

Chromosomal DNA content and C value Paradox

Nuclear DNA content is usually determined by Vicker's integrating microdensitometer. This machine determines nuclear DNA automatically through the use of Feulgen stained squashed root tip cells. In recent years flow cytometry has been successfully used to determine nuclear DNA content using an appropriate fluorescent dye like ethidium bromide and olivomycin. In flow cytometer, DNA from several hundred nuclei can be measured within a second. The data obtained is subjected to statistical analysis using a computer device in order to obtain correct nuclear DNA measurements.

C-value: the amount of DNA (in pico grams) carried in haploid cells. C-values may show large differences in related eukaryotic species. For example, the haploid DNA content in man is 3.2 picogram DNA per haploid genome; Protozoa (*Tetrahymena pyriformis*): 13.6 pg DNA per haploid genome; Maize: 7.5 picogram DNA per haploid genome, Tobacco: 1.2 picogram DNA per haploid genome. Substantial variation in the nuclear DNA content also exists even between closely related species. For instance Bacteria: 0.0016 to 0.011pgDNAper haploid genome; Phages: 2.8 to 46.7 pg DNA per haploid genome 10^{-6} pg to 10^{-5} pg DNA per haploid genome; yeast: 0.245 pg DNA per haploid genome.

The genome size does not uniformly increase with respect to perceived complexity of organisms, for example some Amphibians have more than 10-fold more DNA than do Mammals, including humans. There exists a large variation in C-values between related eukaryotic species and also among closely related species. Moreover, the amount of genomic DNA in complex eukaryotes is much greater than the amount needed to encode proteins. This is termed as **C-value paradox**. C-value paradox probably arises from varying amounts of noncoding DNA such as constitutive heterochromatic regions (repetitive DNA), introns in genes, regulatory elements of genes, pseudogenes, multiple copies of genes (gene duplication), polyploidy in amphibians, intergenic sequences.

Table 28.1. DNA contents in some organisms showing large variation
(1 picogram = 6.1×10^{11} daltons = 965×10^6 base pairs).

Class of organism	Species	Haploid DNA (1C)		
		Picogram	Daltons	Base pairs
1. Phages	$\phi \times 174$ (single strand)	2.8×10^{-6}	1.7×10^6	5,400
	lambda (λ)	46.7×10^{-6}	29×10^6	45,000
	T4	20.7×10^{-5}	126×10^6	200,000
2. Animal virus	adenovirus	21.7×10^{-6}	13×10^6	21,000
3. Prokaryotes	<i>Bacillus subtilis</i>	2.18×10^{-3}	1.3×10^9	2.1×10^6
	<i>Escherichia coli</i>	4.4×10^{-3}	2.7×10^9	4.2×10^6
4. Unicellular eukaryotes	<i>Saccharomyces cerevisiae</i>	14×10^{-3}	8.5×10^9	1.4×10^7
5. Multicellular eukaryotes	<i>Drosophila melanogaster</i>	0.18	0.11×10^{12}	0.17×10^9
	<i>Zea mays</i> (corn)	2.6	1.6×10^{12}	2.5×10^9
	<i>Xenopus laevis</i> (toad)	3.2	1.9×10^{12}	3.1×10^9
	<i>Mus musculus</i> (mouse)	2.7	1.7×10^{12}	2.6×10^9
	<i>Homo sapiens</i> (human)	2.8	1.8×10^{12}	2.8×10^9

DNA content (Genome size) and Adaptability

Correlations of nuclear DNA content with either the altitude or the latitude have been worked out. For instance adaptation to higher altitude has been shown to be correlated with higher DNA content in maize. Similarly in maize, a negative correlation of nuclear DNA content with latitude (lower DNA content at higher latitudes) has been observed. In terms of latitude, the 4C DNA in maize varies about 40% at intraspecific level. At higher latitude 4C DNA content in maize is 9.9 pg and at lower latitude the 4C DNA content is 13.5 pg, a variation of about 40% in DNA content with latitude.

Note: DNA is measured in PICOGRAM (pg). **4C** refers to two homolog chromosomes (diploid) consisting each of two sister chromatids as seen in S-phase of interphase. **2C** refers to two homolog chromosomes that are un-replicated and hence each chromosome with single chromatid as seen in Telophase of mitosis.

Split genes: Genes in eukaryotes such as mammals, birds and amphibians have intervening sequences (non-coding) which are not represented in mRNA transcribed from the gene. Such genes (that is, bits of genomic DNA) with intervening sequences were called split genes. Philip Sharp and Richard Roberts won Nobel Prize for medicine for their work on split genes. They analysed the hybrids of mRNA with corresponding genomic DNA in adenovirus. When mRNA-

DNA hybrids were analyzed in electron microscope, mRNA was found to hybridize with discontinuous stretches of genomic DNA of adenovirus. The intervening sequences called introns were observed as loop and the phenomenon was later described as R-looping. The number of loops correspond to number of introns.

In a given gene, mRNA that is transcribed first is called primary transcript or heterogenous nuclear RNA (hnRNA). The primary transcript has both coding sequences (Exons) and non-coding intervening sequences (Introns). Later introns from the primary transcript were removed by a process called splicing and only exon sequences were joined together in correct order to give the ultimate product called mRNA (messenger RNA). The term introns and exons were used by Gilbert for the first time. Later Chambon proposed GT-AG rule that denote sequences at the boundaries of introns (that is at intron-exon junctions). GT always seen at 5' side of the intron (left splice junction) and AG at 3' side (right splice junction).

Structure of chicken ovalbumin split gene

The structure of **ovalbumin gene** in chicken has been studied in considerable detail and it has been found that in the split gene of ovalbumin there are eight exons and seven introns (Figs. 29.7 and 29.8). The size of ovalbumin gene has been shown to be 7,700 base pairs although the mature mRNA for ovalbumin is only 1,872 nucleotides. It has also been shown, that entire ovalbumin gene having 7,700 base pairs is first transcribed to a precursor RNA to which a cap consisting of 7mG (7 methyl guanosine) is added at the 5' end and poly A tail is added at 3' end. After the addition of cap and tail, the introns are excised, perhaps in two instalments, five introns being excised in the first step and the remaining two in second step. The exons produced due to splicing are then joined with the help of enzyme ligase to produce the mature mRNA of *ovalbumin gene*.

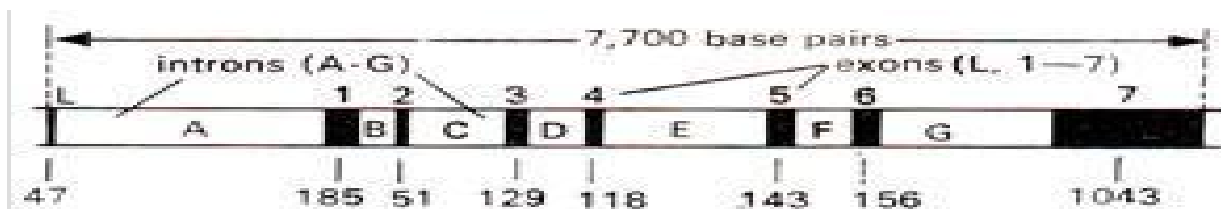


Fig. 29.8. Schematic representation of ovalbumin gene showing 7 introns (A-G) and 8 exons (L, 1-7); number of base pairs in each exon are also given.

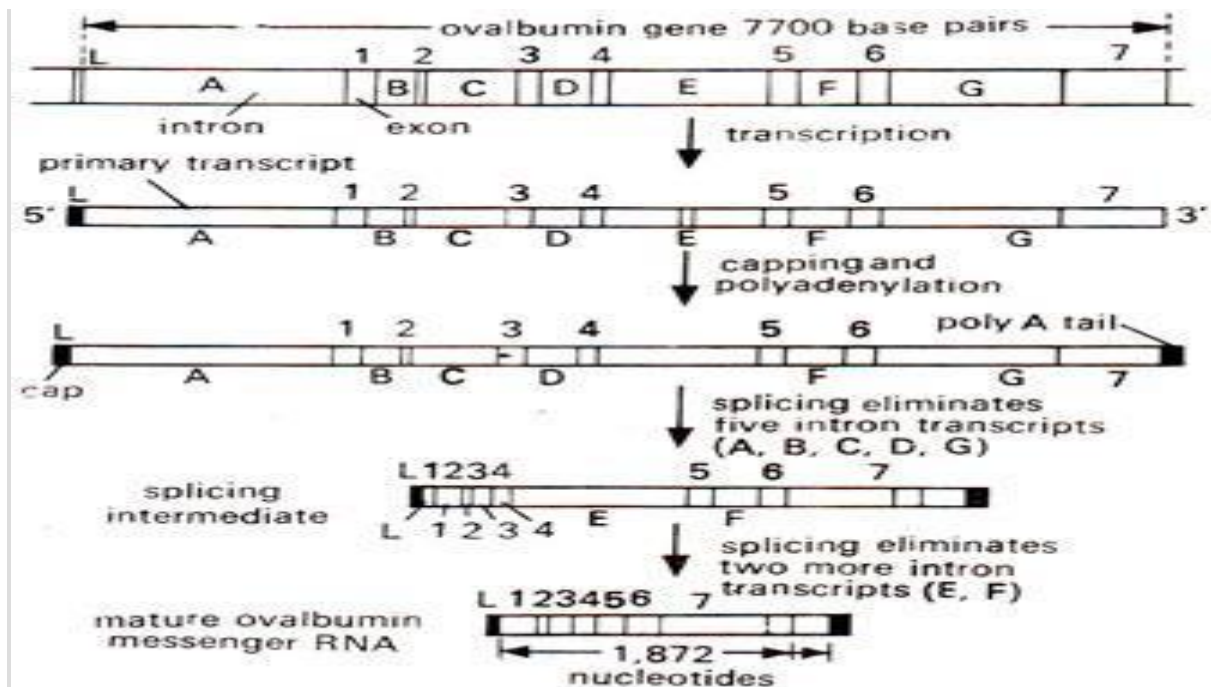


Fig. 29.9. Steps involved in the production of ovalbumin mRNA (1872 nucleotides long) from ovalbumin gene (7700 base pairs long); introns (A-G) and exons (L, 1-7).

When mRNA from chicken ovalbumin gene was hybridized with single stranded genomic DNA, seven distinct loops of DNA at specific sites were observed under electron microscope. This DNA forming loop was missing in mRNA suggesting genomic DNA but not mRNA had intervening sequences. These loops correspond to Introns in genomic DNA and are not represented in mRNA.

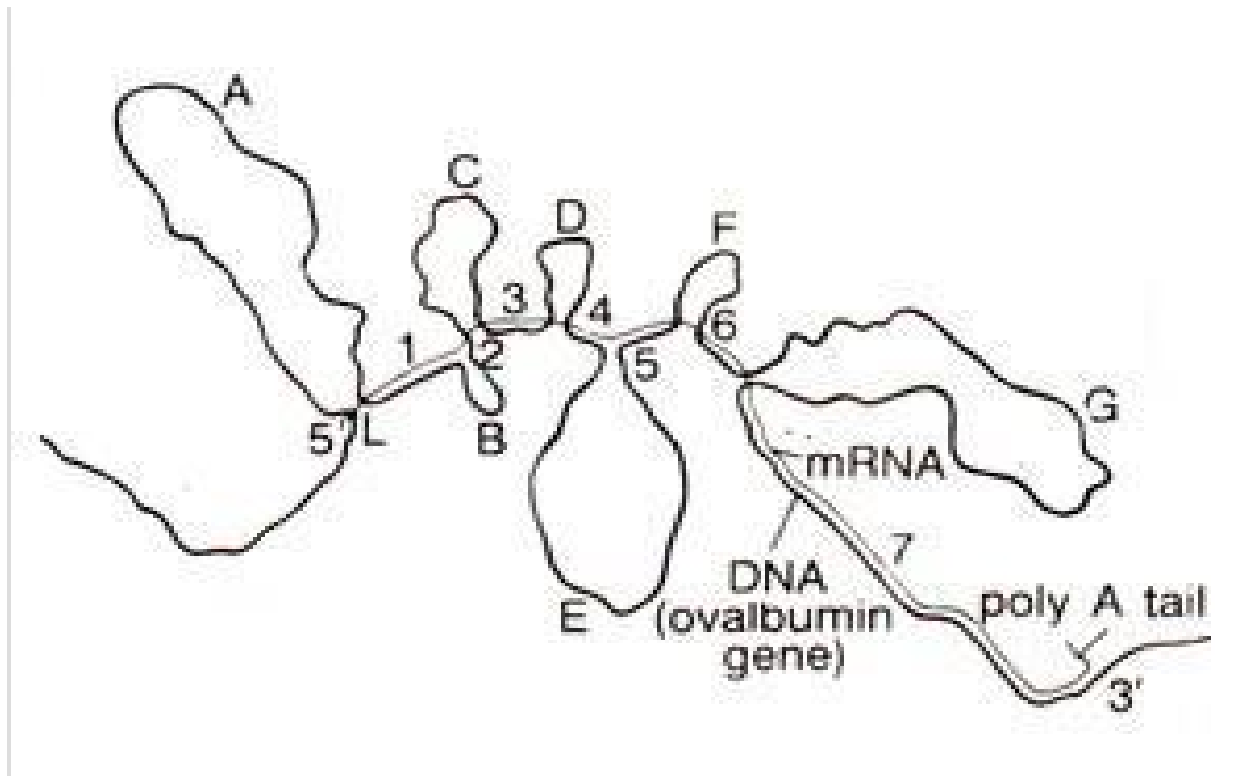


Fig. 29.7. The map of electron micrograph prepared after hybridizing mRNA (thin line) of the ovalbumin gene with its single stranded DNA (thick line). The loops A-G represent 7 different introns and segments L and 1-7 represent 8 exons.

R-loop mapping of interrupted genes

R-loop mapping is a technique in which mRNA is hybridized with double stranded DNA under conditions in which RNA-DNA hybrid is more stable than the DNA duplex. When ds DNA segment belonging to an interrupted gene is used for hybridization with mRNA, the intervening sequence or intron region loops out as double stranded DNA and the exon region loops out as single stranded DNA loops as shown in Figure 29.3. The double stranded DNA loop will appear at the junction of two single stranded loops produced due to displacement by mRNA. These loops can be observed under the electron microscope as has been done for mouse minor β -globin gene showing single intron. This technique is comparable to the hybridization of mRNA with single stranded DNA described earlier.

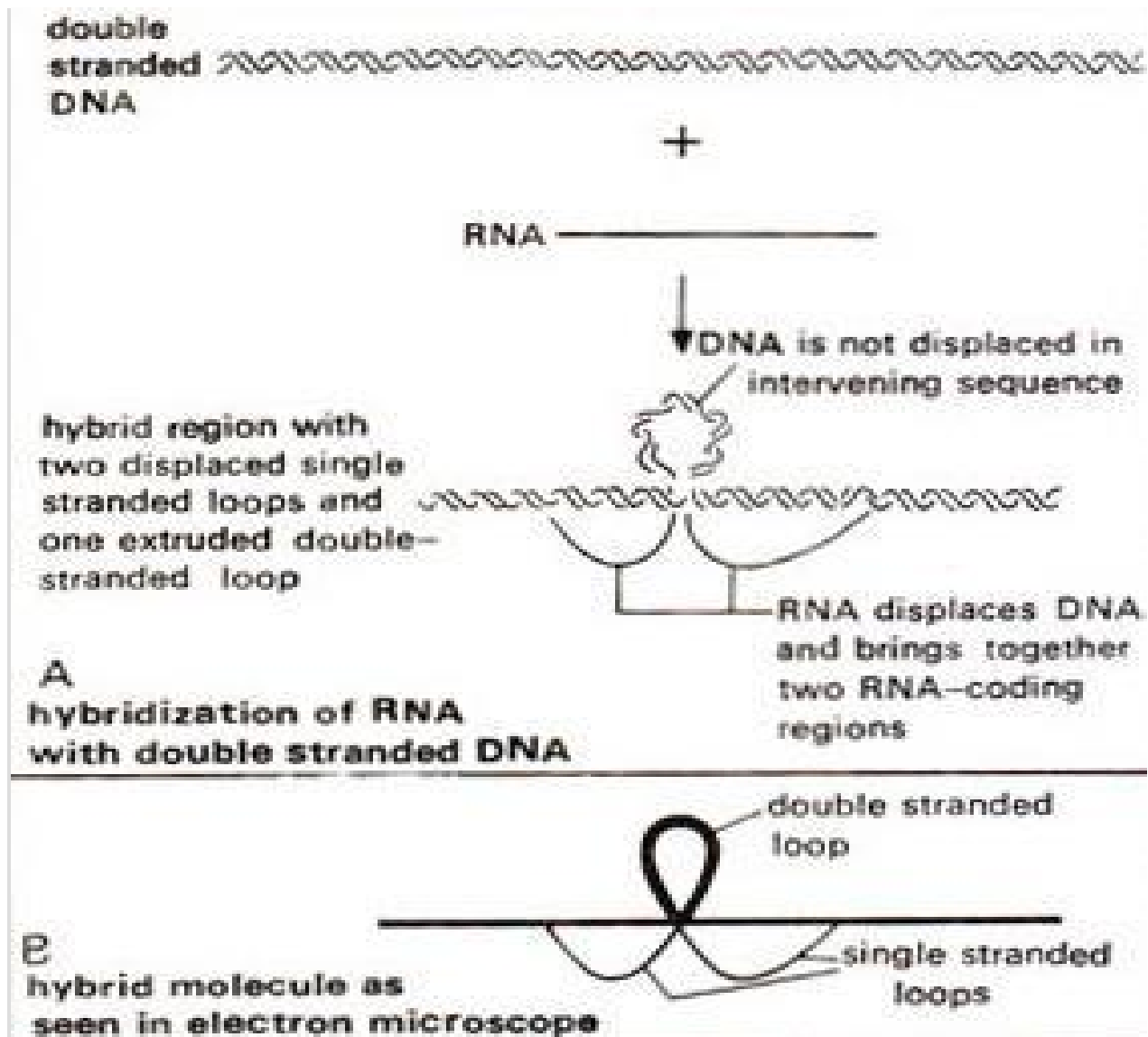


Fig. 29.3. R-looping, where mRNA is hybridized with double stranded DNA (note the double stranded thick loop showing intron region and single stranded thin loops representing the exons).

SPECIAL TYPES OF CHROMOSOMES

Some tissues of certain organisms contain chromosomes which differ significantly from normal chromosomes in terms of either morphology or function. Such chromosomes are referred to as special chromosomes. The following are included under this category:

1. Giant chromosomes or polytene chromosomes: These were first discovered by E. G. Balbiani in 1882 in *Dipteran* salivary glands and hence commonly called salivary gland chromosomes. The giant chromosomes represent bundle of fibrils which arise by repeated cycles of endo-reduplication of single chromatids. These chromosomes replicate repeatedly without cell division and the number of chromonemata (fibrils) keeps on increasing. This phenomenon is known as endomitosis or endoreduplication. Endoreduplication results in the formation of many

stranded giant chromosomes known as polytene chromosomes and the condition is known as polyteny. Their size is 200 times or more than the normal somatic chromosomes (autosomes) and very thick. Hence they are known as giant chromosomes. The homologous chromosomes are continuously paired in the somatic cells.

The polytene chromosome contains two types of bands, dark bands and interbands. The dark bands are darkly stained as it contain more DNA and the inter bands are lightly stained with nuclear stains. The dark bands also called chromomeres. The uncoiling of individual chromomeres in a band causes swelling of bands at certain times to form puffs and exceptionally large puffs are known as balbiani rings. Puffs are the sites of active RNA synthesis. The formation of puffs is called puffing.

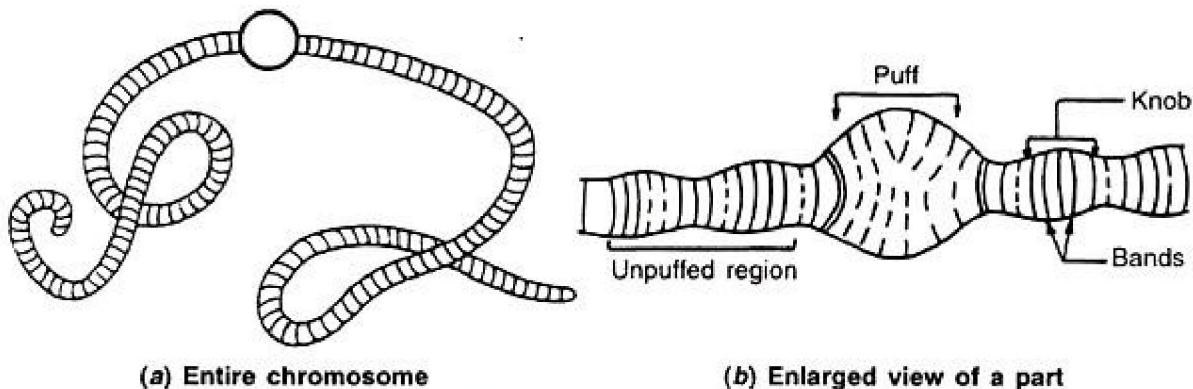


Fig. 4.4. Polytene chromosome of *Drosophila* : (a) whole chromosome, (b) enlarged part showing puffs and knobs.

2. Lamp brush chromosomes: These were first observed by W. Flemming in 1882 and were described in detail in oocytes of sharks by Rukert in 1892. They occur at diplotene stage of meiotic prophase in oocytes of all animal species. Since they are found in meiotic prophase, they are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata at those sites where crossing over has previously occurred. Each bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or chromomeres, each of which have two loop like lateral extensions, one for each chromatid. Thus each loop represents one chromatid of a chromosome and is composed of one DNA double helix. One end of each loop is thinner than the other which is known as thick end. There is extensive RNA synthesis at thin ends of the loop while there is little or no RNA synthesis at the thick ends.

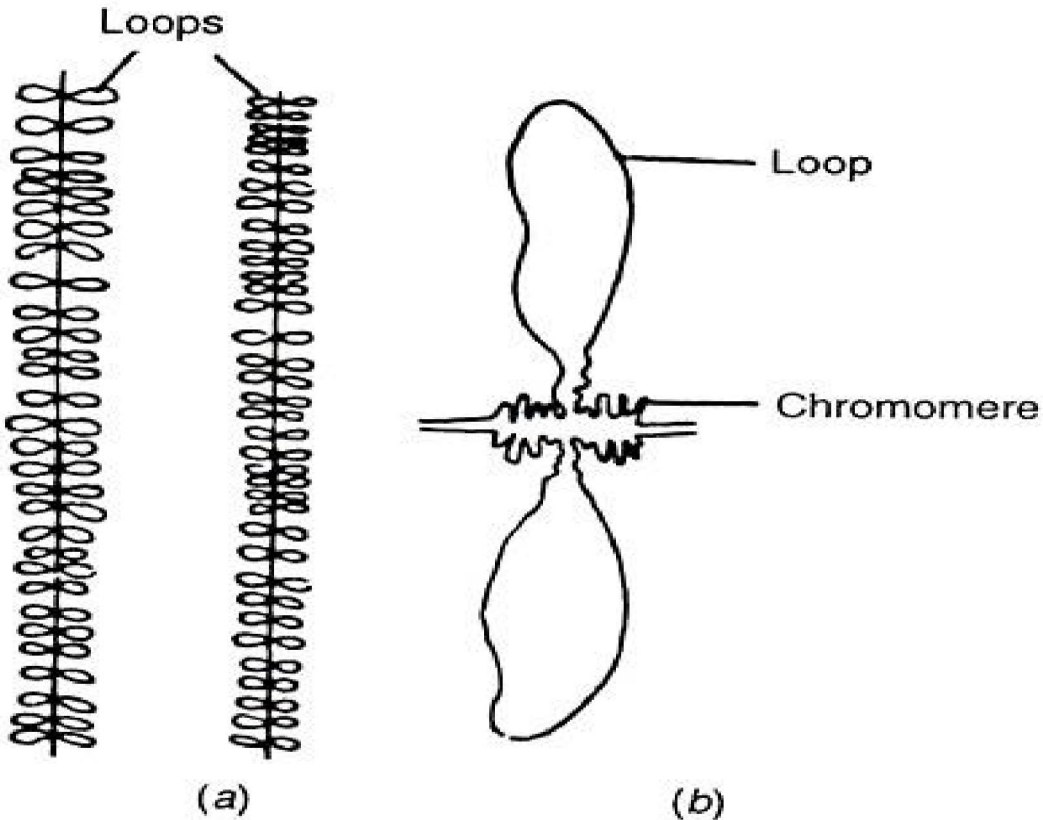


Fig. 4.3. Structure of lampbrush chromosome :
 (a) two homologous chromosomes,
 (b) enlarged view of a single loop.

3. Accessory chromosomes: The normal members of chromosome complements are known as A chromosomes. Some species possess extra chromosomes which are not members of normal chromosome complements. These are called as B chromosomes or supernumerary chromosomes or accessory or extra chromosomes. The following are the characteristics of B-Chromosomes:

1. These chromosomes are generally smaller in size than the normal somatic complement. B chromosomes are believed to originate from A chromosomes.
2. Breakage of A chromosome and subsequent heterochromatinization leads to formation of B chromosomes. However they are genetically inert, but may not be completely devoid of genes.
3. They are non-homologous with any of the basic A chromosomes.
4. When present in high number, they reduce vigour and fertility.
5. Their inheritance is non-Mendelian.

Effects of B-Chromosomes:

In maize, B chromosomes lead to:

- (i) Reduction in vigour,
- (ii) Production of defective seeds with partially developed endosperm, and
- (iii) Increase in the percentage of abortive pollens.

The B chromosomes were first reported in maize and later on in some other species.

4. Isochromosomes: An isochromosome is the one in which two arms are identical with each other in gene content and morphology. In other words, both the arms are mirror images of one another. Every isochromosome is metacentric. The attached 'x' chromosome of *Drosophila* is a classical example of an isochromosome. Isochromosomes can be created in meiosis and mitosis by misdivision of centromere.

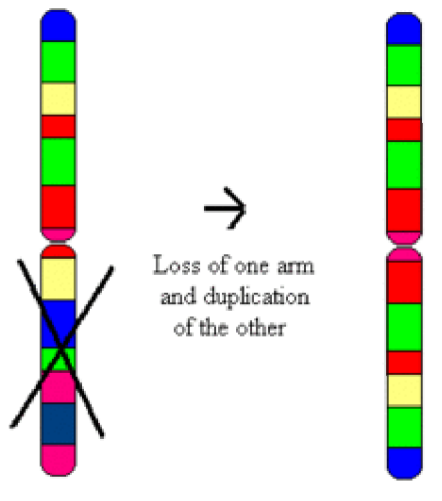


Fig: Isochromosome in which the arms are mirror copies of each other.

5. Allosomes / sex chromosomes: Chromosomes differing in morphology and number in male and female are called allosomes. They are responsible for determination of sex. Eg: X and Y chromosomes in human beings and *Drosophila*. Chromosomes which have no relation with determination of sex and contain genes which determine somatic characters of individuals are called autosomes and are represented by letter 'A'.