BIOLOGY

TEXTBOOK FOR CLASS XII

Punjab School Education Board

Sahibzada Ajit Singh Nagar

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FOREWORD

Punjab School Education Board has continuously been engaged in preparation and review of syllabi and textbooks. In today's scenario, imparting right education to students is the joint responsibility of teachers as well as parents. With a view to carry out entrusted responsibility, some important changes pertaining to present day educational requirements have been made in the textbooks and syllabus in accordance with NCF 2005.

Science has an important place in school curriculum and a good textbook is the first requisite to achieve desired learning outcomes. Therefore, the content matter of Biology for the class XII has been so arranged so as to develop reasoning power of the students and to enhance their understanding of the subject. Graded questions and exercises have been given to suit the mental level of the students. This book is prepared by NCERT, New Delhi for class XII and is being published by Punjab School Education Board with the permission of NCERT, New Delhi. This step was taken to maintain the uniformity in the Biology Subject so that Science student will have no problem while facing the common entrance test at a senior secondary stage.

Every effort has been made to make the book useful for students as well as for the teachers. However, constructive suggestions for its further improvement would be gratefully acknowledged.

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UNIT VI
REPRODUCTION

Chapter 1
Reproduction in Organisms

Chapter 2
Sexual Reproduction in flowering Plants

Chapter 3
Human Reproduction

Chapter 4
Reproductive Health

Biology in essence is the story of life on earth. While individual organisms die without fail, species continue to live through millions of years unless threatened by natural or anthropogenic extinction. Reproduction becomes a vital process without which species cannot survive for long. Each individual leaves its progeny by asexual or sexual means. Sexual mode of reproduction enables creation of new variants, so that survival advantage is enhanced. This unit examines the general principles underlying reproductive processes in living organisms and then explains the details of this process in flowering plants and humans as easy to relate representative examples. A related perspective on human reproductive health and how reproductive ill health can be avoided is also presented to complete our understanding of biology of reproduction.

PANCHANAN MAHESHWARI
(1904-1966)

Born in November 1904 in Jaipur (Rajasthan) Panchanan Maheshwari rose to become one of the most distinguished botanists not only of India but of the entire world. He moved to Allahabad for higher education where he obtained his D.Sc. During his college days, he was inspired by Dr W. Dudgeon, an American missionary teacher, to develop interest in Botany and especially morphology. His teacher once expressed that if his student progresses ahead of him, it will give him a great satisfaction. These words encouraged Panchanan to enquire what he could do for his teacher in return.

He worked on embryological aspects and popularised the use of embryological characters in taxonomy. He established the Department of Botany, University of Delhi as an important centre of research in embryology and tissue culture. He also emphasised the need for initiation of work on artificial culture of immature embryos. These days, tissue culture has become a landmark in science. His work on test tube fertilisation and intra-ovarian pollination won worldwide acclaim.

He was honoured with fellowship of Royal Society of London (FRS), Indian National Science Academy and several other institutions of excellence. He encouraged general education and made a significant contribution to school education by his leadership in bringing out the very first textbooks of Biology for Higher Secondary Schools published by NCERT in 1964.

CHAPTER 1

REPRODUCTION IN ORGANISMS

1.1 *Asexual Reproduction*

1.2 *Sexual Reproduction*

Each and every organism can live only for a certain period of time. The period from birth to the natural death of an organism represents its **life span.** Life spans of a few organisms are given in Figure 1.1. *Several other organisms are drawn for which you should find out their life spans and write in the spaces provided.* Examine the life spans of organisms represented in the Figure 1.1. Isn't it both interesting and intriguing to note that it may be as short as a few days or as long as a few thousand years? Between these two extremes are the life spans of most other living organisms. You may note that life spans of organisms are not necessarily correlated with their sizes; the sizes of crows and parrots are not very different yet their life spans show a wide difference. Similarly, a mango tree has a much shorter life span as compared to a *peepal* tree. Whatever be the life span, death of every individual organism is a certainty, i.e., no individual is immortal, except single-celled organisms. *Why do we say there is no natural death in single-celled organisms?* Given this reality, have you ever wondered how vast number of plant and animal species have existed on earth for several thousands of years? There must be some processes in living organisms that ensure this continuity. Yes, we are talking about reproduction, something that we take for granted.

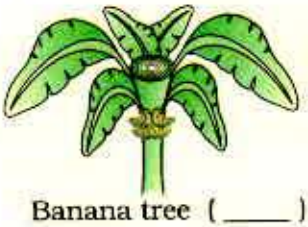
Elephant(_____)

Rose (_____)

Dog (_____)

Butter fly (1-2 weeks)

Crow (15 years)



Banana tree (_____)

Cow (_____)

Parrot (140 years)

Crocodile (60 years)

Horse (_____)

Fruit fly (_____)

Rice plant (_____)

Tortoise (100-150 years)

Banyan tree (_____)

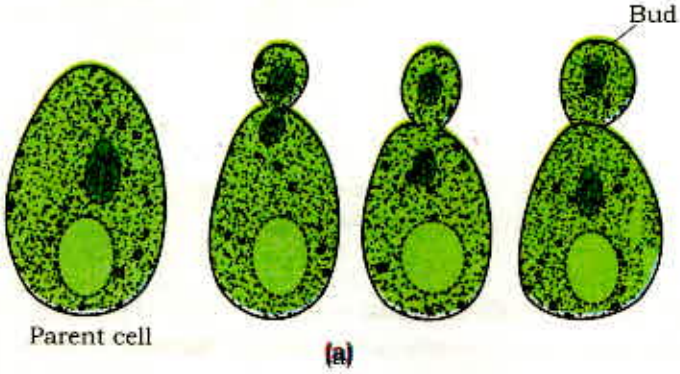
Figure 1.1 Approximate life spans of some organisms

Reproduction is defined as a biological process in which an organism gives rise to young ones (offspring) similar to itself. The offspring grow, mature and in turn produce new offspring. Thus, there is a cycle of birth, growth and death. Reproduction enables the continuity of the species, generation after generation. You will study later in Chapter 5 (Principles of Inheritance and Variation) how genetic variation is created and inherited during reproduction.

There is a large diversity in the biological world and each organism has evolved its own mechanism to multiply and produce offspring. The organism's habitat, its internal physiology and several other factors are collectively responsible for how it reproduces. Based on whether there is participation of one organism or two in the process of reproduction, it is of two types. When offspring is produced by a single parent with or without the involvement of gamete formation, the reproduction is **asexual.** When two parents (opposite sex) participate in the reproductive process and also involve fusion of male and female gametes, it is called **sexual reproduction**.

1.1 Asexual Reproduction

In this method, a single individual (parent) is capable of producing offspring. As a result, the offspring that are produced are not only identical to one another but are also exact copies of their parent. *Are these offspring likely to be genetically identical or different?* The term **clone** is used to describe such morphologically and genetically similar individuals.



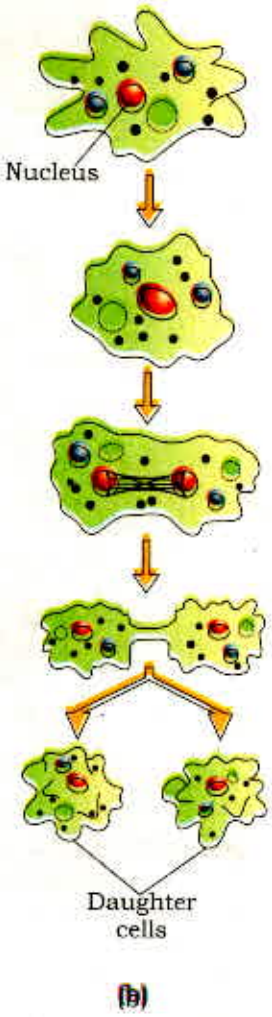


Figure 1.2 Cell division in unicellular organism: (a) Budding in yeast; (b) Binary fission in *Amoeba*

Let us see how widespread asexual reproduction is, among different groups of organisms. Asexual reproduction is common among single-celled organisms, and in plants and animals with relatively simple organisation. In Protists and Monerans, the organism or the parent cell divides into two to give rise to new individuals (Figure 1.2). Thus,

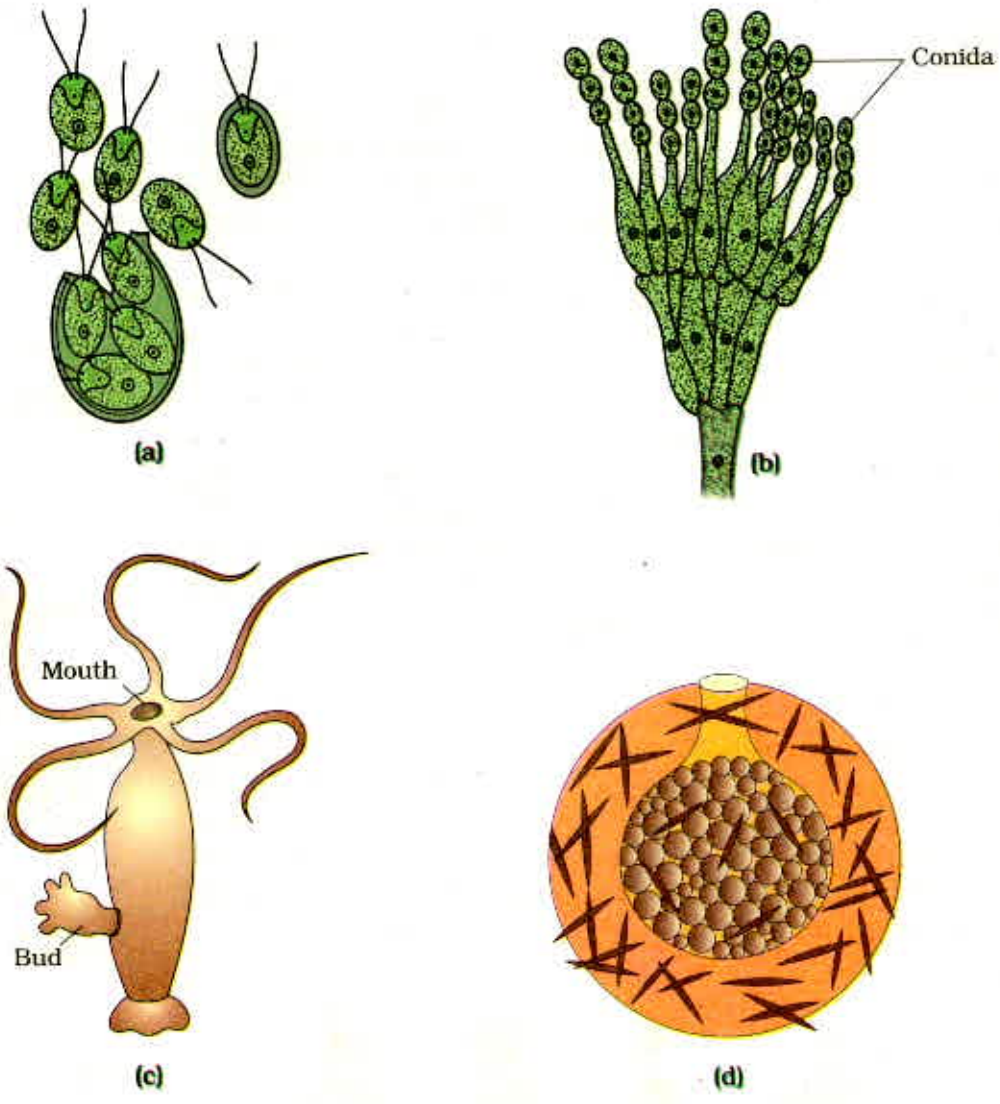


Figure 1.3 Asexual reproductive structures: (a) Zoospores of *Chlamydomonas*; (b) Conidia of *Penicillium*; (c) Buds in *Hydra*; (d) Gemmules in sponge

in these organisms **cell division** is itself a mode of reproduction. Many single-celled organisms reproduce by **binary fission,** where a cell divides into two halves and each rapidly grows into an adult (e.g., *Amoeba, Paramecium*). In yeast, the division is unequal and small **buds** are produced that remain attached initially to the parent cell which, eventually gets separated and mature into new yeast organisms (cells).

Members of the Kingdom Fungi and simple plants such as algae reproduce through special asexual reproductive structures (Figure 1.3). The most common of these structures are **zoospores** that usually are microscopic motile structures. Other common asexual reproductive structures are **conidia** (*Penicillium*), **buds** (*Hydra*) and **gemmules** (*sponge*).

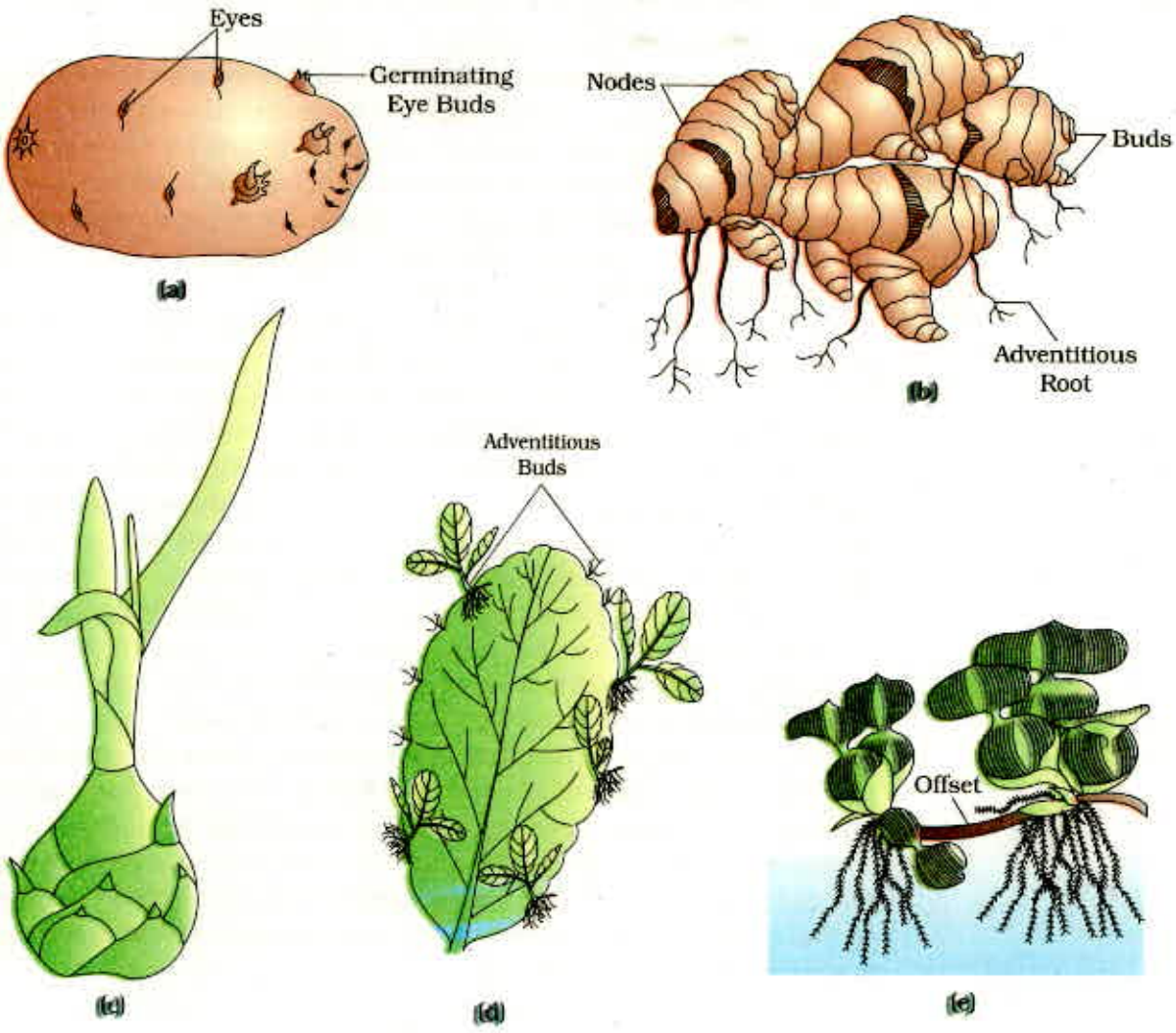


Figure 1.4 Vegetative propagules in angiosperms: (a) Eyes of potato; (b) Rhizome of ginger; (c) Bulbil of *Agave*; (d) Leaf buds of *Bryophyllum*; (e) Offset of water hyacinth

You have learnt about vegetative reproduction in plants in Class XI. What do you think – Is vegetative reproduction also a type of asexual reproduction? Why do you say so? Is the term clone applicable to the offspring formed by vegetative reproduction?

While in animals and other simple organisms the term **asexual** is used unambiguously, in plants, the term **vegetative** reproduction is frequently used. In plants, the units of **vegetative propagation** such as **runner, rhizome, sucker, tuber, offset, bulb** are all capable of giving rise to new offspring (Figure 1.4). These structures are called **vegetative propagules.** Obviously, since the formation of these structures does not involve two parents, the process involved is asexual.

You must have heard about the scourge of the water bodies or about the 'terror of Bengal'. This is nothing but the aquatic plant 'water hyacinth' which is one of the most invasive weeds found growing wherever there is standing water. It drains oxygen from the water, which leads to death of fishes. You will learn more about it in Chapters 13 and 14. You may find it interesting to know that this plant was introduced in India because of its beautiful flowers and shape of leaves. Since it can propagate vegetatively at a phenomenal rate and spread all over the water body in a short period of time, it is very difficult to get rid off them.

Are you aware how plants like potato, sugarcane, banana, ginger, dahlia are cultivated? Have you seen small plants emerging from the buds (called eyes) of the potato tuber, from the rhizomes of banana and ginger? When you carefully try to determine the site of origin of the new plantlets in the plants listed above, you will notice that they invariably arise from the **nodes** present in the modified stems of these plants. When the nodes come in contact with damp soil or water, they produce roots and new plants. Similarly, adventitious buds arise from the notches present at margins of leaves of *Bryophyllum*. This ability is fully exploited by gardeners and farmers for commercial propagation of such plants.

It is interesting to note that asexual reproduction is the common method of reproduction in organisms that have a relatively simple organisation, like algae and fungi and that they shift to sexual method of reproduction just before the onset of adverse conditions. *Find out how sexual reproduction enables these organisms to survive during unfavourable conditions? Why is sexual reproduction favoured under such conditions?* Asexual (vegetative) as well as sexual modes of reproduction are exhibited by the higher plants. On the other hand, only sexual mode of reproduction is present in most of the animals.

1.2 Sexual Reproduction

Sexual reproduction involves formation of the male and female gametes, either by the same individual or by different individuals of the opposite sex. These gametes fuse to form the zygote which develops to form the new organism. It is an elaborate, complex and slow process as compared to asexual reproduction. Because of the fusion of male and female gametes, sexual reproduction results in offspring that are not identical to the parents or amongst themselves.

A study of diverse organisms–plants, animals or fungi–show that though they differ so greatly in external morphology, internal structure and physiology, when it comes to sexual mode of reproduction, surprisingly, they share a similar pattern. Let us first discuss what features are common to these diverse organisms.

All organisms have to reach a certain stage of growth and maturity in their life, before they can reproduce sexually. That period of growth is

called the **juvenile phase**. It is known as **vegetative phase** in plants. This phase is of variable durations in different organisms.

The end of juvenile/vegetative phase which marks the beginning of the reproductive phase can be seen easily in the higher plants when they come to flower. *How long does it take for marigold/rice/wheat/coconut/mango plants to come to flower? In some plants, where flowering occurs more than once, what would you call the inter-flowering period – juvenile or mature?*

Observe a few trees in your area. Do they flower during the same month year after year? Why do you think the availability of fruits like mango, apple, jackfruit, etc., is seasonal? Are there some plants that flower throughout the year and some others that show seasonal flowering? Plants–the annual and biennial types, show clear cut vegetative, reproductive and senescent phases, but in the perennial species it is very difficult to clearly define these phases. A few plants exhibit unusual flowering phenomenon; some of them such as bamboo species flower only once in their life time, generally after 50-100 years, produce large number of fruits and die. Another plant, *Strobilanthus kunthiana* (neelakuranji), flowers once in 12 years. As many of you would know, this plant flowered during September-October 2006. Its mass flowering transformed large tracks of hilly areas in Kerala, Karnataka and Tamil Nadu into blue stretches and attracted a large number of tourists. In animals, the juvenile phase is followed by morphological and physiological changes prior to active reproductive behaviour. The reproductive phase is also of variable duration in different organisms.

Can you list the changes seen in human beings that are indicative of reproductive maturity?

Among animals, for example birds, do they lay eggs all through the year? Or is it a seasonal phenomenon? What about other animals like frogs and lizards? You will notice that, birds living in nature lay eggs only seasonally. However, birds in captivity (as in poultry farms) can be made to lay eggs throughout the year. In this case, laying eggs is not related to reproduction but is a commercial exploitation for human welfare. The females of placental mammals exhibit cyclical changes in the activities of ovaries and accessory ducts as well as hormones during the reproductive phase. In non-primate mammals like cows, sheep, rats, deers, dogs, tiger, etc., such cyclical changes during reproduction are called **oestrus cycle** where as in primates (monkeys, apes, and humans) it is called **menstrual cycle**. Many mammals, especially those living in natural, wild conditions exhibit such cycles only during favourable seasons in their reproductive phase and are therefore called seasonal breeders. Many other mammals are reproductively active throughout their reproductive phase and hence are called continuous breeders.

That we all grow old (if we live long enough), is something that we recognise. *But what is meant by growing old?* The end of reproductive

phase can be considered as one of the parameters of senescence or old age. There are concomitant changes in the body (like slowing of metabolism, etc.) during this last phase of life span. Old age ultimately leads to death.

In both plants and animals, hormones are responsible for the transitions between the three phases. Interaction between hormones and certain environmental factors regulate the reproductive processes and the associated behavioural expressions of organisms.

Events in sexual reproduction : After attainment of maturity, all sexually reproducing organisms exhibit events and processes that have remarkable fundamental similarity, even though the structures associated with sexual reproduction are indeed very different. The events of sexual reproduction though elaborate and complex, follow a regular sequence. Sexual reproduction is characterised by the fusion (or fertilisation) of the male and female gametes, the formation of zygote and embryogenesis. For convenience these sequential events may be grouped into three distinct stages namely, the **pre-fertilisation, fertilisation** and the **post-fertilisation events.**

1.2.1 Pre-fertilisation Events

These include all the events of sexual reproduction prior to the fusion of gametes. The two main pre-fertilisation events are **gametogenesis** and **gamete transfer.**

1.2.1.1 Gametogenesis

As you are already aware, **gametogenesis** refers to the process of formation of the two types of gametes – male and female. Gametes are haploid cells. In some algae the two gametes are so similar in appearance that it is not possible to categorise them into male and female gametes.

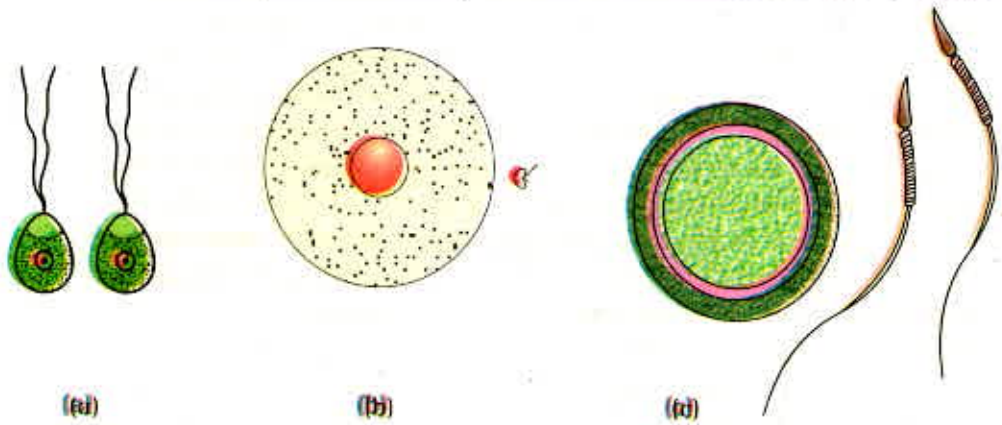


Figure 1.5 Types of gametes: (a) Isogametes of *Cladophora* (an alga); (b) Heterogametes of *Fucus* (an alga); (c) Heterogametes of *Homo sapiens* (Human beings)

They are hence called **homogametes (isogametes)** (Figure 1.5a). However, in a majority of sexually reproducing organisms the gametes produced are of two morphologically distinct types **(heterogametes).** In such organisms the male gamete is called the **antherozoid** or **sperm** and the female gamete is called the **egg** or **ovum** (Figure 1.5 b, c).

Sexuality in organisms: Sexual reproduction in organisms generally involves the fusion of gametes from two different individuals. But this is not always true. *From your recollection of examples studied in Class XI, can you identify cases where self-fertilisation is observed?* Of course, citing such examples in plants is easy.

Plants may have both male and female reproductive structures in the same plant (bisexual) (Figure 1.6 c, e) or on different plants (unisexual) (Figure 1.6d). In several fungi and plants, terms such as **homothallic and monoecious** are used to denote the bisexual condition and **heterothallic** and **dioecious** are the terms used to describe unisexual condition. In flowering plants, the unisexual male flower is **staminate**, i.e., bearing stamens, while the female is **pistillate** or bearing pistils. In some flowering plants, both male and female flowers may be present on the same individual (monoecious) or on separate individuals (dioecious). Some examples of monoecious plants are cucurbits and coconuts and of dioecious plants are papaya and date palm. *Name the type of gametes that are formed in staminate and pistillate flowers.*

But what about animals? Are individuals of all species either male or female (**unisexual**)? Or are there species which possess both the reproductive organs (**bisexual**)? *You probably can make a list of several unisexual animal species.* Earthworms, (Figure 1.6a) sponge, tapeworm and leech, typical examples of bisexual animals that possess both male and female reproductive organs, are **hermaphrodites**. Cockroach (Figure 1.6b) is an example of a unisexual species.

Cell division during gamete formation : Gametes in all heterogametic species are of two types namely, **male** and **female**. Gametes are **haploid** though the parent plant body from which they arise may be either haploid or diploid. A haploid parent produces gametes by mitotic division. *Does this mean that meiosis never occurs in organisms that are haploid?* Carefully examine the flow charts of life cycles of algae that you have studied in Class XI (Chapter 3) to get a suitable answer.

Several organisms belonging to monera, fungi, algae and bryophytes have **haploid** plant body, but in organisms belonging to pteridophytes, gymnosperms, angiosperms and most of the animals including human beings, the parental body is **diploid.** It is obvious that meiosis, the reduction division, has to occur if a diploid body has to produce haploid gametes.

In diploid organisms, specialised cells called **meiocytes** (gamete mother cell) undergo meiosis. At the end of meiosis, only one set of chromosomes

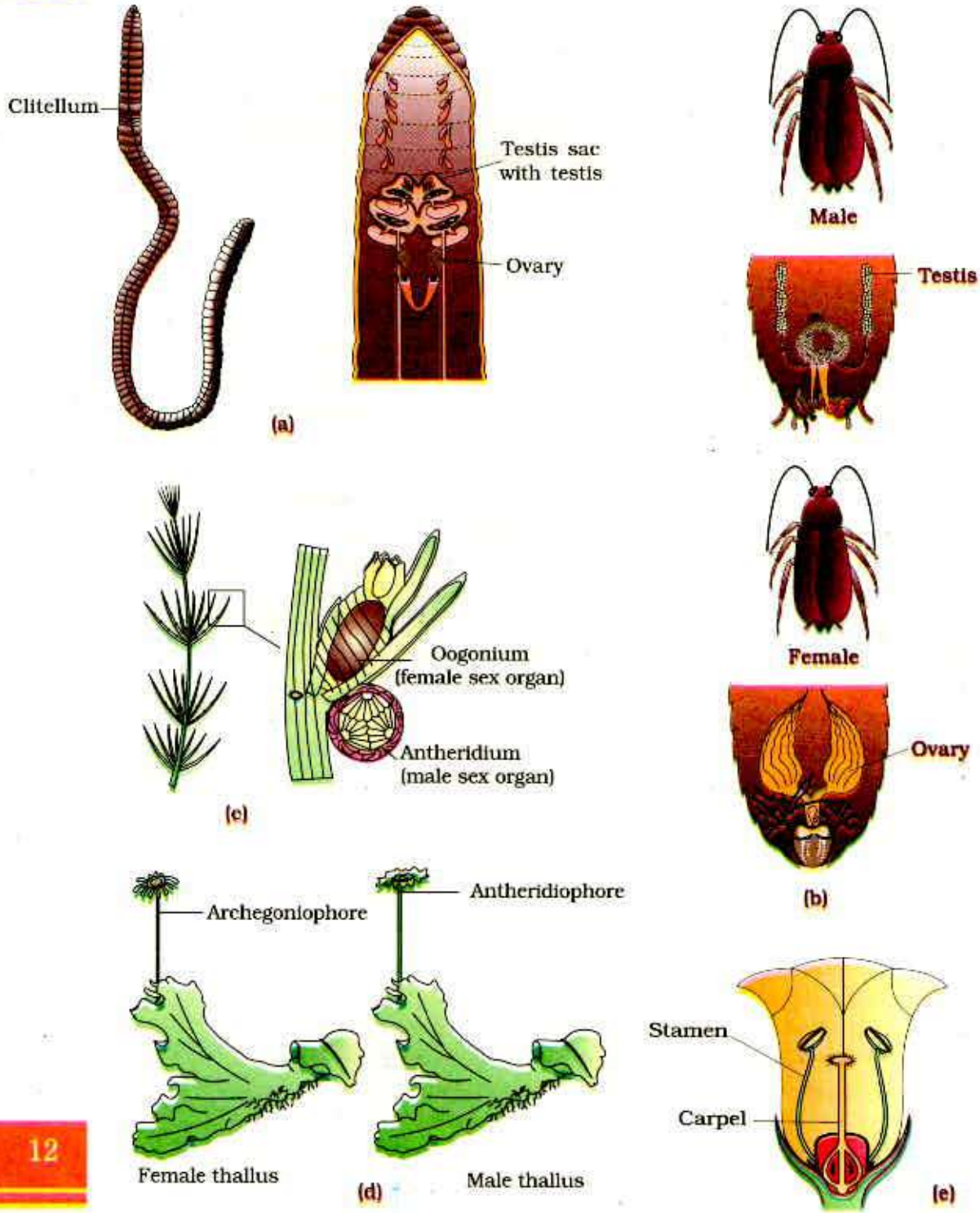


Figure 1.6 Diversity of sexuality in organisms (a) Bisexual animal (Earthworm); (b) Unisexual animal (Cockroach); (c) Monoecious plant *(Chara)*; (d) Dioecious plant *(Marchantia)*; (e) Bisexual flower (sweet potato)

Table 1.1: Chromosome Numbers in Meiocytes (diploid, 2n) and Gametes (haploid, n) of Some Organisms. Fill in the Blank Spaces.

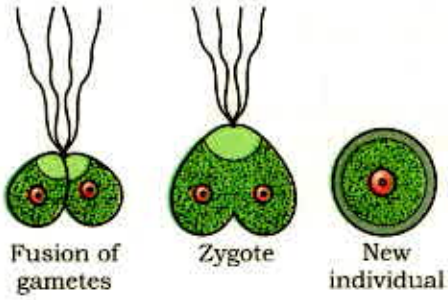
Name of organism	Chromosome number in meiocyte (2n)	Chromosome number in gamete (n)
Human beings	*46*	*23*
House fly	*12*	—
Rat	—	*21*
Dog	*78*	—
Cat	—	*19*
Fruit fly	*8*	—
Ophioglossum (a fern)	—	*630*
Apple	*34*	—
Rice	—	*12*
Maize	*20*	—
Potato	—	*24*
Butterfly	*380*	—
Onion	—	*8*

gets incorporated into each **gamete**. Carefully study Table 1.1 and fill in the diploid and haploid chromosome numbers of organisms. *Is there any relationship in the number of chromosomes of meiocytes and gametes?*

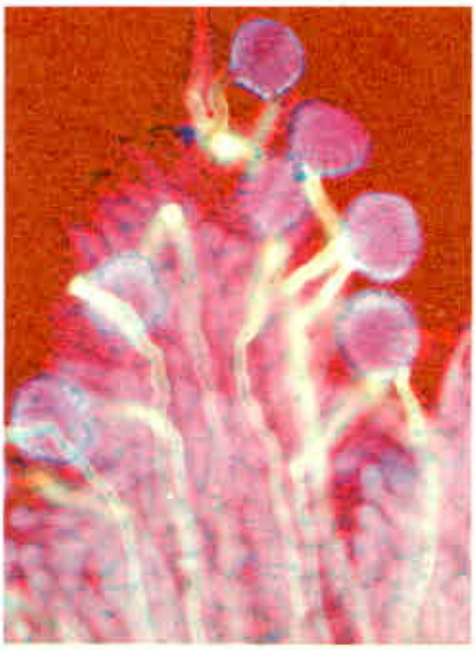
1.2.1.2 Gamete Transfer

After their formation, male and female gametes must be physically brought together to facilitate fusion (fertilisation). *Have you ever wondered how the gametes meet?* In a majority of organisms, male gamete is motile and the female gamete is stationary. Exceptions are a few fungi and algae in which both types of gametes are motile (Figure1.7a). There is a need for a medium through which the male gametes move. In several simple plants like algae, bryophytes and pteridophytes, water is the medium through which this gamete transfer takes place. A large number of the male gametes, however, fail to reach the female gametes. To compensate this loss of male gametes during transport, the number of male gametes produced is several thousand times the number of female gametes produced.

In seed plants, pollen grains are the carriers of male gametes and ovule have the egg. Pollen grains produced in anthers therefore, have to



(a)



(b)

Figure 1.7 (a) Homogametic contact in alga; (b) Germinating pollen grains on the stigma of a flower

be transferred to the stigma before it can lead to fertilisation (Figure 1.7b). In bisexual, self-fertilising plants, e.g., peas, transfer of pollen grains to the stigma is relatively easy as anthers and stigma are located close to each other; pollen grains soon after they are shed, come in contact with the stigma. But in cross pollinating plants (including dioecious plants), a specialised event called **pollination** facilitates transfer of pollen grains to the stigma. Pollen grains germinate on the stigma and the pollen tubes carrying the male gametes reach the ovule and discharge male gametes near the egg. In dioecious animals, since male and female gametes are formed in different individuals, the organism must evolve a special mechanism for gamete transfer. Successful transfer and coming together of gametes is essential for the most critical event in sexual reproduction, the fertilisation.

1.2.2 Fertilisation

The most vital event of sexual reproduction is perhaps the fusion of gametes. This process called **syngamy** results in the formation of a diploid **zygote**. The term **fertilisation** is also often used for this process. The terms syngamy and fertilisation are frequently used though , interchangeably.

What would happen if syngamy does not occur?

However, it has to be mentioned here that in some organisms like rotifers, honeybees and even some lizards and birds (turkey), the female gamete undergoes development to form new organisms without fertilisation. This phenomenon is called **parthenogenesis.**

Where does syngamy occur? In most aquatic organisms, such as a majority of algae and fishes as well as amphibians, syngamy occurs in the external medium (water), i.e., outside the body of the organism. This type of gametic fusion is called **external fertilisation**. Organisms exhibiting external fertilisation show great synchrony between the sexes and release a large number of gametes into the surrounding medium (water) in order to enhance the chances of syngamy. This happens in the bony fishes and frogs where a large number of offspring are produced. A major disadvantage is that the offspring are extremely vulnerable to predators threatening their survival up to adulthood.

In many terrestrial organisms, belonging to fungi, higher animals such as reptiles, birds, mammals and in a majority of plants (bryophytes, pteridophytes, gymnosperms and angiosperms), syngamy occurs inside

the body of the organism, hence the process is called **internal fertilisation.** In all these organisms, egg is formed inside the female body where they fuse with the male gamete. In organisms exhibiting internal fertilisation, the male gamete is motile and has to reach the egg in order to fuse with it. In these even though the number of sperms produced is very large, there is a significant reduction in the number of eggs produced. In seed plants, however, the non-motile male gametes are carried to female gamete by pollen tubes.

1.2.3 Post-fertilisation Events

Events in sexual reproduction after the formation of zygote are called **post-fertilisation events**.

1.2.3.1 The Zygote

Formation of the diploid zygote is universal in all sexually reproducing organisms. In organisms with external fertilisation, zygote is formed in the external medium (usually water), whereas in those exhibiting internal fertilisation, zygote is formed inside the body of the organism.

Further development of the zygote depends on the type of life cycle the organism has and the environment it is exposed to. In organisms belonging to fungi and algae, zygote develops a thick wall that is resistant to dessication and damage. It undergoes a period of rest before germination. In organisms with haplontic life cycle (As you have read in Class XI), zygote divides by meiosis to form haploid spores that grow into haploid individuals. *Consult your Class XI book and find out what kind of development takes place in the zygote in organisms with diplontic and haplo-diplontic life cycles.*

Zygote is the vital link that ensures continuity of species between organisms of one generation and the next. Every sexually reproducing organism, including human beings begin life as a single cell–the zygote.

1.2.3.2 Embryogenesis

Embryogenesis refers to the process of development of **embryo** from the zygote. During embryogenesis, zygote undergoes **cell division** (mitosis) and **cell differentiation**. While cell divisions increase the number of cells in the developing embryo; cell differentiation helps groups of cells to undergo certain modifications to form specialised tissues and organs to form an organism. You have studied about the process of cell division and differentiation in the previous class.

Animals are categorised into **oviparous** and **viviparous** based on whether the development of the zygote takes place outside the body of the female parent or inside, i.e., whether they lay fertilised/unfertilised eggs or give birth to young ones. In oviparous animals like reptiles and birds,

the fertilised eggs covered by hard **calcareous shell** are laid in a safe place in the **environment;** after a period of incubation young ones hatch out. On the other hand, in viviparous animals (majority of mammals including human beings), the zygote develops into a young one inside the body of the female organism. After attaining a certain stage of growth, the young ones are delivered out of the body of the female organism. Because of proper embryonic care and protection, the chances of survival of young ones is greater in viviparous organisms.

In flowering plants, the zygote is formed inside the ovule. After fertilisation the sepals, petals and stamens of the flower wither and fall off. *Can you name a plant in which the sepals remain attached?* The pistil however, remains attached to the plant. The zygote develops into the embryo and the ovules develop into the seed. The **ovary** develops into the **fruit** which develops a thick wall called **pericarp** that is protective in function (Figure 1.8). After dispersal, seeds germinate under favourable conditions to produce new plants.

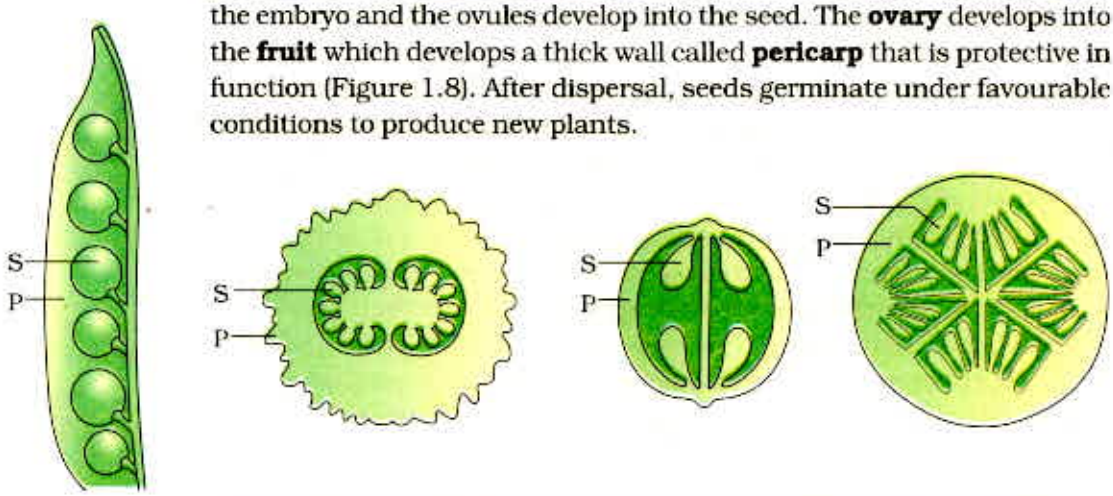


Figure 1.8 A few kinds of fruit showing seeds (S) and protective pericarp (P)

SUMMARY

Reproduction enables a species to live generation after generation. Reproduction in organisms can be broadly classified into asexual and sexual reproduction. Asexual reproduction does not involve the fusion of gametes. It is common in organisms that have a relatively simple organisation such as the fungi, algae and some invertebrate animals. The offspring formed by asexual reproduction are identical and are referred to as clones. Zoospores, conidia, etc., are the most common asexual structures formed in several algae and fungi. Budding and gemmule formation are the common asexual methods seen in animals.

Prokaryotes and unicellular organisms reproduce asexually by cell division or binary fission of the parent cell. In several aquatic and terrestrial species of angiosperms, structures such as runners,

rhizomes, suckers, tubers, offsets, etc., are capable of giving rise to new offspring. This method of asexual reproduction is generally referred to as vegetative propagation.

Sexual reproduction involves the formation and fusion of gametes. It is a complex and slower process as compared to asexual reproduction. Most of the higher animals reproduce almost entirely by sexual method. Events of sexual reproduction may be categorised into pre-fertilisation, fertilisation and post-fertilisation events. Pre-fertilisation events include gametogenesis and gamete transfer while post-fertilisation events include the formation of zygote and embryogenesis.

Organisms may be bisexual or unisexual. Sexuality in plants is varied, particularly in angiosperms, due to the production of diverse types of flowers. Plants are defined as monoecious and dioecious. Flowers may be bisexual or unisexual flowers.

Gametes are haploid in nature and usually a direct product of meiotic division except in haploid organisms where gametes are formed by mitosis.

Transfer of male gametes is an essential event in sexual reproduction. It is relatively easy in bisexual organisms. In unisexual animals it occurs by copulation or simultaneous release. In angiosperms, a special process called pollination ensures transfer of pollen grains which carry the pollen grains to the stigma.

Syngamy (fertilisation) occurs between the male and female gametes. Syngamy may occur either externally, outside the body of organisms or internally, inside the body. Syngamy leads to formation of a specialised cell called zygote.

The process of development of embryo from the zygote is called embryogenesis. In animals, the zygote starts developing soon after its formation. Animals may be either oviparous or viviparous. Embryonal protection and care are better in viviparous organisms.

In flowering plants, after fertilisation, ovary develops into fruit and ovules mature into seeds. Inside the mature seed is the progenitor of the next generation, the embryo.

EXERCISES

1. Why is reproduction essential for organisms?
2. Which is a better mode of reproduction: sexual or asexual? Why?
3. Why is the offspring formed by asexual reproduction referred to as clone?
4. Offspring formed due to sexual reproduction have better chances of survival. Why? Is this statement always true?
5. How does the progeny formed from asexual reproduction differ from those formed by sexual reproduction?
6. Distinguish between asexual and sexual reproduction. Why is vegetative reproduction also considered as a type of asexual reproduction?

7. What is vegetative propagation? Give two suitable examples.
8. Define
   (a) Juvenile phase,
   (b) Reproductive phase,
   (c) Senescent phase.
9. Higher organisms have resorted to sexual reproduction in spite of its complexity. Why?
10. Explain why meiosis and gametogenesis are always interlinked?
11. Identify each part in a flowering plant and write whether it is haploid (n) or diploid (2n).
    (a) Ovary _____
    (b) Anther _____
    (c) Egg _____
    (d) Pollen _____
    (e) Male gamete _____
    (f) Zygote _____
12. Define external fertilisation. Mention its disadvantages.
13. Differentiate between a zoospore and a zygote.
14. Differentiate between gametogenesis from embryogenesis.
15. Describe the post-fertilisation changes in a flower.
16. What is a bisexual flower? Collect five bisexual flowers from your neighbourhood and with the help of your teacher find out their common and scientific names.
17. Examine a few flowers of any cucurbit plant and try to identify the staminate and pistillate flowers. Do you know any other plant that bears unisexual flowers?
18. Why are offspring of oviparous animals at a greater risk as compared to offspring of viviparous animals?

CHAPTER 2

SEXUAL REPRODUCTION IN FLOWERING PLANTS

2.1 *Flower – A Fascinating Organ of Angiosperms*

2.2 *Pre-fertilisation : Structures and Events*

2.3 *Double Fertilisation*

2.4 *Post-fertilisation: Structures and Events*

2.5 *Apomixis and Polyembryony*

Are we not lucky that plants reproduce sexually? The myriads of flowers that we enjoy gazing at, the scents and the perfumes that we swoon over, the rich colours that attract us, are all there as an aid to sexual reproduction. Flowers do not exist only for us to be used for our own selfishness. All flowering plants show sexual reproduction. A look at the diversity of structures of the inflorescences, flowers and floral parts, shows an amazing range of adaptations to ensure formation of the end products of sexual reproduction, the fruits and seeds. In this chapter, let us understand the morphology, structure and the processes of sexual reproduction in flowering plants (angiosperms).

2.1 Flower – A Fascinating Organ of Angiosperms

Human beings have had an intimate relationship with flowers since time immemorial. Flowers are objects of aesthetic, ornamental, social, religious and cultural value – they have always been used as symbols for conveying important human feelings such as love, affection, happiness, grief, mourning, etc. *List at least five flowers of ornamental value that are commonly cultivated at*

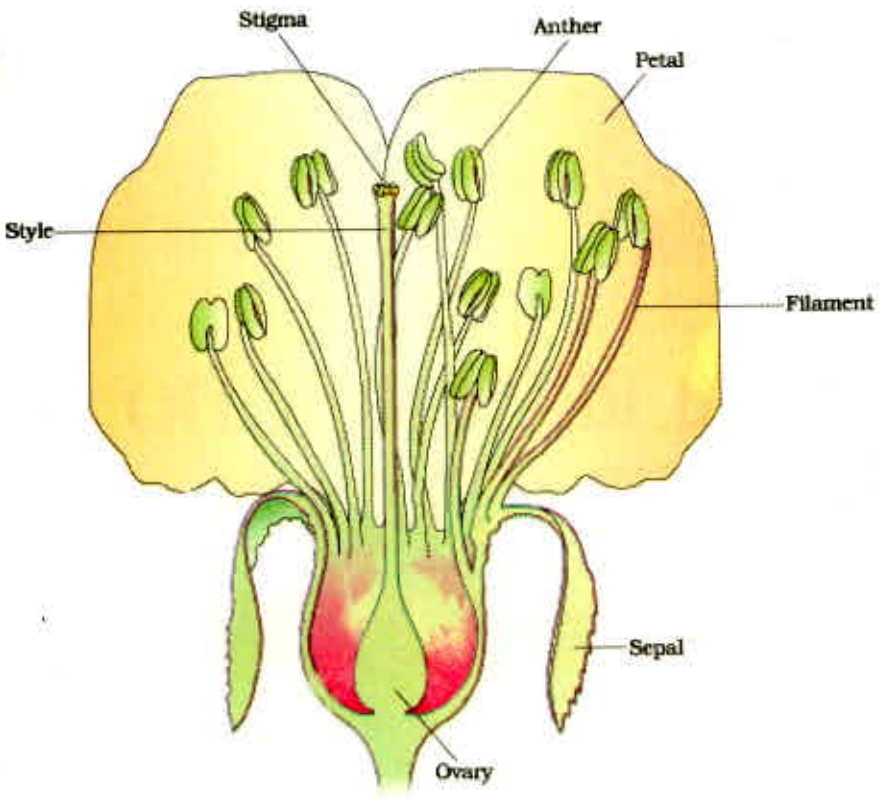


Figure 2.1 A diagrammatic representation of L.S. of a flower

homes and in gardens. Find out the names of five more flowers that are used in social and cultural celebrations in your family. Have you heard of floriculture – what does it refer to?

To a biologist, flowers are morphological and embryological marvels and the sites of sexual reproduction. In class XI, you have read the various parts of a flower. Figure 2.1 will help you recall the parts of a typical flower. Can you name the two parts in a flower in which the two most important units of sexual reproduction develop?

2.2 Pre-fertilisation: Structures and Events

Much before the actual flower is seen on a plant, the decision that the plant is going to flower has taken place. Several hormonal and structural changes are initiated which lead to the differentiation and further development of the floral primordium. Inflorescences are formed which bear the floral buds and then the flowers. In the flower the male and female reproductive structures, the androecium and the gynoecium differentiate and develop. You would recollect that the androecium consists of a whorl of stamens representing the male reproductive organ and the gynoecium represents the female reproductive organ.

2.2.1 Stamen, Microsporangium and Pollen Grain

Figure 2.2a shows the two parts of a typical **stamen** – the long and slender stalk called the **filament**, and the terminal generally bilobed structure called the **anther.** The proximal end of the filament is attached to the thalamus or the petal of the flower. The number and length of stamens are variable in flowers of different species. If you were to collect a stamen each from ten flowers (each from different species) and arrange them on a slide, you would be able to appreciate the large variation in size seen in nature. Careful observation of each stamen under a dissecting microscope and making neat diagrams would elucidate the range in shape and attachment of anthers in different flowers.

A typical angiosperm anther is **bilobed** with each lobe having two theca, i.e., they are **dithecous** (Figure 2.2 b). Often a longitudinal groove runs lengthwise separating the theca. Let us understand the various types of tissues and their organisation in the transverse section of an anther (Figure 2.3 a). The bilobed nature of an anther is very distinct in the transverse section of the anther. The anther is a four-sided (tetragonal) structure consisting of four **microsporangia** located at the corners, two in each lobe.

The microsporangia develop further and become **pollen sacs.** They extend longitudinally all through the length of an anther and are packed with pollen grains.

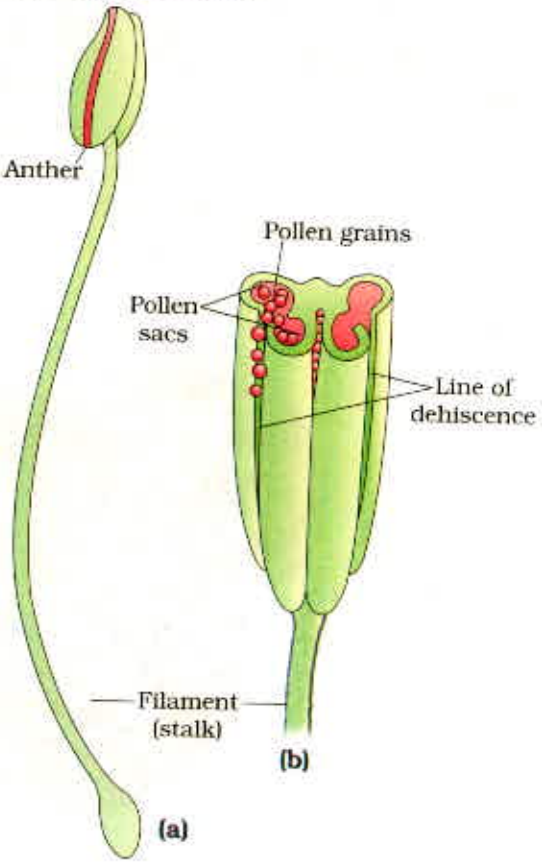


Figure 2.2 (a) A typical stamen; (b) three-dimensional cut section of an anther

Structure of microsporangium: In a transverse section, a typical microsporangium appears near circular in outline. It is generally surrounded by four wall layers (Figure 2.3 b)– the epidermis, endothecium, middle layers and the tapetum. The outer three wall layers perform the function of protection and help in dehiscence of anther to release the pollen. The innermost wall layer is the **tapetum.** It nourishes the developing pollen grains. Cells of the tapetum possess dense cytoplasm and generally have more than one nucleus. *Can you think of how tapetal cells could become bi-nucleate?*

When the anther is young, a group of compactly arranged homogenous cells called the **sporogenous tissue** occupies the centre of each microsporangium.

Microsporogenesis : As the anther develops, the cells of the sporogenous tissue undergo meiotic divisions to form microspore tetrads. *What would be the ploidy of the cells of the tetrad?*

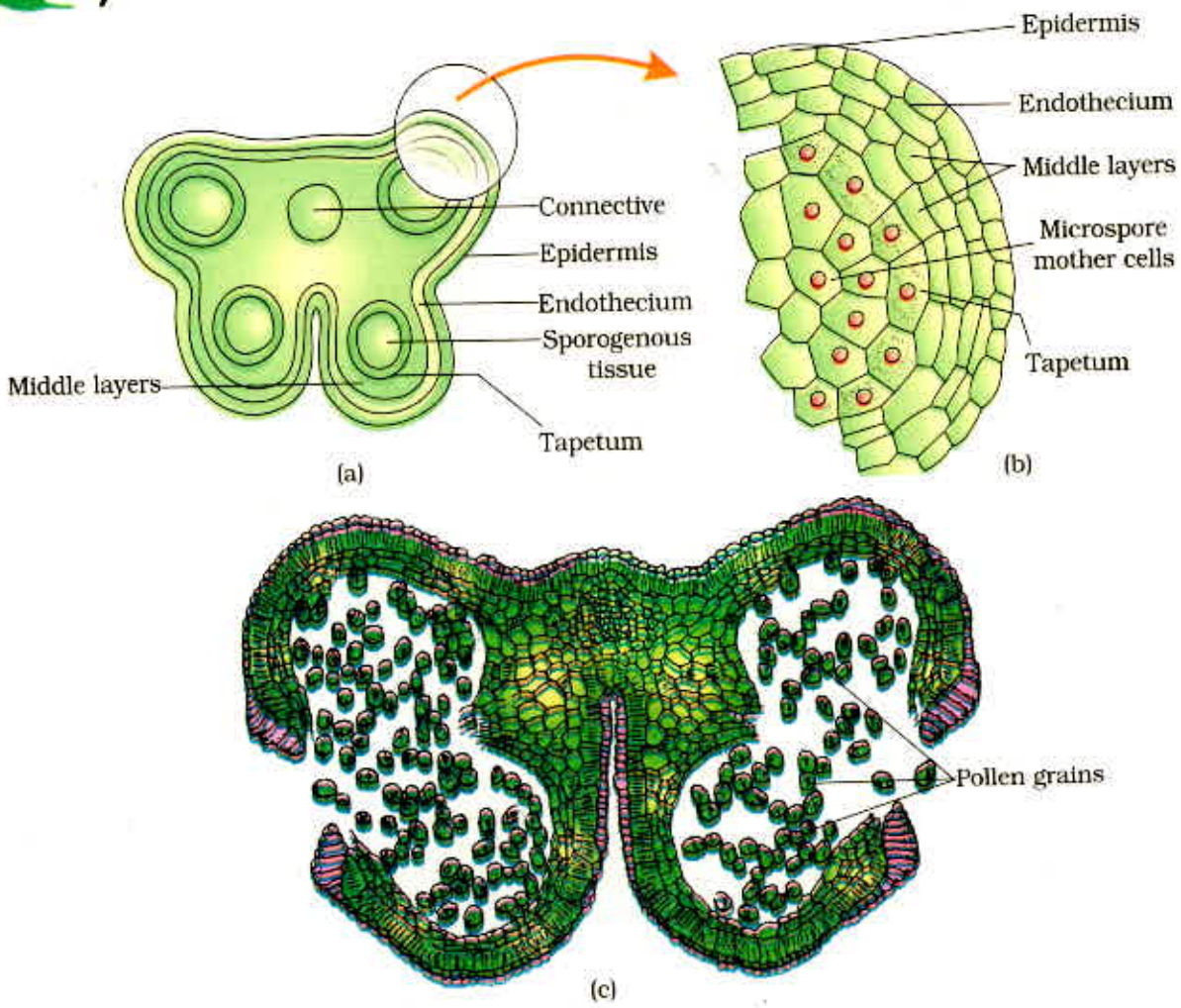


Figure 2.3 (a) Transverse section of a young anther; (b) Enlarged view of one microsporangium showing wall layers; (c) A mature dehisced anther

As each cell of the sporogenous tissue is capable of giving rise to a microspore tetrad. Each one is a potential pollen or microspore mother cell. The process of formation of microspores from a pollen mother cell (PMC) through meiosis is called **microsporogenesis.** The microspores, as they are formed, are arranged in a cluster of four cells–the **microspore tetrad** (Figure 2.3 a). As the anthers mature and dehydrate, the microspores dissociate from each other and develop into **pollen grains** (Figure 2.3 b). Inside each microsporangium several thousands of microspores or pollen grains are formed that are released with the dehiscence of anther (Figure 2.3 c).

Pollen grain: The pollen grains represent the male gametophytes. If you touch the opened anthers of *Hibiscus* or any other flower you would find deposition of yellowish powdery pollen grains on your fingers. Sprinkle these grains on a drop of water taken on a glass slide and observe under

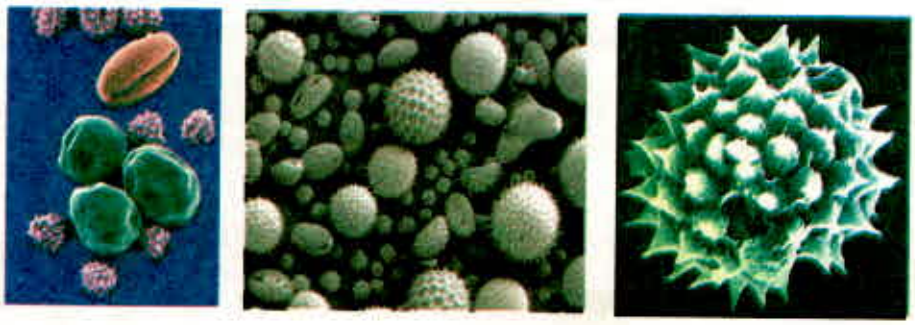


Figure 2.4 Scanning electron micrographs of a few pollen grains

a microscope. You will really be amazed at the variety of architecture – sizes, shapes, colours, designs – seen on the pollen grains from different species (Figure 2.4).

Pollen grains are generally spherical measuring about 25-50 micrometers in diameter. It has a prominent two-layered wall. The hard outer layer called the **exine** is made up of sporopollenin which is one of the most resistant organic material known. It can withstand high temperatures and strong acids and alkali. No enzyme that degrades sporopollenin is so far known. Pollen grain exine has prominent apertures called **germ pores** where sporopollenin is absent. Pollen grains are well-preserved as fossils because of the presence of sporopollenin. The exine exhibits a fascinating array of patterns and designs. *Why do you think the exine should be hard? What is the function of germ pore?* The inner wall of the pollen grain is called the **intine.** It is a thin and continuous layer made up of cellulose and pectin. The cytoplasm of pollen grain is surrounded by a plasma membrane. When the pollen grain is mature it contains two cells, the **vegetative cell** and **generative cell** (Figure 2.5b). The vegetative cell is bigger, has abundant food reserve and a large irregularly shaped nucleus. The **generative cell** is small and floats in the cytoplasm of the vegetative cell. It is spindle shaped with dense cytoplasm and a nucleus. In over 60 per cent of angiosperms, pollen grains are shed at this 2-celled stage. In the remaining species, the generative cell divides mitotically to give rise to the two male gametes before pollen grains are shed (3-celled stage).

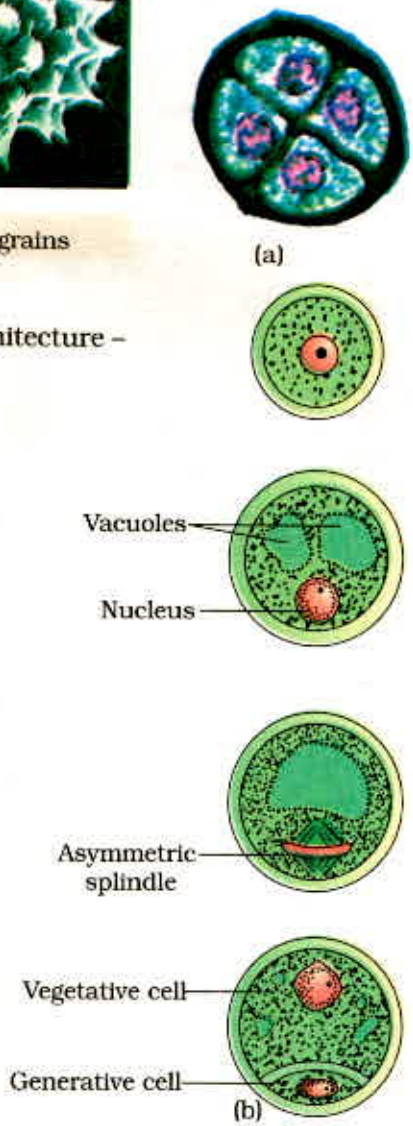


Figure 2.5 (a) Enlarged view of a pollen grain tetrad; (b) stages of a microspore maturing into a pollen grain

Pollen grains of many species cause severe allergies and bronchial afflictions in some people often leading to chronic respiratory disorders– asthma, bronchitis, etc. It may be mentioned that *Parthenium* or carrot grass that came into India as a contaminant with imported wheat, has become ubiquitous in occurrence and causes pollen allergy.

Pollen grains are rich in nutrients. It has become a fashion in recent years to use pollen tablets as food supplements. In western countries, a large number of pollen products in the form of tablets and syrups are available in the market. Pollen consumption has been claimed to increase the performance of athletes and race horses (Figure 2.6).

Figure 2.6 Pollen products

When once they are shed, pollen grains have to land on the stigma before they lose viability if they have to bring about fertilisation. How long do you think the pollen grains retain viability? The period for which pollen grains remain viable is highly variable and to some extent depends on the prevailing temperature and humidity. In some cereals such as rice and wheat, pollen grains lose viability within 30 minutes of their release, and in some members of Rosaceae, Leguminoseae and Solanaceae, they maintain viability for months. You may have heard of storing semen/ sperms of many animals including humans for artificial insemination. It is possible to store pollen grains of a large number of species for years in liquid nitrogen (-196°C). Such stored pollen can be used as pollen banks, similar to seed banks, in crop breeding programmes.

2.2.2 The Pistil, Megasporangium (ovule) and Embryo sac

The gynoecium represents the female reproductive part of the flower. The gynoecium may consist of a single pistil (**monocarpellary**) or may have more than one pistil (**multicarpellary**). When there are more than one, the pistils may be fused together (**syncarpous**) (Figure 2.7b) or may be free (**apocarpous**) (Figure 2.7c). Each pistil has three parts (Figure 2.7a), **the stigma, style and ovary**. The **stigma** serves as a landing platform for pollen grains. The style is the elongated slender part beneath the stigma. The basal bulged part of the pistil is the **ovary.** Inside the ovary is the **ovarian cavity (locule). The placenta** is located inside the ovarian cavity. Recall the definition and types of placentation that you studied in

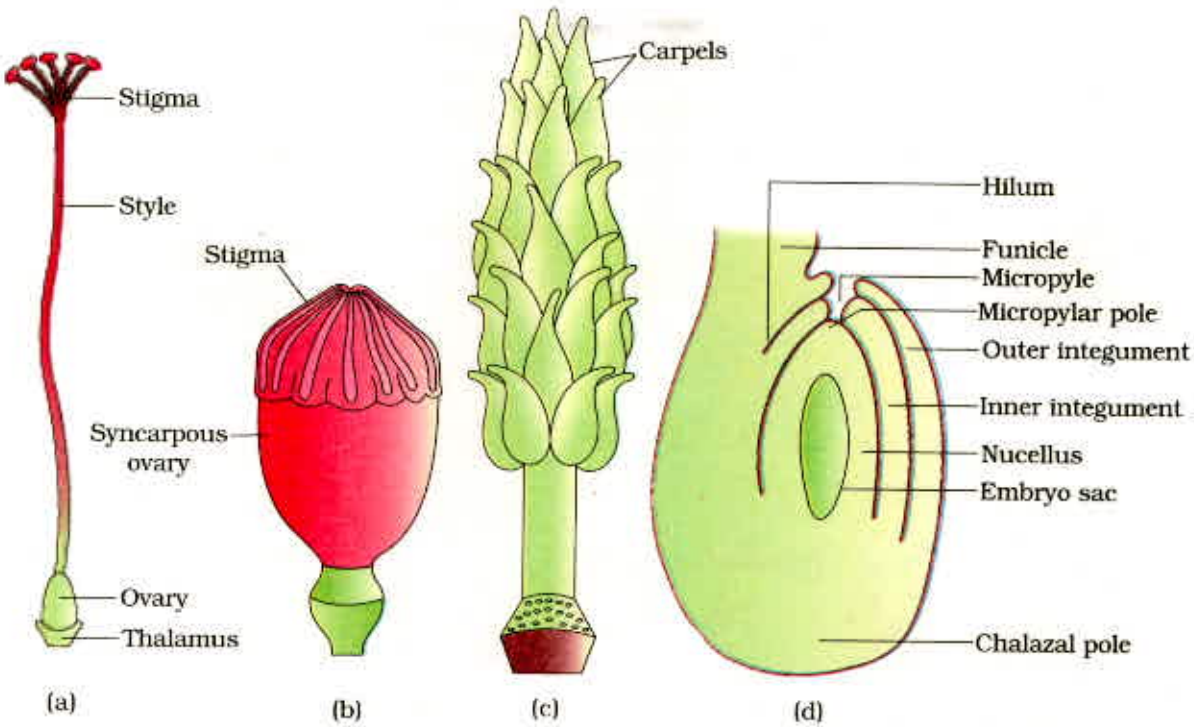


Figure 2.7 (a) A dissected flower of *Hibiscus* showing pistil (other floral parts have been removed); (b) Multicarpellary, syncarpous pistil of *Papaver*; (c) A multicarpellary, apocarpous gynoecium of *Michelia*; (d) A diagrammatic view of a typical anatropous ovule

Class XI. Arising from the placenta are the **megasporangia,** commonly called **ovules.** The number of ovules in an ovary may be one (wheat, paddy, mango) to many (papaya, water melon, orchids).

The Megasporangium (Ovule) : Let us familiarise ourselves with the structure of a typical angiosperm ovule (Figure 2.7d). The ovule is a small structure attached to the placenta by means of a stalk called **funicle**. The body of the ovule fuses with funicle in the region called **hilum.** Thus, hilum represents the junction between ovule and funicle. Each ovule has one or two protective envelopes called **integuments.** Integuments encircle the nucellus except at the tip where a small opening called the **micropyle** is organised. Opposite the micropylar end, is the **chalaza**, representing the basal part of the ovule.

Enclosed within the integuments is a mass of cells called the **nucellus**. Cells of the nucellus have abundant reserve food materials. Located in the nucellus is the **embryo sac** or **female gametophyte**. An ovule generally has a single embryo sac formed from a megaspore.

Megasporogenesis : The process of formation of megaspores from the **megaspore mother cell** is called **megasporogenesis**. Ovules generally differentiate a single megaspore mother cell (MMC) in the micropylar region

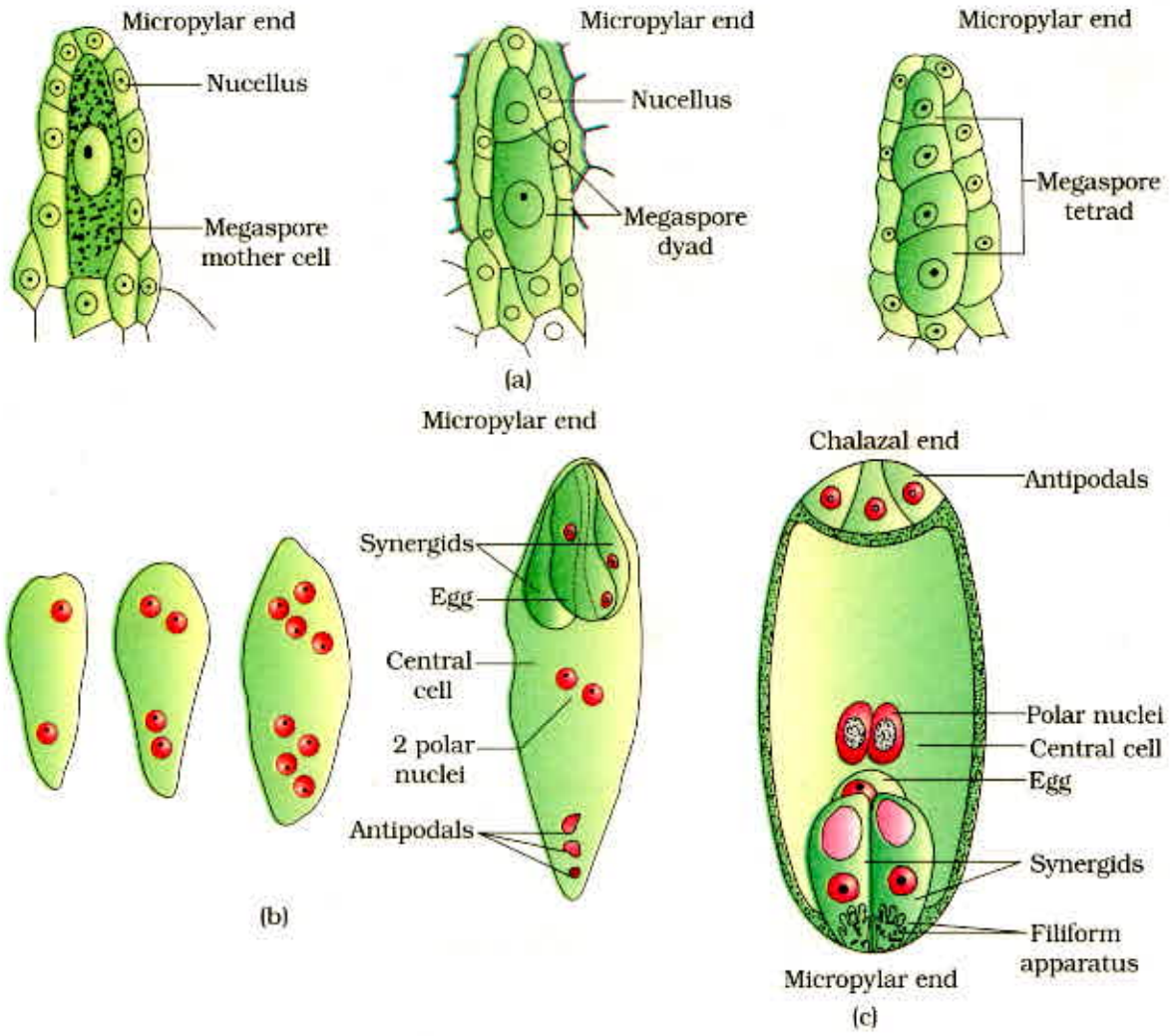


Figure 2.8 (a) Parts of the ovule showing a large megaspore mother cell, a dyad and a tetrad of megaspores; (b) 2, 4, and 8-nucleate stages of embryo sac and a mature embryo sac; (c) A diagrammatic representation of the mature embryo sac.

of the nucellus. It is a large cell containing dense cytoplasm and a prominent nucleus. The MMC undergoes meiotic division. *What is the importance of the MMC undergoing meiosis?* Meiosis results in the production of four **megaspores** (Figure 2.8a).

Female gametophyte : In a majority of flowering plants, one of the megaspores is **functional** while the other three degenerate. Only the **functional megaspore** develops into the **female gametophyte (embryo sac).** This method of embryo sac formation from a single megaspore is termed **monosporic** development. *What will be the ploidy of the cells of the nucellus, MMC, the functional megaspore and female gametophyte?*

Let us study formation of the embryo sac in a little more detail. (Figure 2.8b). The nucleus of the functional megaspore divides mitotically to form two nuclei which move to the opposite poles, forming the **2-nucleate** embryo sac. Two more sequential mitotic nuclear divisions result in the formation of the **4-nucleate** and later the **8-nucleate** stages of the embryo sac. It is of interest to note that these mitotic divisions are strictly free nuclear, that is, nuclear divisions are not followed immediately by cell wall formation. After the 8-nucleate stage, cell walls are laid down leading to the organisation of the typical **female gametophyte** or **embryo sac**. Observe the distribution of cells inside the embryo sac (Figure 2.8b, c). Six of the eight nuclei are surrounded by cell walls and organised into cells; the remaining two nuclei, called polar nuclei are situated below the egg apparatus in the large **central cell.**

There is a characteristic distribution of the cells within the embryo sac. Three cells are grouped together at the micropylar end and constitute the **egg apparatus**. The egg apparatus, in turn, consists of two **synergids** and one **egg cell**. The synergids have special cellular thickenings at the micropylar tip called filiform apparatus, which play an important role in guiding the pollen tubes into the synergid. Three cells are at the chalazal end and are called the **antipodals**. The large central cell, as mentioned earlier, has two polar nuclei. Thus, a typical angiosperm embryo sac, at maturity, though **8-nucleate** is **7-celled**.

2.2.3 Pollination

In the preceding sections you have learnt that the male and female gametes in flowering plants are produced in the pollen grain and embryo sac, respectively. As both types of gametes are non-motile, they have to be brought together for fertilisation to occur. How is this achieved?

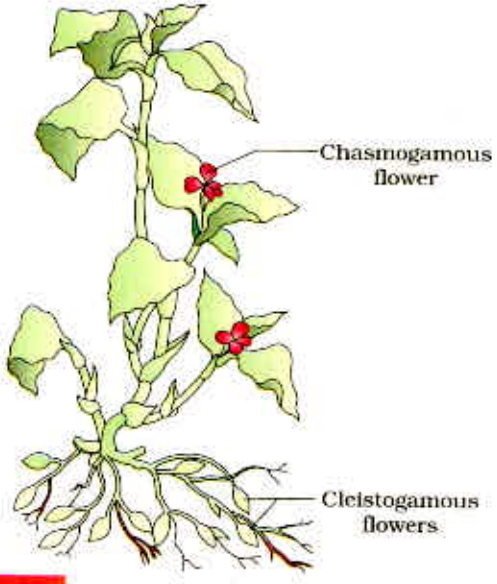
Pollination is the mechanism to achieve this objective. Transfer of pollen grains (shed from the anther) to the stigma of a pistil is termed **pollination**. Flowering plants have evolved an amazing array of adaptations to achieve pollination. They make use of external agents to achieve pollination. *Can you list the possible external agents?*

Kinds of Pollination : Depending on the source of pollen, pollination can be divided into three types.

(i) ***Autogamy*** **:** In this type, pollination is achieved within the same flower. Transfer of pollen grains from the anther to the stigma of the same flower (Figure 2.9a). In a normal flower which opens and exposes the anthers and the stigma, complete autogamy is rather rare. Autogamy in such flowers requires synchrony in pollen release and stigma receptivity and also, the anthers and the stigma should

(a)

(b)



(c)

Figure 2.9 (a) Self-pollinated flowers; (b) Cross pollinated flowers; (c) Cleistogamous flowers

lie close to each other so that self-pollination can occur. Some plants such as *Viola* (common pansy), *Oxalis*, and *Commelina* produce two types of flowers – **chasmogamous** flowers which are similar to flowers of other species with exposed anthers and stigma, and **cleistogamous** flowers which do not open at all (Figure 2.9c). In such flowers, the anthers and stigma lie close to each other. When anthers dehisce in the flower buds, pollen grains come in contact with the stigma to effect pollination. Thus, cleistogamous flowers are invariably autogamous as there is no chance of cross-pollen landing on the stigma. Cleistogamous flowers produce assured seed-set even in the absence of pollinators. Do you think that cleistogamy is advantageous or disadvantageous to the plant? Why?

(ii) ***Geitonogamy*** – Transfer of pollen grains from the anther to the stigma of another flower of the same plant. Although geitonogamy is functionally cross-pollination involving a pollinating agent, genetically it is similar to autogamy since the pollen grains come from the same plant.

(iii) ***Xenogamy*** – Transfer of pollen grains from anther to the stigma of a different plant (Figure 2.9b). This is the only type of pollination which during pollination brings genetically different types of pollen grains to the stigma.

Agents of Pollination : Plants use two abiotic (wind and water) and one biotic (animals) agents to achieve pollination. Majority of plants use biotic agents for pollination. Only a small proportion of plants use abiotic agents. Pollen grains coming in contact with the stigma is a chance factor in both wind and water pollination. To compensate for this uncertainties and associated loss of pollen grains, the flowers produce enormous amount of pollen when compared to the number of ovules available for pollination.

Pollination by wind is more common amongst abiotic pollinations. Wind pollination also requires that the pollen grains are light and non-sticky so that they can be transported in wind currents. They often possess well-exposed stamens (so that the pollens are easily dispersed into wind currents, Figure 2.10) and large often-feathery stigma to easily trap air-borne pollen grains. Wind-pollinated flowers often have a single ovule in each ovary and numerous flowers packed into an inflorescence; a familiar example is the corn cob – the tassels you see are nothing but the stigma and style which wave in the wind to trap pollen grains. Wind-pollination is quite common in grasses.

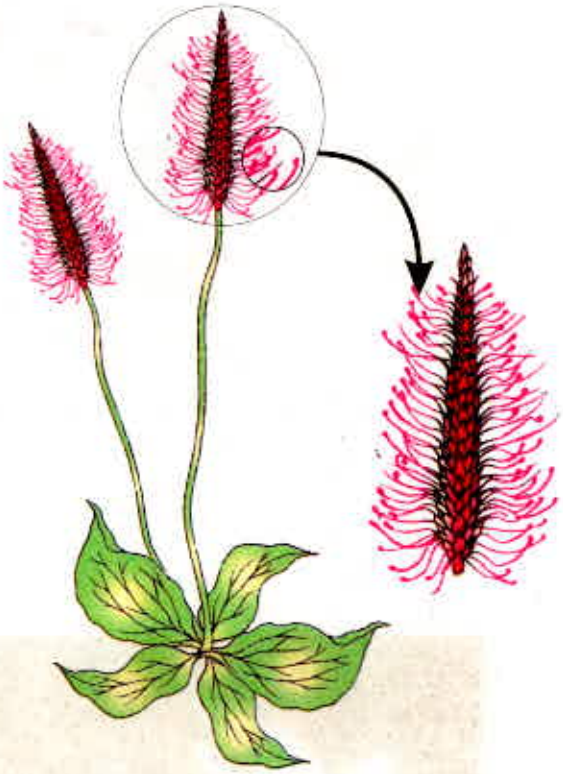


Figure 2.10 A wind-pollinated plant showing compact inflorecence and well-exposed stamens

Pollination by water is quite rare in flowering plants and is limited to about 30 genera, mostly monocotyledons. As against this, you would recall that water is a regular mode of transport for the male gametes among the lower plant groups such as algae, bryophytes and pteridophytes. It is believed, particularly for some bryophytes and pteridophytes, that their distribution is limited because of the need for water for the transport of male gametes and fertilisation. Some examples of water pollinated plants are *Vallisneria* and *Hydrilla* which grow in fresh water and several marine sea-grasses such as *Zostera*. Not all aquatic plants use water for pollination. In a majority of aquatic plants such as water hyacinth and water lily, the flowers emerge above the level of water and are pollinated by insects or wind as in most of the land plants. In *Vallisneria*, the female flower reach the surface of water by the long stalk and the male flowers or pollen grains are released on to the surface of water. They are carried passively by water currents (Figure 2.11a); some of them eventually reach the female flowers and the stigma. In another group of water pollinated plants such as seagrasses, female flowers remain submerged in water and the pollen grains are released inside the water. Pollen grains in many such species are long, ribbon like and they are carried passively inside the water; some of them reach the stigma and achieve pollination. In most of the water-pollinated species, pollen grains are protected from wetting by a mucilaginous covering.

Both wind and water pollinated flowers are not very colourful and do

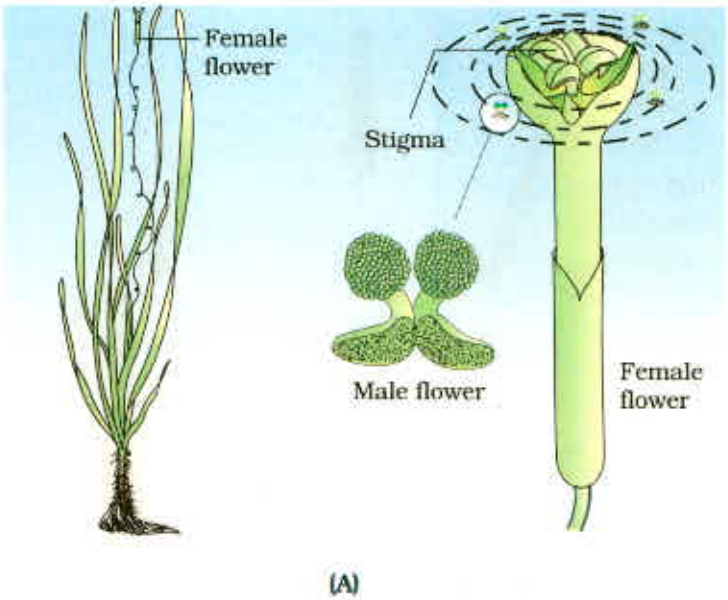


Figure 2.11 (a) Pollination by water in *Vallisneria*; (b) Insect pollination

not produce nectar. *What would be the reason for this?*

Majority of flowering plants use a range of animals as pollinating agents. Bees, butterflies, flies, beetles, wasps, ants, moths, birds (sunbirds and humming birds) and bats are the common pollinating agents. (Figure 2.11b). Among the animals, insects, particularly bees are the dominant biotic pollinating agents. Even larger animals such as some primates (lemurs), arboreal (tree-dwelling) rodents, or even reptiles (gecko lizard and garden lizard) have also been reported as pollinators in some species.

Often flowers of animal-pollinated plants are specifically adapted for a particular species of animal.

Majority of insect-pollinated flowers are large, colourful, fragrant and rich in nectar. When the flowers are small, a number of flowers are clustered into an inflorescence to make them conspicuous. Animals are attracted to flowers by colour and/or fragrance. The flowers pollinated by flies and beetles secrete foul odours to attract these animals. To sustain animal visits, the flowers have to provide rewards to the animals. Nectar and pollen grains are the usual floral rewards. For harvesting the reward(s) from the flower the animal visitor comes in contact with the anthers and the stigma. The body of the animal gets a coating of pollen grains, which are generally sticky in animal pollinated flowers. When the animal carrying pollen on its body comes in contact with the stigma, it brings about pollination.

In some species floral rewards are in providing safe places to lay eggs;

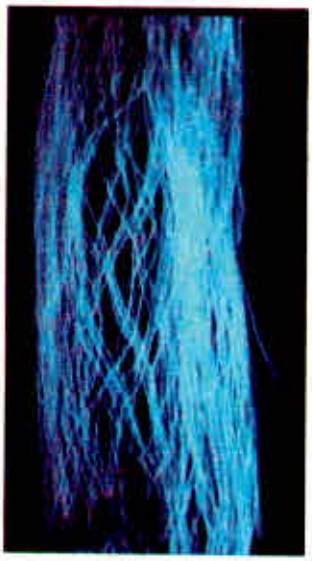
plant – cannot complete their life cycles without each other. The moth deposits its eggs in the locule of the ovary and the flower, in turn, gets pollinated by the moth. The larvae of the moth come out of the eggs as the seeds start developing.

Why don't you observe some flowers of the following plants (or any others available to you): Cucumber, Mango, Peepal, Coriander, Papaya, Onion, Lobia, Cotton, Tobacco, Rose, Lemon, Eucalyptus, Banana? Try to find out which animals visit them and whether they could be pollinators. You'll have to patiently observe the flowers over a few days and at different times of the day. You could also try to see whether there is any correlation in the characteristics of a flower to the animal that visits it. Carefully observe if any of the visitors come in contact with the anthers and the stigma as only such visitors can bring about pollination. Many insects may consume pollen or the nectar without bringing about pollination. Such floral visitors are referred to as pollen/nectar robbers. You may or may not be able to identify the pollinators, but you will surely enjoy your efforts!

Outbreeding Devices : Majority of flowering plants produce hermaphrodite flowers and pollen grains are likely to come in contact with the stigma of the same flower. Continued self-pollination result in inbreeding depression. Flowering plants have developed many devices to discourage self-pollination and to encourage cross-pollination. In some species, pollen release and stigma receptivity are not synchronised. Either the pollen is released before the stigma becomes receptive or stigma becomes receptive much before the release of pollen. In some other species, the anther and stigma are placed at different positions so that the pollen cannot come in contact with the stigma of the same flower. Both these devices prevent autogamy. The third device to prevent inbreeding is self-incompatibility. This is a genetic mechanism and prevents self-pollen (from the same flower or other flowers of the same plant) from fertilising the ovules by inhibiting pollen germination or pollen tube growth in the pistil. Another device to prevent self-pollination is the production of unisexual flowers. If both male and female flowers are present on the same plant such as castor and maize (monoecious), it prevents autogamy but not geitonogamy. In several species such as papaya, male and female flowers are present on different plants, that is each plant is either male or female (dioecy). This condition prevents both autogamy and geitonogamy.

Pollen-pistil Interaction : Pollination does not guarantee the transfer of the right type of pollen (compatible pollen of the same species as the stigma). Often, pollen of the wrong type, either from other species or from the same plant (if it is self-incompatible), also land on the stigma. The pistil has the ability to recognise the pollen, whether it is of the right type (compatible) or of the wrong type (incompatible). If it is of the right type, the pistil accepts the pollen and promotes post-pollination events that

(a)



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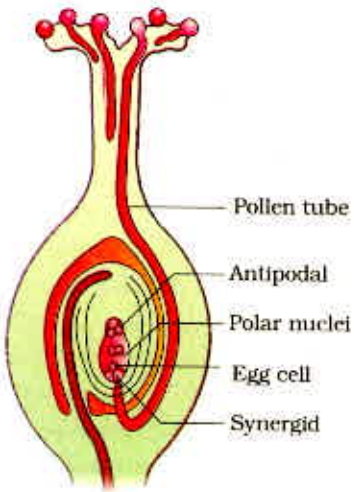
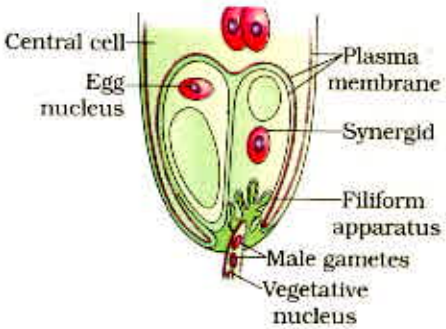
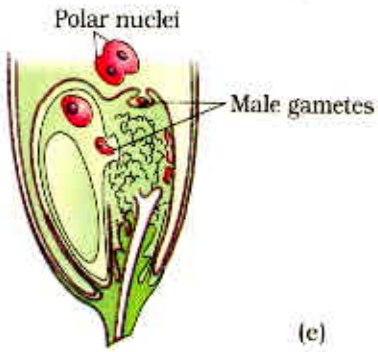


Longitudinal section of a flower showing growth of pollen tube

(c)



(d)



(e)

Figure 2.12 (a) Pollen grains germinating on the stigma; (b) Pollen tubes growing through the style; (c) L.S. of pistil showing path of pollen tube growth; (d) enlarged view of an egg apparatus showing entry of pollen tube into a synergid; (e) Discharge of male gametes into a synergid and the movements of the sperms, one into the egg and the other into the central cell

leads to fertilisation. If the pollen is of the wrong type, the pistil rejects the pollen by preventing pollen germination on the stigma or the pollen tube growth in the style. The ability of the pistil to recognise the pollen followed by its acceptance or rejection is the result of a continuous dialogue between pollen grain and the pistil. This dialogue is mediated by chemical components of the pollen interacting with those of the pistil. It is only in recent years that botanists have been able to identify some of the pollen and pistil components and the interactions leading to the recognition, followed by acceptance or rejection.

As mentioned earlier, following compatible pollination, the pollen grain germinates on the stigma to produce a pollen tube through one of the germ pores (Figure 2.12a). The contents of the pollen grain move into the

pollen tube. Pollen tube grows through the tissues of the stigma and style and reaches the ovary (Figure 2.12b, c). You would recall that in some plants, pollen grains are shed at two-celled condition (a vegetative cell and a generative cell). In such plants, the generative cell divides and forms the two male gametes during the growth of pollen tube in the stigma. In plants which shed pollen in the three-celled condition, pollen tubes carry the two male gametes from the beginning. Pollen tube, after reaching the ovary, enters the ovule through the micropyle and then enters one of the synergids through the filiform apparatus (Figure 2.12d, e). Many recent studies have shown that filiform apparatus present at the micropylar part of the synergids guides the entry of pollen tube. All these events–from pollen deposition on the stigma until pollen tubes enter the ovule–are together referred to as pollen-pistil interaction. As pointed out earlier, pollen-pistil interaction is a dynamic process involving pollen recognition followed by promotion or inhibition of the pollen. The knowledge gained in this area would help the plant breeder in manipulating pollen-pistil interaction, even in incompatible pollinations, to get desired hybrids.

You can easily study pollen germination by dusting some pollen from flowers such as pea, chickpea, *Crotalaria*, balsam and *Vinca* on a glass slide containing a drop of sugar solution (about 10 per cent). After about 15–30 minutes, observe the slide under the low power lens of the microscope. You are likely to see pollen tubes coming out of the pollen grains.

As you shall learn in the chapter on plant breeding (Chapter 9), a breeder is interested in crossing different species and often genera to combine desirable characters to produce commercially 'superior' varieties. **Artificial hybridisation** is one of the major approaches of crop improvement programme. In such crossing experiments it is important to make sure that only the desired pollen grains are used for pollination and the stigma is protected from contamination (from unwanted pollen). This is achieved by emasculation and bagging techniques.

If the female parent bears bisexual flowers, removal of anthers from the flower bud before the anther dehisces using a pair of forceps is necessary. This step is referred to as **emasculation**. Emasculated flowers have to be covered with a bag of suitable size, generally made up of butter paper, to prevent contamination of its stigma with unwanted pollen. This process is called **bagging**. When the stigma of bagged flower attains receptivity, mature pollen grains collected from anthers of the male parent are dusted on the stigma, and the flowers are rebagged, and the fruits allowed to develop.

If the female parent produces unisexual flowers, there is no need for emasculation. The female flower buds are bagged before the flowers open. When the stigma becomes receptive, pollination is carried out using the desired pollen and the flower rebagged.

2.3 Double Fertilisation

After entering one of the synergids, the pollen tube releases the two male gametes into the cytoplasm of the synergid. One of the male gametes moves towards the egg cell and fuses with its nucleus thus completing the **syngamy**. This results in the formation of a diploid cell, the **zygote.** The other male gamete moves towards the two polar nuclei located in the central cell and fuses with them to produce a triploid **primary endosperm nucleus** (PEN) (Figure 2.13a). As this involves the fusion of three haploid nuclei it is termed **triple fusion.** Since two types of fusions, syngamy and triple fusion take place in an embryo sac the phenomenon is termed **double fertilisation**, an event unique to flowering plants. The central cell after triple fusion becomes the **primary endosperm cell** (PEC) and develops into the **endosperm** while the zygote develops into an **embryo.**

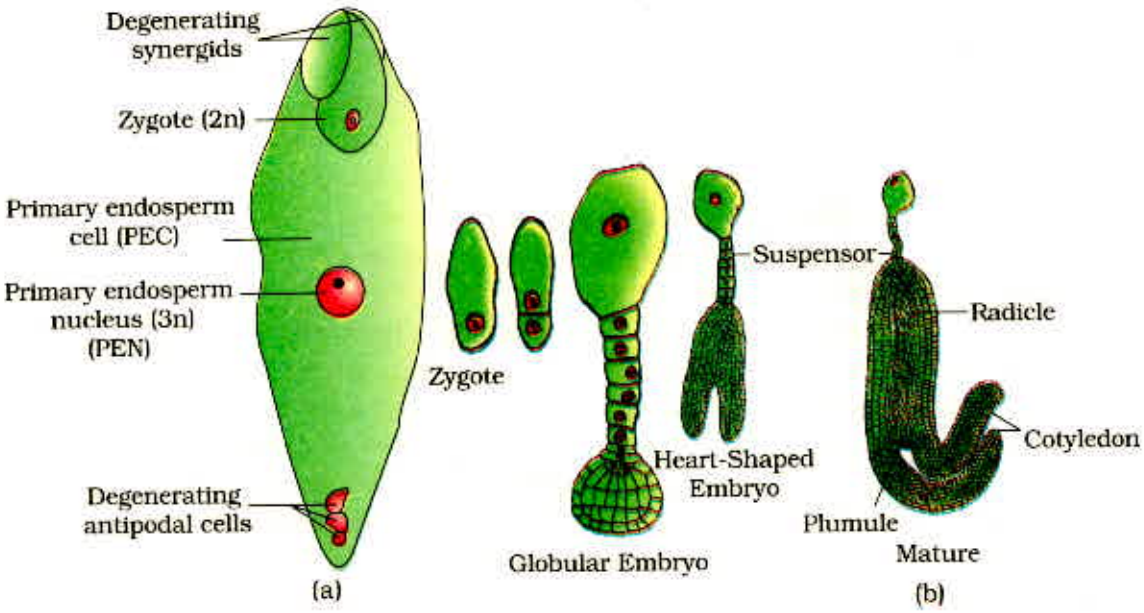


Figure 2.13 (a) Fertilised embryo sac showing zygote and Primary Endosperm Nucleus (PEN); (b) Stages in embryo development in a dicot [shown in reduced size as compared to (a)]

2.4 Post-fertilisation : Structures and Events

Following double fertilisation, events of endosperm and embryo development, maturation of ovule(s) into seed(s) and ovary into fruit, are collectively termed **post-fertilisation events.**

2.4.1 Endosperm

Endosperm development precedes embryo development. *Why?* The primary endosperm cell divides repeatedly and forms a triploid

endosperm tissue. The cells of this tissue are filled with reserve food materials and are used for the nutrition of the developing embryo. In the most common type of endosperm development, the PEN undergoes successive nuclear divisions to give rise to free nuclei. This stage of endosperm development is called free-nuclear endosperm. Subsequently cell wall formation occurs and the endosperm becomes cellular. The number of free nuclei formed before cellularisation varies greatly. The coconut water from tender coconut that you are familiar with, is nothing but free-nuclear endosperm (made up of thousands of nuclei) and the surrounding white kernel is the cellular endosperm.

Endosperm may either be completely consumed by the developing embryo (e.g., pea, groundnut, beans) before seed maturation or it may persist in the mature seed (e.g. castor and coconut) and be used up during seed germination. *Split open some seeds of castor, peas, beans, groundnut, fruit of coconut and look for the endosperm in each case. Find out whether the endosperm is persistent in cereals – wheat, rice and maize.*

2.4.2 Embryo

Embryo develops at the micropylar end of the embryo sac where the zygote is situated. Most zygotes divide only after certain amount of endosperm is formed. This is an adaptation to provide assured nutrition to the developing embryo. Though the seeds differ greatly, the early stages of embryo development **(embryogeny)** are similar in both monocotyledons and dicotyledons. Figure 2.13 depicts the stages of embryogeny in a dicotyledonous embryo. The zygote gives rise to the **proembryo** and subsequently to the **globular, heart-shaped** and **mature embryo**.

A typical dicotyledonous embryo (Figure 2.14a), consists of an **embryonal axis** and two **cotyledons.** The portion of embryonal axis above the level of cotyledons is the **epicotyl,** which terminates with the **plumule** or stem tip. The cylindrical portion below the level of cotyledons is **hypocotyl** that terminates at its lower end in the **radicle** or **root tip.** The root tip is covered with a **root cap.**

Embryos of monocotyledons (Figure 2.14 b) possess only one cotyledon. In the grass family the cotyledon is called **scutellum** that is situated towards one side (lateral) of the embryonal axis. At its lower end, the embryonal axis has the

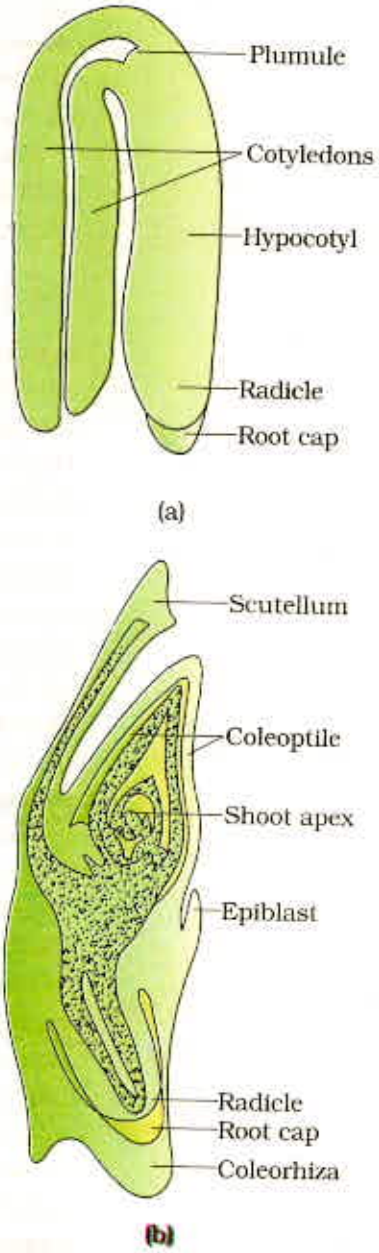


Figure 2.14 (a) A typical dicot embryo; (b) L.S. of an embryo of grass

radical and root cap enclosed in an undifferentiated sheath called **coleorrhiza.** The portion of the embryonal axis above the level of attachment of scutellum is the epicotyl. Epicotyl has a shoot apex and a few leaf primordia enclosed in a hollow foliar structure, the **coleoptile.**

Soak a few seeds in water (say of wheat, maize, peas, chickpeas, ground nut) overnight. Then split the seeds and observe the various parts of the embryo and the seed.

2.4.3 Seed

In angiosperms, the seed is the final product of sexual reproduction. It is often described as a fertilised ovule. Seeds are formed inside fruits. A seed typically consists of seed coat(s), cotyledon(s) and an embryo axis. The cotyledons (Figure 2.15a) of the embryo are simple structures, generally thick and swollen due to storage of food reserves (as in legumes). Mature seeds may be **non-albuminous** or **ex-albuminous**. Non-albuminous seeds have no residual endosperm as it is completely consumed during embryo development (e.g., pea, groundnut). Albuminous seeds retain a part of endosperm as it is not completely used up during embryo development (e.g., wheat, maize, barley, castor). Occasionally, in some seeds such as black pepper and beet, remnants of nucellus are also persistent. This residual, persistent nucellus is the **perisperm.**

Integuments of ovules harden as tough protective seed coats (Figure 2.15a). The micropyle remains as a small pore in the seed coat. This facilitates entry of oxygen and water into the seed during germination. As the seed matures, its water content is reduced and seeds become relatively dry (10-15 per cent moisture by mass). The general metabolic activity of the embryo slows down. The embryo may enter a state of inactivity called **dormancy,** or if favourable conditions are available (adequate moisture, oxygen and suitable temperature), they germinate.

As ovules mature into seeds, the ovary develops into a fruit, i.e., the transformation of ovules into seeds and ovary into fruit proceeds simultaneously. The wall of the ovary develops into the wall of fruit called **pericarp**. The fruits may be fleshy as in guava, orange, mango, etc., or may be dry, as in groundnut, and mustard, etc. Many fruits have evolved mechanisms for dispersal of seeds. Recall the classification of fruits and their dispersal mechanisms that you have studied in an earlier class. *Is there any relationship between number of ovules in an ovary and the number of seeds present in a fruit?*

In most plants, by the time the fruit develops from the ovary, other floral parts degenerate and fall off. However, in a few species such as apple, strawberry, cashew, etc., the thalamus also contributes to fruit formation. Such fruits are called **false fruits** (Figure 2.15b). Most fruits however develop only from the ovary and are called **true fruits**. Although in most of the species, fruits are the results of fertilisation, there are a few species

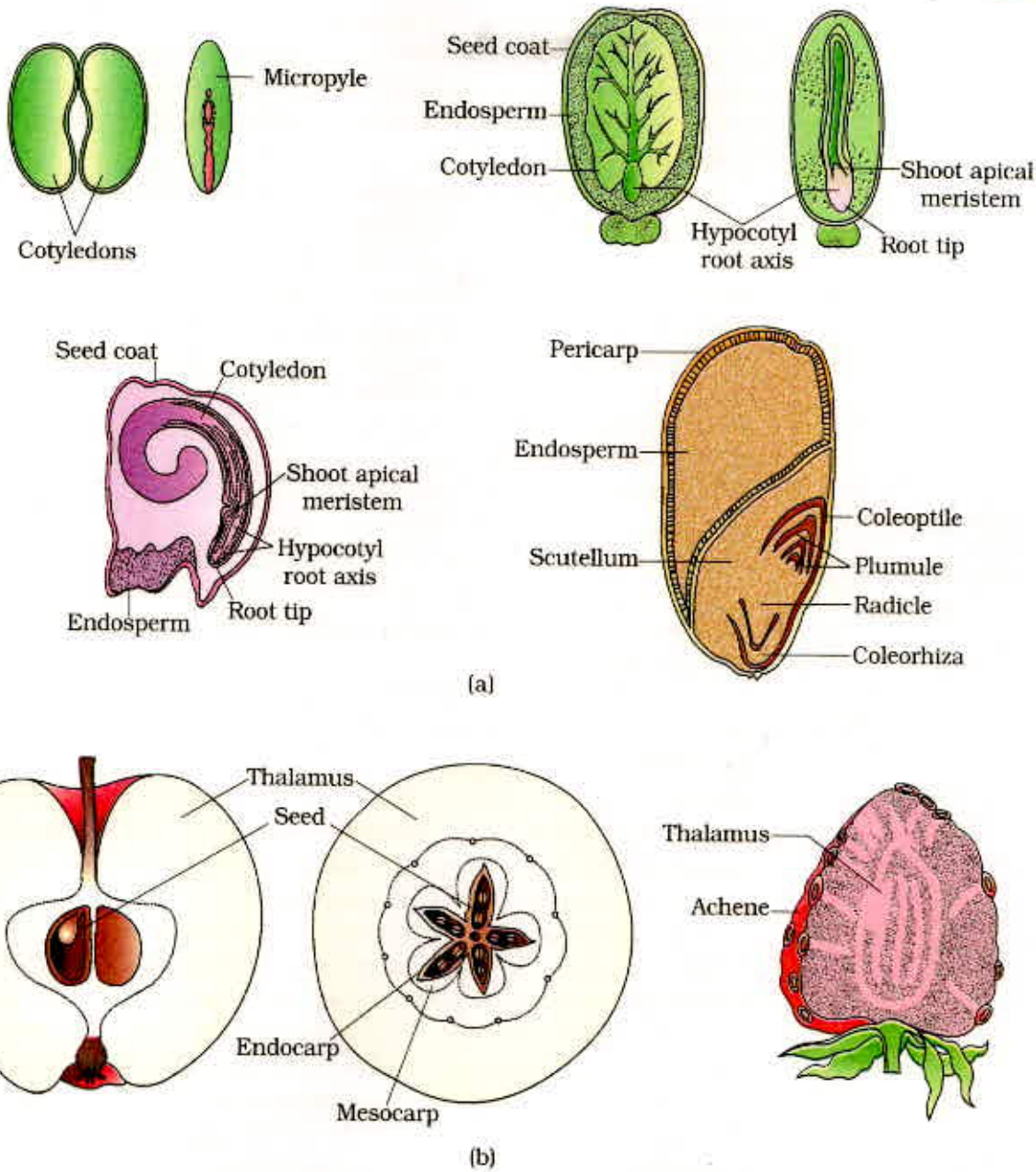


Figure 2.15 (a) Structure of some seeds. (b) False fruits of apple and strawberry

in which fruits develop without fertilisation. Such fruits are called **parthenocarpic fruits.** Banana is one such example. Parthenocarpy can be induced through the application of growth hormones and such fruits are seedless.

Seeds offer several advantages to angiosperms. Firstly, since reproductive processes such as pollination and fertilisation are independent of water, seed formation is more dependable. Also seeds have better adaptive strategies for dispersal to new habitats and help the species

to colonise in other areas. As they have sufficient food reserves, young seedlings are nourished until they are capable of photosynthesis on their own. The hard seed coat provides protection to the young embryo. Being products of sexual reproduction, they generate new genetic combinations leading to variations.

Seed is the basis of our agriculture. Dehydration and dormancy of mature seeds are crucial for storage of seeds which can be used as food throughout the year and also to raise crop in the next season. Can you imagine agriculture in the absence of seeds, or in the presence of seeds which germinate straight away soon after formation and cannot be stored?

How long do the seeds remain alive after they are dispersed? This period again varies greatly. In a few species the seeds lose viability within a few months. Seeds of a large number of species live for several years. Some seeds can remain alive for hundreds of years. There are several records of very old yet viable seeds. The oldest is that of a lupine, *Lupinus arcticus* excavated from Arctic Tundra. The seed germinated and flowered after an estimated record of 10,000 years of dormancy. A recent record of 2000 years old viable seed is of the date palm, *Phoenix dactylifera* discovered during the archeological excavation at King Herod's palace near the Dead Sea.

After completing a brief account of sexual reproduction of flowering plants it would be worth attempting to comprehend the enormous reproductive capacity of some flowering plants by asking the following questions: How many eggs are present in an embryo sac? How many embryo sacs are present in an ovule? How many ovules are present in an ovary? How many ovaries are present in a typical flower? How many flowers are present on a tree? And so on...

Can you think of some plants in which fruits contain very large number of seeds. Orchid fruits are one such category and each fruit contain thousands of tiny seeds. Similar is the case in fruits of some parasitic species such as Orobanche and Striga. Have you seen a tiny seed of Ficus? How large is the tree of Ficus developed from that tiny seed. How many billions of seeds does each Ficus tree produce? Can you imagine any other example in which such a tiny structure can produce such a large biomass over the years?

2.5 Apomixis and Polyembryony

Although seeds, in general are the products of fertilisation, a few flowering plants such as some species of *Asteraceae* and grasses, have evolved a special mechanism, to produce seeds without fertilisation, called **apomixis.** *What is fruit production without fertilisation called?* Thus, apomixis is a form of asexual reproduction that mimics sexual reproduction. There are several ways of development of apomictic seeds. In some species, the diploid egg cell is formed without reduction division and develops into the embryo without fertilisation. More often, as in many *Citrus* and *Mango*

varieties some of the nucellar cells surrounding the embryo sac start dividing, protrude into the embryo sac and develop into the embryos. In such species each ovule contains many embryos. Occurrence of more than one embryo in a seed is referred to as **polyembryony**. *Take out some seeds of orange and squeeze them. Observe the many embryos of different sizes and shapes from each seed. Count the number of embryos in each seed. What would be the genetic nature of apomictic embryos? Can they be called clones?*

Hybrid varieties of several of our food and vegetable crops are being extensively cultivated. Cultivation of hybrids has tremendously increased productivity. One of the problems of hybrids is that hybrid seeds have to be produced every year. If the seeds collected from hybrids are sown, the plants in the progeny will segregate and do not maintain hybrid characters. Production of hybrid seeds is costly and hence the cost of hybrid seeds become too expensive for the farmers. If these hybrids are made into apomicts, there is no segregation of characters in the hybrid progeny. Then the farmers can keep on using the hybrid seeds to raise new crop year after year and he does not have to buy hybrid seeds every year. Because of the importance of apomixis in hybrid seed industry, active research is going on in many laboratories around the world to understand the genetics of apomixis and to transfer apomictic genes into hybrid varieties.

SUMMARY

Flowers are the seat of sexual reproduction in angiosperms. In the flower, androecium consisting of stamens represents the male reproductive organs and gynoecium consisting of pistils represents the female reproductive organs.

A typical anther is bilobed, dithecous and tetrasporangiate. Pollen grains develop inside the microsporangia. Four wall layers, the epidermis, endothecium, middle layers and the tapetum surround the microsporangium. Cells of the sporogenous tissue lying in the centre of the microsporangium, undergo meiosis (microsporogenesis) to form tetrads of microspores. Individual microspores mature into pollen grains.

Pollen grains represents the male gametophytic generation. The pollen grains have a two-layered wall, the outer exine and inner intine. The exine is made up of sporopollenin and has germ pores. Pollen grains may have two cells (a vegetative cell and generative cell) or three cells (a vegetative cell and two male gametes) at the time of shedding.

The pistil has three parts – the stigma, style and the ovary. Ovules are present in the ovary. The ovules have a stalk called funicle, protective integument(s), and an opening called micropyle. The central tissue is the nucellus in which the archesporium differentiates. A cell of the archesporium, the megaspore mother cell divides meiotically and one of the megaspores forms the embryo sac (the female gametophyte). The mature embryo sac is 7-celled and 8-nucleate. At the micropylar end is

the egg apparatus consisting of two synergids and an egg cell. At the chalazal end are three antipodals. At the centre is a large central cell with two polar nuclei.

Pollination is the mechanism to transfer pollen grains from the anther to the stigma. Pollinating agents are either abiotic (wind and water) or biotic (animals).

Pollen-pistil interaction involves all events from the landing of pollen grains on the stigma until the pollen tube enters the embryo sac (when the pollen is compatible) or pollen inhibition (when the pollen is incompatible). Following compatible pollination, pollen grain germinates on the stigma and the resulting pollen tube grow through the style, enter the ovules and finally discharges two male gametes in one of the synergids. Angiosperms exhibit double fertilisation because two fusion events occur in each embryo sac, namely syngamy and triple fusion. The products of these fusions are the diploid zygote and the triploid primary endosperm nucleus (in the primary endosperm cell). Zygote develops into the embryo and the primary endosperm cell forms the endosperm tissue. Formation of endosperm always precedes development of the embryo.

The developing embryo passes through different stages such as the proembryo, globular and heart-shaped stages before maturation. Mature dicotyledonous embryo has two cotyledons and an embryonal axis with epicotyl and hypocotyl. Embryos of monocotyledons have a single cotyledon. After fertilisation, ovary develops into fruit and ovules develop into seeds.

A phenomenon called apomixis is found in some angiosperms, particularly in grasses. It results in the formation of seeds without fertilisation. Apomicts have several advantages in horticulture and agriculture.

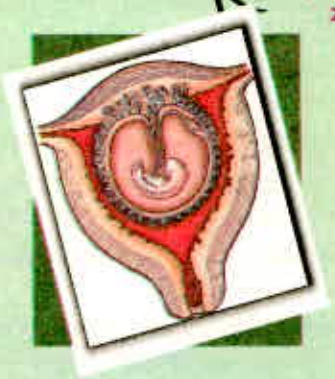
Some angiosperms produce more than one embryo in their seed. This phenomenon is called polyembryony.

EXERCISES

1. Name the parts of an angiosperm flower in which development of male and female gametophyte take place.
2. Differentiate between microsporogenesis and megasporogenesis. Which type of cell division occurs during these events? Name the structures formed at the end of these two events.
3. Arrange the following terms in the correct developmental sequence:

   Pollen grain, sporogenous tissue, microspore tetrad, pollen mother cell, male gametes.
4. With a neat, labelled diagram, describe the parts of a typical angiosperm ovule.
5. What is meant by monosporic development of female gametophyte?
6. With a neat diagram explain the 7-celled, 8-nucleate nature of the female gametophyte.

7. What are chasmogamous flowers? Can cross-pollination occur in cleistogamous flowers? Give reasons for your answer.
8. Mention two strategies evolved to prevent self-pollination in flowers.
9. What is self-incompatibility? Why does self-pollination not lead to seed formation in self-incompatible species?
10. What is bagging technique? How is it useful in a plant breeding programme?
11. What is triple fusion? Where and how does it take place? Name the nuclei involved in triple fusion.
12. Why do you think the zygote is dormant for sometime in a fertilised ovule?
13. Differentiate between:
    (a) hypocotyl and epicotyl;
    (b) coleoptile and coleorrhiza;
    (c) integument and testa;
    (d) perisperm and pericarp.
14. Why is apple called a false fruit? Which part(s) of the flower forms the fruit?
15. What is meant by emasculation? When and why does a plant breeder employ this technique?
16. If one can induce parthenocarpy through the application of growth substances, which fruits would you select to induce parthenocarpy and why?
17. Explain the role of tapetum in the formation of pollen-grain wall.
18. What is apomixis and what is its importance?



CHAPTER 3

HUMAN REPRODUCTION

3.1 *The Male Reproductive System*

3.2 *The Female Reproductive System*

3.3 *Gametogenesis*

3.4 *Menstrual Cycle*

3.5 *Fertilisation and Implantation*

3.6 *Pregnancy and Embryonic Development*

3.7 *Parturition and Lactation*

As you are aware, humans are sexually reproducing and viviparous. The reproductive events in humans include formation of gametes (gametogenesis), i.e., sperms in males and ovum in females, transfer of sperms into the female genital tract (insemination) and fusion of male and female gametes (fertilisation) leading to formation of zygote. This is followed by formation and development of blastocyst and its attachment to the uterine wall (implantation), embryonic development (gestation) and delivery of the baby (parturition). You have learnt that these reproductive events occur after puberty. There are remarkable differences between the reproductive events in the male and in the female, for example, sperm formation continues even in old men, but formation of ovum ceases in women around the age of fifty years. Let us examine the male and female reproductive systems in human.

3.1 The Male Reproductive System

The male reproductive system is located in the pelvis region (Figure 3.1a). It includes a pair of **testes** alongwith **accessory ducts, glands** and the **external genitalia**.

The testes are situated outside the abdominal cavity within a pouch called **scrotum**. The scrotum helps in maintaining the low temperature of the testes (2–2.5° C lower than the normal internal body temperature) necessary for spermatogenesis. In adults, each testis is oval in shape, with a length of about 4 to 5 cm and a width of about 2 to 3 cm. The testis is covered by a dense covering. Each testis has about 250 compartments called **testicular lobules** (Figure 3.1b).

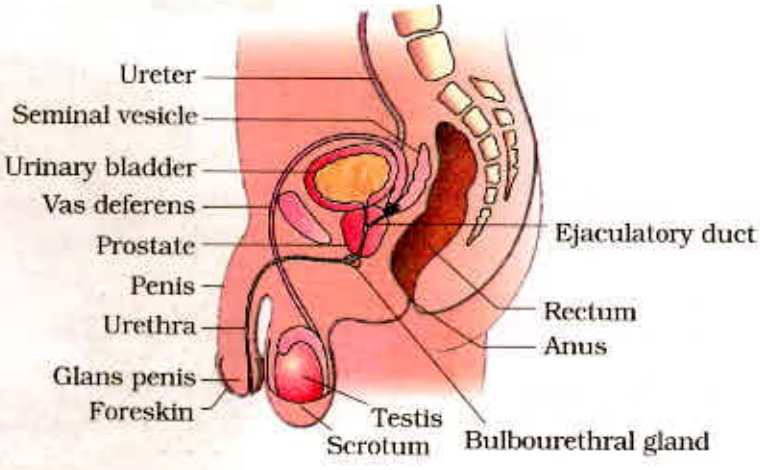


Figure 3.1(a) Diagrammatic sectional view of male pelvis showing reproductive system

Each lobule contains one to three highly coiled **seminiferous tubules** in which sperms are produced. Each seminiferous tubule is lined on its inside by two types of cells called **male germ cells** (*spermatogonia*) and **Sertoli cells** (Figure 3.2). The male germ cells undergo meiotic divisions finally leading to sperm formation, while Sertoli cells provide nutrition to the germ cells. The regions outside the seminiferous tubules called interstitial spaces, contain small blood vessels and **interstitial cells** or **Leydig cells** (Figure 3.2). Leydig cells synthesise and secrete testicular hormones called androgens. Other immunologically competent cells are also present.

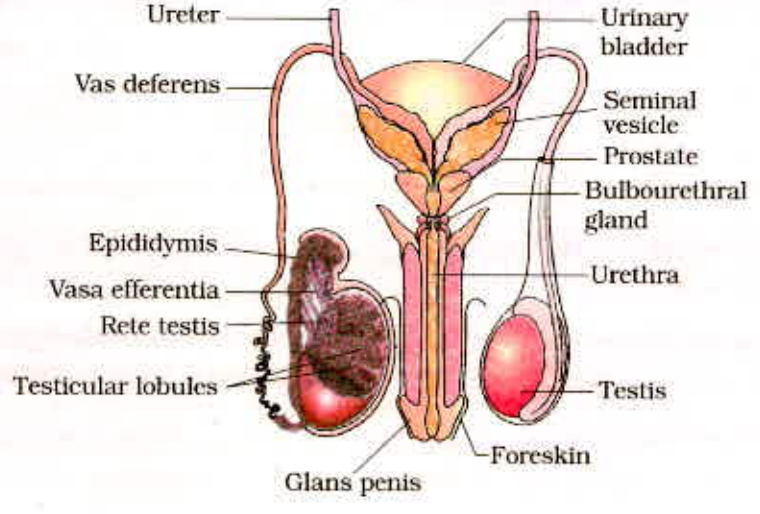


Figure 3.1(b) Diagrammatic view of male reproductive system (part of testis is open to show inner details)

The male sex accessory ducts include **rete testis, vasa efferentia**, **epididymis** and **vas deferens** (Figure 3.1b). The seminiferous tubules of the testis open into the vasa efferentia through rete testis. The vasa efferentia leave the testis and open into epididymis located along the posterior surface of each testis. The epididymis leads to vas deferens that ascends to the abdomen and loops over the urinary bladder. It receives a duct from seminal vesicle and opens into urethra as the ejaculatory duct (Figure 3.1a). These ducts store and transport the sperms from the testis to the outside through urethra. The urethra originates from the urinary bladder and extends through the penis to its external opening called **urethral meatus**.

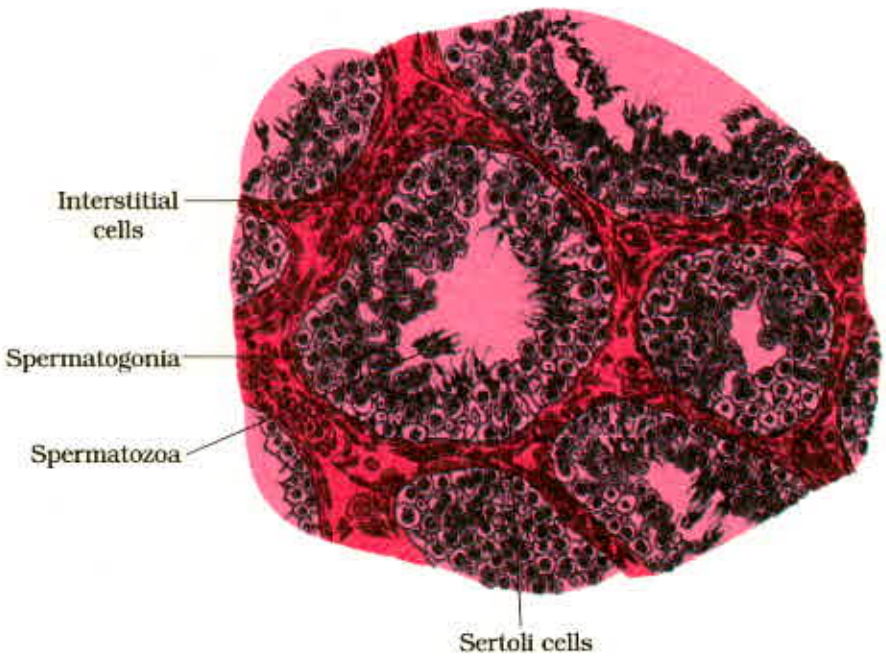


Figure 3.2 Diagrammatic sectional view of seminiferous tubule

The penis is the male external genitalia (Figure 3.1a, b). It is made up of special tissue that helps in erection of the penis to facilitate insemination. The enlarged end of penis called the glans penis is covered by a loose fold of skin called **foreskin**.

The male accessory glands (Figure 3.1a, b) include paired **seminal vesicles**, a **prostate** and paired **bulbourethral** glands. Secretions of these glands constitute the seminal plasma which is rich in fructose, calcium and certain enzymes. The secretions of bulbourethral glands also helps in the lubrication of the penis.

3.2 The Female Reproductive System

The female reproductive system consists of a pair of **ovaries** alongwith a pair of **oviducts, uterus, cervix, vagina** and the **external genitalia** located in pelvic region (Figure 3.3a). These parts of the system alongwith a pair of the **mammary glands** are integrated structurally and functionally to support the processes of ovulation, fertilisation, pregnancy, birth and child care.

Ovaries are the primary female sex organs that produce the female gamete (ovum) and several steroid hormones (ovarian hormones). The ovaries are located one on each side of the lower abdomen (Figure 3.3b). Each ovary is about 2 to 4 cm in length and is connected to the pelvic wall and uterus by ligaments. Each ovary is covered by a thin epithelium which encloses the ovarian stroma. The stroma is divided into two zones – a peripheral cortex and an inner medulla.

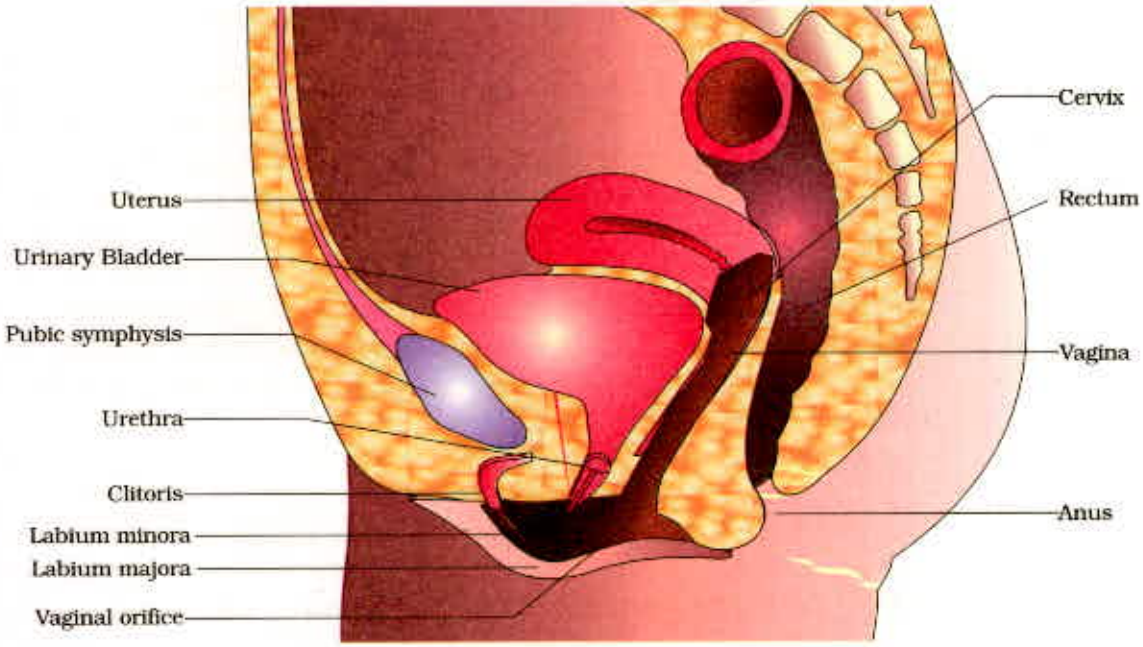


Figure 3.3 (a) Diagrammatic sectional view of female pelvis showing reproductive system

The oviducts (fallopian tubes), uterus and vagina constitute the female accessory ducts. Each fallopian tube is about 10-12 cm long and extends from the periphery of each ovary to the uterus (Figure 3.3b), the part closer to the ovary is the funnel-shaped **infundibulum**. The edges of the infundibulum possess finger-like projections called **fimbriae**, which help in collection of the ovum after ovulation. The infundibulum leads to a wider

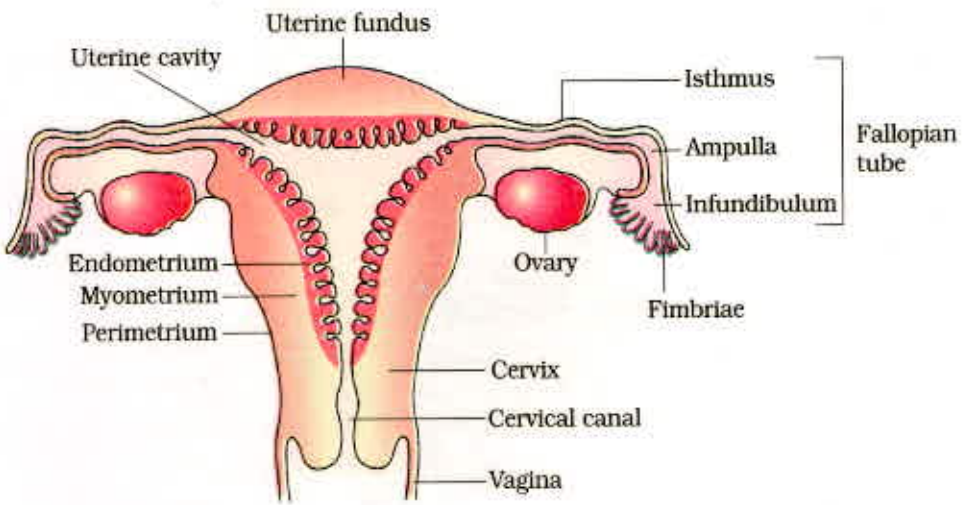


Figure 3.3 (b) Diagrammatic sectional view of the female reproductive system

part of the oviduct called **ampulla**. The last part of the oviduct, **isthmus** has a narrow lumen and it joins the uterus.

The uterus is single and it is also called **womb**. The shape of the uterus is like an inverted pear. It is supported by ligaments attached to the pelvic wall. The uterus opens into vagina through a narrow cervix. The cavity of the cervix is called **cervical canal** (Figure 3.3b) which alongwith vagina forms the birth canal. The wall of the uterus has three layers of tissue. The external thin membranous **perimetrium**, middle thick layer of smooth muscle, **myometrium** and inner glandular layer called **endometrium** that lines the uterine cavity. The endometrium undergoes cyclical changes during menstrual cycle while the myometrium exhibits strong contraction during delivery of the baby.

The female external genitalia include mons pubis, labia majora, labia minora, hymen and clitoris (Figure 3.3a). **Mons pubis** is a cushion of fatty tissue covered by skin and pubic hair. The **labia majora** are fleshy folds of tissue, which extend down from the mons pubis and surround the vaginal opening. The **labia minora** are paired folds of tissue under the labia majora. The opening of the vagina is often covered partially by a membrane called **hymen**. The **clitoris** is a tiny finger-like structure which lies at the upper junction of the two labia minora above the urethral opening. The hymen is often torn during the first coitus (intercourse). *However, it can also be broken by a sudden fall or jolt, insertion of a vaginal tampon, active participation in some sports like horseback riding, cycling, etc. In some women the hymen persists even after coitus. In fact, the presence or absence of hymen is not a reliable indicator of virginity or sexual experience.*

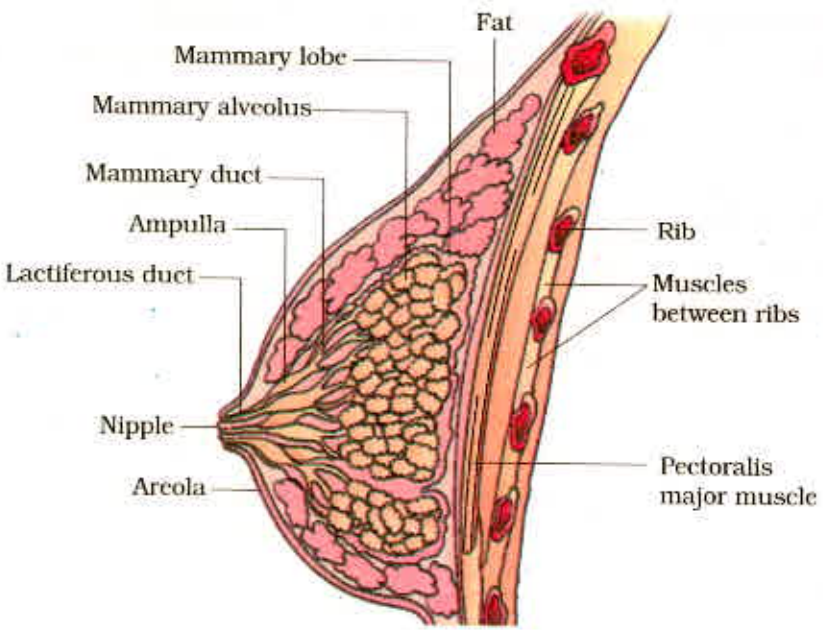


Figure 3.4 A diagrammatic sectional view of Mammary gland

A functional mammary gland is characteristic of all female mammals. The mammary glands are paired structures (breasts) that contain glandular tissue and variable amount of fat. The glandular tissue of each breast is divided into 15-20 **mammary lobes** containing clusters of cells called alveoli (Figure 3.4). The cells of alveoli secrete milk, which is stored in the cavities (lumens) of alveoli. The alveoli open into mammary tubules. The tubules of each lobe join to form a **mammary duct**. Several mammary ducts join to form a wider mammary ampulla which is connected to **lactiferous duct** through which milk is sucked out.

3.3 Gametogenesis

The primary sex organs – the testis in the males and the ovaries in the females – produce gametes, i.e, sperms and ovum, respectively, by the process called gametogenesis. In testis, the immature male germ cells (spermatogonia) produce sperms by **spermatogenesis** that begins at puberty. The **spermatogonia** (sing. spermatogonium) present on the inside wall of seminiferous tubules multiply by mitotic division and increase in numbers. Each spermatogonium is diploid and contains 46 chromosomes. Some of the spermatogonia called **primary spermatocytes** periodically undergo meiosis. A primary spermatocyte completes the first meiotic division (reduction division) leading to formation of two equal, haploid cells called **secondary spermatocytes**, which have only 23 chromosomes each. The secondary spermatocytes undergo the second meiotic division to produce four equal, haploid **spermatids** (Figure 3.5). *What would be the number of chromosome in the spermatids?* The spermatids are transformed into **spermatozoa (sperms)** by the process called **spermiogenesis**. After spermiogenesis, sperm heads become embedded in the **Sertoli cells**, and are finally released from the seminiferous tubules by the process called **spermiation**.

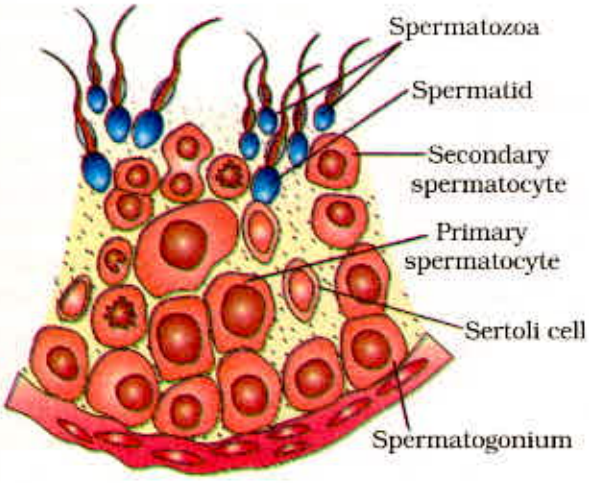


Figure 3.5 Diagrammatic sectional view of a seminiferous tubule (enlarged)

Spermatogenesis starts at the age of puberty due to significant increase in the secretion of gonadotropin releasing hormone (GnRH). This, if you recall, is a hypothalamic hormone. The increased levels of GnRH then acts at the anterior pituitary gland and stimulates secretion of two gonadotropins – luteinising hormone (LH) and follicle stimulating hormone (FSH). LH acts at the Leydig cells and stimulates synthesis and secretion of androgens. Androgens, in turn, stimulate the process of spermatogenesis. FSH acts on the Sertoli cells and stimulates

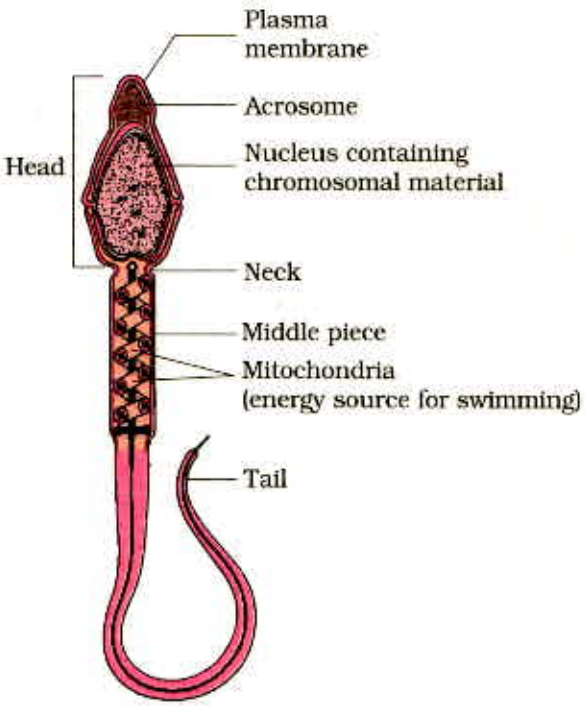


Figure 3.6 Structure of a sperm

secretion of some factors which help in the process of spermiogenesis.

Let us examine the structure of a sperm. It is a microscopic structure composed of a **head**, **neck**, a **middle piece** and a **tail** (Figure 3.6). A plasma membrane envelops the whole body of sperm. The sperm head contains an elongated haploid nucleus, the anterior portion of which is covered by a cap-like structure, **acrosome**. The acrosome is filled with enzymes that help fertilisation of the ovum. The middle piece possesses numerous mitochondria, which produce energy for the movement of tail that facilitate sperm motility essential for fertilisation. The human male ejaculates about 200 to 300 million sperms during a coitus of which, for normal fertility, at least 60 per cent sperms must have normal shape and size and at least 40 per cent of them must show vigorous motility.

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

The process of formation of a mature female gamete is called **oogenesis** which is markedly different from spermatogenesis. Oogenesis is initiated during the embryonic development stage when a couple of million gamete mother cells (**oogonia**) are formed within each fetal ovary; no more oogonia are formed and added after birth. These cells start division and enter into prophase-I of the meiotic division and get temporarily arrested at that stage, called **primary oocytes**. Each primary oocyte then gets surrounded by a layer of granulosa cells and is called the **primary follicle** (Figure 3.7). A large number of these follicles degenerate during the phase from birth to puberty. Therefore, at puberty only 60,000-80,000 primary follicles are left in each ovary. The primary follicles get surrounded by more layers of granulosa cells and a new theca and are called **secondary follicles**.

The secondary follicle soon transforms into a tertiary follicle which is characterised by a fluid filled cavity called **antrum**. The theca layer is organised into an inner theca interna and an outer theca externa. It is important to draw your attention that it is at this stage that the primary oocyte within the tertiary follicle grows in size and completes its first meiotic division. It is an unequal division resulting in the formation of a large haploid **secondary oocyte** and a tiny first polar body (Figure 3.8b). The

secondary oocyte retains bulk of the nutrient rich cytoplasm of the primary oocyte. *Can you think of any advantage for this?* Does the first polar body born out of first meiotic division divide further or degenerate? At present we are not very certain about this. The tertiary follicle further changes into the mature follicle or **Graafian follicle** (Figure 3.7). The secondary oocyte forms a new membrane called **zona pellucida** surrounding it. The Graafian follicle now ruptures to release the secondary oocyte (ovum) from the ovary by the process called **ovulation**. *Can you identify major differences between spermatogenesis and oogenesis?* A diagrammatic representation of spermatogenesis and oogenesis is given below (Figure 3.8).

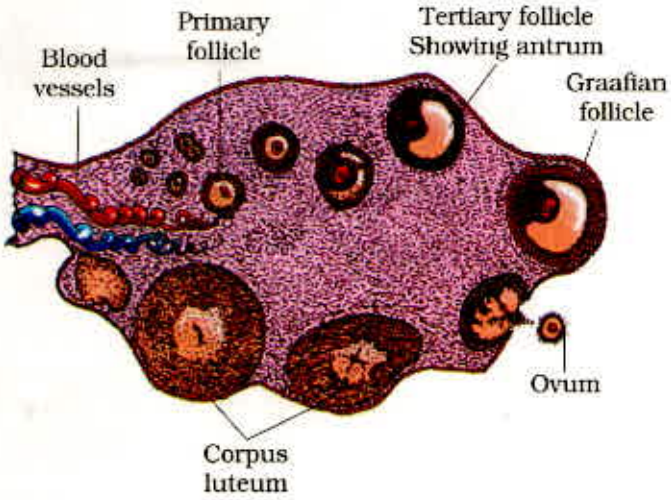


Figure 3.7 Diagrammatic Section view of ovary

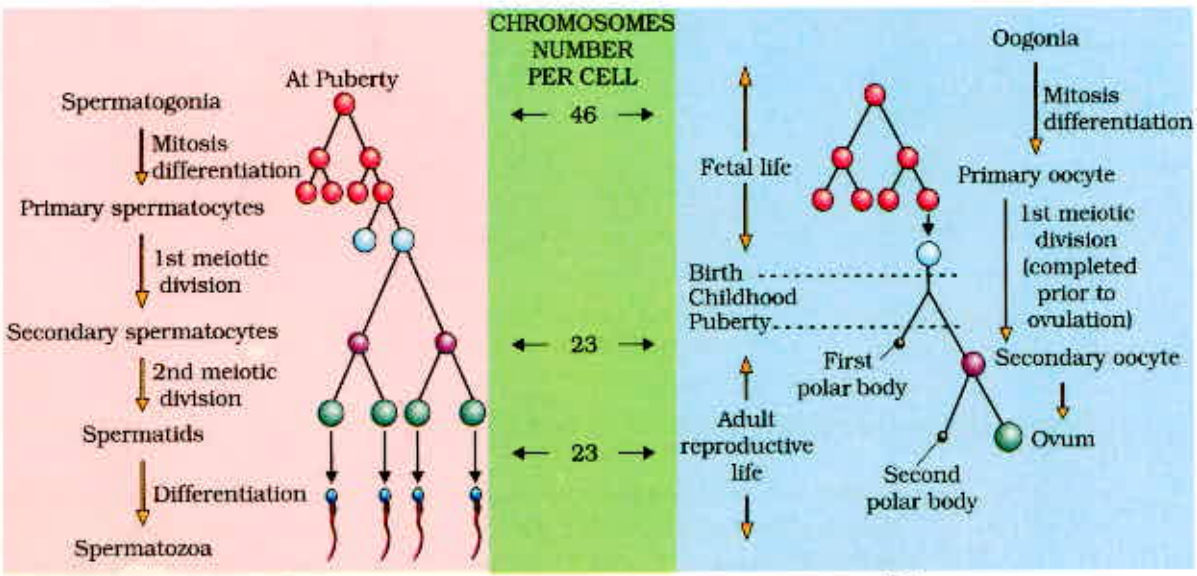


Figure 3.8 Schematic representation of (a) Spermatogenesis; (b) Oogenesis

3.4 Menstrual Cycle

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

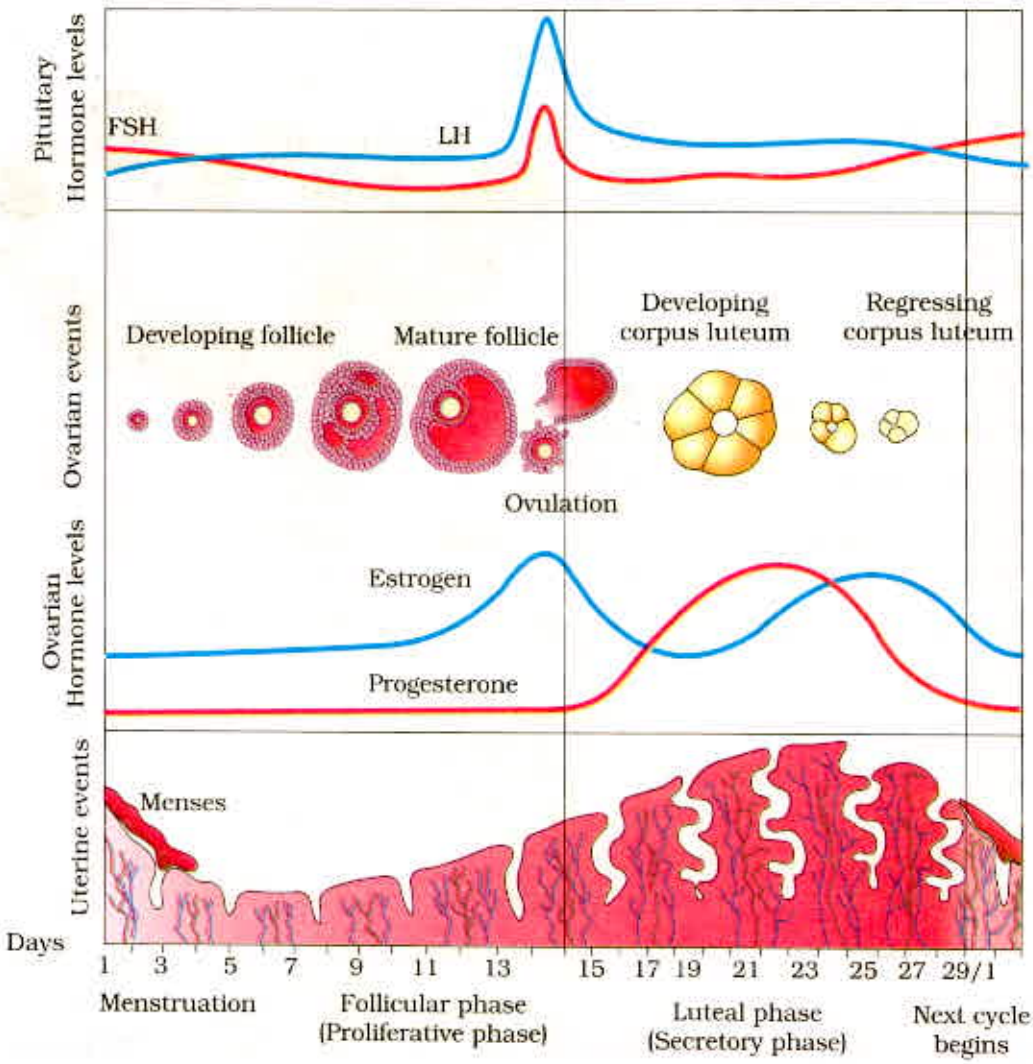


Figure 3.9 Diagrammatic presentation of various events during a menstrual cycle

of each menstrual cycle. The major events of the menstrual cycle are shown in Figure 3.9. The cycle starts with the menstrual phase, when menstrual flow occurs and it lasts for 3-5 days. The menstrual flow results due to breakdown of endometrial lining of the uterus and its blood vessels which forms liquid that comes out through vagina. Menstruation only occurs if the released ovum is not fertilised. Lack of menstruation may be indicative of pregnancy. However, it may also be caused due to some other underlying causes like stress, poor health etc. The menstrual phase is followed by the follicular phase. During this phase, the primary follicles in the ovary grow to become a fully mature Graafian follicle and simultaneously the endometrium of uterus regenerates through proliferation. These changes in the ovary and the uterus are induced by changes in the levels of pituitary and ovarian hormones (Figure 3.9). The secretion of

gonadotropins (LH and FSH) increases gradually during the follicular phase, and stimulates follicular development as well as secretion of estrogens by the growing follicles. Both LH and FSH attain a peak level in the middle of cycle (about 14th day). Rapid secretion of LH leading to its maximum level during the mid-cycle called LH surge induces rupture of Graafian follicle and thereby the release of ovum (**ovulation**). The ovulation (ovulatory phase) is followed by the luteal phase during which the remaining parts of the Graafian follicle transform as the **corpus luteum** (Figure 3.9). The corpus luteum secretes large amounts of progesterone which is essential for maintenance of the endometrium. Such an endometrium is necessary for implantation of the fertilised ovum and other events of pregnancy. During pregnancy all events of the menstrual cycle stop and there is no menstruation. In the absence of fertilisation, the corpus luteum degenerates. This causes disintegration of the endometrium leading to menstruation, marking a new cycle. In human beings, menstrual cycles ceases around 50 years of age; that is termed as **menopause**. Cyclic menstruation is an indicator of normal reproductive phase and extends between menarche and menopause.

3.5 Fertilisation and Implantation

During copulation (coitus) semen is released by the penis into the vagina (insemination). The motile sperms swim rapidly, pass through the cervix, enter into the uterus and finally reach the ampullary region of the fallopian tube (Figure 3.11b). The ovum released by the ovary is also transported to the ampullary region where fertilisation takes place. Fertilisation can only occur if the ovum and sperms are transported simultaneously to the ampullary region. This is the reason why not all copulations lead to fertilisation and pregnancy.

The process of fusion of a sperm with an ovum is called **fertilisation**. During fertilisation, a sperm comes in contact with the *zona pellucida* layer of the ovum (Figure 3.10) and induces changes in the membrane that block the entry of additional sperms. Thus, it ensures that only one sperm can fertilise an ovum. The secretions of the acrosome help the sperm enter into the cytoplasm of the ovum through the zona pellucida and the plasma

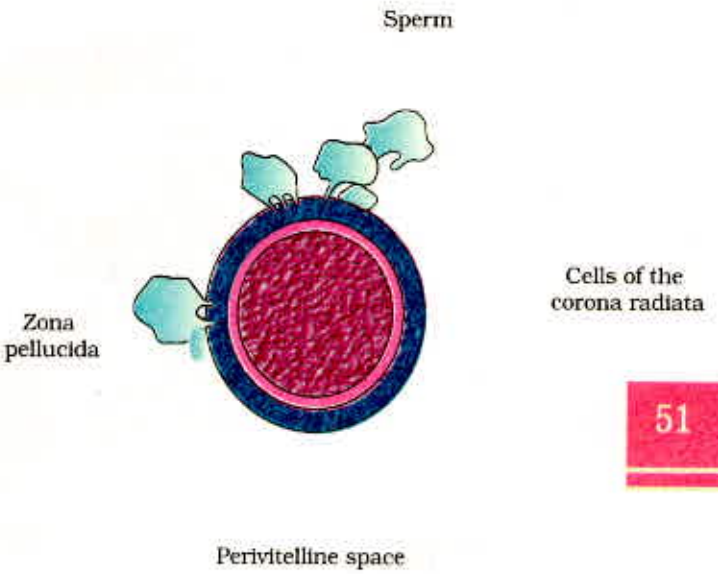


Figure 3.10 Ovum surrounded by few sperms

membrane. This induces the completion of the meiotic division of the secondary oocyte. The second meiotic division is also unequal and results in the formation of a **second polar body** and a haploid ovum (ootid). Soon the haploid nucleus of the sperms and that of the ovum fuse together to form a diploid **zygote**. *How many chromosomes will be there in the zygote?*

One has to remember that the sex of the baby has been decided at this stage itself. Let us see how? As you know the chromosome pattern in the human female is XX and that in the male is XY. Therefore, all the haploid gametes produced by the female (ova) have the sex chromosome X whereas in the male gametes (sperms) the sex chromosome could be either X or Y, hence, 50 per cent of sperms carry the X chromosome while the other 50 per cent carry the Y. After fusion of the male and female gametes the zygote would carry either XX or XY depending on whether the sperm carrying X or Y fertilised the ovum. The zygote carrying XX would develop into a female baby and XY would form a male (you will learn more about the chromosomal patterns in Chapter 5). *That is why, scientifically it is correct to say that the sex of the baby is determined by the father and not by the mother!*

The mitotic division starts as the zygote moves through the isthmus of the oviduct called **cleavage** towards the uterus (Figure 3.11) and forms 2, 4, 8, 16 daughter cells called **blastomeres**. The embryo with 8 to 16

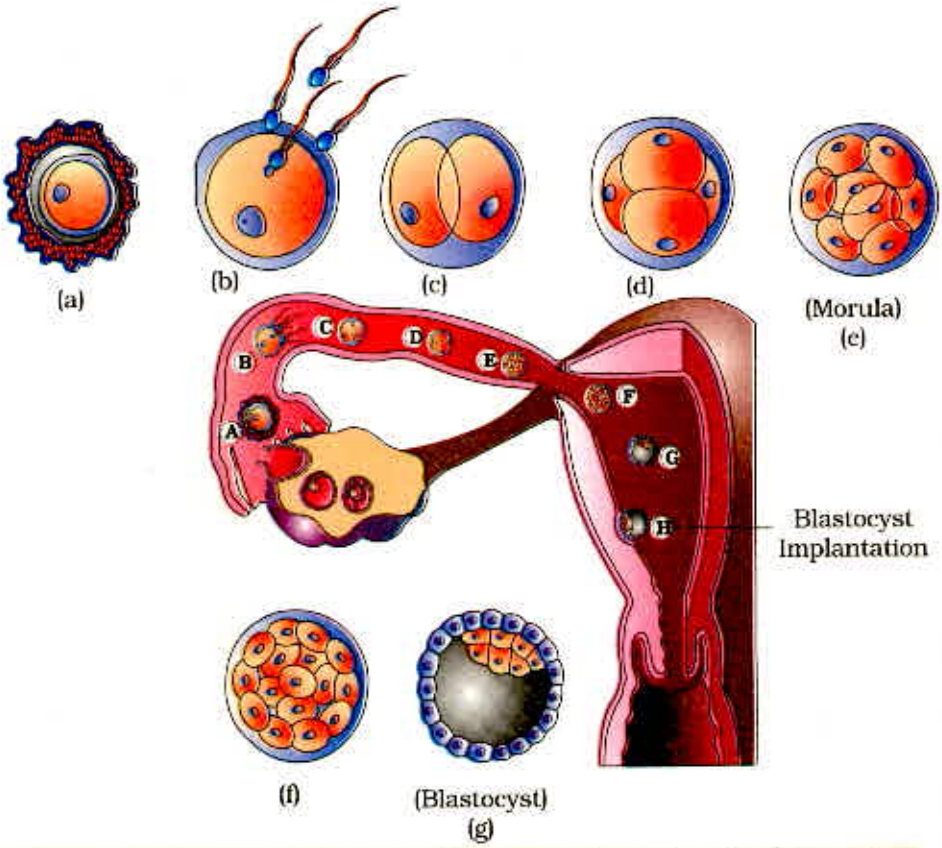


Figure 3.11 Transport of ovum, fertilisation and passage of growing embryo through fallopian tube

blastomeres is called a morula (Figure 3.11e). The morula continues to divide and transforms into blastocyst (Figure 3.11g) as it moves further into the uterus. The blastomeres in the blastocyst are arranged into an outer layer called **trophoblast** and an inner group of cells attached to trophoblast called the **inner cell** mass. The trophoblast layer then gets attached to the endometrium and the inner cell mass gets differentiated as the embryo. After attachment, the uterine cells divide rapidly and covers the blastocyst. As a result, the blastocyst becomes embedded in the endometrium of the uterus (Figure 3.11h). This is called **implantation** and it leads to pregnancy.

3.6 Pregnancy and Embryonic Development

After implantation, finger-like projections appear on the trophoblast called **chorionic villi** which are surrounded by the uterine tissue and maternal blood. The chorionic villi and uterine tissue become interdigitated with each other and jointly form a structural and functional unit between developing embryo (foetus) and maternal body called **placenta** (Figure 3.12).

The placenta facilitate the supply of oxygen and nutrients to the embryo and also removal of carbon dioxide and excretory/waste materials produced by the embryo. The placenta is connected to the embryo through an umbilical cord which helps in the transport of substances to and from the embryo. Placenta also acts as an endocrine tissue and produces several hormones like **human chorionic gonadotropin** (hCG), **human placental lactogen** (hPL), **estrogens, progestogens,** etc. In the later phase of pregnancy, a hormone called **relaxin** is also secreted by the ovary. Let us remember that hCG, hPL and relaxin are produced in women only during pregnancy. In addition, during pregnancy the levels of other hormones like estrogens, progestogens, cortisol, prolactin, thyroxine, etc., are increased several-folds in the maternal blood. Increased production of these hormones is essential for supporting the fetal growth, metabolic changes in the mother and maintenance of pregnancy.

Immediately after implantation, the inner cell mass (embryo) differentiates

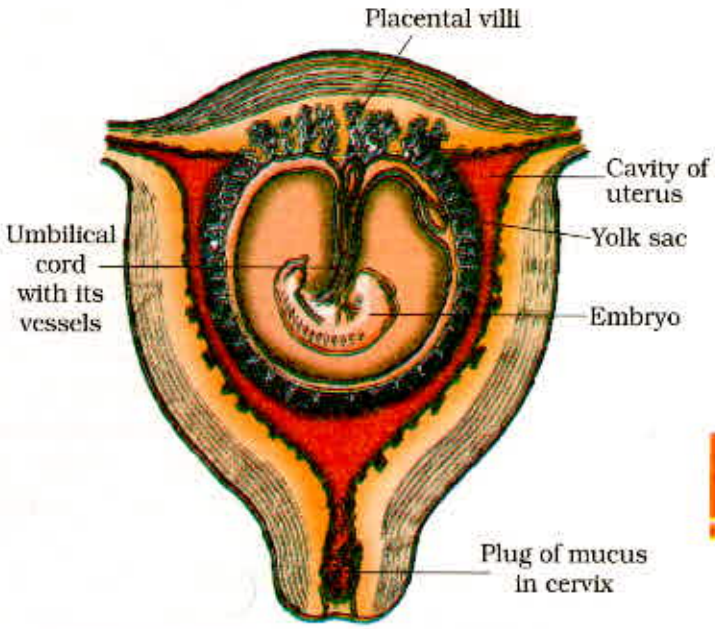


Figure 3.12 The human foetus within the uterus

into an outer layer called **ectoderm** and an inner layer called **endoderm**. A **mesoderm** soon appears between the ectoderm and the endoderm. These three layers give rise to all tissues (organs) in adults. It needs to be mentioned here that the inner cell mass contains certain cells called **stem** cells which have the potency to give rise to all the tissues and organs.

What are the major features of embryonic development at various months of pregnancy? The human pregnancy lasts 9 months. *Do you know for how many months pregnancy last in dogs, elephants, cats?* Find out. In human beings, after one month of pregnancy, the embryo's heart is formed. The first sign of growing foetus may be noticed by listening to the heart sound carefully through the stethoscope. By the end of the second month of pregnancy, the foetus develops limbs and digits. By the end of 12 weeks (first trimester), most of the major organ systems are formed, for example, the limbs and external genital organs are well-developed. The first movements of the foetus and appearance of hair on the head are usually observed during the fifth month. By the end of about 24 weeks (end of second trimester), the body is covered with fine hair, eye-lids separate, and eyelashes are formed. By the end of nine months of pregnancy, the foetus is fully developed and is ready for delivery.

3.7 Parturition and Lactation

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

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

SUMMARY

Humans are sexually reproducing and viviparous. The male reproductive system is composed of a pair of testes, the male sex accessory ducts and the accessory glands and external genitalia. Each testis has about 250 compartments called testicular lobules, and each lobule contains one to three highly coiled seminiferous tubules. Each seminiferous tubule is lined inside by spermatogonia and Sertoli cells. The spermatogonia undergo meiotic divisions leading to sperm formation, while Sertoli cells provide nutrition to the dividing germ cells. The Leydig cells outside the seminiferous tubules, synthesise and secrete testicular hormones called androgens. The male external genitalia is called penis.

The female reproductive system consists of a pair of ovaries, a pair of oviducts, a uterus, a vagina, external genitalia, and a pair of mammary glands. The ovaries produce the female gamete (ovum) and some steroid hormones (ovarian hormones). Ovarian follicles in different stages of development are embedded in the stroma. The oviducts, uterus and vagina are female accessory ducts. The uterus has three layers namely perimetrium, myometrium and endometrium. The female external genitalia includes mons pubis, labia majora, labia minora, hymen and clitoris. The mammary glands are one of the female secondary sexual characteristics.

Spermatogenesis results in the formation of sperms that are transported by the male sex accessory ducts. A normal human sperm is composed of a head, neck, a middle piece and tail. The process of formation of mature female gametes is called oogenesis. The reproductive cycle of female primates is called menstrual cycle. Menstrual cycle starts only after attaining sexual maturation (puberty). During ovulation only one ovum is released per menstrual cycle. The cyclical changes in the ovary and the uterus during menstrual cycle are induced by changes in the levels of pituitary and ovarian hormones. After coitus, sperms are transported to the junction of the isthmus and ampulla, where the sperm fertilises the ovum leading to formation of a diploid zygote. The presence of X or Y chromosome in the sperm determines the sex of the embryo. The zygote undergoes repeated mitotic division to form a blastocyst, which is implanted in the uterus resulting in pregnancy. After nine months of pregnancy, the fully developed foetus is ready for delivery. The process of childbirth is called parturition which is induced by a complex neuroendocrine mechanism involving cortisol, estrogens and oxytocin. Mammary glands differentiate during pregnancy and secrete milk after child-birth. The new-born baby is fed milk by the mother (lactation) during the initial few months of growth.

EXERCISES

1. Fill in the blanks:
   (a) Humans reproduce _____ (asexually/sexually)
   (b) Humans are _____ (oviparous, viviparous, ovoviviparous)
   (c) Fertilisation is _____ in humans (external/internal)
   (d) Male and female gametes are _____ (diploid/haploid)
   (e) Zygote is _____ (diploid/haploid)

(f) The process of release of ovum from a mature follicle is called _____

(g) Ovulation is induced by a hormone called _____

(h) The fusion of male and female gametes is called _____

(i) Fertilisation takes place in _____

(j) Zygote divides to form _____which is implanted in uterus.

(k) The structure which provides vascular connection between foetus and uterus is called _____

2. Draw a labelled diagram of male reproductive system.
3. Draw a labelled diagram of female reproductive system.
4. Write two major functions each of testis and ovary.
5. Describe the structure of a seminiferous tubule.
6. What is spermatogenesis? Briefly describe the process of spermatogenesis.
7. Name the hormones involved in regulation of spermatogenesis.
8. Define spermiogenesis and spermiation.
9. Draw a labelled diagram of sperm.
10. What are the major components of seminal plasma?
11. What are the major functions of male accessory ducts and glands?
12. What is oogenesis? Give a brief account of oogenesis.
13. Draw a labelled diagram of a section through ovary.
14. Draw a labelled diagram of a Graafian follicle?
15. Name the functions of the following:
    (a) Corpus luteum (b) Endometrium
    (c) Acrosome (d) Sperm tail
    (e) Fimbriae
16. Identify True/False statements. Correct each false statement to make it true.
    (a) Androgens are produced by Sertoli cells. (True/False)
    (b) Spermatozoa get nutrition from Sertoli cells. (True/False)
    (c) Leydig cells are found in ovary. (True/False)
    (d) Leydig cells synthesise androgens. (True/False)
    (e) Oogenesis takes place in corpus luteum. (True/False)
    (f) Menstrual cycle ceases during pregnancy. (True/False)
    (g) Presence or absence of hymen is not a reliable indicator of virginity or sexual experience. (True/False)
17. What is menstrual cycle? Which hormones regulate menstrual cycle?
18. What is parturition? Which hormones are involved in induction of parturition?
19. In our society the women are often blamed for giving birth to daughters. Can you explain why this is not correct?
20. How many eggs are released by a human ovary in a month? How many eggs do you think would have been released if the mother gave birth to identical twins? Would your answer change if the twins born were fraternal?
21. How many eggs do you think were released by the ovary of a female dog which gave birth to 6 puppies?

CHAPTER 4

REPRODUCTIVE HEALTH

4.1 *Reproductive Health – Problems and Strategies*

4.2 *Population Explosion and Birth Control*

4.3 *Medical Termination of Pregnancy*

4.4 *Sexually Transmitted Diseases*

4.5 *Infertility*

You have learnt about human reproductive system and its functions in Chapter 3. Now, let's discuss a closely related topic – reproductive health. *What do we understand by this term?* The term simply refers to healthy reproductive organs with normal functions. However, it has a broader perspective and includes the emotional and social aspects of reproduction also. According to the World Health Organisation (WHO), reproductive health means a total well-being in all aspects of reproduction, i.e., physical, emotional, behavioural and social. Therefore, a society with people having physically and functionally normal reproductive organs and normal emotional and behavioural interactions among them in all sex-related aspects might be called reproductively healthy. Why is it significant to maintain reproductive health and what are the methods taken up to achieve it? Let us examine them.

4.1 Reproductive Health – Problems and Strategies

India was amongst the first countries in the world to initiate action plans and programmes at a national level to attain total reproductive health as a social goal. These programmes called 'family planning' were initiated in 1951 and were periodically assessed over the past decades. Improved programmes covering wider

reproduction-related areas are currently in operation under the popular name 'Reproductive and Child Health Care (RCH) programmes'. Creating awareness among people about various reproduction related aspects and providing facilities and support for building up a reproductively healthy society are the major tasks under these programmes.

With the help of audio-visual and the print-media governmental and non-governmental agencies have taken various steps to create awareness among the people about reproduction-related aspects. Parents, other close relatives, teachers and friends, also have a major role in the dissemination of the above information. Introduction of sex education in schools should also be encouraged to provide right information to the young so as to discourage children from believing in myths and having misconceptions about sex-related aspects. Proper information about reproductive organs, adolescence and related changes, safe and hygienic sexual practices, sexually transmitted diseases (STD), AIDS, etc., would help people, especially those in the adolescent age group to lead a reproductively healthy life. Educating people, especially fertile couples and those in marriageable age group, about available birth control options, care of pregnant mothers, post-natal care of the mother and child, importance of breast feeding, equal opportunities for the male and the female child, etc., would address the importance of bringing up socially conscious healthy families of desired size. Awareness of problems due to uncontrolled population growth, social evils like sex-abuse and sex-related crimes, etc., need to be created to enable people to think and take up necessary steps to prevent them and thereby build up a socially responsible and healthy society.

Successful implementation of various action plans to attain reproductive health requires strong infrastructural facilities, professional expertise and material support. These are essential to provide medical assistance and care to people in reproduction-related problems like pregnancy, delivery, STDs, abortions, contraception, menstrual problems, infertility, etc. Implementation of better techniques and new strategies from time to time are also required to provide more efficient care and assistance to people. Statutory ban on **amniocentesis** (a foetal sex determination test based on the chromosomal pattern in the amniotic fluid surrounding the developing embryo) for sex-determination to legally check increasing female foeticides, massive child immunisation, etc., are some programmes that merit mention in this connection.

Research on various reproduction-related areas are encouraged and supported by governmental and non-governmental agencies to find out new methods and/or to improve upon the existing ones. *Do you know that 'Saheli'–a new oral contraceptive for the females–was developed by scientists at Central Drug Research Institute (CDRI) in Lucknow, India?* Better awareness about sex related matters, increased number of medically assisted deliveries and better post-natal care leading to decreased maternal

and infant mortality rates, increased number of couples with small families, better detection and cure of STDs and overall increased medical facilities for all sex-related problems, etc. all indicate improved reproductive health of the society.

4.2 Population Explosion and Birth Control

In the last century an all-round development in various fields significantly improved the quality of life of the people. However, increased health facilities along with better living conditions had an explosive impact on the growth of population. The world population which was around 2 billion (2000 million) in 1900 rocketed to about 6 billions by 2000. A similar trend was observed in India too. Our population which was approximately 350 million at the time of our independence reached close to the billion mark by 2000 and crossed 1 billion in May 2000. That means, every sixth person in the world is an Indian. A rapid decline in death rate, **maternal mortality rate** (MMR) and **infant mortality rate** (IMR) as well as an increase in number of people in reproducible age are probable reasons for this. Through our RCH programmes, though we could bring down the population growth rate, it was only marginal. According to the 2001 census report, the population growth rate was still around 1.7 per cent, i.e., 17/1000/year, a rate at which our population could double in 33 years. Such an alarming growth rate could lead to an absolute scarcity of even the basic requirements, i.e., food, shelter and clothing, in spite of significant progress made in those areas. Therefore, the government was forced to take up serious measures to check this population growth rate.

The most important step to overcome this problem is to motivate smaller families by using various contraceptive methods. You might have seen advertisements in the media as well as posters/bills, etc., showing a happy couple with two children with a slogan *Hum Do Hamare Do* (we two, our two). Many couples, mostly the young, urban, working ones have even adopted an 'one child norm'. Statutory raising of marriageable age of the female to 18 years and that of males to 21 years, and incentives given to couples with small families are two of the other measures taken to tackle this problem. Let us describe some of the commonly used contraceptive methods, which help prevent unwanted pregnancies.

An ideal contraceptive should be user-friendly, easily available, effective and reversible with no or least side-effects. It also should in no way interfere with the sexual drive, desire and/or the sexual act of the user. A wide range of contraceptive methods are presently available which could be broadly grouped into the following categories, namely Natural/Traditional, Barrier, IUDs, Oral contraceptives, Injectables, Implants and Surgical methods.

Natural methods work on the principle of avoiding chances of ovum and sperms meeting. **Periodic abstinence** is one such method in which the couples avoid or abstain from coitus from day 10 to 17 of the menstrual cycle when ovulation could be expected. As chances of fertilisation are

very high during this period, it is called the fertile period. Therefore, by abstaining from coitus during this period, conception could be prevented. **Withdrawal** or **coitus interruptus** is another method in which the male partner withdraws his penis from the vagina just before ejaculation so as to avoid insemination. **Lactational amenorrhea** (absence of menstruation) method is based on the fact that ovulation and therefore the cycle do not occur during the period of intense lactation following parturition. Therefore, as long as the mother breast-feeds the child fully, chances of conception are almost nil. However, this method has been reported to be effective only upto a maximum period of six months following parturition. As no medicines or devices are used in these methods, side effects are almost nil. Chances of failure, though, of this method are also high.

Figure 4.1(a) Condom for male

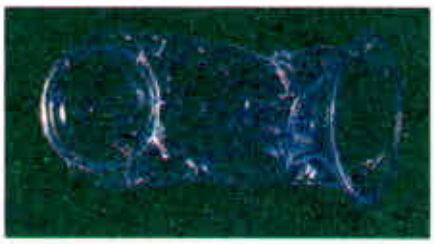


Figure 4.1(b) Condom for female

In **barrier** methods, ovum and sperms are prevented from physically meeting with the help of barriers. Such methods are available for both males and females. **Condoms** (Figure 4.1 a, b) are barriers made of thin rubber/latex sheath that are used to cover the penis in the male or vagina and cervix in the female, just before coitus so that the ejaculated semen would not enter into the female reproductive tract. This can prevent conception. 'Nirodh' is a popular brand of condom for the male. Use of condoms has increased in recent years due to its additional benefit of protecting the user from contracting STDs and AIDS. Both the male and the female condoms are disposable, can be self-inserted and thereby gives privacy to the user. **Diaphragms, cervical caps** and **vaults** are also barriers made of rubber that are inserted into the female reproductive tract to cover the cervix during coitus. They prevent conception by blocking the entry of sperms through the cervix. They are reusable. Spermicidal creams, jellies and foams are usually used alongwith these barriers to increase their contraceptive efficiency.

Figure 4.2. Copper T (CuT)

Another effective and popular method is the use of **Intra Uterine Devices (IUDs)**. These devices are inserted by doctors or expert nurses in the uterus through vagina. These Intra Uterine Devices are presently available as the non-medicated IUDs (e.g., Lippes loop), copper releasing IUDs (CuT, Cu7, Multiload 375) and the hormone releasing IUDs (Progestasert, LNG-20) (Figure 4.2). IUDs increase phagocytosis of sperms within the uterus and the Cu ions released suppress sperm motility and the fertilising capacity of sperms. The hormone releasing IUDs, in addition, make the uterus unsuitable for implantation and the cervix hostile to the sperms. IUDs are ideal contraceptives for the females

who want to delay pregnancy and/or space children. It is one of most widely accepted methods of contraception in India.

Figure 4.3 Implants

Oral administration of small doses of either progestogens or progestogen-estrogen combinations is another contraceptive method used by the females. They are used in the form of tablets and hence are popularly called the **pills**. Pills have to be taken daily for a period of 21 days starting preferably within the first five days of menstrual cycle. After a gap of 7 days (during which menstruation occurs) it has to be repeated in the same pattern till the female desires to prevent conception. They inhibit ovulation and implantation as well as alter the quality of cervical mucus to prevent/retard entry of sperms. Pills are very effective with lesser side effects and are well accepted by the females. *Saheli*–the new oral contraceptive for the females contains a non-steroidal preparation. It is a 'once a week' pill with very few side effects and high contraceptive value.

Progestogens alone or in combination with estrogen can also be used by females as injections or implants under the skin (Figure 4.3). Their mode of action is similar to that of pills and their effective periods are much longer. Administration of progestogens or progestogen-estrogen combinations or IUDs within 72 hours of coitus have been found to be very effective as emergency contraceptives as they could be used to avoid possible pregnancy due to rape or casual unprotected intercourse.

Surgical methods, also called **sterilisation**, are generally advised for the male/female partner as a terminal method to prevent any more pregnancies. Surgical intervention blocks gamete transport and thereby prevent conception. Sterilisation procedure in the male is called 'vasectomy'

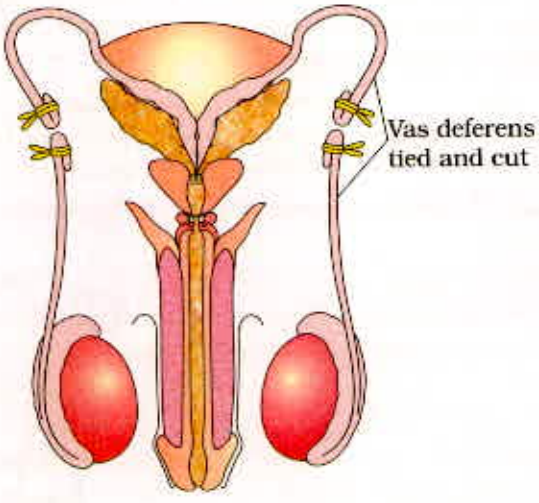


Figure 4.4a Vasectomy

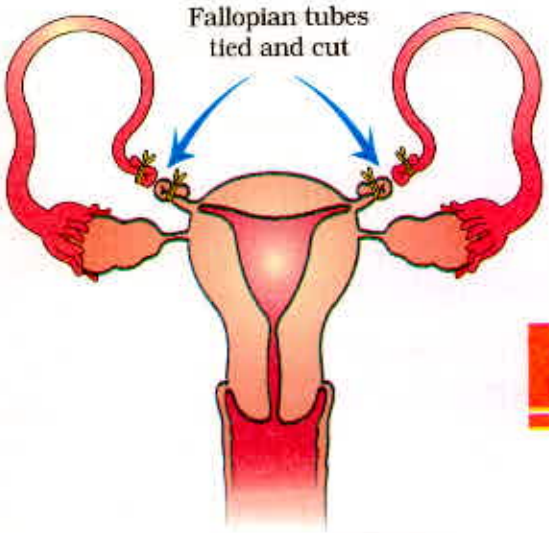


Figure 4.4 (b) Tubectomy

and that in the female, 'tubectomy'. In vasectomy, a small part of the vas deferens is removed or tied up through a small incision on the scrotum (Figure 4.4a) whereas in tubectomy, a small part of the fallopian tube is removed (Figure 4.4b) or tied up through a small incision in the abdomen or through vagina. These techniques are highly effective but their reversibility is very poor.

It needs to be emphasised that the selection of a suitable contraceptive method and its use should always be undertaken in consultation with qualified medical professionals. One must also remember that contraceptives are not regular requirements for the maintenance of reproductive health. In fact, they are practiced against a natural reproductive event, i.e., conception/pregnancy. One is forced to use these methods either to prevent pregnancy or to delay or space pregnancy due to personal reasons. No doubt, the widespread use of these methods have a significant role in checking uncontrolled growth of population. However, their possible ill-effects like nausea, abdominal pain, breakthrough bleeding, irregular menstrual bleeding or even breast cancer, though not very significant, should not be totally ignored.

4.3 Medical Termination of Pregnancy (MTP)

Intentional or voluntary termination of pregnancy before full term is called **medical termination of pregnancy** (MTP) or induced abortion. Nearly 45 to 50 million MTPs are performed in a year all over the world which accounts to 1/5th of the total number of conceived pregnancies in a year. Obviously, MTP has a significant role in decreasing the population though it is not meant for that purpose. Whether to accept / legalise MTP or not is being debated upon in many countries due to emotional, ethical, religious and social issues involved in it. Government of India legalised MTP in 1971 with some strict conditions to avoid its misuse. Such restrictions are all the more important to check indiscriminate and illegal female foeticides which are reported to be high in India.

Why MTP? Obviously the answer is–to get rid of unwanted pregnancies either due to casual unprotected intercourse or failure of the contraceptive used during coitus or rapes. MTPs are also essential in certain cases where continuation of the pregnancy could be harmful or even fatal either to the mother or to the foetus or both.

MTPs are considered relatively safe during the first trimester, i.e., upto 12 weeks of pregnancy. Second trimester abortions are much more riskier. One disturbing trend observed is that a majority of the MTPs are performed illegally by unqualified quacks which are not only unsafe but could be fatal too. Another dangerous trend is the misuse of amniocentesis to determine the sex of the unborn child. Frequently, if the foetus is found to be female, it is followed by MTP- this is totally against what is legal. Such practices should be avoided because these are dangerous both for the young mother and the foetus. Effective counselling on the need to

avoid unprotected coitus and the risk factors involved in illegal abortions as well as providing more health care facilities could reverse the mentioned unhealthy trend.

4.4 Sexually Transmitted Diseases (STDs)

Diseases or infections which are transmitted through sexual intercourse are collectively called sexually transmitted diseases (STD) or venereal diseases (VD) or reproductive tract infections (RTI). Gonorrhoea, syphilis, genital herpes, chlamydiasis, genital warts, trichomoniasis, hepatitis-B and of course, the most discussed infection in the recent years, HIV leading to AIDS are some of the common STDs. Among these, HIV infection is most dangerous and is discussed in detail in Chapter 8.

Some of these infections like hepatitis–B and HIV can also be transmitted by sharing of injection needles, surgical instruments, etc., with infected persons, transfusion of blood, or from an infected mother to the foetus too. Except for hepatitis-B, genital herpes and HIV infections, other diseases are completely curable if detected early and treated properly. Early symptoms of most of these are minor and include itching, fluid discharge, slight pain, swellings, etc., in the genital region. Infected females may often be asymptomatic and hence, may remain undetected for long. Absence or less significant symptoms in the early stages of infection and the social stigma attached to the STDs, deter the infected persons from going for timely detection and proper treatment. This could lead to complications later, which include pelvic inflammatory diseases (PID), abortions, still births, ectopic pregnancies, infertility or even cancer of the reproductive tract. STDs are a major threat to a healthy society. Therefore, prevention or early detection and cure of these diseases are given prime consideration under the reproductive health-care programmes. Though all persons are vulnerable to these infections, their incidences are reported to be very high among persons in the age group of 15-24 years – the age group to which you also belong. There is no reason to panic because prevention is possible. One could be free of these infections by following the simple principles given below:

(i) Avoid sex with unknown partners/multiple partners.

(ii) Always use condoms during coitus.

(iii) In case of doubt, one should go to a qualified doctor for early detection and get complete treatment if diagnosed with disease.

4.5 Infertility

A discussion on reproductive health is incomplete without a mention of infertility. A large number of couples all over the world including India are infertile, i.e., they are unable to produce children inspite of unprotected sexual co-habitation. The reasons for this could be many–physical, congenital, diseases, drugs, immunological or even psychological.

In India, often the female is blamed for the couple being childless, but more often than not, the problem lies in the male partner. Specialised health care units (infertility clinics, etc.) could help in diagnosis and corrective treatment of some of these disorders and enable these couples to have children. However, where such corrections are not possible, the couples could be assisted to have children through certain special techniques commonly known as **assisted reproductive technologies** (ART).

***In vitro* fertilisation** (IVF–fertilisation outside the body in almost similar conditions as that in the body) followed by **embryo transfer** (ET) is one of such methods. In this method, popularly known as **test tube baby** programme, ova from the wife/donor (female) and sperms from the husband/donor (male) are collected and are induced to form zygote under simulated conditions in the laboratory. The zygote or early embryos (with upto 8 blastomeres) could then be transferred into the fallopian tube (ZIFT–**zygote intra fallopian transfer**) and embryos with more than 8 blastomeres, into the uterus (IUT – **intra uterine transfer**), to complete its further development. Embryos formed by **in-vivo fertilisation** (fusion of gametes within the female) also could be used for such transfer to assist those females who cannot conceive.

Transfer of an ovum collected from a donor into the fallopian tube (GIFT – **gamete intra fallopian transfer**) of another female who cannot produce one, but can provide suitable environment for fertilisation and further development is another method attempted. **Intra cytoplasmic sperm injection** (ICSI) is another specialised procedure to form an embryo in the laboratory in which a sperm is directly injected into the ovum. Infertility cases either due to inability of the male partner to inseminate the female or due to very low sperm counts in the ejaculates, could be corrected by **artificial insemination** (AI) technique. In this technique, the semen collected either from the husband or a healthy donor is artificially introduced either into the vagina or into the uterus (IUI – **intra-uterine insemination**) of the female.

Though options are many, all these techniques require extremely high precision handling by specialised professionals and expensive instrumentation. Therefore, these facilities are presently available only in very few centres in the country. Obviously their benefits is affordable to only a limited number of people. Emotional, religious and social factors are also deterrents in the adoption of these methods. Since the ultimate aim of all these procedures is to have children, in India we have so many orphaned and destitute children, who would probably not survive till maturity, unless taken care of. Our laws permit legal adoption and it is as yet, one of the best methods for couples looking for parenthood.

SUMMARY

Reproductive health refers to a total well-being in all aspects of reproduction, i.e., physical, emotional, behavioural and social. Our nation was the first nation in the world to initiate various action plans at national level towards attaining a reproductively healthy society.

Counselling and creating awareness among people about reproductive organs, adolescence and associated changes, safe and hygienic sexual practices, sexually transmitted diseases (STDs) including AIDS, etc., is the primary step towards reproductive health. Providing medical facilities and care to the problems like menstrual irregularities, pregnancy related aspects, delivery, medical termination of pregnancy, STDs, birth control, infertility, post natal child and maternal management is another important aspect of the Reproductive and Child Health Care programmes.

An overall improvement in reproductive health has taken place in our country as indicated by reduced maternal and infant mortality rates, early detection and cure of STDs, assistance to infertile couples, etc. Improved health facilities and better living conditions promoted an explosive growth of population. Such a growth necessitated intense propagation of contraceptive methods. Various contraceptive options are available now such as natural, traditional, barrier, IUDs, pills, injectables, implants and surgical methods. Though contraceptives are not regular requirements for reproductive health, one is forced to use them to avoid pregnancy or to delay or space pregnancy.

Medical termination of pregnancy is legalised in our country. MTP is generally performed to get rid of unwanted pregnancy due to rapes, casual relationship, etc., as also in cases when the continuation of pregnancy could be harmful or even fatal to either the mother, or the foetus or both.

Diseases or infections transmitted through sexual intercourse are called Sexually Transmitted Diseases (STDs). Pelvic Inflammatory Diseases (PIDs), still birth, infertility are some of the complications of them. Early detection facilitate better cure of these diseases. Avoiding sexual intercourse with unknown/multiple partners, use of condoms during coitus are some of the simple precautions to avoid contracting STDs.

Inability to conceive or produce children even after 2 years of unprotected sexual cohabitation is called infertility. Various methods are now available to help such couples. *In Vitro* fertilisation followed by transfer of embryo into the female genital tract is one such method and is commonly known as the 'Test Tube Baby' Programme.

EXERCISES

1. What do you think is the significance of reproductive health in a society?
2. Suggest the aspects of reproductive health which need to be given special attention in the present scenario.
3. Is sex education necessary in schools? Why?
4. Do you think that reproductive health in our country has improved in the past 50 years? If yes, mention some such areas of improvement.
5. What are the suggested reasons for population explosion?
6. Is the use of contraceptives justified? Give reasons.
7. Removal of gonads cannot be considered as a contraceptive option. Why?
8. Amniocentesis for sex determination is banned in our country. Is this ban necessary? Comment.
9. Suggest some methods to assist infertile couples to have children.
10. What are the measures one has to take to prevent from contracting STDs?
11. State True/False with explanation
    (a) Abortions could happen spontaneously too. (True/False)
    (b) Infertility is defined as the inability to produce a viable offspring and is always due to abnormalities/defects in the female partner. (True/False)
    (c) Complete lactation could help as a natural method of contraception. (True/False)
    (d) Creating awareness about sex related aspects is an effective method to improve reproductive health of the people. (True/False)
12. Correct the following statements :
    (a) Surgical methods of contraception prevent gamete formation.
    (b) All sexually transmitted diseases are completely curable.
    (c) Oral pills are very popular contraceptives among the rural women.
    (d) In E. T. techniques, embryos are always transferred into the uterus.

UNIT VII
GENETICS AND EVOLUTION

Chapter 5
Principles of Inheritance and Variation

Chapter 6
Molecular Basis of Inheritance

Chapter 7
Evolution

The work of Mendel and others who followed him gave us an idea of inheritance patterns. However the nature of those 'factors' which determine the phenotype was not very clear. As these 'factors' represent the genetic basis of inheritance, understanding the structure of genetic material and the structural basis of genotype and phenotype conversion became the focus of attention in biology for the next century. The entire body of molecular biology was a consequent development with major contributions from Watson, Crick, Nirenberg, Khorana, Kornbergs (father and son), Benzer, Monod, Brenner, etc. A parallel problem being tackled was the mechanism of evolution. Awareness in the areas of molecular genetics, structural biology and bio informatics have enriched our understanding of the molecular basis of evolution. In this unit the structure and function of DNA and the story and theory of evolution have been examined and explained.

James Dewey Watson was born in Chicago on 6 April 1928. In 1947, he received B.Sc. degree in Zoology. During these years his interest in bird-watching had matured into a serious desire to learn genetics. This became possible when he received a Fellowship for graduate study in Zoology at Indiana University, Bloomington, where he received his Ph.D. degree in 1950 on a study of the effect of hard X-rays on bacteriophage multiplication.

He met Crick and discovered their common interest in solving the DNA structure. Their first serious effort, was unsatisfactory. Their second effort based upon more experimental evidence and better appreciation of the nucleic acid literature, resulted, early in March 1953, in the proposal of the complementary double-helical configuration.

JAMES WATSON
FRANCIS CRICK

Francis Harry Compton Crick was born on 8 June 1916, at Northampton, England. He studied physics at University College, London and obtained a B.Sc. in 1937. He completed Ph.D. in 1954 on a thesis entitled "X-ray Diffraction: Polypeptides and Proteins".

A critical influence in Crick's career was his friendship with J. D. Watson, then a young man of 23, leading in 1953 to the proposal of the double-helical structure for DNA and the replication scheme. Crick was made an F.R.S. in 1959.

The honours to Watson with Crick include: the John Collins Warren Prize of the Massachusetts General Hospital, in 1959; the Lasker Award, in 1960; the Research Corporation Prize, in 1962 and above all, the Nobel Prize in 1962.

CHAPTER 5

PRINCIPLES OF INHERITANCE AND VARIATION

5.1 *Mendel's Laws of Inheritance*

5.2 *Inheritance of One Gene*

5.3 *Inheritance of Two Genes*

5.4 *Sex Determination*

5.5 *Mutation*

5.6 *Genetic Disorders*

Have you ever wondered why an elephant always gives birth only to a baby elephant and not some other animal? Or why a mango seed forms only a mango plant and not any other plant?

Given that they do, are the offspring identical to their parents? Or do they show differences in some of their characteristics? Have you ever wondered why siblings sometimes look so similar to each other? Or sometimes even so different?

These and several related questions are dealt with, scientifically, in a branch of biology known as Genetics. This subject deals with the inheritance, as well as the variation of characters from parents to offspring. Inheritance is the process by which characters are passed on from parent to progeny; it is the basis of heredity. Variation is the degree by which progeny differ from their parents.

Humans knew from as early as 8000-1000 B.C. that one of the causes of variation was hidden in sexual reproduction. They exploited the variations that were naturally present in the wild populations of plants and animals to selectively breed and select for organisms that possessed desirable characters. For example, through artificial selection and domestication from ancestral

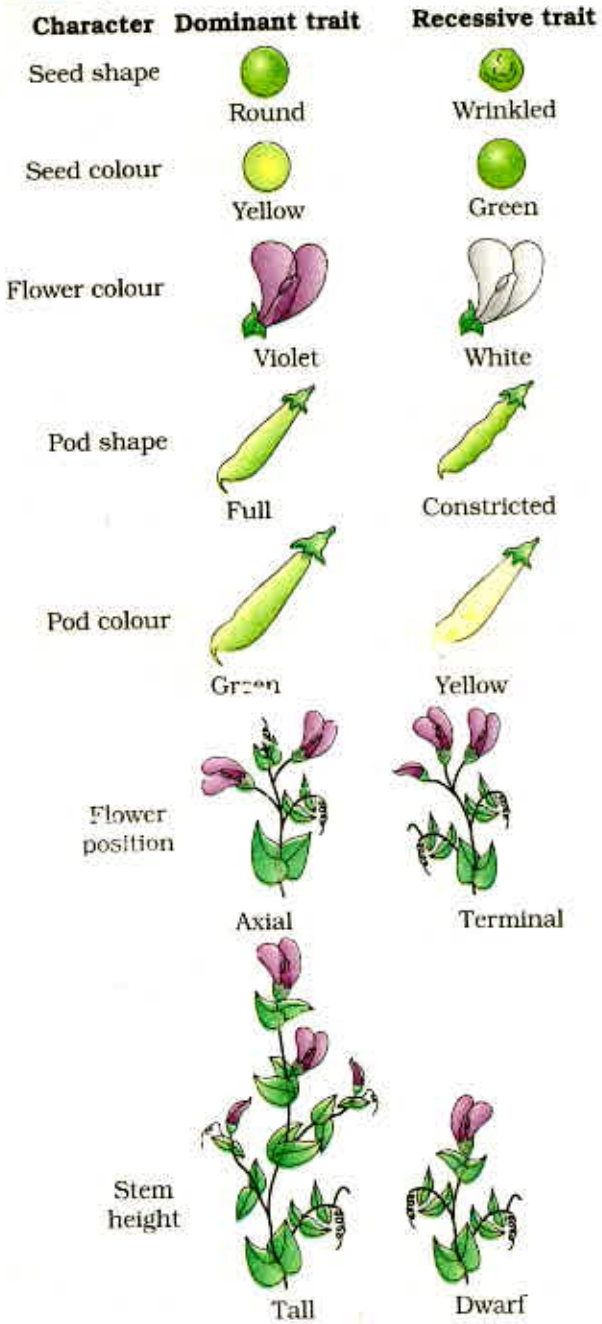


Figure 5.1 Seven pairs of contrasting traits in pea plant studied by Mendel

wild cows, we have well-known Indian breeds, e.g., Sahiwal cows in Punjab. We must, however, recognise that though our ancestors knew about the inheritance of characters and variation, they had very little idea about the scientific basis of these phenomena.

5.1 Mendel's Laws of Inheritance

It was during the mid-nineteenth century that headway was made in the understanding of inheritance. Gregor Mendel, conducted hybridisation experiments on garden peas for seven years (1856-1863) and proposed the laws of inheritance in living organisms. During Mendel's investigations into inheritance patterns it was for the first time that statistical analysis and mathematical logic were applied to problems in biology. His experiments had a large sampling size, which gave greater credibility to the data that he collected. Also, the confirmation of his inferences from experiments on successive generations of his test plants, proved that his results pointed to general rules of inheritance rather than being unsubstantiated ideas. Mendel investigated characters in the garden pea plant that were manifested as two opposing traits, e.g., tall or dwarf plants, yellow or green seeds. This allowed him to set up a basic framework of rules governing inheritance, which was expanded on by later scientists to account for all the diverse natural observations and the complexity inherent in them.

Mendel conducted such artificial pollination/cross pollination experiments using several true-breeding pea lines. A true-breeding line is one that, having undergone continuous self-pollination, shows the stable trait inheritance and expression for several generations. Mendel selected 14 true-breeding pea plant varieties, as pairs which were similar except for one character with contrasting traits. Some of the contrasting traits selected were smooth or wrinkled seeds, yellow or green seeds, inflated (full) or constricted green or yellow pods and tall or dwarf plants (Figure 5.1, Table 5.1).

Table 5.1: Contrasting Traits Studied by Mendel in Pea

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

5.2 Inheritance of One Gene

Let us take the example of one such hybridisation experiment carried out by Mendel where he crossed tall and dwarf pea plants to study the inheritance of one gene (Figure 5.2). He collected the seeds produced as a result of this cross and grew them to generate plants of the first hybrid generation. This generation is also called the **Filial$_1$ progeny** or the **F$_1$**. Mendel observed that all the F$_1$ progeny plants were tall, like one of its parents; none were dwarf (Figure 5.3). He made similar observations for the other pairs of traits – he found that the F$_1$ always resembled either one of the parents, and that the trait of the other parent was not seen in them.

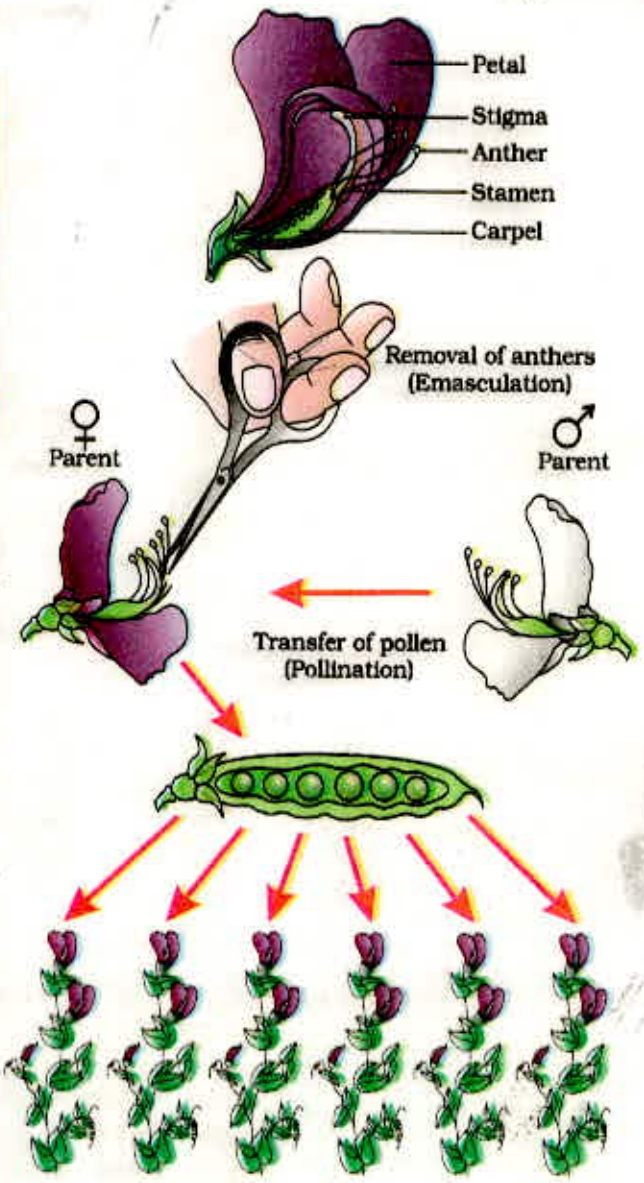


Figure 5.2 Steps in making a cross in pea

Mendel then self-pollinated the tall F$_1$ plants and to his surprise found that in the Filial$_2$ generation some of the offspring were 'dwarf'; the character that was not seen in the F$_1$ generation was now expressed. The proportion of plants that were dwarf were 1/4th of the F$_2$ plants while 3/4th of the F$_2$ plants were tall. The tall and dwarf traits were identical to their parental type and did not show any blending, that is all the offspring were either tall or dwarf, none were of in-between height (Figure 5.3).

Similar results were obtained with the other traits that he studied: only one of the parental traits was expressed in the F$_1$ generation while at the F$_2$ stage both the traits were expressed in the proportion 3:1. The contrasting traits did not show any blending at either F$_1$ or F$_2$ stage.

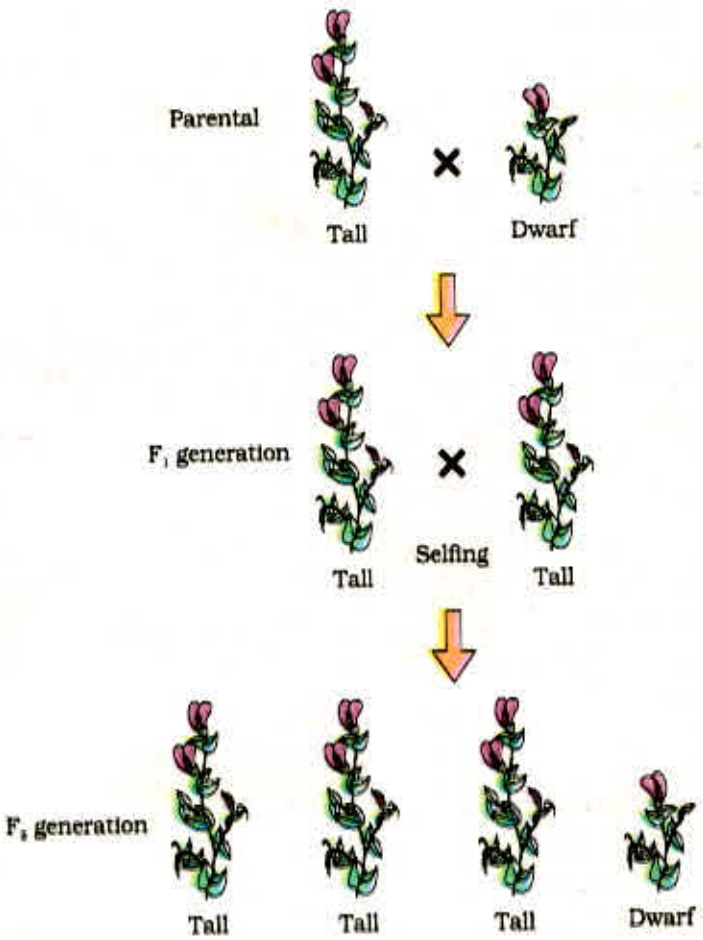


Figure 5.3 Diagrammatic representation of monohybrid cross

Based on these observations, Mendel proposed that something was being stably passed down, unchanged, from parent to offspring through the gametes, over successive generations. He called these things as 'factors'. Now we call them as **genes**. Genes, therefore, are the units of inheritance. They contain the information that is required to express a particular trait in an organism. Genes which code for a pair of contrasting traits are known as **alleles**, i.e., they are slightly different forms of the same gene.

If we use alphabetical symbols for each gene, then the capital letter is used for the trait expressed at the F_1 stage and the small alphabet for the other trait. For example, in case of the character of height, **T** is used for the Tall trait and **t** for the 'dwarf', and **T** and **t** are alleles of each other. Hence, in plants the pair of alleles for height would be **TT**, **Tt** or **tt**. Mendel also proposed that in a true breeding, tall or dwarf pea variety the allelic pair of genes for height are identical or **homozygous**, **TT** and **tt**, respectively. **TT** and **tt** are called the **genotype** of the plant while the descriptive terms **tall** and **dwarf** are the **phenotype**. What then would be the phenotype of a plant that had a genotype **Tt**?

As Mendel found the phenotype of the F_1 heterozygote **Tt** to be exactly like the **TT** parent in appearance, he proposed that in a pair of dissimilar factors, one dominates the other (as in the F_1) and hence is called the **dominant** factor while the other factor is **recessive** . In this case **T** (for tallness) is dominant over **t** (for dwarfness), that is recessive. He observed identical behaviour for all the other characters/trait-pairs that he studied.

It is convenient (and logical) to use the capital and lower case of an alphabetical symbol to remember this concept of dominance and recessiveness. (Do not use **T** for tall and **d** for dwarf because you will find it difficult to remember whether **T** and **d** are alleles of the same gene/character or not). Alleles can be similar as in the case of homozygotes **TT** and **tt** or can be dissimilar as in the case of the heterozygote **Tt**. Since

the **Tt** plant is heterozygous for genes controlling one character (height), it is a **monohybrid** and the cross between **TT** and **tt** is a **monohybrid cross**.

From the observation that the recessive parental trait is expressed without any blending in the F_2 generation, we can infer that, when the tall and dwarf plant produce gametes, by the process of meiosis, the alleles of the parental pair separate or **segregate** from each other and only one allele is transmitted to a gamete. This segregation of alleles is a random process and so there is a 50 per cent chance of a gamete containing either allele, as has been verified by the results of the crossings. In this way the gametes of the tall **TT** plants have the allele **T** and the gametes of the dwarf **tt** plants have the allele **t**. During fertilisation the two alleles, **T** from one parent say, through the pollen, and **t** from the other parent, then through the egg, are united to produce zygotes that have one **T** allele and one **t** allele. In other words the hybrids have **Tt**. Since these hybrids contain alleles which express contrasting traits, the plants are **heterozygous**. The production of gametes by the parents, the formation of the zygotes, the F_1 and F_2 plants can be understood from a diagram called **Punnett Square** as shown in Figure 5.4. It was developed by a British geneticist, Reginald C. Punnett. It is a graphical representation to calculate the probability of all possible genotypes of offspring in a genetic cross. The possible gametes are written on two sides, usually the top row and left columns. All possible combinations are represented in boxes below in the squares, which generates a square output form.

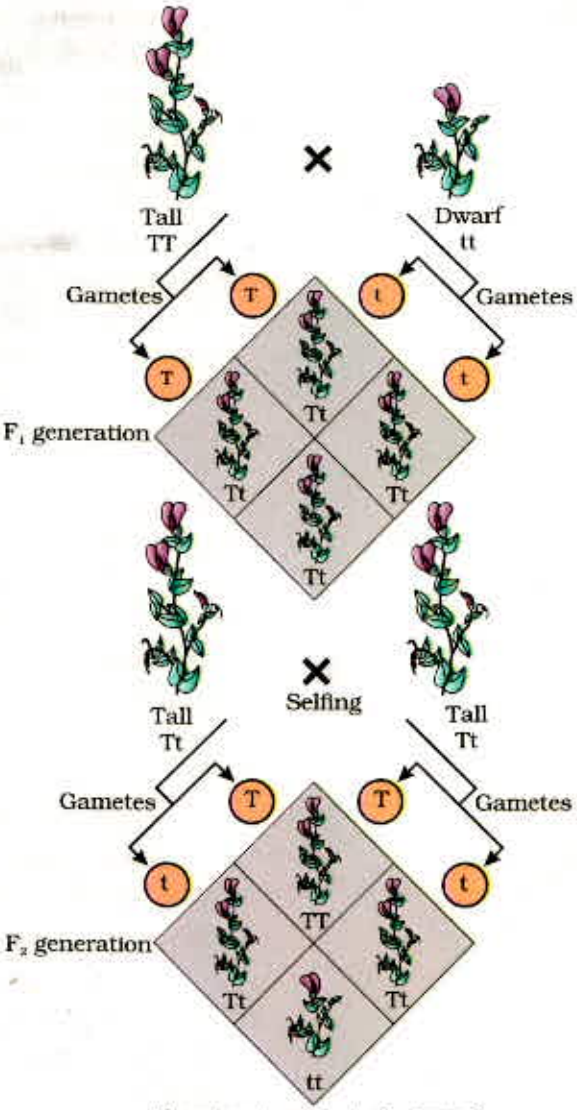


Figure 5.4 **A Punnett square used to understand a typical monohybrid cross conducted by Mendel between true-breeding tall plants and true-breeding dwarf plants**

The Punnett Square shows the parental tall **TT** (male) and dwarf **tt** (female) plants, the gametes produced by them and, the F_1 **Tt** progeny. The F_1 plants of genotype **Tt** are self-pollinated. The symbols ♀ and ♂ are used to denote the female (eggs) and male (pollen) of the F_1 generation, respectively. The F_1 plant of the genotype **Tt** when self-pollinated, produces gametes of the genotype **T** and **t** in equal proportion. When fertilisation takes place, the pollen grains of genotype **T** have a 50 per cent chance to pollinate eggs of the genotype **T**, as well as of genotype **t**. Also pollen grains of genotype **t** have a 50 per cent chance of pollinating eggs of genotype **T**, as well as of

genotype **t**. As a result of random fertilisation, the resultant zygotes can be of the genotypes **TT**, **Tt** or **tt.**

From the Punnett square it is easily seen that $1/4^{th}$ of the random fertilisations lead to **TT**, 1/2 lead to **Tt** and $1/4^{th}$ to **tt**. Though the F_1 have a genotype of **Tt**, but the phenotypic character seen is 'tall'. At F_2, $3/4^{th}$ of the plants are tall, where some of them are **TT** while others are **Tt**. Externally it is not possible to distinguish between the plants with the genotypes **TT** and **Tt**. Hence, within the genopytic pair **Tt** only one character '**T**' tall is expressed. Hence the character **T** or 'tall' is said to dominate over the other allele **t** or 'dwarf' character. It is thus due to this dominance of one character over the other that all the F_1 are tall (though the genotype is **Tt**) and in the F_2 $3/4^{th}$ of the plants are tall (though genotypically 1/2 are **Tt** and only $1/4^{th}$ are **TT**). This leads to a phenotypic ratio of $3/4^{th}$ tall : (1/4 **TT** + 1/2 **Tt**) and $1/4^{th}$ **tt**, i.e., a 3:1 ratio, but a genotypic ratio of 1:2:1.

The 1/4 : 1/2 : 1/4 ratio of **TT**: **Tt**: **tt** is mathematically condensable to the form of the binomial expression $(ax + by)^2$, that has the gametes bearing genes **T** or **t** in equal frequency of ½. The expression is expanded as given below :

$$(1/2\mathbf{T} + 1/2\ \mathbf{t})^2 = (1/2\mathbf{T} + 1/2\mathbf{t}) \times (1/2\mathbf{T} + 1/2\mathbf{t}) = 1/4\ \mathbf{TT} + 1/2\mathbf{Tt} + 1/4\ \mathbf{tt}$$

Mendel self-pollinated the F_2 plants and found that dwarf F_2 plants continued to generate dwarf plants in F_3 and F_4 generations. He concluded that the genotype of the dwarfs was homozygous – **tt**. What do you think he would have got had he self-pollinated a tall F_2 plant?

From the preceeding paragraphs it is clear that though the genotypic ratios can be calculated using mathematical probability, by simply looking at the phenotype of a dominant trait, it is not possible to know the genotypic composition. That is, for example, whether a tall plant from F_1 or F_2 has **TT** or **Tt** composition, cannot be predicted. Therefore, to determine the genotype of a tall plant at F_2, Mendel crossed the tall plant from F_2 with a dwarf plant. This he called a **test cross**. In a typical test cross an organism (pea plants here) showing a dominant phenotype (and whose genotype is to be determined) is crossed with the recessive parent instead of self-crossing. The progenies of such a cross can easily be analysed to predict the genotype of the test organism. Figure 5.5 shows the results of typical test cross where violet colour flower (W) is dominant over white colour flower (w).

Using Punnett square, try to find out the nature of offspring of a test cross.

What ratio did you get?

Using the genotypes of this cross, can you give a general definition for a test cross?

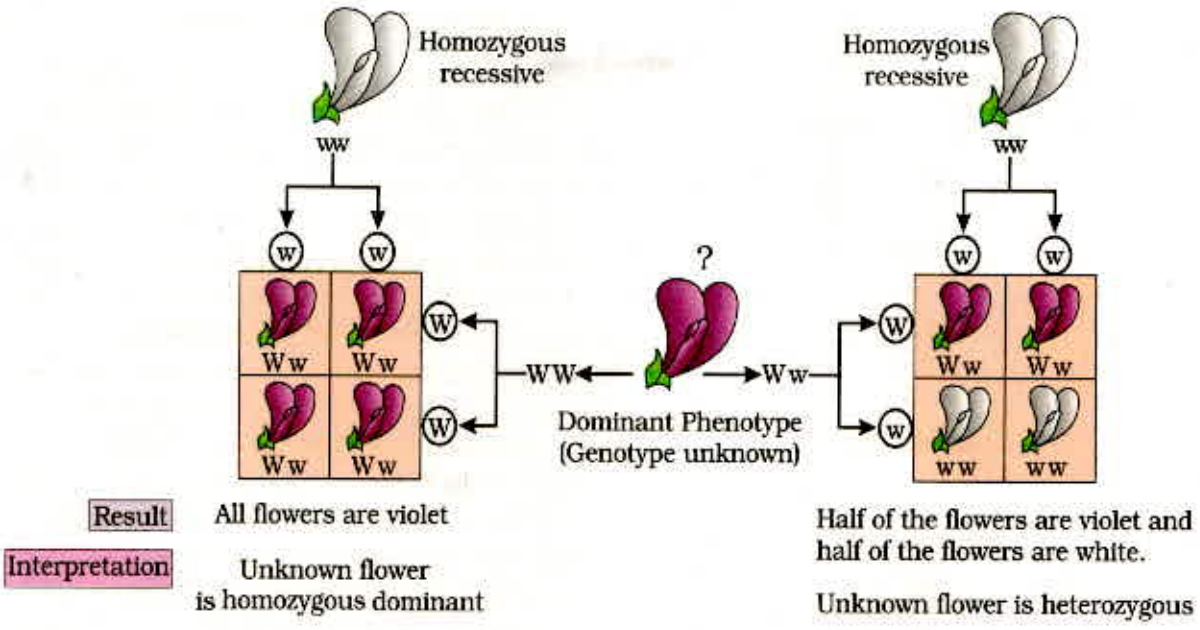


Figure 5.5 Diagrammatic representation of a test cross

Based on his observations on monohybrid crosses Mendel proposed two general rules to consolidate his understanding of inheritance in monohybrid crosses. Today these rules are called the **Principles or Laws of Inheritance:** the First Law or **Law of Dominance** and the Second Law or **Law of Segregation**.

5.2.1 Law of Dominance

(i) Characters are controlled by discrete units called **factors**.

(ii) Factors occur in pairs.

(iii) In a dissimilar pair of factors one member of the pair dominates (dominant) the other (recessive).

The law of dominance is used to explain the expression of only one of the parental characters in a monohybrid cross in the F_1 and the expression of both in the F_2. It also explains the proportion of 3:1 obtained at the F_2.

5.2.2 Law of Segregation

This law is based on the fact that the alleles do not show any blending and that both the characters are recovered as such in the F_2 generation though one of these is not seen at the F_1 stage. Though the parents contain two alleles during gamete formation, the factors or alleles of a pair segregate from each other such that a gamete receives only one of the two factors. Of course, a homozygous parent produces all gametes that are similar while a heterozygous one produces two kinds of gametes each having one allele with equal proportion.

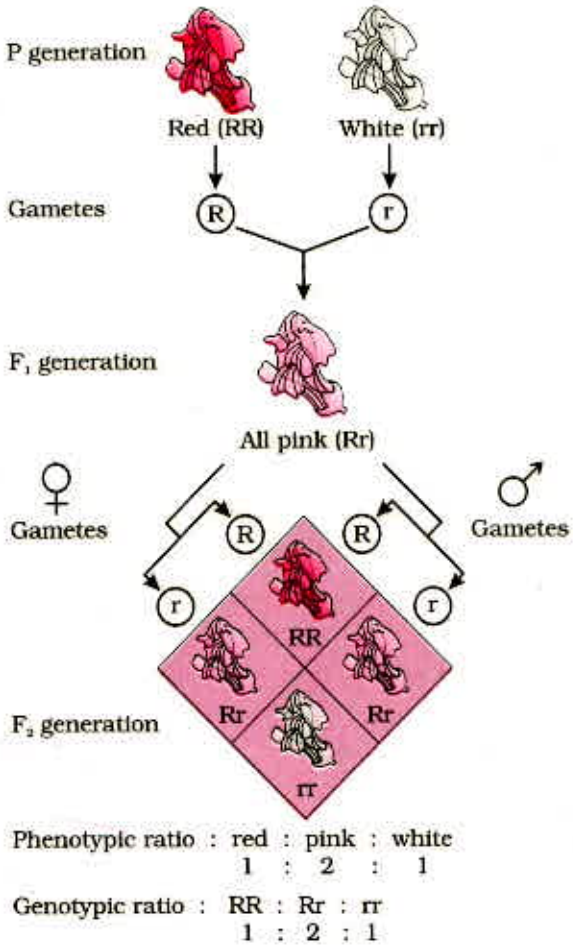


Figure 5.6 Results of monohybrid cross in the plant Snapdragon, where one allele is incompletely dominant over the other allele

5.2.2.1 *Incomplete Dominance*

When experiments on peas were repeated using other traits in other plants, it was found that sometimes the F_1 had a phenotype that did not resemble either of the two parents and was in between the two. The inheritance of flower colour in the dog flower (snapdragon or *Antirrhinum sp.*) is a good example to understand incomplete dominance. In a cross between true-breeding red-flowered **(RR)** and true-breeding white-flowered plants **(rr)**, the F_1 **(Rr)** was pink (Figure 5.6). When the F_1 was self-pollinated the F_2 resulted in the following ratio 1 **(RR)** Red : 2 **(Rr)** Pink : 1 **(rr)** White. Here the genotype ratios were exactly as we would expect in any mendelian monohybrid cross, but the phenotype ratios had changed from the 3:1 dominant : recessive ratio. What happened was that **R** was not completely dominant over **r** and this made it possible to distinguish **Rr** as pink from **RR** (red) and **rr** (white).

Explanation of the concept of dominance: What exactly is dominance? Why are some alleles dominant and some recessive? To tackle these questions, we must understand what a gene does. Every gene, as you know by now, contains the information to express a particular trait. In a diploid organism, there are two copies of each gene, i.e., as a pair of alleles. Now, these two alleles need not always be identical, as in a heterozygote. One of them may be different due to some changes that it has undergone (about which you will read further on, and in the next chapter) which modifies the information that particular allele contains.

Let's take an example of a gene that contains the information for producing an enzyme. Now there are two copies of this gene, the two allelic forms. Let us assume (as is more common) that the normal allele produces the normal enzyme that is needed for the transformation of a substrate S. Theoretically, the modified allele could be responsible for production of –

(i) the normal/less efficient enzyme, or

(ii) a non-functional enzyme, or

(iii) no enzyme at all

In the first case, the modified allele is equivalent to the unmodified allele, i.e., it will produce the same phenotype/trait, i.e., result in the transformation of substrate S. Such equivalent allele pairs are very common. But, if the allele produces a non-functional enzyme or no enzyme, the phenotype may be effected. The phenotype/trait will only be dependent on the functioning of the unmodified allele. The unmodified (functioning) allele, which represents the original phenotype is the dominant allele and the modified allele is generally the recessive allele. Hence, in the example above the recessive trait is seen due to non-functional enzyme or because no enzyme is produced.

5.2.2.2 *Co-dominance*

Till now we were discussing crosses where the F_1 resembled either of the two parents (dominance) or was in-between (incomplete dominance). But, in the case of co-dominance the F_1 generation resembles both parents. A good example is different types of red blood cells that determine ABO blood grouping in human beings. ABO blood groups are controlled by the gene ***I***. The plasma membrane of the red blood cells has sugar polymers that protrude from its surface and the kind of sugar is controlled by the gene. The gene (***I***) has three alleles I^A, I^B and ***i***. The alleles I^A and I^B produce a slightly different form of the sugar while allele ***i*** does not produce any sugar. Because humans are diploid organisms, each person possesses any two of the three ***I*** gene alleles. I^A and I^B are completely dominant over ***i***, in other words when I^A and ***i*** are present only I^A expresses (because ***i*** does not produce any sugar), and when I^B and ***i*** are present I^B expresses. But when I^A and I^B are present together they both express their own types of sugars: this is because of co-dominance. Hence red blood cells have both A and B types of sugars. Since there are three different alleles, there are six different combinations of these three alleles that are possible, and therefore, a total of six different genotypes of the human ABO blood types (Table 5.2). *How many phenotypes are possible?*

Table 5.2: Table Showing the Genetic Basis of Blood Groups in Human Population

Allele from Parent 1	Allele from Parent 2	Genotype of offspring	Blood types of offspring
I^A	I^A	I^AI^A	A
I^A	I^B	I^AI^B	AB
I^A	i	I^Ai	A
I^B	I^A	I^AI^B	AB
I^B	I^B	I^BI^B	B
I^B	i	I^Bi	B
i	i	ii	O

Do you realise that the example of ABO blood grouping also provides a good example of **multiple alleles**? Here you can see that there are more than two, i.e., three alleles, governing the same character. Since in an individual only two alleles can be present, multiple alleles can be found only when population studies are made.

Occasionally, a single gene product may produce more than one effect. For example, starch synthesis in pea seeds is controlled by one gene. It has two alleles (**B** and **b**). Starch is synthesised effectively by **BB** homozygotes and therefore, large starch grains are produced. In contrast, **bb** homozygotes have lesser efficiency in starch synthesis and produce smaller starch grains. After maturation of the seeds, **BB** seeds are round and the **bb** seeds are wrinkled. Heterozygotes produce round seeds, and so **B** seems to be the dominant allele. But, the starch grains produced are of intermediate size in **Bb** seeds. So if starch grain size is considered as the phenotype, then from this angle, the alleles show incomplete dominance.

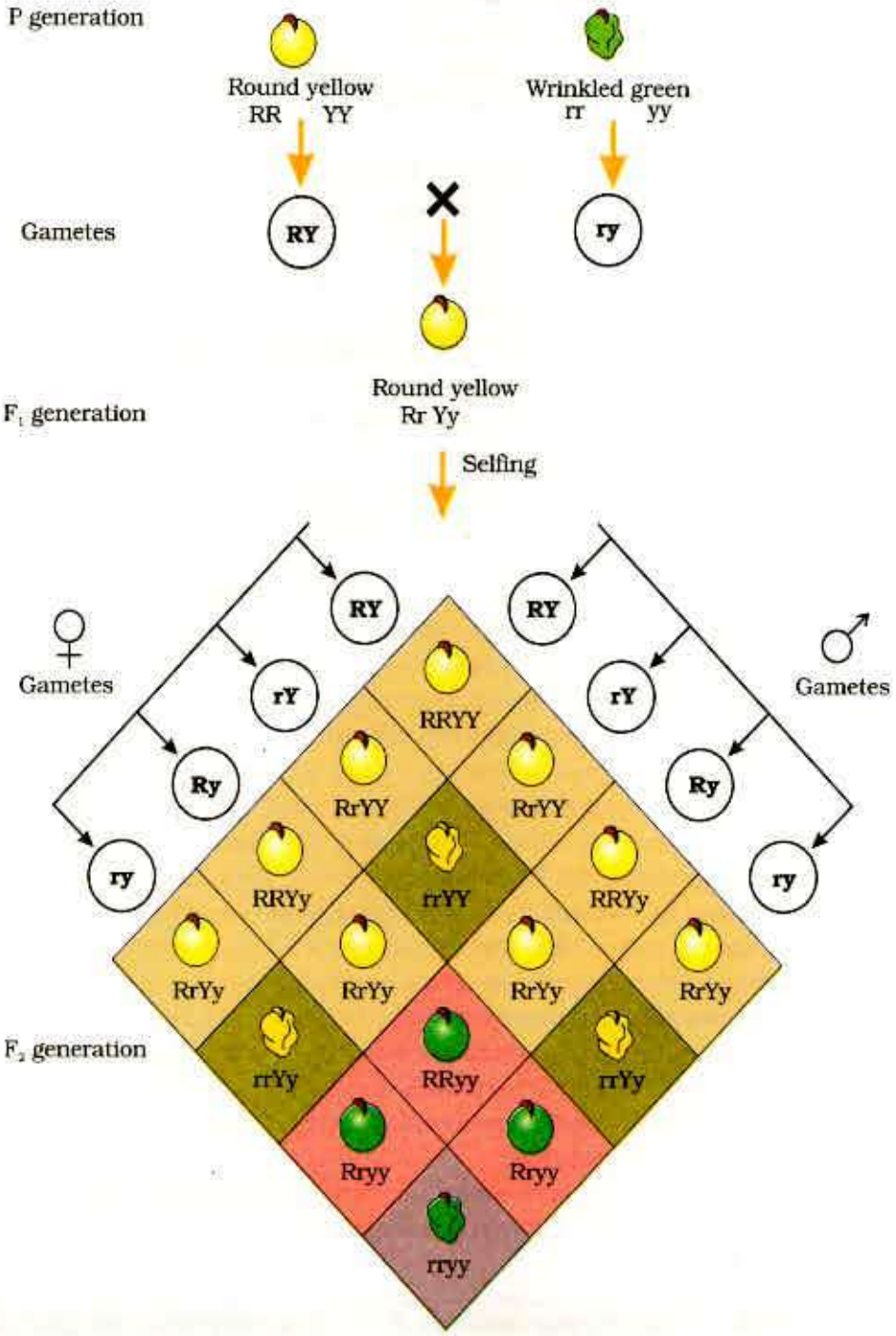
Therefore, dominance is not an autonomous feature of a gene or the product that it has information for. It depends as much on the gene product and the production of a particular phenotype from this product as it does on the particular phenotype that we choose to examine, in case more than one phenotype is influenced by the same gene.

5.3 Inheritance of Two Genes

Mendel also worked with and crossed pea plants that differed in two characters, as is seen in the cross between a pea plant that has seeds with yellow colour and round shape and one that had seeds of green colour and wrinkled shape (Figure5.7). Mendel found that the seeds resulting from the crossing of the parents, had yellow coloured and round shaped seeds. *Here can you tell which of the characters in the pairs yellow/green colour and round/wrinkled shape was dominant?*

Thus, yellow colour was dominant over green and round shape dominant over wrinkled. These results were identical to those that he got when he made separate monohybrid crosses between yellow and green seeded plants and between round and wrinkled seeded plants.

Let us use the genotypic symbols **Y** for dominant yellow seed colour and **y** for recessive green seed colour, **R** for round shaped seeds and **r** for wrinkled seed shape. The genotype of the parents can then be written as **RRYY** and **rryy**. The cross between the two plants can be written down as in Figure 5.7 showing the genotypes of the parent plants. The gametes **RY** and **ry** unite on fertilisation to produce the F_1 hybrid **RrYy**. When Mendel self hybridised the F_1 plants he found that $3/4^{th}$ of F_2 plants had yellow seeds and $1/4^{th}$ had green. The yellow and green colour segregated in a 3:1 ratio. Round and wrinkled seed shape also segregated in a 3:1 ratio; just like in a monohybrid cross.



Phenotypic ratio : round yellow : round green : wrinkled yellow : wrinkled green
9 3 3 1

Figure 5.7 Results of a dihybrid cross where the two parents differed in two pairs of contrasting traits: seed colour and seed shape

5.3.1 Law of Independent Assortment

In the dihybrid cross (Figure 5.7), the phenotypes round, yellow; wrinkled, yellow; round, green and wrinkled, green appeared in the ratio 9:3:3:1. Such a ratio was observed for several pairs of characters that Mendel studied.

The ratio of 9:3:3:1 can be derived as a combination series of 3 yellow: 1 green, with 3 round : 1 wrinkled. This derivation can be written as follows:

(3 Round : 1 Wrinkled) (3 Yellow : 1 Green) = 9 Round, Yellow : 3 Wrinkled, Yellow: 3 Round, Green : 1 Wrinkled, Green

Based upon such observations on **dihybrid crosses** (crosses between plants differing in two traits) Mendel proposed a second set of generalisations that we call Mendel's Law of Independent Assortment. The law states that 'when two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent of the other pair of characters'.

The Punnett square can be effectively used to understand the independent segregation of the two pairs of genes during meiosis and the production of eggs and pollen in the F_1 **RrYy** plant. Consider the segregation of one pair of genes **R** and **r**. Fifty per cent of the gametes have the gene **R** and the other 50 per cent have **r**. Now besides each gamete having either **R** or **r**, it should also have the allele **Y** or **y**. The important thing to remember here is that segregation of 50 per cent **R** and 50 per cent **r** is *independent* from the segregation of 50 per cent **Y** and 50 per cent **y**. Therefore, 50 per cent of the **r** bearing gametes has **Y** and the other 50 per cent has **y**. Similarly, 50 per cent of the **R** bearing gametes has **Y** and the other 50 per cent has **y**. Thus there are four genotypes of gametes (four types of pollen and four types of eggs). The four types are **RY**, **Ry**, **rY** and **ry** each with a frequency of 25 per cent or $1/4^{th}$ of the total gametes produced. When you write down the four types of eggs and pollen on the two sides of a Punnett square it is very easy to derive the composition of the zygotes that give rise to the F_2 plants (Figure 5.7). *Although there are 16 squares how many different types of genotypes and phenotypes are formed?* Note them down in the format given.

Can you, using the Punnett square data work out the genotypic ratio at the F_2 stage and fill in the format given? Is the genotypic ratio also 9:3:3:1?

S.No.	Genotypes found in F_2	Their expected Phenotypes

5.3.2 Chromosomal Theory of Inheritance

Mendel published his work on inheritance of characters in 1865 but for several reasons, it remained unrecognised till 1900. Firstly,

communication was not easy (as it is now) in those days and his work could not be widely publicised. Secondly, his concept of **genes** (or **factors**, in Mendel's words) as stable and discrete units that controlled the expression of traits and, of the pair of alleles which did not 'blend' with each other, was not accepted by his contemporaries as an explanation for the apparently continuous variation seen in nature. Thirdly, Mendel's approach of using mathematics to explain biological phenomena was totally new and unacceptable to many of the biologists of his time. Finally, though Mendel's work suggested that factors (genes) were discrete units, he could not provide any physical proof for the existence of factors or say what they were made of.

In 1900, three Scientists (de Vries, Correns and von Tschermak) independently rediscovered Mendel's results on the inheritance of characters. Also, by this time due to advancements in microscopy that were taking place, scientists were able to carefully observe cell division. This led to the discovery of structures in the nucleus that appeared to double and divide just before each cell division. These were called **chromosomes** (*colored bodies*, as they were visualised by staining). By 1902, the chromosome movement during meiosis had been worked out. Walter Sutton and Theodore Boveri noted that the behaviour of chromosomes was parallel to the behaviour of genes and used chromosome movement (Figure 5.8) to explain Mendel's laws (Table 5.3). Recall that you have studied the behaviour of chromosomes during mitosis (equational division) and during meiosis (reduction division). The important things to remember are that chromosomes as well as genes occur in pairs. The two alleles of a gene pair are located on homologous sites on homologous chromosomes.

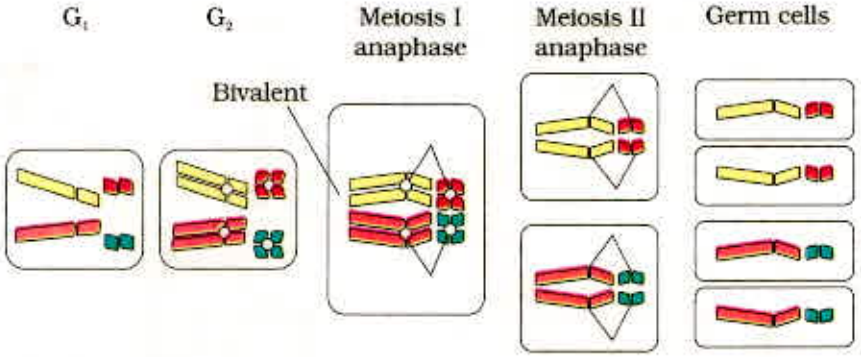


Figure 5.8 Meiosis and germ cell formation in a cell with four chromosomes. Can you see how chromosomes segregate when germ cells are formed?

Table 5.3: A Comparison between the Behaviour of Chromosomes and Genes

A	B
Occur in pairs	*Occur in pairs*
Segregate at the time of gamete formation such that only one of each pair is transmitted to a gamete	*Segregate at gamete formation and only one of each pair is transmitted to a gamete*
Independent pairs segregate independently of each other	*One pair segregates independently of another pair*
Can you tell which of these columns A or B represent the chromosome and which represents the gene? How did you decide?	

During Anaphase of meiosis I, the two chromosome pairs can align at the metaphase plate independently of each other (Figure 5.9). To understand this, compare the chromosomes of four different colour in the left and right columns. In the left column (Possibility I) orange and green is segregating together. But in the right hand column (Possibility II) the orange chromosome is segregating with the red chromosomes.

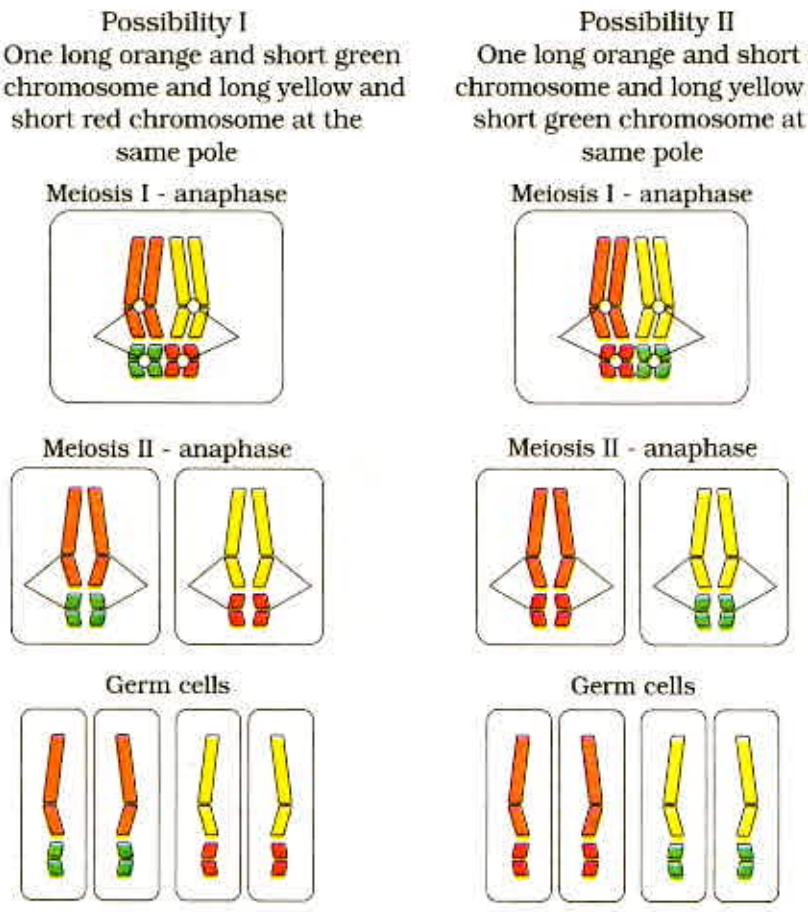


Figure 5.9 Independent assortment of chromosomes

Sutton and Boveri argued that the pairing and separation of a pair of chromosomes would lead to the segregation of a pair of factors they carried. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it the **chromosomal theory of inheritance**.

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

Figure 5.10 *Drosophila melanogaster* (a) Male (b) Female

5.3.3 Linkage and Recombination

Morgan carried out several dihybrid crosses in *Drosophila* to study genes that were sex-linked. The crosses were similar to the dihybrid crosses carried out by Mendel in peas. For example Morgan hybridised yellow-bodied, white-eyed females to brown-bodied, red-eyed males and intercrossed their F_1 progeny. He observed that the two genes did not segregate independently of each other and the F_2 ratio deviated very significantly from the 9:3:3:1 ratio (expected when the two genes are independent).

Morgan and his group knew that the genes were located on the X chromosome (Section 5.4) and saw quickly that when the two genes in a dihybrid cross were situated on the same chromosome, the proportion of parental gene combinations were much higher than the non-parental type. Morgan attributed this due to the physical association or linkage of the two genes and coined the term **linkage** to describe this physical association of genes on a chromosome and the term **recombination** to describe the generation of non-parental gene combinations (Figure 5.11). Morgan and his group also found that even when genes were grouped on the same chromosome, some genes were very tightly linked (showed very low recombination) (Figure 5.11, Cross A) while others were loosely linked (showed higher recombination) (Figure 5.11, Cross B). For example he found that the genes white and yellow were very tightly linked and showed only 1.3 per cent recombination while white and miniature wing showed 37.2 per cent recombination. His student Alfred Sturtevant used the frequency of recombination between gene pairs on the same chromosome as a measure of the distance between genes and 'mapped' their position on the chromosome. Today genetic maps

are extensively used as a starting point in the sequencing of whole genomes as was done in the case of the Human Genome Sequencing Project, described later.

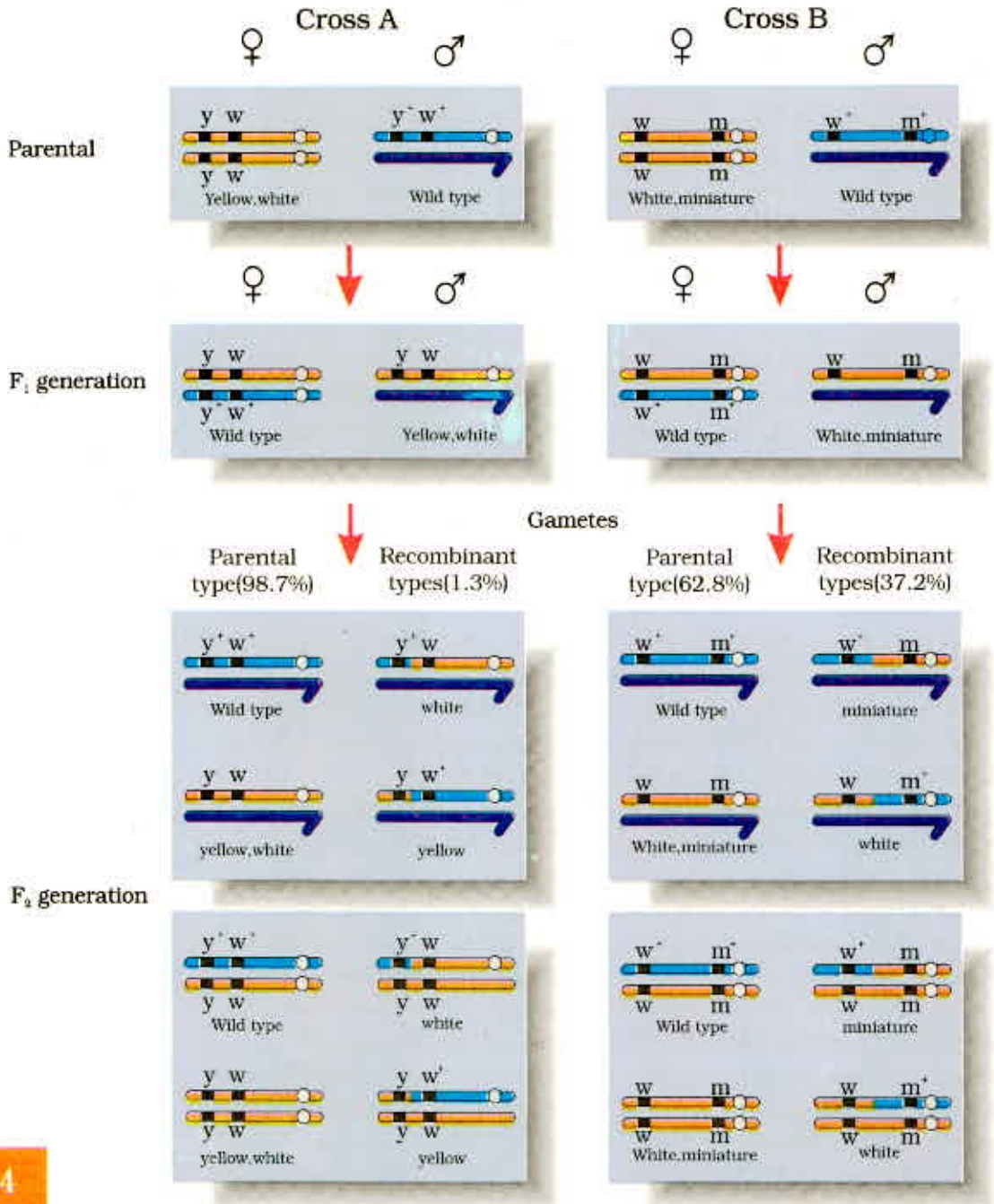


Figure 5.11 Linkage: Results of two dihybrid crosses conducted by Morgan. Cross A shows crossing between gene y and w; Cross B shows crossing between genes w and m. Here dominant wild type alleles are represented with (+) sign in superscript

Note: The strength of linkage between y and w is higher than w and m.

5.4 Sex Determination

The mechanism of sex determination has always been a puzzle before the geneticists. The initial clue about the genetic/chromosomal mechanism of sex determination can be traced back to some of the experiments carried out in insects. In fact, the cytological observations made in a number of insects led to the development of the concept of genetic/chromosomal basis of sex-determination. Henking (1891) could trace a specific nuclear structure all through spermatogenesis in a few insects, and it was also observed by him that 50 per cent of the sperm received this structure after spermatogenesis, whereas the other 50 per cent sperm did not receive it. Henking gave a name to this structure as the **X body** but he could not explain its significance. Further investigations by other scientists led to the conclusion that the 'X body' of Henking was in fact a chromosome and that is why it was given the name X-chromosome. It was also observed that in a large number of insects the mechanism of sex determination is of the XO type, i.e., all eggs bear an additional X-chromosome besides the other chromosomes (autosomes). On the other hand, some of the sperms bear the X-chromosome whereas some do not. Eggs fertilised by sperm having an X-chromosome become females and, those fertilised by sperms that do not have an X-chromosome become males. *Do you think the number of chromosomes in the male and female are equal?* Due to the involvement of the X-chromosome in the determination of sex, it was designated to be the **sex chromosome**, and the rest of the chromosomes were named as **autosomes**. Grasshopper is an example of XO type of sex determination in which the males have only one X-chromosome besides the autosomes, whereas females have a pair of X-chromosomes.

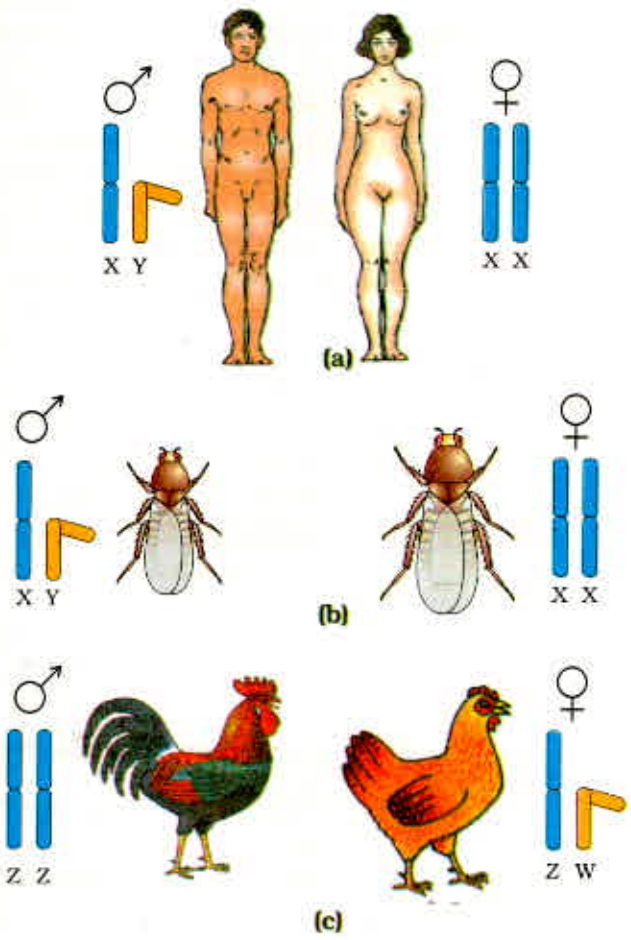


Figure 5.12 Determination of sex by chromosomal differences: (a,b) Both in humans and in *Drosophila*, the female has a pair of XX chromosomes (homogametic) and the male XY (heterogametic) composition; (c) In many birds, female has a pair of dissimilar chromosomes ZW and male two similar ZZ chromosomes

These observations led to the investigation of a number of species to understand the mechanism of sex determination. In a number of other insects and mammals including man, XY type of sex determination is seen where both male and female have same number of chromosomes.

Among the males an X-chromosome is present but its counter part is distinctly smaller and called the Y-chromosome. Females, however, have a pair of X-chromosomes. Both males and females bear same number of autosomes. Hence, the males have autosomes plus XY, while female have autosomes plus XX. In human beings and in *Drosophila* the males have one X and one Y chromosome, whereas females have a pair of X-chromosomes besides autosomes (Figure 5.12 a, b).

In the above description you have studied about two types of sex determining mechanisms, i.e., XO type and XY type. But in both cases males produce two different types of gametes, (a) either with or without X-chromosome or (b) some gametes with X-chromosome and some with Y-chromosome. Such types of sex determination mechanism is designated to be the example of **male heterogamety**. In some other organisms, e.g., birds, a different mechanism of sex determination is observed (Figure 5.12 c). In this case the total number of chromosome is same in both males and females. But two different types of gametes in terms of the sex chromosomes, are produced by females, i.e., **female heterogamety**. In order to have a distinction with the mechanism of sex determination described earlier, the two different sex chromosomes of a female bird has been designated to be the Z and W chromosomes. In these organisms the females have one Z and one W chromosome, whereas males have a pair of Z-chromosomes besides the autosomes.

5.4.1 Sex Determination in Humans

It has already been mentioned that the sex determining mechanism in case of humans is XY type. Out of 23 pairs of chromosomes present, 22 pairs are exactly same in both males and females; these are the autosomes. A pair of X-chromosomes are present in the female, whereas the presence of an X and Y chromosome are determinant of the male characteristic. During spermatogenesis among males, two types of gametes are produced. 50 per cent of the total sperm produced carry the X-chromosome and the rest 50 per cent has Y-chromosome besides the autosomes. Females, however, produce only one type of ovum with an X-chromosome. There is an equal probability of fertilisation of the ovum with the sperm carrying either X or Y chromosome. In case the ovum fertilises with a sperm carrying X-chromosome the zygote develops into a female (XX) and the fertilisation of ovum with Y-chromosome carrying sperm results into a male offspring. Thus, it is evident that it is the genetic makeup of the sperm that determines the sex of the child. It is also evident that in each pregnancy there is always 50 per cent probability of either a male or a female child. It is unfortunate that in our society women are blamed for giving birth to female children and have been ostracised and ill-treated because of this false notion.

How is the sex-determination mechanism different in the birds? *Is the sperm or the egg responsible for the sex of the chicks?*

5.5 Mutation

Mutation is a phenomenon which results in alteration of DNA sequences and consequently results in changes in the genotype and the phenotype of an organism. In addition to recombination, mutation is another phenomenon that leads to variation in DNA.

As you will learn in Chapter 6, one DNA helix runs continuously from one end to the other in each chromatid, in a highly supercoiled form. Therefore loss (deletions) or gain (insertion/duplication) of a segment of DNA, result in alteration in chromosomes. Since genes are known to be located on chromosomes, alteration in chromosomes results in abnormalities or aberrations. Chromosomal aberrations are commonly observed in cancer cells.

In addition to the above, mutation also arise due to change in a single base pair of DNA. This is known as point mutation. A classical example of such a mutation is sickle cell anemia. Deletions and insertions of base pairs of DNA, causes frame-shift mutations (see Chapter 6).

The mechanism of mutation is beyond the scope of this discussion, at this level. However, there are many chemical and physical factors that induce mutations. These are referred to as mutagens. UV radiations can cause mutations in organisms – it is a mutagen.

5.6 Genetic Disorders

5.6.1 Pedigree Analysis

The idea that disorders are inherited has been prevailing in the human society since long. This was based on the heritability of certain characteristic features in families. After the rediscovery of Mendel's work the practice of analysing inheritance pattern of traits in human beings began. Since it is evident that control crosses that can be performed in pea plant or some other organisms, are not possible in case of human beings, study of the family history about inheritance of a particular trait provides an alternative. Such an analysis of traits in a several of generations of a family is called the **pedigree analysis**. In the pedigree analysis the inheritance of a particular trait is represented in the family tree over generations.

In human genetics, pedigree study provides a strong tool, which is utilised to trace the inheritance of a specific trait, abnormality or disease. Some of the important standard symbols used in the pedigree analysis have been shown in Figure 5.13.

As you have studied in this chapter, each and every feature in any organism is controlled by one or the other gene located on the DNA present in the chromosome. DNA is the carrier of genetic information. It is hence transmitted from one generation to the other without any

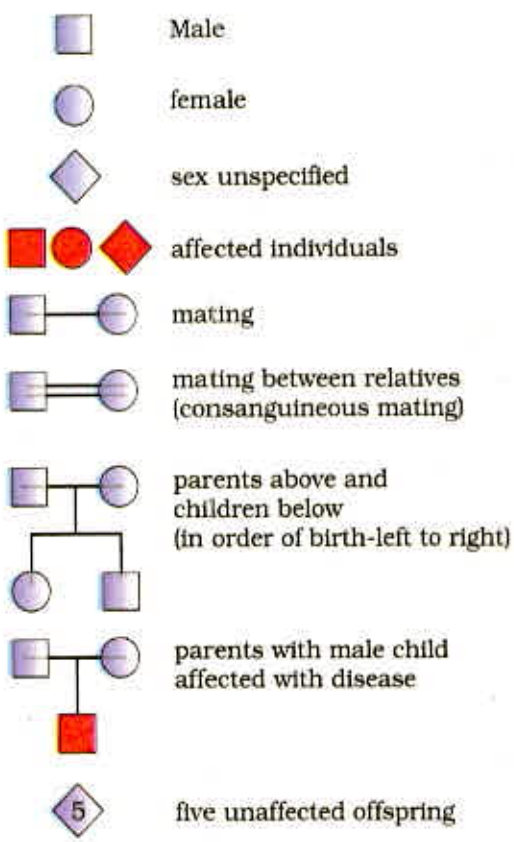


Figure 5.13 Symbols used in the human pedigree analysis

change or alteration. However, changes or alteration do take place occasionally. Such an alteration or change in the genetic material is referred to as mutation. A number of disorders in human beings have been found to be associated with the inheritance of changed or altered genes or chromosomes.

5.6.2 Mendelian Disorders

Broadly, genetic disorders may be grouped into two categories – Mendelian disorders and Chromosomal disorders. Mendelian disorders are mainly determined by alteration or mutation in the single gene. These disorders are transmitted to the offspring on the same lines as we have studied in the principle of inheritance. The pattern of inheritance of such Mendelian disorders can be traced in a family by the pedigree analysis. Most common and prevalent Mendelian disorders are Haemophilia, Cystic fibrosis, Sickle-cell anaemia, Colour blindness, Phenylketonuria, Thalassemia, etc. It is important to mention here that such Mendelian disorders may be dominant or recessive. By pedigree analysis one can easily understand whether the trait in question is dominant or recessive. Similarly, the trait may also be linked to the sex chromosome as in case of haemophilia. It is evident that this X-linked recessive trait shows transmission from carrier female to male progeny. A representative pedigree is shown in Figure 5.14 for dominant and recessive traits. Discuss with your teacher and design pedigrees for characters linked to both autosomes and sex chromosome.

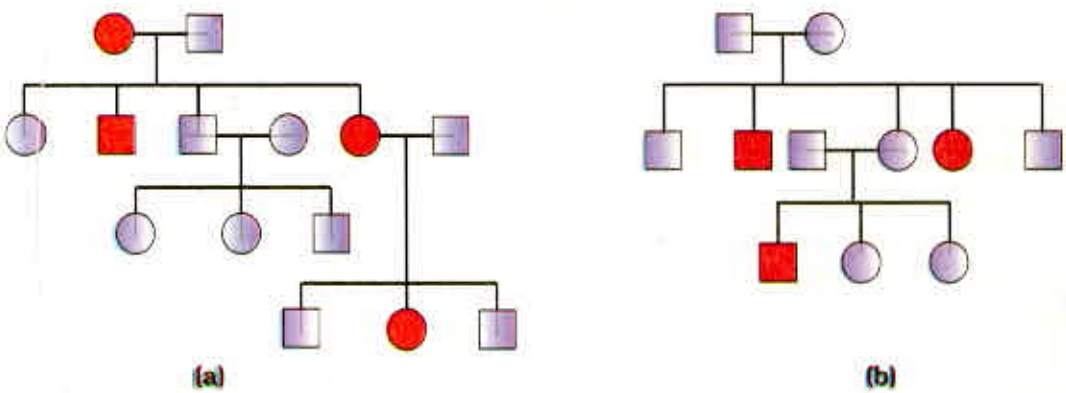


Figure 5.14 Representative pedigree analysis of (a) Autosomal dominant trait (for example: Myotonic dystrophy) (b) Autosomal recessive trait (for example: Sickle-cell anaemia)

Haemophilia : This sex linked recessive disease, which shows its transmission from unaffected carrier female to some of the male progeny has been widely studied. In this disease, a single protein that is a part of the cascade of proteins involved in the clotting of blood is affected. Due to this, in an affected individual a simple cut will result in non-stop bleeding. The heterozygous female (carrier) for haemophilia may transmit the disease to sons. The possibility of a female becoming a haemophilic is extremely rare because mother of such a female has to be at least carrier and the father should be haemophilic (unviable in the later stage of life). The family pedigree of Queen Victoria shows a number of haemophilic descendents as she was a carrier of the disease.

Sickle-cell anaemia : This is an autosome linked recessive trait that can be transmitted from parents to the offspring when both the partners are carrier for the gene (or heterozygous). The disease is controlled by a single pair of allele, Hb^A and Hb^S. Out of the three possible genotypes only homozygous individuals for Hb^S (Hb^SHb^S) show the diseased phenotype. Heterozygous (Hb^AHb^S) individuals appear apparently unaffected but they are carrier of the disease as there is 50 per cent probability of transmission of the mutant gene to the progeny, thus exhibiting sickle-cell trait (Figure 5.15). The defect is caused by the substitution of Glutamic acid (Glu) by

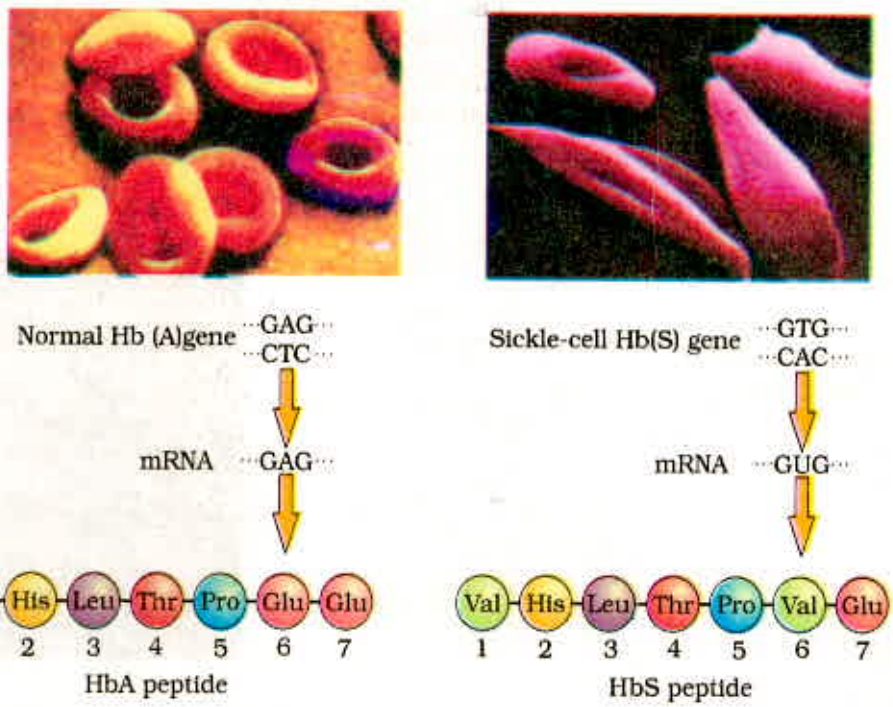


Figure 5.15 Micrograph of the red blood cells and the amino acid composition of the relevant portion of β-chain of haemoglobin: (a) From a normal individual; (b) From an individual with sickle-cell anaemia

Valine (Val) at the sixth position of the beta globin chain of the haemoglobin molecule. The substitution of amino acid in the globin protein results due to the single base substitution at the sixth codon of the beta globin gene from GAG to GUG. The mutant haemoglobin molecule undergoes polymerisation under low oxygen tension causing the change in the shape of the RBC from biconcave disc to elongated sickle like structure (Figure 5.15).

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

5.6.3 Chromosomal disorders

The chromosomal disorders on the other hand are caused due to absence or excess or abnormal arrangement of one or more chromosomes.

Failure of segregation of chromatids during cell division cycle results in the gain or loss of a chromosome(s), called **aneuploidy**. For example, Down's syndrome results in the gain of extra copy of chromosome 21. Similarly, Turner's syndrome results due to loss of an X chromosome in human females. Failure of cytokinesis after telophase stage of cell division results in an increase in a whole set of chromosomes in an organism and,

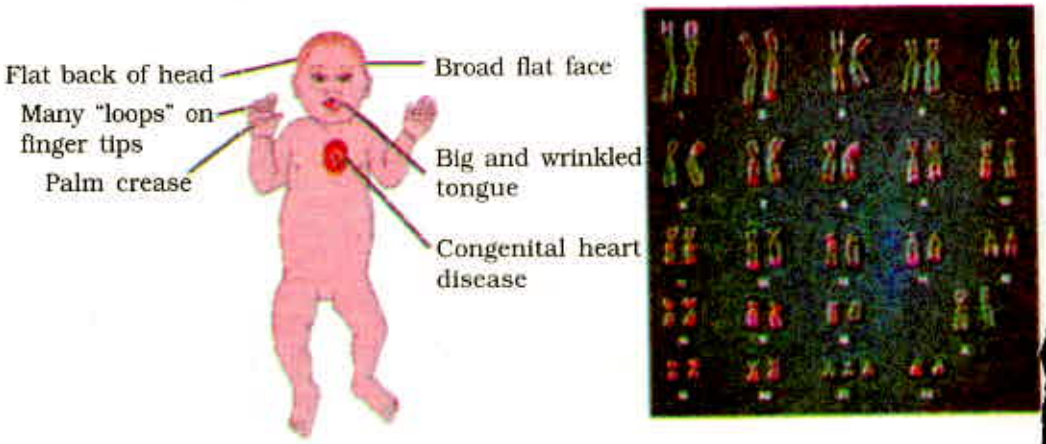


Figure 5.16 *A representative figure showing an individual inflicted with Down's syndrome and the corresponding chromosomes of the individual*

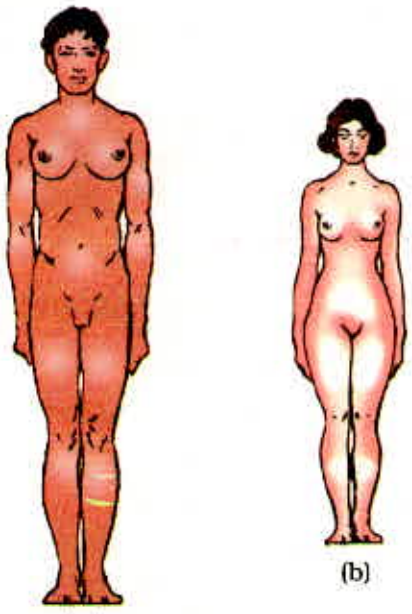
this phenomenon is known as **polyploidy**. This condition is often seen in plants.

The total number of chromosomes in a normal human cell is 46 (23 pairs). Out of these 22 pairs are autosomes and one pair of chromosomes are sex chromosome. Sometimes, though rarely, either an additional copy of a chromosome may be included in an individual or an individual may lack one of any one pair of chromosomes. These situations are known as trisomy or monosomy of a chromosome, respectively. Such a situation leads to very serious consequences in the individual. Down's syndrome, Turner's syndrome, Klinefelter's syndrome are common examples of chromosomal disorders.

Down's Syndrome : The cause of this genetic disorder is the presence of an additional copy of the chromosome number 21 (trisomy of 21). This disorder was first described by Langdon Down (1866). The affected individual is short statured with small round head, furrowed tongue and partially open mouth (Figure 5.16). Palm is broad with characteristic palm crease. Physical, psychomotor and mental development is retarded.

Klinefelter's Syndrome : This genetic disorder is also caused due to the presence of an additional copy of X-chromosome resulting into a karyotype of 47, XXY. Such an individual has overall masculine development , however, the feminine development (development of breast, i.e., Gynaecomastia) is also expressed (Figure 5.17 a). Such individuals are sterile.

Turner's Syndrome : Such a disorder is caused due to the absence of one of the X chromosomes, i.e., 45 with XO, Such females are sterile as ovaries are rudimentary besides other features including lack of other secondary sexual characters (Figure 5.17 b).



(a) Tall stature with feminised character

(b) Short stature and underdeveloped feminine character

Figure 5.17 Diagrammatic representation of genetic disorders due to sex chromosome composition in humans : (a) Klinefelter Syndrome; (b) Turner's Syndrome

SUMMARY

Genetics is a branch of biology which deals with principles of inheritance and its practices. Progeny resembling the parents in morphological and physiological features has attracted the attention of many biologists. Mendel was the first to study this phenomenon systematically. While studying the pattern of inheritance in pea plants of contrasting characters, Mendel proposed the principles of inheritance, which are today referred to as 'Mendel's Laws of Inheritance'. He proposed that the 'factors' (later named as genes) regulating the characters are found in pairs known as alleles. He observed that the expression of the characters in the offspring follow a definite pattern in different–first generations (F_1), second (F_2) and so on. Some characters are dominant over others. The dominant characters are expressed when factors are in heterozygous condition (Law of Dominance). The recessive characters are only expressed in homozygous conditions. The characters never blend in heterozygous condition. A recessive character that was not expressed in heterozygous conditon may be expressed again when it becomes homozygous. Hence, characters segregate while formation of gametes (Law of Segregation).

Not all characters show true dominance. Some characters show incomplete, and some show co-dominance. When Mendel studied the inheritance of two characters together, it was found that the factors independently assort and combine in all permutations and combinations (Law of Independent Assortment). Different combinations of gametes are theoretically represented in a square tabular form known as 'Punnett Square'. The factors (now known as gene) on chromosomes regulating the characters are called the genotype and the physical expression of the chraracters is called phenotype.

After knowing that the genes are located on the chromosomes, a good correlation was drawn between Mendel's laws : segregation and assortment of chromosomes during meiosis. The Mendel's laws were extended in the form of 'Chromosomal Theory of Inheritance'. Later, it was found that Mendel's law of independent assortment does not hold true for the genes that were located on the same chromosomes. These genes were called as 'linked genes'. Closely located genes assorted together, and distantly located genes, due to recombination, assorted independently. Linkage maps, therefore, corresponded to arrangement of genes on a chromosome.

Many genes were linked to sexes also, and called as sex-linked genes. The two sexes (male and female) were found to have a set of chromosomes which were common, and another set which was different. The chromosomes which were different in two sexes were named as sex chromosomes. The remaining set was named as autosomes. In humans, a normal female has 22 pairs of autosomes

and a pair of sex chromosomes (XX). A male has 22 pairs of autosomes and a pair of sex chromosome as XY. In chicken, sex chromosomes in male are ZZ, and in females are ZW.

Mutation is defined as change in the genetic material. A point mutation is a change of a single base pair in DNA. Sickle-cell anemia is caused due to change of one base in the gene coding for beta-chain of hemoglobin. Inheritable mutations can be studied by generating a pedigree of a family. Some mutations involve changes in whole set of chromosomes (polyploidy) or change in a subset of chromosome number (aneuploidy). This helped in understanding the mutational basis of genetic disorders. Down's syndrome is due to trisomy of chromosome 21, where there is an extra copy of chromosome 21 and consequently the total number of chromosome becomes 47. In Turner's syndrome, one X chromosome is missing and the sex chromosome is as XO, and in Klinefelter's syndrome, the condition is XXY. These can be easily studied by analysis of Karyotypes.

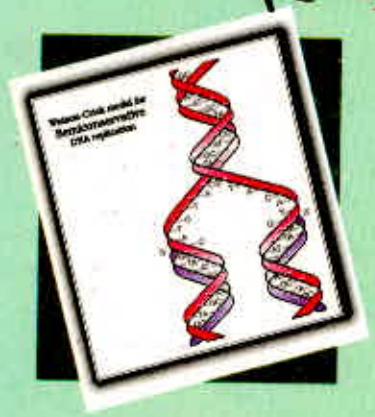
EXERCISES

1. Mention the advantages of selecting pea plant for experiment by Mendel.
2. Differentiate between the following –
   (a) Dominance and Recessive
   (b) Homozygous and Heterozygous
   (c) Monohybrid and Dihybrid.
3. A diploid organism is heterozygous for 4 loci, how many types of gametes can be produced?
4. Explain the Law of Dominance using a monohybrid cross.
5. Define and design a test-cross.
6. Using a Punnett Square, workout the distribution of phenotypic features in the first filial generation after a cross between a homozygous female and a heterozygous male for a single locus.
7. When a cross in made between tall plant with yellow seeds (TtYy) and tall plant with green seed (Ttyy), what proportions of phenotype in the offspring could be expected to be
   (a) tall and green.
   (b) dwarf and green.

8. Two heterozygous parents are crossed. If the two loci are linked what would be the distribution of phenotypic features in F_1 generation for a dibybrid cross?
9. Briefly mention the contribution of T.H. Morgan in genetics.
10. What is pedigree analysis? Suggest how such an analysis, can be useful.
11. How is sex determined in human beings?
12. A child has blood group O. If the father has blood group A and mother blood group B, work out the genotypes of the parents and the possible genotypes of the other offsprings.
13. Explain the following terms with example

    (a) Co-dominance

    (b) Incomplete dominance
14. What is point mutation? Give one example.
15. Who had proposed the chromosomal theory of the inheritance?
16. Mention any two autosomal genetic disorders with their symptoms.



CHAPTER 6

MOLECULAR BASIS OF INHERITANCE

6.1 *The DNA*

6.2 *The Search for Genetic Material*

6.3 *RNA World*

6.4 *Replication*

6.5 *Transcription*

6.6 *Genetic Code*

6.7 *Translation*

6.8 *Regulation of Gene Expression*

6.9 *Human Genome Project*

6.10 *DNA Fingerprinting*

In the previous chapter, you have learnt the inheritance patterns and the genetic basis of such patterns. At the time of Mendel, the nature of those 'factors' regulating the pattern of inheritance was not clear. Over the next hundred years, the nature of the putative genetic material was investigated culminating in the realisation that DNA – deoxyribonucleic acid – is the genetic material, at least for the majority of organisms. In class XI you have learnt that nucleic acids are polymers of nucleotides.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two types of nucleic acids found in living systems. DNA acts as the genetic material in most of the organisms. RNA though it also acts as a genetic material in some viruses, mostly functions as a messenger. RNA has additional roles as well. It functions as adapter, structural, and in some cases as a catalytic molecule. In Class XI you have already learnt the structures of nucleotides and the way these monomer units are linked to form nucleic acid polymers. In this chapter we are going to discuss the structure of DNA, its replication, the process of making RNA from DNA (transcription), the genetic code that determines the sequences of amino acids in proteins, the process of protein synthesis (translation) and elementary basis of their regulation. The determination

of complete nucleotide sequence of human genome during last decade has set in a new era of genomics. In the last section, the essentials of human genome sequencing and its consequences will also be discussed.

Let us begin our discussion by first understanding the structure of the most interesting molecule in the living system, that is, the DNA. In subsequent sections, we will understand that why it is the most abundant genetic material, and what its relationship is with RNA.

6.1 The DNA

DNA is a long polymer of deoxyribonucleotides. The length of DNA is usually defined as number of nucleotides (or a pair of nucleotide referred to as base pairs) present in it. This also is the characteristic of an organism. For example, a bacteriophage known as $\phi \times 174$ has 5386 nucleotides. Bacteriophage lambda has 48502 base pairs (bp), *Escherichia coli* has 4.6×10^6 bp, and haploid content of human DNA is 3.3×10^9 bp. Let us discuss the structure of such a long polymer.

6.1.1 Structure of Polynucleotide Chain

Let us recapitulate the chemical structure of a polynucleotide chain (DNA or RNA). A nucleotide has three components – a nitrogenous base, a pentose sugar (ribose in case of RNA, and deoxyribose for DNA), and a phosphate group. There are two types of nitrogenous bases – Purines (Adenine and Guanine), and Pyrimidines (Cytosine, Uracil and Thymine). Cytosine is common for both DNA and RNA and Thymine is present in DNA. Uracil is present in RNA at the place of Thymine. A nitrogenous base is linked to the pentose sugar through a N-glycosidic linkage to form a nucleoside, such as adenosine or deoxyadenosine, guanosine or deoxyguanosine, cytidine or deoxycytidine and uridine or deoxythymidine. When a phosphate group is linked to 5′-OH of a nucleoside through phosphoester linkage, a corresponding nucleotide (or deoxynucleotide depending upon the type of sugar present) is formed. Two nucleotides are linked through 3′-5′ phosphodiester linkage to form a dinucleotide. More nucleotides can be joined in such a manner to form a polynucleotide chain. A polymer thus formed has at one end a free phosphate moiety at

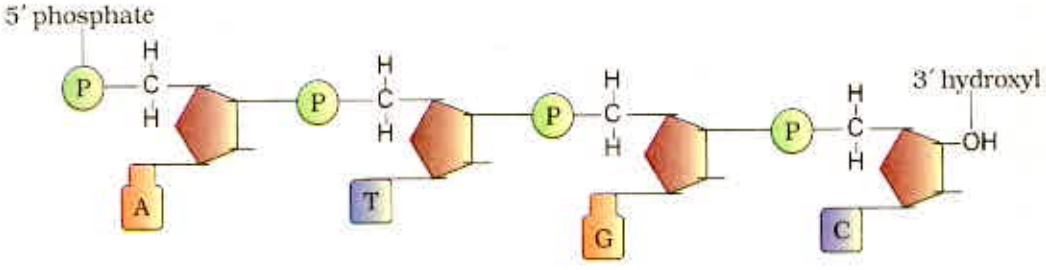


Figure 6.1 A Polynucleotide chain

5′-end of ribose sugar, which is referred to as 5'-end of polynucleotide chain. Similarly, at the other end of the polymer the ribose has a free 3′-OH group which is referred to as 3'-end of the polynucleotide chain. The backbone in a polynucleotide chain is formed due to sugar and phosphates. The nitrogenous bases linked to sugar moiety project from the backbone (Figure 6.1).

In RNA, every nucleotide residue has an additional –OH group present at 2′-position in the ribose. Also, in RNA the uracil is found at the place of thymine (5-methyl uracil, another chemical name for thymine).

DNA as an acidic substance present in nucleus was first identified by Friedrich Meischer in 1869. He named it as 'Nuclein'. However, due to technical limitation in isolating such a long polymer intact, the elucidation of structure of DNA remained elusive for a very long period of time. It was only in 1953 that James Watson and Francis Crick, based on the X-ray diffraction data produced by Maurice Wilkins and Rosalind Franklin, proposed a very simple but famous **Double Helix** model for the structure of DNA. One of the hallmarks of their proposition was base pairing between the two strands of polynucleotide chains. However, this proposition was also based on the observation of Erwin Chargaff that for a double stranded DNA, the ratios between **Adenine** and **Thymine** and **Guanine** and **Cytosine** are constant and equals one.

The base pairing confers a very unique property to the polynucleotide chains. They are said to be complementary to each other, and therefore if the sequence of bases in one strand is known then the sequence in other strand can be predicted. Also, if each strand from a DNA (let us call it as a parental DNA) acts as a template for synthesis of a new strand, the two double stranded DNA (let us call them as daughter DNA) thus, produced would be identical to the parental DNA molecule. Because of this, the genetic implications of the structure of DNA became very clear.

The salient features of the Double-helix structure of DNA are as follows:

(i) It is made of two polynucleotide chains, where the backbone is constituted by sugar-phosphate, and the bases project inside.

(ii) The two chains have anti-parallel polarity. It means, if one chain has the polarity 5′→3′, the other has 3′→5′.

(iii) The bases in two strands are paired through hydrogen bond (H-bonds) forming base pairs (bp). Adenine forms two hydrogen bonds with Thymine from opposite strand and vice-versa. Similarly, Guanine is bonded with Cytosine with three H-bonds. As a result, always a purine comes opposite to a pyrimidine. This generates approximately uniform distance between the two strands of the helix (Figure 6.2).

(iv) The two chains are coiled in a right-handed fashion. The pitch of the helix is 3.4 nm (a nanometre is one billionth of a metre, that is 10^{-9} m) and there are roughly 10 bp in each

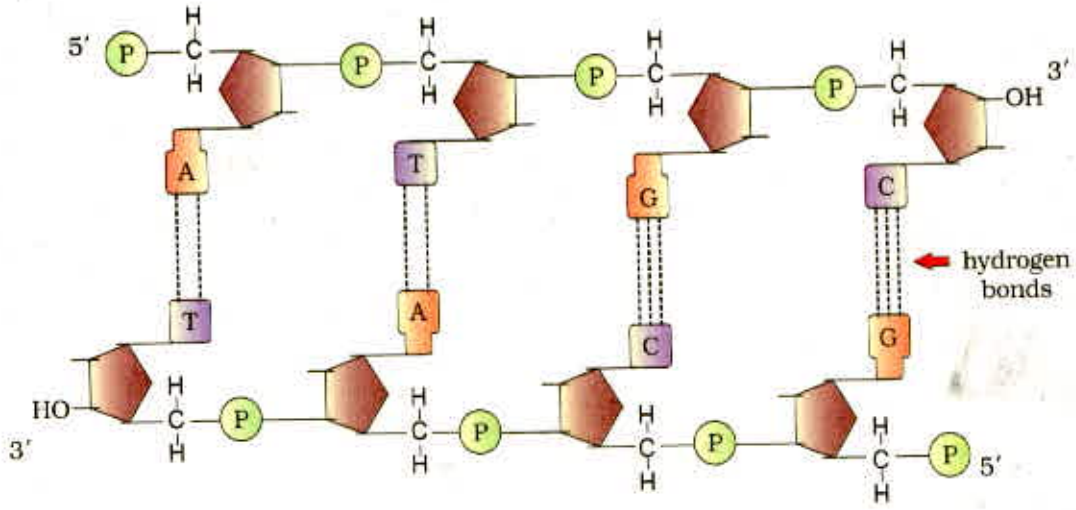


Figure 6.2 Double stranded polynucleotide chain

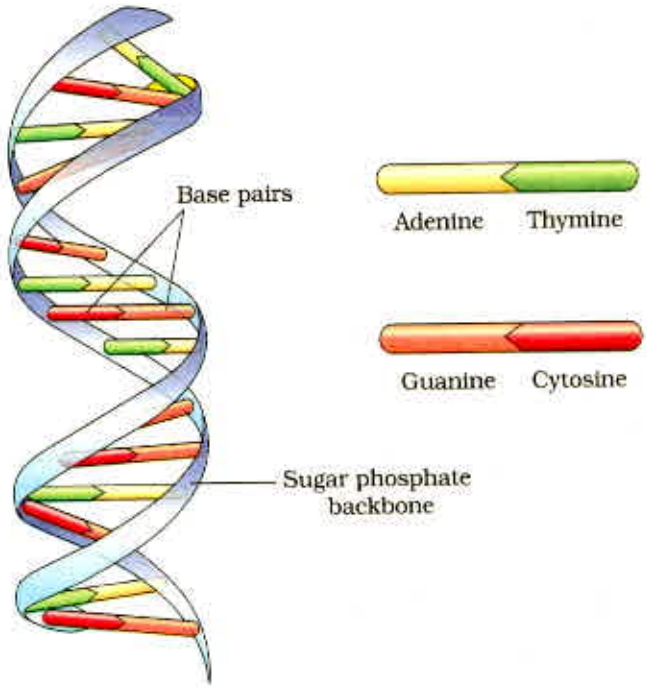


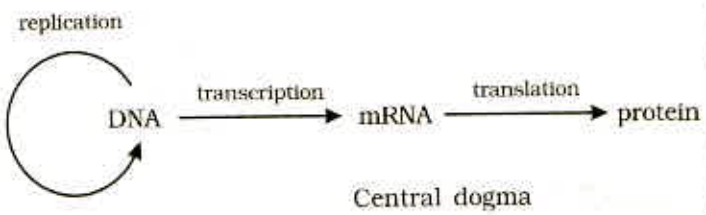
Figure 6.3 DNA double helix

turn. Consequently, the distance between a bp in a helix is approximately equal to 0.34 nm.

(v) The plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure (Figure 6.3).

Compare the structure of purines and pyrimidines. Can you find out why the distance between two polynucleotide chains in DNA remains almost constant?

The proposition of a double helix structure for DNA and its simplicity in explaining the genetic implication became revolutionary. Very soon, Francis Crick proposed the Central dogma in molecular biology, which states that the genetic information flows from DNA→RNA→Protein.



Central dogma

In some viruses the flow of information is in reverse direction, that is, from RNA to DNA. *Can you suggest a simple name to the process?*

6.1.2 Packaging of DNA Helix

Taken the distance between two consecutive base pairs as 0.34 nm (0.34×10^{-9} m), if the length of DNA double helix in a typical mammalian cell is calculated (simply by multiplying the total number of bp with distance between two consecutive bp, that is, 6.6×10^{9} bp $\times$ 0.34×10^{-9}m/bp), it comes out to be approximately 2.2 metres. A length that is far greater than the dimension of a typical nucleus (approximately 10^{-6} m). How is such a long polymer packaged in a cell?

If the length of E. coli DNA is 1.36 mm, can you calculate the number of base pairs in E.coli?

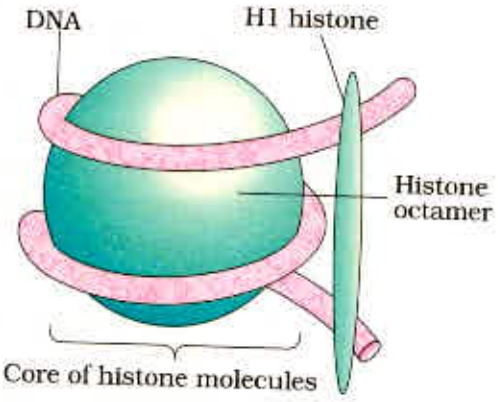


Figure 6.4a Nucleosome

In prokaryotes, such as, *E. coli*, though they do not have a defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region termed as 'nucleoid'. The DNA in nucleoid is organised in large loops held by proteins.

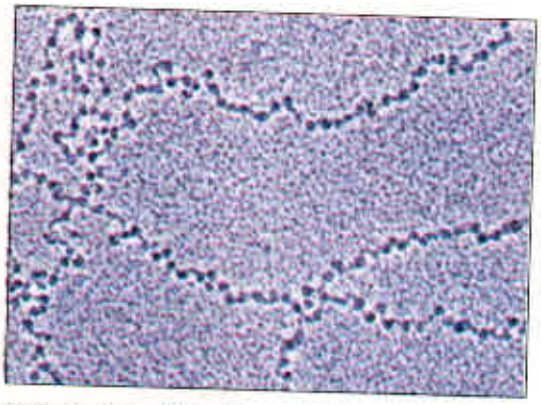


Figure 6.4b EM picture - 'Beads-on-String'

In eukaryotes, this organisation is much more complex. There is a set of positively charged, basic proteins called **histones**. A protein acquires charge depending upon the abundance of amino acids residues with charged side chains. Histones are rich in the basic amino acid residues lysines and arginines. Both the amino acid residues carry positive charges in their side chains. Histones are organised to form a unit of eight molecules called as **histone octamer**. The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called **nucleosome** (Figure 6.4 a). A typical nucleosome contains 200 bp of DNA helix. Nucleosomes constitute the repeating unit of a structure in nucleus called **chromatin**, thread-like stained (coloured) bodies seen in nucleus. The nucleosomes in chromatin are seen as 'beads-on-string' structure when viewed under electron microscope (EM) (Figure 6.4 b).

Theoretically, how many such beads (nucleosomes) do you imagine are present in a mammalian cell?

The beads-on-string structure in chromatin is packaged to form chromatin fibers that are further coiled and condensed at metaphase stage of cell division to form chromosomes. The packaging of chromatin at higher level requires additional set of proteins that collectively are referred to as

Non-histone Chromosomal (NHC) proteins. In a typical nucleus, some region of chromatin are loosely packed (and stains light) and are referred to as **euchromatin**. The chromatin that is more densely packed and stains dark are called as **Heterochromatin**. Euchromatin is said to be transcriptionally active chromatin, whereas heterochromatin is inactive.

6.2 The Search for Genetic Material

Even though the discovery of nuclein by Meischer and the proposition for principles of inheritance by Mendel were almost at the same time, but that the DNA acts as a genetic material took long to be discovered and proven. By 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level. Previous discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan and numerous other scientists had narrowed the search to the chromosomes located in the nucleus of most cells. But the question of what molecule was actually the genetic material, had not been answered.

Transforming Principle

In 1928, Frederick Griffith, in a series of experiments with *Streptococcus pneumoniae* (bacterium responsible for pneumonia), witnessed a miraculous transformation in the bacteria. During the course of his experiment, a living organism (bacteria) had changed in physical form.

When *Streptococcus pneumoniae* (pneumococcus) bacteria are grown on a culture plate, some produce smooth shiny colonies (S) while others produce rough colonies (R). This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not. Mice infected with the S strain (virulent) die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain ⟶ Inject into mice ⟶ Mice die

R strain ⟶ Inject into mice ⟶ Mice live

Griffith was able to kill bacteria by heating them. He observed that heat-killed S strain bacteria injected into mice did not kill them. When he

S strain (heat-killed) ⟶ Inject into mice ⟶ Mice live

S strain (heat-killed) + R strain (live) ⟶ Inject into mice ⟶ Mice die

injected a mixture of heat-killed S and live R bacteria, the mice died. Moreover, he recovered living S bacteria from the dead mice.

He concluded that the R strain bacteria had somehow been **transformed** by the heat-killed S strain bacteria. Some 'transforming principle', transferred from the heat-killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent. This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material was not defined from his experiments.

Biochemical Characterisation of Transforming Principle

Prior to the work of Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44), the genetic material was thought to be a protein. They worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.

They purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material, but not all biologists were convinced.

Can you think of any difference between DNAs and DNase?

6.2.1 The Genetic Material is DNA

The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952). They worked with viruses that infect bacteria called bacteriophages.

The bacteriophage attaches to the bacteria and its genetic material then enters the bacterial cell. The bacterial cell treats the viral genetic material as if it was its own and subsequently manufactures more virus particles. Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.

They grew some viruses on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulfur. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA but not radioactive protein because DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulfur contained radioactive protein but not radioactive DNA because DNA does not contain sulfur.

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Radioactive phages were allowed to attach to *E. coli* bacteria. Then, as the infection proceeded, the viral coats were removed from the bacteria by agitating them in a blender. The virus particles were separated from the bacteria by spinning them in a centrifuge.

Bacteria which was infected with viruses that had radioactive DNA were radioactive, indicating that DNA was the material that passed from the virus to the bacteria. Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did not enter the bacteria from the viruses. DNA is therefore the genetic material that is passed from virus to bacteria (Figure 6.5).

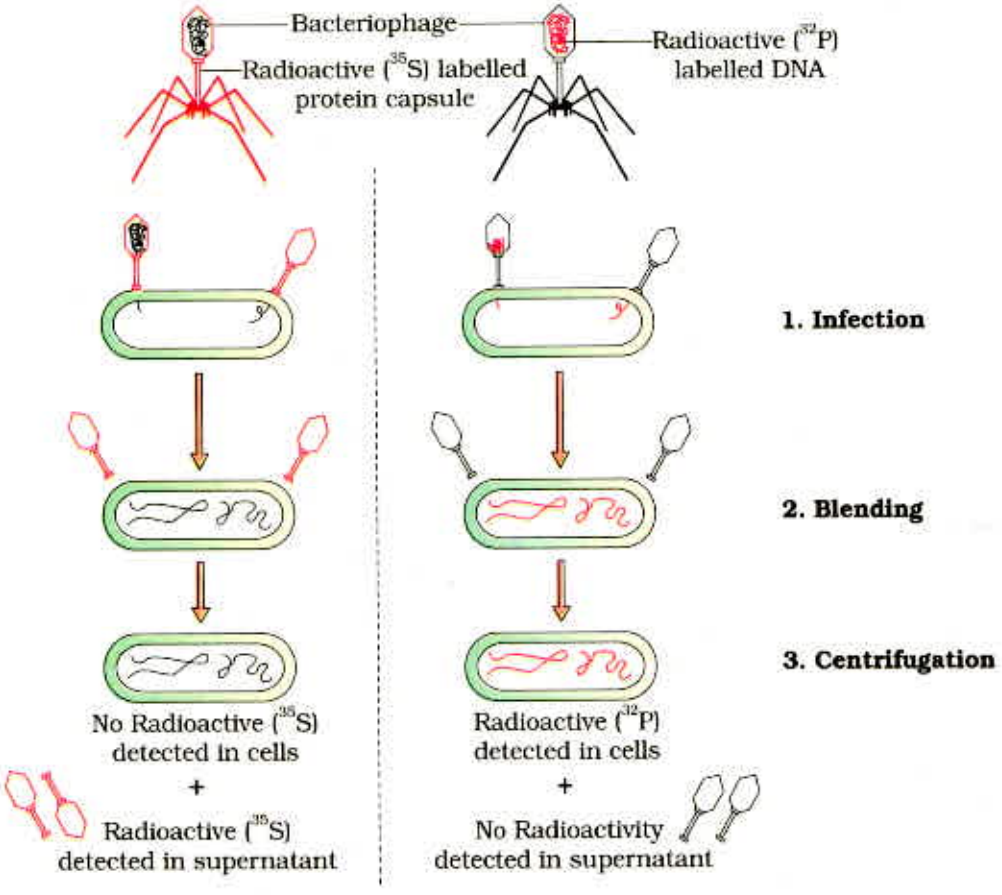

Figure 6.5 The Hershey-Chase experiment

6.2.2 Properties of Genetic Material (DNA versus RNA)

From the foregoing discussion, it is clear that the debate between proteins versus DNA as the genetic material was unequivocally resolved from Hershey-Chase experiment. It became an established fact that it is DNA that acts as genetic material. However, it subsequently became clear that

in some viruses, RNA is the genetic material (for example, Tobacco Mosaic viruses, QB bacteriophage, etc.). Answer to some of the questions such as, why DNA is the predominant genetic material, whereas RNA performs dynamic functions of messenger and adapter has to be found from the differences between chemical structures of the two nucleic acid molecules.

Can you recall the two chemical differences between DNA and RNA?

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

(i) It should be able to generate its replica (Replication).

(ii) It should chemically and structurally be stable.

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

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

If one examines each requirement one by one, because of rule of base pairing and complementarity, both the nucleic acids (DNA and RNA) have the ability to direct their duplications. The other molecules in the living system, such as proteins fail to fulfill first criteria itself.

The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of the properties of genetic material was very evident in Griffith's 'transforming principle' itself that heat, which killed the bacteria, at least did not destroy some of the properties of genetic material. This now can easily be explained in light of the DNA that the two strands being complementary if separated by heating come together, when appropriate conditions are provided. Further, 2'-OH group present at every nucleotide in RNA is a reactive group and makes RNA labile and easily degradable. RNA is also now known to be catalytic, hence reactive. Therefore, DNA chemically is less reactive and structurally more stable when compared to RNA. Therefore, among the two nucleic acids, the DNA is a better genetic material.

In fact, the presence of thymine at the place of uracil also confers additional stability to DNA. (Detailed discussion about this requires understanding of the process of repair in DNA, and you will study these processes in higher classes.)

Both DNA and RNA are able to mutate. In fact, RNA being unstable, mutate at a faster rate. Consequently, viruses having RNA genome and having shorter life span mutate and evolve faster.

RNA can directly code for the synthesis of proteins, hence can easily express the characters. DNA, however, is dependent on RNA for synthesis of proteins. The protein synthesising machinery has evolved around RNA. The above discussion indicate that both RNA and DNA can function as

genetic material, but DNA being more stable is preferred for storage of genetic information. For the transmission of genetic information, RNA is better.

6.3 RNA World

From foregoing discussion, an immediate question becomes evident – which is the first genetic material? It shall be discussed in detail in the chapter on chemical evolution, but briefly, we shall highlight some of the facts and points.

RNA was the first genetic material. There is now enough evidence to suggest that essential life processes (such as metabolism, translation, splicing, etc.), evolved around RNA. RNA used to act as a genetic material as well as a catalyst (there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes). But, RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable. DNA being double stranded and having complementary strand further resists changes by evolving a process of repair.

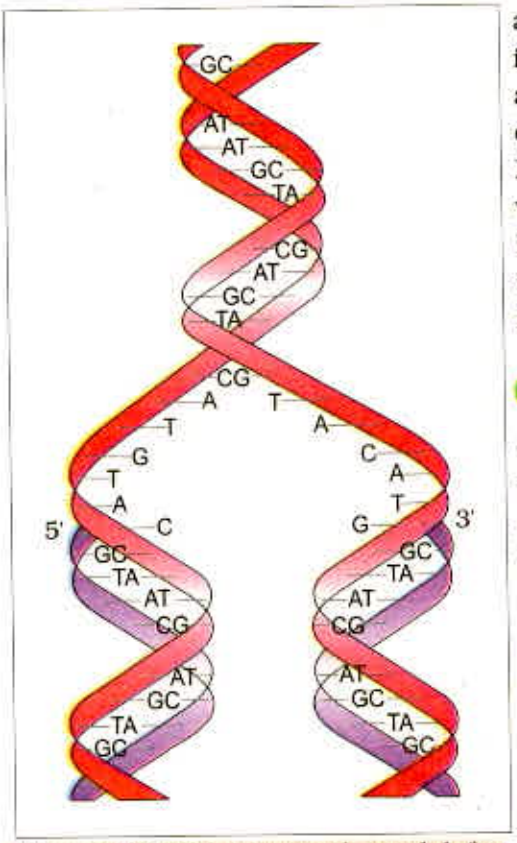

Figure 6.6 Watson-Crick model for semiconservative DNA replication

6.4 Replication

While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).

The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This scheme was termed as **semiconservative** DNA replication (Figure 6.6).

6.4.1 The Experimental Proof

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

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

(i) They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Please note that ^{15}N is not a radioactive isotope, and it can be separated from ^{14}N only based on densities).

(ii) Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA (Figure 6.7).

Can you recall what centrifugal force is, and think why a molecule with higher mass/density would sediment faster?

The results are shown in Figure 6.7.

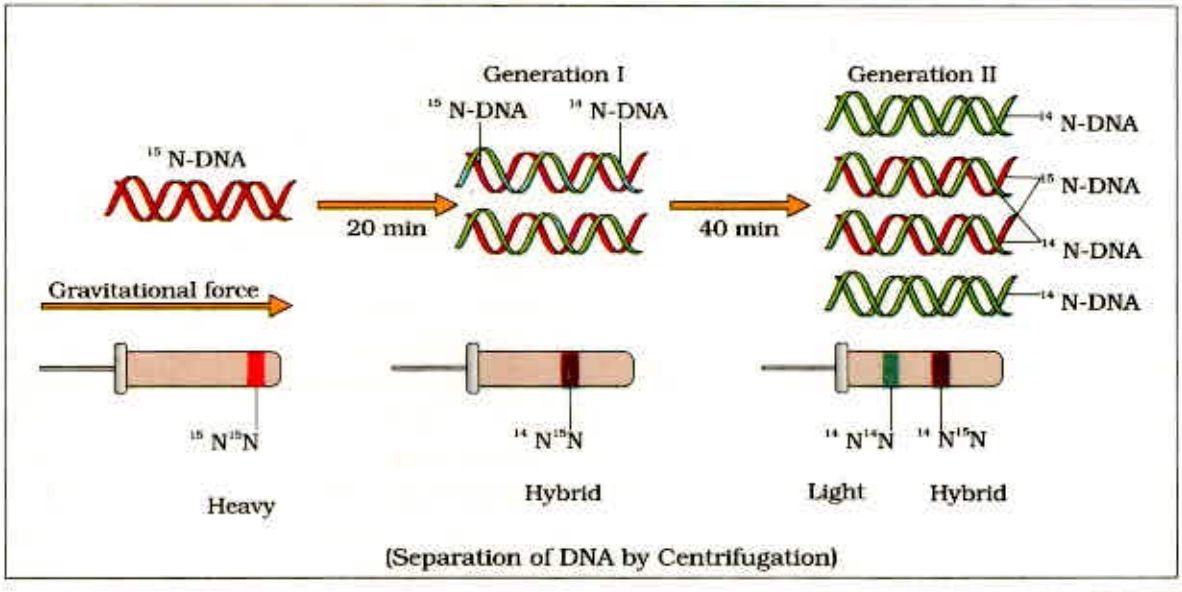

Figure 6.7 Meselson and Stahl's Experiment

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; *E. coli* divides in 20 minutes] had a hybrid or intermediate density. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was

composed of equal amounts of this hybrid DNA and of 'light' DNA.

If E. coli was allowed to grow for 80 minutes then what would be the proportions of light and hybrid densities DNA molecule?

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

6.4.2 The Machinery and the Enzymes

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

In addition to DNA-dependent DNA polymerases, many additional enzymes are required to complete the process of replication with high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated in its entire length (due to very high energy requirement), the replication occur within a small opening of the DNA helix, referred to as **replication fork**. The DNA-dependent DNA polymerases catalyse polymerisation only in one direction, that is 5′→3′. This creates some additional complications at the replicating fork. Consequently, on one strand (the template with polarity 3′→5′), the replication is **continuous**, while on the other (the template with polarity 5′→3′), it is **discontinuous**. The discontinuously synthesised fragments are later joined by the enzyme **DNA ligase** (Figure 6.8).

The DNA polymerases on their own cannot initiate the process of replication. Also the replication does not initiate randomly at any place in DNA. There is a definite region in *E. coli* DNA where the replication originates. Such regions are termed as **origin of replication**. It is

because of the requirement of the origin of replication that a piece of DNA if needed to be propagated during recombinant DNA procedures, requires a vector. The vectors provide the origin of replication.

Further, not every detail of replication is understood well. In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results into polyploidy(a chromosomal anomaly). You will learn the detailed nature of origin and the processes occurring at this site, in higher classes.

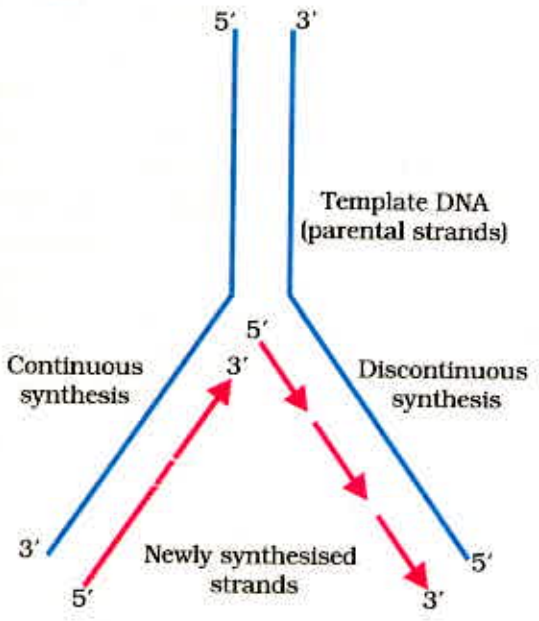

Figure 6.8 Replicating Fork

6.5 Transcription

The process of copying genetic information from one strand of the DNA into RNA is termed as **transcription**. Here also, the principle of complementarity governs the process of transcription, except the adenosine now forms base pair with uracil instead of thymine. However, unlike in the process of replication, which once set in, the total DNA of an organism gets duplicated, in transcription only a segment of DNA and only one of the strands is copied into RNA. This necessitates defining the boundaries that would demarcate the region and the strand of DNA that would be transcribed.

Why both the strands are not copied during transcription has the simple answer. First, if both strands act as a template, they would code for RNA molecule with different sequences (Remember complementarity does not mean identical), and in turn, if they code for proteins, the sequence of amino acids in the proteins would be different. Hence, one segment of the DNA would be coding for two different proteins, and this would complicate the genetic information transfer machinery. Second, the two RNA molecules if produced simultaneously would be complementary to each other, hence would form a double stranded RNA. This would prevent RNA from being translated into protein and the exercise of transcription would become a futile one.

6.5.1 Transcription Unit

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

(i) A Promoter
(ii) The Structural gene
(iii) A Terminator

There is a convention in defining the two strands of the DNA in the structural gene of a transcription unit. Since the two strands have opposite polarity and the **DNA-dependent RNA polymerase** also catalyse the polymerisation in only one direction, that is, 5′→3′, the strand that has the polarity 3′→5′ acts as a template, and is also referred to as **template strand**. The other strand which has the polarity (5′→3′) and the sequence same as RNA (except thymine at the place of uracil), is displaced during transcription. Strangely, this strand (which does not code for anything) is referred to as **coding strand**. All the reference point while defining a transcription unit is made with coding strand. To explain the point, a hypothetical sequence from a transcription unit is represented below:

3′-ATGCATGCATGCATGCATGCATGC-5′ Template Strand

5′-TACGTACGTACGTACGTACGTACG-3′ Coding Strand

Can you now write the sequence of RNA transcribed from the above DNA?

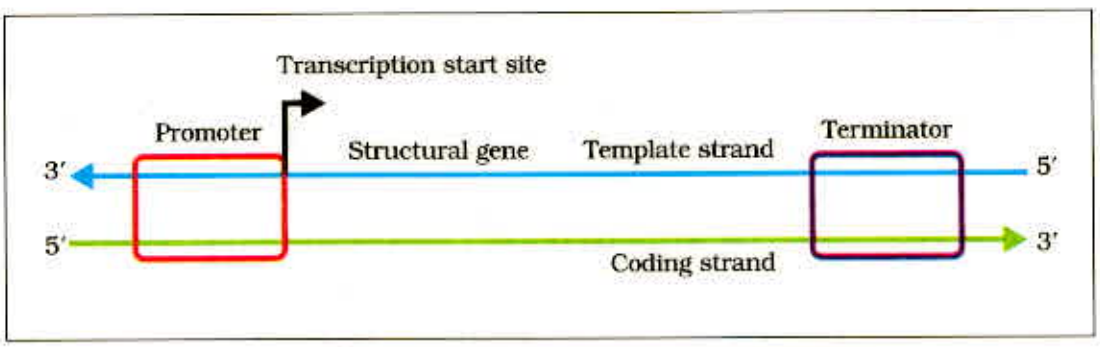

Figure 6.9 Schematic structure of a transcription unit

The **promoter** and **terminator** flank the **structural gene** in a transcription unit. The promoter is said to be located towards 5′-end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides binding site for RNA polymerase, and it is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with terminator, the definition of coding and template strands could be reversed. The terminator is located towards 3′-end (downstream) of the coding strand and it usually defines the end of the process of transcription (Figure 6.9). There are additional regulatory sequences that may be present further upstream or downstream to the promoter. Some of the properties of these sequences shall be discussed while dealing with regulation of gene expression.

6.5.2 Transcription Unit and the Gene

A gene is defined as the functional unit of inheritance. Though there is no ambiguity that the genes are located on the DNA, it is difficult to literally

define a gene in terms of DNA sequence. The DNA sequence coding for tRNA or rRNA molecule also define a gene. However by defining a **cistron** as a segment of DNA coding for a polypeptide, the structural gene in a transcription unit could be said as **monocistronic** (mostly in eukaryotes) or **polycistronic** (mostly in bacteria or prokaryotes). In eukaryotes, the monocistronic structural genes have interrupted coding sequences – the genes in eukaryotes are split. The coding sequences or expressed sequences are defined as **exons**. Exons are said to be those sequence that appear in mature or processed RNA. The exons are interrupted by **introns**. Introns or intervening sequences do not appear in mature or processed RNA. The split-gene arrangement further complicates the definition of a gene in terms of a DNA segment.

Inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as regulatory genes, even though these sequences do not code for any RNA or protein.

6.5.3 Types of RNA and the process of Transcription

In bacteria, there are three major types of RNAs: mRNA (messenger RNA), tRNA (transfer RNA), and rRNA (ribosomal RNA). All three RNAs are needed to synthesise a protein in a cell. The mRNA provides the template, tRNA brings aminoacids and reads the genetic code, and rRNAs play structural and catalytic role during translation. There is single DNA-dependent RNA polymerase that catalyses transcription of all types of RNA in bacteria. RNA polymerase binds to promoter and initiates transcription (**Initiation**). It uses nucleoside triphosphates as substrate

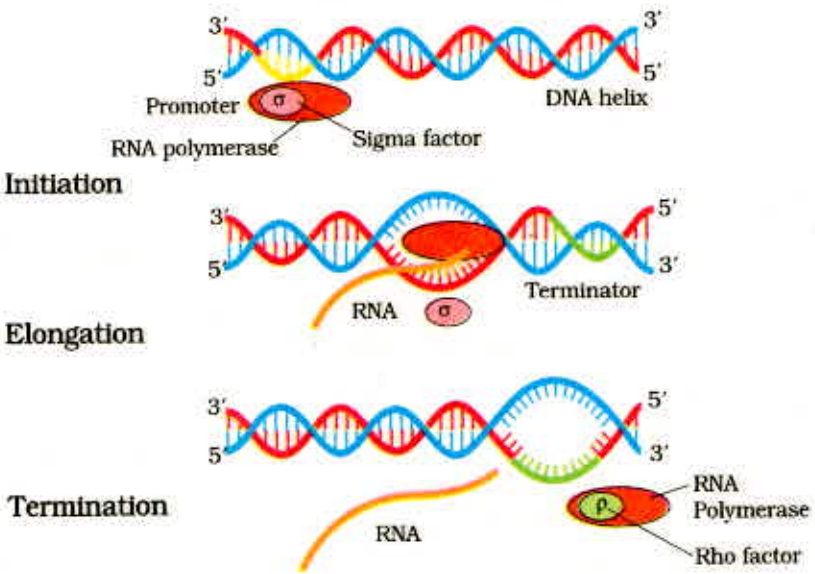

Figure 6.10 Process of Transcription in Bacteria

and polymerises in a template depended fashion following the rule of complementarity. It somehow also facilitates opening of the helix and continues elongation. Only a short stretch of RNA remains bound to the enzyme. Once the polymerases reaches the terminator region, the nascent RNA falls off, so also the RNA polymerase. This results in **termination** of transcription.

An intriguing question is that how is the RNA polymerases able to catalyse all the three steps, which are initiation, elongation and termination. The RNA polymerase is only capable of catalysing the process of elongation. It associates transiently with **initiation-factor** (σ) and **termination-factor** (ρ) to initiate and terminate the transcription, respectively. Association with these factors alter the specificity of the RNA polymerase to either initiate or terminate (Figure 6.10).

In bacteria, since the mRNA does not require any processing to become active, and also since transcription and translation take place in the same compartment (there is no separation of cytosol and nucleus in bacteria), many times the translation can begin much before the mRNA is fully transcribed. Consequently, the transcription and translation can be coupled in bacteria.

In eukaryotes, there are two additional complexities –

(i) There are at least three RNA polymerases in the nucleus (in addition to the RNA polymerase found in the organelles). There is a clear cut division of labour. The RNA polymerase I transcribes **rRNAs**

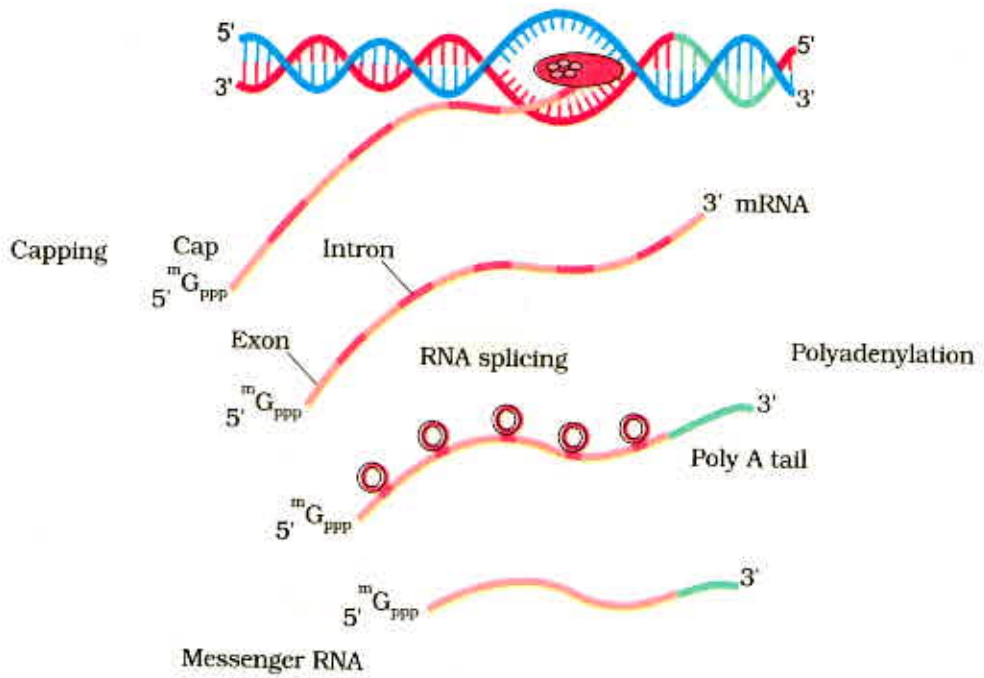

Figure 6.11 Process of Transcription in Eukaryotes

(28S, 18S, and 5.8S), whereas the RNA polymerase III is responsible for transcription of **tRNA**, **5srRNA**, and **snRNAs (small nuclear RNAs)**. The RNA polymerase II transcribes precursor of mRNA, the **heterogeneous nuclear RNA (hnRNA)**.

(ii) The second complexity is that the primary transcripts contain both the exons and the introns and are non-functional. Hence, it is subjected to a process called **splicing** where the introns are removed and exons are joined in a defined order. hnRNA undergoes additional processing called as capping and tailing. In **capping** an unusual nucleotide (methyl guanosine triphosphate) is added to the 5′-end of hnRNA. In **tailing**, adenylate residues (200-300) are added at 3′-end in a template independent manner. It is the fully processed hnRNA, now called mRNA, that is transported out of the nucleus for translation (Figure 6.11).

The significance of such complexities is now beginning to be understood. The split-gene arrangements represent probably an ancient feature of the genome. The presence of introns is reminiscent of antiquity, and the process of splicing represents the dominance of **RNA-world**. In recent times, the understanding of RNA and RNA-dependent processes in the living system have assumed more importance.

6.6 Genetic Code

During replication and transcription a nucleic acid was copied to form another nucleic acid. Hence, these processes are easy to conceptualise on the basis of complementarity. The process of translation requires transfer of genetic information from a polymer of nucleotides to a polymer of amino acids. Neither does any complementarity exist between nucleotides and amino acids, nor could any be drawn theoretically. There existed ample evidences, though, to support the notion that change in nucleic acids (genetic material) were responsible for change in amino acids in proteins. This led to the proposition of a genetic code that could direct the sequence of amino acids during synthesis of proteins.

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

Providing proof that the codon was a triplet, was a more daunting task. The chemical method developed by Har Gobind Khorana was

instrumental in synthesising RNA molecules with defined combinations of bases (homopolymers and copolymers). Marshall Nirenberg's cell-free system for protein synthesis finally helped the code to be deciphered. Severo Ochoa enzyme (polynucleotide phosphorylase) was also helpful in polymerising RNA with defined sequences in a template independent manner (enzymatic synthesis of RNA). Finally a checker-board for genetic code was prepared which is given in Table 6.1.

Table 6.1: The Codons for the Various Amino Acids

First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

The salient features of genetic code are as follows:

(i) The codon is triplet. 61 codons code for amino acids and 3 codons do not code for any amino acids, hence they function as stop codons.

(ii) One codon codes for only one amino acid, hence, it is **unambiguous** and **specific**.

(iii) Some amino acids are coded by more than one codon, hence the code is **degenerate**.

(iv) The codon is read in mRNA in a contiguous fashion. There are no punctuations.

(v) The code is nearly **universal**: for example, from bacteria to human UUU would code for Phenylalanine (phe). Some exceptions to this rule have been found in mitochondrial codons, and in some protozoans.

(vi) AUG has dual functions. It codes for Methionine (met) , and it also act as **initiator** codon.

If following is the sequence of nucleotides in mRNA, predict the sequence of amino acid coded by it (take help of the checkerboard):

-AUG UUU UUC UUC UUU UUU UUC-

Now try the opposite. Following is the sequence of amino acids coded by an mRNA. Predict the nucleotide sequence in the RNA:

Met-Phe-Phe-Phe-Phe-Phe-Phe

Do you face any difficulty in predicting the opposite?

Can you now correlate which two properties of genetic code you have learnt?

6.6.1 Mutations and Genetic Code

The relationships between genes and DNA are best understood by mutation studies. You have studied about mutation and its effect in Chapter 5. Effects of large deletions and rearrangements in a segment of DNA are easy to comprehend. It may result in loss or gain of a gene and so a function. The effect of point mutations will be explained here. A classical example of point mutation is a change of single base pair in the gene for beta globin chain that results in the change of amino acid residue glutamate to valine. It results into a diseased condition called as **sickle cell anemia**. Effect of point mutations that inserts or deletes a base in structural gene can be better understood by following simple example.

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

RAM HAS RED CAP

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

RAM HAS BRE DCA P

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

RAM HAS BIR EDC AP

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

RAM HAS BIG RED CAP

The same exercise can be repeated, by deleting the letters R, E and D, one by one and rearranging the statement to make a triplet word.

RAM HAS EDC AP

RAM HAS DCA P

RAM HAS CAP

The conclusion from the above exercise is very obvious. Insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion. However, such mutations are referred to as

frameshift insertion or **deletion mutations**. Insertion or deletion of three or its multiple bases insert or delete one or multiple codon hence one or multiple amino acids, and reading frame remains unaltered from that point onwards.

6.6.2 tRNA– the Adapter Molecule

From the very beginning of the proposition of code, it was clear to Francis Crick that there has to be a mechanism to read the code and also to link it to the amino acids, because amino acids have no structural specialities to read the code uniquely. He postulated the presence of an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acids. The tRNA, then called sRNA (soluble RNA), was known before the genetic code was postulated. However, its role as an adapter molecule was assigned much later.

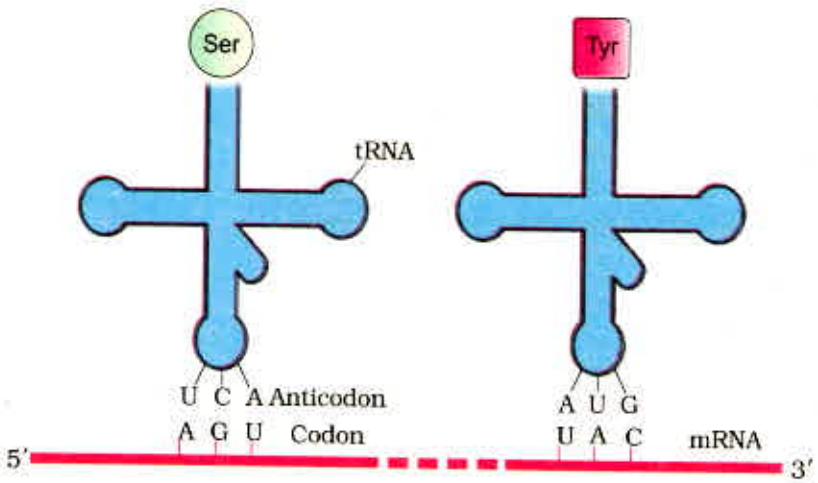

Figure 6.12 tRNA - the adapter molecule

tRNA has an **anticodon loop** that has bases complementary to the code, and it also has an **amino acid acceptor end** to which it binds to amino acids. tRNAs are specific for each amino acid (Figure 6.12). For initiation, there is another specific tRNA that is referred to as **initiator tRNA**. There are no tRNAs for stop codons. In figure 6.12, the secondary structure of tRNA has been depicted that looks like a clover-leaf. In actual structure, the tRNA is a compact molecule which looks like inverted L.

6.7 Translation

Translation refers to the process of polymerisation of amino acids to form a polypeptide (Figure 6.13). The order and sequence of amino acids are defined by the sequence of bases in the mRNA. The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy. Therefore, in the first phase itself amino acids are activated in the presence of ATP and linked to their cognate tRNA–a process commonly called as **charging of tRNA** or **aminoacylation of tRNA** to be more specific. If two such charged tRNAs are brought close enough, the formation of peptide bond between them

would be favoured energetically. The presence of a catalyst would enhance the rate of peptide bond formation.

The cellular factory responsible for synthesising proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In its inactive state, it exists as two subunits; a large subunit and a small subunit. When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. There are two sites in the large subunit, for subsequent amino acids to bind to and thus, be close enough to each other for the formation of a peptide bond. The ribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) for the formation of peptide bond.

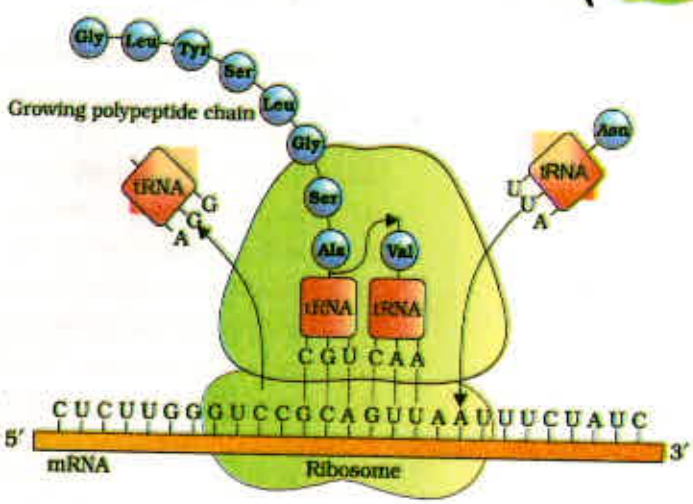

Figure 6.13 Translation

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

For initiation, the ribosome binds to the mRNA at the start codon (AUG) that is recognised only by the initiator tRNA. The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes composed of an amino acid linked to tRNA, sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into Polypeptide sequences dictated by DNA and represented by mRNA. At the end, a **release factor** binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

6.8 Regulation of Gene Expression

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

(i) transcriptional level (formation of primary transcript),
(ii) processing level (regulation of splicing),
(iii) transport of mRNA from nucleus to the cytoplasm,
(iv) translational level.

The genes in a cell are expressed to perform a particular function or a set of functions. For example, if an enzyme called beta-galactosidase is synthesised by *E. coli*, it is used to catalyse the hydrolysis of a disaccharide, lactose into galactose and glucose; the bacteria use them as a source of energy. Hence, if the bacteria do not have lactose around them to be utilised for energy source, they would no longer require the synthesis of the enzyme beta-galactosidase. Therefore, in simple terms, it is the metabolic, physiological or environmental conditions that regulate the expression of genes. The development and differentiation of embryo into adult organisms are also a result of the coordinated regulation of expression of several sets of genes.

In prokaryotes, control of the rate of transcriptional initiation is the predominant site for control of gene expression. In a transcription unit, the activity of RNA polymerase at a given promoter is in turn regulated by interaction with accessory proteins, which affect its ability to recognise start sites. These regulatory proteins can act both positively (activators) and negatively (repressors). The accessibility of promoter regions of prokaryotic DNA is in many cases regulated by the interaction of proteins with sequences termed **operators**. The operator region is adjacent to the promoter elements in most operons and in most cases the sequences of the operator bind a repressor protein. Each operon has its specific operator and specific repressor. For example, *lac* operator is present only in the *lac* operon and it interacts specifically with *lac* repressor only.

6.8.1 The *Lac* operon

The elucidation of the *lac* operon was also a result of a close association between a geneticist, Francois Jacob and a biochemist, Jacque Monod. They were the first to elucidate a transcriptionally regulated system. In *lac* operon (here *lac* refers to lactose), a polycistronic structural gene is regulated by a common promoter and regulatory genes. Such arrangement is very common in bacteria and is referred to as **operon**. To name few such examples, *lac* operon, *trp* operon, *ara* operon, *his* operon, *val* operon, etc.

The *lac* operon consists of one regulatory gene (the *i* gene – here the term *i* does not refer to inducer, rather it is derived from the word inhibitor) and three structural genes (*z*, *y*, and *a*). The *i* gene codes for the repressor of the *lac* operon. The *z* gene codes for beta-galactosidase (β-gal), which is primarily responsible for the hydrolysis of the disaccharide, lactose into its monomeric units, galactose and glucose. The *y* gene codes for permease, which increases permeability of the cell to β-galactosides. The *a* gene encodes a transacetylase. Hence, all the three gene products in *lac* operon are required for metabolism of lactose. In most other operons as well, the genes present in the operon are needed together to function in the same or related metabolic pathway (Figure 6.14).

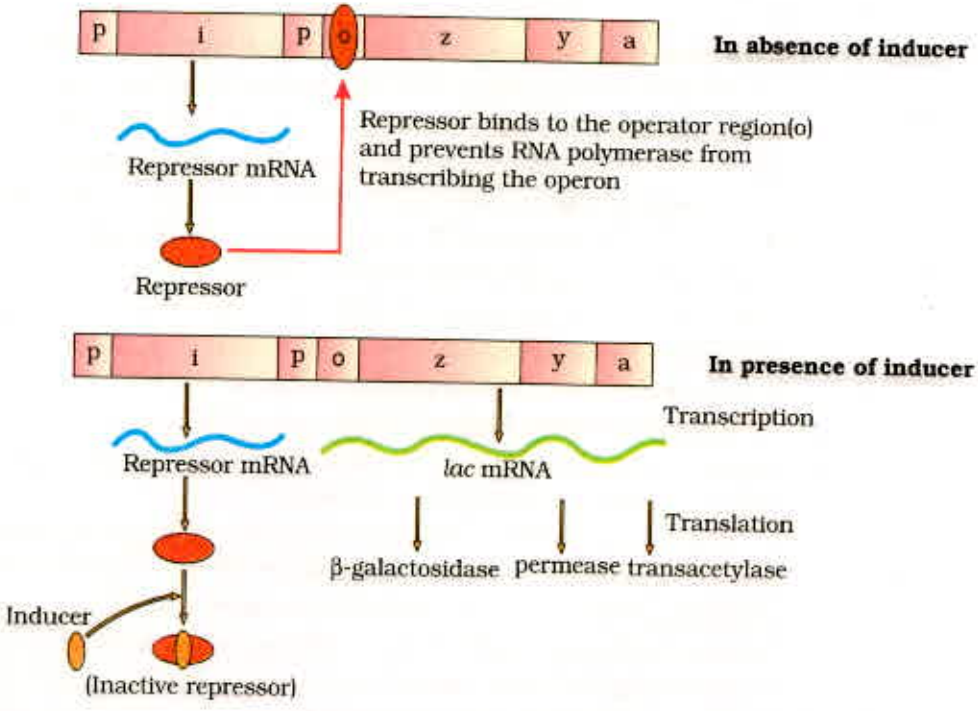

Figure 6.14 The *lac* Operon

Lactose is the substrate for the enzyme beta-galactosidase and it regulates switching on and off of the operon. Hence, it is termed as **inducer**. In the absence of a preferred carbon source such as glucose, if lactose is provided in the growth medium of the bacteria, the lactose is transported into the cells through the action of permease (Remember, a very low level of expression of *lac* operon has to be present in the cell all the time, otherwise lactose cannot enter the cells). The lactose then induces the operon in the following manner.

The repressor of the operon is synthesised (all-the-time – constitutively) from the *i* gene. The repressor protein binds to the operator region of the operon and prevents RNA polymerase from transcribing the operon. In the presence of an inducer, such as lactose or allolactose, the repressor is inactivated by interaction with the inducer. This allows RNA polymerase access to the promoter and transcription proceeds (Figure 6.14). Essentially, regulation of *lac* operon can also be visualised as regulation of enzyme synthesis by its substrate.

Remember, glucose or galactose cannot act as inducers for lac operon. Can you think for how long the lac operon would be expressed in the presence of lactose?

Regulation of *lac* operon by repressor is referred to as **negative regulation**. *Lac* operon is under control of positive regulation as well, but it is beyond the scope of discussion at this level.

6.9 Human Genome Project

In the preceding sections you have learnt that it is the sequence of bases in DNA that determines the genetic information of a given organism. In other words, genetic make-up of an organism or an individual lies in the DNA sequences. If two individuals differ, then their DNA sequences should also be different, at least at some places. These assumptions led to the quest of finding out the complete DNA sequence of human genome. With the establishment of genetic engineering techniques where it was possible to isolate and clone any piece of DNA and availability of simple and fast techniques for determining DNA sequences, a very ambitious project of sequencing human genome was launched in the year 1990.

Human Genome Project (HGP) was called a mega project. You can imagine the magnitude and the requirements for the project if we simply define the aims of the project as follows:

Human genome is said to have approximately 3×10^9 bp, and if the cost of sequencing required is US $ 3 per bp (the estimated cost in the beginning), the total estimated cost of the project would be approximately 9 billion US dollars. Further, if the obtained sequences were to be stored in typed form in books, and if each page of the book contained 1000 letters and each book contained 1000 pages, then 3300 such books would be required to store the information of DNA sequence from a single human cell. The enormous amount of data expected to be generated also necessitated the use of high speed computational devices for data storage and retrieval, and analysis. HGP was closely associated with the rapid development of a new area in biology called **Bioinformatics.**

Goals of HGP

Some of the important goals of HGP were as follows:

(i) Identify all the approximately 20,000-25,000 genes in human DNA;

(ii) Determine the sequences of the 3 billion chemical base pairs that make up human DNA;

(iiii) Store this information in databases;

(iv) Improve tools for data analysis;

(v) Transfer related technologies to other sectors, such as industries;

(vi) Address the ethical, legal, and social issues (ELSI) that may arise from the project.

The Human Genome Project was a 13-year project coordinated by the U.S. Department of Energy and the National Institute of Health. During the early years of the HGP, the Wellcome Trust (U.K.) became a major partner; additional contributions came from Japan, France, Germany, China and others. The project was completed in 2003. Knowledge about the effects of DNA variations among individuals can lead to revolutionary new ways to diagnose, treat and someday prevent the thousands of

disorders that affect human beings. Besides providing clues to understanding human biology, learning about non-human organisms DNA sequences can lead to an understanding of their natural capabilities that can be applied toward solving challenges in health care, agriculture, energy production, environmental remediation. Many non-human model organisms, such as bacteria, yeast, *Caenorhabditis elegans* (a free living non-pathogenic nematode), *Drosophila* (the fruit fly), plants (rice and *Arabidopsis*), etc., have also been sequenced.

Methodologies : The methods involved two major approaches. One approach focused on identifying all the genes that are expressed as RNA (referred to as **Expressed Sequence Tags** (ESTs). The other took the blind approach of simply sequencing the whole set of genome that contained all the coding and non-coding sequence, and later assigning different regions in the sequence with functions (a term referred to as **Sequence Annotation**). For sequencing, the total DNA from a cell is isolated and converted into random fragments of relatively smaller sizes (recall DNA is a very long polymer, and there are technical limitations in sequencing very long pieces of DNA) and cloned in suitable host using specialised vectors. The cloning resulted into amplification of each piece of DNA fragment so that it subsequently could be sequenced with ease. The commonly used hosts were bacteria and yeast, and the vectors were called as **BAC** (bacterial artificial chromosomes), and **YAC** (yeast artificial chromosomes).

The fragments were sequenced using automated DNA sequencers that worked on the principle of a method developed by Frederick Sanger. (Remember, Sanger is also credited for developing method for determination of amino acid sequences in proteins). These sequences were then arranged based on some overlapping regions present in them. This required generation of overlapping fragments for sequencing. Alignment of these sequences was humanly not possible. Therefore, specialised computer based programs were developed (Figure 6.15). These sequences were subsequently annotated and were assigned to each chromosome. The sequence of chromosome 1 was completed only in May 2006 (this was the last of the 24 human chromosomes – 22 autosomes and X and Y – to be

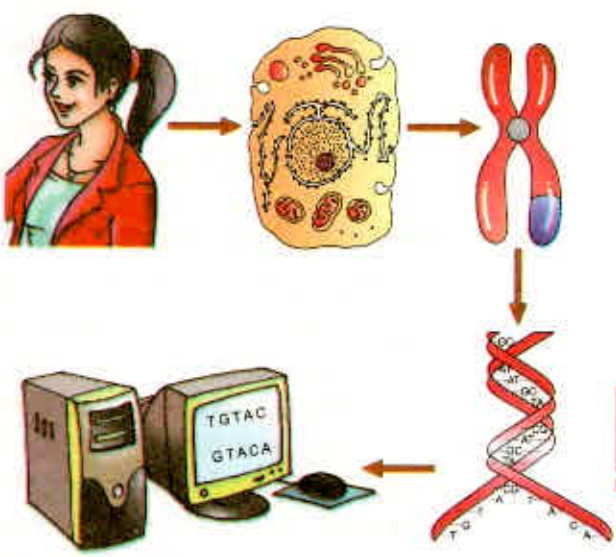

Figure 6.15 A representative diagram of human genome project

sequenced). Another challenging task was assigning the genetic and physical maps on the genome. This was generated using information on polymorphism of restriction endonuclease recognition sites, and some repetitive DNA sequences known as microsatellites (one of the applications of polymorphism in repetitive DNA sequences shall be explained in next section of DNA fingerprinting).

6.9.1 Salient Features of Human Genome

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

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

(ii) The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.

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

(iv) The functions are unknown for over 50 per cent of the discovered genes.

(v) Less than 2 per cent of the genome codes for proteins.

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

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

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

(ix) Scientists have identified about 1.4 million locations where single-base DNA differences (**SNPs – single nucleotide polymorphism**, pronounced as 'snips') occur in humans. This information promises to revolutionise the processes of finding chromosomal locations for disease-associated sequences and tracing human history.

6.9.2 Applications and Future Challenges

Deriving meaningful knowledge from the DNA sequences will define research through the coming decades leading to our understanding of biological systems. This enormous task will require the expertise and creativity of tens of thousands of scientists from varied disciplines in both the public and private sectors worldwide. One of the greatest impacts of having the HG sequence may well be enabling a radically new approach to biological research. In the past, researchers studied one or a few genes at a time. With whole-genome sequences and new high-throughput technologies, we can approach questions systematically and on a much

broader scale. They can study all the genes in a genome, for example, all the transcripts in a particular tissue or organ or tumor, or how tens of thousands of genes and proteins work together in interconnected networks to orchestrate the chemistry of life.

6.10 DNA Fingerprinting

As stated in the preceding section, 99.9 per cent of base sequence among humans is the same. *Assuming human genome as 3×10^9 bp, in how many base sequences would there be differences?* It is these differences in sequence of DNA which make every individual unique in their phenotypic appearance. If one aims to find out genetic differences between two individuals or among individuals of a population, sequencing the DNA every time would be a daunting and expensive task. Imagine trying to compare two sets of 3×10^6 base pairs. DNA fingerprinting is a very quick way to compare the DNA sequences of any two individuals.

DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called as **repetitive DNA**, because in these sequences, a small stretch of DNA is repeated many times. These repetitive DNA are separated from bulk genomic DNA as different peaks during density gradient centrifugation. The bulk DNA forms a major peak and the other small peaks are referred to as **satellite DNA**. Depending on base composition (A : T rich or G:C rich), length of segment, and number of repetitive units, the satellite DNA is classified into many categories, such as micro-satellites, mini-satellites etc. These sequences normally do not code for any proteins, but they form a large portion of human genome. These sequence show high degree of polymorphism and form the basis of DNA fingerprinting. Since DNA from every tissue (such as blood, hair-follicle, skin, bone, saliva, sperm etc.), from an individual show the same degree of polymorphism, they become very useful identification tool in forensic applications. Further, as the polymorphisms are inheritable from parents to children, DNA fingerprinting is the basis of paternity testing, in case of disputes.

As polymorphism in DNA sequence is the basis of genetic mapping of human genome as well as of DNA fingerprinting, it is essential that we understand what DNA polymorphism means in simple terms. **Polymorphism** (variation at genetic level) arises due to mutations. (Recall different kind of mutations and their effects that you have already studied in Chapter 5, and in the preceding sections in this chapter.) New mutations may arise in an individual either in somatic cells or in the germ cells (cells that generate gametes in sexually reproducing organisms). If a germ cell mutation does not seriously impair individual's ability to have offspring who can transmit the mutation, it can spread to

the other members of population (through sexual reproduction). Allelic (again recall the definition of alleles from Chapter 5) sequence variation has traditionally been described as a DNA polymorphism if more than one variant (allele) at a locus occurs in human population with a frequency greater than 0.01. In simple terms, if an **inheritable mutation** is observed in a population at high frequency, it is referred to as **DNA polymorphism**. The probability of such variation to be observed in non-coding DNA sequence would be higher as mutations in these sequences may not have any immediate effect/impact in an individual's reproductive ability. These mutations keep on accumulating generation after generation, and form one of the basis of variability/polymorphism. There is a variety of different types of polymorphisms ranging from single nucleotide change to very large scale changes. For evolution and speciation, such polymorphisms play very important role, and you will study these in details at higher classes.

The technique of DNA Fingerprinting was initially developed by Alec Jeffreys. He used a satellite DNA as probe that shows very high degree of polymorphism. It was called as **Variable Number of Tandem Repeats** (VNTR). The technique, as used earlier, involved Southern blot hybridisation using radiolabelled VNTR as a probe. It included

(i) isolation of DNA,

(ii) digestion of DNA by restriction endonucleases,

(iii) separation of DNA fragments by electrophoresis,

(iv) transferring (blotting) of separated DNA fragments to synthetic membranes, such as nitrocellulose or nylon,

(v) hybridisation using labelled VNTR probe, and

(vi) detection of hybridised DNA fragments by autoradiography. A schematic representation of DNA fingerprinting is shown in Figure 6.16.

The VNTR belongs to a class of satellite DNA referred to as mini-satellite. A small DNA sequence is arranged tandemly in many copy numbers. The copy number varies from chromosome to chromosome in an individual. The numbers of repeat show very high degree of polymorphism. As a result the size of VNTR varies in size from 0.1 to 20 kb. Consequently, after hybridisation with VNTR probe, the autoradiogram gives many bands of differing sizes. These bands give a characteristic pattern for an individual DNA (Figure 6.16). It differs from individual to individual in a population except in the case of monozygotic (identical) twins. The sensitivity of the technique has been increased by use of polymerase chain reaction (PCR–you will study about it in Chapter 11). Consequently, DNA from a single cell is enough to perform DNA fingerprinting analysis. In addition to application in forensic science, it has much wider application, such as in determining population and genetic diversities. Currently, many different probes are used to generate DNA fingerprints.

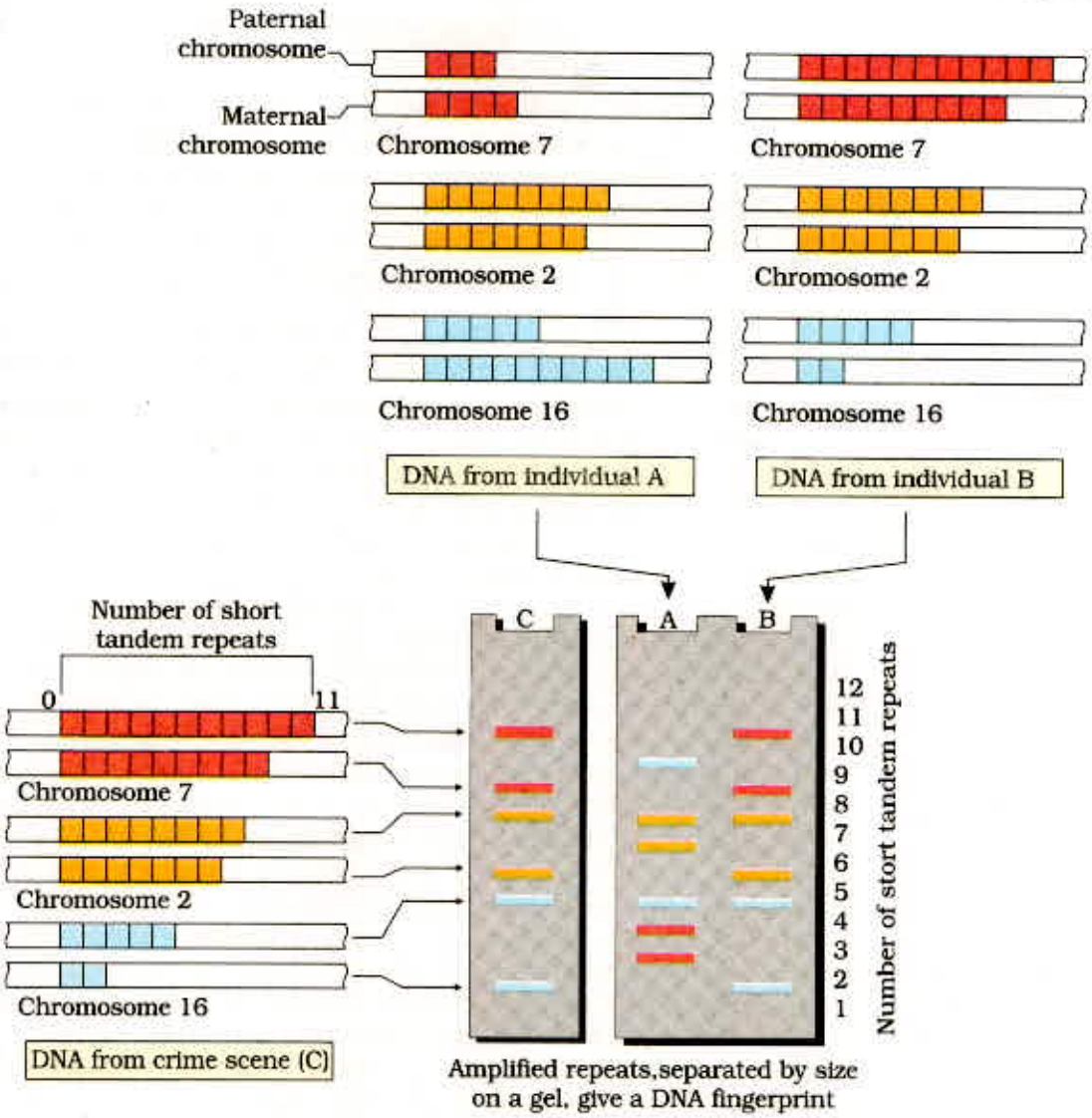

Figure 6.16 Schematic representation of DNA fingerprinting: Few representative chromosomes have been shown to contain different copy number of VNTR. For the sake of understanding different colour schemes have been used to trace the origin of each band in the gel. The two alleles (paternal and maternal) of a chromosome also contain different copy numbers of VNTR. It is clear that the banding pattern of DNA from crime scene matches with individual B, and not with A.

SUMMARY

Nucleic acids are long polymers of nucleotides. While DNA stores genetic information, RNA mostly helps in transfer and expression of information. Though DNA and RNA both function as genetic material, but DNA being chemically and structurally more stable is a better genetic material. However, RNA is the first to evolve and DNA was derived from RNA. The hallmark of the double stranded helical structure of DNA is the hydrogen bonding between the bases from opposite strands. The rule is that Adenine pairs with Thymine through two H-bonds, and Guanine with Cytosine through three H-bonds. This makes one strand complementary to the other. The DNA replicates semiconservatively, the process is guided by the complementary H-bonding. A segment of DNA that codes for RNA may in a simplistic term can be referred as gene. During transcription also, one of the strands of DNA acts a template to direct the synthesis of complementary RNA. In bacteria, the transcribed mRNA is functional, hence can directly be translated. In eukaryotes, the gene is split. The coding sequences, exons, are interrupted by non-coding sequences, introns. Introns are removed and exons are joined to produce functional RNA by splicing. The messenger RNA contains the base sequences that are read in a combination of three (to make triplet genetic code) to code for an amino acid. The genetic code is read again on the principle of complementarity by tRNA that acts as an adapter molecule. There are specific tRNAs for every amino acid. The tRNA binds to specific amino acid at one end and pairs through H-bonding with codes on mRNA through its anticodons. The site of translation (protein synthesis) is ribosomes, which bind to mRNA and provide platform for joining of amino acids. One of the rRNA acts as a catalyst for peptide bond formation, which is an example of RNA enzyme (ribozyme). Translation is a process that has evolved around RNA, indicating that life began around RNA. Since, transcription and translation are energetically very expensive processes, these have to be tightly regulated. Regulation of transcription is the primary step for regulation of gene expression. In bacteria, more than one gene is arranged together and regulated in units called as operons. *Lac* operon is the prototype operon in bacteria, which codes for genes responsible for metabolism of lactose. The operon is regulated by the amount of lactose in the medium where the bacteria are grown. Therefore, this regulation can also be viewed as regulation of enzyme synthesis by its substrate.

Human genome project was a mega project that aimed to sequence every base in human genome. This project has yielded much new information. Many new areas and avenues have opened up as a consequence of the project. DNA Fingerprinting is a technique to find out variations in individuals of a population at DNA level. It works on the principle of polymorphism in DNA sequences. It has immense applications in the field of forensic science, genetic biodiversity and evolutionary biology.

EXERCISES

1. Group the following as nitrogenous bases and nucleosides:
   Adenine, Cytidine, Thymine, Guanosine, Uracil and Cytosine.
2. If a double stranded DNA has 20 per cent of cytosine, calculate the per cent of adenine in the DNA.
3. If the sequence of one strand of DNA is written as follows:

   5′-ATGCATGCATGCATGCATGCATGCATGC-3′

   Write down the sequence of complementary strand in 5′→3′ direction.
4. If the sequence of the coding strand in a transcription unit is written as follows:

   5′-ATGCATGCATGCATGCATGCATGCATGC-3′

   Write down the sequence of mRNA.
5. Which property of DNA double helix led Watson and Crick to hypothesise semi-conservative mode of DNA replication? Explain.
6. Depending upon the chemical nature of the template (DNA or RNA) and the nature of nucleic acids synthesised from it (DNA or RNA), list the types of nucleic acid polymerases.
7. How did Hershey and Chase differentiate between DNA and protein in their experiment while proving that DNA is the genetic material?
8. Differentiate between the followings:
   (a) Repetitive DNA and Satellite DNA
   (b) mRNA and tRNA
   (c) Template strand and Coding strand
9. List two essential roles of ribosome during translation.
10. In the medium where *E. coli* was growing, lactose was added, which induced the *lac* operon. Then, why does lac operon shut down some time after addition of lactose in the medium?
11. Explain (in one or two lines) the function of the followings:
    (a) Promoter
    (b) tRNA
    (c) Exons
12. Why is the Human Genome project called a mega project?
13. What is DNA fingerprinting? Mention its application.
14. Briefly describe the following:
    (a) Transcription
    (b) Polymorphism
    (c) Translation
    (d) Bioinformatics

CHAPTER 7

EVOLUTION

7.1 *Origin of Life*
7.2 *Evolution of Life Forms - A Theory*
7.3 *What are the Evidences for Evolution?*
7.4 *What is Adaptive Radiation?*
7.5 *Biological Evolution*
7.6 *Mechanism of Evolution*
7.7 *Hardy - Weinberg Principle*
7.8 *A Brief Account of Evolution*
7.9 *Origin and Evolution of Man*

Evolutionary Biology is the study of history of life forms on earth. What exactly is evolution? To understand the changes in flora and fauna that have occurred over millions of years on earth, we must have an understanding of the context of origin of life, i.e., evolution of earth, of stars and indeed of the universe itself. What follows is the longest of all the construed and conjectured stories. This is the story of origin of life and evolution of life forms or biodiversity on planet earth in the context of evolution of earth and against the background of evolution of universe itself.

7.1 Origin of Life

When we look at stars on a clear night sky we are, in a way, looking back in time. Stellar distances are measured in light years. What we see today is an object whose emitted light started its journey millions of year back and from trillions of kilometres away and reaching our eyes now. However, when we see objects in our immediate surroundings we see them instantly and hence in the present time. Therefore, when we see stars we apparently are peeping into the past.

The origin of life is considered a unique event in the history of universe. The universe is vast. Relatively speaking

the earth itself is almost only a speck. The universe is very old – almost 20 billion years old. Huge clusters of galaxies comprise the universe. Galaxies contain stars and clouds of gas and dust. Considering the size of universe, earth is indeed a speck. The **Big Bang** theory attempts to explain to us the origin of universe. It talks of a singular huge explosion unimaginable in physical terms. The universe expanded and hence, the temperature came down. Hydrogen and Helium formed sometime later. The gases condensed under gravitation and formed the galaxies of the present day universe. In the solar system of the milky way galaxy, earth was supposed to have been formed about 4.5 billion years back. There was no atmosphere on early earth. Water vapour, methane, carbondioxide and ammonia released from molten mass covered the surface. The UV rays from the sun brokeup water into Hydrogen and Oxygen and the lighter H_2 escaped. Oxygen combined with ammonia and methane to form water, CO_2 and others. The ozone layer was formed. As it cooled, the water vapor fell as rain, to fill all the depressions and form oceans. Life appeared 500 million years after the formation of earth, i.e., almost four billion years back.

Did life come from outerspace? Some scientists believe that it came from outside. Early Greek thinkers thought units of life called **spores** were transferred to different planets including earth. 'Panspermia' is still a favourite idea for some astronomers. For a long time it was also believed that life came out of decaying and rotting matter like straw, mud, etc. This was the theory of spontaneous generation. Louis Pasteur by careful experimentation demonstrated that life comes only from pre-existing life. He showed that in pre-sterilised flasks, life did not come from killed yeast while in another flask open to air, new living organisms arose from 'killed yeast'. Spontaneous generation theory was dismissed once and for all. However, this did not answer how the first life form came on earth.

Oparin of Russia and Haldane of England proposed that the first form of life could have come from pre-existing non-living organic molecules (e.g. RNA, protein, etc.) and that formation of life was preceded by chemical evolution, i.e., formation of diverse organic molecules from inorganic constituents. The conditions on earth were – high temperature, volcanic storms, reducing atmosphere containing CH_4, NH_3, etc. In 1953, S.L. Miller, an American scientist created similar conditions in a laboratory scale (Figure 7.1). He created electric discharge in a closed flask containing CH_4, H_2, NH_3 and water vapour at 800°C. He observed formation of amino acids. In similar experiments others observed, formation of sugars, nitrogen bases, pigment and fats. Analysis of meteorite content also revealed similar compounds indicating that similar processes are occurring elsewhere in space. With this limited evidence, the first part of the conjectured story, i.e., chemical evolution was more or less accepted.

We have no idea about how the first self replicating metabolic capsule of life arose. The first non-cellular forms of life could have originated

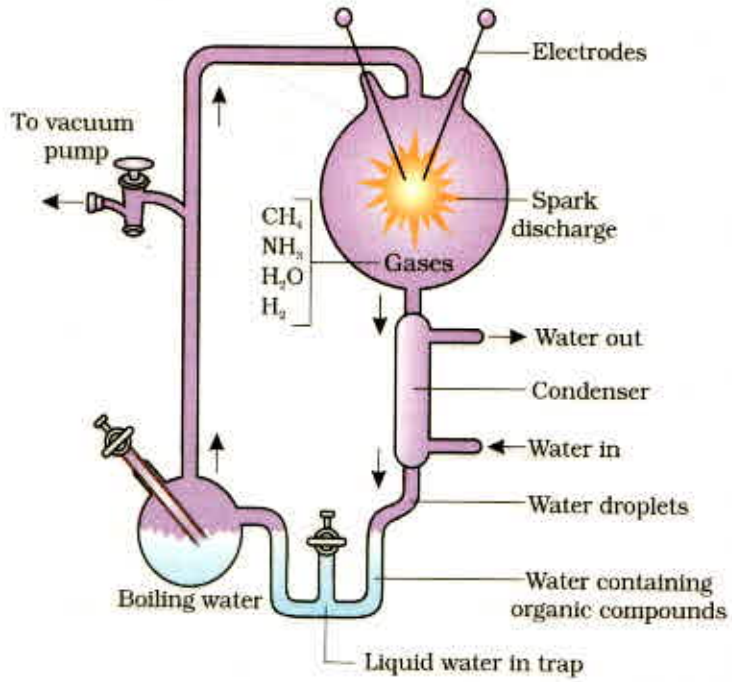

Figure 7.1 Diagrammatic representation of Miller's experiment

3 billion years back. They would have been giant molecules (RNA, Protein, Polysaccharides, etc.). These capsules reproduced their molecules perhaps. The first cellular form of life did not possibly originate till about 2000 million years ago. These were probably single-cells. All life forms were in water environment only. This version of a biogenesis, i.e., the first form of life arose slowly through evolutionary forces from non-living molecules is accepted by majority. However, once formed, how the first cellular forms of life could have evolved into the complex biodiversity of today is the fascinating story that will be discussed below.

7.2 Evolution of Life Forms – A Theory

Conventional religious literature tells us about the theory of special creation. This theory has three connotations. One, that all living organisms (species or types) that we see today were created as such. Two, that the diversity was always the same since creation and will be the same in future also. Three, that earth is about 4000 years old. All these ideas were strongly challenged during the nineteenth century. Based on observations made during a sea voyage in a sail ship called H.M.S. Beagle round the world, Charles Darwin concluded that existing living forms share similarities to varying degrees not only among themselves but also with life forms that existed millions of years ago. Many such life forms do not exist any more. There had been extinctions of different life forms in the

years gone by just as new forms of life arose at different periods of history of earth. There has been gradual evolution of life forms. Any population has built in variation in characteristics. Those characteristics which enable some to survive better in natural conditions (climate, food, physical factors, etc.) would outbreed others that are less-endowed to survive under such natural conditions. Another word used is fitness of the individual or population. The fitness, according to Darwin, refers ultimately and only to reproductive fitness. Hence, those who are better fit in an environment, leave more progeny than others. These, therefore, will survive more and hence are selected by nature. He called it natural selection and implied it as a mechanism of evolution. Let us also remember that Alfred Wallace, a naturalist who worked in Malay Archipelago had also come to similar conclusions around the same time. In due course of time, apparently new types of organisms are recognisable. All the existing life forms share similarities and share common ancestors. However, these ancestors were present at different periods in the history of earth (epochs, periods and eras). The geological history of earth closely correlates with the biological history of earth. A common permissible conclusion is that earth is very old, not thousand of years as was thought earlier but billions of years old.

7.3 What are the Evidences for Evolution?

Evidence that evolution of life forms has indeed taken place on earth has come from many quarters. Fossils are remains of hard parts of life-forms found in rocks. Rocks form sediments and a cross-section of earth's crust indicates the arrangement of sediments one over the other during the long history of earth. Different-aged rock sediments contain fossils of different life-forms who probably died during the formation of the particular sediment. Some of them appear similar to modern organisms (Figure 7.2). They represent extinct organisms (e.g., Dinosaurs). A study of fossils in different sedimentary layers indicates the geological period in which they existed. The study showed that life-forms varied over time and certain life forms are restricted to certain geological time-spans. Hence, new forms of life have arisen at different times in the history of earth. All this is called paleontological evidence. *Do you remember how the ages of the fossils are calculated? Do you recollect the method of radioactive-dating and the principles behind the procedure?*

Comparative anatomy and morphology shows similarities and differences among organisms of today and those that existed years ago. Such similarities can be interpreted to understand whether common ancestors were shared or not. For example whales, bats, Cheetah and human (all mammals) share similarities in the pattern of bones of forelimbs (Figure 7.3b). Though these forelimbs perform different functions in these animals, they have similar anatomical structure – all of them have

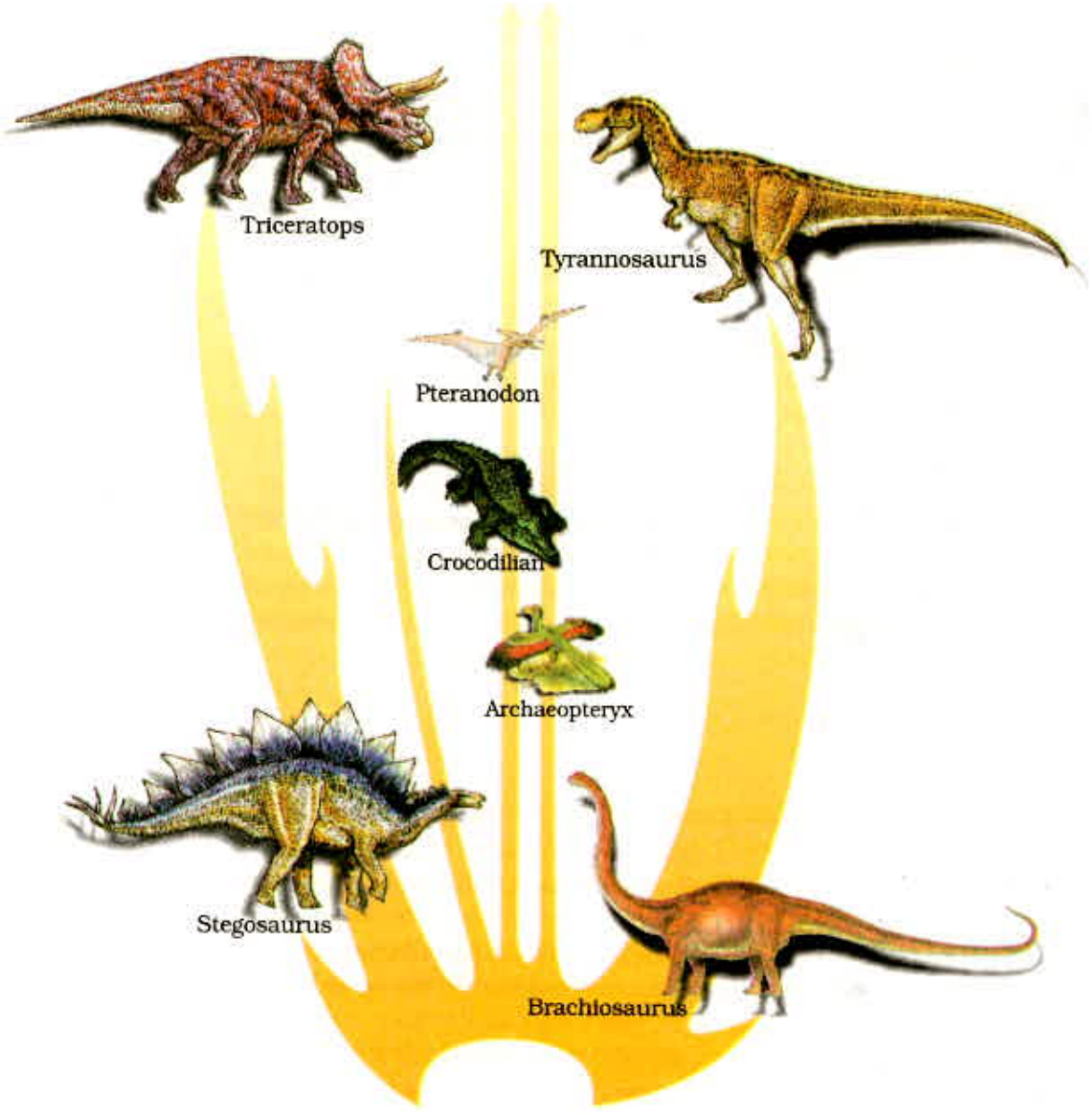

Figure 7.2 A family tree of dinosaurs and their living modern day counterpart organisms like crocodiles and birds

humerus, radius, ulna, carpals, metacarpals and phalanges in their forelimbs. Hence, in these animals, the same structure developed along different directions due to adaptations to different needs. This is **divergent evolution** and these structures are **homologous**. Homology indicates common ancestry. Other examples are vertebrate hearts or brains. In plants also, the thorn and tendrils of *Bougainvillea* and *Cucurbita* represent homology (Figure 7.3a). Homology is based on divergent evolution whereas analogy refers to a situation exactly opposite. Wings of butterfly and of birds look alike. They are not anatomically similar

structures though they perform similar functions. Hence, analogous structures are a result of **convergent evolution** - different structures evolving for the same function and hence having similarity. Other examples of analogy are the eye of the octopus and of mammals or the flippers of Penguins and Dolphins. One can say that it is the similar habitat that has resulted in selection of similar adaptive features in different groups of organisms but toward the same function: Sweet potato (root modification) and potato (stem modification) is another example for analogy.

In the same line of argument, similarities in proteins and genes performing a given function among diverse organisms give clues to common ancestry. These biochemical similarities point to the same shared ancestry as structural similarities among diverse organisms.

Man has bred selected plants and animals for agriculture, horticulture, sport or security. Man has domesticated many wild animals and crops. This intensive breeding programme has created breeds that differ from other breeds (e.g., dogs) but still are of the same group. It is argued that if within hundreds of years, man could create new breeds, could not nature have done the same over millions of years?

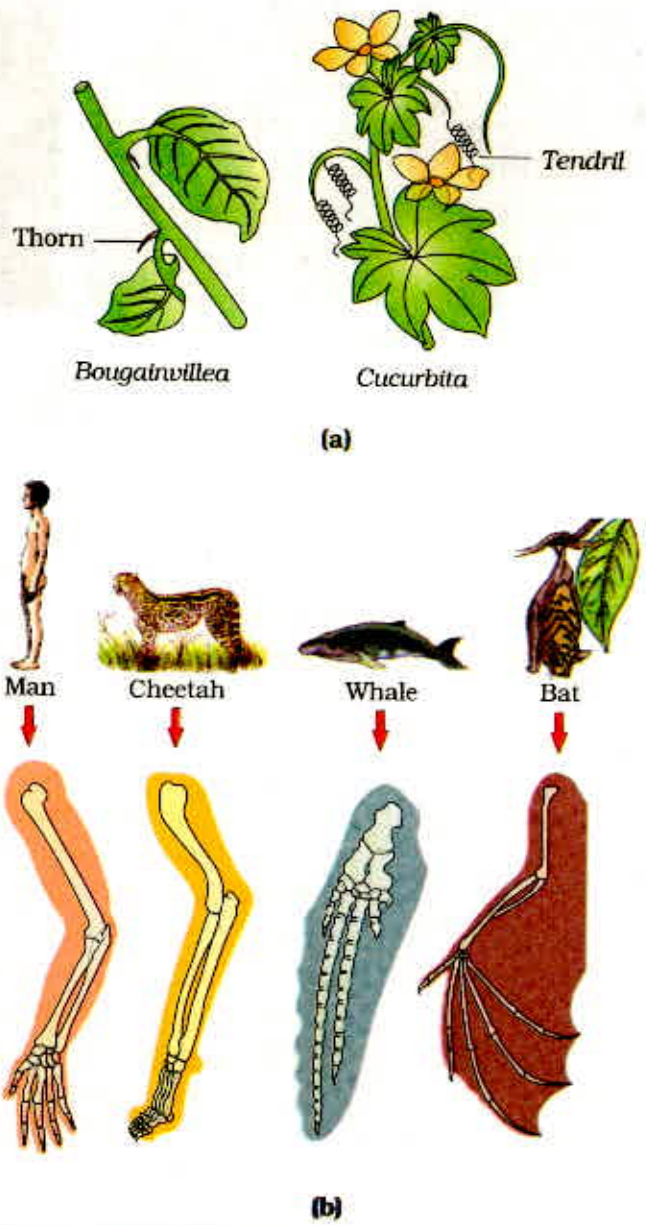

Figure 7.3 Example of homologous organs in (a) Plants and (b) Animals

Another interesting observation supporting evolution by natural selection comes from England. In a collection of moths made in 1850s, i.e., before industrialisation set in, it was observed that there were more white-winged moths on trees than dark-winged or melanised moths. However, in the collection carried out from the same area, but after industrialisation, i.e., in 1920, there were more dark-winged moths in the same area, i.e., the proportion was reversed.

The explanation put forth for this observation was that 'predators will spot a moth against a contrasting background'. During post-industrialisation period, the tree trunks became dark due to industrial smoke and soots. Under this condition the white-winged moth did not

Figure 7.4 Figure showing white - winged moth and dark - winged moth (melanised) on a tree trunk (a) In unpolluted area (b) In polluted area

survive due to predators, dark-winged or melanised moth survived. Before industrialisation set in, thick growth of almost white-coloured lichen covered the trees - in that background the white winged moth survived but the dark-coloured moth were picked out by predators. *Do you know that lichens can be used as industrial pollution indicators?* They will not grow in areas that are polluted. Hence, moths that were able to camouflage themselves, i.e., hide in the background, survived (Figure 7.4). This understanding is supported by the fact that in areas where industrialisation did not occur e.g., in rural areas, the count of melanic moths was low. This showed that in a mixed population, those that can better-adapt, survive and increase in population size. Remember that no variant is completely wiped out.

Similarly, excess use of herbicides, pesticides, etc., has only resulted in selection of resistant varieties in a much lesser time scale. This is also true for microbes against which we employ antibiotics or drugs against eukaryotic organisms/cell. Hence, resistant organisms/cells are appearing in a time scale of months or years and not centuries. These are examples of evolution by anthropogenic action. This also tells us that evolution is not a directed process in the sense of determinism. It is a stochastic process based on chance events in nature and chance mutation in the organisms.

7.4 What is Adaptive Radiation?

During his journey Darwin went to Galapagos Islands. There he observed an amazing diversity of creatures. Of particular interest, small black birds later called Darwin's Finches amazed him. He realised that there were many varieties of finches in the same island. All the varieties, he conjectured, evolved on the island itself. From the original seed-eating features, many other forms with altered beaks arose, enabling them to become insectivorous

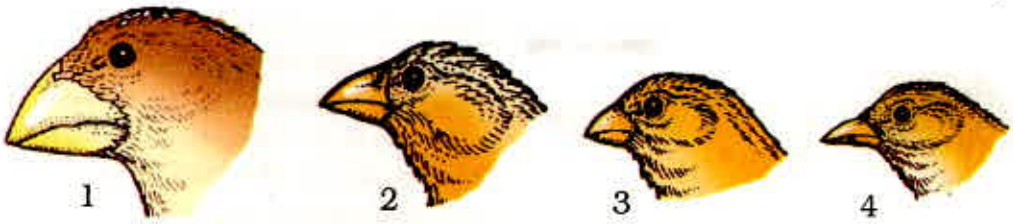

Figure 7.5 Variety of beaks of finches that Darwin found in Galapagos Island

and vegetarian finches (Figure 7.5). This process of evolution of different species in a given geographical area starting from a point and literally radiating to other areas of geography (habitats) is called **adaptive radiation**. Darwin's finches represent one of the best examples of this phenomenon. Another example is Australian marsupials. A number of marsupials, each different from the other (Figure 7.6) evolved from an ancestral stock, but all within the Australian island continent. When more than one adaptive radiation appeared to have occurred in an isolated geographical area (representing different habitats), one can call this convergent evolution.

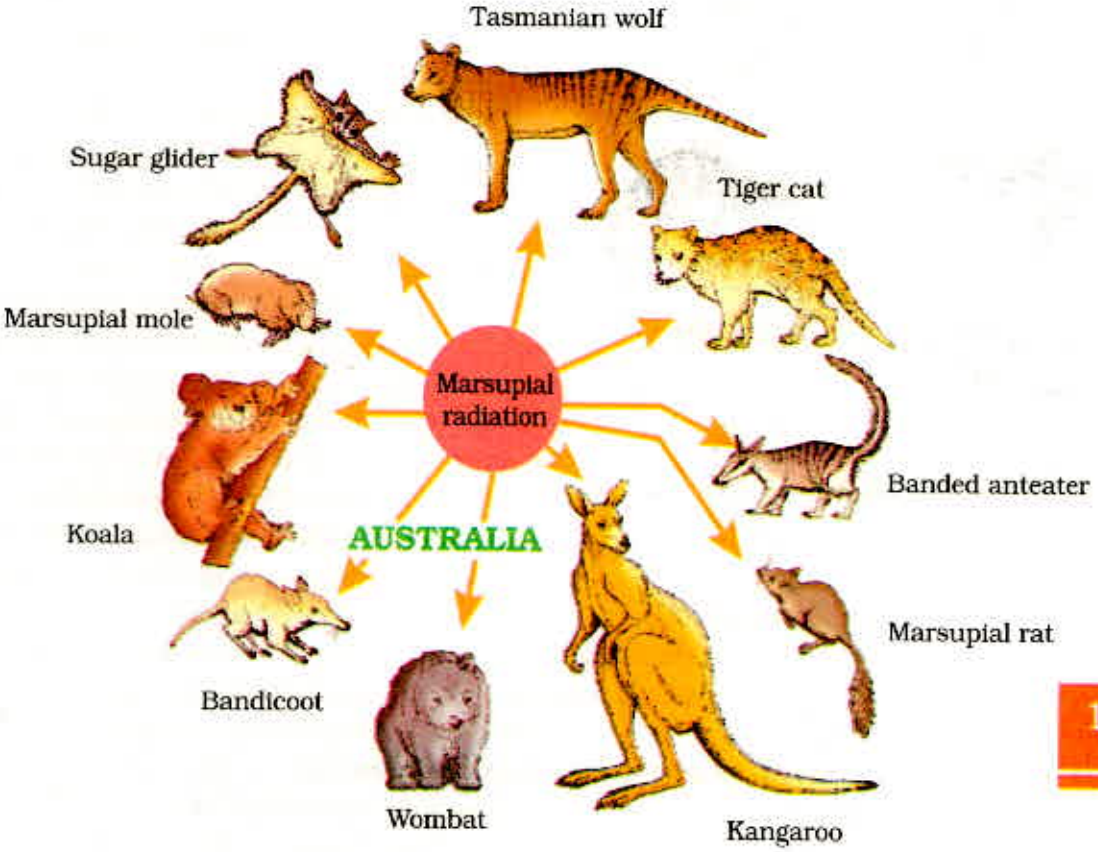

Figure 7.6 Adaptive radiation of marsupials of Australia

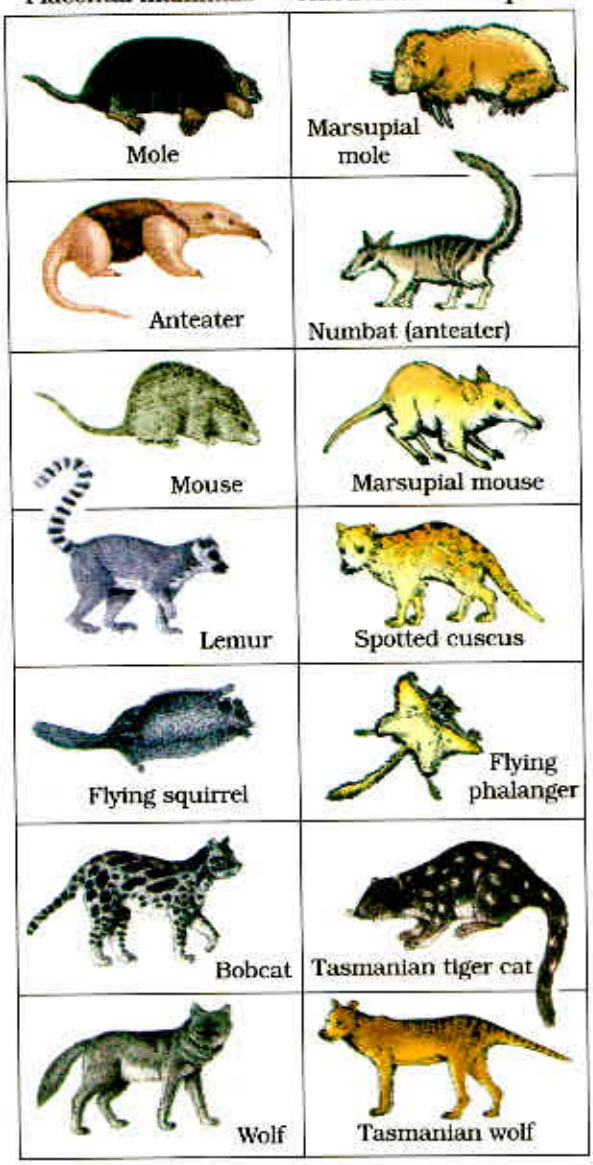

Figure 7.7 Picture showing convergent evolution of Australian Marsupials and placental mammals

Placental mammals in Australia also exhibit adaptive radiation in evolving into varieties of such placental mammals each of which appears to be 'similar' to a corresponding marsupial (e.g., Placental wolf and Tasmanian wolf-marsupial). (Figure 7.7).

7.5 Biological Evolution

Evolution by natural selection, in a true sense would have started when cellular forms of life with differences in metabolic capability originated on earth.

The essence of Darwinian theory about evolution is natural selection. The rate of appearance of new forms is linked to the life cycle or the life span. Microbes that divide fast have the ability to multiply and become millions of individuals within hours. A colony of bacteria (say A) growing on a given medium has built-in variation in terms of ability to utilise a feed component. A change in the medium composition would bring out only that part of the population (say B) that can survive under the new conditions. In due course of time this variant population outgrows the others and appears as new species. This would happen within days. For the same thing to happen in a fish or fowl would take million of years as life spans of these animals are in years. Here we say that fitness of B is better than that of A under the new conditions. Nature selects for fitness. One must remember that the so-called fitness is based on characteristics which are inherited. Hence, there must be a genetic basis for getting selected and to evolve. Another way of saying the same thing is that some organisms are better adapted to survive in an otherwise hostile environment. Adaptive ability is inherited. It has a genetic basis. Fitness is the end result of the ability to adapt and get selected by nature.

Branching descent and **natural selection** are the two key concepts of Darwinian Theory of Evolution (Figures 7.7 and 7.8).

Even before Darwin, a French naturalist Lamarck had said that evolution of life forms had occurred but driven by use and disuse of organs. He gave the examples of Giraffes who in an attempt to forage

leaves on tall trees had to adapt by elongation of their necks. As they passed on this acquired character of elongated neck to succeeding generations, Giraffes, slowly, over the years, came to acquire long necks. Nobody believes this conjecture any more.

Is evolution a process or the result of a process? The world we see, inanimate and animate, is only the success stories of evolution. When we describe the story of this world we describe evolution as a process. On the other hand when we describe the story of life on earth, we treat evolution as a consequence of a process called natural selection. We are still not very clear whether to regard evolution and natural selection as processes or end result of unknown processes.

It is possible that the work of Thomas Malthus on populations influenced Darwin. Natural selection is based on certain observations which are factual. For example, natural resources are limited, populations are stable in size except for seasonal fluctuation, members of a population vary in characteristics (infact no two individuals are alike) even though they look superficially similar, most of variations are inherited etc. The fact that theoretically population size will grow exponentially if everybody reproduced maximally (this fact can be seen in a growing bacterial population) and the fact that population sizes in reality are limited, means that there had been competition for resources. Only some survived and grew at the cost of others that could not flourish. The novelty and brilliant insight of Darwin was this: he asserted that variations, which are heritable and which make resource utilisation better for few (adapted to habitat better) will enable only those to reproduce and leave more progeny. Hence for a period of time, over many generations, survivors will leave more progeny and there would be a change in population characteristic and hence new forms appear to arise.

7.6 Mechanism of Evolution

What is the origin of this variation and how does speciation occur? Even though Mendel had talked of inheritable 'factors' influencing phenotype, Darwin either ignored these observations or kept silence. In the first decade of twentieth century, Hugo deVries based on his work on evening primrose brought forth the idea of mutations – large difference arising suddenly in a population. He believed that it is mutation which causes evolution and not the minor variations (heritable) that Darwin talked about. Mutations are random and directionless while Darwinian variations are small and directional. Evolution for Darwin was gradual while deVries believed mutation caused speciation and hence called it **saltation** (single step large mutation). Studies in population genetics, later, brought out some clarity.

7.7 Hardy-Weinberg Principle

In a given population one can find out the frequency of occurrence of alleles of a gene or a locus. This frequency is supposed to remain fixed and even remain the same through generations. Hardy-Weinberg principle stated it using algebraic equations.

This principle says that allele frequencies in a population are stable and is constant from generation to generation. The gene pool (total genes and their alleles in a population) remains a constant. This is called genetic equilibrium. Sum total of all the allelic frequencies is 1. Individual

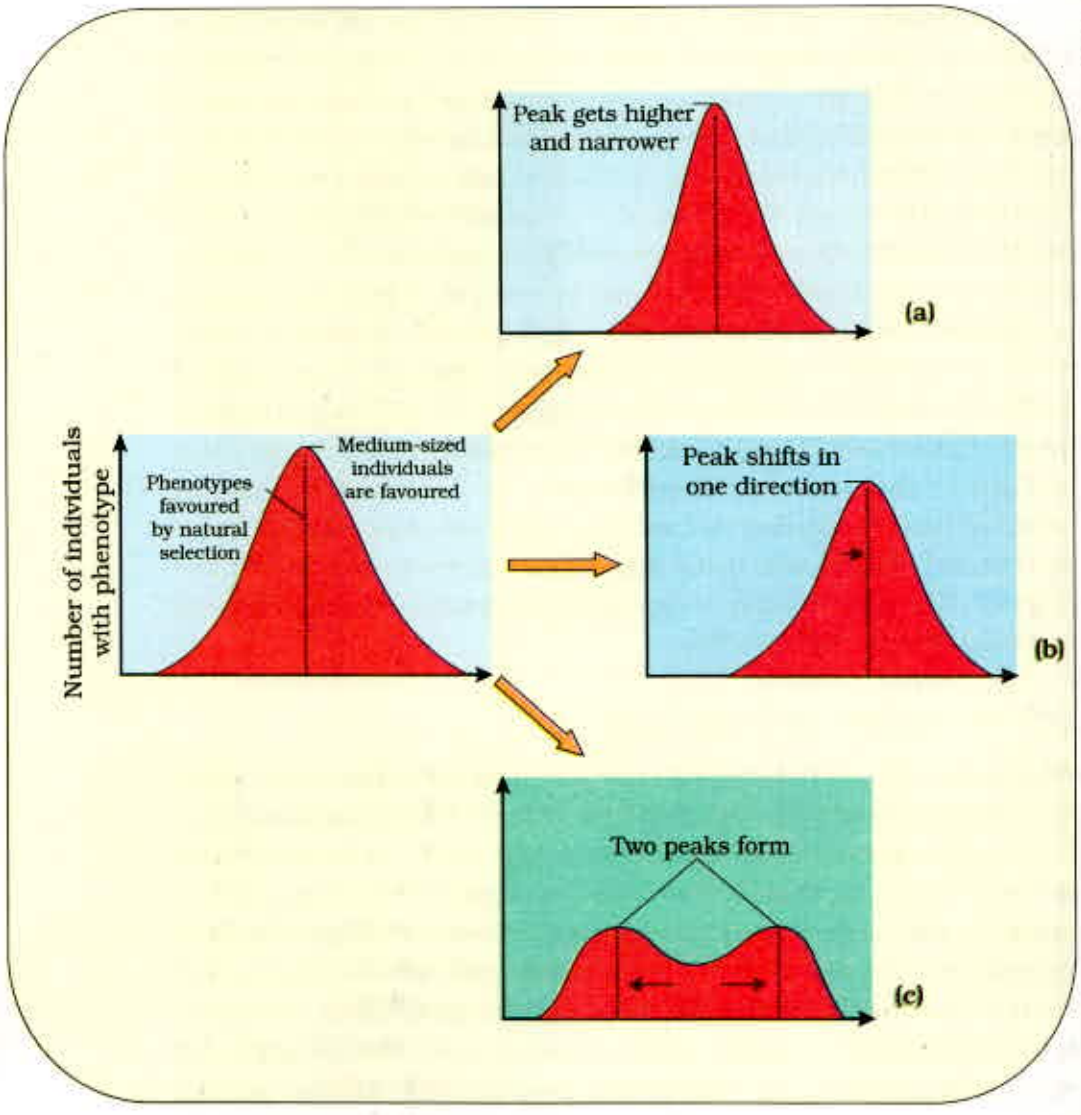

Figure 7.8 Diagrammatic representation of the operation of natural selection on different traits : (a) Stabilising (b) Directional and (c) Disruptive

frequencies, for example, can be named p, q, etc. In a diploid, p and q represent the frequency of allele *A* and allele *a*. The frequency of *AA* individuals in a population is simply p^2. This is simply stated in another ways, i.e., the probability that an allele A with a frequency of p appear on both the chromosomes of a diploid individual is simply the product of the probabilities, i.e., p^2. Similarly of aa is q^2, of Aa 2pq. Hence, $p^2+2pq+q^2=1$. This is a binomial expansion of $(p+q)^2$. When frequency measured, differs from expected values, the difference (direction) indicates the extent of evolutionary change. Disturbance in genetic equilibrium, or Hardy- Weinberg equilibrium, i.e., change of frequency of alleles in a population would then be interpreted as resulting in evolution.

Five factors are known to affect Hardy-Weinberg equilibrium. These are gene migration or gene flow, genetic drift, mutation, genetic recombination and natural selection. When migration of a section of population to another place and population occurs, gene frequencies change in the original as well as in the new population. New genes/alleles are added to the new population and these are lost from the old population. There would be a gene flow if this gene migration, happens multiple times. If the same change occurs by chance, it is called genetic drift. Sometimes the change in allele frequency is so different in the new sample of population that they become a different species. The original drifted population becomes founders and the effect is called **founder effect**.

Microbial experiments show that pre-existing advantageous mutations when selected will result in observation of new phenotypes. Over few generations, this would result in Speciation. Natural selection is a process in which heritable variations enabling better survival are enabled to reproduce and leave greater number of progeny. A critical analysis makes us believe that variation due to mutation or variation due to recombination during gametogenesis, or due to gene flow or genetic drift results in changed frequency of genes and alleles in future generation. Coupled to enhance reproductive success, natural selection makes it look like different population. Natural selection can lead to stabilisation (in which more individuals acquire mean character value), directional change (more individuals acquire value other than the mean character value) or disruption (more individuals acquire peripheral character value at both ends of the distribution curve) (Figure 7.8).

7.8 A Brief Account of Evolution

About 2000 million years ago (mya) the first cellular forms of life appeared on earth. The mechanism of how non-cellular aggregates of giant macromolecules could evolve into cells with membranous envelop is not known. Some of these cells had the ability to release O_2. The reaction

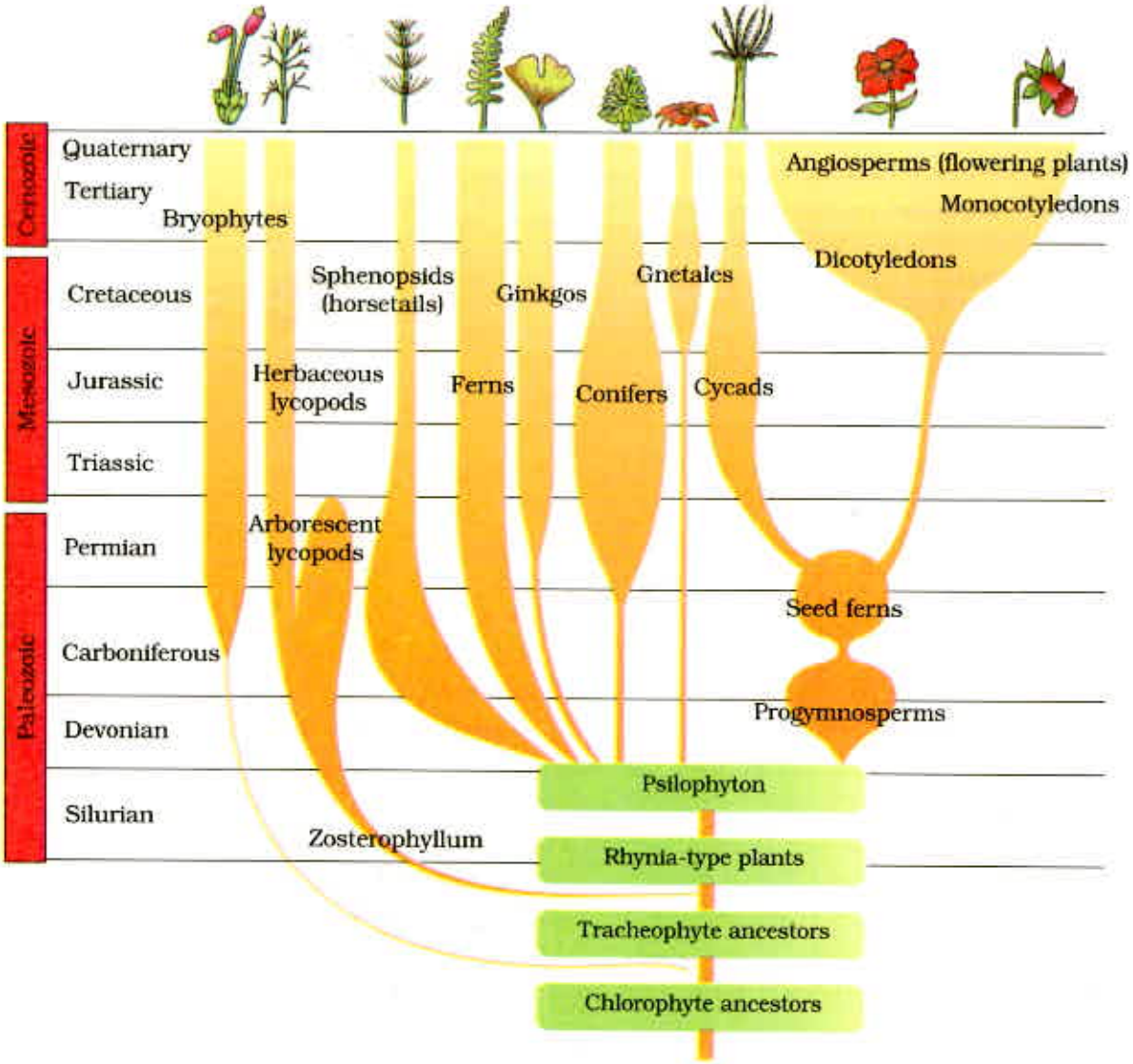

Figure 7.9 A sketch of the evolution of plant forms through geological periods

could have been similar to the light reaction in photosynthesis where water is split with the help of solar energy captured and channelised by appropriate light harvesting pigments. Slowly single-celled organisms became multi-cellular life forms. By the time of 500 mya, invertebrates were formed and active. Jawless fish probably evolved around 350 mya. Sea weeds and few plants existed probably around 320 mya. We are told that the first organisms that invaded land were plants. They were widespread on land when animals invaded land. Fish with stout and strong fins could move on land and go back to water. This was about 350 mya. In 1938, a fish caught in South Africa happened to be a Coelacanth which was thought to be extinct. These animals called lobefins evolved into the

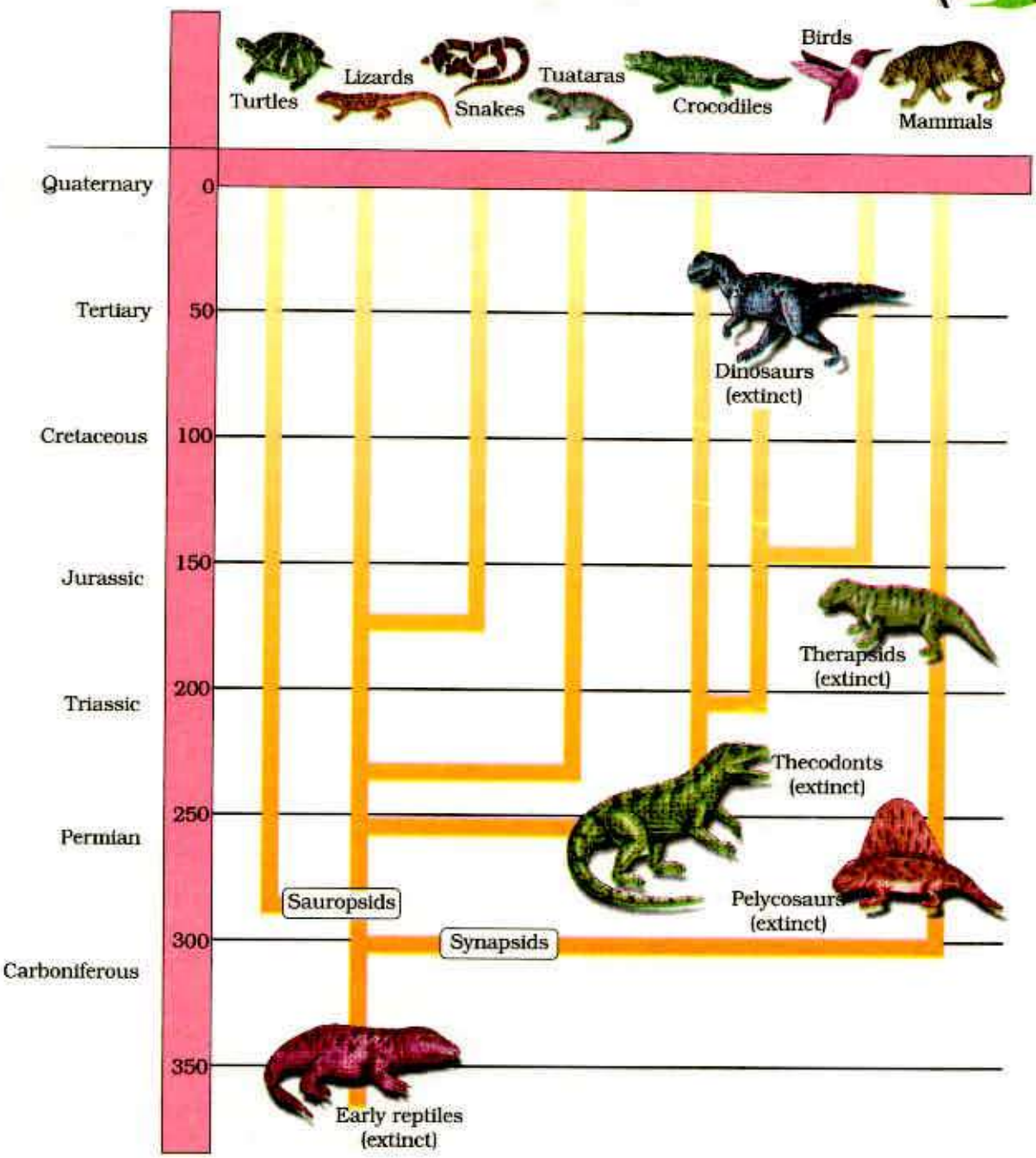

Figure 7.10 Representative evolutionary history of vertebrates through geological periods

first amphibians that lived on both land and water. There are no specimens of these left with us. However, these were ancestors of modern day frogs and salamanders. The amphibians evolved into reptiles. They lay thick-shelled eggs which do not dry up in sun unlike those of amphibians. Again we only see their modern day descendents, the turtles, tortoises and crocodiles. In the next 200 millions years or so, reptiles of different

shapes and sizes dominated on earth. Giant ferns (pteridophytes) were present but they all fell to form coal deposits slowly. Some of these land reptiles went back into water to evolve into fish like reptiles probably 200 mya (e.g. *Ichthyosaurs*). The land reptiles were, of course, the dinosaurs. The biggest of them, i.e., *Tyrannosaurus rex* was about 20 feet in height and had huge fearsome dagger like teeth. About 65 mya, the dinosaurs suddenly disappeared from the earth. We do not know the true reason. Some say climatic changes killed them. Some say most of them evolved into birds. The truth may live in between. Small sized reptiles of that era still exist today.

The first mammals were like shrews. Their fossils are small sized. Mammals were viviparous and protected their unborn young inside the mother's body. Mammals were more intelligent in sensing and avoiding danger at least. When reptiles came down mammals took over this earth. There were in South America mammals resembling horse, hippopotamus, bear, rabbit, etc. Due to continental drift, when South America joined North America, these animals were overridden by North American fauna. Due to the same continental drift pouched mammals of Australia survived because of lack of competition from any other mammal.

Lest we forget, some mammals live wholly in water. Whales, dolphins, seals and sea cows are some examples. Evolution of horse, elephant, dog, etc., are special stories of evolution. You will learn about these in higher classes. The most successful story is the evolution of man with language skills and self-consciousness.

A rough sketch of the evolution of life forms, their times on a geological scale are indicated in (Figure 7.9 and 7.10).

7.9 Origin and Evolution of Man

About 15 mya, primates called *Dryopithecus* and *Ramapithecus* were existing. They were hairy and walked like gorillas and chimpanzees. *Ramapithecus* was more man-like while *Dryopithecus* was more ape-like. Few fossils of man-like bones have been discovered in Ethiopia and Tanzania (Figure 7.11). These revealed hominid features leading to the belief that about 3-4 mya, man-like primates walked in eastern Africa. They were probably not taller than 4 feet but walked up right. Two mya, *Australopithecines* probably lived in East African grasslands. Evidence shows they hunted with stone weapons but essentially ate fruit. Some of the bones among the bones discovered were different. This creature was called the first human-like being the hominid and was called *Homo habilis*. The brain capacities were between 650-800cc. They probably did not eat meat. Fossils discovered in Java in 1891 revealed the next stage, i.e., *Homo*

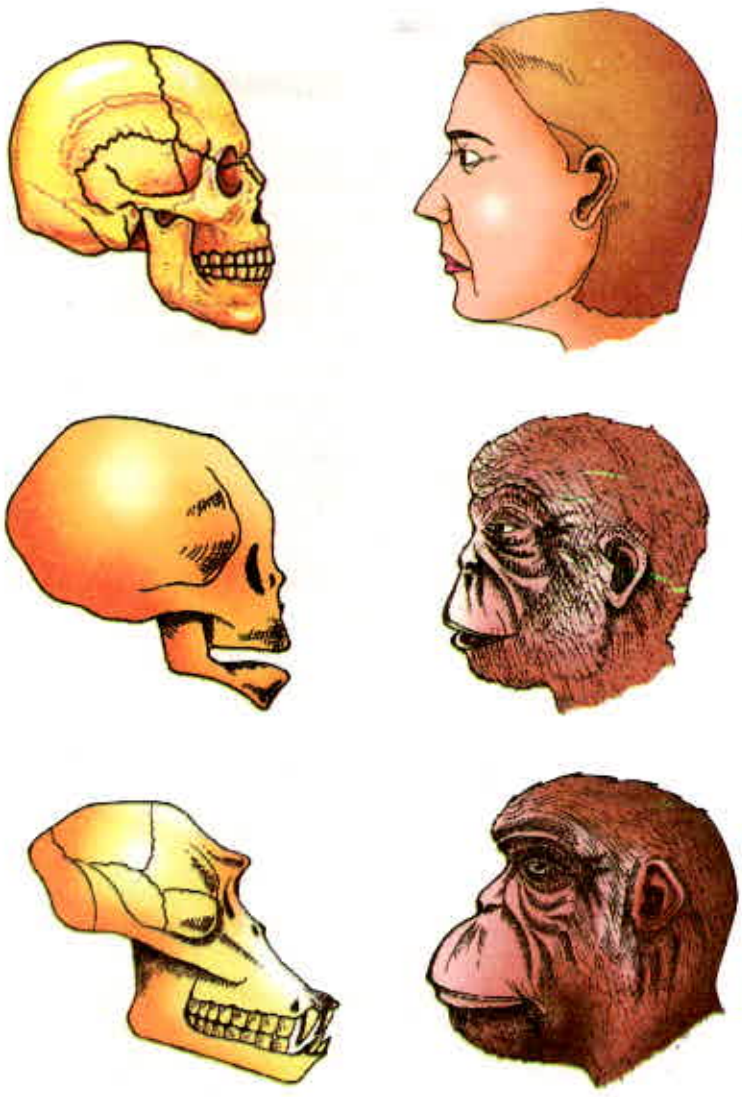

Figure 7.11 A comparison of the skulls of adult modern human being, baby chimpanzee and adult chimpanzee. The skull of baby chimpanzee is more like adult human skull than adult chimpanzee skull

erectus about 1.5 mya. *Homo erectus* had a large brain around 900cc. *Homo erectus* probably ate meat. The Neanderthal man with a brain size of 1400cc lived in near east and central Asia between 1,00,000-40,000 years back. They used hides to protect their body and buried their dead. *Homo sapiens* arose in Africa and moved across continents and developed into distinct races. During ice age between 75,000-10,000 years ago modern *Homo sapiens* arose. Pre-historic cave art developed about 18,000 years ago. Agriculture came around 10,000 years back and human settlements started. The rest of what happened is part of human history of growth and decline of civilisations.