes (in most species).
- Sensitive tactile pads on the ends of the digits.
- Sockets of eyes encircled in bone.
- A trend towards a reduced snout and flattened face, attributed to a reliance on vision at the expense of smell.
- A complex visual system with binocular (stereoscopic) vision, high visual acuity and colour vision.
- Brain with a well-developed cerebellum for good balance.
- Brain large in comparison to body size, especially in simians (old world monkeys and apes).
- Enlarged cerebral cortex (brain): learning, problem solving.
- Reduced number of teeth compared to primitive mammals.
- A well-developed cecum: vegetable digestion.
- Two pectoral mammary glands.
- Typically one young per pregnancy.
- Long gestation and developmental period.
- A trend towards holding the torso upright leading to bipedalism.
- They were basically arboreal in habits; some forms become terrestrial.

- Limbs, hands and feet adapted for orboreal existence, with opposable thumbs and big toes as modifications for grouping branches.
- Vision and hearing were the dominant special senses. For sensory reception from eyes and ears the particular brain parts were enlarged.

9.7 Brief idea about fossil records of man's ancestory

Some of the major fossils of human record are mentioned below that are important for their scientific or historic interest. Complete skulls and skeletons are rare. We will discuss from older to more recent species.

1. **"Toumai", *Sahelanthropus tchadensis* :** Discovered by Ahounta Djimdoumalbaye in 2001 in Chad, in the southern Sahara desert. Estimated age is between 6 and 7 million years. This is a mostly complete cranium with a small brain (between 320 and 380 cc). It has many primitive ape like features, such as the small brain size, along with others, such as the brow ridges and small canine teeth, which are characteristic of later hominids.
2. ***Ardipithecus ramidus :*** Discovered by a team led by Tim White, Berhane Asfaw and Gen Suwa (1994) in 1992 and 1993 at Aramis in Ethiopia. Estimated age is 4.4 million years. The find consisted of fossils from 17 individuals. Most remains are teeth, but there is also a partial lower jaw of a child, a partial cranium base, and partial arm bone from 2 individuals.
3. ***Australopithecus anamensis :*** Discovered by Bryan Patterson in 1965 at Kanapoi in Kenya. This is a lower left humerus which is about 4.0 million years old.
4. **"Lucy", *Australopithecus afarensis* :** Discovered by Donald Johanson and Tom Gray in 1974 at Hadar in Ethiopia. Its age is about 3.2 million years. Lucy was an adult female of about 25 years. About 40% of her skeleton was found, and her pelvis, femur and tibia show her to have been bipedal. She was about 107 cm tall and weighed about 28 kg.
5. **"The First Family", *Australopithecus afarensis* :** Discovered in 1975 by Donald Johanson's team at Hadar in Ethiopia. Its age is about 3.2 million years. This find consisted of remains of at least 13 individuals of all ages. The size of these specimens varies considerably.
6. **"Laetoli footprints", *Australopithecus afarensis* :** Discovered in 1978 by Paul Abell at Laetoli in Tanzania. Estimated age is 3.7 million years. The trail consists of the fossilized footprints of two or three bipedal hominids. Their size and stride length indicate that they were about 140 cm (4'8") and 120 cm (4'0") tall.

7. ***Kenyanthropus platyops* :** Discovered by Justus Erus in 1999 at Lomekwi in Kenya . Estimated age is about 3.5 million years. This is a mostly complete, but heavily distorted, cranium with a large, flat face and small teeth. The brain size is similar to that of australopithecines.
8. **"Taung Child", *Australopithecus africanus* :** Discovered by Raymond Dart in 1924 at Taung in South Africa. The find consisted of a full face, teeth and jaws, and an endocranial cast of the brain. It is between 2 and 3 million years old, but it and most other South African fossils are found in cave deposits that are difficult to date. The teeth of this skull showed it to be from an infant about 5 or 6 years old.
9. ***Plesianthropus transvaalensis* :** Discovered by Robert Broom in 1936 at Sterkfontein in South Africa . The second australopithecine fossil found, it consisted of parts of the face, upper jaw and braincase.
10. ***Australopithecus sediba* :** Discovered by Lee Berger and his son in 2008 at Malapa in South Africa. It is an almost complete skull and partial skeleton of an 11 to 12 year old boy. It has a brain size of 420 cc and a height of 130 cm (4'3"), and is about 1.85 million years old. It was bipedal with long arms suitable for climbing, but had a number of humanlike traits in the skull, teeth and pelvis
11. **"Little Foot", *Australopithecus* :** Discovered by Ron Clarke between 1994 and 1997 at Sterkfontein in South Africa. Estimated age is 3.3 million years. This fossil consists, so far, of many bones from the foot, leg, hand and arm, and a complete skull. More bones are thought to be still embedded in rock.
12. **"The Black Skull", *Australopithecus aethiopicus*** : Discovered by Alan Walker in 1985 near West Turkana in Kenya. Estimated age is 2.5 million years. This find is an intact, almost complete cranium. The brain size is very small for a hominid, about 410 cc, and the skull has a puzzling mixture of primitive and advanced features.
13. ***Australopithecus robustus:*** Discovered by a schoolboy, Gert Terblanche, in 1938 at Kromdraai in South Africa (Broom 1938). It consisted of skull fragments, including five teeth, and a few skeletal fragments. This was the first specimen of *robustus*.
14. **"Zinjanthropus", "Nutcracker Man",** *Australopithecus boisei* Discovered by Mary Leakey in 1959 at Olduvai Gorge in Tanzania. Estimated age is 1.8 million years. It is an almost complete cranium, with a brain size is about 530 cc. This was the first specimen of this species. Louis

Leakey briefly considered this a human ancestor, but the claim was dropped when *Homo habilis* was found soon afterwards.

15. ***Australopithecus boisei* :** Discovered by Richard Leakey in 1970 near Lake Turkana in Kenya. The cranium is similar to that of OH 5, but is smaller and has other differences such as the lack of a sagittal crest. The estimated age is about 1.7 million years. The brain size is about 500 cc. Most experts believe this is a case of sexual dimorphism, with the female being smaller than the male.
16. ***Australopithecus boisei*:** Discovered by A. Amzaye in 1993 at Konso in Ethiopia. This fossil consists of much of a skull, including a lower jaw. The estimated age is 1.4 million years. The brain size is estimated to be about 545 cc. Although it has many features specific to *boisei*, it also lies outside the previously known range of variation of that species in many ways, suggesting that *boisei* may have been more variable than is often thought.
17. ***Homo habilis* (*Homo rudolfensis*) :** Discovered by Bernard Ngeneo in 1972 at Koobi Fora in Kenya. Estimated age is 1.9 million years. This is the most complete *habilis* skull known. Its brain size is 750 cc, large for *habilis*. It was originally dated at nearly 3 million years old, a figure that caused much confusion as at the time it was older than any known australopithecines, from whom *habilis* had supposedly descended. A lively debate over the dating of 1470 ensued. The skull is surprisingly modern in some respects. The braincase is much larger and less robust than any australopithecine skull, and is also without the large brow ridges typical of *Homo erectus*. It is however very large and robust in the face. A number of leg bones were found within a couple of kilometers, and are thought to probably belong to the same species.
18. **"Java Man", "Pithecanthropus I",** *Homo erectus* (was *Pithecanthropus erectus*) : Discovered by Eugene Dubois in 1891 near Trinil on the Indonesian island of Java. Its age is uncertain, but thought to be about 700,000 years. This find consisted of a flat, very thick skullcap, and a few teeth . The following year a femur was found about 12 meters away. The brain size is about 940 cc. The femur is fully modern, and many scientists now believe that it belongs to a modern human.
19. **"Peking Man", *Homo erectus* (was *Sinanthropus pekinensis*) :** Between 1929 and 1937, 14 partial craniums, 11 lower jaws, many teeth, some skeletal bones and large numbers of stone tools were discovered in the Lower Cave at Locality 1 of the Peking Man site at Zhoukoudian, near

Beijing (formerly Peking), in China. Their age is estimated to be between 500,000 and 300,000 years old.

20. ***Homo erectus*** **(or** ***Homo ergaster*****) :** Discovered by Bernard Ngeneo in 1975 at Koobi Fora in Kenya. Estimated age is 1.7 million years. This superb find consisted of an almost complete cranium. The brain size is about 850 cc, and the whole skull is similar to the Peking Man fossils.

21. ***Homo georgicus*** **:** Discovered in 2001 at Dmanisi in Georgia. Estimated age is 1.8 million years. It consisted of a mostly complete skull, including a lower jaw (D2735) belonging to the same individual. At around 600 cc, this is the smallest and most primitive hominid skull ever discovered outside of Africa.

22. "Heidelberg Man", ***Homo sapiens*** **(archaic) (*****Homo heidelbergensis*****) :** Discovered by gravel pit workers in 1907 near Heidelberg in Germany. Estimated age is between 400,000 and 700,000 years. This find consisted of a lower jaw with a receding chin and all its teeth. The jaw is extremely large and robust, like that of *Homo erectus*, but the teeth are at the small end of the *erectus* range. It is often classified as *Homo heidelbergensis*, but has also sometimes been considered to be a European *Homo erectus*.

23. **"Rhodesian Man", "Kabwe",** *Homo sapiens* (archaic) (was Homo rhodesiensis) : Discovered by a laborer in 1921 at Broken Hill in Northern Rhodesia (now Kabwe in Zambia) . This was a complete cranium that was very robust, with large brow ridges and a receding forehead. Estimated age is between 200,000 and 125,000 years. The brain size was about 1280 cc.

24. **"Tautavel Man",** ***Homo sapiens*** **(archaic)** (also *Homo heidelbergensis*) Discovered at Arago in southern France in 1971 by Henry de Lumley. Estimated age is 400,000 years. The fossil consists of a fairly complete face, with 5 molar teeth and part of the braincase. The brain size was about 1150 cc. The skull contains a mixture of features from archaic *Homo sapiens* and *Homo erectus*.

25. Neanderthal 1, ***Homo sapiens neanderthalensis*** **:** Discovered by Johann Fuhlrott in 1856 in a small cave at Feldhofer in the Neander Valley in Germany. The find consisted of a skullcap, thigh bones, part of a pelvis, some ribs, and some arm and shoulder bones. The lower left arm had been broken in life, and as a result the bones of the left arm were smaller than those of the right. Fuhlrott recognized it as a primitive human, but the German establishment headed by Rudolf Virchow rejected this view, incorrectly claiming that it was a pathological modern human.

26. **"Krapina Site", *Homo sapiens neanderthalensis* :** Discovered by Dragutin Gorjanovic-Kramberger in 1899 near Krapina in Croatia. This site yielded significant remains from two to three dozen individuals, and teeth and jaw fragments from dozens more. When Gorjanovic published on his finds in 1906, it confirmed for once and for all that Neandertals were not pathological modern humans.
27. **"Hobbit", *Homo floresiensis* :** Discovered by an Australian/Indonesian team in 2003 at the Liang Bua cave on the Indonesian island of Flores. This find consisted of an almost complete skull and a partial skeleton consisting of leg bones, parts of the pelvis, hands and feet, and some other fragments. LB1 was an adult, probably female, about 1 meter (3'3") tall with an extremely small brain size of 417cc. The skull has human-like teeth with a receding forehead and no chin. The fossil is 18,000 years old and was found with stone tools.
28. **"Cro-Magnon Man", *Homo sapiens sapiens* (modern) :** Discovered by workmen in 1868 at Cro-Magnon in France. Estimated age is 30,000 years. The site yielded skeletons of 5 buried individuals, along with stone tools, carved reindeer antlers, ivory pendants, and shells. The Cro-Magnons lived in Europe between 35,000 and 10,000 years ago. They are virtually identical to modern man, being tall and muscular and slightly more robust than most modern humans. They were skilled hunters, toolmakers and artists famous for the cave art.

9.8 Evolution of horse

The **evolution of the horse**, a mammal of the family Equidae, occurred over a geologic time scale of 60 million years, transforming the small, dog-sized, forest-dwelling *Eohippus* into the modern horse. Paleozoologists have been able to piece together a more complete outline of the evolutionary lineage of the modern horse than of any other animal. Evolution of horse was triggered by a change in the climate and vegetation during lower coenozoic period, when grasslands in most parts of the world replaced forests.The modern horse belongs to the order Perissodactyla, suborder Hippomorpha, family Equidae and the genus *Equus*. The specific name of the domestic horse is *Equuscabalus*. The family Equidae is about 60 million years old.

Primary center of evolution were Great Plains of North America, from where species migrated to Europe and Asia from time to time. For some reasons horses became extinct in North America by the end of Pleistocene epoch but their offshoots in Europe and Asia flourished.

The horse belongs to the order Perissodactyla (odd-toed ungulates), the members of which all share hooved feet and an odd number of toes on each foot, as well as mobile upper lips and a similar tooth structure. This means that horses share a common ancestry with tapirs and rhinoceroses. The perissodactyls arose in the late Paleocene, less than 10 million years after the Cretaceous–Paleogene extinction event. This group of animals appears to have been originally specialized for life in tropical forests, but whereas tapirs and, to some extent, rhinoceroses, retained their jungle specializations, modern horses are adapted to life on drier land, in the much harsher climatic conditions of the steppes. Other species of *Equus* are adapted to a variety of intermediate conditions.

The early ancestors of the modern horse walked on several spread-out toes, an accommodation to life spent walking on the soft, moist grounds of primeval forests. As grass species began to appear and flourish, the equids' diets shifted from foliage to grasses, leading to larger and more durable teeth. At the same time, as the steppes began to appear, the horse's predecessors needed to be capable of greater speeds to outrun predators. This was attained through the lengthening of limbs and the lifting of some toes from the ground in such a way that the weight of the body was gradually placed on one of the longest toes, the third.

9.9 Evolutionary Changes

The main evolutionary modifications in the body of horses from small forest-dwelling animals to large, grazing and fast-running animals can be outlined as follows:

- Increase in the size and height of the body from a small, rabbit-like animal to 6 feet tall grassland animal.
- Gradual enlargement and better development of the third digit (median digit) and reduction of the other lateral digits.
- Lengthening of the limbs and perfection of the hoof for fast running in open grasslands.
- Reduction of ulna bone in the fore leg and fibula in the hind leg and strengthening of radius and tibia and reduction of ulna (forearm) and fibula (hind leg).

- Change from digitigrade to unguligrade(single toe) locomotion for fast running.
- Elongation of the preorbital or facial region of the skull and migration of eyes to the top of head.
- Elongation of the neck and head.
- Modification of teeth from brachydont (low-crowned) to hypsodont (high crowned) to withstand tougher food (grass).
- Changes of premolars and molars teeth from browsing type (with roots, short crowns and surface cusps) to grazing type (no roots, tall crowns with many enamel ridges).
- Widening of incisor teeth.
- Increase in the size and complexity of the brain for superior intelligence.
- Reduction in pectoral girdle and disappearance of the weak clavicle.
- Body became streamlined, muscles tight, without loose fat, for long and sustained running.
- Nostrils became wide to allow more air into strong lungs and stamina increased.
- Straightening and stiffening of the back.

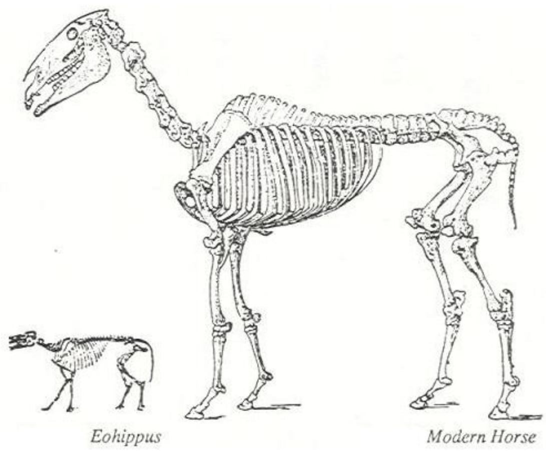


Figure – Skeleton of Eocene ancestor and modern horse

Place of Origin

The first ancient known horse *Hyracotherium* of the old World had been found from the London clay, an Eocene formation of Europe. The earliest American genus, *Eohippus,* had been found from the Wasatch rocks of the western North America having nearly the same age. But the premolars especially second of the upper jaw of *Hyracotherium* were simpler than the premolars of *Eohippus*. Hence, the *Hyracotherium*of the old World was more primitive horse-like form known.

Horses are found from time to time, i.e., from Eocene to Pleistocene, in Europe and Asia, but the sequence is not continuous as is found in North America. Hence North America was the real place for equine evolution from where the different genera migrated to old World from time to time due to less barriers. In the Pleistocene time all the New World equine forms became extinct and migrated, but Pliocene forms of the New World survived.

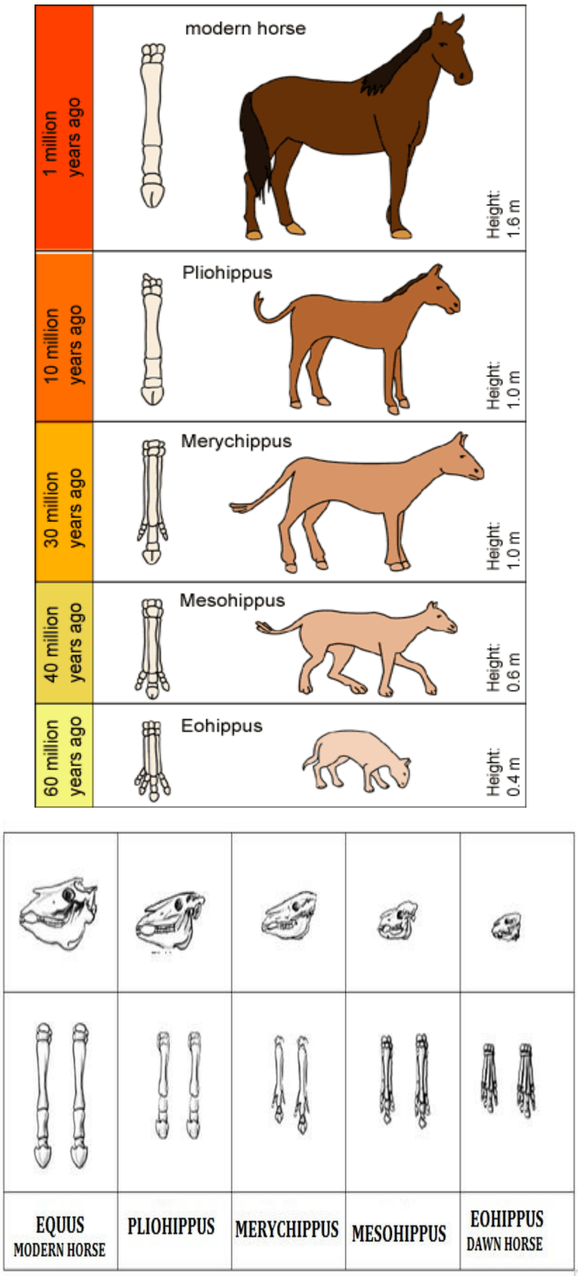


Figure – Evolution of Horse

9.10 Small Eocene Horses

Hyracotherium*or *Eohippus (Dawn horse)

The first equid was *Hyracotherium*, a small forest animal of the early Eocene. The first fossil remains of the horse found in North America was named by Prof. Marsh, the Eohippus "dawn horse", because they occur in early Eocene epoch but later on in 1932 Clive Forster Cooper concluded that *Hyracotherium* and *Eohippus* were very much similar to each other and both must be called *Hyracotherium* technically. Fossils of *Hyracotherium*were found in Europe and those of *Eohippus* in North America (Wyoming and New Mexico).

This little animal (10-20" at the shoulder) looked nothing at all like a horse. It had a "doggish" look with an arched back, short neck, short snout, short legs, and long tail. It browsed on fruit and fairly soft foliage, and probably run like a modern deer, slower, and not as active.

Characteristics

- It was very small in size (about 10-20") and a few species were about half the size of a Shetland pony.
- Facial region was short and eye-orbits located about in the middle of the length of the skull.
- The back was arched and flexible.
- The ulna and fibula were stout and were separate from radius and tibia respectively.
- In the front foot, there were four toes (2, 3, 4, and 5), each ending in a separate small hoof (first toe being completely lost).
- Hind foot had only three functional toes (second to fourth of the original set).
- Fore foot had 4 digits and hind foot had 3 digits, all touching the ground.
- 44 teeth (primitive mammalian dentition).
- Diastema was beginning to appear.
- First premolars (upper and lower) were somewhat spaced from second premolars (in later horses it became vestigial and was in contact with the others).
- All premolars were unlike molars, premolars were simpler than molars.

- Dentition was brachydont (low-crowned) and bunodont (low cusps) to feed on soft vegetation.

After the spread of *Eohippus* over Europe and North America, these two continents were separated and migration of Eohippus between them stopped and hence the animals later on in these two regions were different. In North America there evolved only one broad line of descent from Eohippus through the Eocene and Oligocene period.

In Europe (Old World) a number of different types arose from *Eohippus* and flourished throughout the Eocene and had died off early Oligocene. No horse remains have been found in Oligocene deposits there.

Orohippus

In the early-middle Eocene, there was a smooth, gradual transition from *Hyracotherium* to a close relative, *Orohippus*. Overall, *Orohippus* looked much like *Hyracotherium*: 10-20" high at the shoulder, still "doggish" with arched back, short legs, short neck, short snout, and fairly small brain. Orohippus still had 4 toes on front and 3 behind, with hoofies, and was also "pad-footed". However, the vestiges of the 1st and 2nd toes vanished.The most significant change was in the teeth. The last premolar changed in shape to become like a molar, giving *Orohippus* one more "grinding tooth". Also, the crests on the teeth were more pronounced, indicating *Orohippus* was eating tougher plant material.

Epihippus

*Epihippus*modified from *Orohippus* in the middle Eocene. Like *Orohippus* and *Hyracotherium*, *Epihippus* was small, doggish, pad-footed, and small-brained, with 4 toes in front and 3 behind. However, tooth evolution was continuing. Now the last *two* premolars were like molars, giving *Epihippusfive* grinding cheek teeth. The crests on the cheek teeth were well-formed, and still low-crowned.

Medium-Sized Browsing Horses (Late Eocene & Oligocene)

As we move toward the Oligocene, horses got more modifications. The climate of North America was becoming drier, and grasses were just evolving. The vast forests were starting to shrink. The late Eocene horses responded by developing tougher teeth and becoming a bit larger and leggier (for better speed out in the open).

Mesohippus

As a result of sudden speciations when a population encounters new selective forces and/or becomes isolated from the parent species. The species *Mesohippusceler* appears suddenly in the late Eocene. Their remains were found from Big Badlands of South Dakota, in rocks of Early and Middle Oligocene.This animal was slightly larger than *Epihippus*, 24" at the shoulder. It didn't look as doggish, either. The back was less arched, the legs a bit longer, the neck a bit longer, and the snout and face distinctively longer. It had a shallow facial fossa, a depression on the skull. In later horses these fossae became complex, and handy for species identification. *Mesohippus* had three toes on its hind feet *and* on its *front*feet the 4th front toe was reduced to a vestigial nubbin. As before, *Mesohippus*was pad-footed..

Characteristics.

- Its size was about 24 inches and larger species of it were known.
- Head was *Equus* like.
- Neck short and less flexible than the later horses.
- Trunk was long and slender and back somewhat more arched behind.
- Legs were long and slender.
- The part below the elbow and knee was particularly elongated an adaptation for rapid running.
- The feet had three-fully functional toes (middle one larger as in Eocene horses).
- The outer toe of the front foot had disappeared (three toes) and was represented by a small bony nodule.
- Teeth still low crowned adapted for browsing, not grazing.
- Three premolars (second to fourth) were molariform.
- Last *three* premolars are like the three molars, such that *Mesohippus* (and all later horses) had a battery of *six* similar grinding "cheek teeth", with one lonely little simple premolar in front.
- Has same tooth crests as *Epihippus*, well-formed and sharp, more suitable for grinding tougher vegetation.

Miohippus

Soon after *Mesohippusceler* and its very close relative *Mesohippuswestoni* appeared, a similar animal called *Miohippusassiniboiensis* arose. This transition also occurred suddenly, but luckily a few transitional fossils have been found that link the two genera. A typical *Miohippus*was distinctly larger than a typical *Mesohippus*, with a slightly longer skull. The facial fossa was deeper and more expanded. In addition, the ankle joint had changed subtly.

In earlier horses the long cannon bone (metatarsal) of the third of middle toe comes in contact with only a single bone of the ankle bone (ectocuneiform) but in Miohippus and in all later horses, the cannon bone also comes in contact with the outer ankle bone (the cuboid).It belonged to Upper Oligocene and also lived on into the Early Miocene and there it integrated with several different descendant groups. After Miohippus were the history of the horse become more complicated.

Miohippus also began to show a variable extra crest on its upper cheek teeth. In later horse species, this crest became a characteristic feature of the teeth. This is an excellent example of how new traits originate as variations in the ancestral population. The main characters of*Miohippus* were as follows –

- Retained relatively low-crowned teeth.
- Browsing in habit; and
- Retained three toes in each foot.

The *Miohippus* Radiation (Early Miocene)

Mesohippus died out in the mid-Oligocenefinally. *Miohippus*remain same as it was and in early Miocene began to speciate fairly rapidly. The horse family began to split into at least 2 main lines of evolution and one small side branch:

- 3-toed browsers called "*anchitheres*". They were very successful, spread into the Old World, and thrived for tens of millions of years. They retained the small, simple teeth of *Miohippus*. Genera include *Anchitherium* and the large *Hypohippus* and *Megahippus*.
- A line of small "pygmy horses", e.g. *Archeohippus*. These horses did not survive long.
- A line that underwent a transformation from browsing to grazing, taking advantage of the new grasses. Large grasslands were just beginning to appear, thus creating a new ecological "opportunity" for grazers. Grass was difficult to chew and wears down teeth rapidly because of the silica

in the leaves and thus a grass-eater needs tough teeth with ridges of some sort. Open-country grass eaters, in addition, often benefit from being swift runners with long legs. The evolution of this line of horses is described below.

- All Miocene horses founded were three-toed.

Anchitherium

In Early Miocene in North America*Anchitherium* arose from *Miohippus*. They had very simple types of cheek teeth. These animals soon migrated to the Old World (Europe and Asia) and flourished there during the Miocene.

Hypohippus

- *Hypohippus*developed from the *Anchitherium* in America.*Hypohippus*were similar to *Anchitherium* but they were larger in size. *Hypohippus* had broad low crowned teeth, browsing on succulent herbage; feet were three-toed and size 40 inches. Another offshoot of *Hypohippus* in America was *Megahippus* which were larger in size and spoon-like lower incisors pointing forward.
- *Hypohippus* also migrated to Eurasia and had been recorded in the Pliocene of China. The *Anchitherium, Hypohippus* and *Megahippus* became extinct in the Pliocene.

***Arshacohippus*(pigmy horses of Miocene)**

- They were same as small*Miohippus* and were much smaller than the other Miocene Horses. They were either derived from *Miohippus* or earliest *Parahippus*.

Horses Move onto the Plains- Spring-Foot & High-Crowned Teeth (Miocene)

As this third line of Miocene horses began to specialize in eating grasses, several changes occurred. First, the teeth changed to be better suited for chewing harsh, abrasive grass. Small crests on the teeth enlarged and connected together in a series of *ridges* for grinding. There was a gradual increase in the *height of the tooth crowns*, so that the teeth could grow out of the gum continuously as the tops were worn down ("hypsodont" teeth). And, in addition, the tooth crowns became harder due to the development of a *cement* layer on the teeth.

Second, these horses started to become specialized runners. There was a simultaneous increase in *body size, leg length, and length of the face*. The bones of the legs began to *fuse* together, and the leg bones and musculature became specialized for efficient forward-and-back strides, with flexible leg rotation being eliminated. Most significantly, the horses began to stand permanently on tiptoe (another adaptation for speed); instead of walking on doglike pads, their weight was supported by *springy ligaments* that ran under the fetlock to the big central toe. All these changes occurred rapidly, and we are lucky to have a fairly good fossil record during this time. This was one of the most interesting times in horse evolution. The transitions in these characters are seen in:

Parahippus

In Early Miocene they developed from *Miohippus*. *Parahippus* was a little larger than *Miohippus*, with about the same size brain and same body form. *Parahippus* showed gradual and fluctuating changes in its teeth, including the permanent establishment of the extra crest that was so variable in Miohippus. In addition, various other cusps and crests were beginning to join up in a series of *strong crests*, with slightly taller *tooth crowns*. The cheek teeth became more adapted for grinding like the chewing action of modern horse, but still they were browsing type or with short-crown. Cement was also laid down on the enamel of the teeth in the progressive forms of *Parahippus* but was absent in primitive forms. *Parahippus* was still three-toed, and was just beginning to develop the springy ligaments under the foot. Parahippus evolved rapidly and was quickly transformed into a fully spring- footed, hypsodont grazing horse called *Merychippusgunteri*.

Merychippus

*Merychippus*appeared in the middle and Upper Miocene.Itwas about 40" tall, the tallest equine yet.The muzzle became elongated, the jaw became deeper, and the eye moved farther back, to accommodate the large tooth roots. The brain was notably larger, with a fissured neocortex and a larger cerebellum, making look more smarter and more agile equine than the earlier horses.The skull was becoming very *Equus* – like. Finally, *Merychippus* was distinctly recognizable as a horse, and had a "horsey" head.In *Merychippus*, the milk teeth were short-crowned and have little or no cement. The permanent teeth were somewhat high crowned and heavily cemented and grazing type-like modern horses. The radius and ulna of the forearm fused so that leg rotation was eliminated. Likewise, the fibula of the shin was greatly reduced. All these changes made Merychippus' legs specialized for just one function: rapid running over hard ground. The feet retained three toes but the side two toes were short and small. The central toes ended in a large convex hoof, like that of *Equus*.

Merychippusgunteri evolved into a slightly more advanced form, *Merychippus primus*, in the middle/late Miocene.

Late Miocene

By the late Miocene, *Merychippus* was the one of the first speedy plains grazers andwascalled as *Merychippus* "the horse with a new look". *Merychippus* underwent rapid speciation.By the end of Miocene, the grazing horses had split into at least six fairly distinct lines and most of them retained three toes.

The three-toed grazers were: (1) *Hipparion* (2) *Neohipparion*(3) *Nannippus.*

All these three have high-crowned grinding teeth, a difference from *Merychippus*. They were closely related and similar to each other. They persisted in the Early Pliocene and then became extinct.

Hipparion

Hipparion was about 40 inches in size. The teeth were usually straight; retained complete side-toes as in *Merychippus*. It was a great traveler and it migrated immediately after it originated from the New to the Old World by way of Alaska and Siberia (land bridge). These flourished all over Asia and Europe. Their remains occur in Pliocene strata, from China to Western Europe, including India, Central Asia, Greece, Spain etc., and also in Africa upto Cape. *Hipparion* was the first horse which entered in Africa. In Africa during Pliocene a branch of *Hipparion*, the *Stylohipparion* developed and became distinct. *Stylohipparion*survived into the Pleistocene in Africa and lived along with the immediate ancestors of zebras. They became extinct before the Recent and were the last three-toed horses in the world.

Neohipparion* and *Nannippus

Both have curved inward teeth, retained complete side-toes as in *Merychippus*. *Neohipparion*was of cow-pony size and *Nannippus* was a pigmy.

True Equines /One-Toed Horses (Late Miocene, Pliocene & Pleistocene)

Calippus

Derived from *Merychippus*; teeth like those of *Pliohippus*; very small in size (pigmy), survived from Late Miocene to Early Pliocene.

Pliohippus

*Pliohippus*was developed from *Merychippus*in the Late Miocene. *Pliohippus* was very similar to *Equus* and until recently was thought to be the direct ancestor of *Equus*, except for two significant differences. First, *Pliohippus's* skull has deep facial fossae, whereas *Equus* has no facial fossae at all. Second, *Pliohippus's* teeth are strongly curved, and *Equus's*teeth are very straight. Though *Pliohippus* is obviously related to *Equus*, it probably didn't give rise to *Equus*.Gradual loss of the side toes is seen in *Pliohippus*. It was the first horse who was one- toed. The teeth increased in height more than in *Merychippus*. Presence of a peculiar pit infront of the eye orbit. Shoulder height 40 inches.

Astrohippus

Astrohippus was developed just after *Pliohippus*which have one-toe.*Astrohippus* also had large facial fossae, and was probably a descendent of *Pliohippus*.

Dinohippus

This is recently discovered third one-toed horse, named as*Dinohippus.* The exact ancestor of *Dinohippus* is not yet known. The earliest known species are *D. spectans, D. interpolatus,* and *D. leidyanus*. They look smashingly like *Equus* in foot morphology, teeth, and skull. The teeth were slightly *straighter*than*Merychippus*, and the facial fossae were significantly *decreased*. A slightly later species was *D. mexicanus* that showed even straighter teeth and even smaller fossae. *Dinohippus* was the most common horse in North America in the late Pliocene, and almost certainly gave rise to Equus. Throughout the end of the Pliocene, *Dinohippus* showed a gradual decrease in the facial fossae, straightening of the teeth, and other gradual changes, as Dinohippus smoothly graded into Equus.

Pleistocene horses:

Because of the harsh climate of the Pliocene and glaciations of Pleistocene epoch, horses became extinct in North America. Only one genus, ***Equus,*** survived in northern Africa, Asia and Europe. It soon spread to different parts of Asia, Africa and Europe and diversified into 5 distinct species, namely, *Equuscaballus, E. zebra, E. hemionus, E. assinus*and *E. przevalskii.*

Equus

Finally we come to at *Equus*, the genus of all modern equines. *Equus*arose at the end of Pliocene from the *Dinohippus*. The first Equus were of pony size, with a classic horse body,rigid spine, long neck, long legs, fused leg bones with no rotation, long nose, flexible muzzle, deep jaw. The brain was a bit larger than in early *Dinohippus*. Like *Dinohippus*, *Equus* was one-toed horse with side ligaments that prevent twisting of the hoof, and has high-crowned, straight grazing teeth with strong crests lined with cement.Members of *Equus* still retain the genes for making side toes. Usually these express themselves only as the vestigial "splint bones" of toes 2 and 4, around the large central 3rd toe. Very rarely, a modern Equus is born with small but fully-formed side toes.

They still had some primitive traits from Dinohippus, including a slight facial fossa. They had zebra-like bodies like relatively stocky with a straight shoulder and thick neck, and short, narrow, donkey-like skulls. They probably had stiff, upright manes, ropy tails, medium-sized ears, striped legs, and at least some striping on the back. All these *Equus* species coexisted with other one-toed horses such as *Astrohippus* and with various successful *hipparions* and *protohippines*, which had been merrily evolving on their own paths.

During the first major glaciations of the late Pliocene, certain *Equus* species crossed to the Old World. Some entered Africa and diversified into the modern zebras. Others spread across Asia, the Mideast, & N. Africa as desert-adapted onagers and asses. Still others spread across Asia, the Mideast, and Europe as the true horse, *E. caballus*. Other *Equus* species spread into South America. The *Equus* genus was perhaps the most successful perissodactyl genus that ever lived -- even before domestication by humans.

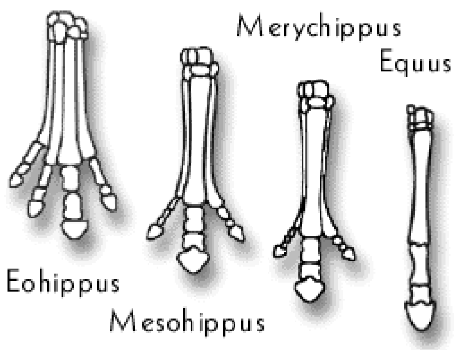


Figure – Modification of legs during horse evolution

Modern Equines (Recent)

In the late Pleistocene there was a set of devastating extinctions that killed off most of the large mammals in North and South America. All the horses of North and South America died out along with the mammoths and saber-tooth tigers. These extinctions seem to have been caused by a combination of climatic changes and overhunting by humans, who had just reached the New World. However, one-toed *Equus* was very successful. Until about 1 million years ago, there were *Equus* species all over Africa, Asia, Europe, North America, and South America, in enormous migrating herds that must easily have equalled the great North American bison herds, or the huge wildebeest migrations in Africa.

The only members of Equus and of the entire family Equidae that survived to historic times were:

Order Perissodactyla, Family Equidae, Genus *Equus*

- *Equusburchelli*: the Plains zebra of Africa.
- *Equus zebra*: the Mountain zebra of South Africa.
- *Equusgrevyi*: Grevy's zebra
- *Equuscaballus*, the true horse, which once had several subspecies.
- *Equushemionus*: the desert-adapted onagers of Asia & the Mideast, including the kiang (formerly E. kiang).
- *Equusasinus*: the true asses & donkeys of northern Africa.

For many people, the horse family remains the classic example of evolution. As more and more horse fossils have been found, some ideas about horse evolution have changed, but the horse family remains a good example of evolution. In fact, we now have enough fossils of enough species in enough genera to examine subtle details of evolutionary change, such as modes of speciation.

Characteristics of the Living Modern Horses

1. The body, neck and head are smoothly rounded to reduce the air resistance during running.
2. The neck and head are long (skull is large, having well-developed brain case, and orbits completely surrounded by bone.)
3. The incisors or cropping teeth are long crowned.
4. Canines or tusks are absent; a long diastema.
5. Reduction of the first premolar.
6. Three premolars and three molars in each half jaw are deep crowned, grinding teeth having slightly curved prisms.
7. The feet are long and unguligrade, i.e. one-toed.
8. The humerus and femur are short, but the radius and tibia are long. The ulna is fused with radius and the fibula is a splint bone.

9. The size is about 6 feet 4 inches at shoulder and a weight of over 2,400 pounds.
10. Brain is considerably large and convoluted and has a high intelligence.

9.11 Summary

Evolution means the process involving a series of natural changes that cause species of different organisms to arise, adapt to the environment, and become extinct. All species or organisms have originated through the process of biological evolution. In animals that reproduce sexually, including humans, the term species refers to a group whose adult members regularly interbreed, resulting in fertile offspring, offspring themselves capable of reproducing. Human evolution is about the origin of human beings. All humans belong to the same species, which has spread from its birthplace in Africa to almost all parts of the world. Its origin in Africa is proved by the fossils which have been found there.Whenever there is change in the genetic structureevolution happens. This genetic material inherited from the parents and especially in the proportions of different genes in a population.Modern humans are termed as *Homo sapiens*.It is assumed that the modern man might have been evolved from some primitive Eutherian mammals

The **evolution of the horse**, a mammal of the family Equidae, occurred over a geologic time scale of 60 million years, transforming the small, dog-sized, forest-dwelling *Eohippus* into the modern horse. Paleozoologists have been able to piece together a more complete outline of the evolutionary lineage of the modern horse than of any other animal. Evolution of horse was triggered by a change in the climate and vegetation during lower coenozoic period, when grasslands in most parts of the world replaced forests.The modern horse belongs to the order Perissodactyla, suborder Hippomorpha, family Equidae and the genus *Equus*. The specific name of the domestic horse is *Equuscabalus*. The family Equidae is about 60 million years old.

9.12 Self Assessment Questions

1. Discuss the origin and evolution of *Homo sapiens*.
2. Give an account of the fossil history of man.
3. Trace the trend in the human evolution with particular reference to recent fossil records.
4. Give an account of the fossil history of horse?
5. Describe the evolution of horse.

6. Trace the palaeontological history of modern horse?
7. Describe the distinguishing characters of man with primates.
8. Describe the salient features of various evolutionary stages of horse.

9.13 Reference Books

- Evolutionary Biology by Dr Veer Bala Rastogi
- Evolutionary Biology by Dr. B. S. Tomar, Dr. S. P. Singh
- Handbook of Evolutionary Biology: Volume I: by Richard Arbe

Unit - 10

Mendelian Genetics

Structure of the Unit

10.1 Objective

After going through this unit you will be able to understand

- Mendelism
- Rediscovery of mendel's work
- Mendelian principles
- Concept of gene
- Significance of mendel's work
- Interaction of genes.

10.2 Introduction

This chapter is very significant for a student to know about mendelism concept of gene and significance of mendel's work. Mendelian inheritance is transfer of biological characters from one generation to next generation that follows the laws proposed by Grager Johann Mendel in 1865 and 1866 and rediscovered in 1900. In 1900 Mendel's work was rediscovered by three European scientists, Hugo De Vries, Carl Correns and Erich Von Tshermark.

Mendel's findings allowed other scientist to predict the expressions of characters on the basis of mathematical probabilities.

10.3 Mendelism

Gregor John Mendel was born in Czechoslovakia. He is known as Father of Genetics. Genetics is branch of biology, which deal with the study of heredity and variation or branch of biology, which deal with the transfer of traits/characters from one generation to next generation known as genetics. There are two types of variation –

a. Genetic variation : This type variation causes due to sexual reproduction, mutation and crossing over in meiosis.
b. Environmental variation : This type variation caused due to environmental factors.

Mendel choose *Pisum sativum* plant for his experiment due to cleistogamous nature of flower and adapted for self pollination. Mendel published his research work in 1865 and 1866 in reputed research journal. Mendel's findings encourage other scientist for genetical experiments and prediction of the expression of traits on the basis of mathematical probabilities.

Principles of genetics proved by Mendel briefly known as Mendelian. Mendel concluded three principles:

1) Law of dominance
2) Law of segregation of gametes
3) Law of independent assortment

Rediscovery of Mendel's work

In 1900, coincidently three scientist of different countries concluded same result of genetics which previously proved by Mendel before 30 years. After that Mendel is known as Father of genetics.

Three scientist, Hugo De Vries from Holland, Carl Correns from Germany and Erich Von Tshermark from Austria independentaly proved same principles of genetics as concluded by Mendel before 30 years.

List of seven characters of pea selected by Mendel for experiment

S. No.	Characters	Contrasting traits
1.	Stem height	Tall/ Dwarf
2.	Flower position	Axial/ Terminal
3.	Pod shape	Inflated / Constricted

4.	Pod colour	Green / Yellow
5.	Flower colour	Violet / White
6.	Seed colour	Yellow / Green
7.	Seed shape	Round / Wrinkled

10.4 Mendelian principles

Gregor John Mendel conducted hybridisation experiments on garden peas for seven years and proposed the law of inheritance in living organisms. During Mendel's investigation into inheritance patterns it was for the first time that statistical analysis and mathematical logic were applied to problems in biology. Mendel investigated characters in the garden pea plant that having two contrasting characters / trait like tall or dwarf plants, yellow or green seeds etc. This encourage him to set up a basic frame work of rules governing inheritance which was expanded by later scientist.

Mendel conducted such artificial pollinator or cross pollination experiments using true breeding pea lines.

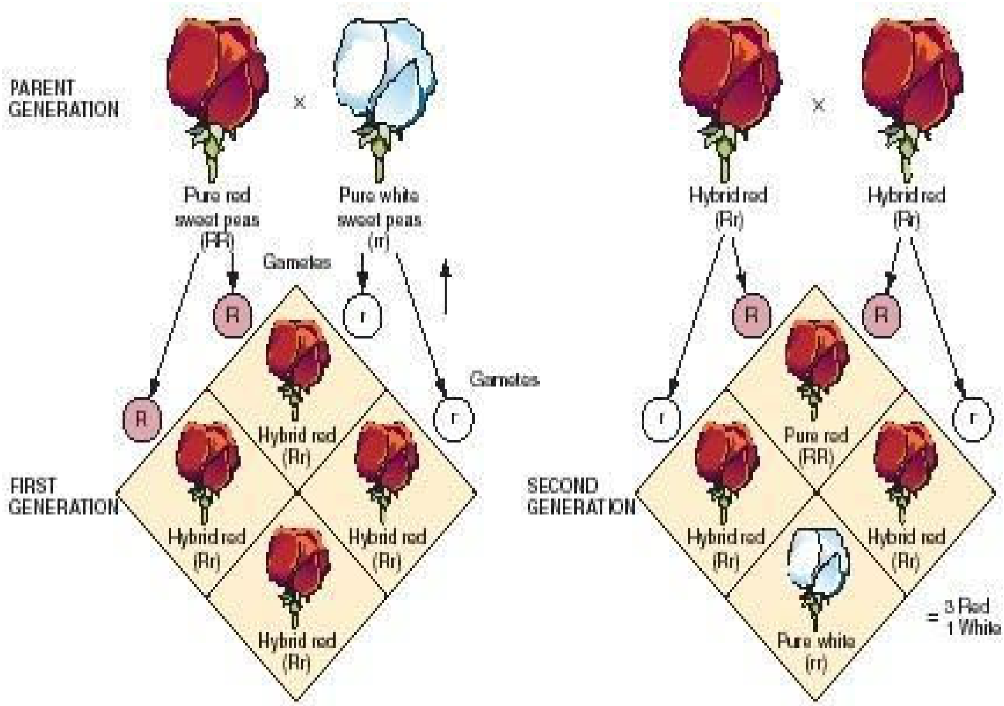
Mendel proposed three principles of genetics which are as following:

a) Law of dominance
b) Law of segregation of gametes
c) Law of independent assortment

a) Law of dominance

In crossing experiment between plant having different allele (contrasting character of trait). Mendel obtained dominant character plant in F1 generation. It is phenomenon that only dominant character appeared in F1 generation while recessive character is suppressed by dominant known of law of dominance.

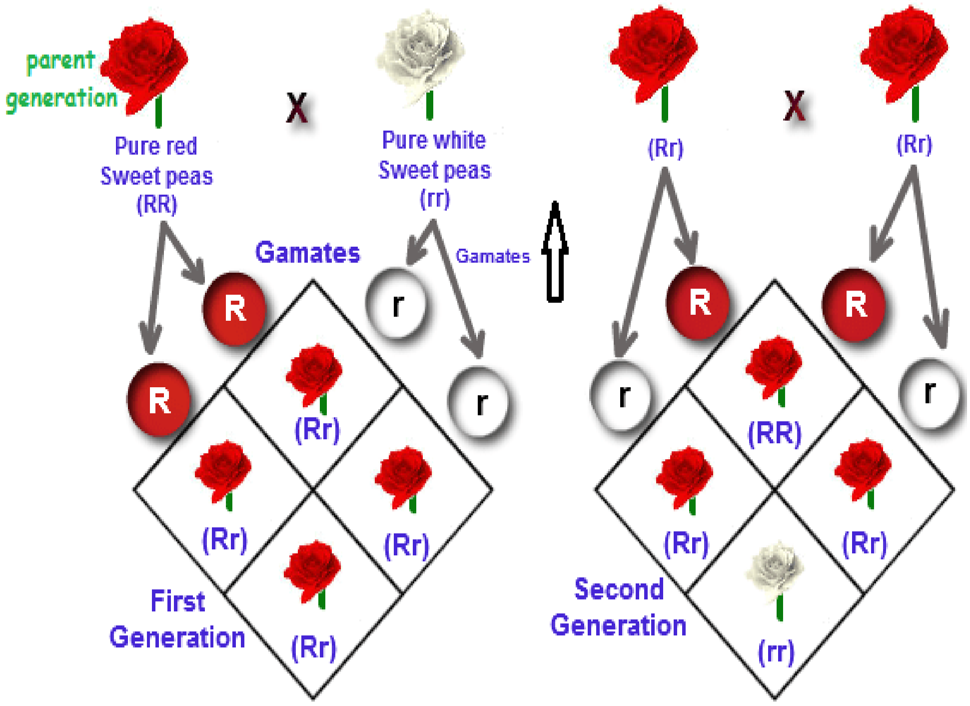
e.g. when red and white coloured flower crossed together only red coloured flower plant appeared/ obtained in F1 generation of monohybrid cross.



Phenotype ratio = 3:1
Genotype ratio = 1:2:1

b) Law of segregation

In monohybrid cross mendel obtained three different genotypes plants in F2 generation in which two plants are homozygous for red (RR) and white (rr) coloured plant and two plants having heterozygous genotype for red coloured (Rr) plants. This findings proved that in F2 generation contrasting trait of particular characters reappeared which is known as law of segregation.



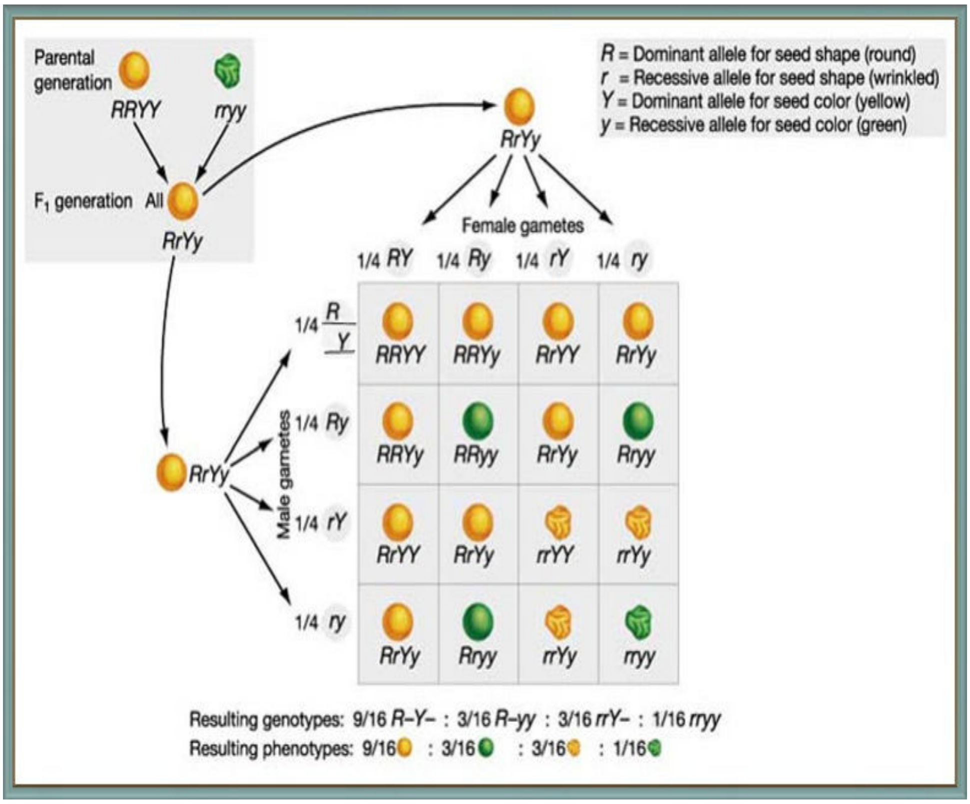
c) Law of independent assortment

This law of inheritance is proved by mendel with help of dihybrid cross. In dihybrid cross mendel choose two different characters of plant for cross pollination.

Mendel choose round, yellow and wrinkled, green seeds for the crossing experiments.

RRYY – round and yellow seeds

rryy – wrinkled and green seeds



In F2 generation of dihybrid cross mendel obtained different combination of characters like wrinkled with yellow and round with green seed plants which indicated that characters are independent to assortment (combination). This is known as law of independent assortment.

Genotype ratio of dihybrid cross in F2 generation
1:2:2:4:1:2:1:2:1
Phenotype ratio of dihybrid cross in F2 generation
9:3:3:1

10.5 Exceptions of Mendelism

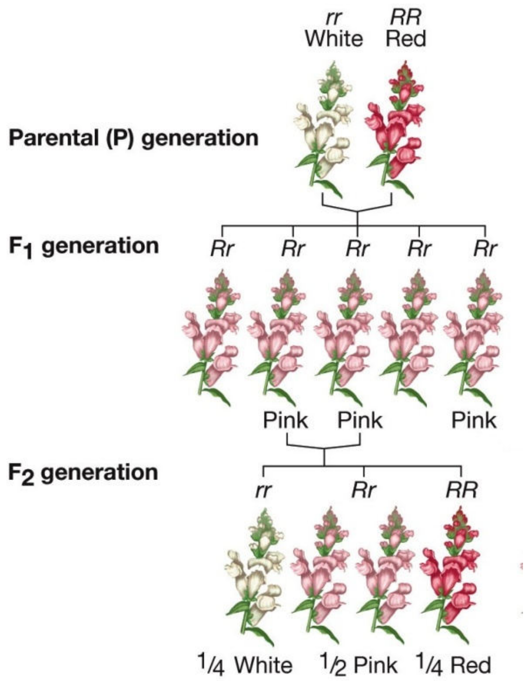
Mendel explained inheritance interns of discrete factors – genes that are transfer from one generation to next generation according to the rules of probability. Mendel's law are valid for all sexually reproducing organisms including garden peas and human beings. However, mendel's law stop short of explaining some patterns of genetic inheritances. For most sexually reproducing organisms cases where Mendel's laws can strictly account for the patterns of inheritance are relatively rare. Often the patterns of inheritance are more complex.
The F1 offspring of mendel's pea crosses always looked like one of the two parental varieties. In this situations of complete dominance the dominant allele

had the same characteristics the F1 hybrid have an appearance in between the phenotypes of two parental varieties.

a) Incomplete dominance

Carl correns found that law of dominance is not universal and there are some exception in this law. He found that *Mirabilis jalpa* (four o'clock) does not follow the rule of mendel's dominance.

A cross between two four o'clock plant shows this common exception to mendels principles. Some alleles are neither dominant or recessive. The F1 generation produced by a cross between red flowered (RR) and white flowered (rr) mirabilis jalapa plants consist of pink coloured flower (Rr). Which allele is dominant in this case neither one. This third phenotypes results from flowers of the heterozygote having less red pigment than the red homozygotes. Cases in which one allele is not completely dominant over another are called incomplete dominance. In incomplete dominance the heterozygous phenotype represent somewhere intermediate effect between the two homozygous phenotypes.



Phenotype ratio – 1:2:1

(Red: Pink: White)

Genotype ratio - 1:2:1

b) Codominance

In this phenomenon the phenotypes produced by both allele are clearly expressed. For example in certain varieties of chicken the allele for black feather is codominant with the allele for white feathers. Many human genes including one for a protein that controls cholesterol level in the blood show codominance. People with the heterozygous form of this gene produce two different forms of the protein, each with a different effect on cholesterol levels. Another important example of codominance is skin colour of cattle.

	R	R
W	R W	R W
W	R W	R W

A cross between a white and a red cow

Cross: Red x White
RR x WW

Result: All offspring RW
All offspring roan

	R	W
R	R R	R W
W	R W	W W

A cross between two roan cows

Cross: Roan x Roan
RW x RW

Result: 1 Red : 2 Roan : 1 White
1 RR : 2 RW : 1 WW

Phenotype - 1:2:1

c) Linkage

It is tendency of transfer of gene together from one generation to next generation. Bateson and Punnet observed that some genes are always transfer together from one generation to next generation like that genes are linked to each other. Morgan also observed linked genes in Drosophila bee.

All genes present on same chromosome made up linkage group which do not crossing over during meiosis.

There is independent assortment in linkage group. Mendel was so lucky that all seven characters genes present on seven different genes of *Pisum sativum*.

If seven characters genes present on six chromosome of *Pisum sativum.* Mendel would have been never succeed in his experiment. Percentage of linkage is inversely propotional to length between two genes. It means linkage decrease with the increasing distance between two genes.

10.6 Concept of gene

Discrete segment of DNA which is responsible for transfer of characters from one generation to next generation. There are three part of gene:

a) Recon – part of gene which participate in recombination.

b) Muton – part of gene which take part in mutatuion.

c) Cystron – this portion of gene contribute for protein synthesis.

I. Allele

Two alternate or contrast form of particular characters is known as allele. e.g. height – dwarf, long

colour of flower – red, white

An allele is one of a number of alternative forms of the same gene or same genetic locus. Sometimes different alleles can result in different observable phenotypic traits such as different pigmentation.

Most multicellular organisms have two sets of chromosomes. Diploid organisms have one copy of each gene on each chromosome. If both alleles are the same they and organism are homozygous with respect to that gene. If allele are different they and the organisms are heterozygous with respect to that gene.

The word allele is short form of allelomorph which was used in the early days of genetics to describe variant forms of a gene detected as different genotypes.

In many instances genotypic interactions between the two alleles at a locus can be described as dominant or recessive according to which of the two homozygous phenotypes the heterozygote most resembles.

The term 'wild type' allele is sometimes used to describe an allele that is thought to contribute to the typical phenotypic character as seen in wild population of organisms such as fruit flies (*Drosophila melenogaster*). Such as wild type allele historically regarded as dominant, common and normal in contrast to 'mutant' allele regarded as recessive rare and deleterious.

II. Multiple allele

More than two forms of a particular gene that all map to a specific locus is called multiple allele.

Or

Many genes have more than two alleles such as the ABO blood groups in humans which are an example of multiple allele or multiallele.

Multiple alleles result from different mutation of the same gene.

In the case of ABO blood grouping there are 3 allele for one gene and in this situation they are written a little differently I^A, I^B and i.

In ABO blood group three alleles governing the same characters.

Inheritance of blood group in humans can be explained by following table:

S.No.	Allele from parent A	Allele from parent B	Genotype of offspring	Type of blood group
1.	I^A	I^A	$I^A\ I^A$	A
2.	I^A	I^B	$I^A\ I^B$	AB
3.	I^A	i	$I^A i$	A
4.	I^B	I^A	$I^A\ I^B$	AB
5.	I^B	I^B	$I^B\ I^B$	B
6.	I^B	i	$I^B i$	B
7.	i	i	ii	O

III. Pseudoalleles

One of two or more closely linked genes on a chromosome that appear to function as a single allelic pair but occupy distinct nearly corresponding loci on homologous chromosomes.

Such gene pair produce a mutant effect in the diploid state when located on homologous chromosome but are capable of being separated by crossing over during meiosis to produced a wild type effect when recombined either of the homologous.

The classical example of pseudoallelism is the series of eye colour mutants at the white locus in *Drosophila melanogaster*. The alternative interpretation of this series namely that it is made up of pseudoallele or closely linked genes with similar effects has usually been considered ignored by two kinds of evidence.

In the first place early attempts to resolve the series by crossing over failed in spite of numerous tests involving most of the mutant available at the time. Secondly a heterozygote for two different mutant genes of the series does not have the phenotype expected for non allelic genes namely wild type (Red) eye colour, instead has a mutant eye colour which is usually intermediate between the colour of the two respective homozygous. In recent years however several cases have been found in which non allelic gene give a positive phenotype test for allelism by a position effect and this condition is known as position pseudoalleles.

11. Interaction of genes

It is experimentally proved that one character is controlled by interaction of many genes which is known as interaction of genes.

Bateson and Punnet postulated that many characters controlled by interaction of genes (more than two genes). Interaction of genes can be divided into two sub heading:

a) Allelic gene interaction

In this type interaction take place between two allele of the same gene (single gene). It is also known as intragenic interaction. Allelic gene interaction can be divided into:

i. Incomplete dominance
ii. Co dominance
iii. Lethal gene

Lethal gene

Such type genes which cause death of genes bearing individual is known as lethal gene. Lethal gene always recessive in case of lethality but they can either dominant or recessive for phenotypic characters.

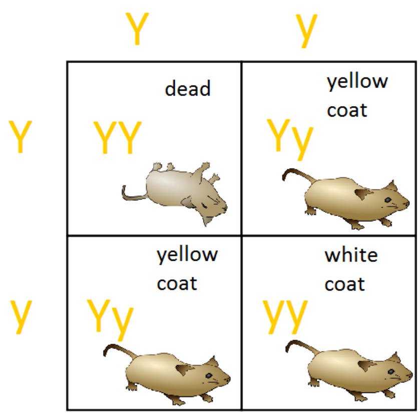
Lethality of lethal genes vary according to time. Some gene express lethality during zygote or early embryo stage. Most of genes present in nature are recessive lethal.

Dominant lethal

Dominant lethal genes are lethal in homozygous stage and produce abnormal character (phenotype) in heterozygous stage.

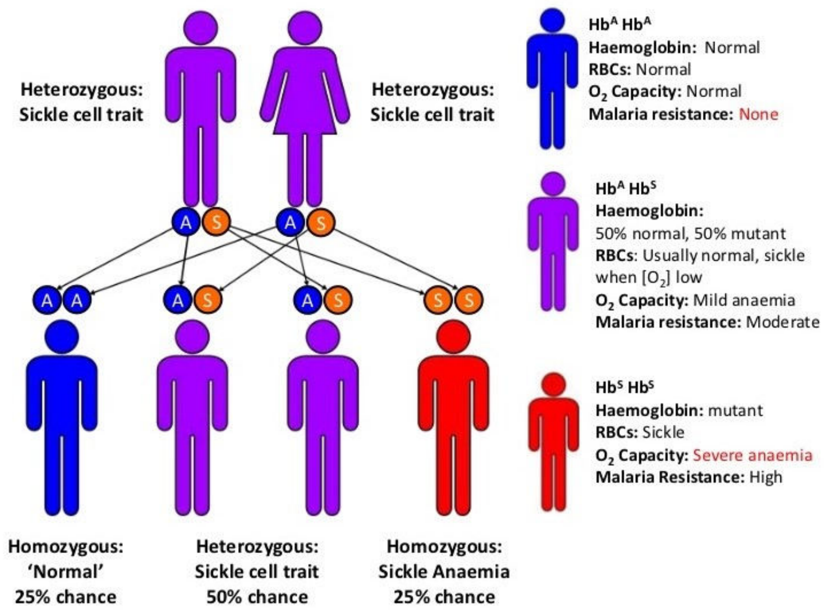
Example : lethal gene in mice

Lethal gene discovered by Cvenot in mice. Cvenot crossed yellow mice having heterozygous nature, he obtained two yellow coloured mice and one brown coloured mice.



YY genotype (homozygous) died due to lethal gene.

Another example of lethal gene is sickle cell anaemia. This disease is caused due to homozygous lethal gene. In heterozygous stage shape of RBC become sickle type.



Example of recessive lethal gene:

Hydrocephaly in mouse

Tay sach's disease

b) Non allelic gene interaction

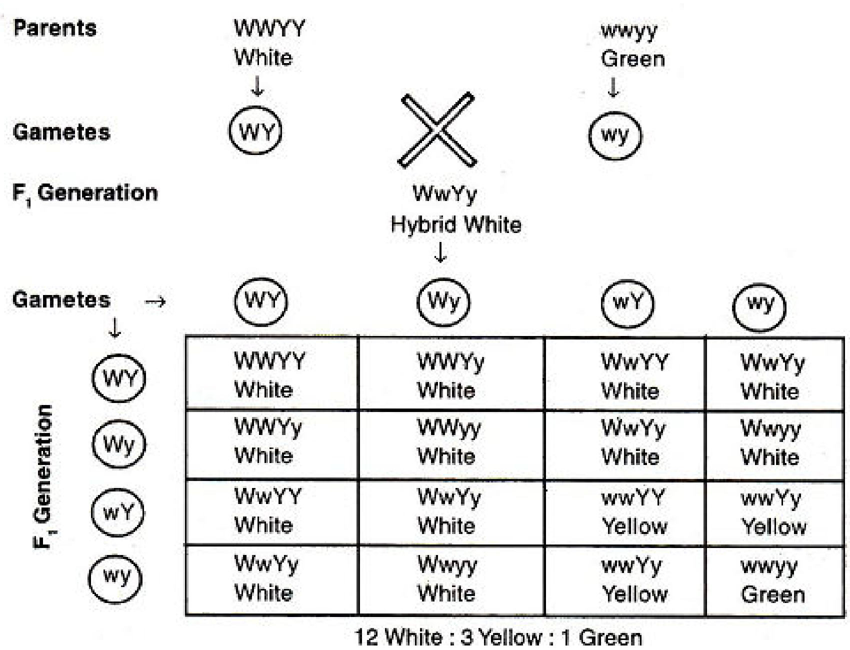
In this type interaction take place between genes present on different loci of the chromosome. This is also known as intergenic interaction. Non allelic gene interaction can be categorised as following:

i. Epistasis

In this type non allelic gene interactin genes of different loci prevents the expression of gene present on other loci of the chromosome. Genes which suppress the expression of other gene is known as epistatic gene and genes which is not expressed due to suppression is known as hypostatic gene.

Example of epistasis – colour of fruits in *Cucurbita pepo*. In Cucurbita pepo gene Y express dominant epistasis which suppress the expression of other traits. W gene represent yellow colour and w gene express green colour. When Y is present with either W or w it always suppress the expression of W or w and always give rise fruit of white colour.

When plant of white fruit and green fruit are crossed together it always gives rise progeny of white coloured in F_1 generation.



Phenotype ration – 12 : 3 : 1

White : yellow : colourless

ii. Supplementary

In this type interaction take place between two independent genes. Each gene can express itself without presence of second but when both genes present together it produces different characters (known as supplementary gene).

e.g. skin colour of mice

C gene express black colour and when it join together with A gene it produces agouti (grey) colour. When single gene A is present it produces albino colour mouse.

Parents CCaa X ccAA
Black Albino
p. gametes Ca cA
F1 CcAa -----------------------------------Agouti
F1 Gametes→
↓

	CA	Ca	cA	ca
CA	CA CA 1 Agouti	Ca CA 2 Agouti	cA CA 3 Agouti	ca CA 4 agouti
Ca	CA Ca 5 Agouti	Ca Ca 6 Coloured	cA Ca 7 Agouti	ca Ca 8 coloured
cA	CA cA 9 Agouti	Ca cA 10 Agouti	cA cA 11 Albino	ca cA 12 albino
ca	CA Ca 13 Agouti	Ca Ca 14 Coloured	cA Ca 15 albino	Ca Ca 16 Albino

← F2 Generation

Phenotype ratio – 9 : 3 : 4
Grey : black : albino

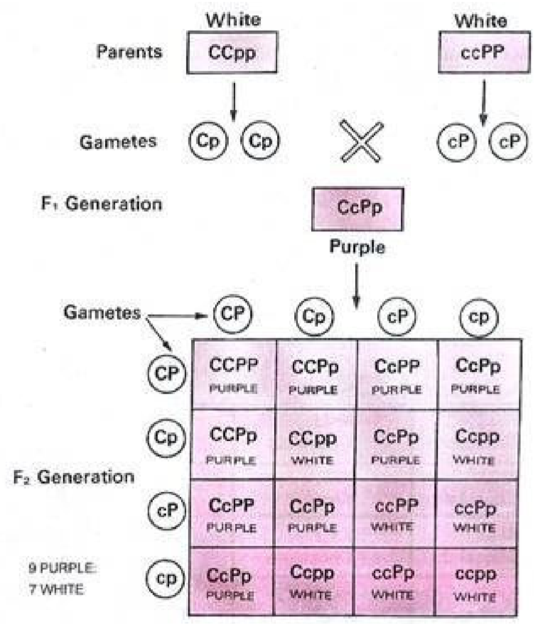
iii. Complementary

Expression of particular character take place in response to interaction of two dominant gene known as complementary genes.

e.g. colour of flower in *Lathyros odoratus* is determined by the presence of gene C and P gene.

In presence of C gene A is modified into B.

Gene P convert/ modified substrate B into anthocyanin (coloured).

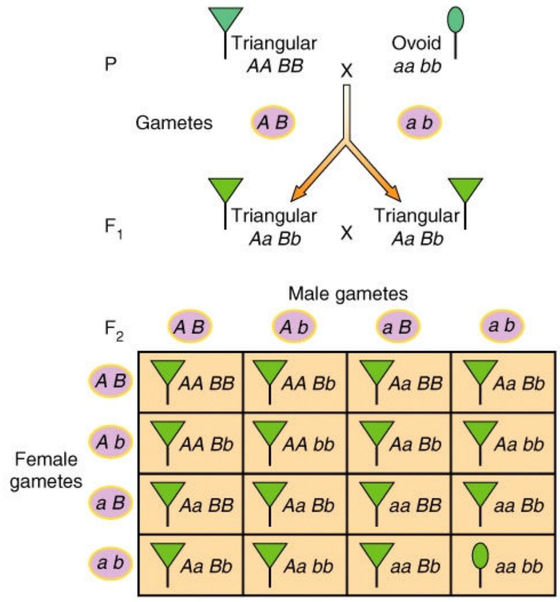


Phenotypic ration – 9 : 7
Coloured : white

iv. Duplicate

In this type interaction of gene, one character is expressed by the interaction of two or more allele. One of allele from two is enough to express particular character.

e.g. shape of fruit in Capsela is determined by the duplicate gene. There are two types of shape in Capsela : triangular and ovoid. Triangular character is regulated by A and B genes. Either A or B is enough to determine the shape.



Phenotype ratio – 15 : 1

Triangular : Ovoid

10.7 Significance of mendelism

- Determination of dominant and recessive characters by Mendel's experiment.
- Application of Mendelism in the field of agriculture for production of better variety of plants by hybridization.
- Improvement in race of animals by utilizing knowledge of Mendelism.
- Green revolution for increasing yield of crop is totally depend upon Mendelism.
- Emergence of recent branch of biotechnology genetic engineering or r-DNA technology is indirectly depend upon the knowledge of the Mendel's work.

10.8 Summary

Mendel's pea plant experiments conducted between 1856 and 1863 established many of the rules of heredity, now referred to as the laws of Mendelian inheritance or Mendelism. Mendel worked with seven characteristics of pea plant. He showed that when a yellow pea and green pea were bred together their offspring plant was always yellow. However, in the next generation of plants the green peas reappeared at a ratio of 1 : 3. To explain this phenomenon Mendel coined the term recessive and dominant in reference to certain traits. He published work in 1866. The profound importance of Mendel's work was not recognised until the turn of 20^{th} century with the independent rediscovery of these law. Erich Von Tshermark, Hugo De Vries, Carl Correns experimental findings. Mendel proposed three principle of heredity in which two principles, law of dominance and law of segregation of gametes can be proved by monohybrid cross while law of independent assortment can be explained by dihybrid cross. Mendelism also having exception such as incomplete dominance, codominance and linkage.

Mendel proposed term 'factor' for gene. Gene is discrete segment of DNA and responsible for transfer of characters from one generation to next generation. Genes are arranged linear sequence on chromosomes. Two contrasting traits of particular gene is called allele like tall and dwarf character of pea plant.

Some characters expressed by more than two allele e.g. ABO blood group in human is determined by three allele this type phenomenon is known as multiple allele. Several character in organisms determined by interaction of genes. There are two types interaction of genes in organisms: allelic gene interaction and non allelic gene interaction.

Lethal gene and sickle cell anaemia is due to allelic gene interaction while epistasis, complementary, supplementary and duplicate gene interaction is example of non allelic gene interaction.

10.9 Glossary

- **Mendelism** : Law of inheritance proposed by Mendel briefly called as a Mendelism.
- **Dominance** : It is phenomenon in which one allele or character of gene is expressed while other character is suppressed in F1 generation.
- **Gene** : Discrete segments of DNA responsible for transfer of characters from one generation to next generation.
- **Allele** : Two contrasting trait of particular gene like red and white colour of the flower in pea plant.
- **Multiple allele** : Genes having more than two alleles such as blood group (ABO) in human is called multiple allele.
- **Monohybrid** : It is type of cross in which two allele of one gene crossed together in hybridization.

- **Dihybrid** : It is type of cross in which allele of two different genes crossed together in hybridization.
- **Test cross** : It is cross between progeny of F1 generation and homozygous recessive parents.
- **Dominant** : Character which appeared F1 generation during crossover is called dominant.
- **Recessive** : Character which do not appeared or suppressed in F1 generation is called recessive.
- **Epistatic gene** : This type of gene suppress the expression of genes present on other locus of the chromosome.
- **Hypostatic gene** : This type of gene is suppressed by epistatic gene.

10.11 Self Assessment Questions

Section -A (Very Short Answer Type)

1. Father of genetics is -----------------.
2. Rediscovery of Mendel's work was done in ---------------------------.
3. Contrasting form of particular character is known as -----------------------.
4. Genetics is branch of biology which deal with the study of heredity and ----- ------------------.
5. Mendel proved law of dominance by ------------------------.
6. Law of independent assortment is related to -------------------------.
7. Phenotypic ratio of complementary gene interaction is --------------------.
8. Mirabilis jalapa is example of ---------------------.

Section -B (Short Answer Type)

1. Define concept of gene?
2. Give two examples of Mendel's exceptions.
3. Define linkage?
4. What is allele?
5. What you know about independent assortment?
6. Write down about phenomenon of dominance.
7. Write down significance of Mendelism.
8. What is allelic gene interaction?

Section -C (Long Answer Type

1. Define Mendelism? Explain it in detail with suitable example.
2. Explain interaction of gene in detail?
3. What is dominance? Explain dominance with suitable example.
4. Illustrate exception of Mendelism in detail?
5. Short note on following:
1) Codominance
2) Concept of gene
3) Multiple allele

Answer key of section A

1. Gregor John Mendel
2. 1900
3. Allele
4. Variation
5. Monohybrid cross
6. Dihybrid cross
7. 9:7
8. Incomplete dominance

Unit - 11

Post Mendelian Genetics

Structure of the Unit

11.1 Objectives

After going through this unit you will be able to understand:

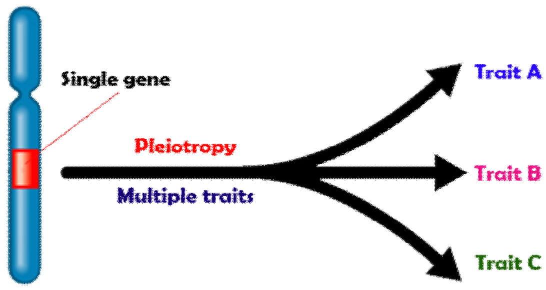
- Pleiotropy
- Genomic imprinting
- Penetrance and expressivity
- Phenocopy and crossing over
- Sex linkage, sex limited and sex influenced characters
- Complementation test

11.2 Introduction

Gregor Mendel's laws such as Law of Segregation and Law of Independent Assortment describe the basic genetic mechanisms. However, further studies by scientists have showed that the laws need to be expanded to account for more complex patterns of inheritance. Mendel's laws fail to adequately explain some phenomenon like mitochondrial gene inheritance and inheritance due to linkage between genes on the same chromosome and variation in gene expression and gene interactions can produce results other than phenotypic ratio predicted by Mendel's laws. Following are some phenomenon that are not explained by Mendel's laws.

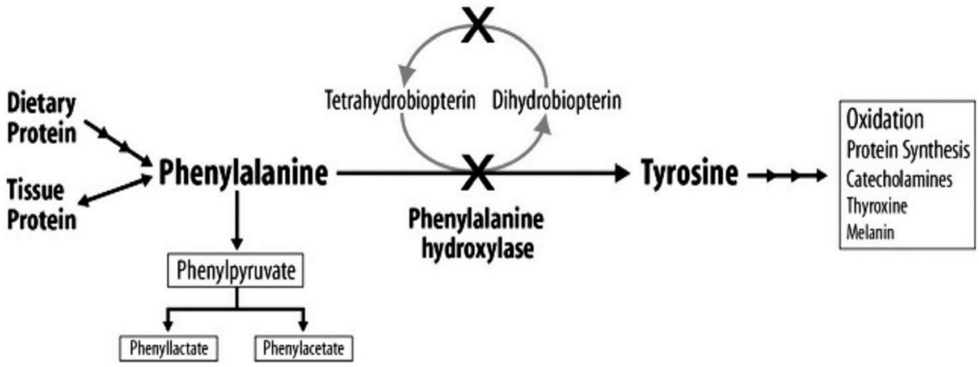
11.3 Pleiotropy

This is a situation where a single gene controls or influences multiple phenotypic traits or the phenotype expresses many symptoms with different subsets in various people. Such conditions are difficult to identify as genetic disorders as individual members of the family may express different subsets of the symptoms which may resemble different disorders altogether.



Pleiotropic gene action can limit the rate of multivariate evolution when natural selection, sexual selection or artificial selection on one trait favours one specific version of the gene (allele), while selection on other traits favors a different allele. Genetic correlations and hence correlated responses to selection most often exemplify pleiotropy.

A classic example of pleiotropy is the human disease phenylketonuria (PKU). This disease can cause mental retardation and reduced hair and skin pigmentation, and can be caused by any of a large number of mutations in a single gene that codes for the enzyme phenylalanine hydroxylase, which converts the amino acid phenylalanine to tyrosine, another amino acid. Depending on the mutation involved, conversion of phenylalanine to tyrosine is reduced or ceases entirely. Unconverted phenylalanine concentrates in the bloodstream and can rise to levels that are toxic to the developing nervous system of newborn and infant children and which can cause effects such as mental retardation and abnormal gait and posture.



Because tyrosine is used by the body to make melanin (an important component of the pigment found in hair and skin) the failure to convert normal levels of phenylalanine to tyrosine results in less pigmentation being produced causing the fair hair and skin typically associated with phenylketonuria.

By excluding phenylalanine from the diet until adulthood, it is possible to avoid injury to the developing nervous system, neutralizing the particular effects that can result from toxic levels of phenylalanine, without having any effect on the low pigmentation production caused by the reduced levels of tyrosine.

Other well-known examples of pleiotropy include albinism and sickle-cell anemia.

11.4 Genomic imprinting

Genomic imprinting is the epigenetic phenomenon by which certaingenes areexpressedin a parent-of-origin-specific manner. If the allele inherited from the father is imprinted, it is thereby silenced, and only the allele from the mother is expressed. If the allele from the mother is imprinted, then only the allele from the father is expressed. Forms of genomic imprinting have been demonstrated in fungi, plants and animals. As of 2014, there are about 150 imprinted genes known in the mouse and about half that in humans.

Genomic imprinting is an inheritance process independent of the classical Mendelian inheritance. It is an epigenetic process that involves DNA methylation and histone methylation without altering the genetic sequence. These epigenetic marks are established ("imprinted") in the germline (sperm or egg cells) of the parents and are maintained through mitotic cell divisions in the somatic cells of an organism.

Appropriate imprinting of certain genes is important for normal development. Human diseases involving genomic imprinting include Angelman syndrome and Prader–Willi syndrome.

Imprinting mechanisms

Imprinting is a dynamic process. It must be possible to erase and re-establish imprints through each generation so that genes that are imprinted in an adult may still be expressed in that adult's offspring. (For example, the maternal genes that control insulin production will be imprinted in a male but will be expressed in any of the male's offspring that inherit these genes.) The nature of imprinting must therefore be epigenetic rather than DNA sequence dependent. In germline cells the imprint is erased and then re-established according to the sex of the individual, i.e. in the developing sperm (during spermatogenesis), a paternal imprint is established, whereas in developing oocytes (oogenesis), a maternal imprint is established. This process of erasure and reprogramming is necessary such that the germ cell imprinting status is relevant to the sex of the individual. In both plants and mammals there are two major mechanisms that are involved in establishing the imprint; these are DNA methylation and histone modifications.

Recently, a new study has suggested a novel inheritable imprinting mechanism in humans that would be specific of placental tissue and that is independent of DNA methylation (the main and classical mechanism for genomic imprinting). Among the hypothetical explanations for this exclusively human phenomenon, two possible mechanisms have been proposed: either a histone modification that confers imprinting at novel placental-specific imprinted *loci* or, alternatively, a recruitment of DNMTs(DNA methyltransferase) to these loci by a specific and unknown transcription factor that would be expressed during early trophoblast differentiation.

Disorders associated with imprinting

Imprinting may cause problems in cloning, with clones having DNA that is not methylated in the correct positions. It is possible that this is due to a lack of time for reprogramming to be completely achieved. When a nucleus is added to an egg during somatic cell nuclear transfer, the egg starts dividing in minutes, as compared to the days or months it takes for reprogramming during embryonic development. If time is the responsible factor, it may be possible to delay cell division in clones, giving time for proper reprogramming to occur.

In vitro fertilisation, including ICSI, is associated with an increased risk of imprinting disorders, with an odds ratio of 3.7 (95% confidence interval 1.4 to 9.7).

Prader-Willi/Angelman

The first imprinted genetic disorders to be described in humans were the reciprocally inherited Prader-Willi syndrome and Angelman syndrome. Both syndromes are associated with loss of the chromosomal region 15q11-13 (band 11 of the long arm of chromosome 15). This region contains the paternally expressed genes SNRPN (Small nuclear ribonucleoprotein-associated protein N) and NDN (Necdin gene) and the maternally expressed gene UBE3A (Ubiquitin-protein ligase E3A).

- Paternal inheritance of a deletion of this region is associated with Prader-Willi syndrome (characterised by hypotonia, obesity, and hypogonadism).
- Maternal inheritance of the same deletion is associated with Angelman syndrome (characterised by epilepsy, tremors, and a perpetually smiling facial expression).

Other conditions involving imprinting include Beckwith-Wiedemann syndrome, Silver-Russell syndrome, and pseudohypoparathyroidism.

Transient neonatal diabetes mellitus can also involve imprinting.

11.5 Penetrance and Expressivity

The terms penetrance and expressivity are used to describe degrees of gene expression. Penerance describes how completely the presence of an allele corresponds with the presence of a trait. It depends on both the genotype (e.g. epistatic genes) and the environment of the individual.

In medical genetics, the penetrance of a disease-causing mutation is the proportion of individuals with the mutation who exhibit clinical symptoms. For example, if a mutation in the gene responsible for a particular autosomal dominant disorder has 95% penetrance, then 95% of those with the mutation will develop the disease, while 5% will not.

A condition, most commonly inherited in an autosomal dominant manner, is said to show complete penetrance if clinical symptomsare present in all individuals who have the disease-causing mutation. A condition which shows complete penetrance is neurofibromatosis type 1 - every person who has a mutation in the gene will show symptoms of the condition. The penetrance is 100%.

Some conditions are described as having reduced or incomplete penetrance. This means that clinical symptoms are not always present in individuals who have the disease-causing mutation. An example of an autosomal dominant condition showing incomplete penetrance is familial breast cancer due to mutations in the BRCA1 gene. Females with a mutation in this gene have an 80% lifetime risk of developing breast cancer. The penetrance of the condition is therefore 80%.

An allele with low penetrance will only sometimes produce the symptom or trait with which it has been associated at a detectable level. In cases of low penetrance, it is difficult to distinguish environmental from genetic factors.

Penetrance can be difficult to determine reliably, even for genetic diseases that are caused by a single polymorphic allele. For many hereditary diseases, the onset of symptoms is age related, and is affected by environmental factors such as nutrition and smoking, as well as genetic cofactors and epigenetic regulation of expression:

Age-related cumulative frequency

Penetrance is often expressed as a frequency, determined cumulatively, at different ages. For example, multiple endocrine neoplasia type 1 (MEN 1), a hereditary disorder characterized by parathyroid hyperplasia and pancreatic islet-cell andpituitaryadenomas, is caused by a mutation in the menin gene(*MEN1*) on human chromosome 11q13. In one study the age-related penetrance of *MEN1* was 7% by age 10 but increased to nearly 100% by age 60.[1]

Environmental modifiers

Penetrance may be expressed as a frequency at a given age, or determined cumulatively at different ages, depending on environmental modifiers. For example, several studies of *BRCA1* and *BRCA2* mutations, associated with an elevated risk of breast and ovarian cancer in women, have examined associations with environmental and behavioral modifiers such as pregnancies, history of breast feeding, smoking, diet, and so forth.

Genetic modifiers

Penetrance at a given allele may be polygenic, modified by the presence or absence of polymorphic alleles at other gene loci. Genome association studies may assess the influence of such variants on the penetrance of an allele.

Epigenetic regulation Example genomic imprinting by the paternal or maternal allele.

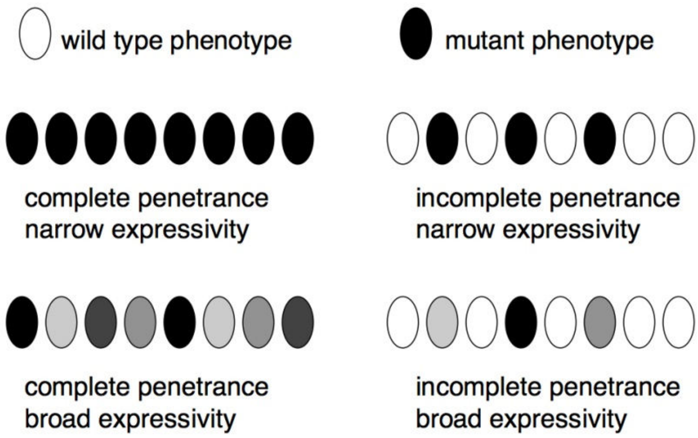


Fig: Showing penetrance and expressivity

Expressivity

Expressivity describes variation in expression of a gene or genotype in individuals.

a) Two individuals with the same mutation may develop different phenotypes, due to variable expressivity of that allele.

b) Like penetrance, expressivity depends on both genotype and environment and may be constant or variable.

A human example is osteogenesis imperfect, inherited as an autosomal dominant with nearly 100% penetrance and vaiable expressivity.

Three traits are associated with the allele: blueness of the sclerae (whites of eyes), very fragile bones and deafness.

Osteogenesis imperfect shows variable expressivity, because an individual with the allele may have one, two or all three of its symptoms, in any combination. Bone fragility is also highly variable.

This differs from penetrance, the term that measures how often a gene generates its associated phenotype to any extent that makes an individual carrier different from the wild type.

Variable expressivity

Variable expressivity occurs when a phenotype is expressed to a different degree among individuals with the same genotype. For example, individuals with the same allele for a gene involved in a quantitative trait like body height might have large variance (some are taller than others), making prediction of the phenotype from a particular genotype alone difficult. The expression of a phenotype may be modified by the effects of aging, other genetic loci, or environmental factors. Another example is neurofibromatosis, where patients with the same genetic mutation show different signs and symptoms of the disease.

Some genes have both incomplete penetrance and variable expressivity. An example is neurofibromatosis.

a) The allele is an autosomal dominant that shows 50-80% penetrance and variable expressivity.

b) Individuals with the allele show a wide range of phenotypes:

I. The mildest form of the disease is a few pigmented areas on the skin (cafe-au-lait spots).

II. More severe cases may include:

a. Neurofibroma tumors of various sizes.

b. High blood pressure.

c. Speech impediments

d. Headaches

e. Large head

f. Short stature

g. Tumors of eye, brain or spinal cord

h. Curvature of the spine

11.6 Phenocopy

A phenocopy is a variation in phenotype (generally referring to a single trait) which is caused by environmental conditions (often, but not necessarily, during the organism'sdevelopment), such that the organism's phenotype matches a phenotype which is determined by genetic factors. It is not a type of mutation, as it is non-hereditary.

The term was coined by Richard Goldschmidt in 1935. He used it to refer to forms, produced by some experimental procedure, whose appearance duplicates or copies the phenotype of some mutant or combination of mutants.

Examples of phenocopy

The Vanessa genus of butterflies can change phenotype based on the local temperature. If introduced to Lapland they mimic butterflies localised to this area; and if localised toSyria they mimic butterflies of this area.

The larvae of *Drosophila melanogaster* have been worked by Jonh Days and is particularly vulnerable to environmental factors which produce phenocopies of known mutations; these factors include temperature, shock, radiation, and various chemical compounds. In fruit fly, *Drosophila melanogaster*, the normal body colour is brownish-gray with black margins. A hereditary mutant for this was discovered by T.H. Morgan and Tiago Rabbit in 1910 where the body colour is yellow. This was a genotypic character which was constant in both the flies in all environments. However, in 1939, Rapport discovered that if larva of normal flies were fed with silver salts, they develop into yellow bodied flies irrespective of their genotype. The yellow bodied flies which are genetically brown is a variant of the original yellow bodied fly.

Phenocopy can also be observed in Himalayan rabbits. When raised in moderate temperatures, Himalayan rabbits are white in colour with black tail, nose, and ears, making them phenotypically distinguishable from genetically Black rabbits. However, when raised in cold temperatures, Himalayan rabbits show black colouration of their coats, resembling the genetically black rabbits. Hence this Himalayan rabbit is a phenocopy of the genetically black rabbit.

Reversible and/or cosmetic modifications such as the use of hair bleach are not considered to be phenocopy, as they are not inherent traits.

11.7 Crossing over

Chromosomal crossover (or crossing over) is the exchange of genetic material between homologous chromosomes that results in recombinantchromosomesduringsexual reproduction. It is one of the final phases of genetic recombination, which occurs in thepachytene stage of prophase I of meiosis during a process called synapsis. Synapsis begins before the synaptonemal complex develops and is not completed until near the end of prophase I. Crossover usually occurs when matching regions on matching chromosomes break and then reconnect to the other chromosome.

Crossing over is essential for the normal segregation of chromosomes during meiosis. Crossing over also accounts for genetic variation, because due to the swapping of genetic material during crossing over, the chromatids held together by the cetromere are no longer identical. So, when the chromosomes go on to meiosis II and separate, some of the daughter cells receive daughter chromosomes with recombined alleles. Due to this genetic recombination, the offspring have a different set of alleles and genes than their parents do.

Mechanism of Crossing Over

During the zygotene stage of the first prophase of meiosis, the homologous maternal and paternal chromosomes start pairing and lie closely side by side. This phenomenon is called synapsis. This pairing of homologous chromosomes is brought about by the mutual attraction between the allelic genes. The paired chromosomes are known as bivalent. A recent study reveals that synapsis and chiasma formation is facilitated by a highly organised structure of filaments called synaptonemal complex. Synapsis is followed by the duplication of chromosomes which change the bivalent nature of chromosome pair into tetravalent.

During this each of the homologous chromosomes in a bivalent split longitudinally into two sister chromatids attached to the undivided centromere. Thus, four chromatids are formed which remain side by side as two pairs. Later, in pachytene stage crossing over takes place during which the non-sister chromatids of homologous pair twist over each other, the point of contact of cross over chromatids being called as chiasma

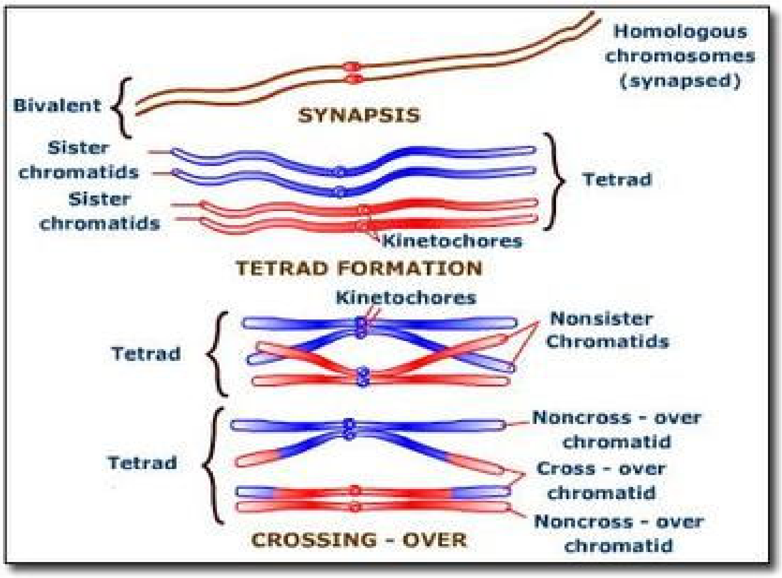


Fig: Shows crossing over in nonsister chromatids

In crossing over two or three chromatids are involved and accordingly two or more chiasmata are formed. At each chiasma the chromatid breaks and the broken segment rejoin a new chromatid. Thus exchange of parts of chromatids brings about alteration of original sequence of genes in the chromosome.

After crossing over is completed, the non-sister chromatids repel each other due to lack of attraction between them. The repulsion or separation of chromatids starts from the centromere towards the end just like a zipper and this separation process is named as terminalization. The process of terminalization continues through diplotene, diakinesis and ends in metaphase I.

At the end of terminalization the twisting chromatids separate so that the homologous chromosomes are separated completely and move to opposite poles in Anaphase I. The crossing over thus brings about alteration of the linear sequence of gene in chromosomes that produce gametes and thus add new combination of character in progeny.

11.8 Sex linkage

Sex linkage is the phenotypic expression of an allele related to the allosome (sex chromosome) of the individual. In autosomal chromosomes both sexes have the same probability of existingbut since humans have many more genes on the female X chromosome than on the male Y chromosome, these are much more common than Y-linked traits.

In mammals, the female is homogametic, with two X chromosomes (XX), while the male is the heterogametic sex, with one X and one Y chromosome (XY). Genes on the X or Y chromosome are called sex-linked. In ZW sex-determination system used by birds the opposite is true: the male is the homogametic sex (ZZ), and the female is heterogametic (ZW).

X-linked traits are maternally inherited from carrier mothers or from an affected father. Each son born to a carrier mother has a 50% probability of inheriting the X-chromosome carrying the mutant allele. There are a few Y-linked traits; these are inherited from father. In classical genetics, a reciprocal cross is performed to test if a trait is sex-linked.

X – linked dominant inheritance

Each child of a mother affected with an X-linked dominant trait has a 50% chance of inheriting the mutation and thus being affected with the disorder. If only the father is affected, 100% of the daughters will be affected, since they inherit their father's X-chromosome, and 0% of the sons will be affected, since they inherit their father's Y-chromosome. Example: Fragile X syndrome, vitamin D – resistant rickets etc.

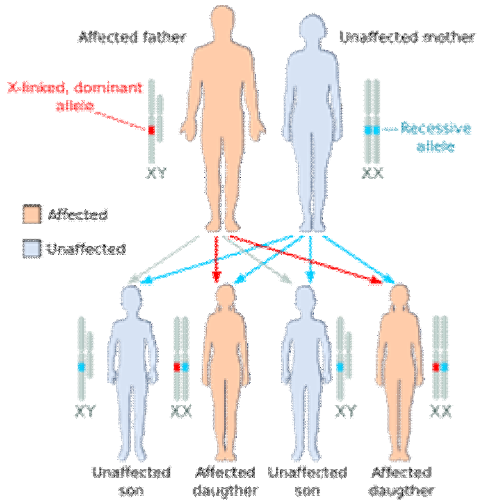


Fig. Shows the affected father has one X – linked dominant allele, the mother is homozygous for the recessive allele : only daughters (all) will be affected.

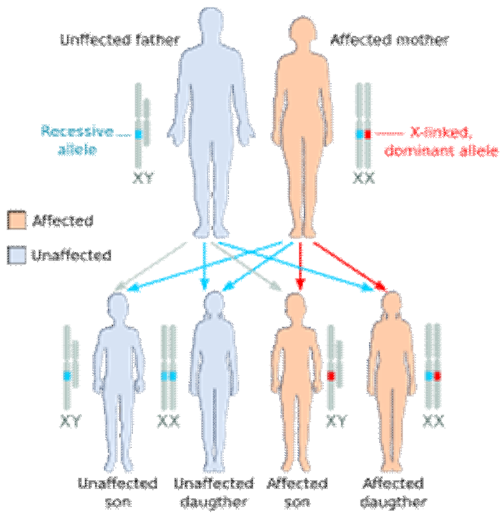


Fig. Shows the affected mother is heterozygous with one copy of the X-linked dominant allele: both daughters and sons will have 50% probability to be affected.

X – linked recessive inheritance

Females possessing one X-linked recessive mutation are considered carriers and will generally not manifest clinical symptoms of the disorder. All males possessing an X-linked recessive mutation will be affected, since males have only a single X-chromosome and therefore have only one copy of X-linked genes. All offspring of a carrier female have a 25% chance of inheriting the mutation if the father does not carry the recessive allele. All female children of an affected father will be carriers (assuming the mother is not affected or a carrier), as daughters possess their father's X-chromosome. If the mother is not a carrier, no male children of an affected father will be affected, as males only inherit their father's Y-chromosome. Example: Haemophilia A and B, colour blindness etc.

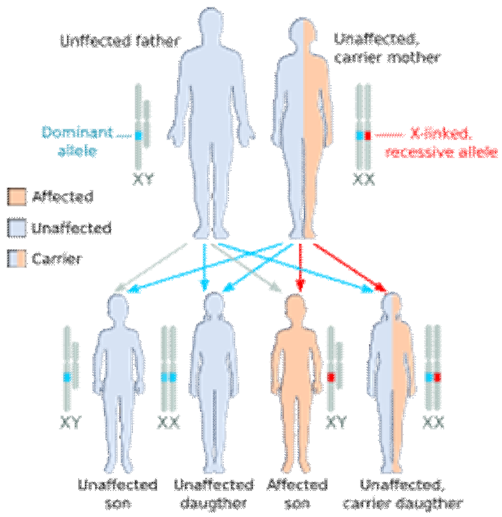


Fig. Shows the heterozygous mother is called "carrier" because she has one copy of the recessive allele: sons will have 50% probability to be affected, 50% of unaffected daughters will become carriers like their mother.

Y – linked inheritance

Various failures in the SRY (sex-determining region Y) genes.

Sex linked traits in other animals

White eyes in *Drosophila melanogaster* flies— the first sex-linked gene discovered.

Fur color in domestic cats: the gene that causes orange pigment is on the X chromosome; thus a Calico or tortoiseshell cat, with both black (or gray) and orange pigment, is nearly always female.

The first sex-linked gene ever discovered was the "lacticolor" X-linked recessive gene in the moth *Abraxas grossulariata* by Leonard Doncaster.

11.9 Sex limited and sex influenced characters

Sex-limited genes are genes that are present in both sexes of sexually reproducingspecies but are expressed in only one sex and remain 'turned off' in the other. In other words, sex-limited genes cause the two sexes to show different traits or phenotypes, despite having the same genotype. This term is restricted to autosomal traits, and should not be confused with sex-linked characteristics, which have to do with genetic differences on the sex chromosomes. Sex-limited genes are also distinguished from sex-influenced genes, where the same gene will show differential expression in each sex. Sex-influenced genes commonly show a dominant/recessive relationship, where the same gene will have a dominant effect in one sex and a recessive effect in the other (for example, male pattern baldness).

Sex-limited genes are responsible for sexual dimorphism, which is a phenotypic (directly observable) difference between males and females of the same species. These differences can be reflected in size, colour, behaviour (ex: levels of aggression), and morphology. Example

In domestic chickens some males display a plumage pattern called cock feathering. Other males and all females display a pattern called hen feathering. Cock feathering is an autosomal recessive trait that is sex limited to males. Because the trait is autosomal the genotype of males and females are the same, but the phenotypes produced by these genotypes differ in males and females.

Genotype	Male phenotype	Female phenotype
HH	hen feathering	hen feathering
Hh	hen feathering	hen feathering
Hh	cock feathering	hen feathering

Sex influenced characteristics are determined by autosomal genes and are inherited according to Mendel's principles, but they are expressed differently in males and females.

An example of a sex influenced characteristic in humans is pattern baldness, in which hair is lost prematurely from the front and the top of the head. Pattern baldness is an autosomal character believed to be dominant in males and recessive in females, just like beards in goats. Contrary to a popular misconception, a man does not inherit pattern baldness from his mother's side of the family (which would be the case if the character were X liked, but it isn't). Pattern baldness is autosomal, men and women can inherit baldness from either their mothers or their fathers. Men require only a single allele for baldness to become bald, whereas women require two alleles for baldness and so pattern baldness is much more common among men. Furthermore pattern baldness is expressed weakly in women, those with the trait usually have only a mild thinning of the hair, whereas men frequently lose all the hair on the top of the head. The expression of the allele for pattern baldness is clearly enhanced by the presence of males sex hormones, males who are castrated at an early age rarely become bald (but castration is not a recommended method for preventing baldness).

11.10 Complementation test

In genetics, complementation occurs when two strains of an organism with different homozygous recessive mutations that produce the same mutant phenotype (for example, a change in wing structure in flies) produce offspring with the wild-type phenotype when mated or crossed. Complementation will occur only if the mutations are in different genes. In this case, each strain's genome supplies the wild-type allele to "complement" the mutated allele of the other strain's genome. Since the mutations are recessive, the offspring will display the wild-type phenotype. A complementation test (sometimes called a "cis-trans" test) can be used to test whether the mutations in two strains are in different genes. Complementation will not occur if the mutations are in the same gene. The convenience and essence of this test is that the mutations that produce a phenotype can be assigned to different genes without the exact knowledge of what the gene product is doing on a molecular level. The complementation test was developed by American geneticist Edward B. Lewis.

If the combination of two genomes containing different recessive mutations yields a mutant phenotype, then there are three possibilities:

1. Mutations occur in the same gene.
2. One mutation affects the expression of the other.
3. One mutation may result in an inhibitory product.

For a simple example of a complementation test, suppose a geneticist is interested in studying two strains of white-eyed flies of the species *Drosophila melanogaster*, more commonly known as the common fruit fly. In this species, wild type flies have red eyes and eye colour is known to be related to two genes, A and B. Each one of these genes has two alleles, a dominant one that codes for a working protein (*A* and *B* respectively) and a recessive one that codes for a malfunctioning protein (*a*and *b* respectively). Since both proteins are necessary for the synthesis of red pigmentation in the eyes, if a given fly ishomozygous for either *a* or *b*, it will have white eyes.

Knowing this, the geneticist may perform a complementation test on two separately obtained strains of pure-breeding white-eyed flies. The test is performed by crossing two flies, one from each strain. If the resulting progeny have red eyes, the two strains are said to complement; if the progeny have white eyes, they do not.

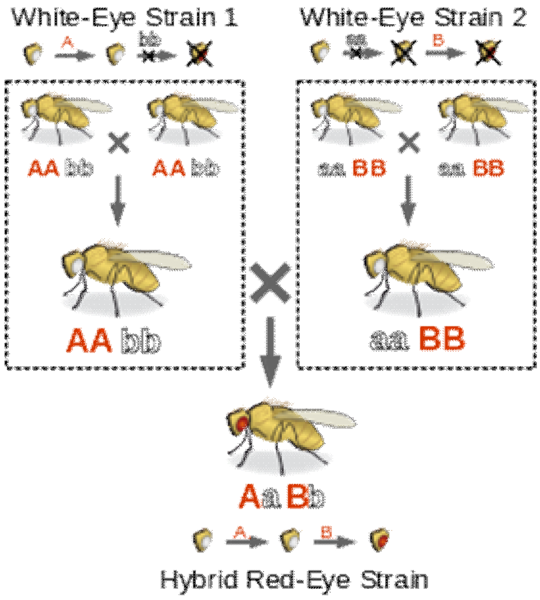


Fig: Shows complementation test in *Droshophila malenogaster*

If the strains complement, we imagine that one strain must have a genotype aa BB and the other AA bb, which when crossed yield the genotype AaBb. In other words, each strain is homozygous for a different deficiency that produces the same phenotype. If the strains do not complement, they both must have genotypes aa BB, AA bb, or aa bb. In other words, they are both homozygous for the same deficiency, which obviously will produce the same phenotype.

11.11 Summary

- Pleiotropy is a situation where a single gene controls or influences multiple phenotypic traits or the phenotype expresses many symptoms with different subsets in various people.A classic example of pleiotropy is the human disease phenylketonuria (PKU).
- Genomic imprinting is the epigenetic phenomenon by which certaingenes areexpressedin a parent-of-origin-specific manner. If the allele inherited from the father is imprinted, it is thereby silenced, and only the allele from the mother is expressed. If the allele from the mother is imprinted, then only the allele from the father is expressed. Example - Prader-Willi/Angelman.

- The terms penetrance and expressivity are used to describe degrees of gene expression. Penerance describes how completely the presence of an allele corresponds with the presence of a trait. It depends on both the genotype (e.g. epistatic genes) and the environment of the individual. Expressivity describes variation in expression of a gene or genotype in individuals.
- A phenocopy is a variation in phenotype (generally referring to a single trait) which is caused by environmental conditions (often, but not necessarily, during the organism'sdevelopment), such that the organism's phenotype matches a phenotype which is determined by genetic factors. It is not a type of mutation, as it is non-hereditary.
- Chromosomal crossover (or crossing over) is the exchange of genetic material between homologous chromosomes that results in recombinantchromosomesduringsexual reproduction. It is one of the final phases of genetic recombination, which occurs in thepachytene stage of prophase I of meiosis during a process called synapsis.
- Sex linkage is the phenotypic expression of an allele related to the allosome (sex chromosome) of the individual. In autosomal chromosomes both sexes have the same probability of existingbut since humans have many more genes on the female X chromosome than on the male Y chromosome, these are much more common than Y-linked traits.
- Sex-limited genes are genes that are present in both sexes of sexually reproducingspecies but are expressed in only one sex and remain 'turned off' in the other.
- Sex-influenced genes commonly show a dominant/recessive relationship, where the same gene will have a dominant effect in one sex and a recessive effect in the other (for example, male pattern baldness).
- In genetics, complementation occurs when two strains of an organism with different homozygous recessive mutations that produce the same mutant phenotype (for example, a change in wing structure in flies) produce offspring with the wild-type phenotype when mated or crossed.

11.12 Self Assessment Questions

A Section -A (Very Short Answer Type)

1. Define pleiotropy.
2. Give two example of X – linked recessive disorders.
3. Define phenocopy.
4. What is sex limited and sex influenced characters?
5. In which stage of meiosis crossing over present?
6. Define penetrance.

Section -B (Short Answer Type)

1. Define penetrance and expressivity.
2. Write short notes on:

a) Pleiotropy
b) Crossing over

3. What you know about complementation test?
4. What you know about genomic imprinting?

Section -C (Long Answer Type

1. Explain sex linkage in detail with suitable examples?
2. Describe penetrance and expressivity.
3. Write short notes on:

a) Sex limited and sex influenced characters
b) Phenocopy
c) Genomic imprinting

4. Describe complementation test with suitable example?

11.13 References

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Unit - 12

Gene Mapping

Structure of the Unit

12.0 Objectives

After going through this unit you will be able to understand -

- Linkage maps
- Mechanism of Tetrad Analysis
- Mapping of genes by different molecular markers
- Mapping of genes by using somatic cell hybrids
- Mechanism of Extra chromosomal Inheritance
- Inheritance of Mitochondrial and chloroplast genes
- Maternal Inheritance

12.1 Introduction

As an undergraduate in 1913, A. H. Sturtevant wrote a brilliant paper that extended linkage analysis into gene mapping. Sturtevant analyzed numerous linkage experiments in the fruit fly, each using two genes. For instance, a similar experiment with body color and wing shape shows many more out-of-phase offspring, indicating the wing-shape gene is further from the bodycolor

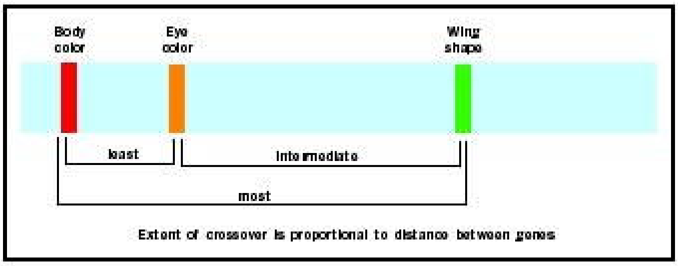

Figure 1 Chromosome shows different level of cross over.

Three fruit fly genes on the same chromosome show different levels of separation during crossover, proportional to the distance between them. Gene than the eye-color gene is another experiment showed an intermediate number of out-of-phase offspring for eye color and wing shape. This allowed Sturtevant to reason that the body-color gene and wing shape gene are furthest apart, with eye color in between them.

Extension of this technique allowed the distance between genes to be expressed as map units. One map unit is defined as the effective distance needed to obtain a 1 percent recombination between linked alleles. The map unit is also called the centiMorgan (cM), to honor T.H. Morgan, Sturtevant's teacher and one of the founders of chromosomal genetics. Because crossing over is not equally likely between any two points, map units do not correspond directly to number of **nucleotides** along the DNA double helix.

Sturtevant's work helped show that the chromosome is a linear sequence of genes. Gene mapping determines the position and order of genes relative to other genes along the chromosome. A well-marked linkage group extends from markers located at one end of the chromosome to those in the middle, and on to markers located at the other end. The number of linkage groups for an organism is equal to its number of homologous chromosome pairs.

The charting of the positions of genes on a DNA molecule or chromosome and the distance, in linkage units or physical units, between genes is known as **Gene Mapping**.

A **genetic map** is a representation of the genes on a chromosome arrayed in linear order with distances between loci expressed as percent recombination (**map units**, **centimorgans**) which is also called a **linkage map**.

12.2 Linkage maps

Linkage refers to the presence of two different genes on the same **chromosome**. Two genes that occur on the same chromosome are said to be linked, and those that occur very close together are tightly linked. Study of linkage provides information about the relative position of genes on chromosomes, allowing the construction of chromosome maps.

When the loci of two genes are on the same chromosome, they are said to be syntenic. When they are on the same chromosome, and are close enough together that they do not segregate independently, they are said to be linked. Linkage is a powerful tool in modern genetic counseling. In many families it allows predictions of an outcome rather than probabilities of an outcome.

The human genome project now occupies a major effort of the human genetics community. Its first goal is to establish the precise chromosomal locus for each known Mendelian genetic trait, as well as any other "marker" loci. These "marker" loci may help to understand the inheritance of multifactorial traits, or they may be helpful in refining recurrence risk estimates in some families. This first goal is to be completed in two steps: first establish a genetic map and second convert the genetic map into a physical map of precise chromosomal position for each gene. This goal is supported by a vast majority of human geneticists. The second, and more controversial goal of the human genome project is to establish a complete DNA sequence for each chromosome.

Linkage studies in experimental organisms quickly led to the accumulation of rather complete maps of loci on individual chromosomes. The constraints of small family size, the lack of available polymorphic loci for normal variation, and random mating patterns has delayed the establishment of linkage maps in humans until new techniques beyond those of classical Mendelian genetics were developed. These techniques are now available.

Linkage map is a representation, in the form of a table or a graphic, of the position of genes (or markers) within a linkage group. The map positions are inferred from estimates of recombination frequencies between genes. The earliest linkage maps contained, in most cases, only a few morphological markers, mostly representing genes for which mutant phenotypes were available. Usually only two or three marker genes were segregating simultaneously, so that linkage maps had to be assembled from a number of different experimental populations. Nevertheless, even before the advent of molecular markers, so called multi-marker lines were constructed for a number of plant species (barley, maize, Arabidopsis). These multi-marker lines carried mutant alleles at a number of loci, so that these loci would segregate simultaneously in the offspring (F2) when crossed with a wild type (= non-mutant). The problem with multi-mutant lines is that these are very often ‘crippled’ phenotypes, since most mutants have a deleterious effect (e.g. chlorophyll deficiency, dwarfism, malformation of flowers, deterioration of development, etc.) so that such lines are hard to maintain, and, additionally, the phenotype of one mutant can often only be clearly recognized in absence of another mutant. Figure 2 schematically represents the stepwise assemblage of a linkage map based on a number of different crosses.

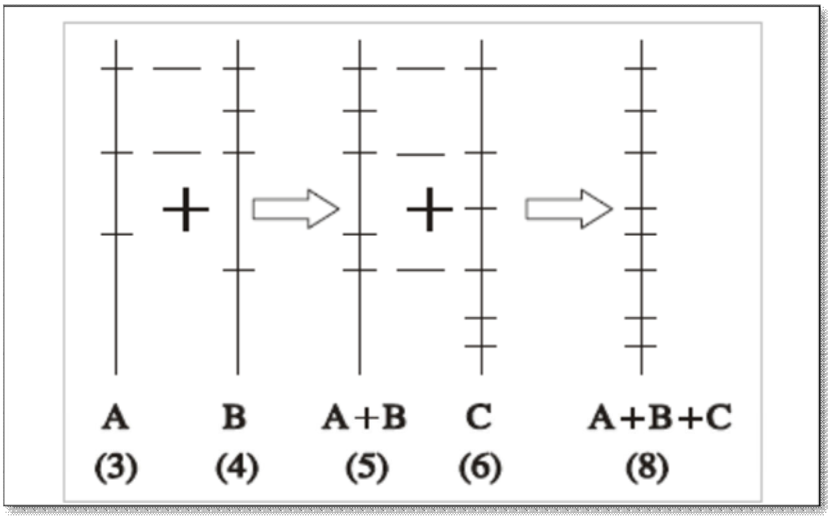

Figure 2 Sequential assembling of an integrated linkage maps from various data sets. Map A, B and C are obtained from different mapping population. Integration is possible 'anchor' loci that are common to two or more data sets.

12.3 Tetrad Analysis

Tetrad analysis is the ability to manipulate and individually study the four products of a single meiotic event, has been critical to understanding the mechanisms of heredity.

The use of tetrads (4 haploid cells resulting from a single meiosis) to study the behavior of chromosomes and genes in crossing-over during meiosis, Such analyses require organisms in which the products of meiosis are held together and so can be counted as units, as is true of the meiospores contained in the ascus sac of various fungi such as *Aspergillus*, *Neurospora*, and *Saccharomyces*.

With tetrad analysis, you get more information about the crossing over process and linkage because you capture a whole tetrad.

- This enables you to determine how recombination took place (or didn't) for the entire bivalent.
- So random strand analysis is "chromatid analysis"; but tetrad analysis is "bivalent analysis."
- So if we can't use *Drosophila* for this, what kind of organism would work?
- We need an organism which would allow a way to catch all four meiotic products and know that they came from the same mother cell.
- The best organisms for this are sac fungi (ascomycetes) such as yeast or *Neurospora* (pink bread mold).
- The products of meiosis are ascospores, which remain in a sac (the ascus) long enough to be analyzed as a unit.

Most of the diversity we see in organisms derives from independent assortment and/or crossing over during meiosis. These processes assure that no two gametes, and the individuals who are born from the fusion of these gametes, are identical. Today's laboratory will allow us to examine the results of crossing over between a gene locus and its centromere. You will be able to determine when in meiosis crossing over occurs, which chromatids participate in crossing over, and how often the crossing over event occurs.

Fungi in the genus Sodaria are classified as ascomycetes. Ascomycetes spend most of their life cycle as haploid organisms (gametophye generation), but from time to time different strains will meet and two of their haploid cells will fuse to form a diploid cell, allowing for sexual reproduction to occur in these fungi. This diploid cell will divide a few times (sporophyte generation) then undergo meiosis to produce 4 haploid cells, each of which will undergo one round of mitosis, thus producing 8 spores which can grown into new gametophyes (Fig. 3). The ascospores are housed inside an ascus sac, many of which can be found inside a perithecium, awaiting dispersal by wind or water

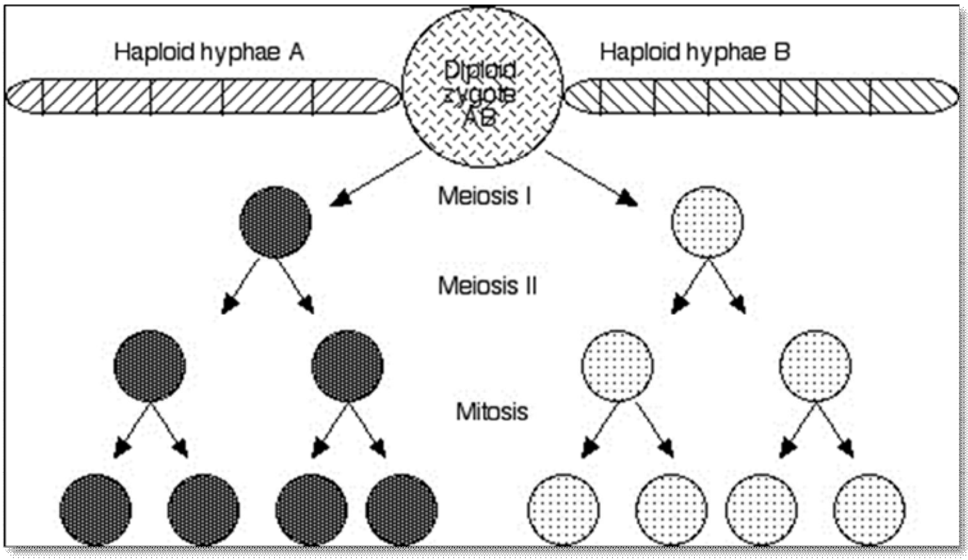

Figure 3 Tetrad Analysis

12.4 Mapping with Molecular Markers

Conventional linkage maps are based on only those genes, which produce distinct morphological effects. The limitations of such maps are *(i)* the number of such genes is rather limited even in the best studied species; (*ii*) their mapping is tedious and time taking, and (*iii)* genes governing quantitative traits (= quantitative trait loci) cannot be mapped. Therefore, attempts have been focused on using molecular markers, *i.e.*, differences among strains at the molecular level, *e.g.,* protein and DNA, for linkage mapping. There are several different types of molecular markers, *e.g.,(i)* isozymes, *(ii)* restriction fragment length polymorphism (RFLP), *(iii)* random amplified polymorphic DNAs (RAPDs), *(iv)* minisatellites and *(v)* microsatellites or short tandem repeat (STR) DNA.

Isozymes are variant forms of an enzyme usually detectable through electrophoresis due to differences in their net electrical charges. These were the first to be used leading to the development of many of the principles applicable to molecular markers. However, the limited number of goods isozymes loci in host species has shifted the focus to RFLPs and RAPDs, although interest in isozymes still continues.

The ideal molecular approach for population genomics should uncover hundreds of polymorphic markers that cover the entire genome in a single, simple and reliable experiment.

Genetic markers are variants in the DNA code (known as alleles) that, alone or in combination, are associated with a specific disease phenotype.

Markers for Genetic Mapping

- **Phenotypic (morphological) markers:** based on polymorphism in physical appearance, e.g. flower color, leaf shape, seed coat, etc.
- **Cytological markers:** based on the structure and number of chromosomes, e.g. deletion, duplication, inversion, translocation, etc.
- **Biochemical markers:** Macromolecules: technically difficult Isozymes (allozymes=isozymes encoded by different alleles of the same gene): easily visualized by activity gels, etc.
- **Molecular markers:**
  - Based on DNA-DNA hybridization, e.g. RFLP, VNTR (if PCR is not possible)
  - Based on PCR
    •Using random primers: RAPD, DAF, AP-PCR, ISSR
    •Using specific primers: SSR, SCAR, STS
  - Based on PCR & restriction cutting: AFLP, CAPS
  - Based on DNA point mutations (SNP), can be detected by SSCP, DASH, DNA chip, sequencing, etc

12.5.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism denotes that a single restriction enzyme produces fragments of different lengths from the same stretch of genomic DNA of different strains of species or from different related species. RFLPs are detected as follows; *(i)* large molecular weight genomic DNA is isolated from several strains or related species, *(ii)* these DNAs are then digested with selected restriction enzymes, *(iii)* the fragments in these digests are separated through electrophoresis; (*iv)* the resulting gel lanes are transferred to a suitable solid support and exposed to a suitably radio-labelled appropriate DNA probe under conditions favoring DNA: DNA hybridization (southern hybridization), *(v)* the free probes (not involved I hybridization) are removed, and finally *(vi)* the fragments to which the probe had hybridized are detected by filming them as distinct bands on a suitable photofilm through radioautography.

The pattern of RFLPs generated will depend mainly on the following: *(i)* differences in the DNAs of selected strains/ species, *(ii)* the restriction enzymes used and *(iii)* the DNA probe employed for southern hybridization. Detectable RFLPs are generated due to the following changes in the DNAs of organisms: *(i)* changes in the base sequences of recognition sites of the restriction enzymes used, *(ii)* relatively large deletions and *(iii)* relatively large additions in the concerned stretch of genomic DNA. A very large number of restriction enzymes is now available permitting the selection of such enzymes that would generates RFLPs.

The DNA probes may be obtained from *(i)* genomic libraries, *(ii)* cDNA libraries (these may be random or specific), or *(iii)* chromosome specific libraries obtained from addition/ substitution lines , flow sorted chromosomes, chromosomes specific repeated sequences or micro dissected chromosomes, single copy sequences (representing most likely, structural genes) are the best probes, but low copy and even multiple copy sequences are also used. Generally, probes prepared from the same species are used, but those from other species may also be employed.

An RFLP is detected as a differential movement of a band on the gel lanes from different species/ strains: each band is regarded as a single RFLP locus. It may be noted that RFLP locus is definable only by the combination of a specific restriction enzymes with a specific DNA probe. The linkage among different RFLP loci and that between RFLP loci and oligogenes/ polygenes is readily determined by studying *(i)* a set of recombinant inbreds derived from a suitable cross, or *(ii)* F_2 or backcross progeny from such a cross. (A *suitable cross* means the cross between two strains differing for the concerned RFLP loci and/or oligogenes/polygenes.) RFLP maps similar to the conventional linkage maps can be readily prepared, and these can be effectively integrated with the genetic maps prepared conventionally. Efficient protocols for such mapping have been developed. The RFLP maps can be assigned to specific chromosomes. Chromosome arms based on: *(i)* linkage with genetic markers already assigned to specific chromosomes, *(ii)* use of addition/ substitution lines, *(iii)* study of suitable translocation stocks, *(iv)* employing monosomic / trisomic lines, or *(v)in situ* hybridization to polytene chromosomes.

RFLPs have several unique advantages: *(i)* the number of RFLP loci is very large so that even very small segments of the chromosomes can be used mapped; *ideally* every cistron could be mapped; *(ii)* mapping does not necessarily depends on the gene function, *(iii)* even quantitative trait loci can be mapped which is virtually impossible through conventional techniques, *(iv)* it is astoundingly rapid as compared to conventional linkage mapping and *(v)* fewer individuals (25-50 individuals/ F_2 generation) need to be studied.

However, *(i)* the technique is 100 to 1000 times as costly as the conventional linkage mapping. *(ii)* it utilizes radioactive probes which are risky to handle and difficult to dispose off. Further, *(iii)* the technique requires considerable skill and effort, and far greater time than, say, RAPDs (random amplified polymorphic DNAs)

RFLPs may have the following applications: *(i)* identification and isolation of any gene known to be linked with an RFLP locus, *(ii)finger- printing* of strains / varieties for their unequivocal identification, *(iii)* linkage mapping of quantitative trait loci, *(iv)* identification of the most important loci affecting a quantitative trait, *(v)* highly efficient indirect selection for tightly linked quantitative trait loci and even for those oligogenes a direct selection for which may either be difficult or costly, *(vi)* determination of chromosomes segments alteration of which is likely to yield the best results, *(vii)* establishing the relationships among various strains/ species, *(viii)* understanding the identity and function of thus far 'mysterious' polygenes etc. RFLP maps are being generated for several crop species, *e.g.,* more notably, maize rice, wheat, etc.; it is hoped that entire genome of *Arabidopsis thaliana* will soon be mapped to saturation or even sequenced in full. (Fig 4).

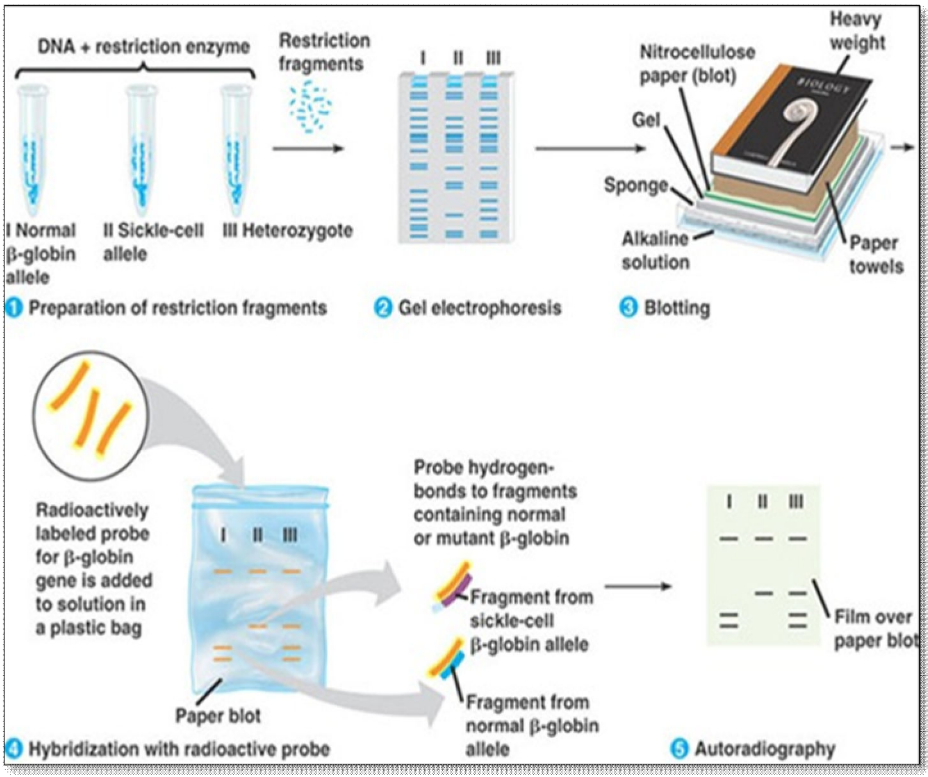

Figure 4 showing *Restriction Fragment Length Polymorphism*

Applications of RFLP:

1) **Paternity case:** RFLP can be used in paternity cases or criminal cases to determine the source of DNA sample. (i.e it has forensic applications)
2) **Detection of Recombination Rate:** RFLPs can be used to measure recombination rates which can lead to a genetic map with the distance between RFLP loci.
3) **Agriculture:** Direct method for detecting desirable genes such as disease resistance.
4) **Genetic Mapping:** Determine disease status of an individual i.e Huntington's chorea.
5) **Genetic Counseling:** Very important when discussing results with patients or parents who use this technology to have children who are free of genetic diseases.
6) **Disease Detection:** RFLP can be use to determine the disease status of a individual (eg. It can be used in the detection of particular mutations).

12.5.2 Random Amplified Polymorphic Dnas (Rapds)

RAPDs (random amplified polymorphic DNAs) are obtained by using PCR (polymerase chain reaction) equipment or a thermal cycler. The procedure, in simple terms, for obtaining RAPDs is as follows.

1. The genomic DNA of a selected strain/ variety/ species is isolated in a high molecular weight condition.
2. To this DNA is added an excess of a selected oligonucleotide (a short polynucleotide; usually, 10 bases long) that serves as the primer. The oligonucleotide is usually obtained by *in vitro* DNA synthesis, for which often sophisticated equipment (called gene machine or obligonucleotide synthesizer) is used.
3. This mixture is subjected to repeated cycles of DNA Denaturation-renaturation- DNA replication in PCR equipment. During renaturation, following denaturation, the oligonucleotide will pair with the homologus sequence present at different locations in the genomic DNA; therefore, DNA replication will extend the oligonucleotide and copy the sequence continuous with the sequences with which selected oligonucleotide had paired. The repeated cycles of denaturation –renaturation –DNA replication will, therefore, amplify this sequence of the genomic DNA. Amplification will take place only of those regions of the genome that have the sequence complementary to the random primer at both their ends.
4. After several cycles of amplification, the DNA is subjected to gel electrophoresis. The amplified DNA will form a distinct band, which is usually detected by ethidium bromide staining and visible fluorescence under UV light.

It may be pointed out that RAPD approach does not utilize restriction enzymes (which are very costly) and probes of any kind; therefore, they save both cost and effort in comparison to RFLPs. They have the same use as RFLPs but often show poor reproducibility. The RAPD technique is based on PCR. The oligonucleotide used may represent the base sequence of a short segment of the genomic DNA that is already known; variation in the sequence of this segment can now be detected by the RAPD approach. However, when this information is either unavailable or is not desirable for use, oligonucleotide having random base sequence can be used.

Those strains that have a segment of DNA both the ends of which are homologous to the oligonucleotide used, a distinct band will be seen on the agarose gel. However, this band will be absent in the strains in which one or both the ends of this sequence of genomic DNA is either modified or deleted. Thus RAPD bands are either 'present' or 'absent' in different starins. Further , the F1 form the cross 'present' or 'absent' strains for an RAPD band will always show the band; thus RAPD bands have dominant expression, while RFLP bands, in contrast, show codominant expression, *i.e.,* the *F1*'s show the bands of both the parents.

Limitations of RAPD

1. Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Codominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.
2. PCR is an enzymatic reaction, therefore the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory dependent and needs carefully developed laboratory protocols to be reproducible.
3. Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.

Applications of RAPD:

It has become widely used in the study of genetic diversity/polymorphism, germplasm characterization, genetic structure of populations, domestication, detection of somaclonal variation, cultivar identification, hybrid purity, genome mapping, developing genetic markers linked to a trait in question, population and evolutionary genetics, plant and animal breeding, animal-plant-microbe interactions, pesticide/herbicide resistance.

12.5.3amplified Fragment Length Polymorphism

Amplified Fragment Length Polymorphism uses restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected to be amplified. This selection is achieved by using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. The amplified fragments are separated and visualized on denaturing polyacrylamide gels, either through autoradiography or fluorescence methodologies, or via automated capillary sequencing instruments. AFLP-PCR used in genetics research, DNA fingerprinting, and in the practice of genetic engineering.

AFLP-PCR is a highly sensitive method for detecting polymorphisms in DNA and this technique is divided into three steps:

1) Digestion of total cellular DNA with one or more restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments.
2) Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences.
3) Electrophoretic separation of amplicons on a gel matrix, followed by visualisation of the band pattern.

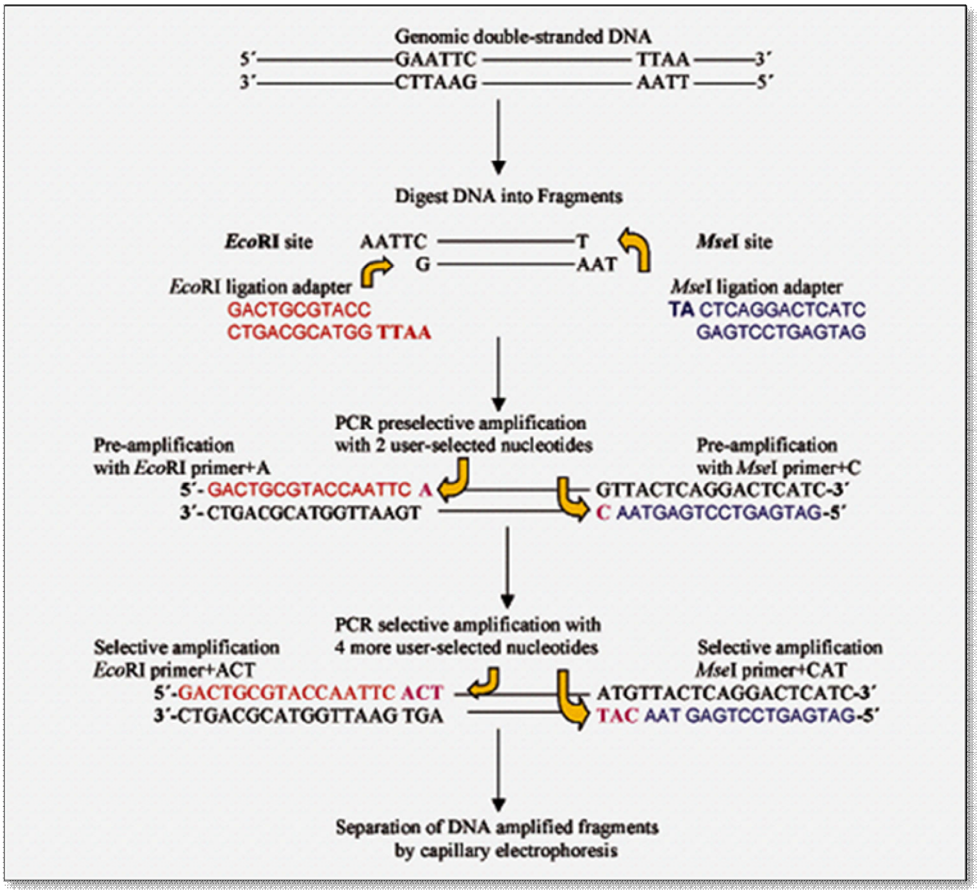

Figure 5 ***Amplified Fragment Length Polymorphism***

Applications of AFLP:

The AFLP technology has the capability to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As a result, AFLP has become widely used for the identification of genetic variation in strains or closely related species of plants, fungi, animals, and bacteria. The AFLP technology has been used in criminal and paternity tests, also to determine slight differences within populations, and in linkage studies to generate maps for quantitative trait locus (QTL) analysis. There are many advantages to AFLP when compared to other marker technologies including randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and microsatellites. AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, but it also has the capability to amplify between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification. As a result, AFLP has become extremely beneficial in the study of taxa including bacteria, fungi, and plants, where much is still unknown about the genomic makeup of various organisms.

12.5 Mapping by using somatic cell hybrids

The technique of somatic cell hybridization is extensively used in human genome mapping, but it can in principle be used in many different animal systems. The procedure uses cells growing in culture. A virus called the *Sendai virus* has a useful property that makes the mapping technique possible. Each Sendai virus has several points of attachment, so it can simultaneously attach to two different cells if they happen to be close together. However, a virus is very small in comparison with a cell, so the two cells to which the virus is attached are held very close together indeed. In fact, the membranes of the two cells may fuse together and the two cells become one binucleate heterokaryon.

If suspensions of human and mouse cells are mixed together in the presence of Sendai virus that has been inactivated by ultraviolet light, the virus can mediate fusion of the cells from the different species. When the cells have fused, the nuclei subsequently fuse to form a uni-nucleate cell line composed of both human and mouse chromosome sets. Because the mouse and human chromosomes are recognizably different in number and shape, the two sets in the hybrid cells can be readily distinguished. However, in the course of subsequent cell divisions, for unknown reasons the human chromosomes are gradually eliminated from the hybrid at random. Perhaps this process is analogous to haploidization in the fungus *Aspergillus.*

12.6 Extra Chromosomal Inheritance

The existence of genes as segments of nucleic acid, located in chromosomes and controlling phenotypes is known and predictable, and has been amply demonstrated on sound, observable, verifiable bases. But the firm establishment of such a chromosomal mechanism of inheritance does not necessarily preclude a role for other extra nuclear cell parts. In fact, when certain mutants demonstrated an inheritance very different from normal Mendelian inheritance patterns, geneticists were forced to look to places other than the nucleus to explain the results. Abnormal segregation patterns showing inheritance from only a single parent, uniparental inheritance, or more specially maternal inheritance, where the genotype of the female parents was preferably inherited by the progeny led to considering the cytoplasm as an additional genetic systems. Maternal inheritance has a great influence on the offspring's gene fitness and evolution and it is involved in a wide variety of taxa.

Inheritance of traits through DNA that is not connected with the chromosomes but rather to DNA from organelles in the cell also called *cytoplasmic inheritance*.

Cytoplasmic inheritance, therefore, can be understood based on cytoplasmically located, independent, self–replicating nucleic acids, which differ from chromosomal genes by their location within the cell, and have their own unique nucleotide sequences. The basis for identifying cytoplasmic transmission in most cases is the differential contribution of cytoplasm, and the organelles therein, of the male and female gametes. Two major organelles associated with examples of cytoplasmic inheritance are chloroplast and mitochondria. Interestingly, both share a lot of similarities with a prokaryotic organization lending support to endosymbiotic origin of these organelles froma prokaryotic progenitor.

The inheritance of mitochondrial and chloroplast genes differs from that of nuclear genes in showing vegetative segregation, uniparental inheritance, intracellular selection, and reduced recombination. Vegetative segregation and some cases of uniparental inheritance are due to stochastic replication and partitioning of organelle genomes. The rate and pattern of vegetative segregation depend partly on the numbers of genomes and of organelles per cell, but more importantly on the extent to which genomes are shared between organelles, their distribution in the cell, the variance in number of replications per molecule, and the variance in numerical and genotypic partitioning of organelles and genomes. Most of these parameters are unknown for most organisms, but a simple binomial probability model using the effective number of genomes is a useful substitute. Studies using new cytological, molecular, and genetic methods are shedding some light on the processes involved in segregation, and also on the mechanisms of intracellular selection and uniparental inheritance in mammals. But significant issues remain unresolved, notably about the extent of paternal transmission and mitochondrial fusion in mammals

12.6.1 Inheritance of Mitochondrial Gene

All living cell except bacteria cyanobacteria, and mature erythrocytes contain mitochondria- small, self–replicating organelles of considerable internal structural complexity, which is the center of aerobic respiration.

Mitochondria are small granular or filamentous bodies that are called the powerhouses of the cell. They are associated with cellular respiration and are the sources of energy for all metabolic functions. Filamentous mitochondrion often swells at the end giving it a club shaped appearance. Pleomorphic forms may contain swellings at both ends. The average length of the mitochondrion is 3-4 μ and the average diameter 0.5 –1.0 micron.

The number of mitochondria varies in different cell types. It is however, rather constant for a particular cell type. The number of mitochondria depends upon the metabolic activity of the cells; cells with high metabolic activity have a high number, while those with low metabolic activity have a lower number. In a normal live cell there are 1000-1600 mitochondria, while renal tubules have 300-400. In sperm they are as far as 20-24. However, the size, number and intracellular volume of the mitochondria undergo variations in response to a variety of physiological and pathological stimuli. Mitochondria may move freely in some cells, carrying ATP wherever required, or may be localized in a particular region, as in muscles and in reptilian venom glands. Movement is less frequent in animals than in plants. Often the movements are rhythmical.

The presence of DNA in mitochondrion has been clearly established. The interesting finding about mitochondrial DNA (mtDNA) is its tremendous variation in size. The three major systems studied so far are human, yeast, and higher plant mtDNA. The human mtDNA contains 16,568 base pairs and has been completely sequenced consisting of 37 genes. Yeast mtDNA is about five times larger than human mtDNA, and maize is about five times larger than yeast mtDNA. The mtDNA encodes several proteins as well as rRNA and tRNAs. A list of mitochondrial proteins and RNAs known to be encoded by the mt DNA is found in Table 3. The human mitochondrial genome has been fully sequenced and mapped. The genome is highly compact such that every base pair is involved in coding for either an mtRNA for a protein, rRNA or a tRNA. The genes appear to be very close, with no noncoding sequences between them. The one DNA strand is rich in guanosine and is called H strand, and the other strand rich in cytosine is referred to as L strand. Furthermore, there is only one major promoter on each strand. The primary transcription product is a full–length copy of each strand. The transcript is then cleaved into the various RNA molecules. At least some of the genes contain introns. It appears that tRNA genes flank almost every major gene and are actually used as spacers between these genes

12.6.2 Inheritance of Chloroplast Gene

Chloroplasts are specialized organelles found in all higher plant cells. These organelles contain the chlorophyll hence provide green color. They are very important for the plant, because they constitute the sites of photosynthesis. These may be spherical, ovoid, or discs shaped and are commonly 4 to 6μm thick. In some algal cells there is only single chloroplast whereas in higher plants the number is much larger (usually 20-40) per cell. The chloroplast is bounded by two membranes, an outer and an inner membrane with an intermembrane space between them. The inner membrane encloses a stroma that contains several enzymes. In higher plants, the stroma contains small cylindrical structures called grana. Most chloroplasts contain 10-100 grana, for example, in spinach chloroplast contains 40-60 grana, and tobacco chloroplast may have 40-80 grana.

Chloroplast genomes resemble large bacterial plasmids or small chromosomes. Their size ranges from 110,000 bp to 160,000 base pairs, depending on the species. Variation rarely appears in size or structure within an individual. The chloroplast genome was the first plant genome to be characterized. Its small size and limited number of repeat elements made it a prime candidate for characterization. Its abundance in foliar tissue made it easy to isolate.

The chloroplast inheritance in the four O'clock plant (*Mirabilis jalapa*) and corn are well documented in the literature. This *Mirabilis jalapa* plant may exist in three forms. Normal green variegated (patches of green and or nongreen tissue), and white (no chlorophyll). Egg cells from green plants carry normal green plastids, those from white plants may have both plastid types or just one type. Pollen (which produces the sperm and rarely contains any plastids) has no effect on progeny phenotype. All that is really involved here is the type of proplastids and plastids present in the egg cytoplasm. If the plastids are defective with regard to chlorophyll synthesis, the F1 plant will be nongreen, if they are not defective, the F1 plant will be green. Variegated parents produce eggs that contain both normal and defective plastids. These eggs give rise to plants, whose cell fortuitously receive a majority of green plastids or whose cells receive larger number of white plastids, which results in variegated plants.

12.6.3 Maternal Inheritance

The maternal transmission of mitochondrial genomes invokes a sex-specific selective sieve, whereby mutations in mitochondrial DNA can only respond to selection acting directly on females. In theory, this enables male-harming mutations to accumulate in mitochondrial genomes when these same mutations are neutral, beneficial, or only slightly deleterious in their effects on females. Ultimately, this evolutionary process could result in the evolution of male-specific mitochondrial mutation loads; an idea previously termed Mother's Curse. Here, we present evidence that the effects of this process are broader than hitherto realized, and that it has resulted in mutation loads affecting patterns of aging in male, but not female *Drosophila melanogaster*. Furthermore, our results indicate that the mitochondrial mutation loads affecting male aging generally comprise numerous mutations over multiple sites. Our findings thus suggest that males are subject to dramatic consequences that result from the maternal transmission of mitochondrial genomes. They implicate the diminutive mitochondrial genome as a hotspot for mutations that affect sex-specific patterns of aging, thus promoting the idea that a sex-specific selective sieve in mitochondrial genome evolution is a contributing factor to sexual dimorphism in aging, commonly observed across species.

To demonstrate that a trait is maternally inherited specific crosses need to be made to generate the required offspring. A number of crosses were made between cultivated tomato (*L. esculentum*) as a female parent and a number of wild species. Chloroplast DNA was obtained from F1 plants of these crosses and digested with different enzymes, in each case the F1 restriction fragment pattern was identical to *L. esculentum* (sample 8). This is conclusive evidence that chloroplast DNA is inherited in a maternal manner.

Uniparental inheritance, as several characters show maternal inheritance both in plants and animals. This was earlier explained to be due to greater amount of cytoplasm in egg than in sperm or male gamete. In *Chlamydomonas*, which is a haploid organism, sexual reproduction is isogamous conjugation, both gametes being similar in morphology and thus having equal amount of cytoplasm, Despite this lack of difference in cytoplasmic content in *Chlamydomonas,* some characters are transferred only from one parent exhibiting what is called uniparental inheritance (Fig. 6) rather than maternal inheritance, because in *Chlamydomonas*, two sexes as two mating

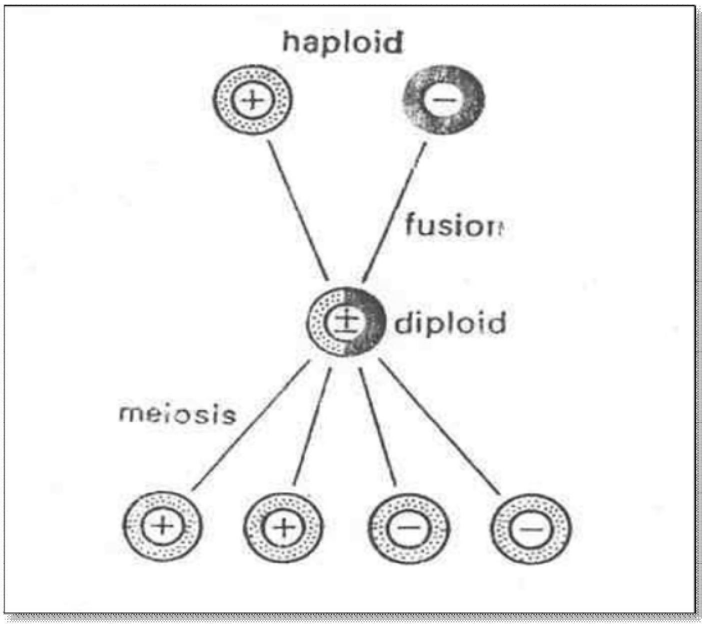

Figure 6 Uniparental inheritance in a haploid organism like *Chlaydomonas*

types, i.e. mt^{+} and mt^{-}, rather than female and male are described. One may notice that in diploids, the male gametes do not contribute cytoplasm (Fig. 7).

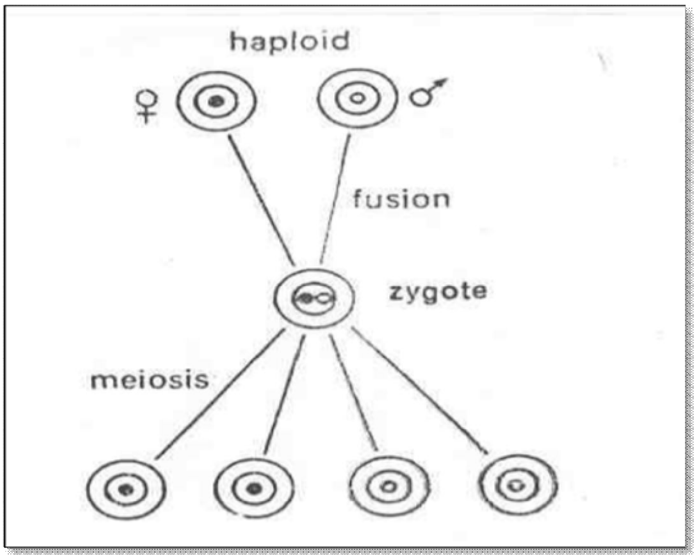

Figure 7 Segregation of nuclear genes in a haploid organism (1:1 ratio)

This way one may also distinguish between the patterns of inheritance between non-chromosomal and chromosomal genes. The inheritance patterns for chromosomal genes in haploid and diploid organisms are shown in Fig. 7 and 8.

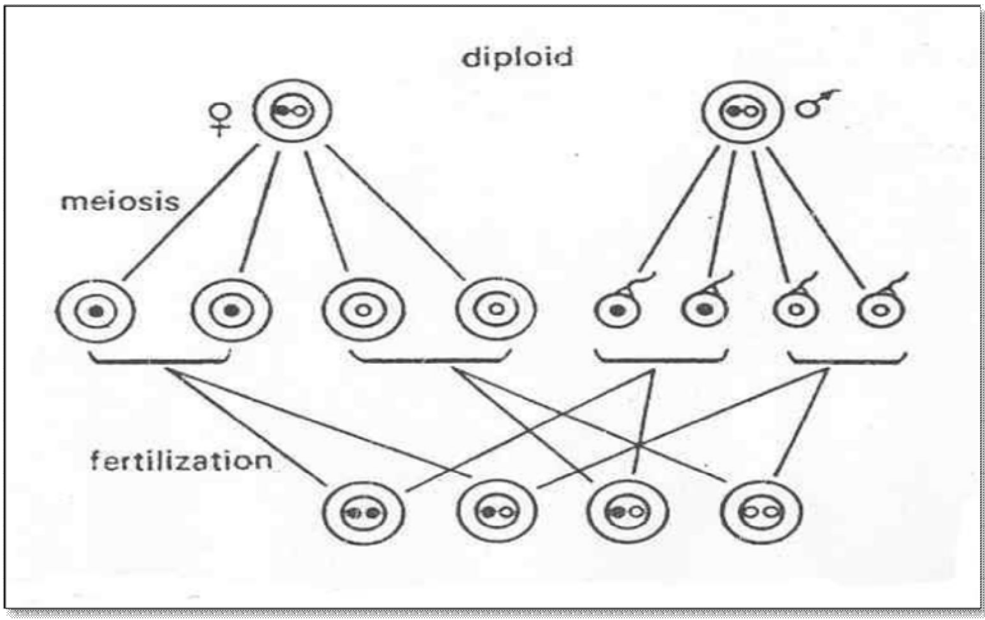

Figure 8 Segregation of nuclear genes in a diploid organism (1:2:1 ratio)

12.7 Summary

In this chapter, we have introduced the techniques and some examples of their applications. Although this only scratches the surface of a swiftly expanding subject, we hope it will have given you some understanding of the fundamental concepts involved, of the advances in knowledge that have already been attained, and of their future possibilities. Some of these possibilities may be detrimental, but in many ways these techniques will play a major role in improvements in health, to use just one example. From your study of this chapter, you should be better placed to understand, and hopefully contribute to, the debate over the use and control of genetic modification through molecular markers and genomic mapping.

12.8 Glossary

- **Ancient DNA:** Preserved DNA from an archaeological or fossil specimen.
- **Bacteria**: One of the two main groups of prokaryotes.

- **Biotechnology** The use of biological processes in industry and technology.
- **cDNA**: A double-stranded DNA copy of an mRNA molecule.
- **Clone**: A group of cells that contain the same recombinant DNA molecule.
- **Comparative genomics** A research strategy that uses information obtained from the study of one genome to make inferences about the map positions and functions of genes in a second genome.
- **Conjugation**: Transfer of DNA between two bacteria that come into physical contact with one another.
- **Conjugation mapping**: A technique for mapping bacterial genes by determining the time it takes for each gene to be transferred during conjugation.
- **Contig**: A contiguous set of overlapping DNA sequences.
- **Denaturation**: Breakdown by chemical or physical means of the non-covalent interactions, such as hydrogen bonding, that maintain the secondary and higher levels of structure of proteins and nucleic acids.
- **DNA**: Deoxyribonucleic acid, one of the two forms of nucleic acid in living cells; the genetic material for all cellular life forms and many viruses.
- **DNA marker:** A DNA sequence that exists as two or more alleles and which can therefore be used in genetic mapping.
- **DNA polymerase:** An enzyme that synthesizes DNA on a DNA or RNA template.
- **DNA profiling** A PCR technique that determines the alleles present at different STR loci
- within a genome in order to use DNA information to identify individuals.
- **DNA sequencing:** Determination of the order of nucleotides in a DNA molecule.
- **Endonuclease**: An enzyme that breaks phosphodiester bonds within a nucleic acid molecule.
- **Expressed sequence tag (EST):** A partial or complete cDNA sequence.

- **Functional genomics** Studies aimed at identifying all the genes in a genome and determining their expression patterns and functions.
- **Gene:** A segment of DNA that codes for an RNA and/or polypeptide molecule.
- **Genetic map:** A genome map that has been obtained by analysing the results of genetic crosses.
- **Genetics:** The branch of biology devoted to the study of genes.
- **Genome**: The entire genetic complement of a living organism.
- **Gene mapping:** Determination of the relative positions of different genes on a DNA molecule.
- **Hybridization**: The formation of double-stranded nucleic acid molecules by the production
- of hydrogen bonds between wholly or partially complementary sequences.
- ***In situ* hybridization:** A technique for gene mapping involving hybridization of a labeled sample of a cloned gene to a large DNA molecule, usually a chromosome.
- **Ligation**: Joining two DNA molecules using DNA ligase.
- **Map**: A chart showing the positions of genetic and/or physical markers in a genome
- **Mapping**: Determination of the position of genes (genetic map),or of physical features such as restriction endonuclease sites (physical map).
- **Microsatellite** A polymorphism comprising tandem copies of, usually, two-, three-, four- or five-nucleotide repeat units. Also called a short tandem repeat (STR).
- **Physical map**: A map of the physical structure of a genome, e.g. showing restriction sites, position of specific clones, or ultimately the complete sequence (c.f. genetic map).
- **Primer**: A specific oligonucleotide, complementary to a defined region of the template
- strand, from which new DNA synthesis will occur.
- **Probe**: A nucleic acid molecule that will hybridize to a specific target sequence.

- **Restriction**: Reduction or prevention of phage infection through the production of
- restriction endonucleases which degrade foreign DNA(see also modification).
- **Restriction endonuclease:** An enzyme that recognizes specific DNA sequences and cuts the DNA, usually at the recognition site.
- **Restriction fragment length polymorphism (RFLP):** Variation between individuals or strains in the size of specific restriction fragments; used for strain typing, and for locating particular genes.
- **Restriction mapping**: Determination of the position of restriction endonuclease recognition
- sites on a DNA molecule.
- **RFLP linkage analysis:** A technique that uses a closely linked RFLP as a marker for the presence of a particular allele in a DNA sample, often as a means of screening individuals for a defective gene responsible for a genetic disease.
- **Selectable marker:** A gene carried by a vector and conferring a recognizable characteristic on a cell containing the vector or a recombinant DNA molecule derived from the vector.
- **Sequence tagged site (STS):** A DNA sequence whose position has been mapped in a genome.
- **Short tandem repeat (STR):** A polymorphism comprising tandem copies of, usually, two-, three-, four- or five-nucleotide repeat units. Also called a microsatellite.
- **Single nucleotide polymorphism (SNP):** A point mutation that is carried by some individuals of a population.

12.9 Self Learning Exercise

Section -A (Very Short Answer Type)

1. Full name of AFLP
2. Expand VNTR
3. Define linkage map
4. Define Cytoplasmic inheritance
5. RAPD is ------------------------ based technique

Section -B (Short Answer Type)

1. Write a note on biochemical markers.
2. Briefly explain linkage map
3. Write a brief note on mitochondrial inheritance.
4. Define Cytoplasmic inheritance with suitable example.

Section -C (Long Answer Type)

1. What are molecular markers explain it in detail.
2. Difference between RAPD and RFLP
3. Discuss RAPD marker and its applications.

12.10 References

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Unit - 13

Human and Quantitative genetics

Structure of the Unit

13.1 Objectives

After going through this unit you will be able to understand

- What are pedigree analysis and karyotype?

- Syndrome associated with genetics?
- Polygenic inheritance its significance and examples?
- What is heritability and how to measure it?

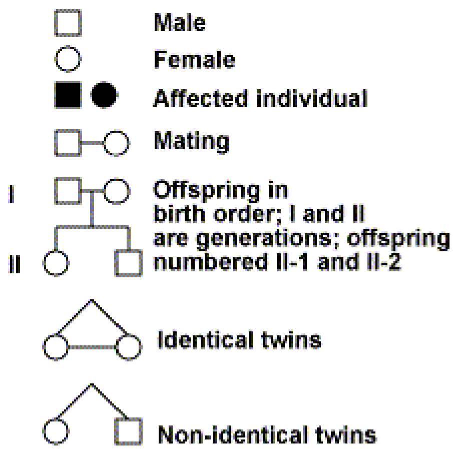
13.2 Introduction

The closely related fields of population genetics and quantitative genetics both focus on the genetic basis of phenotypic variation among the individuals in a population. Population genetics traditionally deals with frequencies of alleles and genotypes, whereas quantitative genetics concentrates on the ways that individual variation in genotype and environment contribute to variance in phenotype. The articles in this topic room represent a blend of both subjects.

The genetic analysis of quantitative or complex traits has been based mainly on statistical quantities such as genetic variances and heritability. These analyses continue to be developed, for example in studies of natural populations. Genomic methods are having an impact on progress and prospects. Actual relationships of individuals can be estimated enabling novel quantitative analyses. Increasing precision of linkage mapping is feasible with dense marker panels and designed stocks allowing multiple generations of recombination, and large SNP panels enable the use of genome wide association analysis utilising historical recombination. Whilst such analyses are identifying many loci for disease genes and traits such as height, typically each individually contributes a small amount of the variation

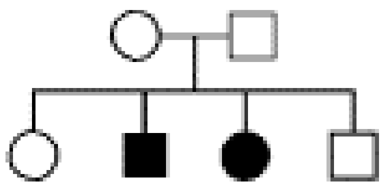
13.3 Human pedigree analysis

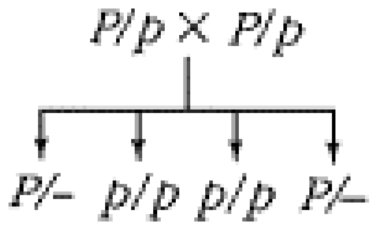
In humans, for genetic maps pedigree chart is utilized because in humans controlled crosses cannot be made, so geneticists must resort to scrutinizing family records in the hope that informative matings have been made that can be used to deduce dominance and distinguish autosomal from X-linked inheritance. The investigator traces the history of some variant phenotype back through the history of the family and draws up a family tree, or pedigree, using the standard symbols given in the Figure The clues in the pedigree have to be interpreted differently depending on whether one of the contrasting phenotypes is a rare disorder or whether both phenotypes of a pair are common morphs of a polymorphism. The genetic disorders of human beings can be dominant or recessive phenotypes and can be either autosomal or X-linked.



13.3.1 Autosomal Recessive Case

The unusual phenotype of a recessive disorder is determined by homozygosity for a recessive allele, and the unaffected phenotype is determined by the corresponding dominant allele. phenylketonuria (PKU) is arecessive phenotype. PKU is determined by an allele that we can call *p*, and the normal condition by *P*. Therefore the diseased genotype *p/p*, and unaffected people are either *P/P* or *P/p*. What patterns in a pedigreewould reveal such an inheritance? Two key points are that generally the disease appears in the progeny of unaffected parents and that the affected progeny include both males and females equally. When we know that both male and female phenotypic proportions are equal, we can assume that we are dealing with autosomal inheritance, not X-linked inheritance. The following typical pedigree illustrates the key point that affected children are born to unaffected parents:



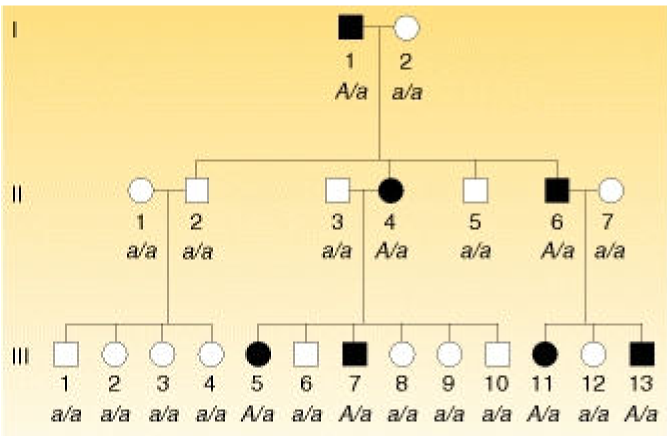


From this pattern we can immediately deduce autosomal inheritance, with the recessive allele responsible for the exceptional phenotype (indicated by shading). Furthermore, we can deduce that the parents must both be heterozygotes,*P/p*. (Both must have a *p* allele because each contributed one to each affected child, and both must have a *P* allele because the people are phenotypically normal.) We can identify the genotypes of the children (in the order shown) as*P/–*, *p/p*, *p/p*, and *P/–*.

13.3.2 Autosomal Dominant Case

In autosomal dominant disorders, the normal allele is recessive and the abnormal allele is dominant. It might seem paradoxical that a rare disorder can be dominant, but remember that dominance and recessiveness are simply reflections of how alleles act and are not defined in terms of *pre*dominance in the population. An example of a rare autosomaldominant phenotype is Huntington's disease. The phenotype is one of neural degeneration, leading to convulsions and premature death. However, it is a late-onset disease, the symptoms generally not appearing until after the person has begun to have children. Each child of a carrier of the abnormal allele stands a 50 percent chance of inheriting the allele and the associated disease. This tragic pattern has led to a drive to find ways of identifying people who carry the abnormal allele before they experience the onset of the disease. The discovery of the molecular nature of the mutant allele, and of neutral DNA mutations that act as "markers" close to the affected allele on thechromosome, has revolutionized this sort of diagnosis.

In pedigree analysis, the main clues for identifying an autosomal dominant disorder are that the phenotype tends to appear in every generation of the pedigree and that affected fathers and mothers transmit the phenotype to both sons and daughters. Again, the representation of both sexes among the affected offspring argues against X-linked inheritance. The phenotype appears in every generation because generally the abnormal allele carried by an individual must have come from a parent in the previous generation. (Abnormal alleles can arise de novo by mutation. This is relatively rare, but must be kept in mind as a possibility.) A typical pedigree for a dominant disorder is shown in below.



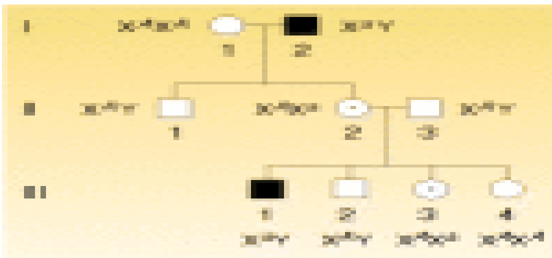
13.3.3 X-Linked recessive Case

Phenotypes with X-linked recessive inheritance typically show the following patterns in pedigrees:

1. Many more males than females show the phenotype under study. This is because a female showing the phenotype can result only from a mating in which both the mother and the father bear the allele (for example, $X^A/X^a \times X^a/Y$), whereas a male with the phenotype can be produced when only the mother carries the allele. If the recessive allele is very rare, almost all individuals showing the phenotype are males.

2. None of the offspring of an affected male are affected, but all his daughters must be heterozygous "carriers" because females must receive one of their X chromosomes from their fathers. Half the sons born to these carrier daughters are affected (Figure 4-24).

   Perhaps the best-known example is hemophilia, a malady in which a person's blood fails to clot. Many proteins must interact in sequence to make blood clot. The most common type of hemophilia is caused by the absence or malfunction of one of these proteins, called *factor VIII.*

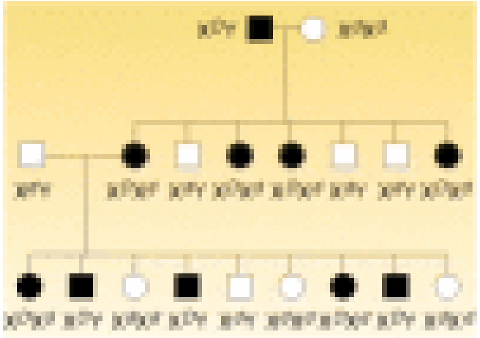


13.3.4 X-Linked dominant Case

Pedigrees of rare X-linked dominant phenotypes show the following characteristics:

1. Affected males pass the condition on to all their daughters but to none of their sons.

2. Females married to unaffected males pass the condition on to half their sons and daughters.



Pedigree of an X-linked dominant disorder.

There are few examples of X-linked dominant phenotypes in humans. One is hypophosphatemia, a type of vitamin D–resistant rickets.

13.4 LOD Score method for estimating linkage distance

The LOD score (logarithm (base 10) of odds), developed by Newton E. Morton, is a statistical test often used for linkage analysis in human, animal, and plant populations. The LOD score compares the likelihood of obtaining the test data if the two loci are indeed linked, to the likelihood of observing the same data purely by chance. Positive LOD scores favor the presence of linkage, whereas negative LOD scores indicates the independent assortment. Computerized LOD score analysis is a simple way to analyze complex family pedigrees in order to determine the linkage between Mendelian traits (or between a trait and a marker, or two markers).

The method is described in greater detail by Strachan and Read. Briefly, it works as follows:

1. Establish a pedigree
2. Make a number of estimates of recombination frequency
3. Calculate a LOD score for each estimate
4. The estimate with the highest LOD score will be considered the best estimate

The LOD score is calculated as follows:

$$LOD = Z = \log_{10}\frac{\text{probability of birth sequence with a given linkage value}}{\text{probability of birth sequence with no linkage}} = \log_{10}\frac{(1-\theta)^{NR}\times\theta^{R}}{0.5^{(NR+R)}}$$

NR denotes the number of non-recombinant offspring, and R denotes the number of recombinant offspring. The reason 0.5 is used in the denominator is that any alleles that are completely unlinked (e.g. alleles on separate chromosomes) have a 50% chance of recombination, due to independent assortment. 'θ' is the recombinant fraction, i.e. the fraction of births in which recombination has happened between the studied genetic marker and the putative gene associated with the disease. Thus, it is equal to R / (NR + R)

By convention, a LOD score greater than 3.0 is considered evidence for linkage, as it indicates 1000 to 1 odds that the linkage being observed did not occur by chance. On the other hand, a LOD score less than -2.0 is considered evidence to exclude linkage.

13.5 Karyotype

A karyotype is the number and appearance of chromosomes in thenucleus of a eukaryotic cell. The term is also used for the complete set of chromosomes in a species, or an individual organism.

Karyotypes describe the chromosome count of an organism, and what these chromosomes look like under a light microscope. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics. The preparation and study of karyotypes is part of cytogenetics.

The study of whole sets of chromosomes is sometimes known as *karyology*. The chromosomes are depicted (by rearranging a photomicrograph) in a standard format known as a *karyogram* or *idiogram*: in pairs, ordered by size and position of centromere for chromosomes of the same size.

The basic number of chromosomes in the somatic cells of an individual or a species is called the *somatic number* and is designated *2n*. Thus, in humans 2n = 46. In the germ-line (the sex cells) the chromosome number is *n* (humans: n = 23).

So, in normal diploid organisms, autosomal chromosomes are present in two copies. There may, or may not, be sex chromosomes. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies.

13.5.1 Importance of karyotyping

The study of karyotypes is important for cell biology and genetics, and the results may be used in evolutionary biology(*karyosystematics*) and medicine. Karyotypes can be used for many purposes; such as to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

13.5.2 Staining for karyotyping

The study of karyotypes is made possible by staining. Usually, a suitable dye, such as Giemsa, is applied after cells have been arrested during cell division by a solution of colchicine usually in metaphase or prometaphase when most condensed. In order for the Giemsa stain to adhere correctly, all chromosomal proteins must be digested and removed. For humans, white blood cells are used most frequently because they are easily induced to divide and grow in tissue culture. Sometimes observations may be made on non-dividing (interphase) cells. The sex of an unborn fetus can be determined by observation of interphase cells.

13.5.3 Observations

Six different characteristics of karyotypes are usually observed and compared:

1. Differences in absolute sizes of chromosomes. Chromosomes can vary in absolute size by as much as twenty-fold between genera of the same family. For example, the legumes *Lotus tenuis* and *Vicia faba* each have six pairs of chromosomes, yet *V. faba* chromosomes are many times larger. These differences probably reflect different amounts of DNA duplication.
2. Differences in the position of centromeres. These differences probably came about through translocations.
3. Differences in relative size of chromosomes. These differences probably arose from segmental interchange of unequal lengths.
4. Differences in basic number of chromosomes. These differences could have resulted from successive unequal translocations which removed all the essential genetic material from a chromosome, permitting its loss without penalty to the organism (the dislocation hypothesis) or through fusion. Humans have one pair fewer chromosomes than the great apes. Human chromosome 2 appears to have resulted from the fusion of two

ancestral chromosomes, and many of the genes of those two original chromosomes have been translocated to other chromosomes.

5. Differences in number and position of satellites. Satellites are small bodies attached to a chromosome by a thin thread.
6. Differences in degree and distribution of heterochromatic regions. Heterochromatin stains darker than euchromatin. Heterochromatin is packed tighter. Heterochromatin consists mainly of genetically inactive and repetitive DNA sequences as well as containing a larger amount of Adenine-Thymine pairs. Euchromatin is usually under active transcription and stains much lighter as it has less affinity for the giemsa stain. Euchromatin regions contain larger amounts of Guanine-Cytosine pairs. The staining technique using giemsa staining is called G banding and therefore produces the typical "G-Bands".

A full account of a karyotype may therefore include the number, type, shape and banding of the chromosomes, as well as other cytogenetic information.

Human karyotype

The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes (allosomes). Normal karyotypes for females contain two X chromosomes and are denoted 46,XX; males have both an X and a Y chromosome denoted 46,XY. Any variation from the standard karyotype may lead to developmental abnormalities.

13.5 Turner syndrome

Turner syndrome (TS) also known as Ullrich–Turner syndrome, gonadal dysgenesis, and 45,X, is a condition in which a female is partly or completely missing an X chromosome. Signs and symptoms vary among those affected. Often, a short and webbed neck, low-set ears, low hairline at the back of the neck, short stature, and swollen hands and feet are seen at birth. Typically they are without menstrual periods, do not develop breasts, and are unable to have children. Heart defects, diabetes, and low thyroid hormone occur more frequently. Most people with TS have normal intelligence. Many, however, have troubles with spatial visualization such as that needed for mathematics. Vision and hearing problems occur more often.

Turner syndrome is not usually inherited from a person's parents. No environmental risks are known and the mother's age does not play a role. Turner syndrome is due to a chromosomal abnormality in which all or part of one of the X chromosomes is missing or altered. While most people have 46 chromosomes, people with TS usually have 45. The chromosomal abnormality may be present in just some cells in which case it is known as TS withmosaicism. In these cases, the symptoms are usually fewer and possibly none occur at all. Diagnosis is based on physical signs and genetic testing.

No cure for Turner syndrome is known. Treatment, however, may help with symptoms. Human growth hormoneinjections during childhood may increase adult height. Estrogen replacement therapy can promote development of thebreasts and hips. Medical care is often required to manage other health problems with which TS is associated. Rarely, Turner syndrome caused by a partial deletion of the X chromosome can be passed from one generation to the next.

13.6 Klinefelter syndrome

Klinefelter's syndrome (KS) also known as 47,XXY or XXY, is the set of symptoms that result from two or more X chromosomes in males. The primary feature is sterility. Often symptoms may be subtle and many people do not realize they are affected. Sometimes symptoms are more prominent and may include weaker muscles, greater height, poor coordination, less body hair, smaller genitals, breast growth, and less interest in sex. Often it is only at puberty that these symptoms are noticed. Intelligence is usually normal; however,reading difficulties and problems with speech are more common. Symptoms are typically more severe if three or more X chromosomes are present.

Genetic changes related to Klinefelter syndrome

Klinefelter syndrome is a condition related to the X and Y chromosomes (the sex chromosomes). People typically have two sex chromosomes in each cell: females have two X chromosomes (46,XX), and males have one X and one Y chromosome (46,XY). Most often, Klinefelter syndrome results from the presence of one extra copy of the X chromosome in each cell (47,XXY). Extra copies of genes on the X chromosome interfere with male sexual development, often preventing the testes from functioning normally and reducing the levels of testosterone. Most people with an extra X chromosome have the features described above, although some have few or no associated signs and symptoms.

Some people with features of Klinefelter syndrome have more than one extra sex chromosome in each cell (for example, 48,XXXY or 49,XXXXY). These conditions, which are often called variants of Klinefelter syndrome, tend to cause more severe signs and symptoms than classic Klinefelter syndrome. In addition to affecting male sexual development, variants of Klinefelter syndrome are associated with intellectual disability, distinctive facial features, skeletal abnormalities, poor coordination, and severe problems with speech. As the number of extra sex chromosomes increases, so does the risk of these health problems.

Some people with features of Klinefelter syndrome have the extra X chromosome in only some of their cells; in these individuals, the condition is described as mosaic Klinefelter syndrome (46,XY/47,XXY). Individuals with mosaic Klinefelter syndrome may have milder signs and symptoms, depending on how many cells have an additional X chromosome.

Inheritence of Klinefelter syndrome

Klinefelter syndrome and its variants are not inherited; these chromosomal changes usually occur as random events during the formation of reproductive cells (eggs and sperm) in a parent. An error in cell division called nondisjunction results in a reproductive cell with an abnormal number of chromosomes. For example, an egg or sperm cell may gain one or more extra copies of the X chromosome as a result of nondisjunction. If one of these atypical reproductive cells contributes to the genetic makeup of a child, the child will have one or more extra X chromosomes in each of the body's cells.

Mosaic 46,XY/47,XXY is also not inherited. It occurs as a random event during cell division early in fetal development. As a result, some of the body's cells have one X chromosome and one Y chromosome (46,XY), and other cells have an extra copy of the X chromosome (47,XXY).

13.7 Polygenic inheritence

A polygene, multiple factor, multiple gene inheritance, or quantitative gene is a group of non-epistatic genes that together influence a phenotypic trait. The precise loci or identities of the non-allelic genes are often unknown to biologists. Advances in statistical methodology and high throughout sequencing are, however, allowing researchers to locate candidate genes for the trait. These genes are generally pleiotropic as well.

Traits with polygenic determinism correspond to the classical quantitative characters, as opposed to the qualitative characters with monogenic or oligogenic determinism. In essence instead of two options, such as freckles or no freckles, there are many variations. Like the color of skin, hair, or even eyes.

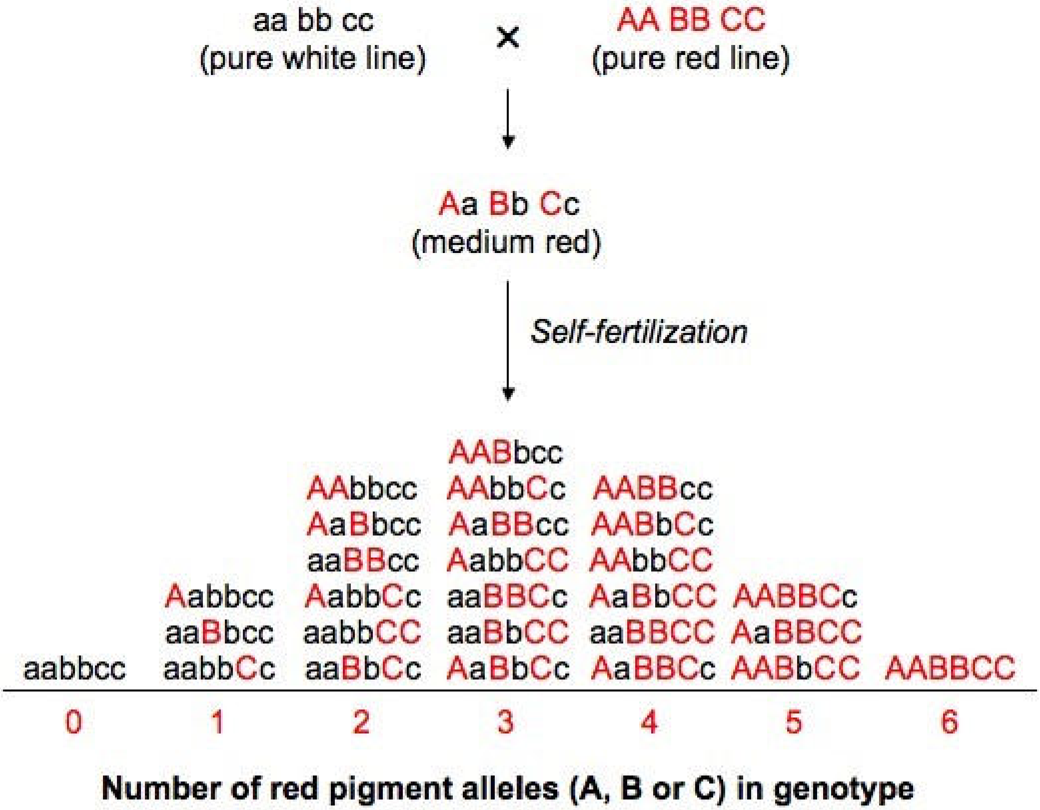
13.7.1 Human skin color is a good example of polygenic (multiple gene) inheritance. Assume that three "dominant" capital letter genes (A, B and C) control dark pigmentation because more melanin is produced. The "recessive"alleles of these three genes (a, b & c) control light pigmentation because lower amounts of melanin are produced. The words dominant and recessive are placed in quotation marks because these pairs of alleles are not truly dominant and recessive as in some of the garden pea traits that Gregor Mendel studied. A genotype with all "dominant" capital genes (AABBCC) has the maximum amount of melanin and very dark skin. A genotype with all "recessive" small case genes (aabbcc) has the lowest amount of melanin and very light skin. Each "dominant" capital gene produces one unit of color, so that a wide range of intermediate skin colors are produced, depending on the number of "dominant" capital genes in the genotype. For example, a genotype with three "dominant" capital genes and three small case "recessive" genes (AaBbCc) has a medium amount of melanin and an intermediate skin color. This latter genotype would be characteristic of a mulatto.

In the following cross between two mulatto genotypes (AaBbCc x AaBbCc), each parent produces eight different types of gametes and these gametes combine with each other in 64 different ways resulting in a total of seven skin colors. The skin colors can be represented by the number of capital letters, ranging from zero (no capital letters) to six (all capital letters). Skin color may involve at least four pairs of alleles with nine (or more) shades of skin color.

13.7.2 Grain Colour in Wheat

Wheat grains vary in colour from white to dark red, depending on the amount of red pigment they contain. Three genes control the colour and each gene has two alleles (one coding for red pigment, the other coding for no pigment). The most frequent combinations have an equal number of 'pigment producing' and 'no pigment' alleles, whereas combinations of one extreme or the other are relatively rare. The overal pattern of inheritance shows continuous variation

Polygenic Inheritance of Grain Wheat Colour



13.8 Heritability and its measurement

If a trait is shown to have some heritability in a population, then it is possible to quantify the degree of heritability. The total phenotypic variance of the population (S^2_p) can then be broken into two parts: the variance between genotypic means (S^2_g) and the remaining variance (S^2_e) The former is called the genetic variance, and the latter is called the environmental variance. Moreover, the breakdown of the phenotypic variance into the sum of environmental and genetic variance leaves out the possibility of some covariance between genotype and environment. For example, suppose it were true (we do not know) that there are genes that influence musical ability. Parents with such genes might themselves be musicians, who would create a more musical environment for their children, who would then have both the genes and the environment promoting musical performance. The result would be an increase in the phenotypic variances of musical ability and an erroneous estimate of genetic and environmental variances. If the phenotype is the sum of a genetic and an environmental effect, $P = G + E$, then, the variance of the phenotype is the sum of the genetic variance, the environmental variance, and twice the covariance between the genotypic and environmental effects.

$$s_p^2 = s_g^2 + s_e^2 + 2\ \text{cov}\ ge$$

If genotypes are not distributed randomly across environments, there will be some covariance between genotype and environmental values, and the covariance will be hidden in the genetic and environmental variances.

The degree of heritability can be defined as the part of the total variance that is due to genetic variance:

$$H^2 = \frac{s_g^2}{s_p^2} = \frac{s_g^2}{s_g^2 + s_e^2}$$

H^2, so defined, is called the broad heritability of the character.

It must be stressed that this measure of "genetic influence" tells us what part of the population's *variation* in phenotypecan be assigned to *variation* in genotype. It does not tell us what parts of an *individual's* phenotype can be ascribed to itsheredity and to its environment. This latter distinction is not a reasonable one. An individual's phenotype is a consequence of the interaction between its genes and its sequence of environments. It clearly would be silly to say that you owe 60 inches of your height to genes and 10 inches to environment. All measures of the "importance" of genes are framed in terms of the proportion of variance ascribable to their variation. This approach is a special application of the more general technique of the analysis of variance for apportioning relative weight to contributing causes. The method was, in fact, invented originally to deal with experiments in which different environmental and genetic factors were influencing the growth of plants.

13.8.1 Methods of estimating heritability

Genetic variance and heritability can be estimated in several ways. Most directly, we can obtain an estimate of (S^2_e) by making a number of homozygous lines from the population, crossing them in pairs to reconstitute individual heterozygotes, and measuring the phenotypic variance *within* each heterozygous genotype. Because there is no genetic variance within a genotypic class, these variances will (when averaged) provide an estimate of (S^2_e). This value can then be subtracted from the value of (S^2_p) in the original population to give (S^2_g). With the use of this method, anycovariance between genotype and environment in the original population will be hidden in the estimate of genetic variance and will inflate it.

1. BROAD SENSE HERITABILITY, H2. This is the proportion of the total phenotypic variance that can attributed to genotypic variance (i.e. to differences within the population in genotype). Mathematically: H2 = VG + VE+ VGI/VT
2. NARROW SENSE HERITABILITY, h2 We are often more interested in variance from additive genes, since genes that show additive inheritance tend to show more intermediate forms for selection to operate on (and thus greater phenotypic variability).
   h2 = VG/ VT

13.9 QTL mapping

Genes involved in common function may be located on same or on different chromosome on different locus.

For example Resistance or susceptibility to a fungal disease is not due to a single gene if four genes are involved in this process designated as ABCD

These genes may be different in their location but they are involved in a common function, the determination of location of these gene on same or on different chromosomes is known as QTL mapping.

13.10 Elementary idea of Human genome project

The Human Genome Project (HGP) is an international scientific research project with the goal of determining the sequence of chemical base pairs which make up human DNA, and of identifying and mapping all of the genes of the human genomefrom both a physical and functional standpoint.[1] It remains the world's largest collaborative biological project. After the idea was picked up in 1984 by the US government the planning started, with the project formally launched in 1990, and finally declared complete in 2003. Funding came from the US government through the National Institutes of Health as well as numerous other groups from around the world. A parallel project was conducted outside of government by the Celera Corporation, or Celera Genomics, which was formally launched in 1998. Most of the government-sponsored sequencing was performed in twenty universities and research centers in the United States, the United Kingdom, Japan, France, Germany, and China.

The Human Genome Project originally aimed to map the nucleotides contained in a human haploid reference genome (more than three billion). The "genome" of any given individual is unique; mapping "the human genome" involves sequencing multiple variations of each gene.

- Genome- Sum total of total genetic material or the genetic information resides in haploid set of chromosomes.
- Mitochondrial and chloroplast genome should be included within the genome although their amount is negligible.
- Total number of genes in human are around 27000 out of this 18000 are protein coding remaining are tRNA and r RNA coding genes.
- The average gene size in case of human is 27KB while in case of prokaryotes is 1KB that means human have 27 times larger genes than prokaryotes but it doesn't means they will code for larger protein because the eukaryotes contains the extra-genic(interons) sequences while the prokaryote not.
- The largest gene in human genome is Destophin but the largest protein is titin.

1. In Turner's syndrome:

1. premature cataract is common
2. the cells contains 45 chromosomes
3. the offsprings have 50% of inheriting the disease
4. there is an increased risk of hypertension

2. In Klinefelter's syndrome:

1. the karyotype is always 47XXY
2. the extra chromosome comes from the mother germ cells
3. there is an increased incidence of lens subluxation
4. the patient is infertile

3.The location on a chromosome where a particular gene is located is known as the:

1.	allele	2.	dihybrid
3.	locus	4.	diploid

1. Give one example of each autosomal dominant and autosomal recessive trait?

2. Describe polygenic inheritance ?
3. What is LOD score and how it will be useful for estimating linkage distances?
4. What is heritability describe methods for estimating the heritability?
5. Describe karyotype and steps of their preparation ?

For a sophisticated but accessible treatment of the analysis of variance written for biologists, see R. Sokal and J. Rohlf, *Biometry,* 3d ed. W. H. Freeman and Company, 1995.)

Unit - 14

Mutation

Structure of the Unit

14.0 Objectives

After going through this unit you will be able to understand -

- What are mutations?
- How many types of mutations are found?
- Various types of Mutagen

- How will you screen and select mutagens?

14.1 Introduction

The development and function of an organism are controlled by genes. Sequence of genes leads to formation of genome which in large part is made-up of DNA. When its structure changes by any of the biological consequences it results in alternation in gene. As a result it leads to biological diversity that exists today. This alternation of gene which is permanent due to change in sequence of DNA is called as mutation. Mutations can lead to changes in the structure of an encoded protein or to a decrease or complete loss in its expression. Because a change in the DNA sequence affects all copies of the encoded protein, mutations can be particularly damaging to a cell or organism. The term mutation was coined by de Vries, according to him "Mutation is the process by which a gene changes structurally". For an observable effect, mutation must occur in gene exon or regulatory elements. Changes in the non-coding regions of DNA (introns and junk DNA) generally do not affect function. Physical or chemical agents that induce mutations in DNA are called mutagens and are said to be mutagenic in nature. In this section we will study about causes of mutation, various types of mutations, mutagenic agents and isolation and identification of mutants.

14.2 Mutation Rates and Mutation Frequency

The frequency with which a gene changes from the wild type to a mutant is referred to as the mutation rate and is generally expressed as the number of mutations per biological unit, which may be mutations per cell division, per gamete, or per round of replication. In contrast mutation frequency is defined as the incidence of a specific type of mutation within a group of individual organisms.

Mutation rates are affected by three factors:

1. The frequency with which primary changes take place in DNA. Primary change may arise from spontaneous molecular changes in DNA or it may be induced by chemical or physical agents in the environment.
2. The probability of repair of mutated DNA.
3. The probability of mutation recognition and recording.

14.3 Causes of Mutation

Mutation can occur either due to exogenous factors or endogenous factors along with this mutations may occur due to errors in the cellular machinery. Those that are a result of natural changes in DNA structure are termed spontaneous mutations, whereas those that result from changes caused by environmental chemicals or radiation are induced mutations.

1. Exogenous factors: Mutations can occur as the result of exposure to environmental factors such as specific chemicals, sunlight and radiation. These agents cause the DNA to break down.
2. Endogenous factors: Errors during DNA replication can lead to genetic changes as can toxic by-products of cellular metabolism.

   Most of the mutations that occur are “naturally-occurring." For example, when a cell divides, it makes a copy of its DNA and sometimes the copy is not quite perfect. That small difference from the original DNA sequence is a mutation.

   As soon as cell recognise any potentially mutation-causing damage its machinery repair it before it becomes a fixed mutation.Mutations contribute to genetic variation within species.Mutations can also be inherited, particularly if they have a positive effect. Mutations can be advantageous and lead to an evolutionary advantage of a certain genotype. Mutations can also be deleterious, causing disease, developmental delays, structural abnormalities or other effects.

14.4 Types of mutations

Mutation can be divided into two major types in multicellular organisms:

a. Somatic mutations : Somatic mutations arise in somatic tissues which do not produce gametes.
b. Germline mutations:Germ-line mutationsarise in cells that ultimately produce gametes. These mutations can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germ-line cells

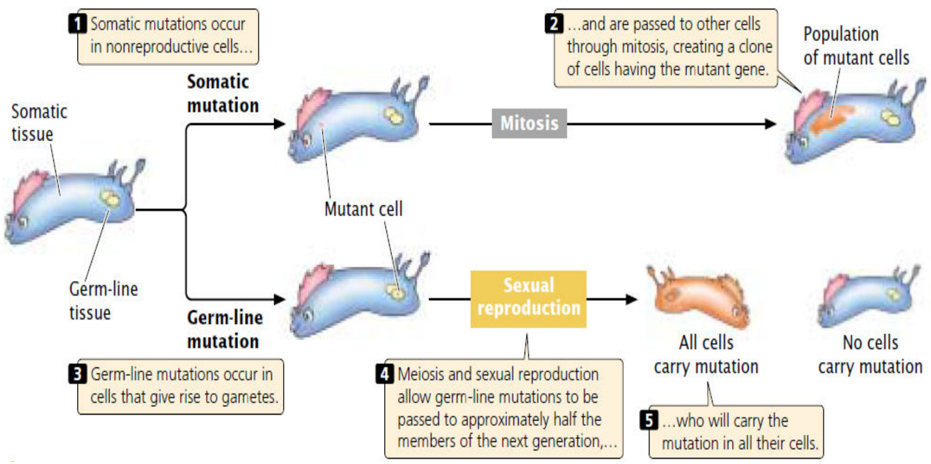


Figure1. Somatic Mutation Vs. Germ line Mutation(Image Source: Benjamin-Pierce-Genetics A Conceptual Approach)

Historically, mutations have been classified into gene mutation and chromosome mutation. Gene mutations are those mutations that affect a single gene and those that affect the number or structure of chromosomes, are called as chromosome mutations. With the advancement in field of molecular biology and development of DNA sequencing, gene mutations and chromosome mutations are distinguished somewhat arbitrarily on the basis of the size of the DNA lesion. Nevertheless, it is useful to use the term chromosome mutation for a large-scale genetic alteration that affects chromosome structure or the number of chromosomes and the term gene mutation for a relatively small DNA lesion that affects a single gene. Here in this chapter we will discuss about gene mutation.

Gene mutations can be classified in number of ways.

1. Gene mutation on the bases of the nature of the phenotypic effect whether the mutation alters the amino acid sequence of the protein.
2. Mutations can also be classified on the bases of the causative agent of the mutation (The agent which cause mutation is called as mutant),
3. On the bases of the molecular nature of the defect.

Gene mutations are broadly classified into mainly substitution and frame shift mutation.

14.4.1 Substitution Mutation

The simplest type of gene mutation is a base substitution. In substitution mutation a single nucleotide is changed into a different nucleotide. Because of the complementary nature of the two DNA strands when the base of one nucleotide is altered, the base of the corresponding nucleotide on the opposite strand also will be altered in the next round of replication. A base substitution therefore usually leads to a base-pair substitution.

Nucleotide substitutions are one of two classes:

1. **Transitions:** a purine nucleotide is replaced with a purine nucleotide, or a pyrimidine nucleotide is replaced with a pyrimidine nucleotide. In other words, the base in the new nucleotide is in the same chemical class as that of the original nucleotide.
2. **Transversions:** The chemical class of the base changes, i.e. a purine nucleotide is replaced with a pyrimidine nucleotide, or a pyrimidine nucleotide is replaced with a purine nucleotide.

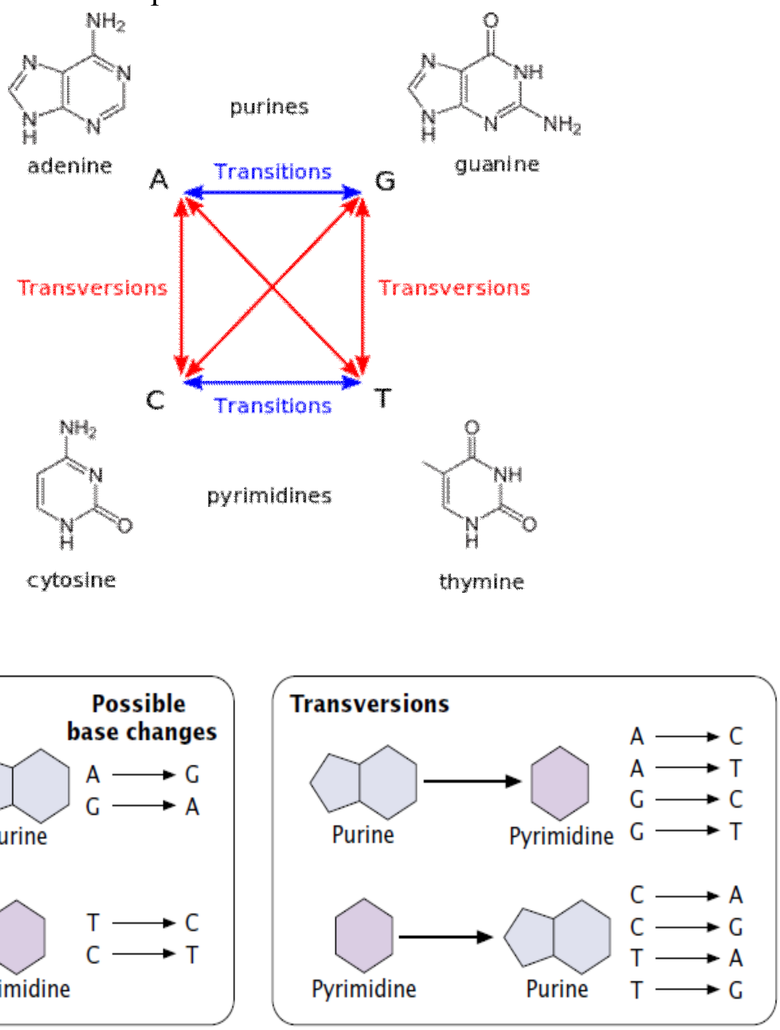


Fig.2 Types of substitutions: transitions and transversions.Image Source: Benjamin-Pierce-Genetics A Conceptual Approach

The consequences of base substitution mutations in protein coding regions of a gene depend on the substitution and its location.

On these bases mutation can be classified as:

a) **Missense Mutation** - A base substitution that results in a change of one amino acid for another could also result in an amino acid substitution. Eg. TCA in DNA sense strand [UCA in mRNA] will specify a Serine residue in the protein; this is altered to TTA in the DNA or UUA in the mRNA, resulting in a leucine residue in the protein chain causing alternation of protein and result in missense mutation.

b) **Nonsense Mutation**- A base substitution that results in a stop codon replacing a normal amino acid codon. It result in formation of incomplete and inactive protein.

c) **Silent Mutation**: A substitution in which a base pair that is altered cause no change in an amino acid in the protein sequence, eg. UCA or UCG codons in mRNA both mean Serine.

d) **Suppressor Mutation**: In such kind of mutation substitution occur in non coding region of DNA or Asuppressor mutationis a genetic change that hides or suppresses the effect of another mutation.

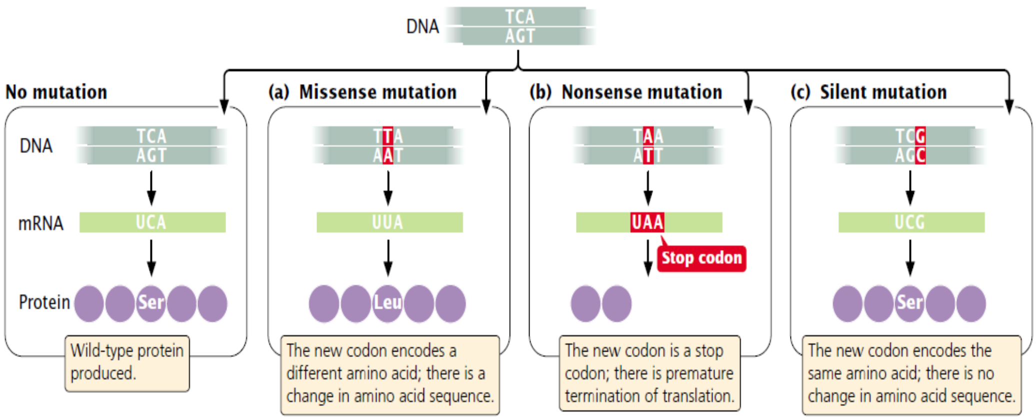


Image Source: Benjamin-Pierce-Genetics A Conceptual Approach

14.4.2 Frame Shift Mutation

Mutations in which a single nucleotide is either deleted or inserted at a single position, such type of mutations are known as frameshift mutation, since it results in altered reading frame for the remainder of the gene. Since the message is read in triplets, with no punctuation, an alteration in the reading frame will result in the synthesis of a totally different protein from that start codon; so frameshift mutations generally have drastic effects on the phenotype because many amino acids can be affected. Not all insertions and deletions lead to frameshifts, however; because codons consist of three nucleotides, insertions and deletions consisting of any multiple of three nucleotides will leave the reading frame intact, although the addition or removal of one or more amino acids may still affect the phenotype.

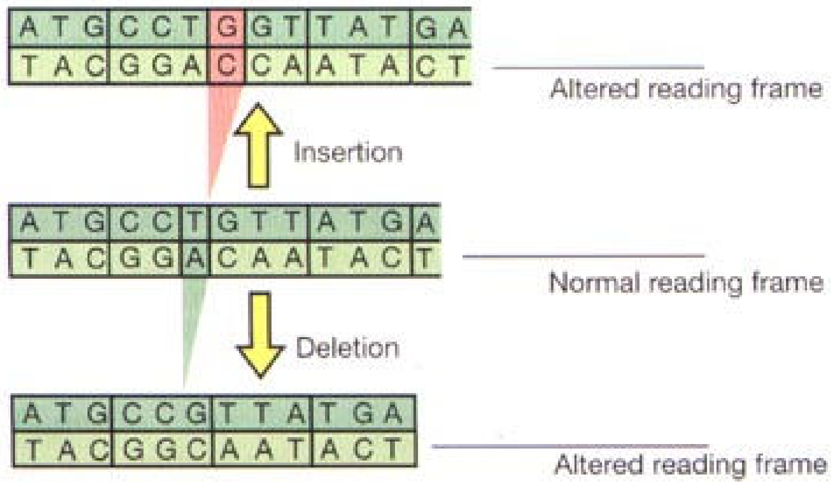


Fig.3 Frame shift Mutation Image source:http://utminers.utep.edu

Mutation can also be categorized into two major categories on the bases of type of insertion into: 1. Spontaneous mutation 2. Induced Mutation

Spontaneous mutation occurs due to:

14.4.3 Spontaneous Mutation

Spontanious mutations are those mutation which are caused by natural changes in DNA structure.These mutation occur due to:

Spontaneous Replication Errors

Spontaneous Chemical Changes

1. **Spontaneous Replication Errors:** Replication is usually correct but less than one in a billion errors are made in the course of DNA synthesis such kind of errors result in spontaneous mutations.

   The primary cause of spontaneous replication errors was thought to be tautomeric shifts, in which the positions of protons in the DNA bases change. Purine and pyrimidine bases exist in different chemical forms called tautomers. The two tautomeric forms of each base are in dynamic equilibrium, although one form remain more common than the other. In normal base pairings Adenine pair with Thymine, and Cytosine with Guanine but, if the bases are in their rare tautomeric forms, other base pairings are possible Watson and Crick proposed that tautomeric shifts might produce mutations, and for many years their proposal was the accepted model for spontaneous replication errors.

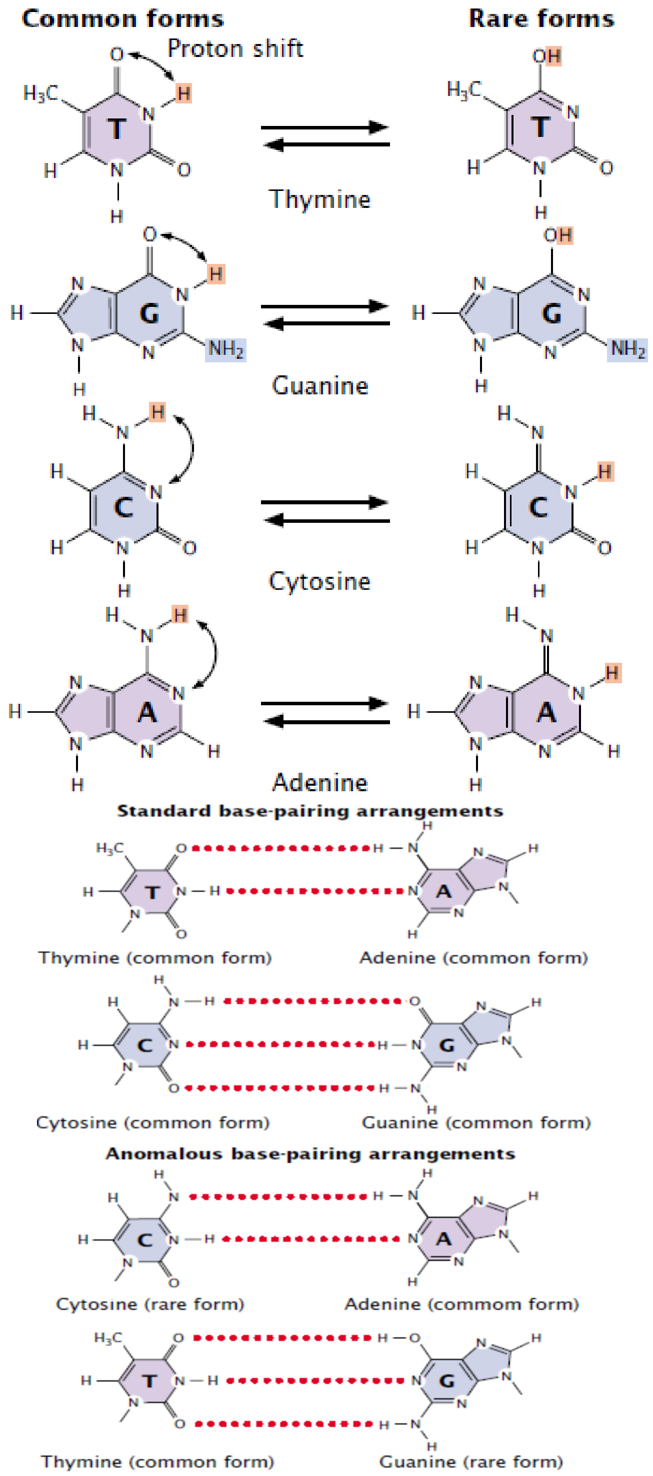
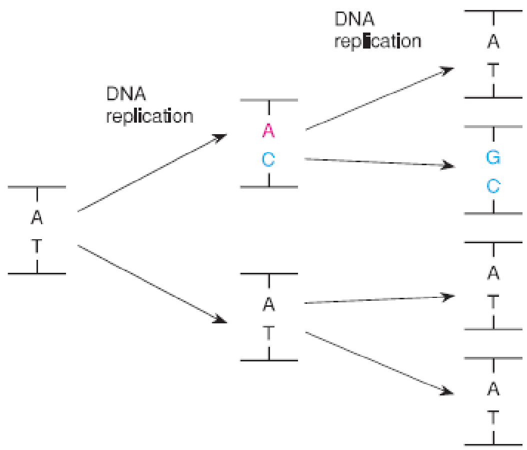
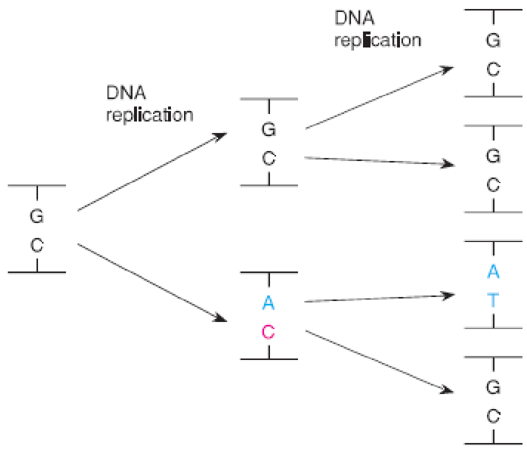


Fig.4 Tautomeric shift due to change in the proton position result in rare tautomeric form which basepair with opposite nitrogenous base



Template transition—tautomerization of adenine in the template



Substrate transition—tautomerization of incoming adenine

Fig.5 Standard and anomalous base-pairing arrangement which may result spontaneous mutation due to replication error

2. **Spontaneous Chemical Changes:** Spontaneous mutation along with replication errors may also be caused by spontaneous chemical changes in DNA. It may be caused by
   1. Depurination
   2. Deamination

Depurination: In this process there is loss of one purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1-carbon atom of the deoxyribose sugar breaks producing an apurinic site (a nucleotide that lacks its purine base) An apurinic site cannot act as a template for a complementary base in replication and in absence of base-pairing constraints, an incorrect nucleotide is incorporated into the newly synthesized DNA strand opposite the apurinic site, this condition leads to error incorporation. This error is then transformed into a replication error in the next round of replication. Depurination is a common cause of spontaneous mutation; a mammalian cell in culture loses approximately 10,000 purines every day.

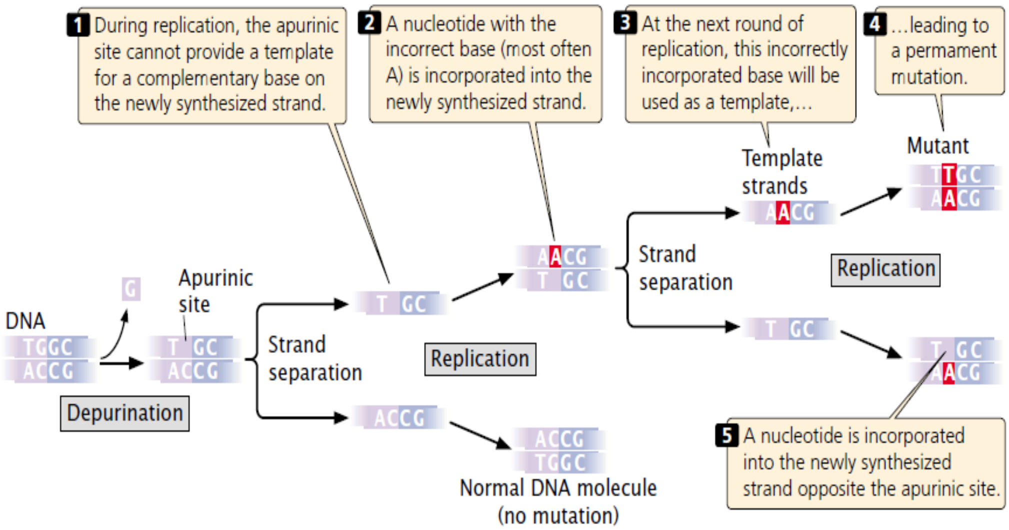


Fig.6 Depurination result in loss of one purine base from a nucleotide and result in mutation.Image Source: Benjamin-Pierce-Genetics A Conceptual Approach

Deamination: Another method of chemical change in nucleotide for mutation is the loss of an amino group (NH_2) from a base, this process is called as deamination. Deamination may occur spontaneously or can be induced by mutagenic chemicals.Deamination may alter the pairing properties of a nitrogenous base. The deamination of cytosine, for example, produces uracil, which pairs with adenine during replication.

After another round of replication, the adenine will pair with thymine, creating a T-A pair in place of the original C-G pair (C-G =>U-A=>T-A); this chemical change is a transition mutation.

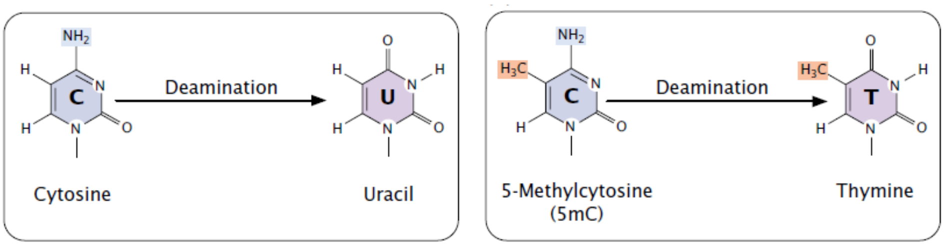


Fig.7 Deamination alters DNA baseImage Source: Benjamin-Pierce-Genetics A Conceptual Approach

Induced Mutation:

Induced mutations are those mutations which result from changes caused by environmental chemicals or radiation.a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a mutagen. Mutagen were first discovered by a German scientist Charlotte Auerbach.

14.5 Mutagen

Mutagens are broadly classified in two categories

1. Physical Mutagen
2. Chemical Mutagen

14.5.1 Physical mutagen

Any agent that damages DNA can in principle lead either to the death of that organism or the survivors to mutation. This is true for physical mutagens which includes irradiation. Many types of irradiation have been used to generate mutations. Broadly such types of radiation are divided into ionizing and non-ionizingradiation.

In 1927, Herman Muller demonstrated that mutations in fruit flies could be induced by X-rays. The results of subsequent studies showed that X-rays greatly increase mutation rates in all organisms. The high energies of X-rays, gamma rays, and cosmic raysare all capable of penetrating tissues and damaging DNA. These forms of radiation, called ionizing radiation, dislodge electrons from the atoms that they encounter and changestable molecules into free radicals and reactive ions, which then alter the structures of bases and break phosphodiesterbonds in DNA. Ionizing radiation also frequently results in double-strand breaks in DNA. Attempts to repair these breaks can produce chromosome mutations. The higher energy rays such as X-rays and gamma rays however require expensive apparatus and safety equipment.

Ultraviolet light has less energy than that of ionizing radiation and does not eject electrons and cause ionization but is nevertheless highly mutagenic. The principal effect of UV irradiation with which we are concerned is the production of pyrimidine dimers (commonly referred to as thymine dimers, although the effect can also occur with cytosine). Where two pyrimidine residues are adjacent on the same DNA strand the result of UV irradiation is the creation of covalent links between them. These pyrimidine dimers cannot be replicated and are therefore lethal to the cell unless it is able to repair the damage.The best defence that is mounted against damage by UV irradiation is known as photoreactivation.

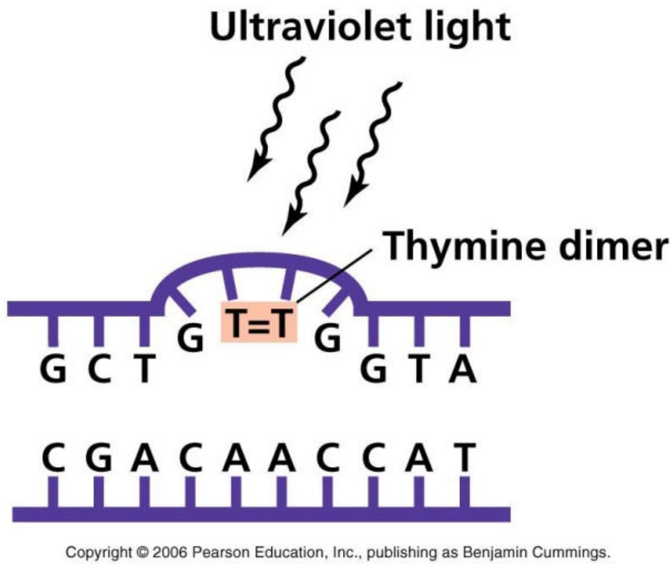


Fig.8 Thymine dimer formation due to UV Light

14.5.2 Chemical mutagen

It includes Base analogues, Alkylating agents, Hydroxylamine, Oxidative reactions, Intercalating agents.

A. Base Analogues: Base analogues are those chemical mutagens which are structurally similar to that of any of the four standard bases of DNA. DNA polymerases cannot distinguish these analogues from the standard bases and due to it if base analogues are present during replication, they may be incorporated into newly synthesized DNA molecules.

Two of the most widely used base analogues are the pyrimidine analogue 5-bromouracil (5BU) and the purine analogue 2-aminopurine (2AP).

The mutagenic mechanisms of both base pair analogues are similar. The 5-bromouracil is incorporated into DNA in place of thymine and acts just like thymine in DNA replication and since it doesn't alter the hydrogen bonding, should induce no mutation. However, it seems that the bromine atom causes 5-bromouracil to tautomerize easily, thus, 5-bromouracil goes from the keto form to the enol form more readily than thymine. In such condition 5-bromouracilmispairs with guanine leading to a transition (T-A => 5BU-A => 5BU-G => C-G), when the enol form of 5-bromouracil pairs with guanine.

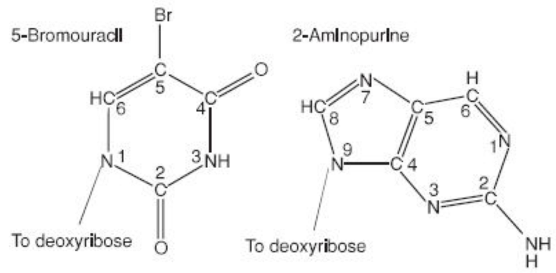


Fig.9 Structure of 5-bromouracil and 2-aminopurine

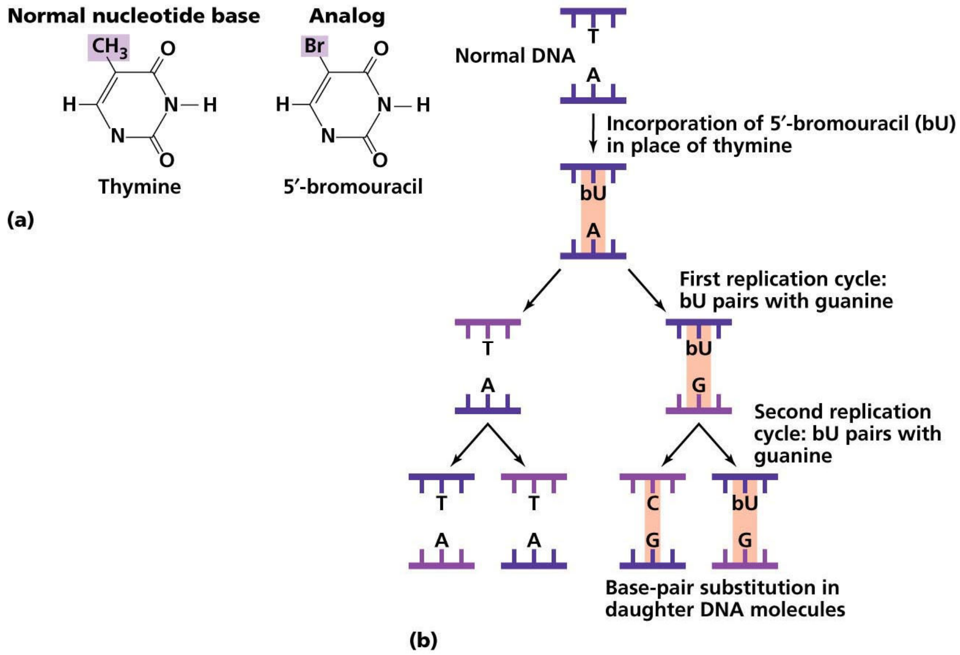


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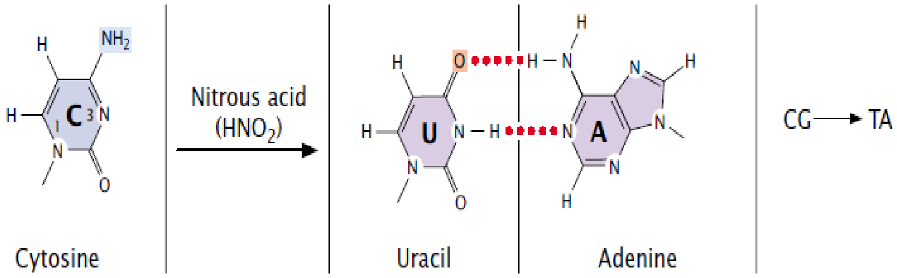
Fig.10 Mutation due to 5-Bromouracil

B. Hydroxylamine: Hydroxylamine is a very specific base modifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine. This conversion increases the frequency of a rare tautomer that pairs with adenine instead of guanine and leads to C-G => T-A transitions. Because hydroxylamine acts only on cytosine, it will *not* generate T-A => C-G transitions.

C. Nitrous acid(HNO_2): Nitrous acid cause deamination of keto group and readily produces transitions by replacing amino groups on nucleotides with keto groups ($–NH_2$ to =O). Nitrous acid deaminates cytosine, result is that cytosine is converted to uracil, which in the next round of replication pairs with adenine, adenine to hypoxanthine which in next replication pair with cytosine, and guanine to xanthine which in next replication basepair with thymine.



D. **Oxidative reactions:** Superoxide radicals, hydrogen peroxide, and hydroxyl radicals are reactive forms of oxygen. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes to DNA. For example, oxidation converts guanine into 8-oxy-7,8-dihydrodeoxyguanine), which frequently mispairs with adenine instead of cytosine, causing a G-C => T-A transversion mutation.

E. **Alkylating agents**: Alkylating agents are chemicals that donate alkyl groups. These agents include methyl (CH_3) and ethyl (CH_3–CH_2) groups, which are added to nucleotide bases by some chemicals. For example, ethylmethanesulfonate (EMS) adds an ethyl group to guanine, producing 6-ethylguanine, which pairs with thymine Thus, EMS produces C-G => T-A transitions. EMS is also capable of adding an ethyl group to thymine, producing 4-ethylthymine, which then pairs with guanine, leading to a TA:CG transition. Because EMS produces both C-G => T-A and T-A => C-G transitions. Mutations produced by EMS can be reversed by additional treatment with EMS. Mustard gas is another alkylating agent.

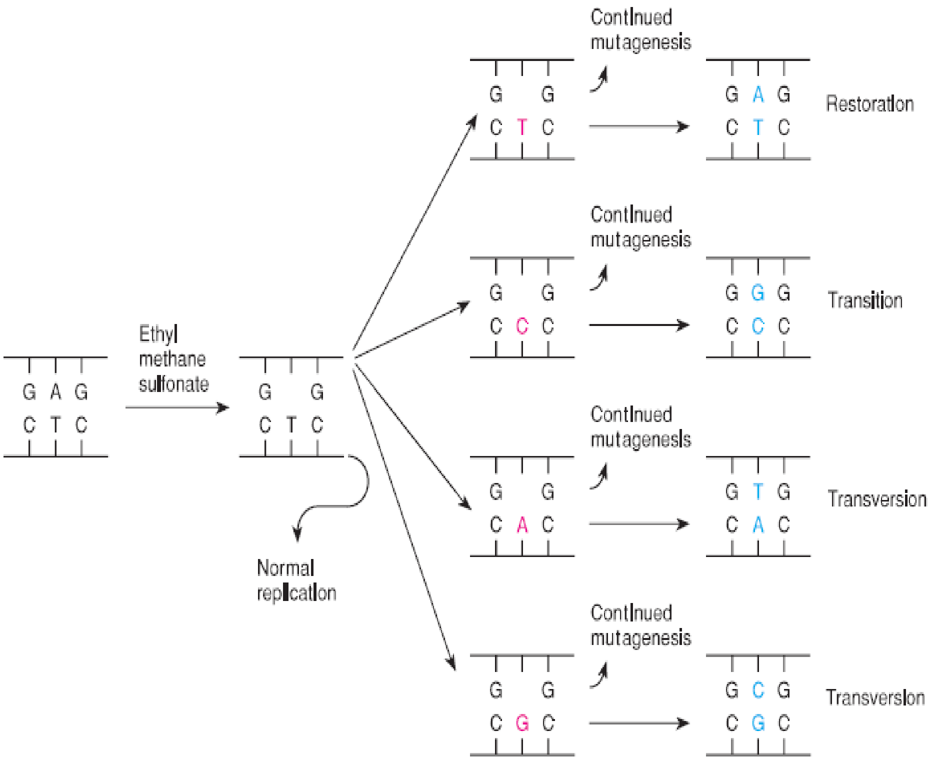
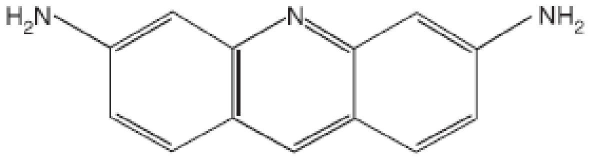
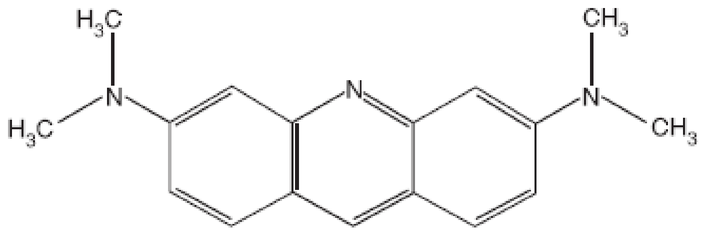


Fig.11 Four possible outcomes due to treatment with alkylating agent

F. **Intercalating agents:** Intercalating agents, produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single- nucleotide insertions and deletions in replication. Example of intercalating dyes are proflavin, acridine orange, ethidium bromide, and dioxin. These dyes are about the same size as a nucleotide. These insertions and deletions frequently produce frameshift mutations.



Proflavin



Acridine orange

14.6 Isolation and identification of mutants

14.6.1 Mutation and selection

The method for selection of a mutant depends on the nature of the Mutation and its consequences for the cell.Mutants on the bases of their properties are divided in three categories:

(1) Mutants that are resistant to antibiotics or to specific bacteriophages, toxic chemicals or any other agents that is usually lethal or inhibitory to the parent cell

(2) Auxotrophs, i.e. mutants that require some additional growth factor, such as an amino acid

(3) Mutants that are unable to use a particular growth substrate (usually a sugar).

Antibiotic resistant mutants are easy to be isolated. The procedure simply involves plating the culture on agar containing the antibiotic at a concentration that will inhibit the parent strain. Very large numbers of bacteria can be used (108–109 cells or 0.1–1 ml of an overnight culture of E. coli), so mutations that occur at a very low frequency can be readily detected.

Auxotrophic mutants cannot usually be selected directly as it is not possible to devise conditions where the mutant will grow but the parent will not. For example, wild type E. coli will grow on a minimal medium consisting of a buffered solution of inorganic salts plus glucose as a carbon/ energy source and ammonium ions as a source of nitrogen. A histidine auxotroph will not grow on this medium but will require the addition of histidine, since it is defective in one of the enzymes of the histidine biosynthetic pathway. The parental strain will also grow quite happily in the presence of histidine. So, although it is not possible to devise a medium on which only the auxotrophic mutant will grow.

14.6.2. Replica Plate Technique

Replica plating is a method of screening to isolate mutant auxotrophs from wild-type bacteria. First, the master plate is imprinted upon a velveteen-covered wood block. Then, using the block+velveteen, replicas of the original plate are made, one on enriched media, and one on minimal media (lacking the factor that the auxotrophs need to grow). When incubated, the plates should grow with colonies at the same places; the spots that are missing on the minimal media correspond to auxotrophic colonies on the enriched media, and thus the auxotrophs can be identified.

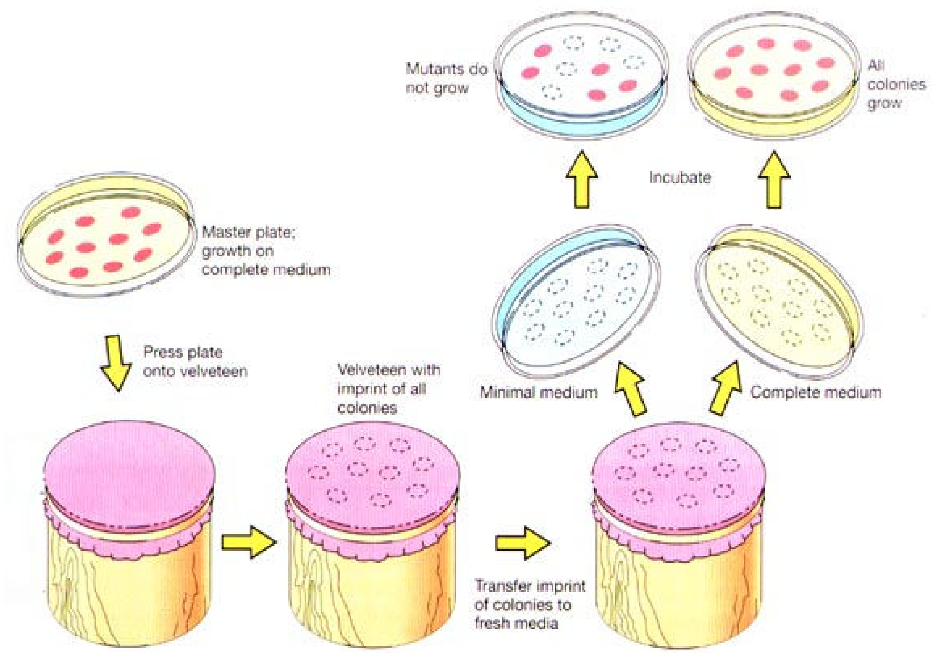


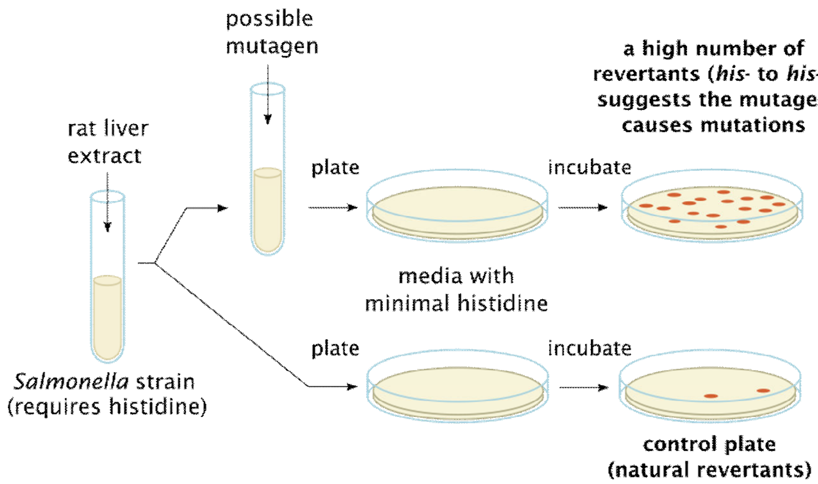
Fig.12 Replica Plate TechniqueImage Source:http://utminers.utep.edu

Because mutations often have detrimental effects, they have been the subject of intense study by geneticists. These studies have included the analysis of reverse mutations, which are often sources of important insight into how mutations cause DNA damage; the development of tests to determine the mutagenic properties of chemical compounds; and the investigation of human populations tragically exposed to high levels of radiation.

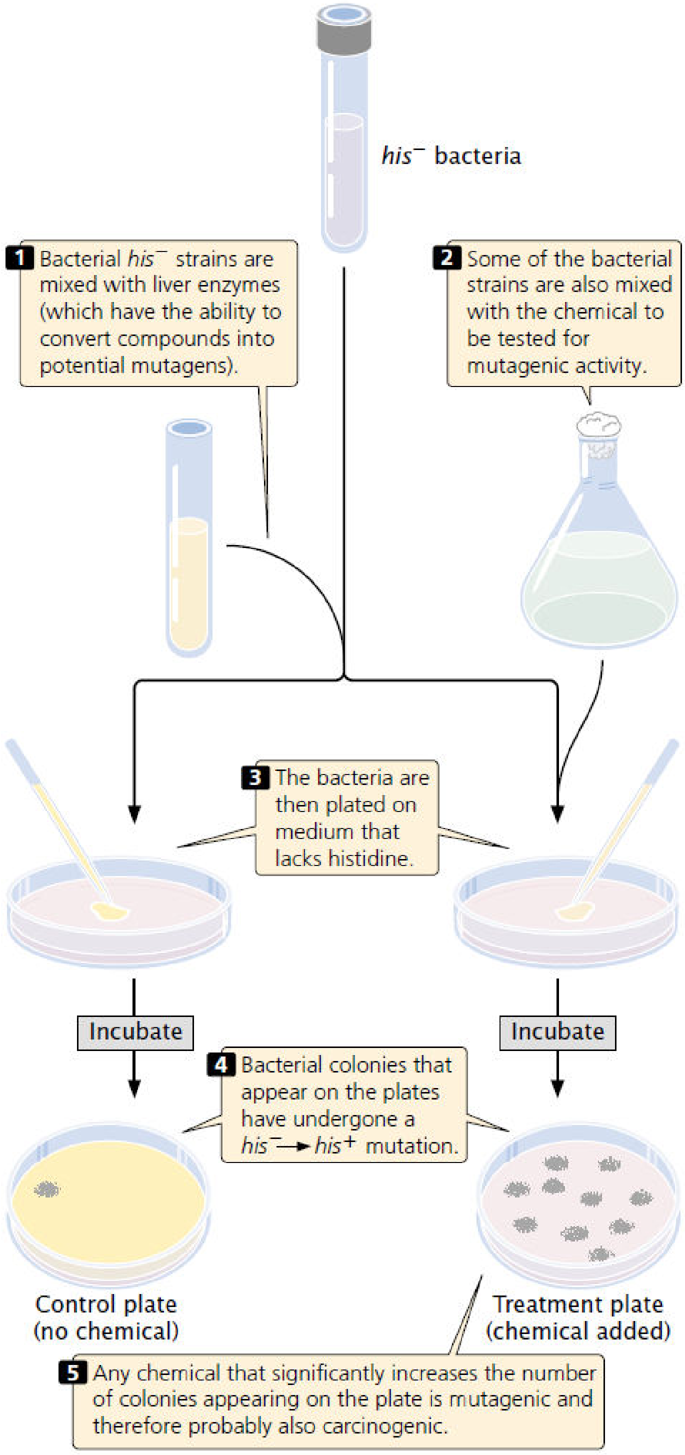
14.6.3 Detecting Mutations with the Ames Test

The Ames test is a widely employed method that uses bacteria to test whether a given chemical can cause mutations in the DNA of the test organism. More formally, it is a biological assay to assess the mutagenic potential of chemical compounds.

A positive test indicates that the chemical is mutagenic and therefore may act as a carcinogen, because cancer is often linked to mutation. The test serves as a quick and convenient assay to estimate the carcinogenic potential of a compound because standard carcinogen assays on mice and rats are time-consuming (taking two to three years to complete) and expensive.



The Ames test uses several strains of the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis. These strains are auxotrophic mutants, i.e. they require histidine for growth, but cannot produce it. The method tests the capability of the tested substance in creating mutations that result in a return to a "prototrophic" state, so that the cells can grow on a histidine-free medium.The bacteria are spread on an agar plate with small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate. When the histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed.



Ames Test used to identify chemical mutagen Image Source: Benjamin-Pierce-Genetics A Conceptual Approach

14.8 Summary

Mutations are heritable changes in genetic information. Gene mutations are genetic alterations that affect a single gene Somatic mutations occur in somatic cells; germ-line mutations occur in cells that give rise to gametes.The simplest type of mutation is a base substitution, a change in a single base pair of DNA.Some mutations occur spontaneously. These mutations include the mispairing of bases in replication and spontaneous depurination and deamination.Base analogs may become incorporated into DNA in replication and pair with the wrong base in subsequent replication events. Alkylating agents and hydroxylamine modify the chemical structure of bases and lead to mutations. Intercalating agents insert into the DNA molecule and cause single-nucleotide additions and deletions. Oxidative reactions alter the chemical structures of bases.Ionizing radiation is mutagenic, altering base structures and breaking phosphodiester bonds. Ultraviolet light produces pyrimidine dimers, which block replication. The analysis of reverse mutations provides information about the molecular nature of the original mutation. The Ames tests uses bacteria to assess the mutagenic potentialof chemical substances.

14.9 Glossary

- **Somatic mutations:** Occur in somatic cells.
- **Base substitution Mutation:** A change in a single base pair of DNA.
- **Insertions:** The addition of nucleotides.
- **Deletions:** The removal of nucleotides.
- **Germline mutations:**Germ-line mutationsarise in cells that ultimately produce gametes.
- **Auxotrophs:** Mutants that require some additional growth factor
- **Transitions:** a purine nucleotide is replaced with a purine nucleotide, or a pyrimidine nucleotide is replaced with a pyrimidine nucleotide.
- **Transversions:** The chemical class of the base changes, i.e. a purine nucleotide is replaced with a pyrimidine nucleotide, or a pyrimidine nucleotide is replaced with a purine nucleotide
- **Missense Mutation -** A base substitution that results in a change of one amino acid for another could also result in an amino acid substitution..
- **Nonsense Mutation-** A base substitution that results in a stop codon replacing a normal amino acid codon.
- **Silent Mutation:** A substitution in which a base pair that is altered cause no change in an amino acid in the protein sequence.
- **Suppressor Mutation:** Asuppressor mutationis a genetic change that hides or suppresses the effect of another mutation.

14.9 Self-Learning Excercise

Section -A (Very Short Answer Type)

1. Define Mutagen.
2. Define Missense Mutation.
3. What is tautomerism
4. What do you mean by Silent Mutation?
5. Mutants that require some additional growth factor are called …………..
6. ------------------ may become incorporated into DNA in replication and pair with the wrong base in subsequent replication events.
7. -----------------------arise in cells that ultimately produce gametes.
8. These reactive forms of oxygen damage DNA and induce ----------------------- by bringing about chemical changes to DNA.

Section -B (Short Answer Type)

1. Write a note on replica Plate technique to isolate mutents.
2. Briefly explain about transition and transversion?
3. What are the causes of mutation?
4. Briefly explain Ames test to isolate mutagenic substances.

Section –C (Long Answer Type)

1. Give a detailed account on various type of gene mutations?
2. Give a detailed account on mutation causing agents?
3. Explain in detail about causes of mutation, mutation rate and mutation frequency?

14.10 References

- Benjamin-Pierce_Genetics_A_Conceptual Approach2nd Edition
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- Robert H. Tamarin- Principals of Genetics 6th Edition

Unit - 15

Alteration of Chromosomes and Recombinations

Structure of the Unit

15.0 Objectives

After going through this unit you will be able to understand -

- What are chromosomal abnormalities?
- How many types of chromosomal abnormalities are found?
- Various types of structural abnormalities in chromosomes
- Importance of aneuploidy and Polyploidy
- How recombination takes place?
- What is the Role of recombination in human genetics

15.1 Introduction

The chromosome set of a species remains relatively stable over long periods of time. However, within populations there can be presence of abnormalities involving the structure or number of chromosomes. Chromosome abnormalities are defined as changes resulting in visible alteration of the chromosomes. Chromosome structure variations result from chromosome breakage and reunion in an abnormal way. Broken chromosomes tend to re-join; if there is more than one break, rejoining occurs at random and not necessarily with the correct ends.The result is structural changes in the chromosomes.

Chromosome breakage can be caused by X-rays, various chemicals and may also occur spontaneously. Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. Their consequences are usually deleterious and give rise to individuals who are unhealthy or sterile, though in rare cases alterations provide new adaptive opportunities that allow evolutionary change to occur.

In fact, the discovery of visible chromosomal differences between species has given rise to the belief that radical restructuring of chromosome architecture has been an important force in evolution.A standard nomenclature has been developed todescribe each of the types of abnormality found in human chromosomes. The current version was developed by the International Standing Committee onHuman Cytogenetic Nomenclature and adopted in 1995 (ISCN, 1995)This lecture will focus on two broad categories of chromosomal abnormalities: numerical (triploidy, trisomy and monosomy) and structural (deletion, duplications and others) aberrations.

15.2 **Types of Chromosomal Abnormalities**:

A chromosomal abnormality can be of two types:

1. **Structural rearrangement**: When the structure ofchromosome is altered,it results in alteration in the structural rearrangement of the chromosome. It can further be divided in various forms such as:
   (1) **Deletions:** A portion of the chromosome is missing or deleted. Well known diseaseexample of such types of rearrangement include Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4 and Jacobsen syndrome, also called the terminal 11q deletion disorder.

(2) **Duplications:** A portion of the chromosome is duplicated, resulting in extra genetic material. Known human disorders include Charcot-Marie-Tooth disease type 1A, which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.

(3) **Translocations:** A portion of one chromosome shifts to a new place and integrates in the genome. There are two main types of translocations:

Reciprocal translocation: Segments from two different chromosomes have been exchanged.

Robertsonian translocation: An entire chromosome has attached to another at the centromere. In humans these only occur with chromosomes 13, 14, 15, 21, and 22.

(4) **Inversions:** A portion of the chromosome turns around 180^{o} and is reinserted into the chromosome, therefore the genetic material is inverted.

(5) **Insertions:** A portion of one chromosome has been deleted from its normal place and inserted into another chromosome.

(6) **Rings**: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material.

(7) **Isochromosome:** Formed by the mirror image copy of a chromosome segment including the centromere.

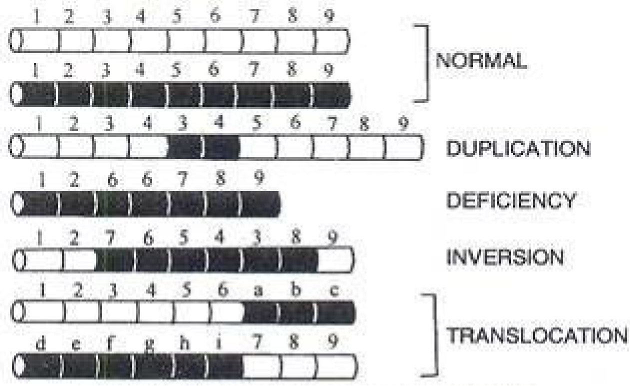
2. **Numerical deviation from the diploid number:** Numerical deviation from the diploid number is called as aneuploidy (an abnormal number of chromosomes). and occurs when an individual either is missing a chromosome from a pair (monosomy) or has more than two chromosomes of a pair (trisomy, tetrasomy, etc.). In humans, an example of a condition caused by a numerical anomaly is Down Syndrome, also known as Trisomy 21 (an individual with Down Syndrome has three copies of chromosome 21, rather than two). Trisomy has been determined to be a function of maternal age.

15.3 Structural abnormalitiesof chromosome:

Structural chromosome abnormalities occur when part of a chromosome is missing, a part of a chromosome is extra, or a part has switched places with another part.Structural abnormalities arise from the incorrect repair of breaks ina chromosome caused by viruses, chemicals, radiations or other events. Although all cells experiences chromosome breakage, in terms of human genetics, only aberrations arising within germ line are important .

The structural aberrations which can ariseare grouped into three major categories:

(1)Deletions/ Deficiency, (2) Inversions (3) Translocations



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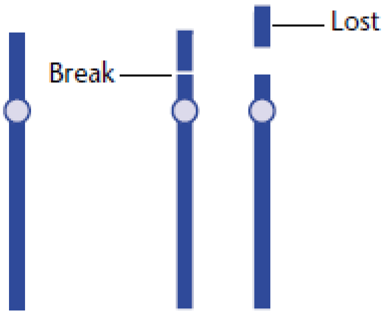
15.3.1 Deletions

The term "deletion" simply means that a part of a chromosome is missing or "deleted." A very small piece of a chromosome can contain many different genes. When genes are missing, there may be errors in the development of a baby, since some of the "instructions" are missing. These abnormalities are due to loss of a chromosome segment. Deletions result in a partial monosomy and are, therefore, unbalanced rearrangements.

Three types of deletions are found:

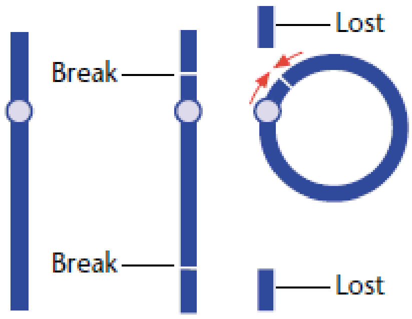
1. **Terminal deletion:** When a single break is found near the end of chromosome it result in terminal deletion. It was first described in maize but otherwise it is not common.There are many terminal deletions in human chromosomes that cause well described syndromes. These require a single break and capping of the broken end with a telomere.

   One of the earliest described and best delineated syndromes due to a terminal deletion is the cri-du-chat syndrome with loss of part of the short arm of chromosome5. This may be due to a very small deletion involving a break at band 5p15.2 or one that includes virtually the entire short arm. The characteristic cat-like cry at birth gives the syndrome its name.



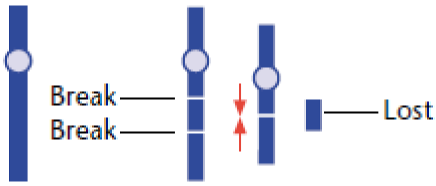
(a) Terminal deletion

2. **Ring chromosomes:** A ring chromosome is formed from two terminal deletions. There is a break in both the short arm and the long arm, with fusion of the ends of the centromeric segment and loss of the two terminal segments. Ring chromosomes represent a form of terminal deletion with the added feature of being mitotically unstable due to mechanical problems during replication. Individuals with ring chromosomes have many of the features of patients with terminal deletions as well as growth retardation.



(b) Ring

3. **Interstitial deletion:** Interstitial deletions require two breaks with loss of the interstitial segment. Chromosome breaks and reunites but the part of it is lost in between. Deletions are detected at the time of homologous pairing. If a part of chromosome is missing then the other chromosome also has to omit it in the form of bulging in order to make synapse. e.g., if a chromosome has 1, 2, 3, 4 genes. The part 2 is missing from one chromosome leaving, 1, 3, 4. The other homologous chromosome at the time of synapse bulge out or form loop atposition 2.



(c) Interstitial deletion

If the missing segment is of physiological importance the individual will not survive. If dominant gene 'A' is missing the recessive allele 'a' may express itself. It is called pseudo dominance.

One interstitial deletion that has been studied extensivelyis a deletion just below the centromere in chromosome 15.This deletion is found in two distinct and clinically verydifferent syndromes, Prader–Willi and Angelman syndromes.Prader–Willi syndrome (PWS) is characterized byintense hyperphagia, obesity, poor muscle tone, hypoplasticgenitalia and moderate intellectual impairment, whileAngelman syndrome (AS) is associated with ataxia,seizures, severe intellectual impairment, delayed or absentspeech, spontaneous outbursts of laughter and characteristicfacial features.

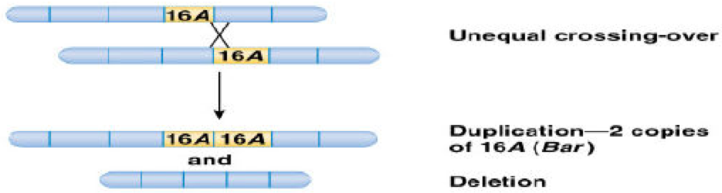
15.3.2 Duplication

Duplication involves attachment of a chromosomal fragment resulting in addition of one ormore genes to a chromosome. Whenever there is duplication in a chromosome, there is a corresponding deletion in another chromosome. Following types of duplications are know:

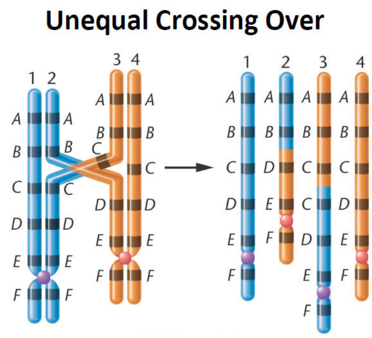
1. **Tandem duplication:** When the extra chromosome segment may be located immediatelyafter the normal segment in precisely the same orientation formsthe tandem duplication.
2. **Reverse tandem duplication:** When the gene sequence in the extra segment of a tandem in the reverse order i.e, inverted , it is known as reverse tandem duplication.
3. **Displaced duplication:**In some cases, the extra segment may be located in the same chromosome but away from the normal segment, it is termed as displaced duplication.
4. **Translocation duplication:** When the additional chromosome segment is located in a non homologous chromosome, it is termed as translocation duplication.

Origin of duplication involves:

1. The Chromosome breakage and reunion of chromosome segment with its homologous chromosome. As a result, one of the two homologous involved in the production of a duplication ends up with a deficiency, while the other has a duplication for the concerned segment.



2. Unequal crossing over between two homologous chromosomes results in deletion in one chromosome and duplication of the same segment in the other.



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15.3.3 Inversion

Inversion arise when a chromosome segment isinverted and turn at 180 degrees, it can be represented as inversion. If a chromosome originally had segments ABCDEFGHIJ, then chromosomeABCDGFEHIJ represents an inversion including segments EFG. In other words It can also b said that two breaks in the same chromosome lead to inversion,in which the middle section is reattached but inthe inverted configuration. An inversion has various properties.

An inversion may break a gene into two parts, with one part moving to a new location and destroying the function of that gene hence individuals with inversions neither loose nor gain any genetic material, there is just the alteration of gene order. Though the chromosome breaks are between genes, the phenotypic effects may arise from the inverted gene order in this type of chromosomal aberration.

When an individual is homozygous for a particular inversion, no special problems arise in meiosis, and the two homologous chromosomes can pair and separate normally. But, when an individual is heterozygous for an inversion, the gene order of the two homologs varies, and the homologous sequences can align and pair only if the two chromosomes form an inversion loop. The presence of an inversion loop in meiosis represent that an inversion have occurred.

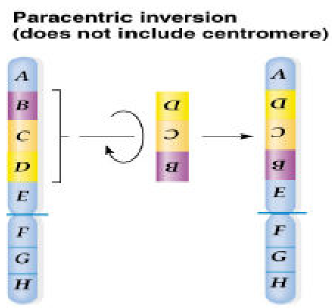
Individuals heterozygous for inversions also exhibit reduced recombination among genes located in the inverted region. The frequency of crossing over within the inversion is not actually diminished but, when crossing over occurs, the outcome is a tendency to produce gametes which are not viable and thus recombinant progeny are not produced.

Inversion can be devided into two types:

1. **Paracentric Inversions:** Inversions that do not include the centromere, are termed paracentric inversions (para meaning "next to").

   A B C D.E F G H IJ

   A B C D.G F E H I J Paracentric inversion



When an individual is heterozygous for an inversion, with one wild-type, unmutated chromosome (ABC•DEFGHIJ) and other inverted chromosome (ABC•DGFEIJ). During prophase I of meiosis, an inversion loop will be formed which will allow the homologous sequences to pair. If a single crossover occurs in the inverted chromosome, the two outer chromatids(which had not participated in crossing over) contain original, non-recombinant gene sequences, andthe two inner chromatids (which did cross over) are highly abnormal: each has two copies of some genes and no copies of others. Furthermore, one of the four chromatids now has two centromeres and is said to be dicentric, the other lacks a centromere and is acentric. In this way during single cross over an unusual structure is formed.

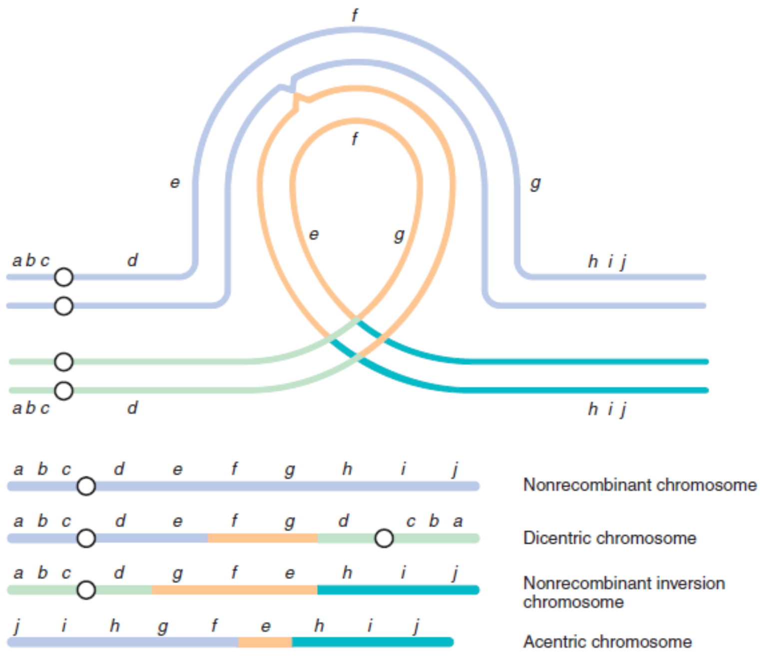


Fig.4 Consequences of a crossover in the loop region of peracentric inversion heterozygote

Image source: Robert H. Tamarin- Principals of Genetics 6th Edition

In anaphase I of meiosis, the centromeres are pulled toward opposite poles and the two homologous chromosomes separate. This stretches the dicentric chromatid across the center of the nucleus and forms a structure called a dicentric bridge.Eventually the dicentric bridge breaks, as the two centromeres are pulled farther apart. The acentric fragment has no centromere. Spindle fibers do not attach to it, and so this fragment does not segregate into a nucleus in meiosis and is usually lost. In the second division of meiosis, the chromatids separate and four gametes are produced. Two of the gametes contain the original, non-recombinant chromosomes (ABC•DEFGHIJ and ABC•DGFEIJ). The other two gametes contain recombinant chromosomes that are missing some genes, which will not produce viable offspring. Thus, when crossing over takes place within a paracentric inversion, no recombinant progeny are formed.

2. **Pericentric Inversions**: Inversions that include the centromere, are termed pericentric inversions (peri meaning“around”).

A B C D.E F G H IJ

A B F E .D C G H I J Pericentric inversion

Recombination is also reduced within a pericentric inversion. No dicentric bridges or acentricfragments are produced, but the recombinant chromosomeshave too many copies of some genes and no copies ofothers; so gametes that receive the recombinant chromosomescannot produce viable progeny.Double crossovers, in whichboth crossovers are on the same two strands (two-strand,double crossovers), result in functional, recombinant chromosomes.Thus, even though the overall rate of recombinationis reduced within an inversion, some viable recombinantprogeny may still be produced through two-strandeddouble crossovers.

Inversion heterozygotes are common in many organisms, including a number of plants, some species of *Drosophila*, mosquitoes, and grasshoppers. Inversions may have an important role in human evolution, G-banding patterns reveal that several human chromosomes differ from those of chimpanzees by only a pericentric inversion.

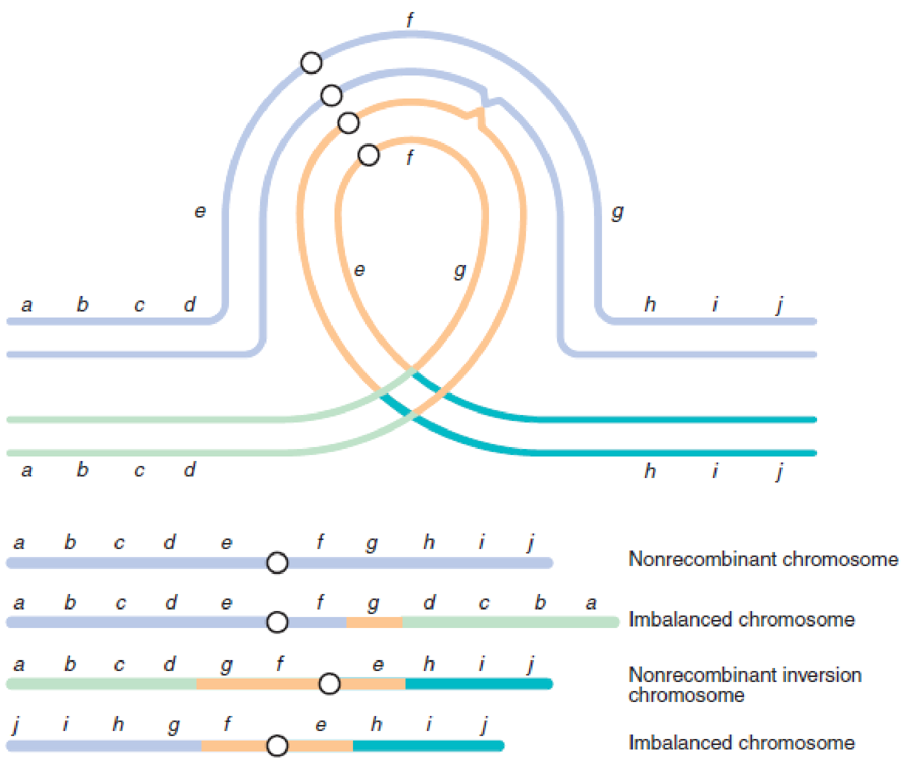


Fig.5 Consequences of a crossover in the loop region of pericentric inversion heterozygote Image source: Robert H. Tamarin- Principals of Genetics 6th Edition.

15.3.4 Translocations

Translocations involve breaks in two different chromosomes along with an exchange of chromosomal segments. Sometimes a segment of a chromosome becomes detached and unites with another non-homologous chromosome. Such inter-chromosomal rearrangement is called translocation.

In humans, there are three major types of translocation:

1. **Simple translocation:** When broken chromosome fragmentbecomes attached to a non-homologous chromosome, it is called as simple translocation. It is a very rare type of translocation.

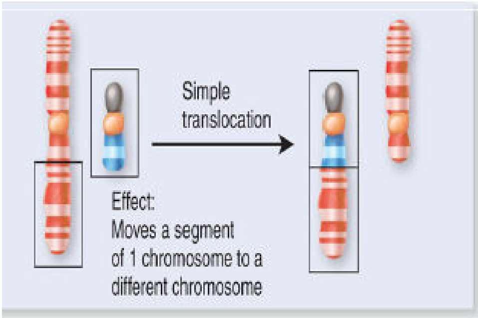


Fig. Simple translocation

2. **Reciprocal translocation:** In this type of translocation there is no visual loss of chromatin. Broken chromosome fragments exchanged between non-homologous chromosome.

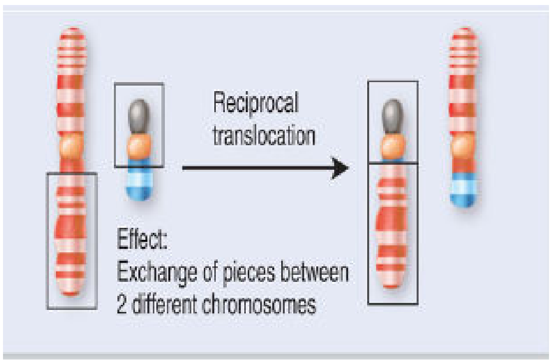


Fig. Reciprocal translocation

3. **Robertsonian translocations:** In this type of translocation the long arms of two acrocentric chromosomes are joined with loss of the two short arms.

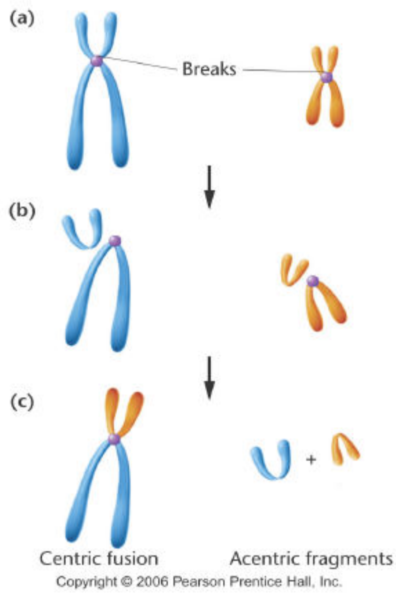


Fig. Robertsonian translocations

Outcome of both reciprocal andRobertsonian translocations is often multiple miscarriages, unbalanced progeny or infertility.

Reciprocal translocations are characterized by an exchangeof chromatin between different chromosomes. A single break occurs in each chromosome, and thenon-centric segments are exchanged without the visibleloss of any chromatin. However, the two newderivative chromosomes may have different morphologydepending on the breakpoints. The carrier of areciprocal translocation generally has no phenotypiceffects due to the rearrangement except for possiblereproductive abnormalities including infertility, spontaneousabortions and abnormal offspring.

Pairing of homologues at meiosis is altered in translocation because thetwo derivative chromosomes and their two normalhomologues pair to form a cross-shaped quadrivalent with each homologous segment pairing with itscounterpart. Pairing and segregation take placeafter DNA replication, so each chromosome consists oftwo chromatids and the quadrivalentconsists of four chromatids.

There are four basic segregation patterns from areciprocal translocation:

Alternate segregation:

Allgametes formed from alternate segregation remain balanced, because both normal chromosomes move to one pole and bothtranslocation chromosomes move to the opposite pole, thus, the chromosomesfound on the diagonals move to the same poles.

Adjacent I segregation: Adjacent nonhomologous centromeres move to the samepole. It will result in an unbalanced chromosomal complementthat will lead to formation of a zygote with partial trisomy of onechromosome and partial monosomy of the other whenfertilized by a normal haploid gamete. The outcome are viable in this segregationpattern .

Adjacent II segregation: Adjacent homologous centromeres move to the same pole and this will result in large amounts of unbalancedchromatin, which is usually incompatible with embryonicsurvival.

Segregation: In this type of translocation three of the four chromosomes move to one pole and onlyone moves to the opposite pole. This type of segregation often occurs when one of thederivative chromosomes is relatively small. Upon fertilizationby a normal gamete, the conceptus will have 47chromosomes

Robertsonian translocations are named for W. R. B. Robertson,who was an insect cytogeneticist and studied numericalchromosome changes in several orthopteran populations(Robertson, 1916). It is a unique types of wholearmtranslocations that result from 'centric fusion' of thelong arms of two acrocentric chromosomes with loss of theshort arms, thus reducing the number of chromosomes by one. The formation of a Robertsoniantranslocation may actually result from breaks in the shortarm, in the long arm or within the centromere of the twochromosomes that form the 'fusion' product. Dependingon the position of the breaks and exchange of chromatinsegments, the resulting derivative chromosome may beeither monocentric or dicentric.

A carrier of a Robertsonian translocation will notgenerally show any physical effects until reproduction. In the case of Robertsoniantranslocations, there are only three chromosomes involved;thus, a trivalent is formed at pachytene. Segregationfrom the trivalent results in the production of six types of gametes:

a. Two of these are normal
b. The other four will produce trisomies or monosomies when fertilized by a normal gamete.

The conceptus may be viable, depending on acrocentric chromosomes involved. Trisomy for chromosomes 13 and 21 are compatible with life, whereas trisomy for the otheracrocentrics (i.e. 14, 15 and 22) will virtually be lost asspontaneous abortions. All the conceptions with monosomieswill also be lost prenatally.

For female carriers of both reciprocal and Robertsoniantranslocations, there is an increased risk for abnormaloffspring as well as an increased risk for miscarriages due toinviable products of conception. The male translocationcarrier has an increased risk for oligospermia or completeazoospermia and often is ascertained through investigationfor infertility.

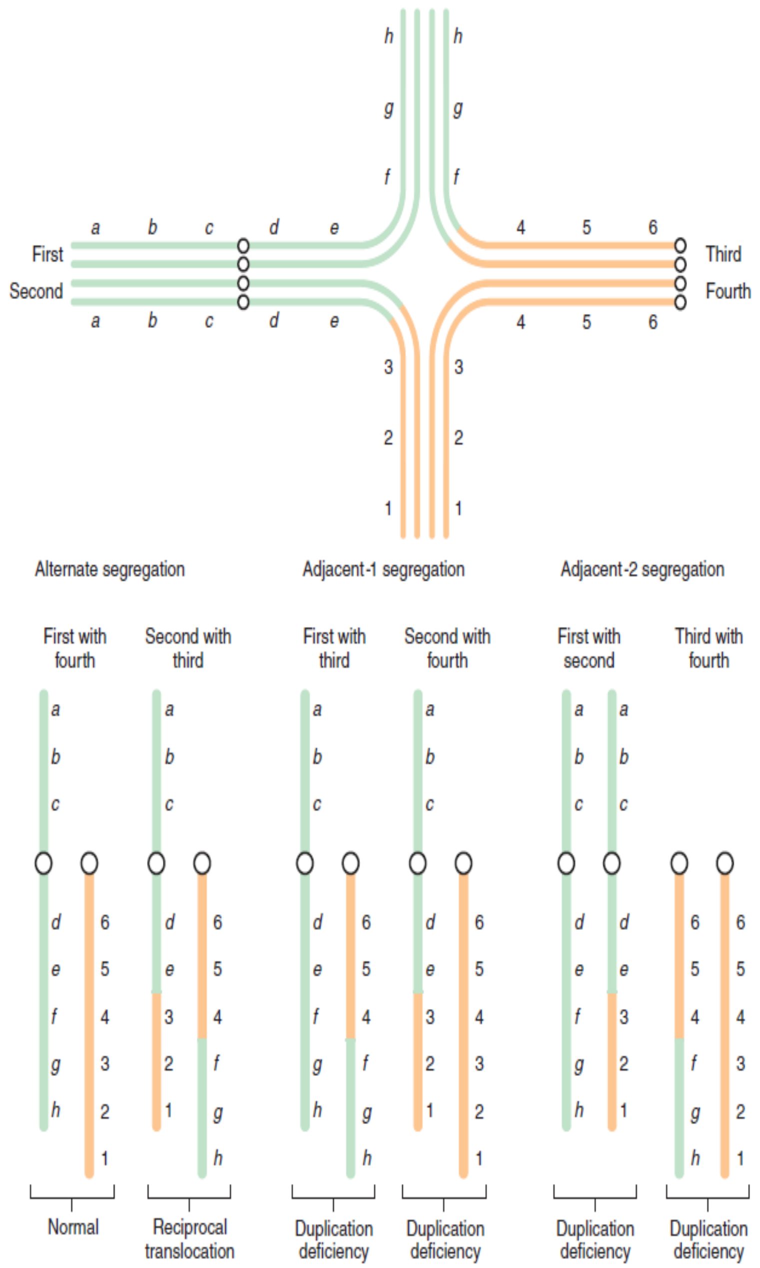


Fig: The possible results of chromatids separation during meiosis in a reciprocal translocation. Image source: Robert H. Tamarin- Principals of Genetics 6th Edition

Numerical changes in Chromosome

Chromosomemutations also include changes in the number of chromosomes.Variations in chromosome number can be classifiedinto two basic types:

Aneuploidy: When the changes are in the number of individual chromosomes, it is called as aneuploidy .

Euploidy: When the changes are in the number of chromosome sets, it is called as polyploidy.

15.3.1 **Aneuploidy**

The aneuploidy arises due to primary and secondry non disjunction. A single chromosome is either added or lost from the compliment

Aneuploidy can arise in several ways:

1. There are chances that chromosome may be lost in the course of mitosis or meiosis. Loss of the centromere prevents the spindle fibers from attachment,due to that the chromosome fails to move to the spindle pole and does not become incorporated into a nucleus after cell division.
2. The loss of small chromosome generated by a Robertsonian translocation during mitosis or meiosis may lead to aneuploidy.
3. Aneuploids may arise when the homologous chromosomes or sister chromatids fail to separate in meiosis or mitosis, through nondisjunction.

Types of Aneuploidy:

There are four types of relatively common aneuploidconditions in diploid individuals: nullisomy, monosomy,trisomy, and tetrasomy.

A. **Nullisomy:** It is the loss of both members of a homologous pair of chromosomes. It can be indicated as 2n - 2, where n refers to the haploid number of chromosomes. Thus,among humans, where 2n = 46 chromosomes, a nullisomic person will have 44 chromosomes.
B. **Monosomy:** When there is the loss of a single chromosome, it is called as monosomy and represented as 2n - 1. A monosomic person will posses 45 chromosomes.
C. **Trisomy:** When there is gain of a single chromosome, it is represented as 2n + 1. A trisomic person will have 47 chromosomes.The gain of a chromosome means that there are threehomologous copies of one chromosome.

D. **Tetrasomy:** When there is the gain of two homologous chromosomes, represented as 2n+ 2. A tetrasomic person has 48 chromosomes. Tetrasomy is not the gain of any two extra chromosomes, but rather the gain of two homologous chromosomes; so there will be fourhomologous copies of a particular chromosome.

More than one aneuploid mutation may occur in the sameindividual. An individual that has an extra copy of two different(nonhomologous) chromosomes is referred to as beingdouble trisomic and represented as 2n+1+1. Similarly, adouble monosomic has two fewer nonhomologous chromosomes (2n- 1- 1), and a double tetrasomic has two extrapairs of homologous chromosomes (2n + 2 + 2).

Effects of Aneuploidy

Aneuploidy usually alters the phenotype drastically. Inmost animals and many plants, aneuploid mutations are lethal, because aneuploidy affects the number of genecopies but not their nucleotide sequences, the effects of aneuploidyare most likely due to abnormal gene dosage.Aneuploidy alters the dosage for some, but not all, genes,disrupting the relative concentrations of gene products andoften interfering with normal development.

One of the first aneuploids to be recognized was a fruit flywith a single X chromosome and no Y chromosome. Another early study of aneuploidy focused onmutants in the Jimson weed, *Datura stramonium*.

Major exception to the relation between gene numberand protein dosage pertains to genes on the mammalian X chromosome. In mammals, X-chromosome inactivationensures that males (who have a single X chromosome) andfemales (who have two X chromosomes) receive the samefunctional dosage for X-linked genes. Extra X chromosomes in mammals are inactivated, so wemight expect that aneuploidy of the sex chromosomeswould be less detrimental in these animals. Y-chromosome aneuploidsare common. Chromosomal abnormalities due to aneuploidy are discussed in detail in another unit.

15.4.2 Polyploidy

Most eukaryotic organisms are diploid (2n) for most of their life cycles and possess two sets of chromosomes. The haploid state is mostly confined to germ cells and it is characterised by the presence of a single set of chromosomes (n). When two haploid germ cells produced by a male and a female parent unite during fertilization a diploid (2n) zygote is formed which give rise to an embryo and than an adult organism. Occasionally, whole sets of chromosomes fail to separate in meiosis or mitosis, leading to polyploidy, the presence of more than two genomic sets of chromosomes. Polyploids include triploids (3n) tetraploids (4n), pentaploids (5n), and even higher numbers of chromosome sets. Polyploidy is common in plants and is a major mechanism by which new plant species have evolved.Polyploidy isless common in animals, but is found in some invertebrates,fishes, salamanders, frogs, and lizards. No naturallyoccurring, viable polyploids are known in birds, but atleast one polyploid mammal (a rat from Argentina) has been reported.

Depending upon the source of the additional chromosome set, polyploids are classified into two types, autopolyploids and allopolyploids which are described below.

(A) **Autopolyploids:**

Autopolyploidy results when accidents of meiosis or mitosis produce extra sets of chromosomes, all derived from a single species. Non-disjunction of all chromosomes in mitosis in an early 2n embryo, for example, doubles the chromosome number and produces an autotetraploid (4n) arise when the additional sets originate from the same species. For example, if the haploid set of a species is designated A, the diploid is AA, triploid AAA, tetraploid AAAA, and so on. Autotetraploids can arise through one of the following ways:

(i) Fertilization of an egg by two or more sperms giving rise to a zygote with three or more sets of chromosomes.

(ii) Normal mitotic division in the diploid zygote in which chromosomes duplicate but cell division fails to occur so that four haploid sets of chromosomes produce a tetraploid nucleus; if the same mitotic error occurs during embryo development it results in some tetraploid tissues in an adult diploid individual

(iii) Failure of meiotic division in germ mother cells so that unreduced diploid gametes are formed instead of haploids.

Although autopolypoids have homologous genomes, yet those having odd numbered setsof chromosomes such as 3n, 5n, 7n, 9n, and so on, show a high degree of sterility.In even-numbered autopolyploids, such as autotetraploids,it is theoretically possible for the homologouschromosomes to form pairs and divide equally. However,this event rarely happens; so these types of autotetraploidsalso produce unbalanced gametes.

The sterility that usually accompanies autopolyploidyhas been exploited in agriculture. Wild diploid bananas(2n = 22), for example, produce seeds that are hard andinedible, but triploid bananas (3n = 33) are sterile, andproduce no seeds, these bananas are sold commercially. Similarly, seedless triploid watermelons have been createdand are now widely sold.

(B) Allopolyploidy:

Allopolyploidy results when the additional set ofchromosomes comes from a different species. For example, suppose a diploid species with twochromosome sets AA crosses naturally or artificially with another species BB. The offspringproduced would be AB which is viable but sterile. This is because during meiosis thechromosomes belonging to the set A do not find homologous partners in chromosomes of B.Due to failure of pairing at anaphase I, the chromosomes move at random towards the twopoles. Thus each gamete gets an unbalanced mixture of A and B chromosomes and results sterility.

There is one way of restoring fertility to a sterile hybrid (AB). If during mitotic divisionin the AB hybrid all the chromosomes are allowed to divide but cell division is inhibited, theresult would be a tetraploid nucleus with two sets of A and two sets of B chromosomes (AABB).Therefore, when meiosis starts, all chromosomes belonging to one set of A will find homologouspartners with the remaining A chromosomes and perfect pairing will result. Similarly, the twosets of B chromosomes will pair with each other and viable fertile gametes would be formed.Such an allopolyploid individual is called an amphidiploid.

George Karpechenko created polyploids experimentallyin the 1920s. Today, as well as in the early twentiethcentury, cabbage (*Brassica oleracea*, 2n= 18) and radishes(*Raphanus sativa*, 2n =18) are agriculturally importantplants, but only the leaves of the cabbage and the roots ofthe radish are normally consumed. Karpechenko wantedto produce a plant that had cabbage leaves and radishroots so that no part of the plant would go to waste.Because both cabbage and radish possess 18 chromosomes,Karpechenko was able to successfully cross them,producing a hybrid with 2n= 18, but, unfortunately, thehybrid was sterile. After several crosses, Karpechenko noticedthat one of his hybrid plants produced a few seeds.When planted, these seeds grew into plants that were viableand fertile. Analysis of their chromosomes revealedthat the plants were allotetraploids, with 2n =36 chromosomes. To Karpechencko's great disappointment, however,the new plants possessed the roots of a cabbage and theleaves of a radish.

The Significance of Polyploidy

The increase in chromosome number in polyploidy is often associated with an increase in cell size, and many polyploids are physically larger than diploids. Breeders have used this effect to produce plants with larger leaves, flowers, fruits, and seeds. The hexaploid (6n= 42) genome of wheat probably contains chromosomes derived from three different wild species. Many other cultivated plants also are polyploidy.

Polyploidy is less common in animals than in plants for several reasons. As discussed, allopolyploids require hybridization between different species, which occurs less frequently in animals than in plants. Animal behavior often prevents interbreeding, and the complexity of animal development causes most interspecific hybrids to be nonviable. Only a few human polyploid babies have been reported, and most died within a few days of birth. Polyploidy, usually triploidy, is seen in about 10% of all spontaneously aborted human fetuses.

Examples of polyploid crop plants

Plant	Type of Polyploidy	Ploidy	Chromosome Number
Potato	Autopolyploid	4n	48
Banana	Autopolyploid	3n	33
Sweet potato	Autopolyploid	6n	90
Peanut	Autopolyploid	4n	40
Tobacco	Allopolyploid	4n	48
Cotton	Allopolyploid	4n	52
Wheat	Allopolyploid	6n	42
Oats	Allopolyploid	6n	42
Sugar cane	Allopolyploid	8n	80
Strawberry	Allopolyploid	8n	56

15.5 Recombination

Recombination is a breakage-and-reunion process. It is the exchange of genetic informationbetween DNA molecules. When the exchange is betweenhomologous DNA molecules, it is called as homologousrecombination. Homologous parts of chromosomes come into appositionand are then reconnected in a crosswise fashion. This process takes place in crossing over, inwhich homologous regions of chromosomes are exchangedand genes are shuffled into new combinations.Recombination is an extremely important geneticprocess because it increases genetic variation.Recombination is also essential for some types of DNA repair.

In 1964, R. Holliday suggested a model of homologous recombinationthat involved simultaneous breaks in onestrand each of the two double helices that were to crossover. In 1983, J. Szostak and colleagues put forth a differentmodel, initiated by a double-strand break in one of thedouble helices. Homologous recombination is a remarkable process: anucleotide strand of one chromosome aligns precisely with anucleotide strand of the homologous chromosome, breaksarise in corresponding regions of different DNA molecules,parts of the molecules precisely change place, and then thepieces are correctly joined. In this complicated series of events,no genetic information is lost or gained.

Homologous recombination requires the formationof heteroduplex DNA consisting of one nucleotidestrand from each of two different chromosomes.In the Holliday model, homologous recombinationis accomplished through a single-strand break inthe DNA, strand displacement, and branchmigration. In the double-strand-break model,recombination is accomplished through doublestrandbreaks, strand displacement, and branchmigration.

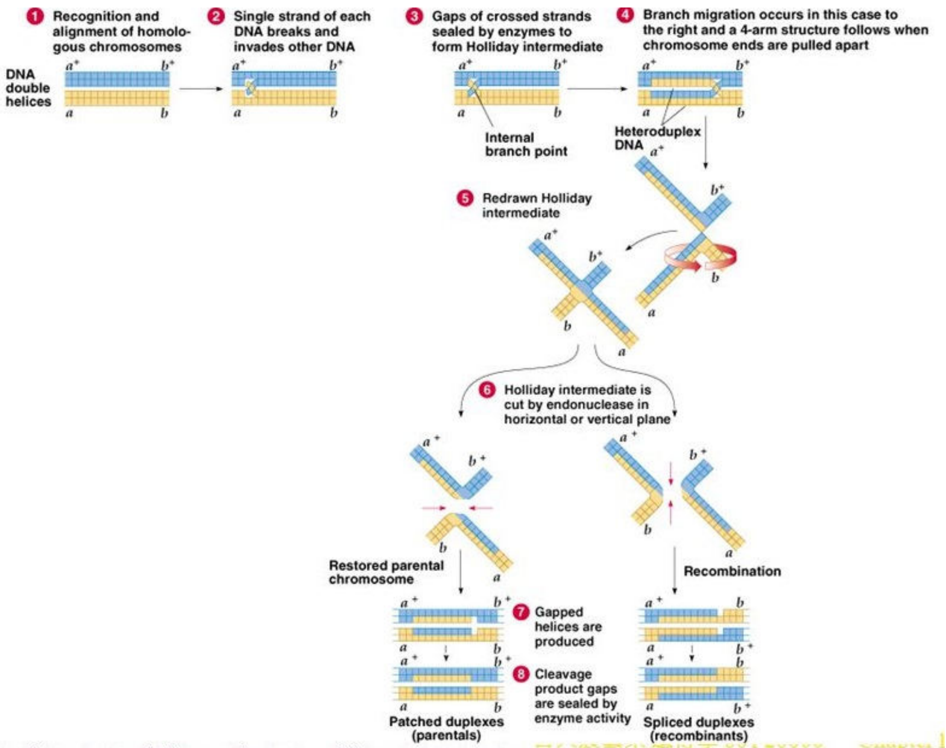
Holliday model, for homologous recombination: It isaccomplished through a single-strand break in each DNA duplex,strand displacement, branch migration, and resolution of asingle Holliday junction. Detail are explained in figure.

The Double-StrandBreak Model: In the Holliday model, recombination starts with singlestrandbreaks at the same positions in two homologousDNA molecules. The double-strand-break model, in contrast,begins with double-strand breaks in one of the twoaligned DNA molecules. Details of this model are explained in figure.

Enzymes Required for Recombination:

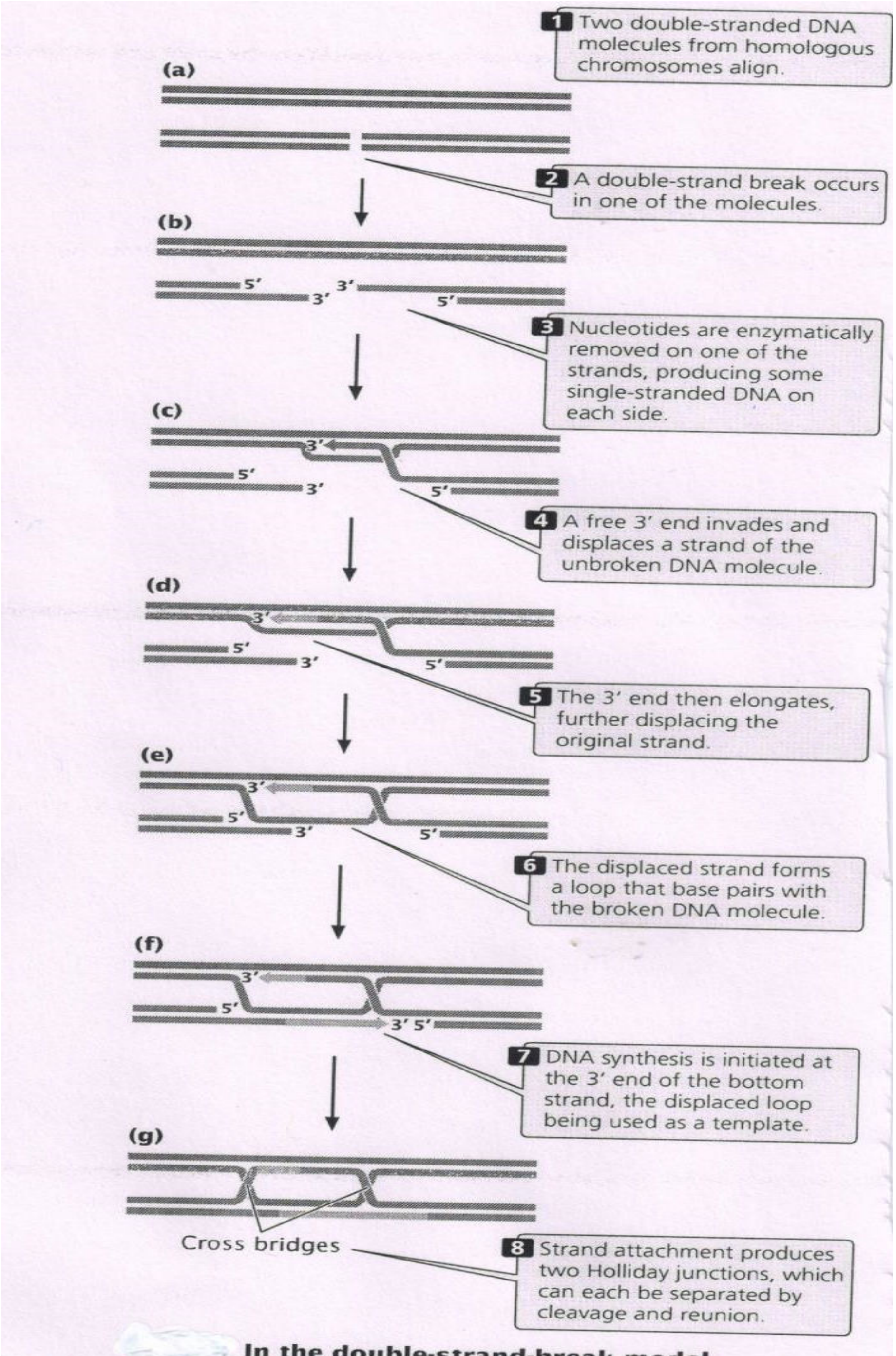
Recombination between DNA molecules requires the unwinding of DNA helices, and then the cleavage of nucleotide strands, strand invasion, and branch migration, followed by further strand cleavage and union to remove cross bridges. Three genes play a pivotal role in *E. coli* recombinationare *recB*, *recC*, and *recD*, which encode threepolypeptides that together form the RecBCD protein. Thisprotein unwinds double-stranded DNA and is capable ofcleaving nucleotide strands. The recA gene encodes theRecA protein that allows a single strand to invade a DNAhelix and the subsequent displacement of one of the original

strands. Thus invasion and displacement are necessaryfor both the single-strand- and the double-strand-breakmodels of homologous recombination.The *ruvA* and *ruvB* genes encode proteins that catalyzebranch migration, and the *ruvC* gene produces a protein,called resolvase, that cleaves Holliday structures. Singlestrand-binding proteins, DNA ligase, DNA polymerases,and DNA gyrase also play roles in various types of recombination,in addition to their functions in DNA replication.



Peter J. Russell, *iGenetics*: Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

Fig. Holliday model for single strand bresk for homologous recombination



In the double-strand-break model, recombination is accomplished through a double-strand break in one DNA duplex, strand displacement, DNA synthesis, and resolution of two Holliday junctions.

Fig.Double-Strand Break Model for genetic recombination Image Source: Benjamin-Pierce-Genetics A Conceptual Approach

15.8 Summary

There are three basic types of chromosomal changes: 1) changes in the structure of chromosomes; 2.) aneuploidyand 3.) polyploidy. Chromosome rearrangements include duplications, deletions,inversions, and translocations.Chromosome duplications arise when a chromosomesegment is doubled. The segment may be adjacent to theoriginal segment or distant from theoriginal segment. Chromosome deletion is the loss of part of a chromosome. In individuals heterozygous for a deletion, one of thechromosomes will loop out during pairing in meiosis. Chromosome inversion is the inversion of achromosome segment. The phenotypiceffects caused by inversions are due to the breaking of genesand their movement to new locations, where they may beinfluenced by different regulatory sequences. Nullisomy refers to the loss of twohomologous chromosomes; monosomy is the loss ofone homologous chromosome; trisomy is the additionof one homologous chromosome; tetrasomy is theaddition of two homologous chromosomes.

Homologous recombination takes place through the exchangeof genetic material between homologous DNA molecules. Inthe Holliday model, homologous recombination begins with

single-strand breaks in both DNA molecules, followed bystrand displacement, branch migration, and Holliday junctionresolution. In the double-strand break model, it begins with adouble-strand-break, followed by strand displacement, DNAsynthesis, and resolution of two Holliday junctions.Homologous recombination in *E. coli* requires a number ofenzymes, including RecA, RecBCD, resolvase, single-strandbindingproteins, ligase, DNA polymerases, and gyrase.

15.7 Glossary

- **Structural rearrangement :** When the chromosome's structure is altered, it results in alteration in the structural rearrangement of the chromosome.
- **Recombination:** Recombination is the exchange of genetic information between DNA molecules.
- **Aneuploidy:** When the changes are in the number of individual chromosomes, it is called as aneuploidy .

- Euploidy: When the changes are in the number of chromosome sets, it is called as polyploidy.
- **Deletions:** A portion of the chromosome is missing or deleted.
- **Duplications:** A portion of the chromosome is duplicated, resulting in extra genetic material.
- **Translocations:** A portion of one chromosome is transferred to another chromosome. There are two main types of translocations:
- **Inversions:** A portion of the chromosome has broken off, turned upside down, and reattached, therefore the genetic material is inverted.
- **Insertions:** A portion of one chromosome has been deleted from its normal place and inserted into another chromosome.

15.8 Self-Learning Excercise

Section -A (Very Short Answer Type)

1. Define deletion.
2. Define duplication.
3. Name type of translocations
4. What do you mean by recombination?
5. ---------------------------is the loss of both members of a homologous pair of chromosomes.
6. Allgametes formed from alternate segregation remain balanced, because both normal chromosomes move to one pole and bothtranslocation chromosomes move to the opposite pole, thus, the chromosomesfound on the diagonals move to the same poles.
7. Inversions that include the centromere, are termed …………………inversions
8. …………………….. Formed by the mirror image copy of a chromosome segment including the centromere.

Section -B (Short Answer Type)

1. Write a note on deletion?.
2. Briefly explain about translocation.
3. Which enzymes are involved in homologous recombination.

4. Briefly explain holiday model of recombination.

Section –C (Long Answer Type)

1. Give a detailed account on structural variation of chromosome?
2. Give a detailed account on numerical variations of chromosome?
3. Explain in detail about recombination.

15.9References

- Benjamin-Pierce-Genetics A Conceptual Approach2nd Edition
- Robert H. Tamarin- Principals of Genetics 6th Edition

Unit -16

Introduction to Microbiology

Structure of the Unit

16.1 Objectives

This unit gives an account for how the micro-organism originate in world their different forms with different morphology and how bacteria different from the eukaryotic organisms. A brief history and introduction about the archaebacteria their sub groups species.

16.2 Introduction

Microbiology often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye - that is the study of microorganisms. Because objects less than about one millimeter in diameter cannot be seen clearly and must be examined with a microscope, microbiology is concerned primarily with organisms and agents. It includes viruses, bacteria, many algae and fungi and protozoa.Yet other members of these groups, particularly some of the algae and fungi, are larger and quite visible. For example, bread molds and filamentous algae are studied by microbiologists, yet are visible to the naked eye. Two bacteria that are visible without a microscope are *Thiomargarita* and *Epulopiscium*, also have been discovered.

Among the great variety of microorganisms that have been identified, bacteria probably have been the most thoroughly studied. The majority of bacteria (singular: bacterium) are single-celled organisms with spherical, rod, or spiral shapes, but a few types form filaments. Most are so small they can be seen with a light microscope only under the highest magnification. Although bacteria are cellular, they do not have a cell nucleus, and they lack the membrane-enclosed intracellular structures found in most other cells. Many bacteria absorb nutrients from their environment, but some make their own nutrients by photosynthesis or other synthetic processes. Some are stationary, and others move about. Bacteria are widely distributed in nature, for example, in aquatic environments and in decaying matter. And some occasionally cause diseases.

16.3 Historical background

Microbiology has had a long, rich history, initially centered in the causes of infectious diseases but now including practical applications of the science. Many individuals have made significant contributions to the development of microbiology.

Early history of microbiology- Historians were unsure who made the first observations of microorganisms, but the microscope was available during the mid 1600s, and a English scientists named Robert Hooke made key observations. He was reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named Anton Van Leeuwenhoek made careful observations of microscopic organisms,which he called **Animalcules**. Until his death in 1723, Van Leeuwenhoek revealed the microscopic world to scientists of the day and was regarded as one of the first to provide accurate descriptions of protozoa, fungi and bacteria.

After Van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of Spontaneous Generation, which stated that microorganism arise from lifeless matter such as beef broth. This theory was disputed by Francesco Redi, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies. An English cleric named John Needham advanced spontaneous generation, but Lazzaro Spallanzani disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

Louis Pasteur and the germ theory- Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine "sick", then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of swan- necked flasks filled with broth (fig.1). He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not) and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulates the germ theory of disease, which states that microorganism are the causes of infectious disease.

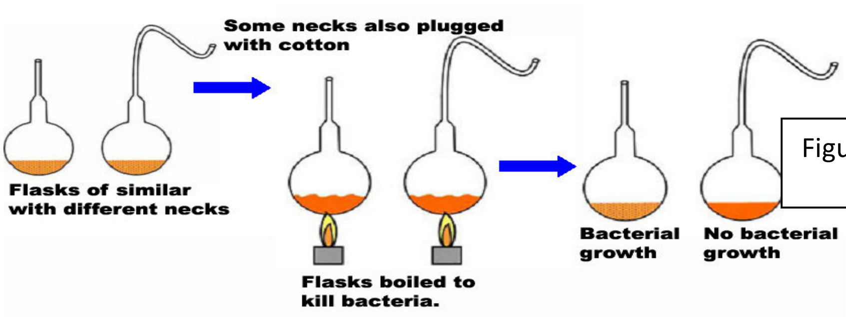


Figure 1

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist Robert Koch provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch's postulates (fig. 2). They provided a set of principles whereby other microorganisms could be related to other diseases.

Koch's postulates

1	The microorganism must be found in all cases of the disease.
2	It must be isolated from the host and grown in pure culture.
3	It must reproduce the original disease when injected into a susceptible host.
4	It must be found in the experimental host so infected.

Figure 2

The development of microbiology- In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a

Golden Age of Microbiology during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial diseases were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after world war II, the antibiotics were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists to see these disease agents. In the 1940s, the electron microscope was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccine in the 1950s and 1960s, such viral diseases as polio, measles, mumps and rubella came under control.

Modern microbiology- Modern microbiology reaches into many fields of human endeavour, including the development of pharmaceutical products, the use of quality control methods in food and dairy product production, the control of disease causing microorganisms in consumable water, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acid, enzymes and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, buttermilk) as well as other fermented foods such as pickles, sauerkraut, breads and alcoholic beverages.

One of the major areas of applied microbiology is biotechnology. In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be re-engineered to increase plant resistance to insects and frost and biotechnology will represent a major application of microorganisms in the next century.

Year	Investigator	Events
1665	Hooke	First observation of cells
1673	Van leeuwenhoek	First observation of live microorganisms
1735	L.Jnnaeus	Nomenclature for organisms
1798	Jenner	First vaccine
1835	Bassi	Silkworm fungus
1840	Semmelweis	Childbirth fever
1653	DeBary	Fungal plant disease
1857	Pasteur	Fermentation
1861	Pasteur	Disproved spontaneous generation
1866	Pasteur	Pasteurization
1867	Lister	Aseptic surgery
1876	Koch	Germ theory of disease
1879	Neisser	*Neisseria gonorrhoeae*
1881	Koch	Pure culture
1882	Koch	*Mycobacterium tuberculosis*
1883	Koch	*Vibrio cholerae*
1884	Metchnikoff	Phagocytosis
1887	Petri	Petri dish
1889	Kitasato	*Clostridium tetani*
1890	Von Behring	Diphtheria antitoxin
1890	Ehrlich	Theory of immunity
1892	Winogradsky	Sulfur cycle

1898	Shiga	*Shigella dysenteriae*
1908	Ehrlich	Syphilis
1910	Chagas	*Trypanosoma cruzi*
1911 (1966 Nobel Prize)	Rous	Tumor-causing virus
1928	Fleming, Chain,Florey	Penicillin
1928	Griffith	Transformation in bacteria
1934	Lancefield	Streptococcal antigens
1935	Stanley, Northrup,Sumner	Crystallized virus
1941	Beadle and Tatum	Relationship between genes and enzymes
1943	Delbruck and luria	Viral infection of bacteria
1944	Avery. Macleod, McCarty	Genetic material is DNA
1946	Lederberg and Tatum	Bacterial conjugation
1953	Watson and Crick	DNA structure
1957	Jacob and Monad	Protein synthesis regulation
1959	Stewart	Viral cause of human cancer
1962	Edelman and Porter	Antibodies
1964	Epstein,Achong, Barr	Epstein-Barr virus as cause of human cancer
1971	Nathans,Smith, Arber	Restriction enzymes (used for recombinant
		DNA technology)
1973	Berg, Boyer, Cohen	Genetic engineering

1975	Dulbecco, Temin, Baltimore	Reverse transcriptase
1978	Mitchell	Chemiosmotic mechanism
1981	Margulis	Origin of eukaryotic cells
1982	Klug	Structure of tobacco mosaic virus
1983	McClintock	Transposons
1988	Deisenhofer, Huber, Michel	Bacterial photosynthesis pigments
1994	Cano	Reported to have cultured 40, million year old
		bacteria.
1997	Rusiner	Prions

16.4 Classification of Bacteria

Bacterial classification-

In science, accurate and standardized names are important. So because of the great diversity of organisms, biologists use the characteristics of different organisms to describe specific forms of life and to identify new ones. The grouping of related organisms together is the basis of classification.

The reasons for classification are:

(1) to establish the criteria for identifying organisms.

(2) to arrange related organisms into groups.

(3) to provide important information on how organisms evolved.

Taxonomy is the science of classification. It provides an orderly basis for the naming of organisms and for placing organisms into a category, or taxon (plural: taxa). Organisms classified in any particular group have certain common characteristics that is, they have unity with respect to these characteristics. For example, *Escherichia coli* cells are rod-shaped and have a Gram-negative cell wall. Members of the same species display variations in size, shape, and other characteristics. Humans vary in height, weight, hair and eye color, and facial features. Certain kinds of bacteria vary somewhat in shape and in their ability to form specific structures, such as endospores. Likewise, nearly all bacteria have a cell wall, but in some the wall is Gram-positive and in others it is Gram-negative.

The eighteenth-century Swedish botanist **Carolus Linnaeus** is credited with founding the science of taxonomy. He originated binomial nomenclature, the system that is still used today to name all living things. In the binomial, or ‘‘two-name,’’ system, the first name designates the genus (plural:genera) of an organism, and its first letter is capitalized. The second name is the specific epithet, and it is not capitalized even when derived from the name of the person who discovered it. Together the genus and specific epithet identify the species to which the organism belongs. Both words are italicized in print but underlined when handwritten.When there is no danger of confusion, the genus name may be abbreviated to a single letter. Thus, *Escherichia coli* is often written *E. Coli*.

Now, there are some general ways in which classification systems can be constructed. Organisms can be grouped together based on overall similarity to form a phenetic system or they can be grouped based on probable evolutionary relationships to produce a phylogenetic system. Computers may be used to analyze data for the production of phenetic classifications. The process is called numerical taxonomy. This section briefly discusses phenetic and phylogenetic classifications, and describes numerical taxonomy.

Phenetic Classification

A good classification should bring order to biological diversity and may even clarify the function of a morphological structure. For example, if motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility. When viewed in this way, the best natural classification system may be a phenetic system, one that groups organisms together based on the mutual similarity of their phenotypic characteristics.

Numerical Taxonomy

The development of computers has made possible the quantitative approach known as numerical taxonomy. Peter H. A. Sneath and Robert Sokal have defined numerical taxonomy as “the grouping by numerical methods of taxonomic units into taxa on the basis of their character states.” Information about the properties of organisms is converted into a form suitable for numerical analysis and then compared by means of a computer. The resulting classification is based on general similarity as judged by comparison of many characteristics, each given equal weight. Example., morphological, biochemical, and physiological characteristics.

After character analysis, an association coefficient that is a function that measures the agreement between characters possessed by two organisms, is calculated for each pair of organisms in the_group. **The simple matching coefficient (S_{SM})**, the most commonly used coefficient in bacteriology, is the proportion of characters that match regardless of whether the attribute is present or absent. Sometimes the **Jaccard coefficient (SJ)** is calculated by ignoring any characters that both organisms lack. Both coefficients increase linearly in value from 0.0 (no matches) to 1.0 (100% matches). The simple matching coefficients, or other association coefficients, are then arranged to form a similarity matrix. This is a matrix in which the rows and columns represent organisms, and each value is an association coefficient measuring the similarity of two different organisms. Organisms with great similarity are grouped together and separated from dissimilar organisms.

The Calculation of Association Coefficients for Two Organisms

In this example, organisms A and B are compared in terms of the characters they do and do not share. The terms in the association coefficient equations are defined as follows:

Organism B

	1	**0**	
Organism A	**a**	**b**	**1**
	c	**d**	**0**

a= number of characters coded as present (1) for both organisms

b and c = numbers of characters differing (1,0 or 0,1) between the two organisms

d= number of characters absent (0) in both organisms

Total number of characters compared = a+b+c+d

The simple matching coefficient (S_{SM}) = $$\frac{a+d}{a+b+c+d}$$

The Jaccard coefficient (S_J) = $$\frac{a}{a+b+c}$$

Phylogenetic Classification

Following the publication in 1859 of **Darwin's** On the Origin of Species, biologists began trying to develop phylogenetic or phyletic classification systems. These are systems based on evolutionary relationships rather than general resemblance (the term phylogeny [Greek phylon, tribe or race, and genesis, generation or origin] refers to the evolutionary development of a species). This has proven difficult for procaryotes and other microorganisms, primarily because of the lack of a good fossil record. The direct comparison of genetic material and gene products such as RNA and proteins overcomes many of these problems.

Genetic Relatedness

This classification is based on the degree of genetic relatedness between organisms. The method is the most objective of all and is based on the most fundamental aspect of organisms, their hereditary material (DNA). In the 1960s the development of that branch of science known as molecular biology provided techniques by which the DNA of one organism could be compared with that of other organisms. At first only crude comparisons could be made. Based on mol% G+C values. It is true that two organisms of the same or similar species that are very closely related will have very similar mol% G+C values and it is also true that two organisms having quite different mol% G+C values are not very closely related. However, it is important to realize that organisms that are completely unrelated may have similar mol% G+C values. Therefore much more precise methods of comparison were needed- namely methods by which the DNA molecules from various organisms could be compared with respect to the sequence of that component nucleotides. This sequence is the most fundamental characteristics of an organism. Modern techniques have now made it possible to make such a comparison.

Bergey's Manual of Systematic Bacteriology

The taxonomic classification scheme for prokaryotes is found in ***Bergey's Manual of Systematic Bacteriology***. In *Bergey's Manual*, prokaryotes are divided into two domains: Bacteria and Archaea. Each domain is divided into phyla. Remember, the classification is based on similarities in nucleotide sequences in rRNA. Classes are divided into orders; orders, into families; families, into genera; and genera, into species. A prokaryotic species is defined somewhat differently than a eukaryotic species, which is a group of closely related organisms that can interbreed. Unlike reproduction in eukaryotic organisms, cell division in bacteria is not directly tied to sexual conjugation, which is infrequent and does not always need to be species-specific. A prokaryotic species therefore, is defined simply as a population of cells with similar characteristics. The members of a bacterial species are essentially indistinguishable from each other but are distinguishable from members of other species, usually on the basis of several features. As you know, bacteria grown at a given time in media are called a **culture**. A pure culture is often a **clone**, that is, a population of cells derived from a single parent cell. All cells in the clone should be **identical**. However, in some cases, pure cultures of the same species are not identical in all ways. Each such group is called a **strain**. Strains are identified by numbers, letters, or names that follow the specific epithet. *Bergey's Manual* provides a reference for identifying bacteria in the laboratory, as well as a classification scheme for bacteria.

In this scheme, bacteria are classified on the basis of many characteristics. **Cell shape**, **nature of multicell aggregates**, **motility**, **formation of spores**, and **reaction to the Gram stain** are important. These morphological features, including the shape and colour of bacterial colonies, are not always constant and can be influenced by environmental conditions. Important in the identification of a genus and species of bacteria are biochemical tests, including the determination of the kinds of nutrients a cell can use, the products of its metabolism, the response to specific chemicals, and the presence of particular characteristic enzymes. Other criteria used for the identification of some types of bacteria might be their antigenic composition, habitat, disease production, and requirement for specific nutrients. Some tests are based on the ultrastructure of the bacteria revealed under the electron microscope by negative staining and preparation of thin sections.

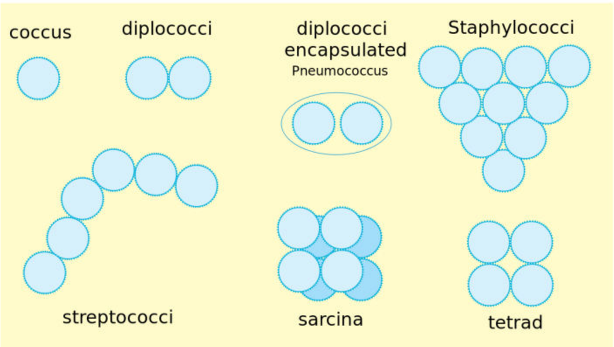
Characters used to classify Bacteria:

- **Classification based on Shape**

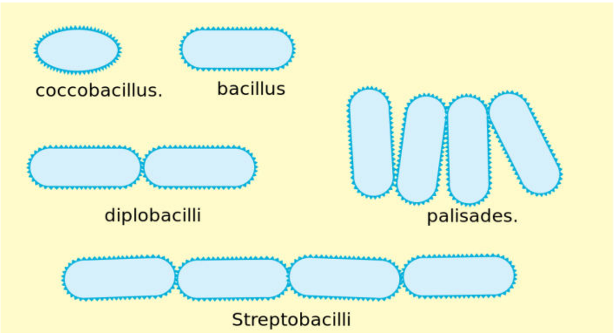
  Bacteria can be classified in four groups based on shape :

  (a) **Cocci**: These are classified according to their mode of cell grouping.

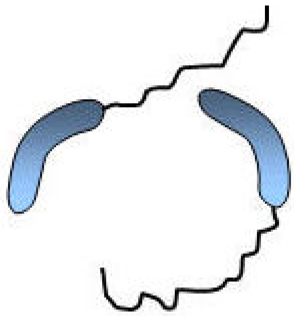
  1. *Staphylococcus* and *Micrococcus*; arranged in irregular clusters. Example., *Staphylococcus aureus*.
  2. *Streptococcus* ; arranged in chains. Example., *Streptococcus pyogenes*.
  3. *Sarcina* ; arranged in cubical packets of eight. Example., *Sarcina lutea*.
  4. *Neisseria*; arranged in pairs and slightly elongated. Example ., *Neisseria meningidits*.
  5. *Veillonella*; very small cocci arranged in clusters and pairs. Example., *Veillonella parvula*.



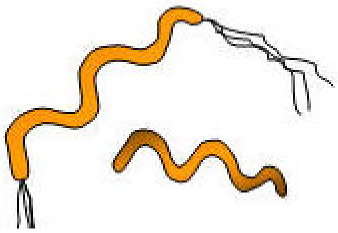
  (b) **Bacilli**: These are rod shaped : Examples., *E.coli*, *Lactobacilli*.



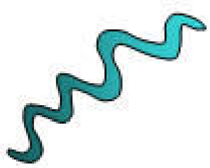
(c) **Vibrio** : These are curved rod shaped cells (comma shaped) they are short, non-flexuous, gram negative, motile, polar flagellum. Example ., *Vibrio cholerae*.



(d) **Spirilla** : These are spirally twisted, non flexuous, gram negative. Example., *S.minus*.



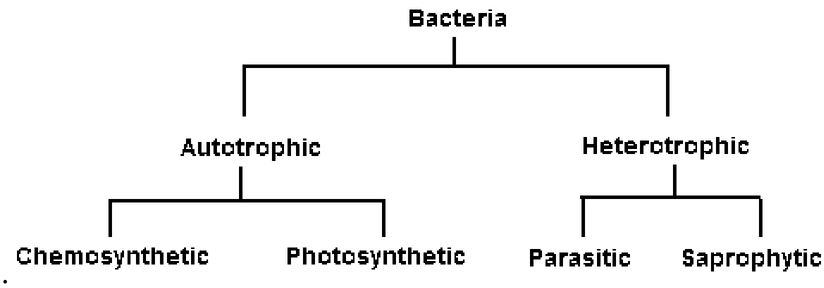
(e) **Spirochaetes** : These are slender, flexuous spiral filament, gram negative, motile without the possession of flagella. The following are the genera under spirochaete *–Borrelia, Treponema, Leptospira.*



Treponema pallidum

- **Classification based on mode of nutrition**

Based on the mode of nutrition bacteria are broadly classified into Autotrophic and Heterotrophic



Autotrophic bacteria -Bacteria which prepare their own food are autotrophic. (Example., *Cyanobacteria*)

Heterotrophic bacteria- Bacteria which are dependent on other organisms for their food are heterotrophic. (Example., *Escherichia coli*).

Autotrophic bacteria can be Chemosynthetic or Photosynthetic.

Chemosynthetic bacteria are those which prepare their food with the help of inorganic substrates.

Photosynthetic bacteria are autotropic bacteria which prepare their own food by the process of photosynthesis.

Cyanobacteria (Blue-green algae) - They have chlorophyll similar to plants and hence they are photosynthetic autotrophs. The marine and terrestrial and they may be unicellular, colonial or filamentous. The colonies are surrounded by gelatinous sheath. They can also fix atmospheric nitrogen. ***Example:*** *Nostoc* and *Anaebena.*

Chemosynthetic autotrophs - these organisms oxidise substances like nitrites, nitrates, ammonia etc. The help in recycling substances like nitrogen, sulphur, iron etc.

Heterotrophic bacteria are those which are dependent on other organism either directly or indirectly for their nutrition. They are most abundant and are important decomposers. Some are helpful in curdling milk, production of antibiotics, in nitrogen fixation and some are pathogens. Heterotrophic bacteria can be parasitic and saprophytic. Parasitic bacteria are those which depend on the host for nutrition and cause harm to the host. Saprophytic bacteria feed on dead and decaying matter.

Symbiotic - it is a type where the bacteria are in mutual relation with other organisms. Symbiosis is of two types mutualism and commensalism. **Mutualism** is where the bacteria and the other organism are benefited due to the relationship. **Commensalism** is a relationship where the bacteria is benefited while the other organism is not affected by the relationship.

- **Classification based on presence and absence of spores**

  Gram positive – spore forming bacteria: These may be either aerobic (*Bacillus*) or anaerobic (*Clostridia*).

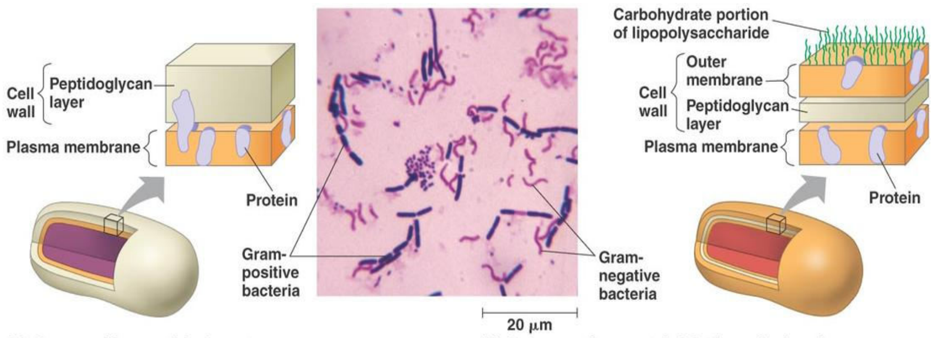
  Gram positive – non spore forming bacteria: These include *Corynebacteria*, these are club shaped slightly curved. *Erysipelothrix*, grows in chain or filaments. *Lactobacilli*, grow in filament but anaerobic. *Listeria*, these have flagella and motile.

- **Classification based on Gram's staining**

  Gram's staining is a test on cell walls developed by Hans Christian Gram. This method helps classifying bacteria into Gram positive bacteria and Gram negative bacteria.

  Gram positive bacteria - The bacteria's cell wall is made up of protein-sugar complex that takes on purple color during gram staining.

  Gram Negative Bacteria - The gram negative bacteria has an extra layer of lipid on the outside of the cell wall and appear pink during the Gram staining procedure.



(a) Gram-positive: peptidoglycan traps crystal violet.

(b) Gram-negative: crystal violet is easily rinsed away, revealing red dye.

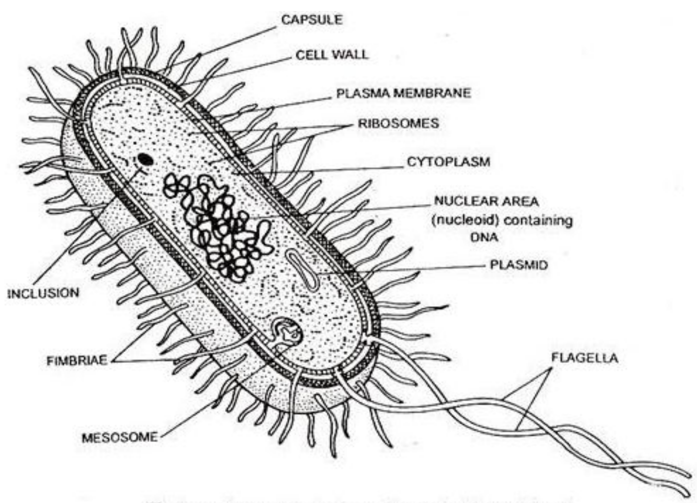
16.5 Difference between Prokaryotic and Eukaryotic Organisms

Characteristic **Genetic Structures:**	**Prokaryotic Cells**	**Eukaryotic Cells**
Genetic material (DNA)	Usually found in single circular chromosome	Typically found in paired chromosomes
Location of genetic information	Nuclear region (nucleoid)	Membrane-enclosed nucleus
Nucleolus	Absent	Present
Histones	Absent	Present
Extrachromosomal DNA	In plasmids	In organelles, such as mitochondria and chloroplasts
Intracellular Structures:		
Mitotic spindle	Absent	Present during cell division
Plasma membrane	Fluid-mosaic structure lacking sterols	Fluid - mosaic structure containing sterols
Internal membranes	Only in photosynthetic organisms	Numerous membrane-enclosed organelles
Endoplasmic reticulum	Absent	Present
Respiratory enzymes	Cell membrane	Mitochondria
Chromatophores	Present in	Absent

	photosynthetic bacteria	
Chloroplasts	Absent	Present in some
Golgi apparatus	Absent	Present
Lysosomes	Absent	Present
Peroxisomes	Absent	Present
Ribosomes	70S	80S in cytoplasm and on endoplasmic reticulum and 70S in organelle like mitochondria and chloroplast.
Cytoskeleton	Absent	Present
Extracellular Structures:		
Cell wall	Peptidoglycan found on most cells	Cellulose, chitin, or both found on plant and fungal cells
External layer	Capsule or slime layer	Pellicle, test, or shell in certain protists
Flagella	When present, consist of fibrils of flagellin	When present consist of complex membrane enclosed structure with 9+2 microtubule arrangement
Cilia	Absent	Present as structures shorter than, but similar to, flagella in some eukaryotic cells

Pili	Present as attachment or conjugation pili in some prokaryotic cells	Absent
Reproductive Process:		
Cell division	Binary fission	Mitosis and/or meiosis
Sexual exchange of genetic material	Not part of reproduction	Meiosis
Sexual or asexual reproduction	Only asexual reproduction	Sexual or asexual reproduction

16.6 General Structure and Features of Bacteria



Electron microscopic structure of a typical bacterial cell.

AN OVERVIEW OF STRUCTURE

Structurally, bacterial cells consist of the following:

1. A cell membrane, usually surrounded by a cell wall and sometimes by an additional outer layer.

2. An internal cytoplasm with ribosomes, a nuclear region, and in some case granules and/or vesicles.

3. A variety of external structures, such as capsules, flagella, and pili.

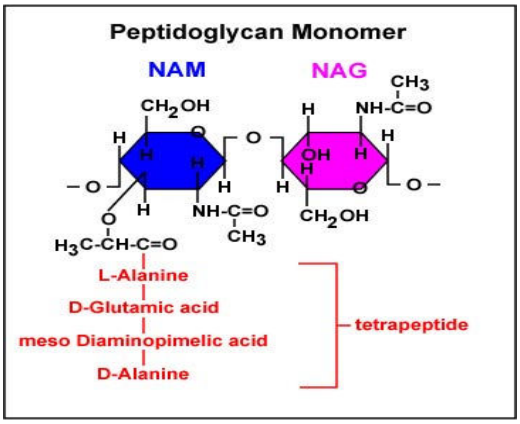
THE CELL WALL:

The semi rigid cell wall lies outside the cell membrane in nearly all bacteria. It performs two important functions. First, it maintains the characteristic shape of the cell. Second, it prevents the cell from bursting when fluids flow into the cell by osmosis.

Components of Cell Walls-

Peptidoglycan. It is also called murein (from murus-wall), is the single most important component of the bacterial cell wall. It forms a supporting net around a bacterium. In the peptidoglycan polymer, molecules of N-acetylglucosamine (gluNAc) alternate with molecules of N-acetylmuramic acid (murNAc). These molecules are cross-linked by tetra-peptides, chains of four amino acids. In most Gram-positive organisms, the third amino acid is lysine; in most Gram-negative organisms, it is diaminopimelic acid.

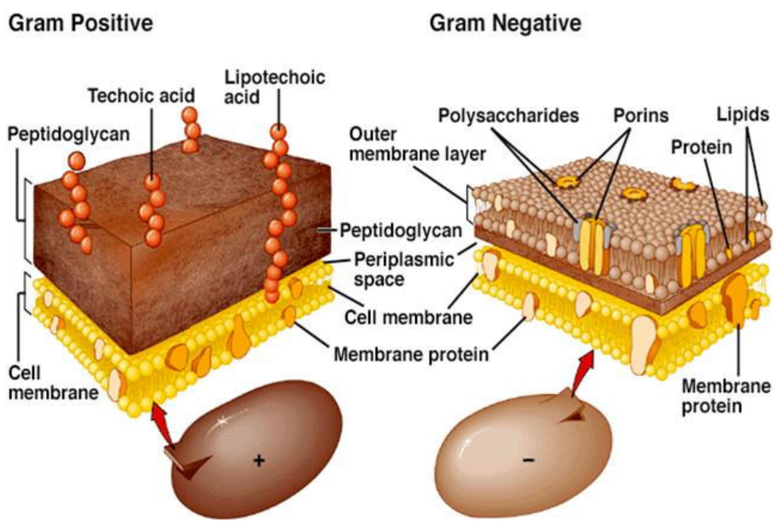
Cell walls of Gram-positive organisms have an additional molecule, teichoic acid. **Teichoic acid**, which consists of glycerol, phosphates, and the sugar alcohol ribitol, occurs in polymers up to 30 units long. These polymers extend beyond the rest of the cell wall, even beyond the capsule in encapsulated bacteria. teichoic acid furnishes attachment sites for bacteriophages (viruses that infect bacteria) and probably serves as a passageway for movement of ions into and out of the cell.



Outer Membrane. The outer membrane, found primarily in Gram-negative bacteria, is a bilayer membrane. It forms the outermost layer of the cell wall and is attached to the peptidoglycan by an almost continuous layer of small lipoprotein molecules (proteins combined with a lipid). The lipoproteins are embedded in the outer membrane and covalently bonded to the peptidoglycan. The outer membrane acts as a coarse sieve and exerts little control over the movement of substances into and out of the cell. Proteins called **porins** form channels through the outer membrane. Gram-negatives are less sensitive to penicillin than are Gram-positives, in part because the outer membrane inhibits entrance of penicillin into the cell.

Lipopolysaccharide (LPS) also called endotoxin is an important part of the outer membrane and can be used to identify Gram-negative bacteria. LPS consists of polysaccharides and lipid A. The lipid A portion is responsible for the toxic properties that make any Gram-negative infection.

Periplasmic Space. The gap between the cell membrane and the cell wall. The gap is most easily observed by electron microscopy of Gram-negative bacteria. It represents a very active area of cell metabolism. Periplasmic spaces are rarely observed in Gram-positive bacteria.



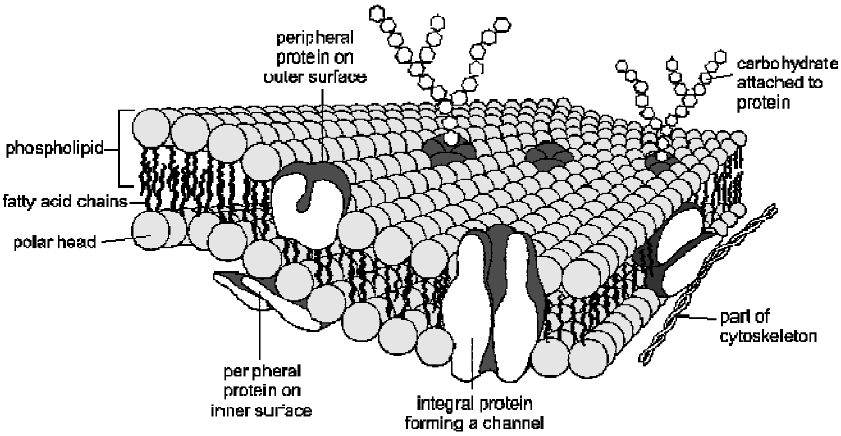
Distinguishing Bacteria by Cell Walls

Certain properties of cell walls produce different staining reactions. Gram-positive, Gram-negative, and acid-fast bacteria can be distinguished on the basis of these reactions.

Characteristics of the Cell Walls of Gram-Positive, Gram-Negative, and Acid-Fast Bacteria

Characteristic	**Gram-Positive Bacteria**	**Gram-Negative Bacteria**	**Acid-Fast Bacteria**
Peptidoglycan	Thick layer	Thin layer	Very small
Teichoic acid	Often present	Absent	Absent
Lipids	Very little present	Lipopolysaccharide	Mycolic acid and other waxes and glycolipids
Outer membrane	Absent	Present	Absent
Periplasmic space	Absent	Present	Absent
Cell shape	Always rigid	Rigid or flexible	Rigid or flexible
Results of enzyme digestion	Protoplast	Spheroplast	Difficult to digest
Sensitivity to dyes and antibiotics	Most sensitive	Moderately sensitive	Least sensitive

THE CELL MEMBRANE



The cell membrane or plasma membrane, is a living membrane that forms the boundary between a cell and its environment. Bacterial cell membranes have the same general structure as the membranes of all other cells. Such membranes, formerly called unit membranes, consist mainly of phospholipids and proteins. The fluid-mosaic model represents the current understanding of the structure of such a membrane. The model's name is derived from the fact that phospholipids in the membrane are in a fluid state and that proteins are dispersed among the lipid molecules in the membrane, forming a mosaic pattern.

Membrane phospholipids form a bilayer, or two adjacent layers. In each layer, the phosphate ends of the lipid molecules extend toward the membrane surface, and the fatty acid ends extend inward. Protein molecules are interspersed among the lipid molecules and some protein molecules extend through the entire membrane and act as carriers or form pores or channels through which materials enter and leave the cell. Exception few bacteria, such as *Mycoplasmas*, have cholesterol molecules in their cell membranes, *Mycoplasmas* lack cell walls; cholesterol molecules add rigidity to their cell membranes.

Cell membranes are dynamic, constantly changing entities. Materials constantly move through pores and through the lipids themselves, although selectively. Some antibiotics a disinfectants kill bacteria by causing their cell membranes to leak.

The main function of the cell membrane is to regulate the movement of materials into and out of a cell by transport mechanisms. In bacteria this membrane also performs some functions carried out by other structures in eukaryotic cells. It synthesizes cell wall components, assists with DNA replication, secretes proteins, carries on respiration, and captures energy as ATP. It also contains bases of appendages called flagella; the actions of the bases cause the flagella to move. Finally, some proteins in the bacterial cell membrane respond to chemical substances in the environment.

INTERNAL STRUCTURE

Bacterial cells typically contain ribosomes, a nucleoid, and a variety of vacuoles within their cytoplasm. Certain bacteria sometimes contain endospores as well.

Cytoplasm The cytoplasm of prokaryotic cells is the semi fluid substance inside the cell membrane. Because these cells typically have only a few clearly defined structures, such as one, two, or three chromosomes and some ribosomes, they consist mainly of cytoplasm. Cytoplasm is about four-fifths water and one-fifth substances dissolved or suspended in the water. These substances include enzymes and other proteins, carbohydrates, lipids, and a variety of inorganic ions. Many chemical reactions, both anabolic and catabolic, occur in the cytoplasm. Unlike eukaryotic cytoplasm, that of prokaryotes does not carry out the movement known as ‘‘streaming.’’

Ribosomes Ribosomes consist of RNA and protein. They are abundant in the cytoplasm of bacteria, often grouped in long chains called polyribosomes. Ribosomes are nearly spherical, stain densely, and contain a large subunit and a small subunit. Ribosomes serve as sites for protein synthesis. The relative sizes of ribosomes and their subunits can be determined by measuring their sedimentation rates-the rates at which they move toward the bottom of a tube when the tube is rapidly spun in an instrument called a centrifuge. Sedimentation rates, which generally vary with molecular size, are expressed in terms of **Svedberg(S) units**. Whole bacterial ribosomes, which are smaller than eukaryotic ribosomes, have a rate of 70S; their subunits have rates of 30S and 50S. Certain antibiotics, such as streptomycin and erythromycin, bind specifically to 70S ribosomes and disrupt bacterial protein synthesis. Because those antibiotics do not affect the larger 80S ribosomes found in eukaryotic cells, they kill bacteria without harming host cells.

Nuclear Region One of the key features differentiating prokaryotic cells from eukaryotic cells is the absence of a nucleus bounded by a nuclear membrane. Instead of a nucleus, bacteria have a nuclear region, or nucleoid. The centrally located nuclear region consists mainly of DNA, but has some RNA and protein associated with it. It was long believed that the DNA was always arranged in one large, circular chromosome. Then in 1989, two circular chromosomes were found in the aquatic photosynthetic bacterium ***Rhodobacter sphaeroides***. ***Agrobacterium rhizogenes*** likewise has two circular chromosomes, but its close relative ***Agrobacterium tumefaciens***, which causes tumors in plants, has one circular chromosome and a second chromosome that is linear. The cholera-causing bacterium, ***Vibrio cholerae***, has two circular chromosomes: one large, the other about one-fourth the size of the first. Both are essential for reproduction. Some bacteria also contain smaller circular molecules of DNA called plasmids.

Internal Membrane Systems Photosynthetic bacteria and *Cyanobacteria* contain internal membrane systems, sometimes known as **chromatophores**. The membranes of the chromatophores, derived from the cell membrane, contain the pigments used to capture light energy for the synthesis of sugars. Nitrifying bacteria, soil organisms that convert nitrogen compounds into forms usable by green plants, also have internal membranes. They house the enzymes used in deriving energy from the oxidation of nitrogen compounds.There are large infoldings of the cell membrane called **mesosomes**.

Inclusions Bacteria can have within their cytoplasm a variety of small bodies collectively referred to as **inclusions**. Some are called granules; others are called vesicles.

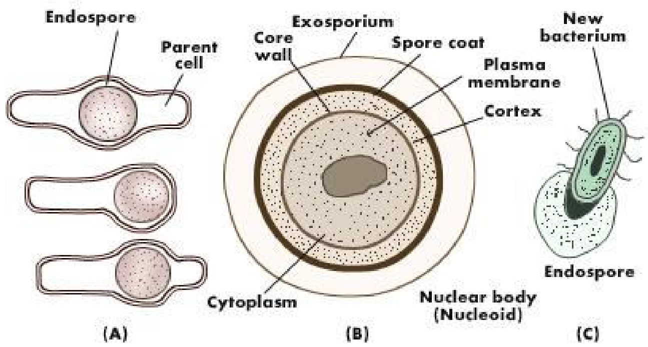
Granules, although not bounded by membrane, contain substances so densely compacted that they do not easily dissolve in cytoplasm. Each granule contains a specific substance, such as glycogen or polyphosphate. In some bacteria, these granules become depleted during starvation. Bacteria that obtain energy by the metabolism of sulfur may contain reserve granules of sulfur in their cytoplastm.

Certain bacteria have specialized membrane-enclosed structures called **vesicles** (or vacuoles). Some aquatic photosynthetic bacteria and *Cyanobacteria* have rigid gas-filled vacuoles. Another type of vesicle, found only in bacteria, contains deposits of poly-b-hydroxybutyrate. These lipid deposits serve as store houses of energy and as sources of carbon for building new molecules.

Endospores Vegetative cells of some bacteria, such as ***Bacillus*** and ***Clostridium***, produce resting stages called endospores. Although bacterial endospores are commonly referred to simply as spores, do not confuse them with fungal spores. A bacterium produces a single endospore, which merely helps that organism survive and is not a means of reproduction. A fungus produces numerous spores, which help the organism survive and provide a means of reproduction.

Endospores, which are formed within cells, contain very little water and are highly resistant to heat, drying, acids, bases, certain disinfectants, and even radiation. The depletion of a nutrient will usually induce a large number of cells to produce spores.

Structurally, an endospore consists of a core, surrounded by a cortex, a spore coat, and in some species a delicately thin layer called the exosporium. The core has an outer core wall, a cell membrane, nuclear region, and other cell components. Unlike vegetative cells, endospores contain dipicolinic acid and a large quantity of calcium ions (Ca^{2+}). These materials, which are probably stored in the core, appear to contribute to the heat resistance of endospores. Endospores are capable of surviving adverse environ-mental conditions for long periods of time, some for over 10,000 years. When conditions become more favorable, endospores germinate, or begin to develop into functional vegetative cells.

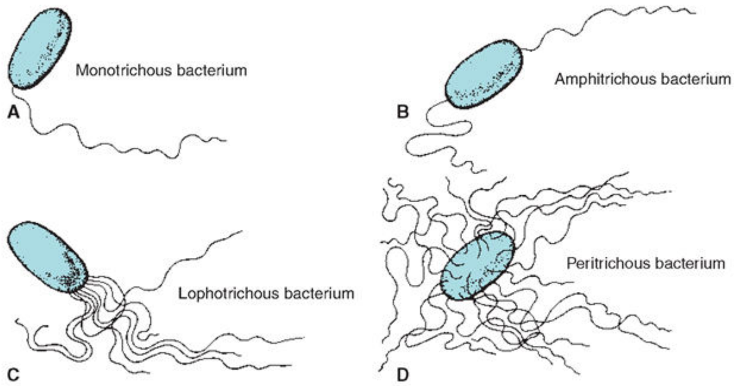


Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

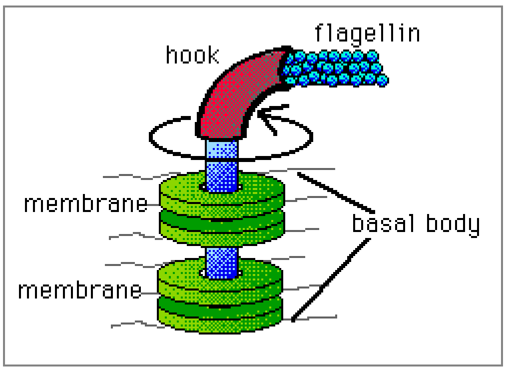
EXTERNAL STRUCTURE

In addition to cell walls, many bacteria have structures that extend beyond or surround the cell wall. Flagella and pili extend from the cell membrane through the cell wall and beyond it. Capsules and slime layers surround the cell wall.

Flagella About half of all known bacteria are motile, or capable of movement. They often move with speed and apparent purpose, and they usually move by means of long, thin, helical appendages called flagella (singular: flagellum). A bacterium can have one flagellum or two or many flagella. Bacteria with a single polar flagellum located at one end, or pole, are said to be **monotrichous** bacteria with two flagella, one at each end, are **amphitrichous** both types are said to bipolar. Bacteria with two or more flagella at one or both ends are **lophotrichous** and those with flagella all over the surface are **peritrichous**. Bacteria without flagella are **atrichous**. Cocci rarely have flagella.



The diameter of a prokaryote's flagellum is about one-tenth that of a eukaryote's flagellum. It is made of protein subunits called **flagellin**. Each flagellum is attached to the cell membrane by a basal region consisting of a protein other than flagellin. The basal region has a hook like structure and a complex basal body. The basal body consists of a central rod or shaft surrounded by a set of rings. Gram-negative bacteria have a pair of rings embedded in the cell membrane and another pair of rings associated with the peptidoglycan and lipopolysaccharide layers of the cell wall. Gram-positive bacteria have one ring embedded in the cell membrane and another in the cell wall.

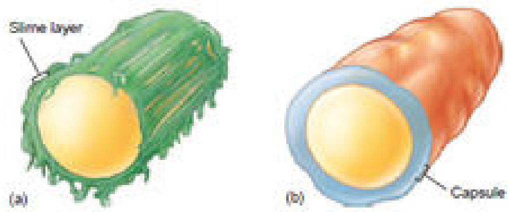


Pili Pili (singular: pilus) are tiny, hollow projections. They are used to attach bacteria to surfaces and are not involved in movement. A pilus is composed of subunits of the protein **pilin**. Bacteria can have two kinds of pili: (1) long conjugation pili, or F pili (also called sex pili), and (2) short attachment pili, or fimbriae (singular:fimbria).

Conjugation Pili. Conjugation pili (or sex pili), found only in certain groups of bacteria, attach two cells and may furnish a pathway for the transfer of the genetic material DNA. This transfer process is called conjugation.

Attachment Pili. Attachment pili,or fimbriae, help bacteria adhere to surfaces, such as cell surfaces and the interface of water and air. They contribute to the pathogenicity of certain bacteria,their ability to produce disease by enhancing colonization (the development of colonies) on the surfaces of the cells of other organisms. For example, some bacteria adhere to red blood cells by attachment pili and cause the blood cells to clump, a process called hemagglutination.

Glycocalyx Glycocalyx is the currently accepted term used to refer to all polysaccharide containing substances found external to the cell wall, from the thickest capsules to the thinnest slime layers.



- **Capsule.** A capsule is a protective structure outside the cell wall of the organism that secretes it. Only certain bacteria are capable of forming capsules, and not all members of a species have capsules. For example, the bacterium that causes anthrax, a disease naturally found mainly in cattle, does not produce a capsule when it grows outside an organism but does when it infects an animal. Capsules typically consist of complex polysaccharide molecules arranged in a loose gel.
- **Slime Layer.** A slime layer is less tightly bound to the cell wall and is usually thinner than a capsule. When present, it protects the cell against drying, helps trap nutrients near the cell, and sometimes binds cells

together. Slime layers allow bacteria to adhere to objects in their environments, such as rock surfaces or the root hairs of plants, so that they can remain near sources of nutrients or oxygen. This ''biofilm'' protects bacteria on the bottom of the layers from environmental or man-made chemicals. Some oral bacteria, for example, adhere by their slime layers and form dental plaque. The slime layer keeps the bacteria in close proximity to the tooth surface, where they can cause dental caries. Plaque is extremely tightly bound to tooth surfaces. If not removed regularly by brushing, it can be removed only by a dental professional in a procedure called scaling.

Archaebacteria

Introduction to the Archaea

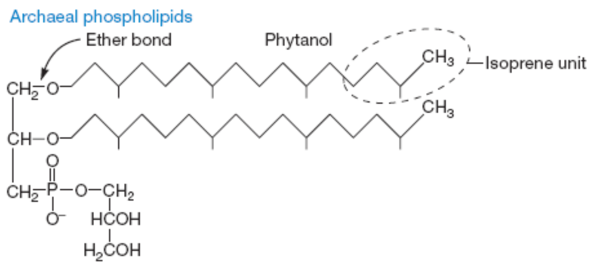
The Archaea [Greek archaios, ancient] are quite diverse, both in morphology and physiology. They can stain either gram positive or gram negative and may be spherical, rod-shaped, spiral, lobed, plate-shaped, irregularly shaped, or pleomorphic. Some are single cells, whereas others form filaments or aggregates. They range in diameter from 0.1 to over 15 um, and some filaments can grow up to 200 um in length. Multiplication may be by binary fission, budding, fragmentation, or other mechanisms. Archaea are just as diverse physiologically. They can be aerobic, facultatively anaerobic, or strictly anaerobic. Nutritionally they range from chemolithoautotrophs to organotrophs. Some are mesophiles; others are hyperthermophiles that can grow above 100°C.

Archaea often are found in extreme aquatic and terrestrial habitats. They are often present in anaerobic, hypersaline, or high-temperature environments. Recently archaea have been discovered in cold environments. It appears that they constitute up to 34% of the procaryotic biomass in coastal Antarctic surface waters. A few are symbionts in animal digestive systems.

Archaeal Cell Walls. The chemistry of archaeal cell walls is also quite different from that of the bacteria. None have the muramic acid and D-amino acids characteristic of bacterial peptidoglycan. Not surprisingly, all archaea resist attack by lysozyme and β-lactam antibiotics such as penicillin. Gram-positive archaea can have a variety of complex polymers in their walls. *Methanobacterium* and some other *Methanogens* have walls containing pseudomurein, a peptidoglycan like polymer that has L-amino acids in its cross-links, **N-acetyltalosaminuronic acid** instead of N-acetylmuramic acid, and β(1→3) glycosidic bonds instead of β(1→4) glycosidic bonds. *Methanosarcina* and *Halococcus* lack pseudomurein and contain complex polysaccharides similar to the chondroitin sulfate of animal connective tissue. Other heteropolysaccharides are also found in gram-positive walls.

Gram-negative archaea have a layer of protein or glycoprotein outside their plasma membrane. The layer may be as thick as 20 to 40 nm. Sometimes there are two layers, a sheath surrounding an electron-dense layer. The chemical content of these walls varies considerably. Some Methanogens (*Methanolobus*), *Halobacterium*, and several extreme thermophiles (*Sulfolobus*, *Thermoproteus*, and *Pyrodictium*) have glycoproteins in their walls. In contrast, other methanogens (*Methanococcus*, *Methanomicrobium*, and *Methanogenium*) and the extreme thermophile *Desulfurococcus* have protein walls.

Archael lipids and membranes. Polar lipids are present in archaeobacterial membranes: phospholipids, sulfolipids and glycolipids. From 7 to 30% of the membrane lipids are nonpolar lipids which usually are derivatives of squalene. These lipids can be combined in various ways to yield membranes of different rigidity and thickness. For example., the C_{20} diethers can be used to make a regular bilayer membrane. A much more rigid monolayer membrane may be constructed of C_{40} tetraether.



Archael genetics and molecular biology. Chromosome of archaeobacteria is a single closed DNA circle, however their genomes are significantly smaller than the normal eubacterium ***E.coli*** DNA has a size of about **2.5 x 10^9** daltons whereas ***Thermoplasma acidophilum*** DNA is about **0.8 x 10^9** daltons and ***Methanobacterium thermoautotrophicum*** DNA is **1.1 x 10^9**daltons. The variation in G+C content is great from about 21 to 68 mol% and is another sign of archaeobacterial diversity.

Halophiles:

The extreme halophiles or halobacteria, class *Halobacteria*, are another major group of archaea, currently with 15 genera in one family, the Halobacteriaceae. They are aerobic chemoheterotrophs with respiratory metabolism and require complex nutrients, usually proteins and amino acids, for growth. Species are either non motile or motile by lophotrichous flagella.

The most obvious distinguishing trait of this family is its absolute dependence on a high concentration of NaCl. These procaryotes require at least **1.5 M NaCl**, and usually have a growth optimum at about **3 to 4 M NaCl**. They will grow at salt concentrations approaching saturation (about 36%). *Halobacterium's* cell wall is so dependent on the presence of NaCl that it disintegrates when the NaCl concentration drops to about 1.5 M. Thus *Halobacteria* only grow in high-salinity habitats such as marine salterns and salt lakes such as the Dead Sea between Israel and Jordan, and the Great Salt Lake in Utah. They also can grow in food products such as salted fish and cause spoilage. *Halobacteria* often have red-to-yellow pigmentation from carotenoids that are probably used as protection against strong sunlight. They can reach such high population levels that salt lakes, salterns, and salted fish actually turn red.

Halobacterium salinarium (*H. halobium*), this procaryote is unusual because it can trap light energy photosynthetically without the presence of chlorophyll. When exposed to low oxygen concentrations, some strains of *Halobacterium* synthesize a modified cell membrane called the purple membrane, which contains the protein bacteriorhodopsin. ATP is produced by a unique type of photosynthesis without the participation of bacteriochlorophyll or chlorophyll.

Methanogens:

Methanogens are strict anaerobes that obtain energy by converting CO_2 , H_2, formate, methanol, acetate, and other compounds to either methane or methane and CO_2. They are autotrophic when growing on H_2 and CO_2. This is the largest group of archaea. There are five orders (*Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*) and 26 genera, which differ greatly in overall shape, 16S rRNA sequence, cell wall chemistry and structure, membrane lipids, and other features. For example, methanogens construct three different types of cell walls.

One of the most unusual methanogenic groups is the class ***Methanopyri***. It has one order, *Methanopyrales*, one family and a single genus, ***Methanopyrus***. This extremely thermophilic rod-shaped methanogen has been isolated from a marine hydrothermal vent.

Methanogens has the ability to produce methane anaerobically, their metabolism is unusual. These procaryotes contain several unique cofactors: tetrahydromethanopterin (H4MPT), methanofuran (MFR), coenzyme M, coenzyme F420, and coenzyme F430. It appears that ATP synthesis is linked with **methanogenesis** by electron transport, proton pumping, and a chemiosmotic mechanism. Some methanogens can live autotrophically by forming acetyl-CoA from two molecules of CO_2 and then converting the acetyl-CoA to pyruvate and other products.

Methanogens thrive in anaerobic environments rich in organic matter: the rumen and intestinal system of animals, freshwater and marine sediments, swamps and marshes, hot springs, anaerobic sludge digesters, and even within anaerobic protozoa. Methanogens often are of ecological significance. The rate of methane production can be so great that bubbles of methane will sometimes rise to the surface of a lake or pond. Rumen methanogens are so active that a cow can belch 200 to 400 liters of methane a day.

Methanogenic archaea are potentially of great practical importance since methane is a clean-burning fuel and an excellent energy source. For many years sewage treatment plants have been using the methane they produce as a source of energy for heat and electricity. Anaerobic digester microbes will degrade particulate wastes such as sewage sludge to H_2, CO_2 and acetate. CO_2-reducing methanogens form CH_4 from CO_2 and H_2, while aceticlastic methanogens cleave acetate to CO_2 and CH_4. future research will greatly increase the efficiency of methane production and make methanogenesis an important source of pollution-free energy.

Methanogenesis also can be an ecological problem. Methane absorbs infrared radiation and thus is a green house gas. There is evidence that atmospheric methane concentrations have been rising over the last 200 years. Methane production may significantly promote future global warming. Recently it has been discovered that methanogens can oxidize Fe^0 and use it to produce methane and energy. This means that methanogens growing around buried or submerged iron pipes and other objects may contribute significantly to iron corrosion.

Hyperthermophilic archaea: This physiological group contains the class ***Thermococci***, with one order, *Thermococcales*. The *Thermococcales* are strictly anaerobic and can reduce sulphur to sulfide. They are motile by flagella and have optimum growth temperatures around 88° to 100°C. The order contains one family and two genera, ***Thermococcus*** and ***Pyrococcus***.

Sulfate-Reducing Archaea- Archaeal sulfate reducers are found in the class *Archaeoglobi* and the order *Archaeoglobales*. This order has only one family and one genus. ***Archaeoglobus*** contains gram-negative, irregular coccoid cells with walls consisting of glycoprotein subunits. It can extract electrons from a variety of electron donors (e.g., H_2, lactate, glucose) and reduce sulfate, sulfite, or thiosulfate to sulfide. Elemental sulfur is not used as an acceptor. ***Archaeoglobusis*** extremely thermophilic (the optimum is about 83°C) and can be isolated from marine hydrothermal vents. The organism is not only unusual in being able to reduce sulfate, unlike other archaea, but it also possesses the methanogen coenzymes F420 and methanopterin.

Thermoplasma: Procaryotes in the class *Thermoplasmata* are thermoacidophiles that lack cell walls. At present, only two genera, ***Thermoplasma*** and ***Picrophilus***, are known. They are sufficiently different from one another to be placed in separate families, *Thermoplasmataceae* and *Picrophilaceae*.

Thermoplasma grows in refuse piles of coal mines. These piles contain large amounts of iron pyrite (FeS), which is oxidized to sulfuric acid by chemolithotrophic bacteria. As a result the piles become very hot and acidic. This is an ideal habitat for *Thermoplasma* since it grows best at 55° to 59°C and pH 1 to 2. Although it lacks a cell wall, its plasma membrane is strengthened by large quantities of diglycerol tetraethers, lipopolysaccharides, and glycoproteins. The organism's DNA is stabilized by association with a special histone like protein that condenses the DNA into particles resembling eucaryotic nucleosomes. At 59°C, *Thermoplasma* takes the form of an irregular filament, whereas at lower temperatures it is spherical. The cells may be flagellated and motile.

Picrophilus is even more unusual than *Thermoplasma*. It originally was isolated from moderately hot solfataric fields in Japan. Although it lacks a regular cell wall, *Picrophilus* has an S-layer outside its plasma membrane. The cells grow as irregularly shaped cocci, around 1 to 1.5µm in diameter and have large cytoplasmic cavities that are not membrane bounded. *Picrophilus* is aerobic and grows between 47° and 65°C with an optimum of 60°C. It is most remarkable in its pH requirements. The organism will grow only below pH 3.5 and has a growth optimum at pH 0.7.

16.8 Summary

Introduction

- Microbiology often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye- that is the study of microorganisms.
- It includes viruses, bacteria, many algae and fungi and protozoa.
- Bacteria are cellular, they do not have a cell nucleus, and they lack the membrane-enclosed intracellular structures found in most other cells.

Historical background

- Microbiology has had a long, rich history, initially centered in the causes of infectious diseases but now including practical applications of the science.
- **Early history of microbiology-** Historians were unsure who made the first observations of microorganisms, but the microscope was available during the mid 1600s, and a English scientists named Robert Hooke made key observations.
- Anton Van Leeuwenhoek made careful observations of microscopic organisms, which he called **Animalcules**.
- Pasteur postulates the germ theory of disease, which states that microorganism are the causes of infectious disease.
- Koch developed four postulates that aided in the definitive establishment of the germ theory of disease.
- Golden Age of Microbiology during which many agents of different infectious diseases were identified.
- Modern microbiology reaches into many fields of human endeavour, including the development of pharmaceutical products, the use of quality control methods in food and dairy product production, the control of disease causing microorganisms in consumable water, and the industrial applications of microorganisms.

Bacterial classification

- **Taxonomy** is the science of classification. It provides an orderly basis for the naming of organisms and for placing organisms into a category, or taxon (plural: taxa).
- **Carolus Linnaeus** is credited with founding the science of taxonomy.
- Organisms can be grouped together based on overall similarity to form a phenetic system or they can be grouped based on probable evolutionary relationships to produce a phylogenetic system.
- Computers may be used to analyze data for the production of phenetic classifications. The process is called numerical taxonomy.
- Classification is based on the degree of genetic relatedness between organisms. The method is the most objective of all and is based on the most fundamental aspect of organisms, their hereditary material (DNA).

Bergey's Manual of Systematic Bacteriology

- In *Bergey's Manual*, prokaryotes are divided into two domains: Bacteria and Archaea. Each domain is divided into phyla.
- Classes are divided into orders; orders, into families; families, into genera; and genera, into species.
- In this scheme, bacteria are classified on the basis of many characteristics. Cell shape, nature of multicell aggregates, motility, formation of spores, and reaction to the Gram stain are important.

Difference between prokaryotes and eukaryotes

- Both prokaryotic cells and eukaryotic cells have membranes that define the bounds of the living cell, and both contain genetic information stored in DNA.
- Prokaryotic cells differ from eukaryotic cells in that they lack a defined nucleus and membrane enclosed organelles (except for a few simple membrane-covered bodies in certain types of prokaryotes).

General Structure and Features of Bacteria

- The semi rigid cell wall lies outside the cell membrane in nearly all bacteria.
- Components of Cell Walls : Peptidoglycan, Outer Membrane, Periplasmic Space.

- Distinguishing Bacteria by Cell Walls.
- Gram-positive, Gram-negative, and acid-fast bacteria can be distinguished on the basis of some reactions.
- Bacterial cell membranes have the same general structure as the membranes of all other cells. Such membranes, formerly called unit membranes, consist mainly of phospholipids and proteins.
- Bacterial cells typically contain ribosomes, a nucleoid, and a variety of vacuoles within their cytoplasm. Certain bacteria sometimes contain endospores as well.
- In addition to cell walls, many bacteria have structures that extend beyond or surround the cell wall. Flagella and pili extend from the cell membrane through the cell wall and beyond it. Capsules and slime layers surround the cell wall.

Introduction to the Archaea

- The Archaea are quite diverse, both in morphology and physiology.
- They can be aerobic, facultatively anaerobic, or strictly anaerobic. Nutritionally they range from chemolithoautotrophs to organotrophs. Some are mesophiles; others are hyperthermophiles that can grow above 100°C.
- Gram-positive archaea can have a variety of complex polymers in their walls.
- Gram-negative archaea have a layer of protein or glycoprotein outside their plasma membrane.
- Polar lipids are present in archaeobacterial membranes: phospholipids, sulfolipids and glycolipids.
- The variation in G+C content is great from about 21 to 68 mol% and is another sign of archaeobacterial diversity.
- Halophiles are aerobic chemoheterotrophs with respiratory metabolism and require complex nutrients, usually proteins and amino acids, for growth. Species are either non motile or motile by lophotrichous flagella.
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- Methanogens has the ability to produce methane anaerobically, their metabolism is unusual.
- Methanogenesis an important source of pollution-free energy.
- Hyperthermophilic archaea, they are motile by flagella and have optimum growth temperatures around 88^{o} to 100°C.
- Sulfate-Reducing Archaea- Archaeal sulfate reducers are found in the class *Archaeoglobi* and the order *Archaeoglobales*.
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Unit- 17

Microbial Growth

Structure of the Unit

17.1 Objectives

After going through this unit you will be able to understand

- The growth of Microbes

- Expression of growth with growth curve
- Measurement of growth and growth yields
- Synchronous growth with Continuous culture
- Growth affected by environmental factors like temperature, acidity, alkalinity, water availability and oxygen

17.2 Microbial Growth: An introduction

Microbial growth is a growth in cell number and their genetic material in a microbial population. Generally this process is completed by binary fission where two daughter cells are produced from a single cell. The growth process is a complex process involving numerous anabolic (synthesis of cell constituents and metabolites) and catabolic (breakdown of cell constituents and metabolites) reactions.

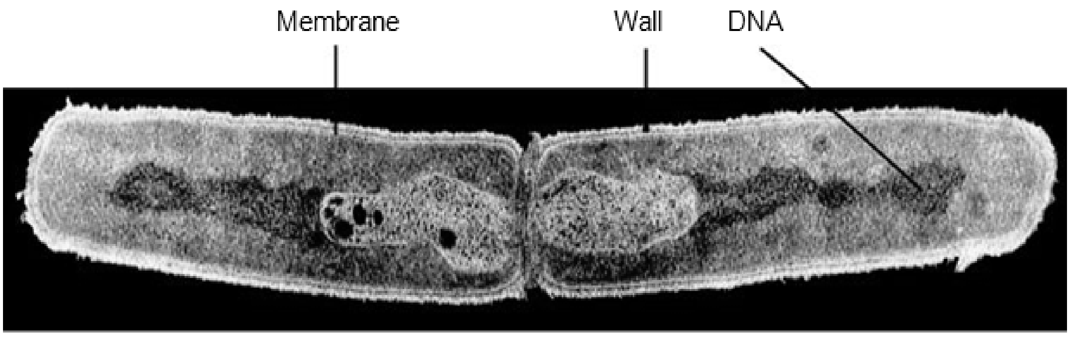

Figure-1: Electron micrograph ofdivision stage in gram-positive bacterium.

From Maier,2009

17.3 Mathematical expression of growth

During the exponential phase, each microorganism is doubled at constant intervals. Thus the population doubles in number during a specific length of time called the generation (doubling) time. The resulting population increase is exponential i.e. logarithmic.In growth, all chemical components of the cell increase with the same speed, a unicellular bacterium increases in cell number exponentially.

These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

So: $N_t = N_0 \times 2^n$

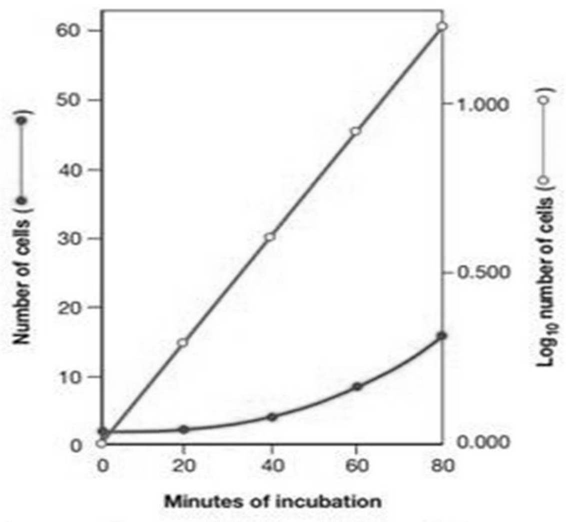

Figure-2: Exponential Microbial Growth curve.(Source Prescott, 2002)

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\text{Log}\, N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the mean growth rate constant(k).Growth rate is one important way of expressing the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it. The duration of exponential phase in cultures depends upon the size of the inoculums, the growth rate and the capacity of the medium and culturing conditions to support algal growth. This is the number of generations per unit time, oftenexpressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log \mathrm{N_t} - \log \mathrm{N_0}}{0.301\mathrm{t}}$$

The time it takes a population to double in size—that is, the meangeneration (doubling) time (g)—can now be calculated. If thepopulation doubles ($t = g$), then

$N_{t=}\quad 2N_0$

Substitute $2N_0$ into the mean growth rate equation and solve for k:

$$k = \frac{\log(2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (g) can be determined directly from a semi logarithmic plot of the growth data and the growth rate constant calculated from the g value.

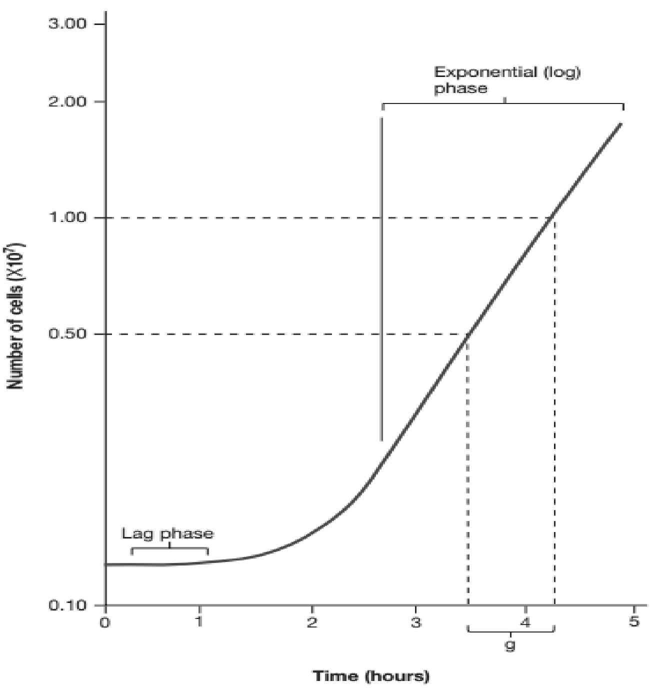

Figure-3: Generation Time Determination. (Source Prescott, 2002)

The generation time also may be calculated directly from the previous equations. Itcan vary basis on the species of microorganism and environmental conditions. The range relates from less than 10 minutes (0.17 hours) to several days. Generation times in nature are usually much longer than in culture.

17.4 Growth curve

The growth curve is a curve that explains the changes in size of growth or bacterial mass ofmicrobial population over time in a sterile nutrient medium culture. The bacteria are cultured in and incubated at the optimum temperature for growth. Several distinct growth phases can be observed within a growth curve. These include the lag phase, the exponential or log phase, the stationary phase, and the death phase. Each of these phases represents a distinct period of growth that is associated with typical physiological changes in the cell culture.

The Lag Phase: The Lag Phase is first phase to be observed under batch conditions in which the growth rate is essentially zero. When inoculums are placed into fresh medium, growth begins after a period of time called the lag phase. The lag phase is defined to transition to the exponential phase after the initial population has doubled. The lag phase involves a time requirement for induction of specific messenger RNA (mRNA) and protein synthesis to meet new culture requirements. The lag phase usually can take time in minutes to several hours. The length of the lag phase can be controlled to some extent because it is dependent on the type of medium as well as on the initial inoculums size.

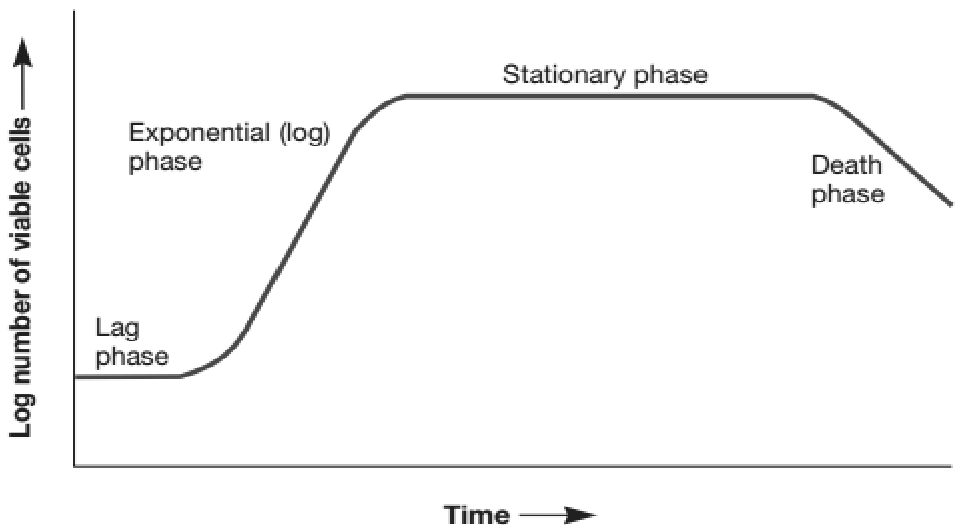

Figure-4: AMicrobial Growth Curve in a Closed System.(Source Prescott, 2002)

The Exponential Phase: The second phase of growth is the exponential phase in a culture system. The exponential phase is characterized by a period of the exponential growth—the most rapid growth possible under the conditions present in the batch system. During exponential growth the rate of increase of cells in the culture is proportional to the number of cells present at any particular time.

The Stationary Phase: The third phase of growth is the stationary phase. The stationary phase in a batch culture can be defined as a state of no net growth. Although there is no net growth in stationary phase, cells still grow and divide with simply balanced by an equal number of cells dying. The certain phase is occurred due to the carbon and energy source or an essential nutrient becomes completely used up. When a carbon source is used up it does not necessarily mean that all growth stops. This is because dying cells can lyses and provides a source of nutrients. The phase also known as endogenous metabolism that occurs throughout the growth cycle, but it can be best observed during stationary phase when growth is measured in terms of oxygen uptake or evolution of carbon dioxide.

The Death Phase: The final phase of the growth curve is the death phase, which is characterized by a net loss of cultivable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are lost than are gained so there is a net loss of viable cells. The death phase is often exponential, although the rate of cell death is usually slower than the rate of growth during the exponential phase.

17.5 Measurement of growth and growth yields

Population growth is measured by following the changes in the number of cells or in the level of cellular component such as protein, nucleic acids, or the dry weight of the cells.

Microscopic Counts: A total count of microbial numbers can be achieved using a microscope to observe and enumerate the cells present in a culture or natural sample. The method is simple, but the results can be unreliable. The most common total count method is the microscopic cell count. Microscopic counts can be done on either sample dried on slides or on samples in liquid. Dried samples can be stained to increase contrast between cells and their background. With liquid samples, specially designed counting chambers are used. In such a counting chamber, a grid with squares of known area is marked on the surface of a glass slide. When the cover slip is placed on the chamber, each square on the grid has a precisely measured volume. The number of cells per unit area of grid can be counted under the microscope, giving a measure of the number of cells per small chamber volume. The number of cells per milliliter of suspension is calculated by employing a conversion factor based on the volume of the chamber sample. Microscopic counting is a quick and easy way of estimating microbial cell number. However, it has several limitations such as:

- Without special staining techniques, dead cells cannot be distinguished from live cells.
- Small cells are difficult to see under the microscope, and some cells are inevitably missed.
- Precision is difficult to achieve.
- A phase-contrast microscope is required if the sample is not stained.
- Motile cells must be immobilized before counting.

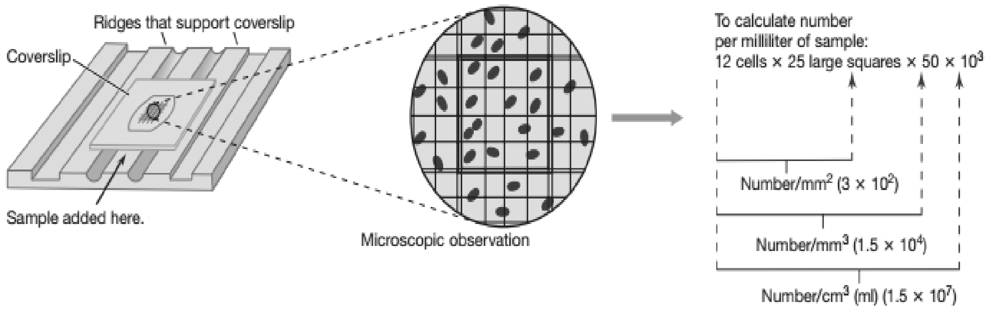

Figure-5: Direct microscopic counting procedure using the petroff–Hausser counting chamber. (Source: Madigan et al., 2012)

Flow-Cytometry: It is a method to enumerating cells in liquid samples that employs a laser beam and complex electronics to count individual cells. Flow cytometry is rarely used for the routine counting of microbial cells, but has applications in the medical field for counting and differentiating blood cells and other cell types from clinical samples. It has also been used in microbial ecology to separate different types of cells for isolation purposes.

Viable Counts: A viable cell is one that is able to divide and form offspring, and in most cell-counting situations that can be identify via use of viable counting method. In method, the numbers of cells count in a sample capable of forming colonies on a suitable agar medium. So the viable count is also called a plate count. The assumption made in the viable counting procedure is that each viable cell can grow and divide to yield one colony. Thus, colony numbers are a reflection of cell numbers. There are at least two ways of performing a plate count: the spread-plate method and the pour-plate method. In the spread-plate method, a volume (usually 0.1 ml or less) of an appropriately diluted culture is spread over the surface of an agar plate using a sterile glass spreader. The plate is then incubated until colonies appear, and the number of colonies is counted.

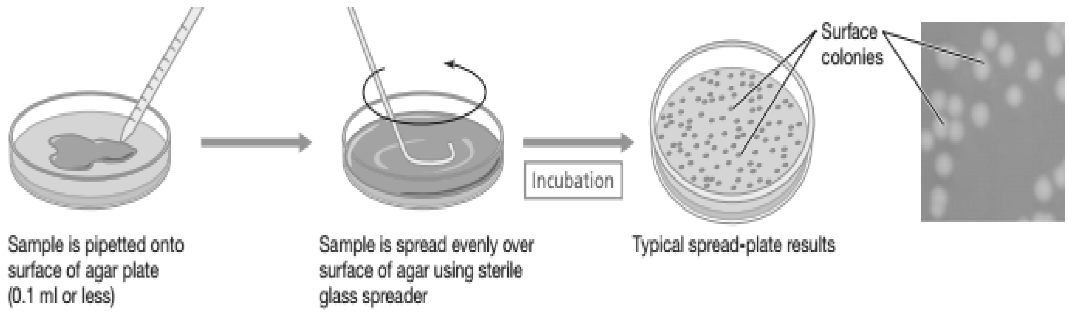

Figure-6: Spread-plate method for the viable count. Source Madigan et al., 2012

In the pour-plate method, a known volume (usually 0.1–1.0 ml) of culture is pipetted into a sterile Petri plate. Melted agar medium, tempered to just about gelling temperature, is then added and mixed well by gently swirling the plate on the bench top. Because the sample is mixed with the molten agar medium, a larger volume can be used than with the spread plate. However, with this method the organism to be counted must be able to withstand brief exposure to the temperature of molten agar (+45–50 C). Here, colonies form throughout the medium and not just on the agar surface as in the spread-plate method. The plate must therefore be examined closely to make sure all colonies are counted.

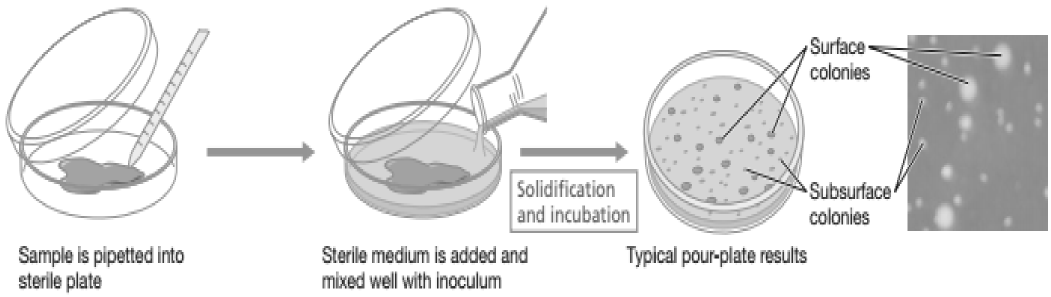

Figure-7: The Pour-plate method for the viable count. (Source Madigan et al., 2012)

Targeted Plate Counts: The use of highly selective culture media and growth conditions in viable counting procedures allows one to target only particular species, or in some cases even a single species, in a mixed population of microorganisms present in the sample. In practical applications such as in the food industry, viable counting on both complex and selective media allows for both quantitative and qualitative assessments of the microorganisms present in a food product. That is, with a single sample one medium may be employed for a total count and a second medium used to target a particular organism, such as a specific pathogen. Targeted counting is common in wastewater and other water analyses.

Turbidimetric Methods: During exponential growth, the cellsreproduce in proportion to the increase in cell numbers. Similarly, cells are objects instead of dissolved substances, cells scatter light, and a rapid and quite useful method of estimating cell numbers based on this property is turbidity. A suspension of cells looks cloudy (turbid) to the eye because cells scatter light passing through the suspension. Cell mass is proportional to cell number, turbidity can be used as a measure of cell numbers and can also be used to follow an increase in cell numbers of a growing culture. It is measured with a spectrophotometer, an instrument that passes light through a cell suspension and measures the unscattered light that emerges in the cell suspension. Commonly used wavelengths for bacterial turbidity measurements include 480 nm (blue), 540 nm (green), 600 nm (orange), and 660 nm (red). The unit of turbidity is called optical density (OD) at the wavelength specified.

17.6 Synchronous growth curve

Synchronous growth of a microbial population is that during which all cells of the population are physiologically identical and in the same stage of cell division cycle at a given time. Synchronous growth helps studying particular stages or the cell division cycle and their interrelations. In most of the cultures the stages of growth and cell division cycle are completely random and thus it becomes difficult to understand the properties during the course of division cycle using such cultures. To solve certain problem, the microbiologists have developed synchronous culture techniques to find synchronous growth of microbial population. A synchronous culture can be obtained either by manipulating environmental conditions such as by repeatedly changing the temperature or by adding fresh nutrients to cultures as soon as they enter the stationary phase, or by physical separation of cells by centrifugation or filtration. An excellent and most widely used method to obtain synchronous cultures is the Helmstetter-Cummings Technique in which an unsynchronized microbial culture is filtered through cellulose nitrate membrane filter. Hence, all cells in the effluent are newly formed and are, therefore, at the same stage of growth and division cycle. The effluent thus represents a synchronous culture.

Measurements made on synchronized cultures are equivalent to measurements made on individual cells. A number of techniques have been devised to obtain bacterial populations at the same stage in the cell cycle. Some techniques involve manipulation of environmental parameters which induces the population to start or stop grow that the same point in the cell cycle, while others are physical methods for selection of cells that have just completed the process of binary fission. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division.

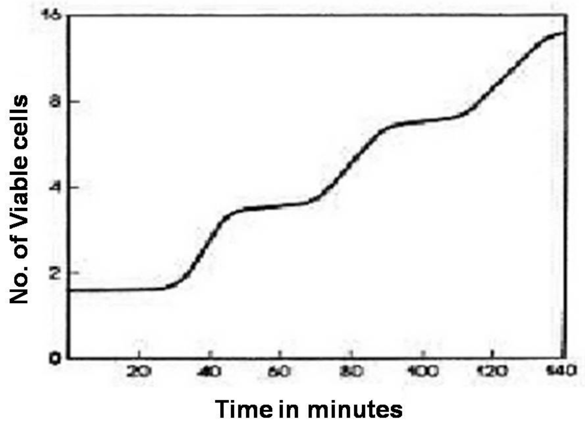

Figure-9: The synchronous growth of a bacterial population.

Synchronous cultures rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time. The degree of synchrony that exists in a cell culture can be assessed by following changes in the culture's mitotic index, which is a measure of the fraction of cells undergoing division at any instant and is given by the formula:

IM =NM/N

Where IM is the mitotic index, NM is the total number of cells visibly in mitosis, and N is the total number of cells present. During the exponential expansion of a cell culture, IM remains constant; however, during synchronous growth, IM changes from some minimal value (ideally 0) to some maximum value during a short time interval.

17.7 Continuous culture

The continuous culture is an open system that is designed for long-term operation with a continuous feed of influent solution that contains nutrients and substrate, as well as a continuous drain of effluent solution that contains cells, metabolites, waste products, and any unused nutrients and substrate. The vessel that is used as a growth container in continuous culture is called a bioreactor or a chemostat. In a bioreactor, we can control the flow rate, maintain a constant substrate concentration, as well as provide continuous control of pH, temperature, and oxygen levels. Bioreactor cultures are also being used to aid in study of the functional genomics of growth, nutrient limitation, and stress responses at the whole-organism level. The advantage of the bioreactor in such studies lies in the constant removal of metabolites, including signal molecules or secondary metabolites that may mask or subtly alter physiological conditions under batch culture conditions. Dilution rate and influent substrate concentration are the two parameters controlled in a bioreactor to study microbial growth or to optimize metabolite production. In the bioreactor the number of cells produced will be directly proportional to the amount of substrate provided. Bioreactor can also produce microbial products more efficiently than batch fermentations. This is because a bioreactor can essentially hold a culture in the exponential phase of growth for extended periods. Despite these advantages, bioreactors are not yet widely used to produce commercial products because it is often difficult to maintain sterile conditions over time.

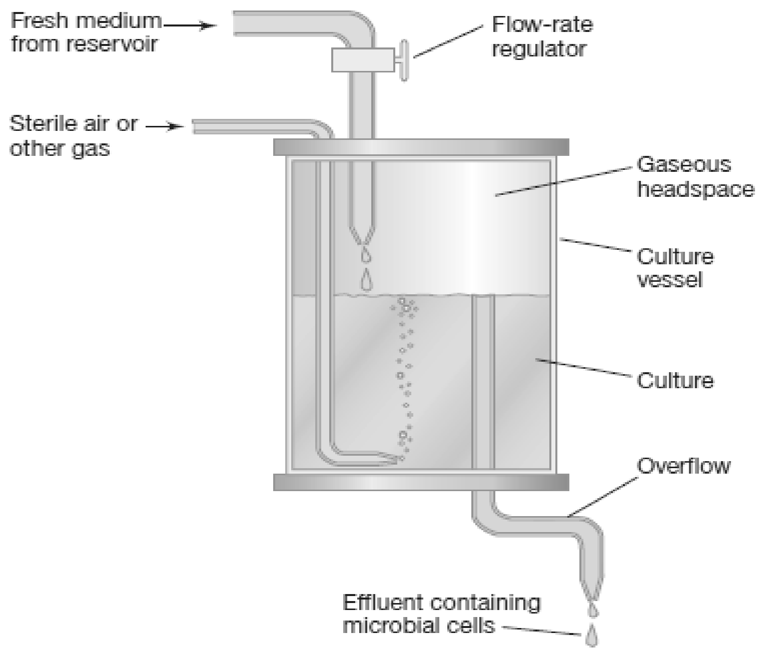

Figure-10: Schematic for a continuous culture chamber or Bioreactor.

(Source Madigan et al., 2012)

In a bioreactor, the growth medium undergoes constant dilution with respect to cells due to the influx of nutrient solution. The combination of growth and dilution within the bioreactor will ultimately determine growth. Thus, in a bioreactor, the change in biomass with time is

$$\frac{dX}{dt} = \mu X - DX \qquad Eq\text{-}1$$

Where X is the cell mass (mass/volume), μ is the specific growth rate (1/time), and D is the dilution rate (1/time).

The Eq. 1 shows that a steady state (no increase or decrease in biomass) will be reached when $\mu = D$. If $\mu > D$, the utilization of substrate will exceed the supply of substrate, causing the growth rate to slow until it is equal to the dilution rate. If $\mu < D$, the amount of substrate added will exceed the amount utilized.

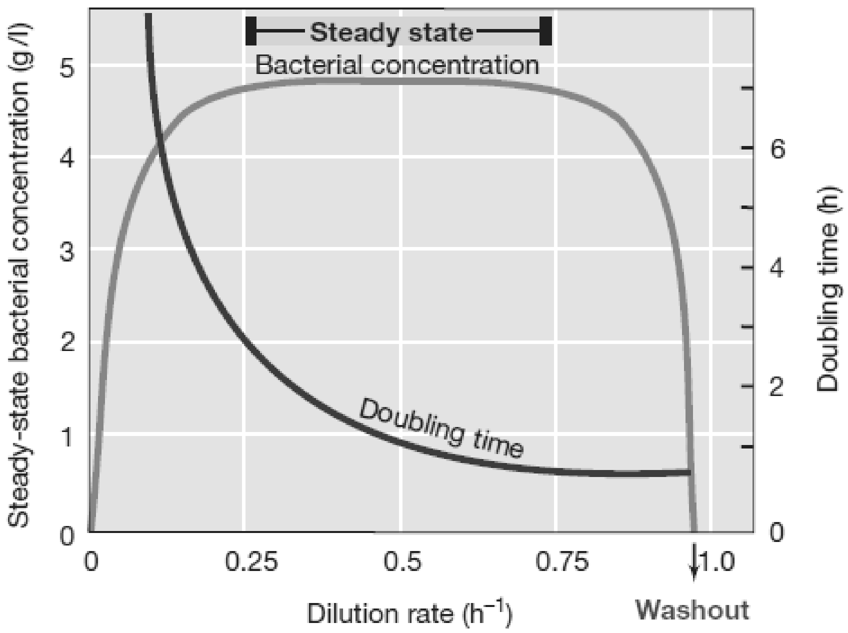

Figure-11: Steady state relationships in the Bioreactor.(Source Madigan et al., 2012)

According to Monod equation, that was developed by Jacques Monod in the 1940s.

$$\mu = \frac{\mu_{max} S}{K_S + S} \qquad \text{Eq. -2}$$

Where μ is the specific growth rate (1/time), μmax is the maximum specific growth rate (1/time) for the culture, S is the substrate concentration (mass/volume), and KS is the half-saturation constant (mass/volume) also known as the affinity constant.

Therefore the growth rate will increase until it is equal to the dilution rate. In either case, given time, a steady state will be established where

$$\mu = D \quad \text{Eq. -3}$$

Such a steady state can be achieved and maintained as long as the dilution rate does not exceed a critical rate, Dc. The critical dilution rate can be determined by combining Eq. 2 and 3.

$$DC = \mu Max \frac{S}{Ks+sS} \quad \text{Eq.- 4}$$

Where S is substrate or nutrient concentration in the bioreactor and KS is the half-saturation constant (this equation assumes Monod kinetics).

17.8 Effects of environmental factors on Growth

The growths of microorganisms are greatly affected by the chemical and physical state of their environment. Many environmental factors can be considered as temperature, pH, water availability, pressure, radiation, and oxygen. Clearly, each microbe must have evolved adaptations that allow it to adjust its physiology within its preferred range, and it may also have adaptations that protect it in environments outside this range.

17.8.1. Temperature

Temperature is probably the most important environmental factor affecting the growth and survival of microorganisms. At either too cold or too hot a temperature, microorganisms will not be able to grow and may even die. The minimum and maximum temperatures for growth vary greatly among different microorganisms and usually reflect the temperature range and average

- **Minimum temperature** - below growth does not occur may be due to the stiffening of the cytoplasmic membrane.
- **Optimum temperature** - where the growth rate is maximum.
- **Maximum temperature**- above which growth does not occur which reflects when proteins may be denatured, nucleic acids and other cellular components are irreversibly damaged.

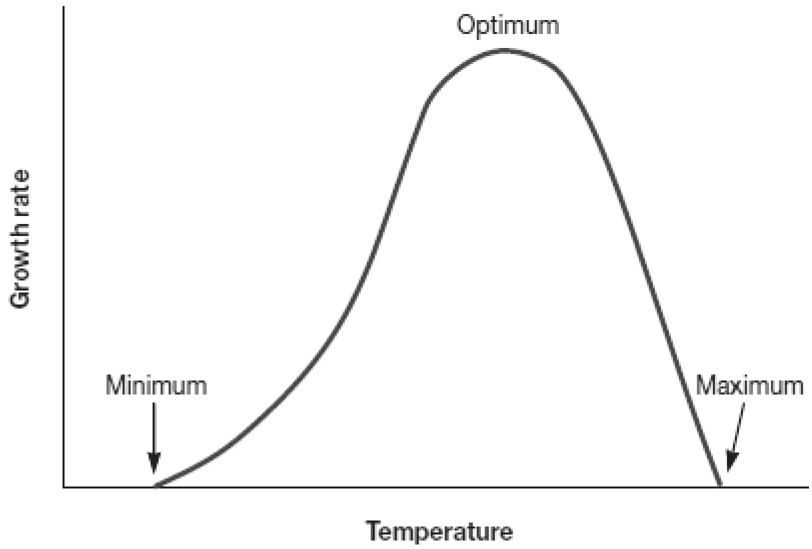

Figure-12: Effect of Temperature on Growth.(Source Prescott, 2002)

Temperature Classes of Organisms

Although there is a continuum of organisms, from those with very low temperature optima to those with high temperature optima, it is possible to distinguish few classes of microorganisms in relation to their growth temperature optima.

- **psychrophiles** - low temperature optima <15º C - may even be killed by brief warming or thawing.
- **mesophiles** - midrange temperature optima 25 - 40º C.
- **thermophiles** - high temperature optima 40 - 80º C.
- **hyperthermophiles** - very high temperature optima >80º C.
- **Psychrophiles** open ocean water is between 1 and 3º C. Artic and Antartic regions are cold.

17.8.2. Acidity and alkalinity

pH is a measure of the relative acidity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$pH = -\log [H^+] = \log(1/[H^+])$

Every microorganism has a pH range within which growth is possible and typically shows a well-defined growth pH optimum. Acidity or alkalinity of a solution is expressed by its pH on a scale on which neutrality is pH 7. The pH values less than 7 are acidic and those greater than 7 are alkaline. Most organisms show a growth range of 2–3 pH units. Most natural environments have a pH between 4 and 9, and organisms with optima in this range are most commonly encountered. Only a few species can grow at pH values of lower than 3 or greater than 9.

pHClasses of Organisms:

- **Acidophiles:** These are live at low pH in range pH 2 to 5.5. A critical factor governing acidophily is the stability of the cytoplasmic membrane. When the pH is raised to neutrality, the cytoplasmic membranes of strongly acidophilic bacteria are destroyed and the cells lyse. This indicates that these organisms are not just acid-tolerant but that high concentrations of hydrogen ions are actually required for membrane stability.
- **Alkaliphiles:** These are live at high pH ranging >8 pH such as soda lakes and carbonate soils. They are Important in biotechnology for production of hydolytic proteases that function at alkaline pH and are used in household cleaners.
- **Neutrophiles:** These Organisms grow optimally at a pH value in the range termed circumneutral (pH 5.5 to 7.9).

17.8.3. Water availability

Water is the solvent of life, and water availability is an important factor affecting the growth of microorganisms. Water availability not only depends on the absolute water content of an environment, that is, how moist or dry it is, but it is also a function of the concentration of solutes such as salts, sugars, or other substances that are dissolved in the water. Dissolved substances have an affinity for water, which makes the water associated with solutes Water availability is expressed in physical terms as water activity.

Water diffuses from regions of high water concentration (low solute concentration) to regions of lower water concentration (higher solute concentration) in the process of osmosis. The cytoplasm of a cell typically has a higher solute concentration. However, when a cell finds itself in an environment where the solute concentration exceeds that of the cytoplasm, water will flow out of the cell. This can cause serious problems if a cell has no way to counteract it because a dehydrated cell cannot grow. Water moves from high water activity values to lower values in the process of osmosis. Different bacteria have different tolerances towards low water activities. In fact, preservation process takes advantage of lower water activity which causes plasmolysis or pulling away of the membrane from the cell wall.

17.8.4. Oxygen

Animals require molecular oxygen (O2), it is easy to assume that all organisms require O2. However, this is not true; many microorganisms can, and some must, live in the total absence of oxygen. Oxygen is poorly soluble in water, and because of the constant respiratory activities of microorganisms in aquatic habitats, O2 can quickly become exhausted. Thus, anoxic (O2-free) microbial habitats are common in nature and include muds and other sediments, bogs, marshes, water-logged soils, intestinal tracts of animals, sewage sludge, the deep subsurface of Earth, and many other environments. In these anoxic habitats, microorganisms, particularly prokaryotes, thrive.

Oxygen Classes of Microorganisms:

- **Aerobes:** Theyrequire oxygen up to 21% as in air.
- **Microaerophilic:** These bacteria require reduced levels of oxygen.
- **Strict** or obligate **anaerobes:** They grow only in the absence of oxygen.
- **Facultative anaerobes:** These Microbes can grow aerobically if oxygen is present and switch to fermentation or anaerobic respiration if oxygen is absent.
- **Aerotolerant anaerobes:** These Microbes don't use oxygen for growth but tolerate its presence. They can grow on the surface of solid medium without the special anaerobic conditions required for the strict anaerobes.

17.8.5. Radiation

Sunlight is the major source of radiation on Earth. It includes visible light, ultraviolet (UV) radiation, infrared rays, and radio waves. Visible light is a most conspicuous and important aspect of our environment: most life depends on the ability of photosynthetic organisms to trap the light energy of the sun. Almost 60% of the sun's radiation is in the infrared region rather than the visible portion of the spectrum.

In nature, various types' radiations are bombarded with electromagnetic field. They behave as if it were composed of waves moving through space like waves traveling on the surface of water. The distance between two wave crests or troughs is the wavelength. As the wavelength of electromagnetic radiation, gamma rays and X-rays are much more energetic than visible light or infrared waves. The X-rays are artificially produced, and gamma rays are emitted during radioisotope decay. These electromagnetic radiation are true of ionizing radiation havingvery short wavelength and high energy, which can cause atoms to lose electrons (ionize). Low levels of ionizing radiation may produce mutations and may indirectly result in death, whereas higher levels are directly lethal.

Ultraviolet (UV) radiations have potential tokill microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimmers in DNA. Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function.

17.8.6. Pressure

Organisms that spend their lives on land or the surface of water are always subjected to a pressure of 1 atmosphere (atm) and are never affected significantly by pressure. The high hydrostatic pressure affects membrane fluidity and membrane associated function.

17.9 Summary

A Growth of organism is an increase in cellular constituents and results in an increase in cell size, cell number, or both by binary fission. When microorganisms are grown in a batch culture, the resulting growth curve usually has four phases: lag, exponential (log),stationary, and death**.** In the exponential phase, the population number of cells undergoing binary fission doubles at a constant interval called the generation or doubling time**.** The mean growth rate constant (k*)* is the reciprocal of the generation time.A Continuous Culture of Microorganisms is an open system in which nutrients are constantly provided and wastes removed with maintain a microbial population in log phase.

Microbial populations can be counted directly with counting chambers, electronic counters, or Viable counting techniques such as the spread plate, the pour plate, or the membrane filter can be employed. It is affected by temperature, pH, water availability, pressure, radiation, and oxygen.

17.10 Glossary

- **Acidophile:** An organism that grows best at acidic pH values.

- **Aerobe:** an organism that grows in the presence of O_2; may be facultative, obligate or micro-aerophilic.
- **Anaerobe:** An organism that grows in the absence of O_2; some may even be killed by O_2.
- **Anoxic:** Oxygen-free. Usually used in reference to a microbial habitat.
- ***Bacteria:***Phylogenetically related prokaryotes distinct from Archaea.
- **Batch culture:** A closed-system microbial culture of fixed volume.
- **Chemostat:** A continuous culture device controlled by the concentration of limiting nutrient and dilution rate.
- **Culture:** A particular strain or kind of organism growing in a laboratory medium.
- **Culture medium:** An aqueous solution of various nutrients suitable for the growth of microorganisms.
- **Doubling time:** The time needed for a population to double.
- **Exponential growth:** Growth of a microbial population in which the cell number doubles within a fixed time period.
- **Exponential phase:** A period during the growth cycle of a population in which growth increases at an exponential rate.
- **Extremophile:** An organism that grows optimally under one or more chemical or physical extremes, such as high or low temperature or pH.
- **Extremozyme:** An enzyme able to function in one or more chemical or physical extremes, for example, high temperature or low pH.
- **Facultative:** Indicates that an organism is able to grow in either the presence or absence of an environmental factor (for example, “facultative aerobe”).
- **Flow cytometry:** A technique for counting and examining microscopic particles by suspending them in a stream of fluid and passing them by an electronic detection device.
- **Generation time:** The time required for a cell population to double
- **Lag phase:** The period after inoculation of a culture before growth begins.

- **Microorganism:** A microscopic organism consisting of a single cell or cell cluster, also including the viruses, which are not cellular.
- **Osmosis:** The diffusion of water through a membrane from a region of low solute concentration to one of higher concentration.
- **pH:** The negative logarithm of the hydrogen ion (H1) concentration of a solution.
- **Plate count:** A viable counting method in which the number of colonies on a plate is used as a measure of cell number.
- **Population:** A group of organisms of the same species in the same place at the same time.
- **Thermophile:** An organism with a growth temperature optimum between 45 and 80°C.
- **Turbidity:** A measurement of suspended solids in water.
- **Viable:** Alive; able to reproduce.
- **Viable count:** A measurement of the concentration of live cells in a microbial population.
- **Xerophile:** An organism adapted to growth at very low water potentials.

17.11 Self-Learning Exercise

Section -A (Very Short Answer Type):

1. Growth is ---------------------------------
2. Mesophiles are ---------------------.
3. Log phase is found in ---------------------------------culture system.
4. Monod equation is -----------------------
5. Generation time is a --------------------------------
6. Xerophiles are ---------------------------------------

Section -B (Short Answer Type):

1. Define the difference between Lag and Log phase.
2. Define the Exponential growth phase.
3. A Short note on Microbial growth.
4. Explain a key note Synchronous growth curve.

5. Describe a key note on mathematical expression of growth.

Section -C (Long Answer Type)

1. Define the Growth with explaining growth curve & expression of growth.
2. Write about Continuous culture. How it is differs with Synchronous growth.
3. Explain the effects of environmental factors on Growth with Types of factors.
4. Write about Measurement of growth and growth yields.

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Unit - 18

Microbial Physiology

Structure of the Unit

18.13 Objectives

This unit gives an account for the nutrients utilizes by the microorganism for their growth and metabolism and other activity.Microorganisms require about 10 elements inlarge quantities, in part because they are used to construct carbohydrates, lipids, proteins, and nucleic acids. Beside this several other elements are needed in very small amounts and are parts of enzymes and cofactors. All microorganisms can be placed in one of a few nutritional categories on the basis of their requirements for carbon, energy, and hydrogen atoms or electrons, and how different microorganism fix the atmospheric nitrogen and convert into ammonia. Lastly this unit explain the role of microorganism as biofertilizers in which how microorganism improves the fertility of soil.

18.2 Introduction

Nutrients are substances used in biosynthesis and energy production and therefore are required for microbial growth. To obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients.

18.3 The Common Nutrient Requirements

1. The Common Nutrient Requirements:

Microbial cell composition shows that over 95% of celldry weight,which is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called **macroelements**or **macronutrients**because they are required by microorganisms in relatively largeamounts.The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining fourmacroelements exist in the cell as cations and play a variety of roles.For example, potassium **(K^+)** is required for activity by a number ofenzymes, including some of those involved in protein synthesis. Calcium **(Ca^{2+}),** among other functions, contributes to the heat resistance of bacterial endospores. Magnesium **(Mg^{2+})** serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomesand cell membranes. Iron **(Fe^{2+}andFe^{3+})** is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

All organisms, including microorganisms, require severalmicronutrientsortrace elementsbesides macroelements. The micronutrients- manganese, zinc, cobalt, molybdenum, nickel, and copper- are needed by most cells.However, cells require such small amounts that contaminants in water, glassware, and regular media components often are adequate for growth.Micronutrients are normally a part ofenzymes and cofactors, and they aid in the catalysis of reactionsand maintenance of protein structure. For example, zinc **(Zn^{2+})** ispresent at the active site of some enzymes but is also involved inthe association of regulatory and catalytic subunits in ***E. coli*** aspartate carbamoyltransferase.Manganese**(Mn^{2+})** aids many enzymes catalyzing the transfer of phosphate groups. Molybdenum **(Mo^{2+})** is required for nitrogen fixation,and cobalt **(Co^{2+})** is a component of vitamin **B_{12}.**

Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect thespecial nature of their morphology or environment. Diatomsneed silicic acid **(H_4SiO_4)** to construct their cell walls of silica **[$(SiO_2)n$]**.Although most bacteria do notrequire large amounts of sodium, many bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion **(Na^+).**Finally, it must be emphasized that microorganisms requirea balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of the concentrations of other nutrients.

18.4 Requirements for Carbon, Hydrogen, and Oxygen

The requirements for carbon, hydrogen, and oxygen often are satisfied together. Carbon is needed for the skeleton or backbone ofall organic molecules, and molecules serving as carbon sourcesnormally also contribute both oxygen and hydrogen atoms. Theyare the source of all three elements. Because these organic nutrients are almost always reduced and have electrons that they candonate to other molecules, they also can serve as energy sources.Indeed, the more reduced organic molecules are, the higher theirenergy content (e.g., lipids have a higher energy content than carbohydrates). This is because, electron transfers release energy when the electrons move from reduced donorswith more negative reduction potentials to oxidized electron acceptors with more positive potentials.One important carbon source that does not supply hydrogenor energy is carbon dioxide (CO_2). This is because CO_2 is oxidized and lacks hydrogen. Probably all microorganisms can fixCO_2 - that is, reduce it and incorporate it into organic molecules.Only autotrophscan use CO_2as theirsole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and uselight as their energy source. Some autotrophs oxidize inorganicmolecules and derive energy from electron transfers.

The reduction of CO_2 is a very energy expensive process.Thus many microorganisms cannot use CO_2as their sole carbon source but must rely on the presence of more reduced, complex molecules such as glucose for a supply of carbon. Organisms that use reduced, preformed organic molecules as carbon sources are heterotrophs(these preformed molecules normally come from other organisms).

Most heterotrophsuse reduced organic compounds as sources of both carbon and energy. For example, the glycolytic pathway produces carbon skeletons for use in biosynthesis and also releases energy as ATP andNADH.

A most remarkable nutritional characteristic of microorganisms is their extraordinary flexibility with respect to carbonsources. Laboratory experiments indicate that there is no naturally occurring organic molecule that cannot be used by some microorganism. ***Actinomycetes*** will degrade amyl alcohol, paraffin,and even rubber. Some bacteria seem able to employ almost anything as a carbon source; for example,***Burkholderia cepaciacan***use over 100 different carbon compounds. In contrast to thesebacterial omnivores, some bacteria are exceedingly fastidious andcatabolize only a few carbon compounds. Cultures of methylotrophic bacteria metabolize methane, methanol, carbon monoxide, formic acid, and related one-carbon molecules. Parasitic members of the genus ***Leptospira***use only long-chain fattyacids as their major source of carbon and energy.

It appears that in natural environments complex populations ofmicroorganisms often will metabolize even relatively indigestiblehuman-made substances such as pesticides. Indigestible moleculessometimes are oxidized and degraded in the presence of a growth-promoting nutrient that is metabolized at the same time, a process called **cometabolism**. The products of this breakdown process canthen be used as nutrients by other microorganisms.

18.5 Nutritional Classification Of Microorganisms

Microorganisms can be grouped into nutritional classes based onhow they satisfy all these requirements (**table 1**). We have alreadyseen that microorganisms can be classified as either heterotrophs orautotrophs with respect to their preferred source of carbon. There areonly two sources of energy available to organisms: (1) light energy,and (2) the energy derived from oxidizing organic or inorganic molecules.**Phototrophs**use light as their energy source; **chemotrophs**obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources forelectrons.**Lithotrophs** (i.e., “rock-eaters”) use reduced inorganicsubstances as their electron source, whereas **organotrophs** extract electrons from organic compounds.

Despite the great metabolic diversity seen in microorganisms,most may be placed in one of four nutritional classes based ontheir primary sources of carbon, energy, and electrons (**table 2**). The large majority of microorganisms thus far studied are eitherphotolithotrophic autotrophs or chemoorganotrophic heterotrophs.**Photolithotrophic autotrophs**(often called photoautotrophsorphotolithoautotrophs) use light energy and have CO_2 as their car-bon source. Eukaryotic algae and cyanobacteria employ water asthe electron donor and release oxygen. Purple and green sulphur bacteria cannot oxidize water but extract electrons from inorganicdonors like hydrogen, hydrogen sulfide, and elemental sulfur.**Chemoorganotrophic heterotrophs** (often called chemo heterotrophs,chemoorganoheterotrophs, or even heterotrophs)use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy all these requirements. It should be noted that essentially allpathogenic microorganisms are chemoheterotrophs.

The other two nutritional classes have fewer microorganismsbut often are very important ecologically. Some purple and greenbacteria are photosynthetic and use organic matter as their electrondonor and carbon source. These **photoorganotrophic heterotrophs**(photoorganoheterotrophs) are common inhabitants ofpolluted lakes and streams. Some of these bacteria also can grow asphotoautotrophs with molecular hydrogen as an electron donor. Thefourth group, the **chemolithotrophic autotrophs** (chemolithoautotrophs), oxidizes reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons forbiosynthesis. Carbon dioxide is the carbon source. A fewchemolithotrophs can derive their carbon from organic sources andthus are heterotrophic. Chemolithotrophs contribute greatly to thechemical transformations of elements (e.g., the conversion of ammonia to nitrate or sulfur to sulfate) that continually occur in theecosystem.

Although a particular species usually belongs in only one ofthe four nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes. For example, many purple nonsulfur bacteria act as photoorganotrophic heterotrophs in the absence of oxygen but oxidize organic molecules and functionchemotrophically at normal oxygen levels. When oxygen is low,photosynthesis and oxidative metabolism may function simultaneously. Another example is provided by bacteria such as ***Beggiatoa*** that rely on inorganic energy sources and organic (or sometimes CO_2) carbon sources. These microbes aresometimes called **mixotrophic** because they combinechemolithoautotrophic and heterotrophic metabolic processes.This sort of flexibility seems complex and confusing, yet it givesits possessor a definite advantage if environmental conditionsfrequently change.

TABLE. 1 : Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Light
Chemotrophs	Oxidation of organic or inorganic compounds
Electron Sources	
Lithotrophs	Reduced inorganic molecules
Organotrophs	Organic molecules

TABLE. 2 : Major Nutritional Types of Microorganisms

Major Nutritional TypesSources of Energy, Hydrogen/Electrons Representative Microorganisms

and Carbon

Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy	Algae
	Inorganic hydrogen/electron (H/e^-) donor	Purple and green sulfur bacteria
	CO_2carbon source	Cyanobacteria
Photoorganotrophic	Light energy	Purple nonsulfur

heterotrophy (Photoorganoheterotrophy)		bacteria
	Organic H/e$^-$ donor	Green nonsulfur bacteria
	Organic carbon source (CO_2 may also be used)	
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic)	Sulfur-oxidizing bacteria
	Inorganic H/e$^-$ donor	Hydrogen bacteria
	CO_2 carbon source	Nitrifying bacteria
		Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic)	Protozoa
	Organic H/e$^-$ donor	Fungi
	Organic carbon source	Most nonphotosynthetic bacteria (including most pathogens)

18.6 Requirements for Nitrogen, Phosphorus, and Sulfur

To grow, a microorganism must be able to incorporate large quantities of nitrogen, phosphorus, and sulfur. Although these elementsmay be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well.

Nitrogen is needed for the synthesis of amino acids, purines,pyrimidines, some carbohydrates and lipids, enzyme cofactors,and other substances. Many microorganisms can use the nitrogenin amino acids, and ammonia often is directly incorporatedthrough the action of such enzymes as glutamate dehydrogenaseor glutamine synthetase and glutamate synthase. Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammoniain assimilatory nitrate reduction. A variety of bacteria (e.g., many ***Cyanobacteria*** and the symbiotic bacterium***Rhizobium***) can reduce and assimilate atmospheric nitrogen usingthe nitrogenase system.

Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and othercell components. Almost all microorganisms use inorganicphosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth inmany aquatic environments. Phosphate uptake by ***E. coli***has been intensively studied. This bacterium can use both organicand inorganic phosphate. Some organophosphates such as hexose 6-phosphates can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasmby the enzyme alkaline phosphatase to produce inorganic phosphate, which then is transported across the plasma membrane.When inorganic phosphate is outside the bacterium, it crossesthe outer membrane by the use of a porin protein channel. Oneof two transport systems subsequently moves the phosphateacross the plasma membrane. At high phosphate concentrations,transport probably is due to the Pit system. When phosphateconcentrations are low, the PST, (phosphate-specific transport)system is more important. The PST system has higher affinityfor phosphate; it is an ABC transporter and usesa periplasmic binding protein.

Sulfur is needed for the synthesis of substances like theamino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a sourceof sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

18.7 Growth Factors

Microorganisms often grow and reproduce when minerals andsources of energy, carbon, nitrogen, phosphorus, and sulfur aresupplied. These organisms have the enzymes and pathways necessary to synthesize all cell components required for their well-being. Many microorganisms, on the other hand, lack one or moreessential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursorsfrom the environment. Organic compounds required because theyare essential cell components or precursors of such componentsand cannot be synthesized by the organism are called growth factors.There are three major classes of growth factors: (1) aminoacids, (2) purines and pyrimidines, and (3) vitamins. Amino acidsare needed for protein synthesis, purines and pyrimidines for nucleic acid synthesis.

Vitaminsare small organic molecules thatusually make up all or part of enzyme cofactors and only very small amounts sustain growth. The functions of selected vitamins, and examples of microorganisms requiring them, are given in (**table 3.).**Some microorganisms require many vitamins; for example,***Enterococcus faecalis*** needs eight different vitamins for growth. Other growth factors are also seen; heme(from hemoglobin or cytochromes) is required by ***Haemophilusinfluenzae***,and some mycoplasmas need cholesterol.

Knowledge of the specific growth factor requirements ofmany microorganisms makes possible quantitative growth-response assays for a variety of substances. For example, speciesfrom the bacterial genera ***Lactobacillus***and***Streptococcus***can beused in microbiological assays of most vitamins and amino acids.

The observation that many microorganisms can synthesizelarge quantities of vitamins has led to their use in industry. Severalwater-soluble and fat-soluble vitamins are produced partly or completely using industrial fermentations. Good examples of such vitamins and the microorganisms that synthesize them are riboflavin(***Clostridium***, ***Candida***, ***Ashbya***, ***Eremothecium***), coenzyme A(***Brevibacterium***), vitamin B_{12}(***Streptomyces***,***Propionibacterium***,***Pseudomonas)***, vitamin C(***Gluconobacte***r, ***Erwinia***, ***Corynebacterium***), β-carotene (***Dunaliella***), and vitamin D (***Saccharomyces***).

TABLE. 3 : Functions of Some Common Vitamins in Microorganisms
Vitamin FunctionExamples of Microorganisms Requiring Vitamin [a]

Biotin	Carboxylation (CO_2 fixation) One-carbon metabolism	*Leuconostoc mesenteroides*(B) *Saccharomyces cerevisiae*(F) *Ochromonas malhamensis*(A) *Acanthamoeba castellanii*(P)
Cyanocobalamin(B12)	Molecular rearrangements One-carbon metabolism-carries methyl groups	*Lactobacillus spp.* (B) *Euglena gracilis*(A) Diatoms and many other algae (A) *Acanthamoeba castellanii*(P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis*(B) *Tetrahymena pyriformis*(P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei*(B) *Tetrahymena spp.* (P)
Pantothenic acid	Precursor of coenzyme A-carries acyl groups(pyruvate oxidation, fatty acid metabolism)	*Proteus morganii*(B) *Hanseniaspora spp.* (F) *Paramecium spp.* (P)
Pyridoxine (B6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus spp.* (B) *Tetrahymena pyriformis* (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP-carry electrons and hydrogen atoms	*Brucella abortus*, *Haemophilus influenzae*(B) *Blastocladia pringsheimii*(F) *Crithidia fasciculate*(P)

Riboflavin (B2)	Precursor of FAD and FMN-carry electrons or hydrogen atoms	*Caulobacter vibrioides*(B) *Dictyostelium spp.* (F) *Tetrahymena pyriformis*(P)
Thiamine (B1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis*(B) *Phycomyces blakesleeanus*(F) *Ochromonas malhamensis*(A) *Colpidium campylum*(P)

([a])The representative microorganisms are members of the following groups: bacteria (B), fungi (F), algae (A), and protozoa (P).

18.7 Nitrogen fixation

The reduction of atmospheric gaseous nitrogen to ammonia iscalled nitrogen fixation. Because ammonia and nitrate levels often are low and only a few prokaryotes can carry out nitrogen fixation (eukaryotic cells completely lack this ability), the rate ofthis process limits plant growth in many situations. Nitrogen fixation occurs in (1) free-living bacteria (e.g.,***Azotobacter***, **Klebsiella**, ***Clostridium***,and***Methanococcus***), (2) bacteria living insymbiotic association with plants such as legumes (***Rhizobium***), and (3) ***Cyanobacteria*** (***Nostoc and Anabaena***).

The reduction of nitrogen to ammonia is catalyzed by the enzyme**nitrogenase**.Although the enzyme-bound intermediates inthis process are still unknown, it is believed that nitrogen is reducedby two-electron additions in a way similar to that illustrated in**figure 1.**

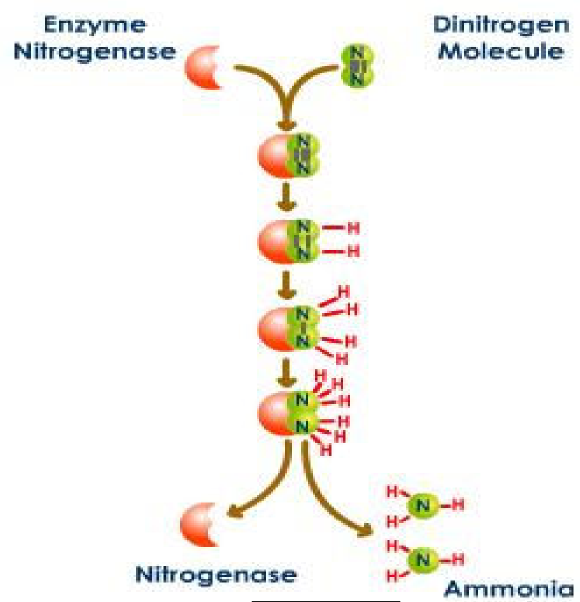


Figure. 1

The reduction of molecular nitrogen to ammonia isquite exergonic, but the reaction has a high activation energy because molecular nitrogen is an unreactive gas with a triple bond between the two nitrogen atoms. Therefore nitrogen reduction is expensive and requires a large ATP expenditure. At least 8 electronsand 16 ATP molecules, 4 ATPs per pair of electrons, are required.

$$\mathbf{N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16\ Pi}$$

The electrons come from ferredoxin that has been reduced in a variety of ways: by photosynthesis in ***Cyanobacteria***, respiratory processes in aerobic nitrogen fixers, or fermentations in anaerobic bacteria.For example,***Clostridium pasteurianum*** (an anaerobic bacterium) reduces ferredoxin during pyruvate oxidation,whereas the aerobic ***Azotobacter***uses electrons from NADPH toreduce ferredoxin.

Nitrogenase is a complex system consisting of two major protein components, a MoFe protein (MW 220,000) joined with oneor two Fe proteins (MW 64,000). The MoFe protein contains 2atoms of molybdenum and 28 to 32 atoms of iron; the Fe protein has 4 iron atoms (**figure 2**.). Fe protein is first reduced by ferredoxin, then it binds ATP (**figure 3.**). ATP binding changes the conformation of the Fe protein and lowers its reduction potential, enabling it to reduce the MoFe protein. ATP is hydrolyzed when this electron transfer occurs. Finally, reduced MoFe protein donates electrons to atomic nitrogen. Nitrogenase is quite sensitive to $O_2$and must be protected from O_2inactivation within the cell. In many ***Cyanobacteria***, this protection against oxygen is provided by a special structure called the heterocyst.

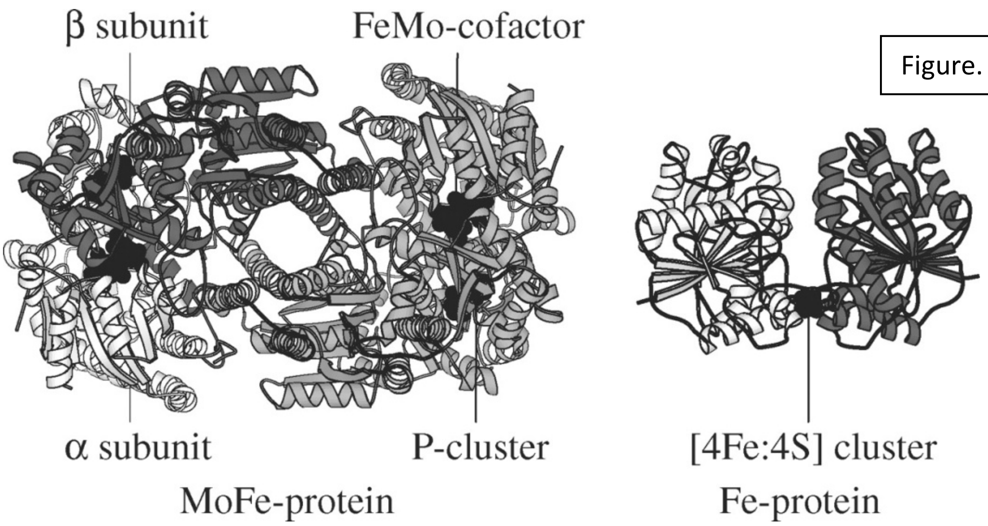


Figure. 2

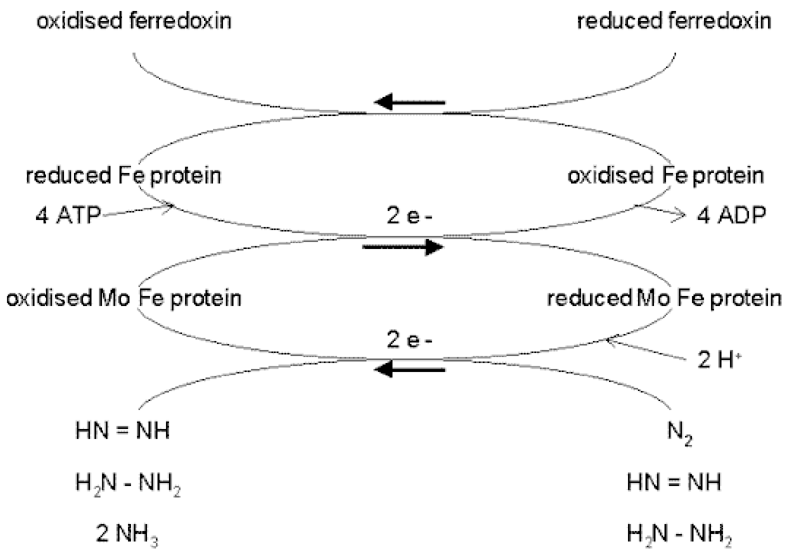


Figure. 3

The reduction of $N_2$to NH_3occurs in three steps, each ofwhich requires an electron pair (**figures 1 and 3**). Six electron transfers take place, and this requires a total 12 ATPs per N_2reduced. The overall process actually requires at least 8 electronsand 16 ATPs because nitrogenase also reduces protons to H_2. TheH_2reacts with diimine (HN—NH) to form $N_2$and H_2. This futilecycle produces some N_2 even under favorable conditions andmakes nitrogen fixation even more expensive. Symbiotic nitrogen-fixing bacteria can consume almost 20% of the ATP produced bythe host plant.

Nitrogenase can reduce a variety of molecules containingtriple bonds (e.g., acetylene, cyanide, and azide).

$$\mathbf{HC \equiv CH + 2H^{+} + 2e \longrightarrow H_2C = CH_2}$$

The rate of reduction of acetylene to ethylene is even used to estimate nitrogenase activity.

Once molecular nitrogen has been reduced to ammonia, theammonia can be incorporated into organic compounds. In the symbiotic nitrogen fixer ***Rhizobium***,it appears that ammonia diffusesout of the bacterial cell and is assimilated in the surrounding legumecell. The primary route of ammonia assimilation seems to be thesynthesis of glutamine by the glutamine synthetase- glutamate synthase system (**figure 4.**). However, substances such asthe purine derivatives allantoin and allantoic acid also are synthesized and used for the transport of nitrogen to other part.

<u>**Glutamine synthetase reaction**</u>

Figure. 4

		O	
		‖	
COOH		C — NH_2	
CH_2		CH_2	
CH_2	+ NH_3 + ATP ⟶	CH_2	+ ADP +Pi
$CHNH_2$		CH — NH_2	
COOH		COOH	
Glutamic acid		**Glutamine**	

<u>**Glutamate synthase reaction**</u>

COOH	COOH	COOH	COOH
C = O	$CHNH_2$	$CHNH_2$	$CHNH_2$
CH_2+	CH_2+**NADPH + H**$^{+}$$CH_2$+ ⟶		CH_2 +
NADP^{+}			

CH_2 $\quad$ CH_2 $\quad$ CH_2 $\quad$ CH_2

COOH $\quad$ C — NH_2 $\quad$ COOH $\quad$ COOH

‖

O

α-Ketoglutaric acidGlutamineTwo glutamic acids

(1) Free-living bacteria

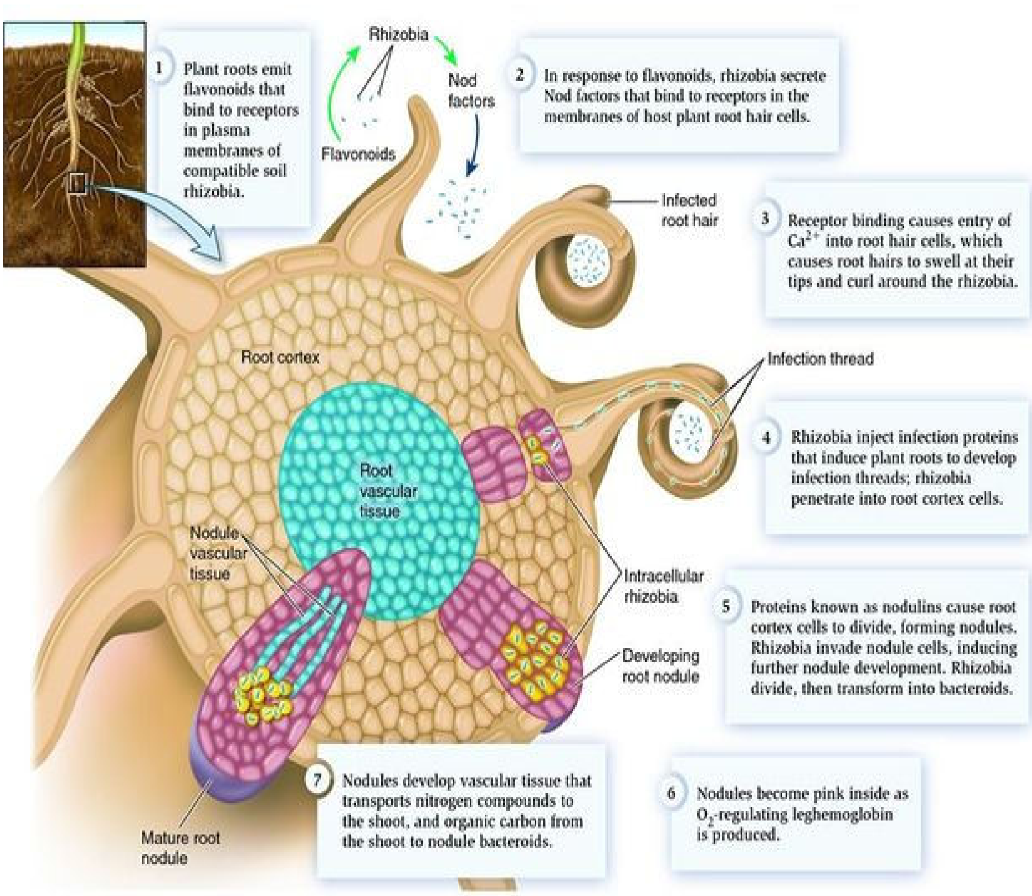
Free-living aerobic nitrogen fixers include several species of genus ***Azotobacter***and some methylotrophic bacteria. ***Cyanobacteria*** are also free-living nitrogen fixers.***Azotobacter***is found in soils and is a versatile heterotroph whose growth is limited by the amount of organic carbon available.Methylotrophic bacteriacan fix nitrogen when provided with methane, methanol, or hydrogen from various substrates. ***Cyanobacteria*** can fix nitrogen by using hydrogen from hydrogen sulfide, sothey increase the availability of nitrogen in sulfurous environments.Nitrogen-fixing facultative anaerobes include species of***Klebsiella***, ***Enterobacter***, ***Citrobacter***, and ***Bacillus***.In addition, a number of obligate anaerobes, including photosynthetic *Rhodospirillaceae* and bacteria of the genera ***Clostridium***, ***Desulfovibrio***,and***Desulfotomaculum***, also fix nitrogen. Several species of***Klebsiella*** capable of fixing nitrogen are found inrhizomes(subsurface stems) of legumes such as peas and beans and in the intestines of humans and other animals. Nitrogen fixation has been observed in about 12% of***Klebsiella pneumonia*** organisms from patients. Various species of ***Clostridium*** are found in soils and muds. They use a variety of organic substances for energy and withstand unfavorable conditions as spores. They tolerate a range of pH from 4.5 to 8.5 but fix nitrogen best at pH 5.5 to 6.5.***Desulfovibrio*** and***Desulfotomaculum***, anaerobic sulfate reducers that live in mud and soil sediments, fix nitrogen at pH 7 to 8.

(2) Bacteria living in symbiotic association with plants such as legumes

Rhizobiumis the primary symbiotic nitrogen fixer. It lives innodules, growths extending from the roots of certain plants, usually legumes (**figure 5.**).In this symbioticrelationship, the plant benefits by receiving nitrogen in a usable form, and the bacteria benefit byreceiving nutrients needed for growth. When paired with legumes,***Rhizobium***can fix 150 to 200 kg of nitrogen per hectare of land per year. In the absence of legumes, the bacteria fix only about 3.5 kg of nitrogen per hectare per year - less than 2% of that fixed in the symbiotic relationship. Farmers often mix nitrogen-fixing bacteria with seed peas and beans before planting to ensure that nitrogen fixation will be adequate for their crops to thrive. The mechanisms by which ***Rhizobium***establishes a symbiotic relationship with a legume has been the subject of many research studies. ***Rhizobium***multiplies in the vicinity of legume roots, probably under the influence of root secretions. As the rhizobia increase in numbers, they release enzymes that digest cellulose and the substances that cement cellulose fibers together in the root cell walls. The rhizobia then change from their free-living rod shape and become spherical, flagellated cells called**swarmer cells**. These cells are thought to produce indoleacetic acid, a plant growth hormone that causes curling of root hairs. The swarmer cells then invade the root hairs and form hyphalike networks, killing some root cells and proliferating in others. Swarmer cells become large, irregularly shaped cells called **bacteroids**, which are tightly packed into root cells, probably under the influence of chemical substances in the plant cells. Accumulations of bacteroids in adjacent root cells form nodules on the plant roots.Bacteroids contain the enzyme **nitrogenase**, which catalyzes the following reaction:

$$N_2 + 3H_2 \longrightarrow 2NH_3$$

However, this enzyme is inactivated by oxygen, so nitrogen fixation can occur only when oxygen is preventedfrom reaching the enzyme. Nitrogenase is protected from oxygen by a kind of hemoglobin, a red pigment that binds oxygen. This particular hemoglobin is synthesized only in root nodules containing bacteroids, because part of the genetic information for its synthesis is in the bacteroids and part is in the plant cells. Synthesisof nitrogenase is repressed in the presence of excess ammonium ($NH4^+$) and derepressed in the presence of free nitrogen. Thus, fixation occurs only when ‘‘fixed’’ nitrogen is in short supply and free nitrogen is available.***Rhizobium***species vary in their capacity to invade particular legumes and in their capacity to fix nitrogen once they have invaded. Some species cannot invade any legumes, whereas others invade only certain legumes. This invasive specificity is determined genetically (probably by a single gene or a group of closely related genes) and can be altered by genetic transformation. Sucha transformation mightenable a species of***Rhizobium***to invade a group of legumes it previously could not colonize. Although symbiotic nitrogen fixation occurs mainly in association between rhizobia and legumes, other such associations are known. The **alder tree**, which grows in soils low in nitrogen, has root nodules similar to those formed by rhizobia. These nodules contain nitrogen fixing actinomycetes of the genus***Frankia***.



(3) Cyanobacteria

Some nitrogen fixers are found in symbiotic association with other organisms, which provide them with organic carbon sources. For example, the Cyanobacterium***Anabaena*** is found in pores of the leaves ofAzolla, a small water fern found in many parts of the world. The nitrogen fixing***Anabaena*** supplies the necessary nitrogen; the fern supplies substrates for energy capture in ATP and reductants for nitrogen fixation. Together, they fix 100 kg of nitrogen per hectare (about two football fields in area) per year and are used as ‘‘green manure’’ in rice cultivation in southeast Asia.

18.9 Microbes used as bio-fertilizer

A number of microorganisms (bacteria fungi and algae) are considered as beneficial for agriculture and used as **biofertilizers**.

These fertilizers are not harmful to crops or other plants like the chemical fertilizers. They are actually taken from the animal wastes along with the microbial mixtures. Microorganisms are used to increase the level of nutrients in the plants. They let the plants grow in a healthy environment. They are also environment friendly and do not cause the pollution of any sort. Use of biofertilizers in the soil, makes the plants healthy as well as protect them from getting any diseases.

Biofertilizers are supposed to be a safe alternative to chemical fertilizers to minimize the ecological disturbance. Biofertilizers are cost effective, eco-friendly and when they are required in bulk can be generated at the farm itself. They increase crop yield upto 10-40% and fix nitrogen upto 40-50 Kg. The other plus point is that after using 3-4 years continuously there is no need of application of biofertilizers because parental inoculums are sufficient for growth and multiplication. They improve soil texture, pH, and other properties of soil. They produces plant growth promoting substances IAA amino acids, vitamins etc. They have 75% moisture and it could be applied to the field directly.

Microbes are effective in inducing plant growth as they secrets plant growth promoters (auxins, abscisic acid, gibberellic acid, cytokinis, ethylene) and enhance seed germination and root growth. They also play a considerable role in decomposition of organic materials and enrichment of compost.

Nitrogen fixing Bacteria

1. Rhizobia:

Legumes plants have root nodules, where atmospheric nitrogen fixation is done by bacteria belonging to genera, ***Rhizobium***, ***Bradyrhizodium***, ***Sinorhizobium***, ***Azorhizobium*** and ***Mesorhizobium*** collectively called as rhizobia. Rhizobia, as these bacteria are commonly known, are specially adapted to particular leguminous plant species, on which they form root nodules. Nitrogen is then fixed by a symbiotic process of the plant and the bacteria. The plant furnishes anaerobic conditions and growth nutrients for the bacteria, and the bacteria fix nitrogen that can be incorporated into plant protein.

2. ***Azorhizobium*:**

It is a stem nodule forming bacteria and fixes nitrogen symbionts of the stem nodule also produce large amount of IAA that promotes plant growth.

3. ***Bradyrhizobium*:**

Bradyrhizobium is reported a good nitrogen fixer. ***Bradyrhizobium*** strain inoculation with Mucuna seeds enhances total organic carbon, N_2, phosphorus and potassium in the soil, increases plant growth and consequently plant biomass, reduction in the weed population and increased soil microbial population.

Diazotrophs

These are aerobic chemolithotrophs and anaerobic photoautotrophs. These are non nodule forming bacteria. They include numbers of the families.

Azotobacteracae: e.g. ***Azotobacter*:**They are the free living aerobic, photoautotrophic, non-symbiotic bacteria. They secretes vitamin-B complex, gibberellins, napthalene, acetic acid and other substances that inhibit certain root pathogens and improves root growth and uptake of plant nutrients. It occurs in the roots of *Paspalum notatum* (tropical grasses) and other spp. and adds 15-93 Kg N/ha/annum on *P. notatum* roots. ***Azotobacterindicum*** occurs in acidic soil in sugarcane plant roots. It can apply in cereals, millets, vegetables and flowers through seed, seedlings soil treatment.

Spirillaceae: e.g. ***Azospirillum*** **and** ***Herbaspirillum*:**These are gram negative, free living, associative symbiotic and non-nodule forming, aerobic bacteria, occurs in the roots of dicots and monocot plants i.e. corn, sorghum, wheat etc. It is easy to culture and identify. ***Azospirillum*** is found to be very effective in increasing 10-15% yield of cereal crops and fixes N_2 upto 20-40% Kg/ha. Different ***A. brasiliense*** strains inoculation in the wheat seed causes increase in seed germination, plant growth, plumule and radicle length. ***Herbaspirillum*** species occurs in roots, stems and leaves of sugarcane and rice. They produce growth promoters (IAA, Gibberillins, Cytokinins) and enhance root development and uptake of plant nutrients (N, P & K).

Acetobacter diazotrophicus*:** Another diazotroph is ***Acetobacter diazotrophicus occurs in roots, stem and leaves of sugarcane and sugar beet crops as nitrogen fixer and applied through soil treatment. It also produces growth promoters e.g. IAA and helps in nutrients uptake, seed germination, and root growth. This bacterium fixes nitrogen upto 15kg /ha/year and enhance upto 0.5 – 1% crop yield.

***Cyanobacteria* (Blue green algae):**

Nostoc, ***Anabaena***, ***Oscillatoria***, ***Aulosira***, ***Lyngbya*** etc. are the prokaryotic organisms and phototropic in nature. They play an important role in enriching paddy field soil by fixing atmospheric nitrogen and supply vitamin B complex and growth promoting substance which makes the plant grow vigorously. ***Cyanobacteria*** fixes 20-30 Kg/N/ha and increase10-15% crop yield when applied at 10 Kg/ha.

Azolla (Anabaena symbiosis)

It is a free floating, aquatic fern found on water surface having a cyanobacterial symbiont ***Anabaena azollae*** in their leaves. It fixes atmospheric nitrogen in paddy field and excrete organic nitrogen in water during its growth and also immediately upon trampling. Azolla contributes nitrogen, phosphorus (15-20 Kg/ha/month), potassium (20-25 kg/ha/month) and organic carbon etc. and increases 10-20% yield of paddy crops and also suppresses weed growth. Azolla also absorbs traces of potassium from irrigation water and can be used as green manure before rice planting. Azolla spp. are metal tolerant hence, can be applied near heavy metal polluted areas.

Phosphate Solubilising Bacteria

Pseudomonas fluorescens, ***Bacillus megatherium*** var. ***phosphaticum***, ***Acrobacter acrogens***, ***nitrobacter*spp.**, ***Escherichia freundii***, ***Serratia*spp.**, ***Pseudomonas striata***, ***Bacillus polymyxa*** are the bacteria have phosphate solubilising ability. 'Phosphobacterin' are the bacterial fertilizers containing cells of ***Bacillus megatherium*** var**.** ***phosphaticum***, prepared firstly by USSR scientists. They increased about 10 to 20 % crop yield and also produces plant growth promoting hormones which helps in phosphate solubilising activity of soil.

Phosphate solubilizing fungi

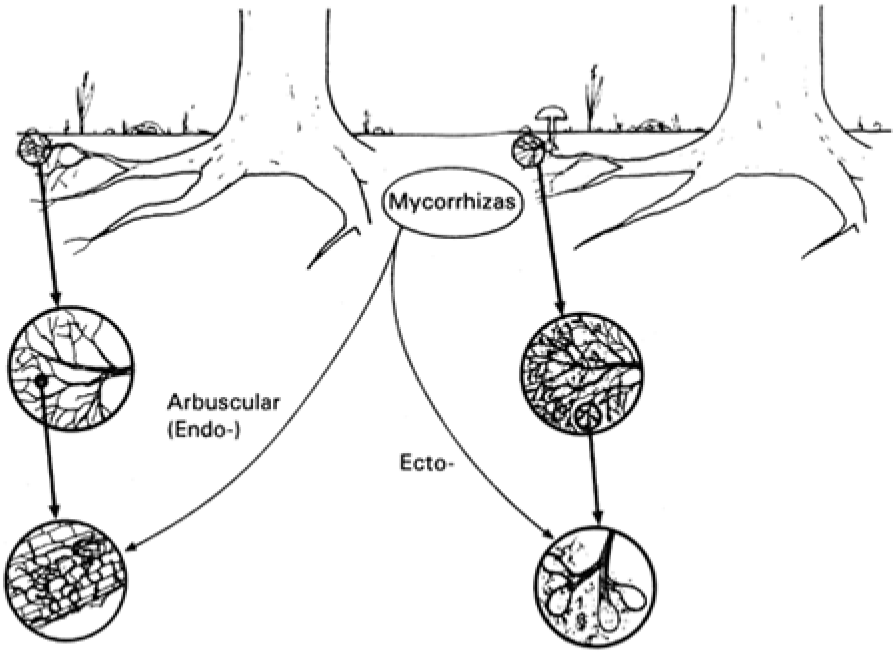
Some fungi also have phosphate dissolving ability e.g. ***Aspergillus niger***, ***Aspergillus awamori***, ***Penicillium digitatum*** etc.

Plant Growth Promoting Rhizobacteria (PGPR)

They are also called as microbial pesticides e.g. ***Bacillus spp***. and ***Pseudomonas fluorescence***. ***Serratia spp.*** and ***Ochrobactrum spp***. are able to promote growth of plants. ***Pseudomonas fluorescence*** application to the Black pepper enhanced uptake of nutrients which increased plant biomass. ***Fluorescent rhizobacteria*** improve the growth of *H. brasiliensis*.

Mycorrhiza

Mycorrhizas are developed due to the symbiosis between some specific root inhabiting fungi and plant roots as in the (**figure 6.)** and used as biofertilizers. They absorb nutrients such as manganese, phosphorus, iron, sulphur, zinc etc. from the soil and pass it to the plant. Mycorrhizal fungus increases the yield of crops by 30-40% and also produces plant growth promoting substances.



VAM fungi or Endomycorrhiza

They occur commonly in the roots of crop plants as in the (**figure 6.**). VAM fungal hyphae enhance the uptake of phosphorus and other nutrients that are responsible for plant growth stimulation including roots and shoot length. VAM also enhances the growth of black pepper and protects from *Phytophthora capsici*, *Radopholussimilis* and *Melvidogyne incognita*. VAM fungi enhance water uptake in plants and also provide heavy metals tolerance to plants.

18.10 Summary

Nutrients are substances used in biosynthesis and energy production and therefore are required for microbial growth. To obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients.

The Common Nutrient Requirements

Microbial cell composition shows that over 95% of celldry weight, which is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called **macroelements**or **macronutrients.**

All organisms, including microorganisms, require severalmicronutrientsortrace elementsbesides macroelements. The micronutrients- manganese, zinc, cobalt, molybdenum, nickel, and copper- are needed by most cells.

Requirement For Carbon, Hydrogen, And Oxygen

The requirements for carbon, hydrogen, and oxygen often are satisfied together. Carbon is needed for the skeleton or backbone ofall organic molecules, and molecules serving as carbon sourcesnormally also contribute both oxygen and hydrogen atoms.Many microorganisms are autotrophic, and most of these carry out photosynthesis and uselight as their energy source. Some autotrophs oxidize inorganicmolecules and derive energy from electron transfers.Thus many microorganisms cannot use CO_2as their sole carbon source but must rely on the presence of more reduced, complex molecules such as glucose for a supply of carbon.

Nutritional Classification Of Microorganisms

Microorganisms can be classified as either heterotrophs orautotrophs with respect to their preferred source of carbon.

Phototrophsuse light as their energy source; chemotrophsobtain energy from the oxidation of chemical compounds.

Microorganisms also have only two sources forelectrons.Lithotrophs(i.e., “rock-eaters”) use reduced inorganicsubstances as their electron source, whereas organotrophs extract electrons from organic compounds.

The large majority of microorganisms thus far studied are eitherphotolithotrophic autotrophs or chemoorganotrophic heterotrophs.

Some purple and greenbacteria are photosynthetic and use organic matter as their electrondonor and carbon source. These photoorganotrophic heterotrophs(photoorganoheterotrophs) are common inhabitants ofpolluted lakes and streams.

Thefourth group, the chemolithotrophic autotrophs (chemolithoautotrophs), oxidizes reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons forbiosynthesis.

Requirements For Nitrogen, Phosphorus And Sulfur

Nitrogen is needed for the synthesis of amino acids, purines,pyrimidines, some carbohydrates and lipids, enzyme cofactors,and other substances. Many microorganisms can use the nitrogenin amino acids, and ammonia often is directly incorporatedthrough the action of such enzymes as glutamate dehydrogenaseor glutamine synthetase and glutamate synthase.

Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and othercell components.

Sulfur is needed for the synthesis of substances like theamino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a sourceof sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

Growth Factors

Organic compounds required because theyare essential cell components or precursors of such componentsand cannot be synthesized by the organism are called growth factors.There are three major classes of growth factors: (1) aminoacids, (2) purines and pyrimidines, and (3) vitamins. Amino acidsare needed for protein synthesis, purines and pyrimidines for nucleic acid synthesis.

Knowledge of the specific growth factor requirements ofmany microorganisms makes possible quantitative growth-response assays for a variety of substances.

Nitrogen Fixation

The reduction of atmospheric gaseous nitrogen to ammonia iscalled nitrogen fixation.Nitrogen fixation occurs in (1) free-living bacteria (e.g.,***Azotobacter***, ***Klebsiella***, ***Clostridium***,and***Methanococcus***), (2) bacteria living insymbiotic association with plants such as legumes (***Rhizobium***), and (3) ***Cyanobacteria*** (***Nostoc and Anabaena***).

Nitrogen reduction is expensive and requires a large ATP expenditure. At least 8 electronsand 16 ATP molecules, 4 ATPs per pair of electrons, are required.The reduction of nitrogen to ammonia is catalyzed by the enzyme**nitrogenase.**Nitrogenase is a complex system consisting of two major protein components, a MoFe protein (MW 220,000) joined with oneor two Fe proteins (MW 64,000). The MoFe protein contains 2atoms of molybdenum and

28 to 32 atoms of iron; the Fe protein has 4 iron atoms.Nitrogenase is quite sensitive to $O_2$and must be protected from O_2inactivation within the cell.

The reduction of $N_2$to NH_3occurs in three steps, each ofwhich requires an electron pair.

The primary route of ammonia assimilation seems to be thesynthesis of glutamine by the glutamine synthetase- glutamatesynthase system.

(1) Free-living bacteria

Free-living aerobic nitrogen fixers include several species of genus ***Azotobacter***and some methylotrophic bacteria. ***Cyanobacteria*** are also free-living nitrogen fixers.

(2) Bacteria living in symbiotic association with plants such as legumes

Rhizobiumis the primary symbiotic nitrogen fixer. It lives innodules, growths extending from the roots of certain plants, usually legumes.In this symbioticrelationship, the plant benefits by receiving nitrogen in a usable form, and the bacteria benefit byreceiving nutrients needed for growth.***Rhizobium***can fix 150 to 200 kg of nitrogen per hectare of land per year, when paired with legume.***Rhizobium***multiplies in the vicinity of legume roots.Bacteroids contain the enzyme **nitrogenase.**

(3) Cyanobacteria

Some nitrogen fixers are found in symbiotic association with other organisms, which provide them with organic carbon sources. For example, the Cyanobacterium***Anabaena*** is found in pores of the leaves ofAzolla.

Microbes Used As Bio-Fertilizer

A number of microorganisms (bacteria fungi and algae) are considered as beneficial for agriculture and used as **biofertilizers.**Use of biofertilizers in the soil, makes the plants healthy as well as protect them from getting any diseases.Biofertilizers are cost effective, eco-friendly.They produces plant growth promoting substances IAA amino acids, vitamins etc.

Nitrogen fixing Bacteria

Rhizobia*, *Azorhizobium, Bradyrhizobium, these bacteria fix nitrogen that can be incorporated into plant protein.

Diazotrophs

These are aerobic chemolithotrophs and anaerobic photoautotrophs. These are non nodule forming bacteria. ***Azotobacterindicum*** occurs in acidic soil in sugarcane plant roots. It can apply in cereals, millets, vegetables and flowers through seed, seedlings soil treatment. ***Azospirillum*** is found to be very effective in increasing 10-15% yield of cereal crops and fixes N_2 upto 20-40% Kg/ha.Another diazotroph is ***Acetobacter diazotrophicus*** occurs in roots, stem and leaves of sugarcane and sugar beet crops as nitrogen fixer and applied through soil treatment.

***Cyanobacteria* (Blue green algae)**

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Mycorrhiza

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VAM fungi or Endomycorrhiza

VAM fungal hyphae enhance the uptake of phosphorus and other nutrients that are responsible for plant growth stimulation including roots and shoot length

18.11 Glossary

- **Actinomycete**- An aerobic, gram-positive bacterium that forms branching filaments (hyphae) and asexual spores.
- **Actinorhizae**- Associations between actinomycetes and plant roots.
- **Active transport**- The transport of solute molecules across a membrane against an electrochemical gradient; it requires a carrier protein and the input of energy.
- **Adenosine 5′-triphosphate (ATP)**- The triphosphate of the nucleoside adenosine, which is a high energy molecule or has high phosphate group transfer potential and serves as the cell's major form of energy currency.
- **Adenosine diphosphate (ADP)**- The nucleoside diphosphate usually formed upon the breakdown of ATP when it provides energy for work.
- **Adhesion**- A molecular component on the surface of a microorganism that is involved in adhesion to a substratum or cell. Adhesion to a specific host tissue usually is a preliminary stage in pathogenesis, and adhesins are important virulence factors.
- **Arbuscular mycorrhizal (AM) fungi**- The mycorrhizal fungi in a symbiotic fungus-root association that penetrate the outer layer of the root, grow intracellularly, and form characteristic much-branched hyphal structures called arbuscules.
- **Arbuscules**- Branched, treelike structures formed in cells of plant roots colonized by endotrophic mycorrhizal fungi.
- **Autotroph**- An organism that uses CO_2 as its sole or principal source of carbon.
- **Bacillus**- A rod-shaped bacterium.
- **Bacteroid**- A modified, often pleomorphic, bacterial cell within the root nodule cells of legumes; after transformation into a symbiosome it carries out nitrogen fixation.
- **Chemolithotrophic Autotrophs**- Microorganisms that oxidize reduced inorganic compounds to derive both energy and electrons; $CO_2$is their carbon source. Also called chemolithoautotrophs.
- **Chemoorganotrophic Heterotrophs**- Organisms that use organic compounds as sources of energy, hydrogen, electrons, and carbon for biosynthesis.

- **Chemoreceptors**- Special protein receptors in the plasma membrane or periplasmic space that bind chemicals and trigger the appropriate chemotaxic response.
- **Chemotrophs**- Organisms that obtain energy from the oxidation of chemical compounds.
- **Cometabolism**- The modification of a compound not used for growth by a microorganism, which occurs in the presence of another organic material that serves as a carbon and energy source.
- **Cyanobacteria**- A large group of bacteria that carry out oxygenic photosynthesis using a system like that present in photosynthetic eukaryotes.
- **Ectomycorrhizal**- Referring to a mutualistic association between fungi and plant roots in which the fungus surrounds the root tip with a sheath.
- **Endomycorrhizal**- Referring to a mutualistic association of fungi and plant roots in which the fungus penetrates into the root cells and arbuscules and vesicles are formed.
- **Endosymbiont**- An organism that lives within the body of another organism in a symbiotic association.
- **Endosymbiosis**- A type of symbiosis in which one organism is found within another organism.
- **Enzyme**- A protein catalyst with specificity for both the reaction catalyzed and its substrates.
- **Fixation**- The process in which the internal and external structures of cells and organisms are preserved and fixed in position.
- **Growth**- An increase in cellular constituents.
- **Growth factors**- Organic compounds that must be supplied in the diet for growth because they are essential cell components or precursors of such components and cannot be synthesized by the organism.
- **Heterocysts**- Specialized cells produced by ***Cyanobacteria*** that are the sites of nitrogen fixation.
- **Heterotroph**- An organism that uses reduced, preformed organic molecules as its principal carbon source.

- **Infection**- The invasion of a host by a microorganism with subsequent establishment and multiplication of the agent. An infection may or may not lead to overt disease
- **Infection thread**- A tubular structure formed during the infection of a root by nitrogen-fixing bacteria. The bacteria enter the root by way of the infection thread and stimulate the formation of the root nodule.
- **Infectivity**- Infectiousness; the state or quality of being infectious or communicable.
- **Invasiveness**- The ability of a microorganism to enter a host, grow and reproduce within the host, and spread throughout its body.
- **Lithotroph**- An organism that uses reduced inorganic compounds as its electron source.
- **Macromolecule**- A large molecule that is a polymer of smaller units joined together
- **Metabolism**- The total of all chemical reactions in the cell; almost all are enzyme catalyzed.
- **Microenvironment-** The immediate environment surrounding a microbial cell or other structure, such as a root.
- **Micronutrients**- Nutrients such as zinc, manganese, and copper that are required in very small quantities for growth and reproduction. Also called trace elements.
- **Mixotrophic**- Refers to microorganisms that combine autotrophic and heterotrophic metabolic processes (they use inorganic electron sources and organic carbon sources).
- **Nicotinamide Adenine Dinucleotide (NAD)**- An electron-carrying coenzyme; it is particularly important in catabolic processes and usually donates its electrons to the electron transport chain under aerobic conditions.
- **Nicotinamide Adenine Dinucleotide Phosphate (NADP)**- An electron-carrying coenzyme that most often participates as an electron carrier in biosynthetic metabolism.
- **Nitrifying bacteria**- Chemolithotrophic, gram-negative bacteria that are members of the family Nitrobacteriaceae and convert ammonia to nitrate and nitrite to nitrate.

- **Nitrogen fixation**- The metabolic process in which atmospheric molecular nitrogen is reduced to ammonia; carried out by ***Cyanobacteria, Rhizobium***, and other nitrogen-fixing bacteria.
- **Nitrogen saturation point**- The point at which mineral nitrogen, when added to an ecosystem, can no longer be incorporated into organic matter through biological processes.
- **Nitrogenase**- The enzyme that catalyzes biological nitrogen fixation.
- **Nutrient**- A substance that supports growth and reproduction.
- **Organotrophs**- Organisms that use reduced organic compounds as their electron source
- **Oxidizing agent or oxidant**- The electron acceptor in an oxidation-reduction reaction
- **Photolithotrophic Autotrophs**- Organisms that use light energy, an inorganic electron source (e.g., H_2O, H_2,H_2S), and CO_2 as a carbon source.
- **Photoorganotrophic Heterotrophs**- Microorganisms that use light energy and organic electron donors, and also employ simple organic molecules rather than CO_2 as their carbon source.
- **Phototrophs**- Organisms that use light as their energy source
- **Reducing agent or reductant**- The electron donor in an oxidation-reduction reaction.
- **Rhizosphere**- A region around the plant root where materials released from the root increase the microbial population and its activities.
- **Root nodule**- Gall-like structures on roots that contain endosymbiotic nitrogen-fixing bacteria (e.g.,***Rhizobium***or***Bradyrhizobium***is present in legume nodules).
- **Species(spp.)**-Species of higher organisms are groups of interbreeding or potentially interbreeding natural populations that are reproductively isolated. Bacterial species are collections of strains that have many stable properties in common and differ significantly from other groups of strains.
- **Stem-nodulating rhizobia**- Rhizobia (members of the genera ***Rhizobium***, ***Bradyrhizobium***,and ***Azorhizobium***) that produce nitrogen-

fixing structures above the soil surface on plant stems. These most often are observed in tropical plants and produced by ***Azorhizobium***.

- **Subsurface biosphere**- The region below the plant root zone where microbial populations can grow and function.
- **Sulfate reduction**- The process of sulphate use as an oxidizing agent, which results in the accumulation of reduced forms of sulfur such as sulfide, or incorporation of sulfur into organic molecules, usually as sulfhydryl groups
- **Symbiosis**- The living together or close association of two dissimilar organisms, each of these organisms being known as a symbiont.
- **Symbiosome**- The final nitrogen-fixing form of ***Rhizobium***that is active within root nodule cells.
- **Vitamin**- An organic compound required by organisms in minute quantities for growth and reproduction because it cannot be synthesized by the organism; vitamins often serve as enzyme cofactors or parts of cofactors.

18.12 Questions

Define the following:

1. Bacteroid
2. Cometabolism
3. Mixotrophs
4. Swarmer cells
5. Biofertilizers

Very short questions:

1. What are macroelements ? Name them and explain their role.
2. What are microelements ? Name them and explain their role.
3. What are growth factors? List three examples of growth factors.
4. Define the process called nitrogen fixation.
5. Name the two transport system through which phosphate moves across the plasma membrane.
6. Give names of two free living bacteria use in nitrogen fixation.
7. Write the reaction in which nitrogen is converted into ammonia by nirogenase enzyme.

8.
   a. Write the function of heterocyst and in which bacteria it is present.
9. Name any two sulfate reducer under anaerobic condition that can also able to fix nitrogen.
10. Name the aerobic nitrogen fixer which can fix N_2 in presence of oxygen.
11. Name the plant growth promoter which cause curling of roots
12. Give an example of an inorganic and an organic source if nitrogen for organisms.

Describe following:

1. What is nitrogen fixation? Briefly describe the structure and mechanism of action of nitrogenase.
2. Distinguish between autotrophs, heterotrophs, and decomposers.
3. How microorganism utilize nitrogen phosphorus and sulfur.
4. Give examples of microorganisms which synthesize riboflavin, vitamin B_{12}, vitamin C.
5. Write the function of some vitamins used by the microorganism. Give name of any two microorganisms with each fuction.

a) Biotin
b) Cyanocobalamin(B12)
c) Pantothenic acid
d) Niacin
e) Thiamine (B1)

6. Explain the structure of nitrogenase enzyme with its use.
7. Make the diagrammatic representation in with bacteria living in symbiotic association with plants such as legumes.
8. How much amount of nitrogen is fix by bacteria when it live with symbiotic in presence and absence of legumes.
9. Explain the role of *Frankia* in alder tree.
10. Explain in short the role of microorganism in biofertilizers. Give name of any two microorganism used as biofertilizer.

11. Explain the role of some nitrogen fixing bacteria use as biofertilizers.
12. What are diazotrophs. Explain with examples of different microorganisms.
13. What are Cyanobacteria. write its role as biofertilizer with examples.
14. What due you mean by azolla- anabaena symbiosis. Explain.
15. Give the names of phosphate solubilising bacteria.
16. What is meant byMycorrhiza. Explain with example.

Briefly explain following:

1. List the four nutritional groups into which all organisms can be placed according to the carbon and energy source they utilize.Define each of these groups as to what kind of carbon and energy source they utilize. Give an example of organisms that belong in each of these groups.
2. Explain the nitrogen fixation with diagram and reaction.
3. Explain the role of microorganism as biofertilizer with examples.
4. Explain the role of common nutrients require by microorganism.

Unit – 19

Food Microbiology

Structure of the Unit

19.1 Objectives

This unit gives an account for the foods which often provide an ideal environment formicrobial survival and growth.Microbial growth in foods involves successional changes, with intrinsic, or food-related, and extrinsic, or environmental, factors interacting with the microbial community over time.Food spoilage is a major problem in all societies. This can occur at any point in the course of food production, transport, storage, or preparation. Foods can be preserved by physical, chemical, and biological processes. Refrigeration does not significantly reduce microbial populations but only retards spoilage. Pasteurization results in a pathogen-free product with a longer shelf life. Some products can be fermented by help of microorganisms such as bread, sauerkraut, sufu, pickles, cheese wine, beer, spirit and many other foods also involves the use of fermentation processes.

19.2 Introduction

Foods, microorganisms, and humans have had a long and interesting association that developed long before the beginning of recorded history. Foods are not only of nutritionalvalue to those who consume them but often are ideal culture media for microbial growth. Fermentation by some microorganisms can lead to food preservation instead of food spoilage.

Microorganisms can be used to transform raw foods into gastronomic delights, including cheeses, pickles, sausages, and soysauce. Wines, beers, and other alcoholic products also are produced through microbial activity. On the other hand, foods alsocan serve as vehicles for disease transmission, and the detection and control of pathogens and food spoilage microorganisms are important parts of food microbiology. During the entire sequence of food handling, from the producer to the final consumer, microorganisms can affect food quality and human health.

Microbial growth in foods can result in either preservation orspoilage, depending on the microorganisms involved and the foodstorage conditions. Contamination by disease causing microorganisms can occur at any point in the food-handling sequence.

Foods, because they provide nutrients for us, also are excellentenvironments for the growth of microorganisms. Microbialgrowth is controlled by factors related to the food itself, or intrinsic factors,and also to the environment where the food is being stored. **The intrinsic or food-related factors** include pH, moisturecontent, water activity or availability, oxidation-reduction potential, physical structure of the food, available nutrients, and the possible presence of natural antimicrobial agents. **Extrinsic or environmental factors** include temperature, relative humidity, gases(CO_2,O_2) present, and the types and numbers of microorganismspresent in the food.

19.3 Important microbes involved in spoilage of food, meat, poultry, fish and sea food, vegetables and dairy products

Anything that people eat or drink can be used asfood by microorganisms, too. Most substances consumedby humans are derived from plants,which of course grow in soil, or from animals, which livein contact with soil and so have soil organisms on them.

Although soil organisms usually are not human pathogens, many can cause food spoilage. The handling offoods from harvest or slaughter to human consumptionprovides many more opportunities for the foods to become contaminated with microorganisms. Unsanitary practices by foodhandlers and unsanitary working conditions frequently lead to contamination of foods with pathogens. Improperstorage and preparation procedures athome and especially in restaurants canlead to further contamination with pathogens. Improperrefrigeration of prepared foods is a major source of foodpoisoning. Microbial growth in foods can lead to visible changes, including a variety of colors caused by spoilage organisms.

Meats And Poultry

Meat, with their high nutritional value and the presence of easily usable carbohydrates, fats, and proteins, provide ideal environments for microbial spoilage. Proteolysis and putrefaction are typical results of microbial spoilage of such high-protein materials.

Meat:

Meat animals arrive at slaughterhouses with numerous and varied microorganisms in the gut and feces, on hides and hoofs, and sometimes in tissues. At least 70 pathogens have been identified among these microbes. Nearly all animal carcasses in slaughterhouses in the United States are inspected by a veterinarian or a trained inspector, and those found to be diseased are condemned and discarded.

Some of the mostcommon diseases identified in slaughterhouses areabscesses,pneumonia, septicemia,enteritis, toxemia,nephritis, andpericarditis. Lymphadenitis, an inflammation of the lymph nodes, is especially common in sheepand lambs.

Even after animals are slaughtered and the carcasses are hung in refrigerated rooms to age, microorganisms sometimes spoil the meat. Several moldsgrow on refrigerated meats, and ***Cladosporium herbarum***can grow on frozen meats. Mycelia of***Rhizopus*** and***Mucor***produce a fluffy, white growth referred to as ‘‘whiskers’’ on the surfaces of hanging carcasses. The bacterium ***Pseudomonasmephitica***releases hydrogen sulfide and causes green discoloration on refrigerated meat under low oxygen conditions. Several species of ***Clostridium***cause putrefaction called**bone stink**deep in the tissues of large carcasses.Ground meats sometimes contain helminth eggs andalways contain large numbers of lactobacilli and molds.In most areas butcher shops are required to maintaintwo separate meat-grinding machines - one for pork andone for other meats. This practice is encouraged becauseit is difficult to clean a machine thoroughly enough toeliminate any possibility of transmitting raw pork bits,which may carry the helminth***Trichinella spiralis***, thecause of trichinosis, to other meats***Lactobacilli*** in ground meats produce acidsthat retard the growth of enteric pathogens. Nevertheless, ground meats are subject to spoilage even whenrefrigerated and should be frozen if they are not to beused within a day or two. All meats, but especiallyground meats, should be cooked thoroughly to killpathogens.More than 20 bacterial genera have been found ondressed poultry, and mishandling of poultry in restaurants accounts for many foodborne infections. Nearlyhalf these infections have been traced to ***Salmonella***and a fourth each to ***Clostridium perfringens*** and***Staphylococcus aureus***. Freezing fails to rid poultry of***Salmonella***. **Pseudomonads** and several other Gram-negative bacteria are common contaminants of poultry,where they cause slime and offensive odors.

Poultry:

You might suppose that eggs, with their hard shells,would be free of microbial contamination. Most are, but the shells are porous, and pseudomonads and some other bacteria, as well as fungi such as ***Penicillium***, ***Cladosporium***,and***Sporotrichum***, grow on eggshells. These microbes can pass through pores in the shells to infect the inside of eggs.***Salmonella***also survives on eggshells and can enter broken eggs or be deposited with bits of shell in foods. Hens infected with***S. pullorum***lay infected eggs. Those of you who have ever cleaned the internal organs out of a chicken have no doubt seen that the shell is not laid down around an egg until it has traveled a goodly distance down the oviduct. The sperm fertilize the egg at the top of the oviduct where there is no shell. Bacteria such as***Salmonella***can also get inside the egg before the shell is laid down. The shell does not have to be cracked for an egg to be filled with ***Salmonella***. The CDC reports that 1 out of every 10,000 eggs has***Salmonella***inside the shell. Any pathogens on eggshells or in eggs can be transmitted to humans unless eggs and foods that contain them are cooked thoroughly. Eating raw eggs, such as in eggnog, is a calculated risk.

Fish And Sea Food

Algal toxinscontaminatefish and thus affect the health of marine animals higher in the food chain; they also can contaminate shellfish and fin fish, whichare later consumed by humans. Most toxins are produced by dinoflagellates, but some diatoms also are toxic. Major human diseases that result from algal toxins in marine products include amnesic, diarrhetic, and neurotoxicshellfish poisoning. The complex algal toxins, which are temperature stable, are known to cause peripheral neurological system effects, often in less than one hour after ingestion.

Fish:

Fresh fish abound with microorganisms. Several speciesof enteric bacteria and clostridia, enteroviruses, andparasitic worms are commonly found on or in fresh fish.Many of these organisms survive shipment of fishpacked in crushed ice, especially if the fish are packedtoo tightly together or are pressed against the slats ofcontaminated crates.

Sea Food:

Shellfish, such as oysters and clams, carry many of the same organisms as fish. Raw oysters typically carry ***Salmonella typhimurium*** and sometimes***Vibrio cholerae***. Clams are especially likely sources of human infection because they are filter feeders - that is, they obtain food by filtering water and extracting microbes. If clams are exposed to increased levels of sewage, red tides, and other sources of large numbers of pathogens or toxin producers, clamming may be prohibited until the numbers of organisms decrease. Scallops are less likely to transmit diseases to humans because only the muscular part of the organism, and not its digestive tract, is eaten.

Among crustaceans, shrimp are extremely likely tobe contaminated. Some studies have shown that overhalf the breaded shrimp on the market contain in excess of 1 million bacteria (and more than 5,000 coliforms) per gram. Such high bacterial counts probablyresult from growth of bacteria during processing beforethe shrimp are frozen. Lobsters and crabs are evenmore perishable than shrimp and can carry a variety ofenteric pathogens. Along the U.S. Gulf Coast, improperly cooked crabs have transmitted cholera. Crabsalso carry ***Clostridium botulinum*** and the pathogenic fungi ***Cryptococcus***and***Candida***. Keep in mind, however, that the mere presence of microorganisms in seafoods or in any other foods does not necessarily mean that the foods arespoiled or contaminated with pathogens. In fact,***Lactobacillus bulgaricus***, which produces hydrogen peroxide,can be used to inhibit growth of other organisms foundon seafood.

Vegetable And Dairy Products

Vegetables And Fruits:

Millions of commensal bacteria, especially***Pseudomonas fluorescens***, are found on the surfaces of fruits and vegetables. These foods also easily become contaminated with organisms from soil, animals, air, irrigation water, and equipment used to pick, transport, store, or process them. Pathogens such as ***Salmonella***, ***Shigella***, ***Entamoeba histolytica***, ***Ascaris***, and a variety of viruses can be transmitted on the surfaces of fruits and vegetables. However, the skins of most plant foods contain waxes and release antimicrobial substances,both of which tend to prevent microbial invasion of internal tissues.

Cantaloupes (musk melon) present a special problem. Their nettedsurfaces develop biofilms of***Salmonella***, which grow intothe fissures beneath the netting. Fimbriaeandcellulosesecretions attach the bacteria firmly andthickly to the surface of the cantaloupe rind. The upperlayers of the biofilm protect bacteria located deeperwithin the film from the effects of sanitizing solutions.About 5% of melons imported into the United Statesfrom Mexico have such layers of***Salmonella***. When youcut through the rind, you can drag bacteria over the cutsurfaces of the flesh.Certainvegetables are particularly vulnerable to microbial attack and spoilage. Leafy vegetables and potatoes are susceptible to bacterial soft rot by ***Erwiniacarotovora***. The fungus ***Phytophthora infestans***causedthe Irish potato famine of 1846.

Fruits are likewise susceptible to spoilage by microbial action. Tomatoes, cucumbers, and melons can bedamaged by the fungus***Fusarium***, which causes soft rotand cracking of tomato skins. Fruit flies pick up thefungus from infected tomatoes and transmit it tohealthy ones as they deposit eggs in surface cracks.Other insects pierce tomatoes to feed on them and atthe same time introduce ***Rhizopus***, which breaks downpectin and can turn a tomato into abag of water. Fresh fruit juices,because of their high sugar and acidcontent, provide an excellent medium for growth of molds, yeasts,and bacteria of the genera ***Leuconostoc*** and ***Lactobacillus***.Grapesand berries are damaged by a widevariety of fungi, and large numbersof stone fruits, such as peaches, canbe destroyed overnight by brown rotdue to***Moniliafructicola***. ***Penicilliumexpansum***, which grows on apples, produces the toxinpatulin, which can easily contaminate cider. Other***Penicillium*** species produce blue and green mold on citrusfruits.

Dairy Products:

Modern mechanized milking and milk handling has greatly reduced the microbial content of raw milk. However, breeding dairy cattle for increased milk productionhas resulted in exceptionally large udders and teats that easily admit bacteria. The first few milliliters of milk drawn from such cows can contain as many as 15,000 bacteria per milliliter, whereas the last milk drawn is free of microorganisms. Most microorganisms in freshly drawn milk are ***Staphylococcusepidermidis***and***Micrococcus***, but ***Pseudomonas***, ***Flavobacterium***, ***Erwinia***, and some fungi also can be present. Microorganisms have many opportunities to enter milk before it is consumed. Hand milking, as opposed to mechanical milking, allows organisms from the body of the cow to enter the milk. These include ***Escherichiacoli***, which give milk a fecal flavor, and ***Acinetobacterjohnsoni*** (formerly known as ***Alcaligenesviscolactis***), which is especially abundant during summer months and causes a viscous slime to form in milk. Storing, transporting, and processing milk allow for contamination with any organisms in containers and for growth of those already present. Infectious organisms in milk usually come either from infected cows or from unsanitary practices of milk handlers. Diseased cattle can transmit ***Mycobacterium bovis*** and ***Brucella*** species to their milk. Dairy herds are tested for tuberculosis (with infected animals removed from the herd) and vaccinated against brucellosis (undulant fever); hence the risk of transmitting these diseases to humans is small. ***Staphylococcus aureus***, ***Salmonella*** species, and other enteric bacteria can enter milk through unsanitary handling. Bacteria and molds will grow even in dried milk, which is made from pasteurized liquid milk but is unsterilized, if the water content of the powder reaches 10%.Some microorganisms, such as certain ***Pseudomonas*** species and some soil organisms, grow in refrigerated milk. These organisms are psychrophilic; although they normally grow at higher temperatures, they can grow at 5^0C (refrigerator temperature). They also survive the concentration of chlorine normally used to purify drinking water. Organisms that sour milk include***Streptococcuslactis***and species of***Lactobacillus***. When these microbes release enough lactic acid to bring the pH below 4.8, the milk proteins coagulate, and the milk is said to have soured. Souring of milk does not mean that the milk is unsafe for human consumption, but it does greatly alter the taste and appearance of the milk.

19.4 Food Poisoning

Many microorganisms contaminating food and water can causeacute gastroenteritis or inflammation of the stomach and intestinal lining. When food is the source of the pathogen, the condition is often called food poisoning.

Gastroenteritis can arise intwo ways. The microorganisms may actually produce a food-borne infection.That is, they may first colonize the gastrointestinal tract and grow within it, then either invade host tissues orsecrete exotoxins. Alternatively the pathogen may secrete an exotoxin that contaminates the food and is then ingested by the host.This is sometimes referred to as a **food intoxication**because thetoxin is ingested and the presence of living microorganisms is notrequired. Because these toxins disrupt the functioning of the intestinal mucosa they are called enterotoxins. Common symptoms of enterotoxin poisoning are nausea, vomiting, and diarrhea.

Causes of food poisoning

a) Poisons derived from plant and animal sources.
b) Such standard chemicals added to the food.
c) Excess use of preservation in food.
d) Presence of higher population of microorganisms in food.
e) Toxins produced by various types of microorganisms.

Types of food poisoning

There are two types of food poisoning.

1. Food intoxication. eg) Botulism, Staphylococcal food poisoning.
2. Food infection. eg) Shigellosis (Bacillary dysentry), Enteropathogen Escherichia, Cholera, Brucellosis.

Botulism : Botulism is a food borne disease due to exotoxin produced by the bacterium ***Clostridium botulinum***. The main sources of this disease are canned food and preserved foods. This disease affects the nervous system so it is called neurotoxin.

Staphylococcal food poisoning or staphylococcal enterotoxemia : The causative organism for the disease is ***Staphylococcusaureus***. The main sources for the disease are potato salad, cream-filled bakery goods and dry skim milk. The disease is characterized by sudden nausea, vomiting and diarrhoea.

Enterococcus food poisoning : The causative organism for this disease is ***Strepcococcusfaecalis***. It is frequently found in the intestinal tract of human and animal. The disease is characterized by nausea, frequently vomiting, colicky pain and diarrhoea.

Reason for food poisoning caused by bacteria:

If food is not preserved properly, then it gets spoiled due to the action of enzymes and also by the action of bacteria and fungi which grow in it. Generally, but not always we can come to know about the stale food from its unpleasant smell and bad taste. Such a food when eaten leads to food poisoning. Bacteria and spores of fungi are always present in air. They are also sticking to the containers of the food, to our unwashed hands, and unwashed vegetables and fruits. They grow and multiply by eating the nutrients present in the food and in the process spoil it. Canned food products, meats, fish, pastry, milk are the common food products, which generally get spoiled and cause food poisoning. Some examples of food poisoning caused by certain bacteria.

Bacteria That Cause Acute Bacterial Diarrheas and Food Poisonings

Organism	**Incubation Period (Hours)**	**Vomiting**	**Diarrhea**	**Fever**	**Epidemiology**	**Pathogenesis**
Staphylococcus aureus	1– 8	+ + +	+	–	Staphylococci grow in meats, dairy and bakery products and produce enterotoxins.	Enterotoxins act on receptors in gut that transmit impulse to medullary centers; may also act as superantigens
Bacillus cereus	2–16	+ + +	+ +	–	Reheated fried rice causes vomiting or diarrhea	Enterotoxins formed in food or in gut from growth of *B. cereus.*
Clostridium perfringens	8–16	±	+ + +	–	Clostridia grow in rewarmed meat dishes.	Enterotoxin produced during sporulation

					Huge numbers ingested	in gut, causes hypersecretion
Clostridium botulinum	18–24	±	Rare	–	Clostridia grow in anaerobic foods and produce toxin	Toxin absorbed from gut and blocks acetylcholine release a neuromuscular junction
Escherichia coli	24 –72	±	+ +	–	Organisms grow in gut and are a major cause of traveler's diarrhea	Heat-labile (LT) and heat-stable(ST) enterotoxins cause hypersecretion in small intestine
Vibrio parahaemolyticus	6–96	+	+ +	±	Organisms grow in seafood and in gut and produce toxin, or invade	Toxin causes hypersecretion;vibrios invade epithelium; stools may be bloody
Vibrio cholerae	24 –72	+	+ + +	–	Organisms grow in gut and produce	Toxin causes hypersecre

					toxin.	tion in small intestine. Infective dose >10^5 vibrios
Shigella spp.	24 –72	±	+ +	+	Organisms grow in superficialgut epithelium. *S. dysenteriae* produces toxin	Organisms invade epithelialcells; blood, mucus, andneutrophils in stools. Infective dose <10^3 organisms
Salmonella spp. (gastroenteritis)	8 –48	±	++	+	Organisms grow in gut	Superficial infection of gut,little invasion. Infective dose>10^5 organisms
Salmonella typhi(typhoid fever)	10–14 days	±	±	+ +	Bacteria invade the gutepithelium and reach thelymph nodes, liver, spleen,and gallbladder	Symptoms probably due toendotoxins and tissueinflammation. Infective dose ≥10^7 organisms

Clostridium difficile	Days to weeks	–	+ + +	+	Antibiotic-associated colitis	Toxin causes epithelial necrosisin colon; pseudomembranouscolitis
Campylobacter jejuni	2–10 days	–	+ + +	+ +	Infection by oral route fromfoods, pets. Organism growsin small intestine	Invasion of mucous membrane. Toxin production uncertain
Yersinia enterocolitica	4–7 days	±	+ +	+	Fecal-oral transmission Food-borne. Animals infected	Gastroenteritis or mesenteric adenitis. Occasional bacteremia. Toxin producedoccasionally.

19.5 Food preservation

Principle:

Foods originate as biological material which hasbeen growing and developing prior to harvest; post-harvest, foods continue to change. These changes caninclude:

- Enzymic developments such as ripening which insome circumstances occurs off the plant. While ripening itself is desirable, sometimes undesirable enzymic changes may occur in foods.
- Drying out of the food.
- Physical damage through poor transportation andhandling, and the ensuing enzymic changes that may result.
- Microbial attack resulting in spoilage.
- Physical damage by pests such as insects or rodents and the subsequent microbial damage thatwill likely follow.
- Chemical changes such as oxidation.
  Food preservation aims at controlling such naturalprocesses of decay in order to maintain high quality, safe and nutritious foods with an extended shelf life than if the food was left at ambient conditions. Thus foods are available out of season and in regions of the world where they are not indigenous, both adding to consumer choice. Some processes of food preservation maintain the food in a native state. Other processes transform the food into a different product which may be desirable in its own right.

Methods:

Many of the methods of food preservation, they include canning using moist heat; refrigeration, freezing; lyophilization and drying; and theuse of radiation. A number of chemical food additivesalso are used to retard spoilage.

Canning - The most common method of food preservation is canning - the use of moist heat under pressure. Thismethod, analogous to laboratory autoclaving, is used topreserve fruits, vegetables, and meats in metal cans orglass jars. If properly carried out, canningdestroysallharmful spoilage microorganisms, includingmost heat-resistant endospores; prevents spoilage;and averts any hazard of disease transmission. Foods sotreated may remain edible for years.

Some thermophilic anaerobic endospores, such asthose of ***Bacillusstearothermophilus***, may remain aliveeven after commercial canning. Hence canned foodshould not be stored in a hot environment, such as a cartrunk or hot attic. At elevated temperatures, the endospores can germinate, grow, and cause spoilage.Usually this produces gas, which causes the ends of cansto bulge so that the ends can be pressed up and down.Such spoilage also usually produces acid, which gives asour flavor. Such changes are called thermophilic anaerobic spoilage. In some cases, however, spoilage due togrowth of such spores does not cause cans to bulge withgas; such spoilage is calledflat sour spoilage. Cans canalso bulge due to mesophilic spoilage, which occurswhen canning procedures were improperly followed orthe seal has been broken. This type of spoilage can takeplace at room temperature, unlike flat sour and thermophilic spoilage, which occur only in properly processedand sealed cans that have been stored at hightemperatures.

Because of the danger of botulism and other kindsof spoilage in improperly processed canned foods,theU.S. Department of Agriculture (USDA) now recommends that all low-acid foods be processed by pressure cooking. The USDA also recommends that jelliesand jams, formerly packed hot and sealed with wax, beprocessed in a boiling water bath and sealed with lidslike other canned goods to prevent accumulation of moldtoxins.

Sugar in jelliesand jams increases osmotic pressure and retards growthof microorganisms. (Saccharin does not have this effect,so artificially sweetened products may require more stringent precautions to prevent spoilage than do those with ahigh sugar content.) Likewise, the high acidity of picklesand other sour foods helps prevent microbial growth.

Refrigeration and Freezing- Refrigeration at temperatures slightly above freezing(about 4^0C) is suitable for preserving foods for only a fewdays. It does not prevent growth of psychrophilic organisms that can cause food poisoning. Freezing, anothercommon method of food preservation, involves storage offoods at temperatures below freezing (about -10^0Cinmost home freezers). All kinds of foods can be preservedby freezing for several months and some for much longerperiods of time. An advantage of freezing is that it preserves the natural flavor of foods better than does canning.However, freezing has two disadvantages: (1) It causessome foods, especially fruits and watery vegetables, to become somewhat soft upon thawing and detracts from theirappearance. (2) Although freezing prevents growth ofmost microorganisms, it does not destroy them. As soon asfood begins to thaw, microorganisms begin to grow. Infact, freezing and thawing of foods actually promotesgrowth of microorganisms. Ice crystals puncture cell andplasma membranes and cell walls, allowing nutrients to escape from foods. These nutrients are then readily availableto support microbial growth. Consequently, it is importantnever to thaw and refreeze foods.

Drying and Lyophilization -Drying (dessication, or dehydration) is one of the oldestmethods used for food preservation. A certain level ofwater activity is necessary for microbial growth. Ideally,if more than 90% of the water is removed, the food canbe stored in that state. Drying stops microbial growthbut does not kill all microorganisms in or on food. Foodscan be dehydrated by natural means, such as sun drying,or by artificial means, by passing heated air over thefood with controlled humidity. Addition of salt, highconcentrations of sugar, or chemical preservatives thatalter osmotic pressure and reduce water content are often included in the drying process.

Currently, lyophilization (freeze-drying) is employed in the food industry almost exclusively for thepreparation of instant coffee and dry yeast for bread-making. (The technique is also used to preserve bacterial cultures. Lyophilizationinvolves drying frozen food in a vacuum. The processyields higher quality foods than are produced by ordinarydrying methods.

Irradiation- Irradiation of food as a method of preservation is stillnew and quite controversial due to public concern aboutthe hazards of radiation. There are two categories ofradiation used to control microorganisms in food: non-ionizing and ionizing.

Ultraviolet (UV) radiation, a form ofnonionizingradiation, is limited by poor penetrating power. The radiation wavelength and exposure time also determinethe effectiveness of this method of preservation. UVradiation is effective as a sanitizing agent for food-processing equipment and other surfaces. Microwaves,another form of nonionizing radiation, are useful forfood preparation, cooking, and processing but not forpreservation. Microwaves do not kill microorganismsdirectly, but the heat generated during cooking canbe microbicidal. Uneven heating can interfere withthis antimicrobial activity. Therefore, frequent rotation of food is necessary during microwaving.

Ionizing radiation, such as gamma rays, has great penetrating ability and is microbicidal. This type of radiationcan be utilized either before or after packaging, depending on the foodstuff. Gamma radiation from cobalt-60 orcesium-137 has been used in Japan and some Europeancountries for food preservation for a number of years.The U.S. Food and Drug Administration (FDA) has declared such irradiation safe for preserving certain foods.Radiation has successfully been used to control microbialgrowth on fresh fish during transport to market and to killinsects in spices. It has also proved effective in reducingspoilage of fresh fruits and vegetables. Most recently theUSDA proposed rules providing for the irradiation offresh poultry. The public has much skepticism about irradiated foods. It should be emphasized that irradiationkills microorganisms but does not cause the food itself tobecome radioactive.

Chemical Additives-A large number of chemical compounds are added to various foods to kill microorganisms or retard their growth.A few examples and their uses are described here.

Organic acids, some of which occur naturally insome foods, lower the pH of foods enough to preventgrowth of human pathogens and toxin-producing bacteria. Acids such as benzoic, sorbic, and propionic inhibit growth of yeast and other fungi in margarine, fruitjuices, breads, and other baked goods.

Alkylating agents such as ethylene oxide and propylene oxide are used only in nuts and spices. Sulfur dioxide, which is most effective at an acidic pH, is used in theUnited States only to bleach dried fruits and to eliminate bacteria and undesired yeasts in wineries. Ozone, ahighly reactive form of oxygen, is used to kill coliformbacteria in shellfish and to treat water used in beverages.

It has the advantage of leaving no residue. The disadvantages of ozone are that it tends to give foods a rancid taste by oxidizing fats, and it can damage molecules,especially lung lysozyme, if inhaled.

Sodium chloride, perhaps one of the first food additives, increases osmotic pressure in foods, keeping mostmicroorganisms from growing. Salt used to cure meats isespecially useful in preventing growth of clostridia deep intissues, although fungi eventually grow on the surfaces ofsalted foods. Salt dehydrates bacteria and makes it difficultfor them to take in water and nutrients. A recent discoverysuggests that salt, in addition to increasing osmotic pressure, may create electric charges on the surface of meats,preventing bacteria from adhering to the surfaces.

Still other chemical additives have special uses. Halogen compounds, such as sodium hypochlorite, disinfectwater and food surfaces. Gaseous chlorine preventsgrowth of microorganisms on food-processing equipment. Nitrates and nitrites suppress microbial growth inmeats, especially ground meats and cold cuts. Duringcooking, however, they can be converted to nitrosamines, which are carcinogenic and toxic to the liver.We continue to use nitrates and nitrites because we haveno good alternatives, particularly for sausage. The namebotulismcomes from the Latin word for sausage, socommon was food poisoning from sausage in the daysbefore the use of nitrites. Another reason nitrites areused is to keep colors bright, especially the red color offresh meat. Carbon dioxide kills microorganisms, exceptfor some fungi, in carbonated beverages. It also retardsmaturation of fruits and decreases spoilage during shipment. Finally, quaternary ammonium compounds(quats) can be used to sanitize many objects - utensils,cows' udders, fresh vegetables, and the surfaces of egg-shells, which they do not penetrate.

Antibiotics - Antibiotics are added to foods and milk in some countries. In the United States only the anticlostridial agent**nisin**, a bacteriocin produced naturally during fermentation of milk by ***Streptococcuslactis***, may be used. Antibiotic use in foods and milk is prohibited for thefollowing good reasons:

- The antibiotics might be relied on instead of goodsanitation.
- Pathogenic microorganismsmight develop resistanceto the antibiotics, so treatment of the diseases theycause would become difficult or impossible.
- Humans might be sensitized to the antibiotics andsubsequently suffer allergic reactions.
- The antibiotics might interfere with the activitiesof microorganisms essential for fermenting milkand making cheese.

Pasteurization of milk

Prevention of spoilage and disease transmission in milkbegins with the maintenance of health in both dairy animals and in milk handlers. In the past, bovine tuberculosis was sometimes transmitted to humans fromcows' milk. Many children were infected early in life andusually died by age 15. Establishment of mandatory testing of dairy herds for tuberculosis every 3 years (thetime it takes an infection to progress to a transmissiblestage) has greatly decreased incidence of the disease inthe United States.

Milk is collected under clean, but not sterile, conditions and is usually subjected to pasteurization. Twomethods of pasteurization are currently in use:

- In high-temperature short-time **(HTST)** pasteurization,orflash pasteurization, milk is heated to 71.6^0C for at least 15 seconds.
- Inlow-temperature long-time **(LTLT)** pasteurization, or the holding method, milk is heated to62.9^0C for at least 30 minutes.

Both methods destroy vegetative cells of pathogenslikely to be found in milk and decrease the number oforganisms that can cause souring.

Following pasteurization, milk is quickly cooled and refrigerated in sealedcontainers until it is used.

Milk can be preserved and kept safe to drink bymeans other than pasteurization. In Europe and increasingly in parts of the United States, milk may be sterilizedbyultra-high temperature (UHT) treatmentrather thansimple pasteurization. UHT milk is heated to 87.8^{0}Cfor 3seconds. Such milk can be kept in sealed paper containers - called aseptic packaging and stay unrefrigeratedfor about 6 months. Milk can also be preserved as cannedcondensed milk, which also is sterilized. It is reconstitutedby adding an equal volume of water. Although sterilizedmilk is completely free of microorganisms, the heat treatment necessary to render it sterile alters its flavor.Various chemical additives are sometimes used inmilk. Addition of hydrogen peroxide to milk reduces thetemperature required to destroy most pathogens. However, it fails to kill mycobacteria, and its use in milk hasbeen banned in the United States for this reason.

19.6 Standardization of fish products and agencies in india

1. **Microbes In Milk**

In addition to being a nutritious food for humans, milk provides a favourable environment for the growth of microorganisms. Yeasts, molds and a broad spectrum of bacteria can grow in milk, particularly at temperatures above 16°C.

Sources of microorganisms in milk:

Milk secreted into the udder is sterile. The first few strippings of milk contain more amount of bacteria and the population of bacteria gradually decreases. It is observed that last strippings of milk from the udder seems to be free from bacteria. This clearly indicates that most of the microorganisms found in the milk are from external source. The different sources of microorganism in milk are from 1) the udder of the cow, 2) skin of the cow, 3) utensils and equipment, 4) feeds, 5) air of the cow shed, 6) milking persons and 7) water.

1) **Udder of the cow** : The milk producing animals should be kept neat and clean. More care should be taken to keep the flanks, udder and teats clean. The interior of the teats of the udder is warm and contains the last remains of the milk which has more microbes which would have entered through opening of teat and multiplied.

2) **Skin of the cow** : Soil, faeces and dirt adhere to the skin and hairs of the cow. Hair, dirt and dust fall in to milking utensils or into the teat cups of milking machines. Most of the organisms from these sources are gas producers and putrefactive types. Faeces contain enormous quantity of organisms and most of them are pathogenic microorganisms.
3) **Utensils and equipments** : Milking utensils and equipments are the major sources of contamination of milk. They have to be washed properly with detergent. Further the utensils and equipments should be cleaned with hot water, air and steam to remove all the spore forming, fluorescent and coliform microorganisms.
4) **Feeds** : Microorganisms are found everywhere. They are present in abundant in vegetation and soil. Dry feeds have more amount of bacteria and less amount of fungi. These organisms contaminate the milk.
5) **Air of the cow shed** : The air of the cow shed is greatly contaminated by dry dirt and dust. During the mixing of feeds and during the cleaning process of the floor, the air of the cow shed is highly contaminated and it is passed on to the milk.
6) **Milking persons** : Pathogenic microorganisms may enter into the milk through milking persons. They should wear clean clothes and properly wash their hands before milking. Nails should be cleaned and trimmed. Discharge from sneezing, coughing and nose blowing should not reach the atmosphere, equipment or the milk. Some of the organisms may be carriers of diseases.
7) **Water** : Pure water should be used for cleaning purposes. Water exposed to contamination spreads the microorganisms. Water should be free from coliform organisms. Chlorination of water prevents such contamination.

Once microorganisms get into the milk their numbers increase rapidly. It is more effective to exclude microorganisms than to try to control microbial growth once they have entered the milk. Milking equipment should be washed thoroughly before and after use - rinsing is not enough.

Bacterial types commonly associated with milk are given in Table .

Pseudomonas	Spoilage
Brucella	Pathogenic

Enterobacteriaceae	Pathogenic and Spoilage
Staphylococci	
Staphylococcus aureus	Pathogenic
Streptococcus	
S. agalactiae	Pathogenic
S. thermophilus	Acid fermentation
S. lactis	Acid fermentation
S. lactis-diacetyllatic	Flavour production
S. cremoris	Acid fermentation
Leuconostoc lactis	Acid fermentation
Bacillus cereus	Spoilage
Lactobacillus	
L. lactis	Acid production
L. bulgaricus	Acid production
L. acidophilus	Acid production
Propionibacterium	Acid production
Mycobacterium tuberculosis	Pathogenic

19.7 Role of microbes in production of various food products

The use of microorganisms in making bread, cheese, andwine is as old as civilization itself. Long before any microorganisms were identified, milk was being made into cheese and fermented beverages, and bread was being leavened by microbes. In modern food production, specific organisms are purposely used to make a variety of foods.

Bread

In bread, yeast is used as aleavening agent—that is, to produce gas that makes the dough rise. A particular strain of***Saccharomycescerevisiae***isadded to a mixture of flour, water, salt, sugar, and shortening. The mixture is allowed to ferment at about 25^0C for several hours. During fermentation, yeast cells produce a little alcohol and much carbon dioxide. As the carbon dioxide bubbles become trapped in the dough, they cause the dough to increase in bulk and acquire a lighter, finer texture. When the dough is baked, the alcohol and carbon dioxide are driven off. The bread becomes light and porous because of spaces created by carbon dioxide bubbles. Home bakers often use activated dry yeast, a product that is prepared by the lyophilization of yeast cells.

Dairy Products

Microorganisms are used in making a wide varietyof dairy products. Cultured buttermilk, popular in the United States, is made by adding***Streptococcus cremoris*** to pasteurized skim milk and allowing fermentation to occur until the desired consistency, flavor, and acidity are reached. Other organisms - ***Streptococcus lactis***, ***S. diacetylactis***and ***Leuconostoccitrovorum***, ***L. cremoris***,or***L. dextranicum*** - make buttermilk with different flavors because of variations in fermentation products. Sour cream is made by adding one of these organisms to cream. Yogurt is made by adding***Streptococcus thermophilus*** and ***Lactobacillusbulgaricus***to milk. These organisms release still other products, and so yogurt has a different texture and flavour.

Fermented milk beverages have been made forcenturies in various countries, especially Eastern European countries, by adding specific organisms or groups of organisms to milk. The products vary in acidity and alcohol content. Acidophilus milk is made by adding ***Lactobacillus acidophilus*** to sterile milk. Sterilization prevents uncontrolled fermentation by organisms that might already be present in nonsterilized milk. Bulgarian milk is made by***L. bulgaricus***; it is similar to buttermilk except that it is more acidic andlacks the flavor imparted by the **leuconostocs**. The Balkanproduct kefir is made from the milk of cows, goats, or sheep, and the fermentation is usually carried out in goatskin bags. The Russian product koumiss is made from mare's milk. In kefir and koumiss, ***Streptococcuslactis***, ***L. bulgaricus***, and **yeasts** are responsible for theproduction of lactic acid, alcohol, and other products. These products are usually made by continuous fermentation - fresh milk is added as fermented product is removed.

Cheeses

The first step in making almost any cheese is to add lactic acid bacteria and either rennin (an enzyme fromcalves' stomachs) or bacterial enzymes to milk. The bacteria sour the milk, and the enzymes coagulate the milk protein casein. The solid portion – the curd is used to make cheese, and the liquid portion – the whey is a waste product of this process. Sometimes lacticacidisextracted from whey. In separating the curd and the whey, different amounts of moisture are removed according to the kind of cheese being made. For soft cheeses, the whey is simply allowed to drain from the curd; for harder cheeses, heat and pressure are used to extract more moisture. Nearly all cheeses are salted. Salting helps remove water, prevents growth of undesired microorganisms, and contributes to the flavor of the cheese.

A few cheeses, such as cream cheese, cottagecheese, and ricotta, are unripened, but most cheeses are ripened. Ripening involves the action of microorganisms on the curd after it is pressed into a particular form. Soft cheeses are ripened by the action of microorganisms that occur naturally or are inoculated onto the surface of the pressed curd. Because the enzymes that ripen thecheese must diffuse from the surface of the center of thecheese, soft cheeses are relatively small. In contrast,hard cheeses are ripened by the action of microorganisms distributed through the curd. Because microbial action does not depend on diffusion, these cheeses can be quite large. Cheese is ripened by microorganisms in a cool, moist environment. Many modernfactories have environmentally controlled rooms, butsome still use natural caves similar to those where cheeses were first ripened.

Cheeses can be classified by their consistency (softto hard), by the kind of microorganisms involved in the ripening process, and by the length of time required for ripening. The ripening period is shorter for softcheeses (1to 5 months) than for hard cheeses (2 to 16 months).

Several microbial actions, such as decomposition ofthe curd and fermentation, occur during the ripening of cheeses. Prior to ripening, the curd consists of protein, lactose, and if the cheese is made from whole milk, fat. As microorganisms act on the curd, they first break down the lactose to lactic acid and other products, such as alcohols and volatile acids. Proteolytic enzymes break down protein, more extensively in soft than in hard cheese.***Brevibacteriumlinens***and the mold***Penicilliumcamemberti***are especially adept at releasing proteolytic enzymes. Lipase, particularly in***Penicilliumroqueforti***, releases short-chain fatty acids such as butyric, caproic, and caprylic acids. These acids and their oxidation products contribute significantly to the flavor of the cheeses. The effects of fermentation in cheese ripening are most easily seen in Swiss cheese. Bacteria of the genus***Propionibacterium*** ferment lactic acid and produce propionic acid, acetic acid, and carbon dioxide. The acids flavor the cheese, and the carbon dioxide, which becomes trapped in the curd, produces the characteristic holes in the cheese.

Other Products

A great variety of fermented foods and food productsare made throughout the world;

Vinegar : Vinegar is made from ethyl alcohol by theaceticacidbacterium, ***Acetobacteraceti***, which oxidizesthe alcohol to acetic acid. Commercially produced vinegar contains about 4% acetic acid. Cider vinegar is made from alcohol in fermented apple cider; wine vinegar is made from alcohol in wine.

Sauerkraut : Sauerkraut was first made in Europe inthesixteenthcentury. Bacteria naturally present on cabbage leaves act on shredded cabbage placed in layers in large crocks. Enough dry salt is placed between the layers to make a 2 to 3% salt solution as the salt drawswater out of the cabbage. The cabbage is firmly packedand weighted down to create an anaerobic environment.Although many organisms cannot tolerate such an environment, anaerobic, halophilic species of***Lactobacillus***and***Leuconostoc***can carry out fermentation under theseconditions. These microbes produce lactic acid, acetic acid, carbon dioxide, alcohol, and small amounts of other substances. After 2 to 4 weeks at room temperature, the cabbage is changed by fermentation to sauerkraut, which can be refrigerated until eaten or preserved by canning.

Pickles : Pickles are made by essentially the same processasthat used to make sauerkraut. Fresh cucumbers,either whole, sliced, or ground, are packed in brine and allowed to ferment for a few days to a few weeks.***Leuconostocmesenteroides***is the major fermenting organism in low-salt brines (less than 5% salt), whereas species of***Pediococcus***are more active in high-salt brines (5% orhigher salt concentration). A problem in pickle making is to prevent a yeast film from forming on the surface of vats. Direct sunlight and ultraviolet light help solve this problem. After fermentation, vinegar and spices are added to sour pickles; sugar also is added to sweet pickles. Most pickles are pasteurized. Some pickles, such as sweet pickles and pickle relish, are made without fermentation. After the pickles have been soaked in brine for a few hours, they are seasoned with vinegar and spices, heat-processed, and sealed. The entire operation can be done in a day in any kitchen.

Olives : Olives are treated with lye to hydrolyze oleuropein,a verybitter phenolic glucoside they contain thatgives an undesirable flavor. Green olives are fermented in a 5 to 8% salt solution by ***Leuconostocmesenteroides*** and***Lactobacillusplantarum***. They are packed in waterand pasteurized. Ripe olives are picked when reddish incolor but not fully ripe. They are oxidized with tannins to blacken them, fermented in dilute (less than 5%) salt solution, packed in water in cans, and processed at 116^{0}C for 60 minutes.

Poi : Poi, a common food in the South Pacific, is madefrom ground rootsof the taro plant. A paste of ground roots is allowed to ferment through the action of a succession of naturally occurring organisms. **Pseudomonads**and **coliforms** begin the fermentation process, and lactobacilli later take over. Yeasts add alcohol to the mixture.

Soy Sauce : Soy sauce is made in a stepwise process. A salted mixture of crushed soybeans and wheatis treated with the mold ***Aspergillusoryzae***to break down starch into fermentable glucose. The product, called**koji**, is mixed with an equal quantity of salt solution to make a mixture called moromi. Moromi is fermented for 8 to 12 months at low temperature and with occasional stirring. The fermenting organisms are mainly the bacterium***Pediococcus soyae***and the yeasts ***Saccharomyces rouxii***and***Torulopsis***species. Lactic acid, other acids, and alcohol are produced. Upon completion of fermentation, the liquid and solid parts of the moromi are separated. The liquid part is bottled as soy sauce, and the solid part is sometimes used as animal food.

Soy Products : Other soy products include miso, tofu,andsufu. Misois afermented paste of soybeans made like soy sauce. Tofuis a soft curd of soybeans. It ismade from soybeans ground to make a milk, which isboiled to inactivate enzymes. The curd is precipitated with calcium or magnesium sulfate and pressed into a soft mass similar in consistency to soft cheese. Sufuis made by action of a fungus on soy curd. Cubes of curd are dipped in a mixture of salt and citric acid, inoculated with***Mucor***, and incubated until coated with mycelia. The fungus-coated cubes are aged 6 weeks in a rice-wine brine.

Fermented Meats : Microbes such as***Lactobacillusplantarum***and***Pediococcus cerevisiae*** add flavor by fermenting meats such as salami, summer sausage, and Lebanon bologna. The heterolactic acid fermentation helps preserve the meat and also gives it a tangy flavor. Fungi such as***Penicillium***and***Aspergillus***, growing naturally on the surfaces of country hams, help to produce their distinctive flavor.

19.8 Fermented food products

Beer and wine are made by fermenting sugaryjuices; spirits, such as whiskey, gin, and rum, are made by fermenting juices and distilling the fermented product. Distillationseparates alcohol and other volatile substances from solid and nonvolatile substances. Strains of***Saccharomyces*** are the fermenters for all alcoholic beverages. Many different strains have been developed, each having distinctive characteristics. Both the organisms and how they are used are carefully guarded brewers' secrets.

To make beer, cereal grains (usually barley) aremalted(partially germinated) to increase the concentration of starch-digesting enzymes that provide the sugar for fermentation. Malted grain is crushedandmixed with hot water (about 65^0C), producing mash. After a few hours, a liquid extract called wort is separated from the mix. Hops(flower cones from the hop plant) are added to the wort for flavoring, and the mixture is boiled to stop enzyme action and precipitate proteins. A strain of***Saccharomyces*** is added. Fermentation produces ethyl alcohol, carbon dioxide, and other substances, including amyl and isoamyl alcohols and acetic and butyric acids, which add to the flavor of the beer. After fermentation, the yeast is removed, and the beer is filtered, pasteurized, and bottledMost wine is made from juice extracted from grapes, although it can be made from any fruit andeven from nuts or dandelion blossoms. Juice is treated with sulfur dioxide to kill any wild yeasts that may already be present. Sugar and a strain of ***Saccharomyces*** are then added, and fermentation proceeds. Although ethyl alcohol is the main product of fermentation, other products similar to those in beer add to the flavor of the wine. In both beer and wine, the particular characteristics of the juice and the yeast strain determine the flavor of the final product. When fermentation is completed, liquid wine is siphoned to separate it from yeast sediment and, if necessary, cleared with agents such as charcoal to remove suspended particles. Finally, it is bottled and aged in a cool place.

Spirits are made from the fermentation of a varietyof foods, including malted barley (Scotch whiskey), rye(rye whiskey, gin), corn (bourbon), wine or fruit juice(brandy), potatoes (vodka), and molasses (rum). Afterfermentation, distilling separates alcohol and other volatile substances that impart flavor from the solid and non-volatile substances. Because of distillation, the alcoholcontent of spirits ranges from 40 to 50% - much higher than the typical 12% for wine and 6% for beer. (Winesdo not contain more alcohol because when the alcoholconcentration reaches 12 to 15%, it poisons the yeastscarrying out the fermentation. To produce fortifiedwines such as sherry and cognac, extra alcohol is added after fermentation.)

19.9 Summary

Introduction

- Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth
- Fermentation by some microorganisms can lead to food preservation instead of food spoilage.
- Microbialgrowth is controlled by factors related to the food itself, or intrinsic factors,and also to the environment where the food is being stored

Important Microbes Involved in Spoilage of Food, Meat, Poultry, Fish, Sea Food, Vegetables and Dairy Products

- Anything that people eat or drink can be used asfood by microorganisms, too
- Microbial growth in foods can lead to visible changes, including a variety of colors caused by spoilage organisms.

Meat:

- Meat animals arrive at slaughterhouses with numerous and varied microorganisms in the gut and feces, on hides and hoofs, and sometimes in tissues. At least 70 pathogens have been identified among these microbes
- Some of the mostcommon diseases identified in slaughterhouses areabscesses, pneumonia, septicemia, enteritis, toxemia,nephritis, and pericarditis.

Poultry:

- Eggs, with their hard shells,would be free of microbial contamination, but the shells are porous, and pseudomonads and some other bacteria, as well as fungi such as ***Penicillium***, ***Cladosporium***,and***Sporotrichum***, grow on eggshells

- The CDC reports that 1 out of every 10,000 eggs has***Salmonella***inside the shell

Fish:

- Fresh fish abound with microorganisms. Several speciesof enteric bacteria and clostridia, enteroviruses, andparasitic worms are commonly found on or in fresh fish

Sea Food:

- Shellfish, such as oysters and clams, carry many of the same organisms as fish
- Among crustaceans, shrimp are extremely likely tobe contaminated. Some studies have shown that overhalf the breaded shrimp on the market contain in excess of 1 million bacteria

Vegetables And Fruits:

- Millions of commensal bacteria, especially***Pseudomonas fluorescens***, are found on the surfaces of fruits and vegetables. These foods also easily become contaminated with organisms from soil, animals, air, irrigation water, and equipment used to pick, transport, store, or process them

Dairy Products:

- Modern mechanized milking and milk handling has greatly reduced the microbial content of raw milk
- Most microorganisms in freshly drawn milk are ***Staphylococcusepidermidis***and***Micrococcus***, but ***Pseudomonas***, ***Flavobacterium***, ***Erwinia***, and some fungi also can be present.
- Dairy herds are tested for tuberculosis (with infected animals removed from the herd) and vaccinated against brucellosis (undulant fever); hence the risk of transmitting these diseases to humans is small.

Food Poisoning

- Many microorganisms contaminating food and water can causeacute gastroenteritis or inflammation of the stomach and intestinal lining.

When food is the source of the pathogen, the condition is often called food poisoning.

Types of food poisoning

1. Food intoxication
2. Food infection

Reason for food poisoning caused by bacteria:

- If food is not preserved properly, then it gets spoiled due to the action of enzymes and also by the action of bacteria and fungi which grow in it.

Food Preservation

Principle:

Food preservation aims at controlling such natural processes of decay in order to maintain high quality, safe and nutritious foods with an extended shelf life than if the food was left at ambient conditions.

Methods:

Many of the methods of food preservation, they include canning using moist heat; refrigeration, freezing; lyophilization and drying; and theuse of radiation. A number of chemical food additivesalso are used to retard spoilage.

Standardization Of Fish Products And Agencies In India

Microbes In Milk

- In addition to being a nutritious food for humans, milk provides a favourable environment for the growth of microorganisms

Sources of microorganisms in milk:

- The different sources of microorganism in milk are from 1) the udder of the cow, 2) skin of the cow, 3) utensils and equipment, 4) feeds, 5) air of the cow shed, 6) milking persons and 7) water.

Role of Microbes in Production of Various Food Products

- The use of microorganisms in making bread, cheese, andwine is as old as civilization itself. Long before any microorganisms were identified, milk was being made into cheese and fermented beverages, and bread was being leavened by microbes.
- Yeast is used to leaven bread

- The fermentative capabilities of certain bacteria are used tomakedairyproductssuch as buttermilk, sour cream, yogurt,variety of fermented beverages, and cheeses
- In cheese making, thewheyof milkisdiscarded, and microorganisms ferment the curd and impart flavor and texture to acheese.
- Other foods produced by microbial fermentation includevinegar,sauerkraut, pickles,olives, poi, soy sauce, other soy products, salami, Lebanon bologna, and summer sausage.

Fermented Food Products

- Beer and wine are made by fermenting sugaryjuices; spirits, such as whiskey, gin, and rum, are made by fermenting juices and distilling the fermented product.
- Beer is made frommaltedgrains; hops areadded, and themixture is fermented.
- Wine is made by fermenting fruit juices
- Spirits are made by fermenting various substances and distillingtheproducts.

19.10 Glossary

- **Abscess**- An accumulation of pus in a cavityhollowed out by tissue damage.
- **Acid** - A substance that releases hydrogenions when it is dissolved in water.
- **Acidophile**- An acid-loving organism thatgrows best in an environment with a pH of 4.0 to 5.4.
- **Aerobe**- An organism that uses oxygen, including ones that must have oxygen.
- **Aerosol**- A cloud of tiny liquid dropletssuspended in air.
- **Alcoholic fermentation**- Fermentation inwhich pyruvic acid is reduced to ethyl alcohol by electrons from reduced NAD (NADH).
- **Algae** (singular: alga) - Photosynthesis, eukaryotic organisms in the kingdoms Protista and Plantae.
- **Alkaline** (also called basic) - Conditioncaused by an abundance of hydroxyl ions resulting in a pH of greater than 7.0.

- **Anaerobe**- An organism that does not useoxygen, including some organisms that are killed by exposure to oxygen.
- **Anaerobic respiration**- Respiration in whichthe final electron acceptor in the electron transport chain is an inorganic molecule other than oxygen (e.g., a sulfate, nitrate).
- **Antibiotic**- A chemical substance producedby microorganisms that can inhibit the growth of or destroy other microorganism.
- **Aseptic technique**- A set of procedures usedto minimize chances that cultures will be contaminated by organisms from the environment.
- **Asthma**- Repiratory anaphylaxis caused byinhaled or ingested allergens or by hypersensitivity to endogenous microorganisms
- **Bacillus** (plural:bacilli) - A rodlike bacterium.
- **Bacteriocin**- A protein released by somebacteria that inhibits the growth of other strains of the same or closely related species.
- **Botulism**- Disease caused by***Clostridium botulinum***. The most common form, food-borne botulism, results from ingestion of preformed toxin and is, therefore, an in-toxication rather than an infection.
- **Bread mold** (also called Zygomycota orconjugation fungus) - A fungus with complex mycelia composed of aseptate hyphae with chitinous cross walls.
- **Canning**- The use of moist heat under pres-sure to preserve food.
- **Carbohydrate**- A compound composed of car-bon, hydrogen, and oxygen that serves as themain source of energy for most living things.
- **Coliform bacterium**- Gram-negative, non-spore-forming, aerobic or facultativelyanaerobic bacterium that ferments lactose and produces acid and gas; significant numbers may indicate water pollution.
- **Commensal**- An organism that lives in or onanother organism without harming it and that benefits from the relationship.
- **Contamination**- The presence of microorganisms on inanimate objects or surfacesof the skin and mucous membranes.
- **Corynebacteria**- Club-shaped, irregular, non-pore-forming, Gram-positive rods.
- **Curd**- The solid portion of milk resultingfrom bacterial enzyme addition and used to make cheese.

- **Diarrhea**- Excessive frequency and looseness of bowel movements.
- **Dinoflagellate**- An alga or plantlike protist,usually with two flagella.
- **Diphtheria**- A severe upper respiratory disease caused by***Corynebacterium diphtheriae***; can produce subsequent myocarditisand polyneuritis.
- **Dysentery**- A severe diarrhea that often contains mucus and sometimes blood or pus.
- **Endotoxin** (also called lipopolysaccharide) - A toxin incorporated in Gram-negative bacterial cell walls and released when the bacterium dies.
- **Enteric bacteria**- Members of the familyEnterobacteriaceae, many of which are intestinal; small facultatively anaerobic Gram-negative rods with peritrichous flagella.
- **Enteric fever**- Systemic infection, such astyphoid fever, spread throughout the body from the intestinal mucosa.
- **Enteritis**- An inflammation of the intestine.
- **Enterocolitis**- Disease caused by***Salmonella typhimurium*** and***S. paratyphi***that invade intestinal tissue and produce bacteremia.
- **Enterotoxin**- An exotoxin that acts on tissues of the gut.
- **Enzyme**- A protein catalyst that controls therate of chemical reactions in cells.
- **Exotoxin**- A soluble toxin secreted by microbes into their surroundings, includinghost tissues.
- **Fatty acid**- A long chain of carbon atoms andtheir associated hydrogens with a carboxyl group at one end.
- **Feces**- Solid waste produced in the large intestine and stored in the rectum until eliminated from the body.
- **Fermentation**- Anaerobic metabolism of thepyruvic acid produced in glycolysis.
- **Fever**- A body temperature that is abnormally high.
- **Flat sour spoilage**- Spoilage due to the growthof spores that does not cause cans to bulge with gas.

- **Food poisoning** (also called enterotoxicosis) - A gastrointestinal disease caused by ingestion of foods contaminated with preformed toxins or other toxic substances.
- **Helminth**- A worm, with bilateral symmetry;includes the roundworms and flatworms.
- **Infection**- The multiplication of a parasiteorganism, usually microscopic, within or upon the host's body.
- **Intoxication**- The ingestion of a microbialtoxin that leads to a disease.
- **Lactobacilli**- Type of regular, nonsporing,Gram-positive rods found in many foods;used in production of cheeses, yogurt, sourdough, and other fermented foods.
- **Leavening agent**- An agent, such as yeast,that produces gas to make dough rise.
- **Low-temperature long-time (LTLT) pasteurization** (also called holding method)- Procedure in which milk is heated to 62.9^0 C for at least 30 minutes.
- **Lyophilisation**- Freeze drying, a means ofpreservation of cultures.
- **Medium**- A mixture of nutritional substanceson or in which microorganisms grow.
- **Mesophilic spoilage**- Spoilage due to improper canning procedures or because theseal has been broken.
- **Neurotoxin**- A toxin that acts on nervoussystem tissues.
- **Pasteurization**- Mildheating to destroypathogens and other organisms that cause spoilage.
- **Pathogen**- Any organism capable of causingdisease in its host.
- **Pericarditis**- An inflammation of the protective membrane around the heart.
- **pH** - A means of expressing the hydrogen-ion concentration, and thus the acidity, of asolution.
- **Physical factor**- Factor in the environment,such as temperature, moisture, pressure, or radiation, that influences the kinds of organisms found and their growth.

- **Pneumonia**- An inflammation of lung tissuecaused by bacteria, viruses, or fungi.
- **Potable water**- Water that is fit for humanconsumption.
- **Putrefaction**- The microbialdecomposition of organic matter, especially the anaerobic breakdown of proteins, with the production of foul-smelling compounds such as hydrogen sulfide and amines.
- **Rennin**- An enzyme from calves' stomachsused in cheese manufacture.
- **Salmonellosis**- A common enteritis characterized by abdominal pain, fever, anddiarrhea with blood and mucus; caused by Salmonellaspecies.
- **Septicemia**- A disease associatedwith the presence in the blood of pathogens or bacterial toxins.
- **Shigellosis** (also called bacillary dysentery)- Gastrointestinal disease caused by several strains ofShigella that invade intestinal lining cells.
- **Sterilization**- The killing or removal of allmicroorganisms in a material or on an object.
- **Streptococci**- Aerotolerant anaerobes thatform pairs, tetrads, or chains by dividing in one or two planes; most lack the enzyme catalase.
- **Toxemia**- The condition caused bytoxins in the blood of the host.
- **Toxic shock syndrome (TSS)** - Conditioncaused by infection with certain toxigenic strains of***Staphylococcus aureus***; often associated with the use of superabsorbent but abrasive tampons.
- **Toxin**- Any substance that is poisonous toother organisms.
- **Traveler's diarrhea**- Gastrointestinal disorder generally caused by pathogenicstrains of***Escherichia coli***.
- **Typhoid fever**- An epidemic enteric infection caused by ***Salmonella typhi***; uncommon in areas with good sanitation.
- **Ultra-high temperature (UHT) processing** - A method of sterilizing milk and dairy pro-ducts by raising the temperature to 87.8^{0}C for 3 seconds.
- **Vibriosis**- An enteritis caused by***Vibrio parahaemolyticus***, acquired from eating contaminated fish and shellfish that have not been thoroughly cooked.

- **Viral enteritis**- Gastrointestinal diseasecaused by rotaviruses, characterized by diarrhea.
- **Whey**- The liquid portion (waste product) ofmilk resulting from bacterial enzyme addition.

19.11 Questions

Define the following:

1. Food poisoning
2. Food intoxication
3. Enterotoxin
4. Food preservation
5. Botulism

Section -A (Very Short Answer Type)

1. What are the intrinsic factors?
2. What are the extrinsic factors?
3. Examples of food products associated with microbial activity.
4. Examples of any two food with their high nutritional value.
5. What are the diseases found in slaughter houses.
6. What is meant by Lymphadenitis.
7. What is role of rhizopus and mucor in food spoilage of meat.
8. Name the helminth which is found in ground meat and what disease it cause.
9. Name the three fungi which grows on eggshell.
10. Name the fungus and its toxin which can contaminate the cider.
11. What do you mean by souring of milk and which microorganisms cause this souring.
12. Write any two cause of food poisoning.
13. What is role of sodium chloride in food preservation.
14. Name any two microorganism which can cause acid fermentation in milk.
15. What do you mean by leavening agent.

Describe following:

1. How the spoilage occur in egg and what are the different microbes in spoilage of egg.
2. What organisms are most likely to contaminate milk.
3. What is bone stink?

4. What pathogenic bacteria are associated with eggs?
5. What is flat sour spoilage?
6. How does thawing frozen foods make them moresusceptible to microbial food spoilage?
7. Why are there different methods of pasteurization?
8. Describe the process of cheese production
9. How gasteroenteritis cause by microorganism.
10. Describe the types of food poisoning with examples.
11. Name any 5 microorganism which are responsible for acute bacterial diarrheas and food poisonings with their pathogenesis.
12. Describe the principle of food preservation.
13. Describe any two methods of food preservation.
14. What do you mean by Lyophilization. Explain its role in food preservation.
15. Why antibiotics added in food and milk is prohibited. Give reasons.
16. Explain any two sources from which microorganism enter in milk.
17. Explain the process of beer making.

Briefly explain following:

1. Briefly explain the spoilage of vegetables and dairy products.
2. What do you meant by food poisoning. What are the causes of food poisoning. Explain the different types of food poisoning.
3. What do you meant by food preservation. Explain its principle and also explain its any two methods.
4. Explain the different sources of microorganisms in milk.
5. Explain the role of microorganism in making of dairy products.
6. Explain the making of fermented food products from microorganism:
    a) Vinegar
    b) Sauerkraut
    c) Pickles
    d) Poi

Unit-20

Environmental Microbiology

Structure of the Unit

20.1 Objectives

After going through this unit you will be able to understand

- Universal connections of Environmental microbiology with beneficiary for Human
- Biodegradation- A Potential and Procumbent
- Bio-pesticides- The Eco Friendly progress & classes
- Bio-Fertilizers: nourishment proceed in crop cultivation
- Bioremediation: Waste Management Performance
- Biosensors: Biological Recognizer

20.2 Introduction

Environmental microbiology is the study of microbial processes in the environment, microbial communities and microbial interactions.Environmental microbiology is also known as microbial ecology that is relationship with one another with their environment. They play an important role in regulating biogeochemical systems in our planet's environments, including from frozen environments and acidic lakes, to hydrothermal vents at the bottom of deepest oceans.

20.3 Biodegradation

Biodegradation is biologically catalyzed humiliations of organic substances that are broken down into smaller compounds by living microbes through enzymatic processes. Biodegradation is based on growth and co-metabolism processes. In growth, organic impurities are used as sole source of carbon and energy that results in a complete degradation of organic pollutants. Co-metabolism is also a metabolic process where the presence of a growth substrate used the primary carbon and energy source. Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process.

Bioremediation process divides into three phases. First is usual reduction where contaminants are reduced by native microorganisms without any human augmentation. Second, bio-stimulation where nutrients and oxygen are applied for improves their effectiveness and accelerates biodegradation. The last is bio-augmentation in which microorganisms are added to the systems.

20.3.1. Bacterial degradation

Many bacteria are able to metabolize organic pollutants by possess the enzymatic ability in polluted soil. Generally, around 80 bacterial strains take part in degradation with belonging to 10 genuses as follows: Bacillus, Corynebacterium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella and Enterobacter, Aeromonas in nature.

20.3.2. PGPR and PGPB degradation

Plant growth promoting rhizobacteria (PGPR) are naturally presenting soil bacteria that colonize in plant roots and benefit them by providing growth promotion. Plant associated bacteria are endophytic bacteria and rhizospheric bacteria has contributed to biodegradation of toxic organic compounds in contaminated soil.

20.3.3. Fungi and Mycorrhiza degradation

Fungi also metabolize to dissolve organic matter. They are principal organisms responsible for the decomposition of carbon in the biosphere. They play role especially in breaking down the natural polymeric compounds or the recycling of recalcitrant polymers (e.g., lignin) and for the elimination of hazardous wastes from the environment.

20.3.4. Algae and Protozoa

In spite of algae and protozoa, they are the important members of the microbial community in both aquatic and terrestrial ecosystems; reports are scanty regarding their involvement in hydrocarbon biodegradation.

10.4 Bio-pesticides

India is a developing country reliable on Agriculture. Apart from fulfilling the food requirement of the growing Indian population, the Agriculture plays a role in improving economy of the country. Bio-pesticides are eco friendly pesticides which are obtained from naturally occurring substances, microbes and plants.

They are a type of pest management intervention through predatory, parasitic, or chemical relationships. Since from the past decades, Agriculture is dependent upon synthetic or organic insecticides for crop protection. It was started with introduction of arsenical insecticides and Bordex mixtures as grape fungicide. The hazardous effect of synthetic chemicals on agriculture has been so dramatic that there was urgent need to search out a new biological control from nature to improve conventional agriculture system. Today, biological control is widely used as an attractive technique for controlling insects, due to its minimal environmental impact and prevention from resistance in the vectors and agricultural pests. The biological control is presently being used as attractants for trapping and monitoring of insects and for mating disruption. The biological control can separated into (a) classical biological control, where exotic natural enemies are introduced against an exotic or native pest, or (b) augmentative biological control, where human intervention occurs to enhance the effectiveness of the natural enemies already present in an area through manipulation of the environment.

The main advantages are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. The most commonly used bio-pesticides are living organisms, which are pathogenic for the pest of interest. These include biofungicides (Trichoderma), bioherbicides (Phytopthora) and bioinsecticides (*Bacillus thuringiensis*). Especially the bio-toxin-producing strains of *Bacillus thuringiensis var. israelensis or B. sphaericus* have been used throughout the world to suppress the larval stages of mosquitoes, filariasis or certain arboviruses and larval stages of *Simuliumspp*. The grasshopper pathogen Nosema locustae Canning is the only species that has been registered and commercially developed.

Bio-pesticides are divided into three major classes:

20.4.1. Microbial pesticides

They consist of bacteria, entomopathogenic fungi or viruses.

20.4.2. Biochemical pesticides or herbal pesticides

These are naturally occurring substances that control pests and microbial diseases.

20.4.3. Plant-incorporated protectants (PIPs)

These have genetic material from other species incorporated into their genetic material (i.e. GM crops). Their use is controversial, especially in many

European countries. RNAi pesticides are also there in which some of topical are absorbed by the crop.

The main advantages of bio-pesticides are based on associated products as:

- Essentially less harmful and less environmental load
- Designed to affect only one specific pest or, in some cases, a few target organisms.
- Often effective in very small quantities and decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems and When used as a component of Integrated Pest Management (IPM) programs, bio-pesticides can contribute greatly.

10.5 Bio-Fertilizers

A Bio fertilizer is a substance which contains living microorganisms that promotes growth by raising the supply of major nutrients to host plants. They applied to seeds, plant surfaces, or soil where colonizes at the rhizosphere or the interior of the plant. They accelerate certain microbial processes in the soil which supplement the availability of nutrients in a form easily assimilated by plants. The Bio-fertilizers provide nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The microorganisms restore the soil's natural nutrient cycle and build soil organic matter. Bio-fertilizers such as Rhizobium, Azotobacter, Azospirilium and blue green algae (BGA) have been in use a long time.

Isolation of microbes from the soil

↑↓

Laboratory screening of microbes for plant growth

↑↓

Greenhouse screening of microbes to promote growth in potted soil

↑↓

Field screening of most effective microbes in cropped soil

(Crop variety and different soil types examined)

↑↓

Refinement of inoculum

↑↓

Production of biofertilizer

Figure1: Production of Bio-Fertilizers (Bhattacharjee and Dey, 2014)**.**

Types of Bio-Fertilizers:

20.5.1. Rhizobium

Rhizobium is a Gram-negative soil bacterium that forms an endosymbiotic nitrogen fixing relationship with roots of legumes and Parasponia. The bacterium colonize in plant root nodules where exchange atmospheric nitrogen into ammonia and finally in organic nitrogenous compounds such as glutamine or ureides to the plant. Around 40-250 kg N / ha / year is fixed by the microbial activities of Rhizobium in different legume crops.

20.5.2. Azotobacter

Azotobacter is generally motile, oval or spherical, aerobic, free-living soil bacteria that have vital role in the nitrogen cycle in nature. Azotobacter also plays important role in nitrogen fixation and synthesizes biologically active substances, including auxins. Their nitrogen fixation ability increases the soil fertility and stimulates plant growth. Azotobacter species are widely used particularly in nitrogen biofertilizers such as azotobacterin. They are used for all non leguminous plants especially rice, cotton, vegetables etc.

20.5.3. Azospirillum

Azospirillum belongs to the best characterized genus of plant growth-promoting rhizobacteria (PGPR). These free-living diazotrophs presents in association with plant roots. They fix the considerable quantity of nitrogen in the range of 20- 40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, Oilseeds, cotton etc.

20.5.4. Cyanobacteria

Cyanobacteria are blue-green algae present in all latitudes, widespread in freshwater, marine, and terrestrial ecosystems, and in most extreme niches such as hot springs, salt works, and hypersaline bays habitats. They are engage as Photoautotrophic, oxygen-producing cyanobacteria.

20.5.5. Azolla

Azolla is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. Azolla founds as sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. Azolla has been used as a fodder for domesticated animals such as pigs and ducks. In recent days, Azolla is very much used as a sustainable feed substitute for livestock especially dairy cattle, poultry, piggery and fish.

20.5.6. Phosphate solubilizing microorganisms (PSM)

Phosphate solubilizing bacteria (PSB) are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds.

20.5.7. AM fungi

An arbuscular mycorrhiza (AM Fungi) is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant.

20.5.8. **Silicate solubilizing bacteria** (SSB)

Microorganisms are capable of degrading silicates and aluminum silicates. During the metabolism of microbes several organic acids are produced and these have a dual role in silicate weathering.

20.5.9. **Plant Growth Promoting Rhizobacteria** (PGPR)

The group of bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR).

Benefits: A bio-fertilizer provides protection against drought and some soil-borne diseases. Bio-fertilizers are cost-effective relative to chemical fertilizers.

They have lower manufacturing costs, especially regarding nitrogen and phosphorus use.

20.6 Bioremediation

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. It is also a “treatment that uses naturally occurring organisms to break hazardous substances into less toxic or non toxic substances”. It have two types as In situ bioremediation that engage treating of contaminated material at the site, while ex situ involves the removal of the contaminated material to be treated elsewhere. Some bioremediation technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration and biostimulation.

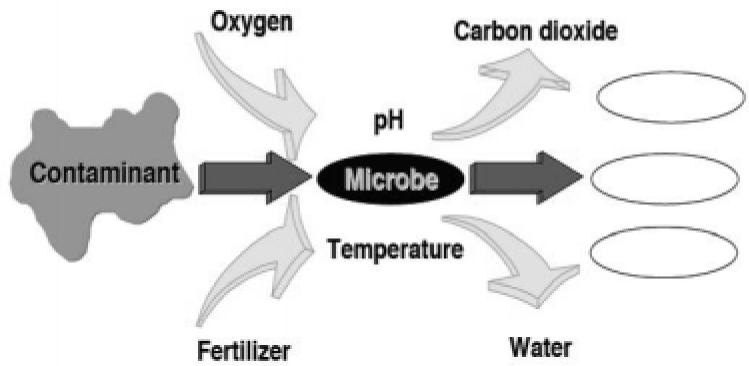

Figure: 2-: Process of bioremediation

The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments. They certainly accelerate the development of bioremediation technologies and biotransformation processes. Phytoremediation is a cost-effective plant-based approach to remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. In addition to these redox (oxidation / reduction) reactions, bioremediations have also developed a myriad of other strategies enabling them to detoxify their environment.

Toxic heavy metals and organic pollutants are the major targets for phyto-remediation.

Advantages:

- The cost of the remediation is lower than that of traditional processes both in situ and ex situ.
- The process can be easily monitored
- The possibility of the recovery and re-use of valuable metals

Limitations:

- with plant-based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater
- Bio-accumulation of contaminants, especially metals, into the plants which then pass into the food chain, from primary level consumers upwards or requires the safe disposal of the affected plant material.

20.7 Biosensors

A Biosensor is independent device that have capacity to provide specific quantitative or semi-quantitative logical data using a biological recognition element which is in direct contact with a transducer element or it is also an analytical device used for the detection of an analytic combination of biological component with physicochemical detector.

The first biosensor was illustrated by Clark and Lyons in 1962. They immobilized Glucose oxidase (GOD) on an amperometric oxygen electrode surface semi permeable dialysis membrane in order to quantify glucose concentration in a blood sample directly.

Biosensor system:

A biosensor consists of a bio-recognition component, bio-transducer component, and electronic system that comprise a signal amplifier, processor, and display unit. The recognition component or Bio-receptor uses Bio-molecules from biological systems to interact with the analyte of interest. This communication is calculated by the bio-transducer that results as measurable signals present in the target analyte of test. In genosensors, the nucleic acid recognized based on the principle of complementary base pairing in DNA.

Types of Biosensors:

20.7.2.1. Resonant Biosensors

This type of biosensor consists of an audio wave transducer coupled with a Bio-element. During attachment of membrane with analyte molecule, the mass of membrane is changed that subsequently changes as resonant frequency of the transducer which can measure.

20.7.2.2. Optical-detection Biosensors

In this type of biosensor, the output transducer signals are measured in light. The biosensor is made based on optical diffraction in which, a silicon wafer is coated with a protein via covalent bonds. The wafer is exposed to UV light through a photo-mask and the antibodies become inactive in the exposed regions. When the diced wafer chips are incubated in an analyte, where create a diffraction grating. This grating produces a diffraction signal which can be measured for improved sensitivity.

20.7.2.3. Thermal-detection Biosensors

This biosensor exploits one of the biological reactions such as absorption or invention of heat where the temperature of the medium will transform. When an analyte associates with immobilized enzyme, the heat reaction of enzyme is calculated and regulated adjacent to the analyte concentration. In results, the total heat produced or absorbed is relative to the molar enthalpy and the total number of molecules.

20.7.2.4. Ion-Sensitive Biosensors

In this type of biosensor, semiconductor FETs have an ion-sensitive surface where electrical potential changes. This change in the potential can be subsequently measured. The Ion Sensitive Field Effect Transistor constructs by covering the sensor electrode with a polymer layer. The ions disperse through the polymer layer and cause change in the FET surface potential. This type of biosensor is also called an Enzyme Field Effect Transistor.

20.7.2.5. Electrochemical Biosensors

The Electrochemical biosensors are utilized in exposure of hybridized DNA, DNA-binding drugs, glucose concentration. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) conductimetric, (2) amperometric (3) potentiometric.

Where the Conductimetric is the electrical conductance that produce ions or electrons, the overall conductivity or resistivity of the solution changes. This change is measured and calibrated to a proper scale.

similarly Amperometric is sensitivity biosensor that detect electroactive species present in biological test samples. And Potentiometric sensor is used to measure oxidation or reduction potential of an electrochemical reaction.

Applicationsof Biosensors:

The major necessities of biosensors are in research and commercial appliance as recognition of target molecule, accessibility of biological recognition element and possible for disposable portable detection systems. Some other Applicationsof Biosensors are as:

- In Glucose monitoring in diabetes patients
- In Environmental pollutants detection such as pesticides and in water contaminations.
- Remote sensing of airborne bacterial activities
- Detection of pathogens
- Detection of toxic substances before and after bioremediation
- Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid
- Determination of drug residues in food
- Drug discovery and evaluation of biological activity of new compounds

Disadvantages of Biosensors:

- Heat sterilization is not achievable as denaturalization of biological material
- Constancy of biological substance depends on the natural molecule that can denaturalized under environmental circumstances
- The cells in a biosensor can inebriated by other molecules that are capable of diffusing through the membrane.

20.8 Summaries

Microbes are used in Environment to study microbial interactions and microbial communities of microbial processes in the environment. There are several major approaches such as Biodegradation, Bio-pesticides, Bio-fertilizers, Bio-remediation, and Bio-sensors in nature that have broad appliance strategies for human.

20.9 Glossary

- **Absorption**: The soaking up of one substance into the body of another by molecular or chemical action (as tree roots absorb dissolved nutrients in the soil).
- **Aeration**: The process of adding air to water. In wastewater treatment, air is added to freshen wastewater and to keep solids in suspension.
- **Aerobe**: An organism that requires free oxygen for growth.
- **Ambient temperature**: Temperature of the surroundings.
- **Anaerobe**: An organism that lives and reproduces in the absence of dissolved oxygen, instead deriving oxygen from the breakdown of complex substances.
- **Biodegradable**: Organic matter that can be broken down by bacteria to more stable forms which will not create a nuisance.
- **Biomass**: A mass or clump of living organisms feeding on the wastes in wastewater, dead organisms and other debris.
- **Degradation**: A growth phase in which the availability of food begins to limit cell growth.
- **Denitrification**: An anaerobic biological reduction of nitrate nitrogen to nitrogen gas, the removal of total nitrogen from a system.
- **Effluent**: Wastewater or other liquid - raw (untreated), partially or completely treated - flowing from a reservoir, basin, treatment process, or treatment plant.
- **Enzyme:** Organic substances (proteins) produced by living organisms and act as catalysts to speed up chemical changes.
- **Heterotroph:** A microorganism which uses organic matter for energy and growth.
- **Influent**: The liquid - raw (untreated) or partially treated - flowing into a reservoir, basin, treatment process or treatment plant.
- **Metabolism**: All of the processes or chemical changes in an organism or a single cell by which food is built up (anabolism) into living protoplasm and by which protoplasm is broken down (catabolism) into simpler compounds with the exchange of energy.

- **Nitrification**: An aerobic process in which bacteria change the ammonia and organic nitrogen in wastewater into oxidized nitrogen (usually nitrate).
- **Nitrifying Bacteria**: Bacteria that change the ammonia and organic nitrogen in wastewater into oxidized nitrogen.
- **Nitrogen Fixation**: Conversion of atmospheric nitrogen into organic nitrogen compounds available to green plants; a process that can be carried out only by certain strains of soil bacteria.
- **Polymer**: A chemical formed by the union of many monomers (a molecule of low molecular weight).
- **Rhizosphere**: Soil surrounding plant roots.
- **Wastewater**: The used water and solids from a community that flow to a treatment plant.

20.10 Self-Learning Exercise

Section -A (Very Short Answer Type):

1. PGPR is --------------------------------
2. Major Bio-fertilizers are ---------------------.
3. PIP is found in -----------------------------------
4. Founder of Biosensor was----------------------
5. Rhizofiltration is a type of -------------------------------
6. Biosensor system is composed of -------------------------------------

Section -B (Short Answer Type):

1. Define the difference between Bioremediation and phytoremediation.
2. What is Plant Growth Promoting Rhizobacteria?
3. A Short note on Biodegradation.
4. Explain a key note on Applications of Biosensors.
5. What is Biosensors? Describe a key note on structure of Biosensors.
6. What is Role of microbes in Environmental Microbiology?

Section -C (Long Answer Type)

1. Define the Biodegradation with discussing the process of Bioremediation and microorganisms to be added to the systems.
2. Write about Bio-fertilizers with their types.

3. Describe Types of Bio-sensors, Give suitable Applications with important threats.
4. Write about Bio-pesticides.

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Unit-21

Virology

Structure of the Unit

21.1 Objectives

After going through this unit you will be able to understand

- Universal Interaction of virus with Human
- Viral Shape & Classification
- Bacteriophage: Theaggressor toward Microorganisms
- Plant viral Diseases
- Human viral Diseases

21.2 Virus

21.2.1. Introduction

The virus is an infectious, intercellular, obligate parasitic element of DNA or RNA characteristically surrounded by structural proteins. It replicates inside the living cells of the host such as animals, plants, bacteria. The word virus is from the Latin that referring to poison and other noxious liquids [10}

21.2.2. Discovery

The virus discovery was initiated with Louis Pasteur who was unable to find a causative agent for rabies. In 1892, the first virus was tobacco mosaic virus by Dmitri Ivanovsky with the help of Chamberland-Pasteur filter from infected tobacco plants. In the same year, Friedrich Loeffler and Paul Frosch also passed the first animal viral agent for foot-and-mouth disease (aphthovirus). In the 20th century, the Frederick Twort discovered a group of the virus and called bacteriophages. In 1949, John Franklin Enders, Thomas Weller, and Frederick Robbinsisolated polio virus in cultured human embryo cells.This work enabled Jonas Salk to make an effective polio vaccine. In 1965, Howard Temin and David Baltimoredescribed the first retrovirus which produces Reverse transcriptase enzyme that uses to make DNA copies of their RNA.

21.2.3. Viral Shape & Structure

Viruses exhibit a large diversity in shapes and sizes. In common, viruses are much smaller than bacteria with a diameter of 20 and 300nm which can be visualized by scanning and transmission electron microscopes. All viruses have a core of nucleic acid surrounded by protein (capsid). The nucleic acid, either DNA or RNA genome may be linear or circular, and single-stranded or double-stranded. These are formed from identical protein subunits called capsomeres. In some viruses, specialized enzymes are amassed within the capsid. In general, there are four main morphological virus types:

21.2.3.1. **Helical**

These viruses are composed of capsomeres stacked around the nucleic acid in a central axis to form a helical structure with a central cavity or hollow tube that is made of proteins arranged in a circular fashion, creating a disc-like a shape. They have usually 15-19nm wide and 300 to 500nm range in length depending on the genome size. The structure may be smooth and highly rigid, or long and very flexible. In the genetic material single-stranded RNA, as well as ssDNA, bounded into the protein helix by interactions between the negatively charged nucleic acid and positively charged protein. The good example of the helical virus is tobacco mosaic virus.

21.2.3.2. **Icosahedral**

The icosahedral structure is enormously widespread among viruses with size differences and slight variations between virions. In regular icosahedrons, twelve each subunit of capsomeres are arranged in five pentameric symmetry shape in an equivalent manner. The same protein may act as the subunit of both the pentamers and hexamers or may be composed of different proteins. The common examples are as rotavirus, poliovirus, rhinovirus, and adenovirus.

21.2.3.3. **Prolate**

Prolate is an icosahedron lengthened alongside the fivefold axis. It is present as a common arrangement of the heads of bacteriophages. This structure is formed by a cylinder with a cap at either end. The cylinder is composed of 10 triangles. The caps are classified by the T number.

21.2.3.4. **Envelope**

Some species of virus are enveloped by one of the cell membranes, either the outer membrane of the infected host cell or internal membranes such as endoplasmic reticulum. These membranes are studded with proteins coded by the viral and host genome. The influenza virus and HIV use this strategy. The virus structure is a conventional icosahedral or helical structure.The most well-known examples of enveloped viruses are the influenza virus, Hepatitis C and HIV.

21.2.3.5. **Complex**

These viral structures have a combination of icosahedral and helical shape and have a complex outer wall or head-tail morphology. They also possess extra structures such as protein tails or complex outer wall. Some bacteriophages have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibers. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.

21.3. **Virus classification**

Virus classification is a nomenclature process for viruses to put them into a taxonomic system. They are classified on the basis of phenotypic characteristics such as morphology, Nucleic acid type, Mode of replication, Host, and the type of disease caused by them. The taxonomy system encloses mainly new illustrated viruses to be labeled. At present, majorly two systems are used to classify viruses: International Committee on Taxonomy of Viruses (ICTV) system and Baltimore classification system. Both systems put viruses into one of seven groups.

21.3.1. **ICTV classification:** The ICTV (International Committee on Taxonomy of Viruses) was started to create and implement certain rules for the naming and classification of viruses. The ICTV is regulated by the International

Union of Microbiological Societies with the developing, refining, and maintaining worldwide virus taxonomy. The Viral classification system starts with taxon structure such as the level of order with the suffixes as follows: Order (-virales); Family (-viridae); Subfamily (-virinae); Genus (-virus); Species. The Species names generally obtain from the virus disease. Currently, there are seven orders with following 96 families, 22 subfamilies, 420 genera, and 2,618 species of viruses have been listed by the ICTV. The Key orders are mainly Caudovirales, Herpesvirales, Ligamenvirales, Mononegavirales, Nidovirales, Picornavirales, and Tymovirales. These orders cover viruses with varying host ranges.

21.3.2. **Baltimore System:**The Baltimore System was first defined in 1971 by David Baltimore, a Nobel Prize-winning biologist, to place viruses into one of seven groups depending on their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Viruses can be placed in one of the seven following groups.

- I: Double-stranded DNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: Single-stranded DNA viruses (+ strand or "sense") DNA (e.g. Parvoviruses)
- III: Double-stranded RNA viruses (e.g. Reoviruses)
- IV: (+) Single-stranded RNA viruses (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)
- V: (−) Single-stranded RNA viruses (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: Single-stranded RNA-RT viruses (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: Double-stranded DNA-RT viruses (e.g. Hepadnaviruses)

❖ **DNA VIRUSES:**

- **Group I: Double-stranded DNA viruses:**These types of viruses generally enter in the host nucleus before it is able to replicate. Furthermore, these viruses require host cell polymerases to replicate the viral genome and, are highly dependent on the cell cycle. The Examples of this group are Herpesviridae, Adenoviridae, and Papovaviridae.

- **Group II: Single-stranded DNA viruses:**This group contains mainly *Anelloviridae, Circoviridae, Parvoviridae, Geminiviridae, Nanoviridae* and *Microviridae*. Most of them have circular genomes.

❖ **RNA VIRUSES**

- **Group III: Double-stranded RNA viruses:** These viruses have double-stranded RNA genomes in their "Core" capsid. This group includes 2 major families, the Reoviridae and Birnaviridae. The key character of this group is Replication that is monocistronic, includes individual, segmented genomes, where each of the genes codes for only one protein, unlike other viruses that reveal complex translation.
- **Group IV & V: Single-stranded RNA viruses:**The Single-stranded RNA viruses can classify basis on negative sense or positive sense according to the sense of polarity of RNA in Baltimore classification.
- **Group IV: Single-stranded RNA viruses - Positive-sense:** Viruses possess positive-sense single-stranded RNA genomes. Many well-known viruses are found in this group, including the picornaviruses, SARS virus, hepatitis C virus, yellow fever virus, and rubella virus. The positive-sense RNA viruses defined as positive-sense can be directly accessed by host ribosomes to immediately form proteins. In Process, the Viruses contains polycistronic mRNA where the genome RNA forms the mRNA and is translated into a polyprotein product. Examples of this class include the families *Astroviridae, Caliciviridae, Coronaviridae, Flaviviridae, Picornaviridae, Arteriviridae,* and *Togaviridae.*
- **Group V: Single-stranded RNA viruses - Negative-sense:** This group of the virus has negative-sense single-stranded RNA genomes. The Negative-sense cannot be directly accessed by host ribosome to immediately form proteins. Instead, they have to be transcribed by viral polymerases into a "readable" appearance, which is the positive-sense reciprocal. Common examples in this class belong to the families *Arenaviridae, Orthomyxoviridae, Paramyxoviridae, Bunyaviridae, Filoviridae,* and *Rhabdoviridae.*
- **Group VI: viruses possess single-stranded RNA viruses that replicate through a DNA intermediate:** This group of the virus has a feature to use reverse transcriptase enzyme to convert the positive-sense RNA into DNA. With replacing to the RNA template, they use DNA to create the templates, which is spliced into the host genome using integrase. A well-studied example of this group is the retroviruses or HIV.

- **Group VII: Double-stranded DNA viruses that replicate through a single-stranded RNA intermediate:** This small group of viruses has a double-stranded genome that is subsequently filled in to form a covalently closed circle DNA (cccDNA) that serves as a template for production of viral mRNAs and a subgenomic RNA. The hepatitis B virus can be found in the *Hepadnaviridae* family in this group.

21.3.3. Holmes classification

Holmes (1948) was also used binomial nomenclature to classify viruses into 3 groups under one order, Virales. They are placed as follows:

- Group I: *Phaginae* (attacks bacteria)
- Group II: *Phytophaginae* (attacks plants)
- Group III: *Zoophaginae* (attacks animals)

21.4. Bacteriophage

21.4.1. Introduction

The Bacteriophages are viruses that attack to bacteria. It was discovered by Frederick Twort (1915) in England and by Felix d'Herelle (1917) at the Pasteur Institute in France. They are also commonly known as "phage". It can cause a complete lysis of a susceptible bacterial culture at the virulent stage. They can obtain from widely from feces and sewage in nature. There are mainly 12 groups of bacteriophages. The common examples of phages are T-even phages (T2, T4 and T6), temperate phages (λand Mμ), Spherical phages (ΦX174), Filamentous phages (M13) and Q-β phase.

21.4.2. Morphology

Most phages have a range from 24-200 nm in length that contains a head structure, which can vary in size and shape. Some of the phages are icosahedral (20 sides) and others are filamentous. The head part encloses nucleic acid. The nucleic acid can be either DNA or RNA but not both. The tail part is a hollow tube through which the nucleic acid passes during infection. At the end of the tail, phages have a base plate and one or more tail fibers attached to the host.

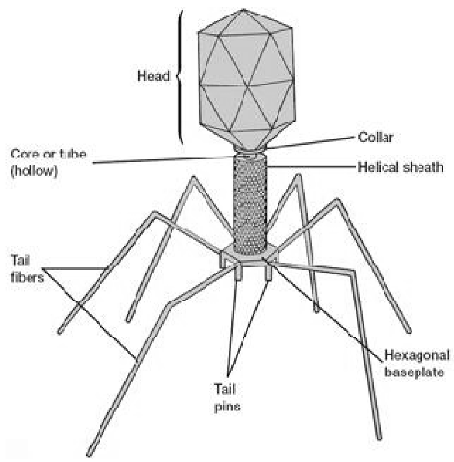

Figure -1: Structure of Bacteriophage.

21.4.3. Phage Genetics

The transfer of genetic materials from one bacterium to another by a bacteriophage is termed as transduction. It can be generalized or specialized. In the generalized transduction, the lytic cycle is completed where fragments of bacterial DNA are packaged with replacing to phage DNA inside phage capsid. When such phages infect new bacterial cells, the bacterial DNA is injected inside. In specialized transduction, only those genes that are adjacent to the prophage are transferred.

21.4.4. Reproduction

In the initiation of transduction, the first step in the infection process is the adsorption of the phage to the bacterial cell. This step is regulated by the tail fibers on phages. After the same, the hollow tail fiber is pushed through the bacterial envelope. Some phages enzymes also help to digest various components of the bacterial envelope. Nucleic acid from the head passes through the hollow tail and enters the bacterial cell. Depending on the life cycle, phages can reproduce via either by lytic (virulent) or lysogenic (temperate).

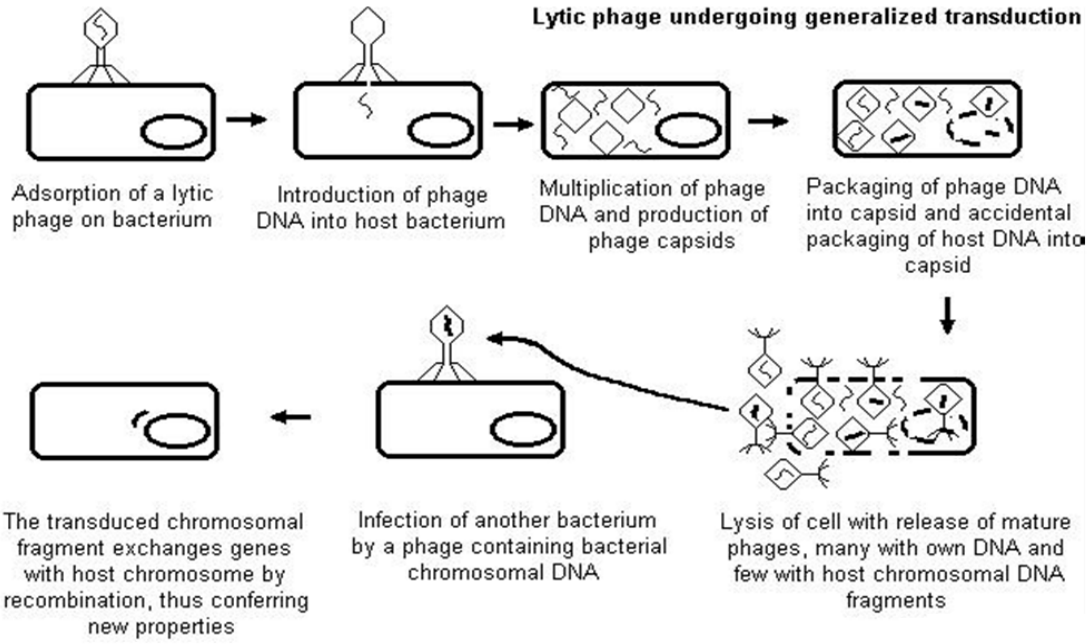

Figure-2: Life cycle of Bacteriophage including Lytic cycle and Lysogenic cycle.

21.4.4.1. **Lytic cycle**

The lytic phages kill the cells they infect; temperate phages establish a persistent infection of the cell without killing it. In lytic cycle, the subsequent steps are the synthesis of phage components, assembly, maturation and release. After the injection of nucleic acid, the phage cycle is started to be in eclipse period. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell. The Structural proteins (head, tail) that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized. The Nucleic acid is then packaged inside the head and then the tail is added to the head. In Lysis and Release Phase the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium. The number of particles released per infected bacteria may be as high as 1000. The average yield of phages per infected bacterial cell is known as burst size.

21.4.4.2. **Lysogenic cycle**

In Lysogenic phages, the phage DNA integrates into the host chromosome and is replicated along with the host chromosome. Then it is passed on to the daughter cells. This process is known as lysogeny and the bacteria harboring prophage are called lysogenic bacteria. This process is known as lysogenic conversion or phage conversion. The lysogenic state of a bacterium can get terminated anytime when it exposed to adverse conditions. Conditions that favor the termination of the lysogenic state include desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals, etc.

21.5 Tobacco mosaic virus

21.5.1. Introduction

The Tobacco mosaic virus (TMV) is a positive-sense single-stranded RNA virus that infects to tobacco and other members of the family Solanaceae. In 1892, the first virus was tobacco mosaic virus by Dmitri Ivanovsky with the help of Chamberland-Pasteur filter from infected tobacco plants. In 1886, Adolf Mayer first described the tobacco mosaic disease that could be transferred between plants, similar to bacterialinfections. Schelsinger (1933) was the first to study the chemical composition of the virus. He showed that the bacteriophage consisted of only nucleic acids ant proteins. A few years later Stanley (1935) isolated tobacco mosaic virus if paracrystalline form. Later Bawden and Pirie (1938) identified the chemical nature of TMV and other viruses. Gierr and Schramm (1956) showed that the nucleic acid in viruses is the actual infective agent. Viroids and satellite viruses were discovered by Kassanis (1966) and Dicner and Raymer (1967) respectively.

21.5.2. The structure of TMV

The TMV virus has a rod-like appearance. Its capsid is made from 2130 molecules of coat protein and one molecule of genomic single strand RNA. The coat protein self-assembles into a rod-like helical structure around the RNA which forms a hairpin loop structure. The TMV genome consists of a 6.3-6.5 kb single-stranded (ss) RNA that encodes a replicase with methyltransferase [MT] and RNA helicase [Hel] domains, an RNA-dependent RNA polymerase, and a capsid protein (CP).

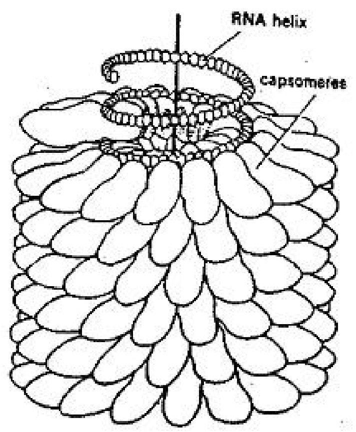

Figure -3: Structure of TMV.

21.5.3. **Host and symptoms**

The TMV has a very wide host range and has different effects depending on the host being infected. The first symptom of this virus disease is a light green coloration between the veins of young leaves. This is followed quickly by the development of a "mosaic" or mottled pattern of light and dark green areas in the leaves. Rugosity may also be seen where the infected plant leaves display small localized random wrinkles. These symptoms develop quickly and are more pronounced on younger leaves. Lower leaves are subjected to "mosaic burn" especially during periods of hot and dry weather. In these cases, large dead areas develop in the leaves. Infected leaves may be crinkled, puckered, or elongated.

21.5.4. **Treatment and management**

The most common control of TMV is sanitation that includes removing infected plants, washing hands in between each planting and Crop rotation.

21.6. **Cauliflower Mosaic Virus**

21.6.1. **Introduction**

Cauliflower mosaic virus is a member of the *Caulimoviridae* family, which was described in 1937. It infects *Brassicaceae* family's plants through reverse transcription like retroviruses. The major difference is that viral particles contain DNA instead of RNA. Some CaMV strains (D4 and W260) are also able to infect Solanaceae species of the genera Datura and Nicotiana. The CaMV genome contains 35S RNA promoter and a subgenomic 19S RNA sequence for the transcription.

21.6.2. **Structure**

The CaMV particle is an icosahedron structure with a diameter of 52 nm built from 420 capsid protein (CP) subunits arranged with a triangulation T = 7, which surrounds a solvent-filled central cavity. It contains a circular double-stranded DNA molecule of about 8.0 kilobases. After entering the host cell, these single-stranded "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones.

21.6.3. **Symptoms**

CaMV induces a variety of systemic symptoms such as mosaic, necrotic lesions on leaf surfaces, stunted growth, and deformation of the overall plant structure.

The symptoms exhibited vary depending on the viral strain, host ecotype, and environmental conditions. CaMV is transmitted in a non-circulatory manner by aphid species such as *Myzus persicae*. Once introduced into a plant host cell, virions migrate to the nuclear envelope of the plant cell.

21.6.4. **Treatment and management**

The most common control of CaMV is sanitation that includes removing infected plants, washing hands in between each planting and Crop rotation.

21.7. **Rubella**

21.7.1. Introduction

Rubella is a universal virus that known as German measles or three-day measles. It leads to each year about in 100,000 cases around the world. It was described by Friedrich Hoffmann in 1740 which was confirmed by De Bergen in 1752 and Orlow in 1758. The disease is usually spread by a togavirus. This virus has an envelope structure and a single-stranded RNA genome that is transmitted by the respiratory route, nasopharynx and lymph nodes. The virus is infected to the blood in initial 5 to 7 days and then spreads throughout the body. The virus has also teratogenic nature which has the capacity to cross the placenta and infects the fetus where it stops cells from developing or destroys them.

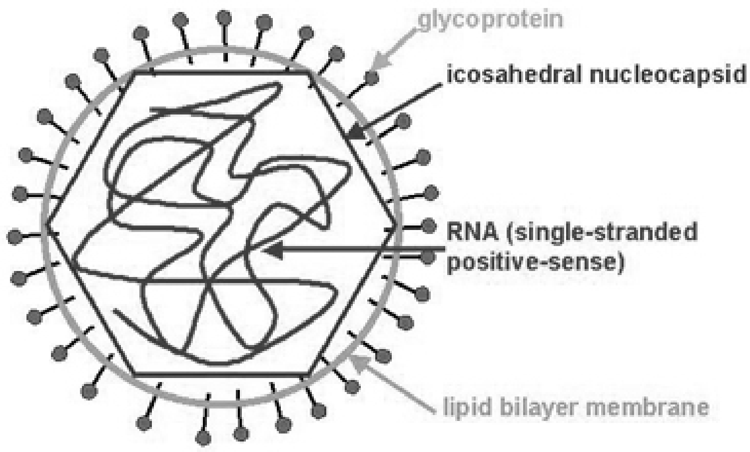

Figure -4: Structure of Rubella virus.

21.7.2. Signs and symptoms

The primary symptoms are similar to the flu. The inflammation starts around two weeks after exposure and lasts for three days. It usually starts on the face and spreads to the whole body. The Swollen lymph nodes are occurring & a fever, sore throat, and fatigue symptoms may also appear. In adults joint pain is common but some Complications as bleeding problems, testicular swelling, and inflammation of nerves may occur. The Rubella Infection during early pregnancy cause congenital rubella syndrome (CRS) or miscarriage in a child born. Late stage in rubella may convert into pneumonia as either direct viral pneumonia or secondary bacterial pneumonia, and bronchitis. The disease can be confirmed by identifying the virus in the blood, throat, or urine.

21.7.3. Prevention and treatment

Rubella may be curable with the rubella vaccine is given with the combination of the measles vaccine and mumps vaccine, commonly called as the MMR vaccine.

21.8. Herpes simplex virus

21.8.1. Introduction

Herpes simplex virus is a member of the Herpesviridae family which infects to human beings. They spread via an infected person who is produced and shredded the virus. It may also circulate through saliva contact, such as sharing drinks. The Herpes Viruses encloses a double-stranded, linear DNA genome encapsulated with an icosahedral protein capsid that is wrapped by lipid bilayer or the envelope.

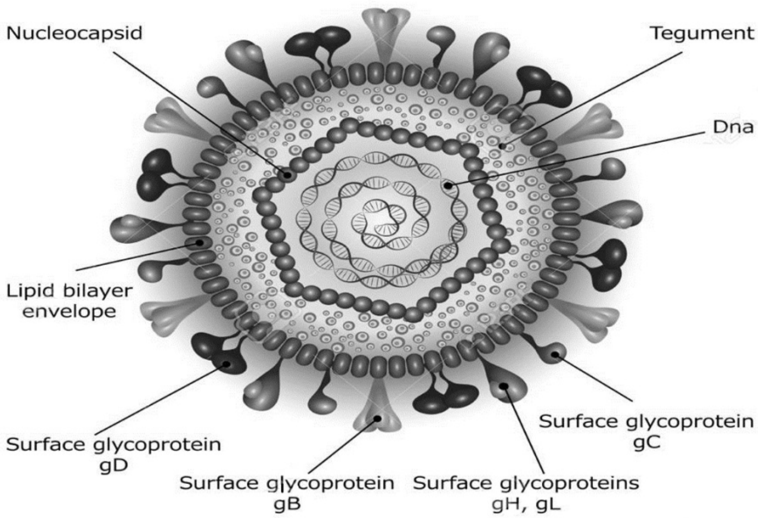

Figure-5 : Structure of Herpes Simplex virus.

21.8.2. There are two types of HSV as HSV-1 and HSV-2 that each contains at least 74 genes within their genomes. These genes encode a variety of proteins to form the capsid, tegument, and envelope of the virus, as well as controlling the replication and infectivity of the virus. The virus is entered into the host cell with interactions of several glycoproteins on the surface of the enveloped virus.

21.8.3. Symptoms:

This viral infection is one of the most universal sexually transmitted infections. It includes watery wound on the skin or mucous membranes of the mouth, lips or genitals tract. However, the neurotropic and neuroinvasive viruses (HSV-1 and HSV-2) also persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. Initially the virus active in a nerve cell and is transported through the neuron's axon to the skin, where virus replication and shedding occur and cause new sores. The risk of infection is minimal if the mother has no symptoms or exposed blisters during delivery. The risk is considerable when the mother is infected with the virus for the first time during late pregnancy.

21.8.4. Treatment:

Herpesviruses establish lifelong infections, and cannot eliminate from the body. Treatment can be maintained via antiviral drugs such as aciclovir and valaciclovir that hold up the viral replication & reduce the physical severity of outbreak-associated lesions, and lower the chance of transmission to others.

21.9. Severe Acute Respiratory Syndrome

21.9.1. Introduction

Severe Acute Respiratory Syndrome (SARS) is a respiratory viral disease caused by the SARS coronavirus. The first case of SARS was reported in Shunde, Foshan, Guangdong in 2002. Histological Phylogenetic analysis of these viruses indicated that the SARS virus was originated in bats and spread to humans either directly or through animals.

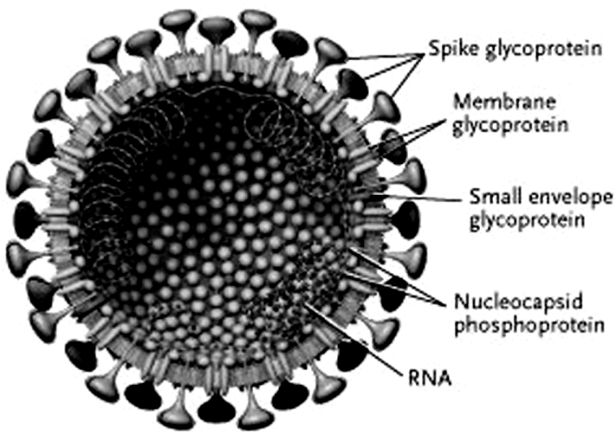

Figure-6: Structure of Severe Acute Respiratory Syndrome

21.9.2. Symptoms and Diagnosis

Initial symptoms are flu-like such as fever, myalgia, lethargy symptoms, cough, sore throat, and other nonspecific symptoms. Patient has symptoms as cold in the primary stage but Shortness of breath, influenza and pneumonia. This disease also includes other things as pulmonary fibrosis, osteoporosis, and femoral necrosis which led to the complete loss of working ability or even self-care ability. The Disease can be identified by approved tests as chest X-ray, ELISA, immune-fluorescence or PCR.

21.9.3. Treatment

SARS viral disease can be controlled by antipyretics, supplemental oxygen and mechanical ventilation. There is no cure or protective vaccine for SARS that is safe for use in humans but the monoclonal antibody therapy have been demonstrated successfully in animal models.

21.10. Summaries

Virology is a branch of science that is of immense relevance to mankind for a host of reasons, not least of which the threats to human health are caused by viruses, such as HIV, hepatitis B virus, measles and influenza viruses, to mention just a few. The virus is a parasitic element of Nucleic acid typically surrounded by structural proteins.The present study revealed on the fundamental facts of the virus with details about the Bacteriophage, plant viruses and some human parasitic viruses.

21.11. Glossary

2. **Agent (of disease):** Factor such as a microorganism whose presence is essential for the occurrence of a disease.
3. **Antiretroviral:** A substance that stops or suppresses the activity of a retrovirus such as HIV.
4. **Attachment:**Binding of a virion to specific receptors on the host cell.
5. **Bacteriophage (phage):** A type of virus that infects bacteria
6. **Baltimore classification:** A scheme that classifies viruses into seven groups on the basis of the nature of the genome and the way in which it is transcribed.
7. **Biosafety:** Safety with respect to the effects of biological research on humans and the environment.
8. **Communicable disease:** An infectious disease transmissible (as from person to person) by direct contact with an infected individual or the individual's discharges or by indirect means (as by a vector).

9. **Coronavirus:** Any of a family (*Coronaviridae*) of single-stranded RNA viruses that have a lipid envelope with club-shaped projections and include some causing respiratory symptoms in humans.
10. **Disease:** As used in this report, refers to a situation in which infection has elicited signs and symptoms in the infected individual; the infection has become clinically apparent.
11. **Envelope:** A lipid bilayer and associated protein forming the outer component of an enveloped virion.
12. **Epidemiology:** Study of the distribution and determinants of health-related states or events in specified populations. Epidemiology is the basic quantitative science of public health.
13. **Host (disease):** Person or another living animal that affords subsistence or lodgment to an infectious agent under natural conditions.
14. **Lysis:** Destruction of a cell caused by the rupture of its membrane and release of contents, e.g. the bursting of a cell and release of progeny virions at the end of the replication cycle.
15. **Lysogeny:** Latent infection of a bacterial cell with a phage.
16. **Monoclonal antibody:** A single type of antibody produced by a clone of identical cells.
17. **Syndrome:** A group or recognizable pattern of symptoms or abnormalities that indicate a particular trait or disease. (http://www.genome.gov/glossary.cfm?key= syndrome)
18. **Vaccine:** A preparation of living, attenuated, or killed bacteria or viruses, fractions thereof, or synthesized or recombinant antigens identical or similar to those found in the disease-causing organism that is administered to raise immunity to a particular microorganism.
19. **Virion:** Virus particle
20. **Virulence:** The ability of any infectious agent to produce disease. The virulence of a microorganism (such as a bacterium or virus) is a measure of the severity of the disease it is capable of causing.

21.12. Self-Learning Exercise

Section -A (Very Short Answer Type):

1. Capsomeres is --------------------------------

2. ICTV means ---------------------.

3. Bacteriophages are found in ------------------------------------

4. Founder of Virus was----------------------

5. SARS is a group in family -------------------------------
6. Two plant virus examples are --------------------------------------

Section -B (Short Answer Type):

1. Define the difference between Icosahedral and Prolate.
2. Define the Means of Virus.
3. A Short note on Capsid.
4. Explain a keynote Vaccine.
5. Describe a keynote on structure of TMV.
6. Explain a keynote about Rubella virus?

Section -C (Long Answer Type)

1. Define the Classification System for Virus.
2. Write about Plant Virus with appropriate examples.
3. Define the virus with chronological preface and Types of virus structure.
4. Write about Bacteriophage & their Lifecycle.

21.13 References

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