



**FACULTY OF AGRICULTURE SCIENCES AND
ALLIED INDUSTRIES**

(Principles of Cytogenetics)

For

M.Sc. Ag (GPB)



RAMA
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Course Instructor

Dr Shiv Prakash Shrivastav

FASAI(Genetics and Plant Breeding)

Rama University, Kanpur

Study of chromosome structure, morphology, number and types - Karyotype and Idiogram

A chromosome is a structure that occurs within cells and that contains the cell's genetic material. That genetic material, which determines how an organism develops, is a molecule of deoxyribonucleic acid (DNA). A molecule of DNA is a very long, coiled structure that contains many identifiable subunits known as genes. In prokaryotes, or cells without a nucleus, the chromosome is merely a circle of DNA. In eukaryotes, or cells with a distinct nucleus, chromosomes are much more complex in structure.

Historical background

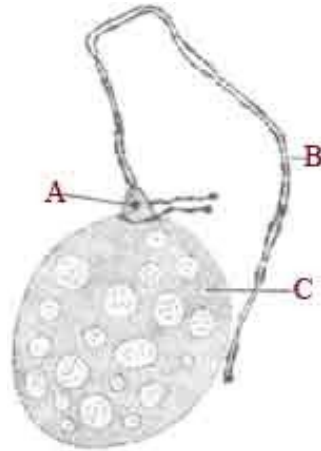
The terms chromosome and gene were used long before biologists really understood what these structures were. When the Austrian monk and biologist Gregor Mendel (1822–1884) developed the basic ideas of heredity, he assumed that genetic traits were somehow transmitted from parents to offspring in some kind of tiny "package." That package was later given the name "gene." When the term was first suggested, no one had any idea as to what a gene might look like. The term was used simply to convey the idea that traits are transmitted from one generation to the next in certain discrete units.

The term "chromosome" was first suggested in 1888 by the German anatomist Heinrich Wilhelm Gottfried von Waldeyer-Hartz (1836–1921). Waldeyer-Hartz used the term to describe certain structures that form during the process of cell division (reproduction).

One of the greatest breakthroughs in the history of biology occurred in 1953 when American biologist James Watson and English chemist Francis Crick discovered the chemical structure of a class of compounds known as deoxyribonucleic acids (DNA). The Watson and Crick discovery made it possible to express biological concepts (such as the gene) and structures (such as the chromosome) in concrete chemical terms.

According to the classical cytological studies, each chromosome structurally consists of a limiting membrane called pellicle, an amorphous matrix and two very thin, highly coiled filaments called chromonema or chromonemata. Each chromonemata is 800Å thick and contains 8-microfibrils, each of which in its turn contains two double helices of DNA. Both chromonematae remain intimately coiled in spiral manner with each other and have a series of microscopically visible bead-like swelling along its length called chromomeres. The early geneticists have attached great significance to the chromomeres and erroneously considered them as hereditary unit, hereditary or Mendelian factors or genes; but modern cytological investigations have confirmed that the chromomeres are not genes but the regions of super-imposed coils.

The recent cytological findings have also condemned the view that chromosomes have pellicle, matrix and chromonemata.

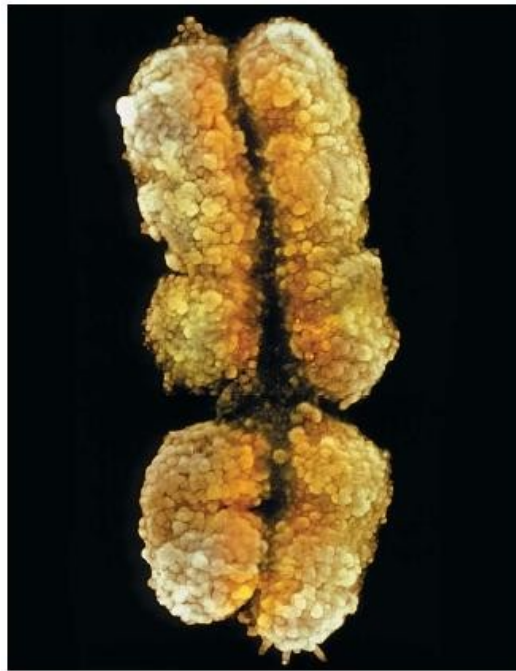


A. Nucleolus organizer B. Chromosome C.

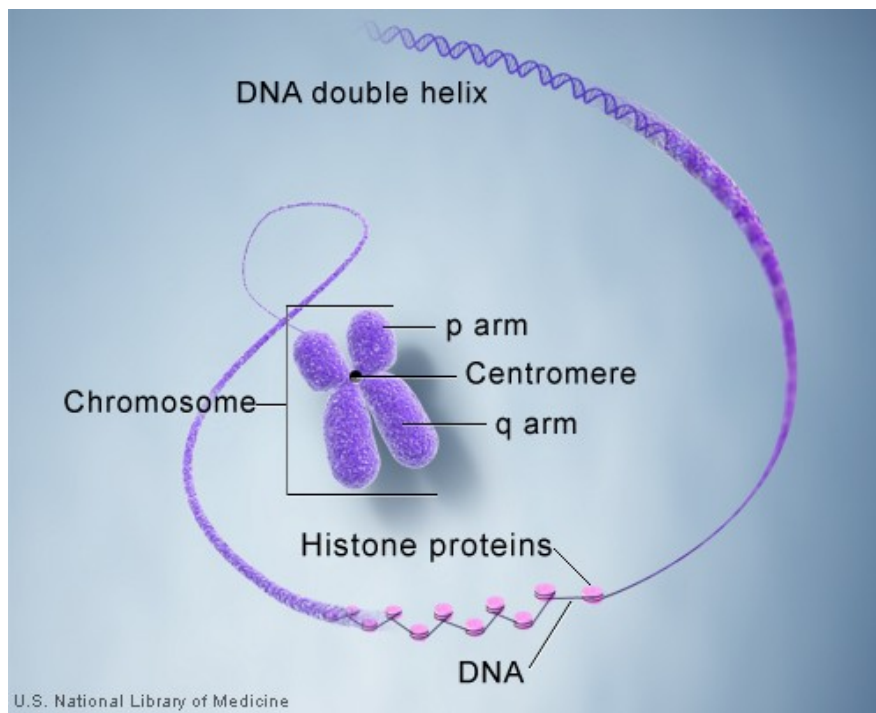
Nucleolus The structure of chromosomes and genes

A chromosome is an organized structure of DNA and protein that is found in cells. A chromosome is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word *chromosome* comes from the Greek *chroma* - color and *soma* - body due to their property of being very strongly stained by particular dyes. Chromosomes vary widely between different organisms. The DNA molecule may be circular or linear, and can be composed of 10,000 to 1,000,000,000 nucleotides in a long chain. Typically eukaryotic cells (cells with nuclei) have large linear chromosomes and prokaryotic cells (cells without defined nuclei) have smaller circular chromosomes, although there are many exceptions to this rule.

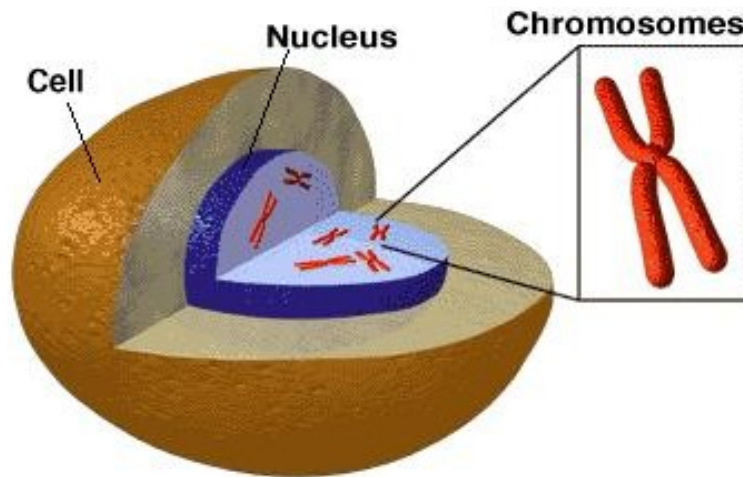
Today we know that a chromosome contains a single molecule of DNA along with several kinds of proteins. A molecule of DNA, in turn, consists of thousands and thousands of subunits, known as nucleotides, joined to each other in very long chains. A single molecule of DNA within a chromosome may be as long as 8.5 centimeters (3.3 inches). To fit within a chromosome, the DNA molecule has to be twisted and folded into a very complex shape.



Each chromosome has a constriction point called the centromere, which divides the chromosome into two sections, or “arms.” The short arm of the chromosome is labeled the “p arm.” The long arm of the chromosome is labeled the “q arm.” The location of the centromere on each chromosome gives the chromosome its characteristic shape, and can be used to help describe the location of specific genes.



The arrangement of packets of genetic information in a chromosome is as follows:



Furthermore, cells may contain more than one type of chromosome; for example, mitochondria in most eukaryotes and chloroplasts in plants have their own small chromosomes. The following are the different types of chromosomes

Viral Chromosomes

The chromosomes of viruses are called viral chromosomes. They occur singly in a viral species and chemically may contain either DNA or RNA. The DNA containing viral chromosomes may be either of linear shape (e.g., T2, T3, T4, T5, bacteriophages) or circular shape (e.g., most animal viruses and certain bacteriophages). The RNA containing viral chromosomes are composed of a linear, single-stranded RNA molecule and occur in some animal viruses (e.g., poliomyelitis virus, influenza virus, etc.); most plant viruses, (e.g., tobacco mosaic virus, TMV) and some bacteriophages. Both types of viral chromosomes are either tightly packed within the capsids of mature virus particles (virions) or occur freely inside the host cell.

Prokaryotic Chromosomes

The prokaryotes usually consists of a single giant and circular chromosome in each of their nucleoids. Each prokaryotic chromosome consists of a single circular, double-stranded DNA molecule; but has no protein and RNA around the DNA molecule like eukaryotes. Different prokaryotic species have different sizes of chromosome.

Eukaryotic Chromosomes

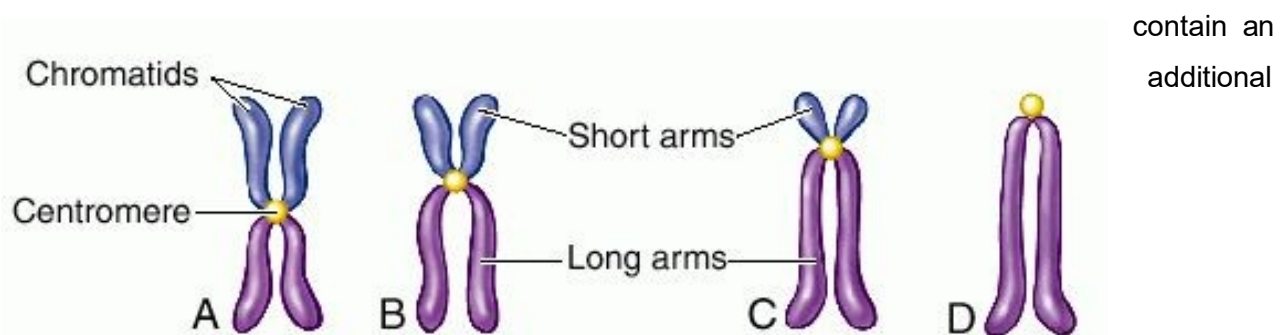
The eukaryotic chromosomes differ from the prokaryotic chromosomes in morphology, chemical composition and molecular structure. The eukaryotes (plants and animals) usually contain much more genetic informations than the viruses and prokaryotes, therefore, contain a great amount of genetic material, DNA molecule which here may not occur as a single unit, but,

as many units called chromosomes. Different species of eukaryotes have different but always constant and characteristic number of chromosomes. In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The shape of the eukaryotic chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division. Chromosomes are the essential unit for cellular division and must be replicated, divided, and passed successfully to their daughter cells so as to ensure the genetic diversity and survival of their progeny. They are thin, coiled, elastic, contractile thread-like structures during the interphase (when no division of cell occurs) and are called chromatin threads which under low magnification look like a compact stainable mass, often called as chromatin substance or material. During metaphase stage of mitosis and prophase of meiosis, these chromatin threads become highly coiled and folded to form compact and individually distinct ribbon-shaped chromosomes. These chromosomes contain a clear zone called kinetochore or centromere along their length.

Eukaryotes (cells with nuclei such as plants, yeast, and animals) possess multiple large linear chromosomes contained in the cell's nucleus. Each chromosome has one centromere, with one or two arms projecting from the centromere, although, under most circumstances, these arms are not visible as such. In addition, most eukaryotes have a small circular mitochondrial genome, and some eukaryotes may have additional small circular or linear cytoplasmic chromosomes.

The number and position of centromeres is variable, but is definite in a specific chromosome of all the cells and in all the individuals of the same species. Thus, according to the number of the centromere the eukaryotic chromosomes may be acentric (without any centromere), monocentric (with one centromere), dicentric (with two centromeres) or polycentric (with more than two centromeres). The centromere has small granules or spherules and divides the chromosomes into two or more equal or unequal chromosomal arms.

According to the position of the centromere, the eukaryotic chromosomes may be rod-shaped (telocentric and acrocentric), J-shaped (submetacentric) and V-shaped (metacentric). During the cell divisions the microtubules of the spindle are get attached with the chromosomal centromeres and move them towards the opposite poles of cell. Beside centromere, the chromosomes may bear terminal unipolar segments called telomeres. Certain chromosomes



specialized segment, the nucleolus organizer, which is associated with the nucleolus.

Position of the centromere in (A) metacentric; (B) submetacentric; (C) acrocentric; and

(D) telocentric chromosomes.



A. Centromere

Acrocentric

Telocentric

Metacentric

Sub metacentric

In the nuclear chromosomes of eukaryotes, the uncondensed DNA exists in a semi-ordered structure, where it is wrapped around histones (structural proteins), forming a composite material called chromatin. Chromatin is the complex of DNA and protein found in the eukaryotic nucleus which packages chromosomes. The structure of chromatin varies significantly between different stages of the cell cycle, according to the requirements of the DNA.

Interphase chromatin

During interphase (the period of the cell cycle where the cell is not dividing), two types of chromatin can be distinguished. The density of the chromatin that makes up each chromosome (that is, how tightly it is packed) varies along the length of the chromosome.

dense regions are called heterochromatin

less dense regions are called euchromatin.

- Euchromatin, which consists of DNA that is active, e.g., being expressed as protein.
- Heterochromatin, which consists of mostly inactive DNA. It seems to serve structural purposes during the chromosomal stages. Heterochromatin can be further distinguished into two types:
 - Constitutive heterochromatin, which is never expressed. It is located around the centromere and usually contains repetitive sequences.

- Facultative heterochromatin, which is sometimes expressed.

Individual chromosomes cannot be distinguished at this stage - they appear in the nucleus as a homogeneous tangled mix of DNA and protein.

Diploids and Haploids

In contrast to prokaryotes, most eukaryotes are diploids, i.e., each somatic cell of them contains one set of chromosomes inherited from the maternal (female) parent and a comparable set of chromosomes (called homologous chromosomes) from the paternal (male) parent. The number of chromosomes in a dual set of a diploid somatic cell is called the diploid number ($2n$). The sex cells (sperms and ova) of a diploid eukaryote cell contain half the number of chromosomal sets found in the somatic cells and are known as haploid (n) cells. A haploid set of chromosome is also called genome. The fertilization process restores the diploid number of a diploid species.

Chemical Structure of Chromosomes

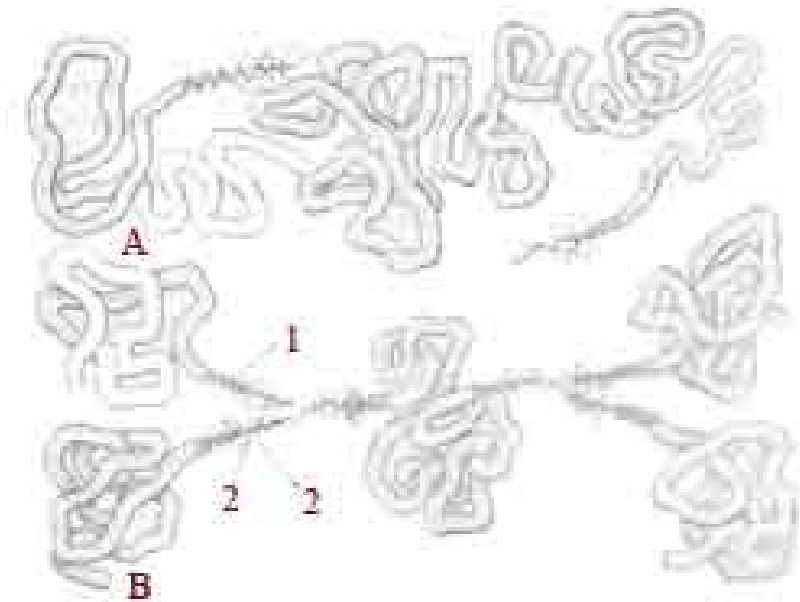
Chemically, the eukaryotic chromosomes are composed of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), histone and non-histone proteins and certain metallic ions. The histone proteins have basic properties and have significant role in controlling or regulating the functions of chromosomal DNA. The non-histone proteins are mostly acidic and have been considered more important than histones as regulatory molecules. Some non-histone proteins also have enzymatic activities. The most important enzymatic proteins of chromosomes are phosphoproteins, DNA polymerase, RNA-polymerase, DPN-pyrophosphorylase, and nucleoside triphosphatase. The metal ions as Ca^{+} and Mg^{+} are supposed to maintain the organization of chromosomes intact.

Molecular Structure of Chromosomes

According to the recent and widely accepted theory of Dupraw (1965, 1970) and Hans Ris (1967) called unistranded theory, each eukaryotic chromosome is composed of a single, greatly elongated and highly folded nucleoprotein fibre of 100Å thick. This nucleoprotein fibre in its turn is composed of a single, linear, double stranded DNA molecule which remains wrapped in equal amounts of histone and non-histone proteins and variable amounts of different kinds of RNA. Dupraw produced a "folded-fibre Model" to show the ultrastructure of chromosome.

FIBRE FOLDED MODEL

This model shows a highly folded nucleoprotein fibre in a chromosome and also suggests that how the nucleoprotein fibre of a chromosome replicates during cell division and how the nucleoprotein fibre of both chromatids remain held at the centromere by a unreplicated fibre segment to DNA until the anaphase.



A-B- The folded fibre model of Dupraw for chromosomes in Interphase

1. DNA molecule

2. Protein molecules



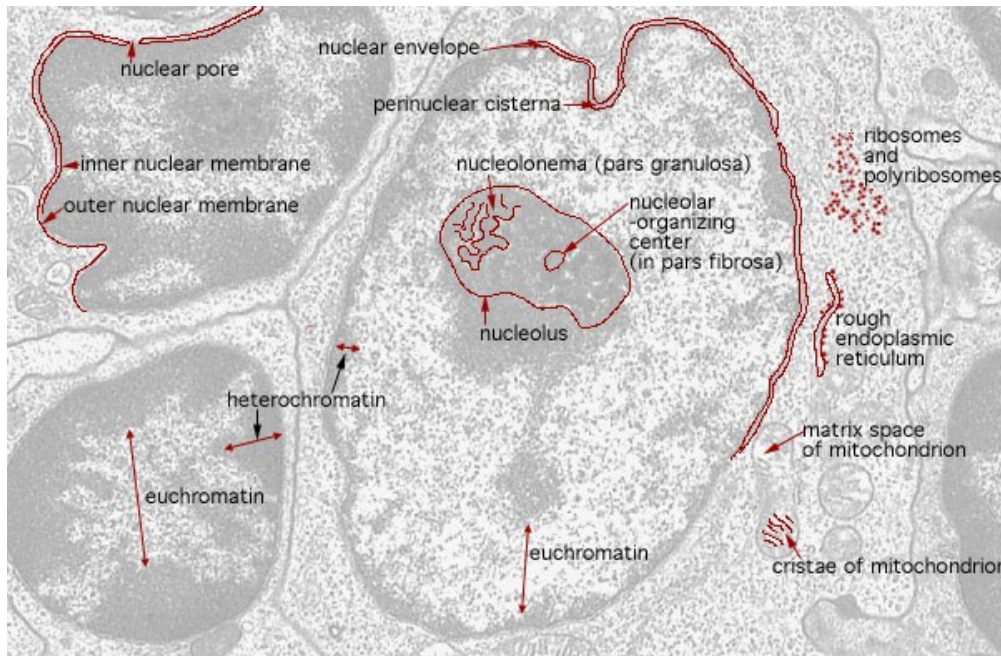
3. Chromatids

Material of Chromosomes

The chromatin material of the eukaryotic chromosomes according to its percentage of DNA, RNA and proteins and consequently due to its, staining property has been classified into following by classical cytologists:

1. Euchromatin

The euchromatin is the extended form of chromatin and it forms the major, portion of chromosomes. The euchromatin has special affinity for basic stains and is genetically active because its component DNA molecule synthesizes RNA molecules only in the extended form of chromatin.

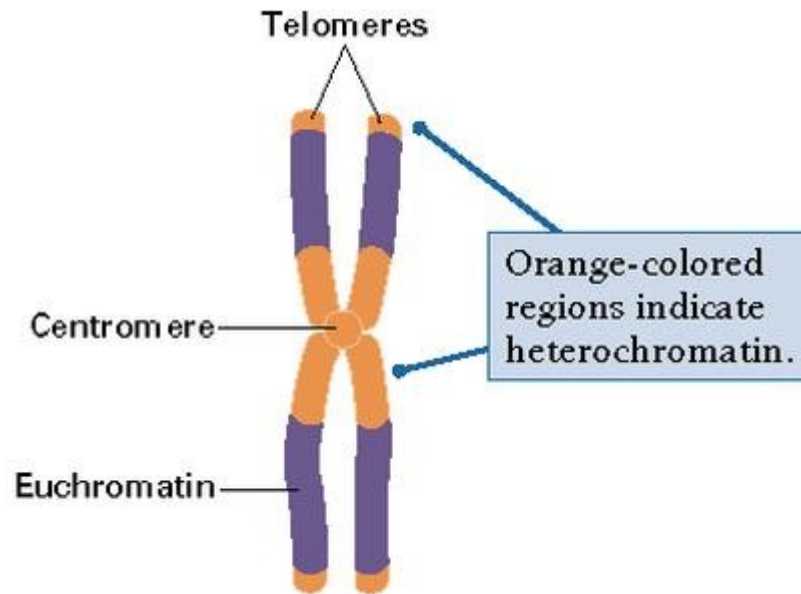


2. Heterochromatin

The heterochromatin is a condensed intercoiled state of chromatin, containing two to three times more DNA than euchromatin. However, it is genetically inert as it does not direct synthesize RNA (i.e., transcription) and protein and is often replicated at a different time from the rest of the DNA.

Recent molecular biological studies have identified three kinds of heterochromatins, namely constitutive, facultative and condensed heterochromatin. The constitutive heterochromatin is present at all times and in the nuclei of virtually all the cells of an organism. In a interphase nucleus, it tends to clump together to form chromocentre or false nucleoli. In *Drosophila*, for example, most pupal, larval and adult cells contain large blocks of constitutive heterochromatin that lie adjacent to centromeres. Constitutive heterochromatin contain highly repetitive satellite DNA which is late replicating, it fails to replicate until late in the S-phase and is then replicated during a brief period just before the G2. The facultative heterochromatin reflect the existence of a regulatory device designed to adjust the "dosages" of certain genes in the nucleus

It is originated during the process called facultative heterochromatization : a process in which a chromosome or a set of chromosome becomes heterochromatic (turned off) in the cells of one sex, while remaining become heterochromatic (turned on) in the cells of opposite sex. In other words, it remains indirectly related to, sexual differentiation. The condensed heterochromatin is deeply staining tightly coiled chromatin which does not resemble with two other kinds of chromatin, has some specific role in gene regulation and is found in many interphase nuclei.



Kinds of Chromosomes

The eukaryotic chromosomes have been classified into autosomes and sex chromosomes. The autosomes have nothing to do with the determination of sex and exceed in number than sex chromosomes. The sex chromosomes determine the sex of their bearer. They are usually two in number and are usually of two kinds: X chromosomes and Y chromosomes.

Special Types of Chromosomes

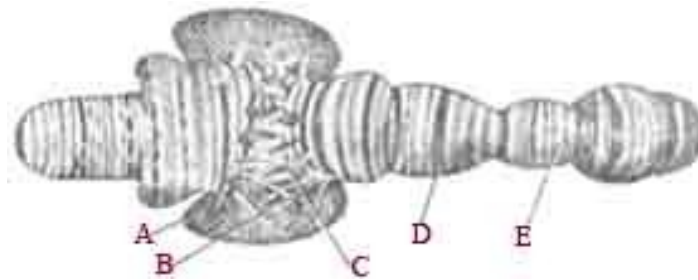
The eukaryotes besides possessing the usual type of chromosomes in their body cells, contain some unusual and special types of chromosomes in some body cells or at some particular stage of their life cycle. The special type of eukaryotic chromosomes are following:

Polytene chromosomes

The nuclei of the salivary gland cells of the larvae of dipterans like *Drosophila* have unusually long and wide chromosomes, 100 or 200 times in size of the chromosomes in meiosis and mitosis of the same species. This is particularly surprising, since the salivary gland cells do

not divide after the glands are formed, yet their chromosomes replicate several times (a process called endomitosis) and become exceptionally giant-sized to be called polytene or multistranded chromosomes (discovered by Balbiani (1881) and named by Koller). The endomitosis process result in the production of 2^X chromosomes, where X gives the number of multiplication cycle.

The polytene Chromosomes of the salivary gland cells of *D. melanogaster* contain 1000 to 2000 chromosomes, which are formed by nine or ten consecutive multiplication cycles and remain associated parallel to each other. Further, the polytene chromosomes have alternating dark and light bands along their length. The dark bands are comparable with the chromomeres of a simple chromosomes and are disc-shaped structures occupying the whole diameter of chromosome. They contain euchromatin. The light bands or inter bands are fibrillar and composed of heterochromatin.



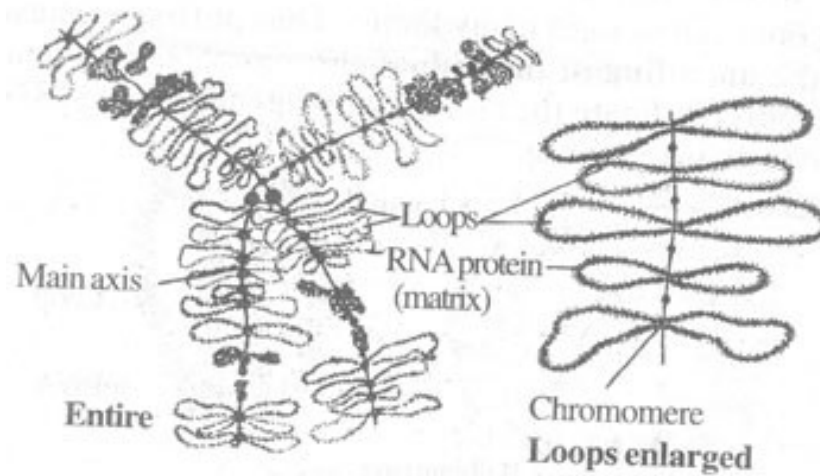
- A. mRNA
- B. Chromosome puff
- C. Chromonemata
- D. Dark band
- E. Inter band

If the polytene chromosomes of dipteran larval salivary glands are examined at several stages of development; it is seen that specific areas (sets of bands) enlarge or "puff". Such puffs change location as development proceed, those at specific locations being correlated with particular developmental stages. This temporal puffing indicates changes in gene activity and involves several processes such as the accumulation of acidic proteins, despiralization of DNA, formation of chromonemal loops called Balbiani rings at the lateral sides of dark bands, synthesis of mRNA (messenger RNA) and storage (accumulation) of newly synthesized mRNA around the Balbiani rings.

Lampbrush chromosomes

In diplotene stage of meiosis, the yolk rich oocytes of vertebrates contain the nuclei with many lamp brush shaped chromosomes of exceptionally large sizes. The lampbrush chromosomes (discovered by Ruckert in 1892) are formed during the active synthesis of mRNA

molecules for the future use by the egg during cleavage when no synthesis of mRNA molecules is possible due to active involvement of chromosomes in the mitotic cell division.



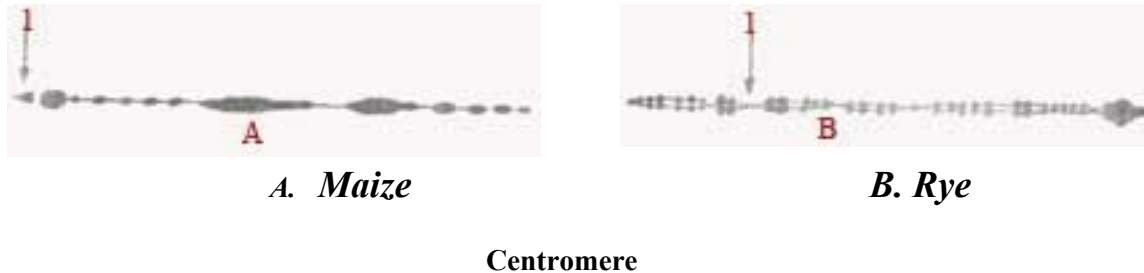
A lampbrush chromosome contains a main axis whose chromonemal fibres (DNA molecule) gives out lateral loops throughout its length. The loops produce the mRNA molecules of different kinds. In a mature egg, as the chromosome contracts the lateral loops disappear.

B-chromosomes

Many plant (maize, etc.) and animal (such as insects and small mammals) species, besides having autosomes (A-chromosomes) and sex-chromosomes possess a special category of chromosomes called B-chromosomes without obvious genetic function. These B-chromosomes (also called supernumerary chromosomes, accessory chromosomes, accessory fragments, etc.) usually have a normal structure, are somewhat smaller than the autosomes and can be predominantly, heterochromatic (many insects, maize, etc.) or pro-dominantly euchromatic (rye).

In maize, their number per cell can vary from 0 to 30 and they adversely affect, development and fertility only when occur, in large amount. In animals, the B-chromosomes disappear from the non-reproductive (somatic) tissue and are maintained only in the cell-lines that lead to the reproductive organs. B-chromosomes have negative consequences for the organism, as they have deleterious effect because of abnormal crossing over during the meiosis of animals and abnormal nucleus divisions of the gametophyte plants. In animals, B-chromosomes occur more frequently in females and the basis is non-disjunction. The non-

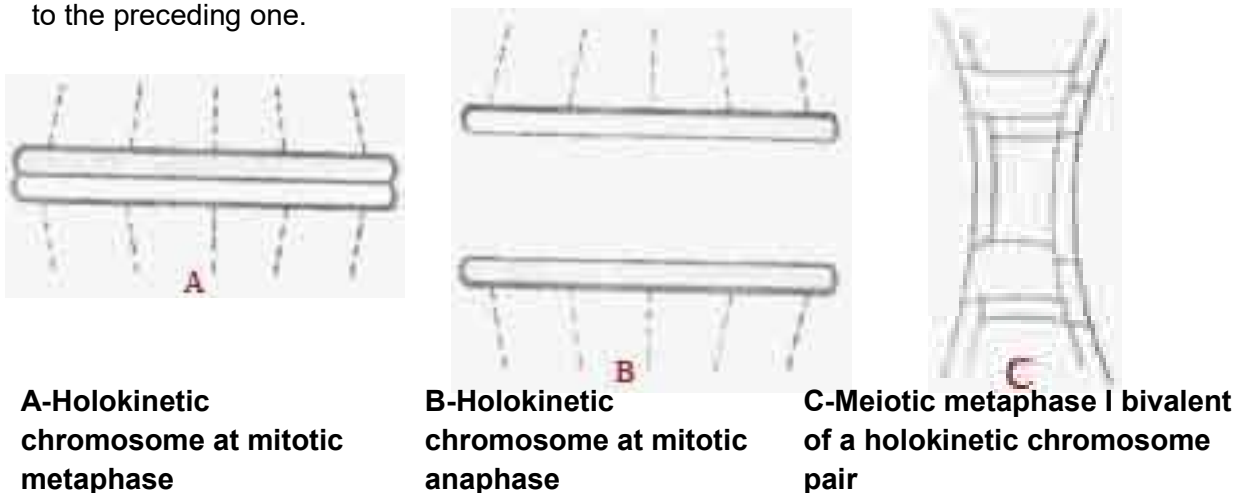
disjunction of B-chromosomes of rye plant is found to be caused due to the presence of a heterochromatic knob at the end of long arm of B-chromosome.



The origin of the B-chromosomes is uncertain. In some animals they may be derivatives of sex chromosomes, but this is not the rule. They generally do not show any pairing affinity with the A-chromosomes.

Holokinetic chromosomes

The chromosomes of most plants and animals have centromeres that are situated at one specific position in each chromosome. In a number of animals, especially in insects of the order Hemiptera and a few, mostly monocotyledonous plants (Juncales, Cyperales), the kinetic activity is distributed over the entire chromosome and such chromosomes are called Holokinetic chromosomes (Sybenga, 1972). The term -diffuse centromere has been used as an alternative but is not quite logical. In mitotic metaphase, the chromatids of a Holokinetic chromosome orient parallel in the equator: one chromatid towards one pole the other towards the other pole. This is also the way they separate in anaphase and they maintain this orientation until they arrive at the poles. Probably kinetic activity starts at one point and proceed from there on, orienting each unit to the preceding one.



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Genetic Significance of Chromosomes

The chromosomes are considered as the organs of heredity because of following reasons:

- (i) They form the only link between two generations.
- (ii) A diploid chromosome set consists of two morphologically similar (except the X and Y sex chromosomes) sets, one is derived from the mother and another from the father at fertilization.
- (iii) The genetic material, DNA or RNA is localized in the chromosome and its contents are relatively constant from one generation to the next.
- (iv) The chromosomes maintain and replicate the genetic informations contained in their DNA molecule and this information is transcribed at the right time in proper sequence into the specific types of RNA molecules which directs the synthesis of different types of proteins to form a body form like the parents.

KARYOTYPE

A karyotype is the characteristic chromosome complement of a eukaryote species. The preparation and study of karyotypes is part of cytogenetics. The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated $2n$. Thus, in humans $2n=46$. In the germ-line (the sex cells) the chromosome number is n (humans: $n=23$). So, in normal diploid organisms, autosomal chromosomes are present in two copies. There may, or may not, be sex chromosomes. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies. The study of whole sets of chromosomes is sometimes known as karyology. The chromosomes are depicted (by rearranging a microphotograph) in a standard format known as a karyogram or idiogram: in pairs, ordered by size and position of centromere for chromosomes of the same size. Karyotypes can be used for

many purposes; such as, to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

Idiogram

Staining

The study of karyotypes is made possible by staining. Usually, a suitable dye is applied after cells have been arrested during cell division by a solution of colchicine. For humans, white blood cells are used most frequently because they are easily induced to divide and grow in tissue culture. Sometimes observations may be made on non-dividing (interphase) cells. The sex of an unborn fetus can be determined by observation of interphase cells (see amniotic centesis and Barr body).

Most (but not all) species have a standard karyotype. The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes. Normal karyotypes for females contain two X chromosomes and are denoted 46, XX; males have both an X and a Y chromosome denoted 46,XY. Any variation from the standard karyotype may lead to developmental abnormalities.

Six different characteristics of karyotypes are usually observed and compared:

1. differences in absolute sizes of chromosomes. Chromosomes can vary in absolute size by as much as twenty-fold between genera of the same family: *Lotus tenuis* and *Vicia faba* (legumes), both have six pairs of chromosomes ($n=6$) yet *V. faba* chromosomes are many times larger. This feature probably reflects different amounts of DNA duplication.
2. differences in the position of centromeres. This is brought about by translocations.
3. differences in relative size of chromosomes can only be caused by segmental interchange of unequal lengths.
4. differences in basic number of chromosomes may occur due to successive unequal translocations which finally remove all the essential genetic material from a chromosome, permitting its loss without penalty to the organism (the dislocation hypothesis). Humans have one pair fewer chromosomes than the great apes, but the genes have been mostly translocated (added) to other chromosomes.
5. differences in number and position of satellites, which (when they occur) are small bodies attached to a chromosome by a thin thread.
6. differences in degree and distribution of heterochromatic regions. Heterochromatin stains darker than euchromatin, indicating tighter packing, and mainly consists of genetically inactive repetitive DNA sequences.

A full account of a karyotype may therefore include the number, type, shape and banding of the chromosomes, as well as other cytogenetic information.

Variation is often found:

1. between the sexes
2. between the germ-line and soma (between gametes and the rest of the body)
3. between members of a population (chromosome polymorphism)
4. geographical variation between races
5. mosaics or otherwise abnormal individuals

IDEOGRAMS

Ideograms are a schematic representation of chromosomes. They show the relative size of the chromosomes and their banding patterns. A banding pattern appears when a tightly coiled chromosome is stained with specific chemical solutions and then viewed under a microscope. Some parts of the chromosome are stained (G-bands) while others refuse to adopt the dye (R-bands). The resulting alternating stained parts form a characteristic banding pattern which can be used to identify a chromosome. The bands can also be used to describe the location of genes or interspersed elements on a chromosome.

Below is an ideogram of each human chromosome. Next to the known schematic representation a chromosome was added that was rendered from DNA data. The G-bands, areas with proportional more A-T base pairs, are normally colored black in schematic representations. To compare the schematic ideograms the rendered chromosomes, were colored the A-T bases black and the G-C bases white. Blue areas in the rendered chromosomes identify bases not known yet.

The results are interesting. When comparing the schematic ideograms with the rendered chromosomes from our project, a significant conformancy can be seen. Most black areas on the left side show also black areas on the right side and white areas are also white on the "digital" chromosomes.

