AIM: Study of Polytene chromosomes from Chironomous / Drosophila larvae

14.1 INTRODUCTION

Chromosomes are the carriers of genetic information from one cell generation to another. They are composed of nucleic acids and histones. The chromosomes are thread like structures and have different shapes depending upon the position of the centromere. However, in some species the size of these chromosomes become very large, and they are called **giant chromosomes**.

Polytene chromosomes were first recognized as giant bodies by an Italian cytologist E. Balbiani in cells having large nuclei in the salivary glands of the midge *Chironomus* (Diptera). They were named as polytene chromosomes by Kollar. Polytene chromosomes in the larval salivary glands of *Drosophila* are about 100 times longer than the chromosomes of those normally found at the metaphase of mitosis. This happens because cells undergo repeated rounds of DNA replication without cell division. The DNA of the giant chromosome repeatedly replicates but the daughter strands do not separate. Hence the chromosome number remains constant but the DNA content is amplified many fold. The fully developed chromosomes are nearly 0.25 to 0.55 mm long. The chromosomes show alternating dark and light bands, and each band represents a gene. The giant polytene chromosomes display about 5000

bands when stained with dyes. The bands may also show **chromosomal puffs** or **Balbiani Rings** which are the localized swellings of the chromosome due to high DNA transcription activity. The amplification of DNA of salivary gland chromosomes in the insect larvae is also known as *polytenisation* and the chromosomes are referred to as polytene chromosomes.

Lampbrush chromosomes are a special type of chromosome found in the growing oocytes (immature eggs) of most animals. They were first observed and described in 1882 by Flemming in amphibian oocytes. A detailed study was made by Ruckert (1892) on the oocytes of Shark. Lampbrush chromosomes are larger than the polytene chromosomes. Chromosomes transform into such form during the diplotene stage of meiotic prophase I due to an active transcription of many genes. The chromosomes have loops projecting in pairs which give lampbrush chromosome its characteristic appearance.

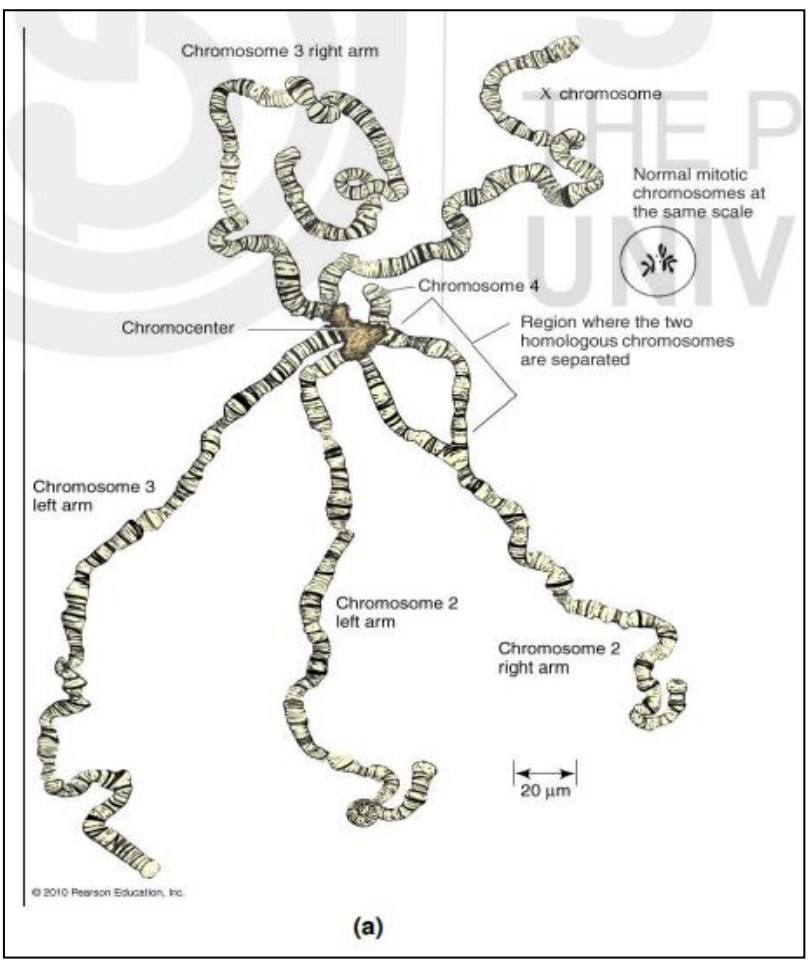
14.5 OBSERVATIONS

You have been provided with permanent slides and the photographs of Polytene and Lampbrush chromosomes. Observe each one of them carefully and note down their characteristic features.

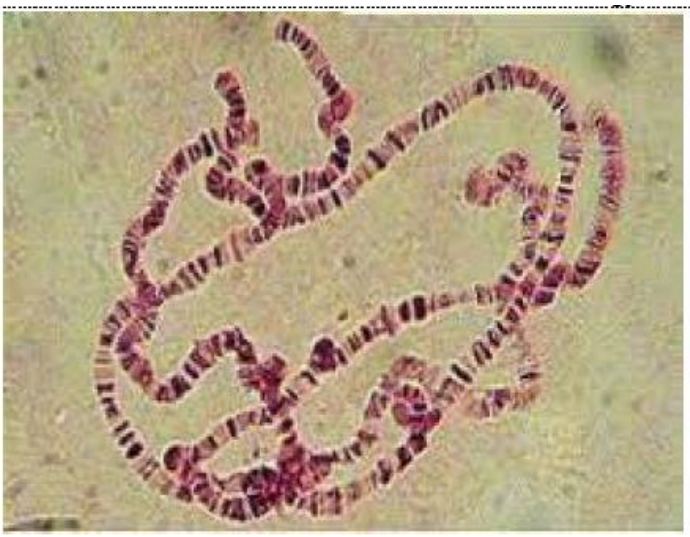
14.5.1 Polytene Chromosomes

A micrograph of the salivary gland cells of *Drosophila* or a dipteran *Chironomus* exhibits the following structural features:

- The salivary gland chromosomes are exceptional as they are clearly visible during interphase.
- They are about 200 times longer than the normal chromosomes. They attain a large size due to **polyteny**.
- Regions with more tightly coiled DNA fibres are represented as darkly stained **bands**. Such bands alternate with relatively clear zones lightly stained zones called **interbands** which are regions of low DNA density (Fig. 14.1).



(a)



(b)

Fig. 14.1: a) A polytene chromosome; b) Polytene chromosome as seen in *Drosophila melanogaster*.

- In certain areas of the bands, the tightly coiled chromosomal fibers open out; the DNA unfolds into loops because of intense mRNA synthesis (gene transcription). This results in a swelling in regions due to increase in area. Such zones are called chromosomal puffs or Balbiani rings (Fig.14.2).
- In *D. melanogaster*, the polytene chromosomes are seen as a complex of five long and one short arm radiating out from a deeply staining, amorphous region called the **chromocenter**. This region is formed to the fusion of the centromeric regions of all the chromosomes (the entire Y chromosome in males). The maternal and paternal homologous chromosomes remain associated side by side. This is called **somatic pairing**.

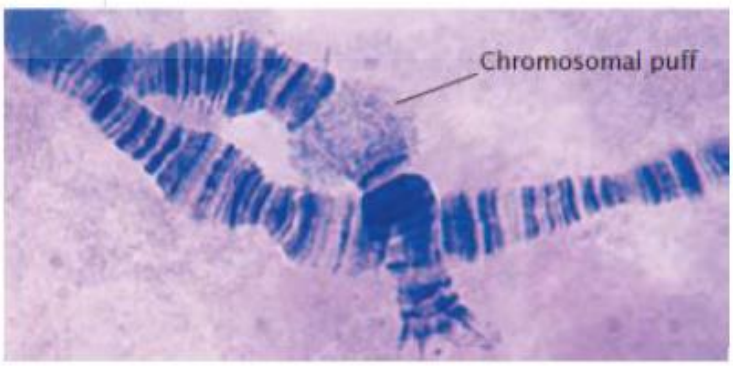


Fig. 14.2: Photomicrograph of giant polytene chromosomes isolated from the salivary glands of larva of *Drosophila*. The chromosomal puffs depict regions of relaxed chromatin with active transcription.

Aim: To estimate the DNA using spectrophotometer.

Principle: The DNA absorbs radiation strongly in the UV region of spectrum due to the conjugated bonding system of the constituent purine and pyrimidines. They show characteristic maxima at 260 nm.

Requirments:

DNA Sample, spectrophotometer *etc.*,

Procedure:

1. Pipette out 3 ml of the given sample in cuvette
2. Measure the absorbance at 260 nm against distilled water/saline. Theoretically one OD at 260 nm corresponds to 50 µg/ml of dsDNA and it is 33 µg/ml for ssDNA
3. Then from the following formula calculate the concentration of DNA in the given sample.

Result: The given unknown sample contains ----µg DNA/ml.

Observations and Calculations

Optical density of the given DNA sample is ------ OD

The DNA concentration (µg/ml)= Absorbance at 260 X 50