

Session-2

Composition of chromosomes: The material of which chromosomes are composed is called chromatin. N.Fleming introduced the term chromatin in 1879. Chromatin = DNA + Histone proteins. The basic unit of chromatin is Nucleosome. Chromatin was classified into two groups by cytologists when chromosomes are stained with stains such as basic dyes like acetocarmine or feulgen (basic fuchsin) reagent at prophase. The darkly stained regions were called heterochromatin, while lightly stained regions were called euchromatin. This differential staining capacity of different parts of chromosomes is known as 'heteropycnosis'. In general heterochromatin is found in centromeric and telomeric regions and these regions of chromosome generally replicate later than the euchromatic regions of chromosomes. The genes within the heterochromatic regions are usually inactive. Most of the genome of an active cell is euchromatic and the genes within this euchromatic region are expressed. Heterochromatin is further classified into two groups:

a) Constitutive and b) Facultative

a) **Constitutive heterochromatin:** It is present in all cells at identical positions on both homologous chromosomes of a pair.

b) **Facultative heterochromatin:** It varies in state in different cell types, at different stages or sometimes, from one homologous chromosome to another. A well known example of facultative heterochromatin is the Barr body, an inactivated X chromosome in somatic cells of mammalian female (XX).

Differences between Heterochromatin and euchromatin:

	Heterochromatin	Euchromatin
1.	Represent darkly stained regions of the chromatin due to tight coiled DNA	Lightly stained regions of the chromatin due to loosely coiled DNA
2.	Usually found near centromere and telomere	Found in the middle of chromosome between centromere and telomere
3.	Genes in heterochromatin are transcriptionally inactive due to tightly coiled DNA	Contains lot of active genes and plays active role in transcription
4	Two types – Constitutive and facultative	Only one type
5	Examples are chromomeres, chromocentre (occur near centromere of giant polytene chromosomes) and Knobs of maize (spherical heterochromatin bodies seen in pachytene stage that serve as valuable chromosome markers for distinguishing related species and races)	

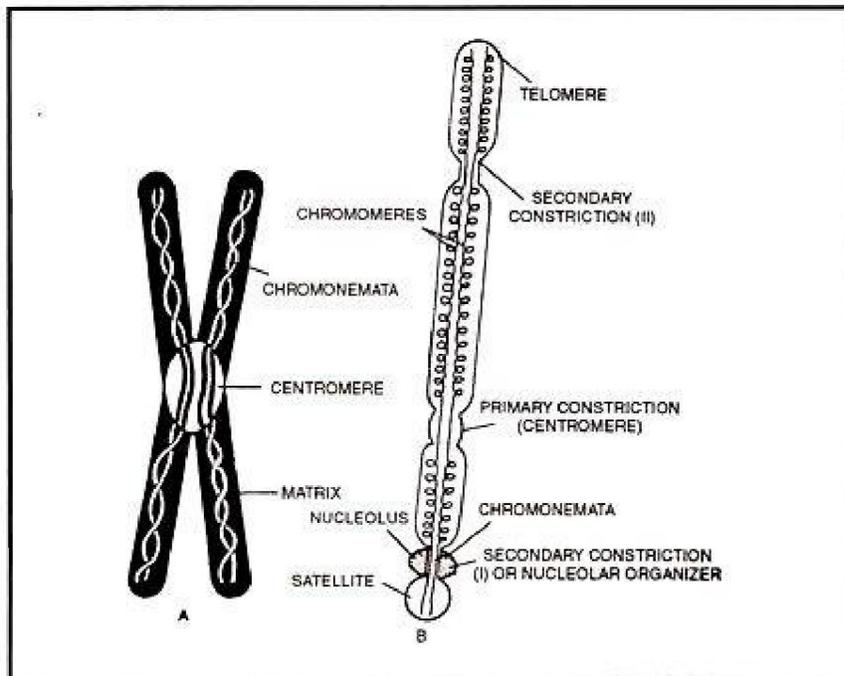


Fig. 49. Structure of chromosome (in metaphase stage)

Characteristic Features of Chromosomes:

i. Karyotype:

Karyotype refers to the characteristic morphological features of chromosomes of a species such as chromosome Number, Size, Position of centromere, Position of satellite, and degree and distribution of heterochromatin. Generally, karyotype is represented by arranging the haploid set of chromosomes an organism in descending order of size, keeping their centromeres in the same line. Thus the largest chromosome is placed on extreme left and the shortest on extreme right. The diagrammatic representation of karyotype of a species is known as ideogram or ideotype (Fig. 4.2). Hence, the karyotype represents haploid (gametic) chromosome number in a given

species.

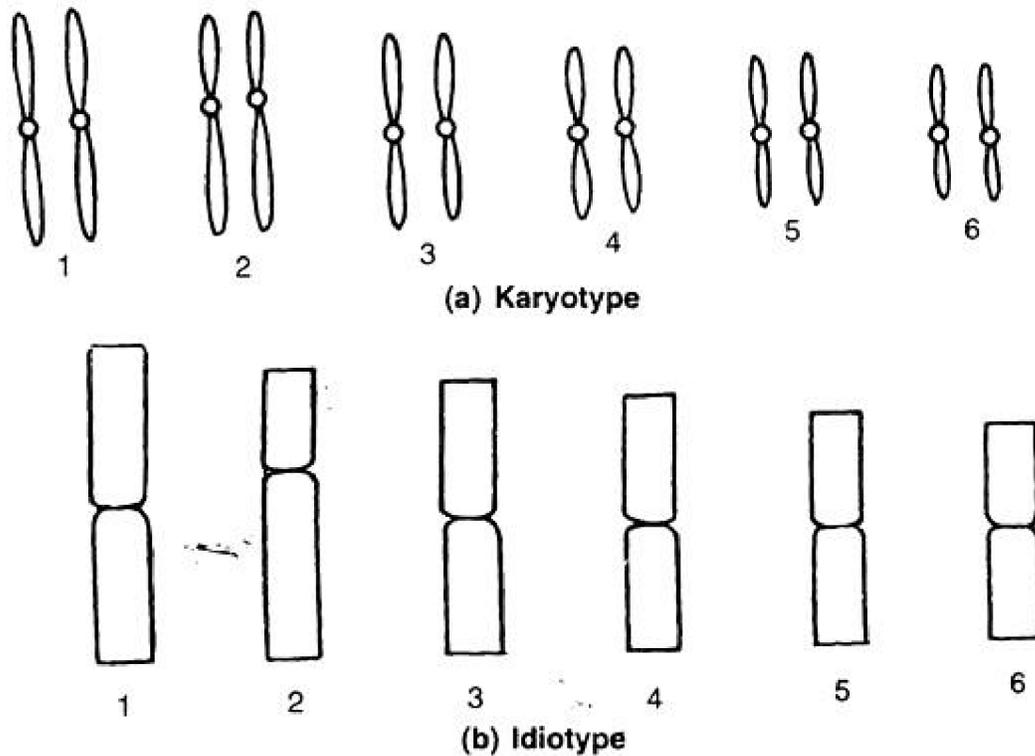


Fig. 4.2. A schematic representation of (a) Karyotype, and (b) idiotype using $2n = 12$.

Importance of karyotype study:

1. The karyotype is generally identical for a species, but it differs from species to species. Hence karyotype represents evolutionary relationships.
2. KARYOTYPE suggest primitive and advanced feature of an organism. Karyotype is of two types, viz., symmetrical and asymmetrical. In the symmetrical karyotype, all the chromosomes are mostly Meta centric chromosomes and less variation in the size of chromosomes. Plant species with this type of karyotype are considered as primitive ones. In case of asymmetrical karyotype, the chromosomes show large differences between the size of the smallest and the longest chromosome and have fewer metacentric chromosomes. Plant species with asymmetrical karyotype are considered advanced from evolution point of view. Flowering plants having specialized zygomorphic flowers show asymmetrical karyotype. Thus, study of karyotype helps in understanding the evolutionary process.

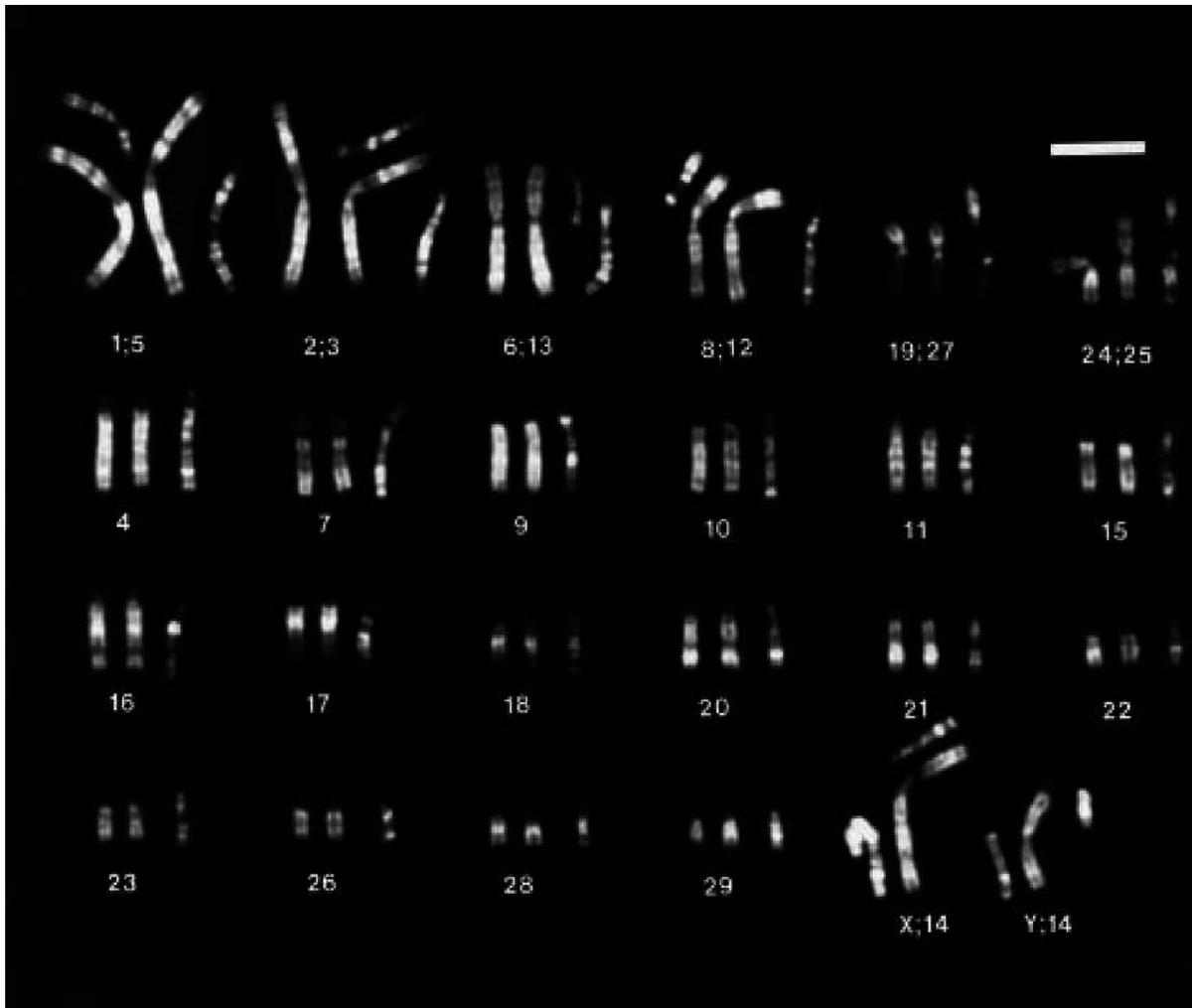
“Banding pattern for identification of Chromosomes

A Band is defined as a part of a chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter. Each chromosome has a unique banding pattern that is specific to each chromosome and are reproducible landmarks of chromosomes.

Uses of Chromosome Banding

1. Once karyotype is constructed, chromosome aberrations such as partial deletions and translocations in genetic diseases can be identified by banding techniques. Secondly specific chromosomes and also small chromosome fragments can be identified. This is because the chromosome banding pattern is constant and highly specific for each chromosome that allow distinction of similar sized chromosomes in a karyotype.
2. Similar sized chromosomes in a karyotype.

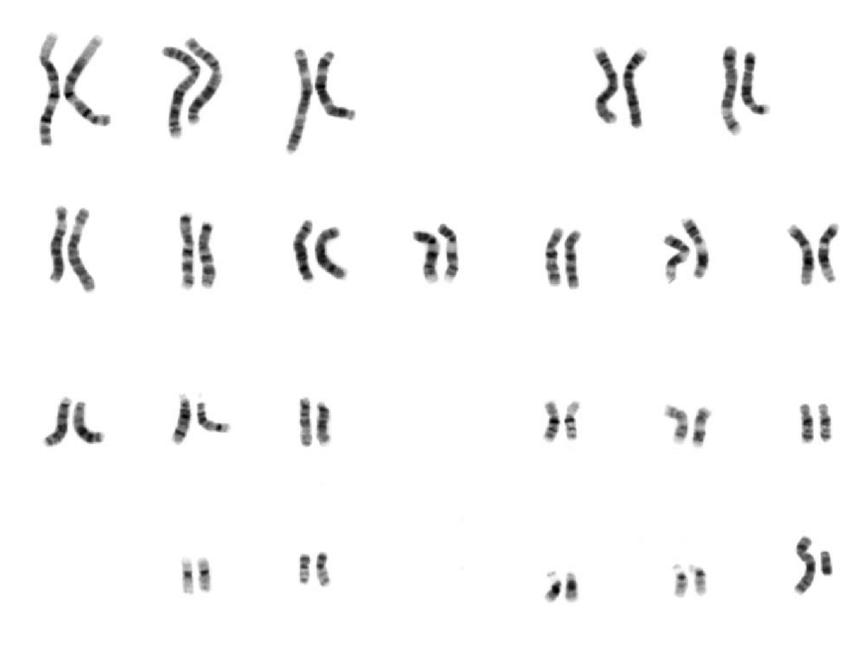
Quinacrine banding (Q-banding) (Caspersson et al.,) is obtained when chromosomes are stained with quinacrine mustard (QM) dye, a fluorochrome. The chromosomes display bright and dark bands when viewed under UV light and every chromosome pair can be identified by this fluorescence characteristics. The banding pattern is specific for each pair of chromosomes. Q-banding is most commonly used in plants.



Q-band karyotype

Giemsa banding (G-banding): It involves pre-treatment of the condensed metaphase chromosomes with trypsin (to partially digest the chromosomes), followed by staining with Giemsa. Giemsa is Romanowsky type dye mixture consisting of various dyes such as methylene blue, Azure A, B & C, Eosin and thionin. These dye components binds only to DNA that are rich in the base pairs Adenine (A) and Thymine (T) producing a dark band. Heterochromatic regions that are rich in A-T, replicates late and relatively gene poor stain more darkly in G-banding. G-banding shows alternate regions of heavy staining (called G bands) and light staining (interbands). The pattern of bands are numbered on each arm of the chromosome starting from centromere to the telomere, G-banding is most commonly used in analyzing vertebrate chromosomes but not plant chromosomes as plant chromosomes contain more DNA than vertebrates. The region where a transition occurs between a dark and light band is known as

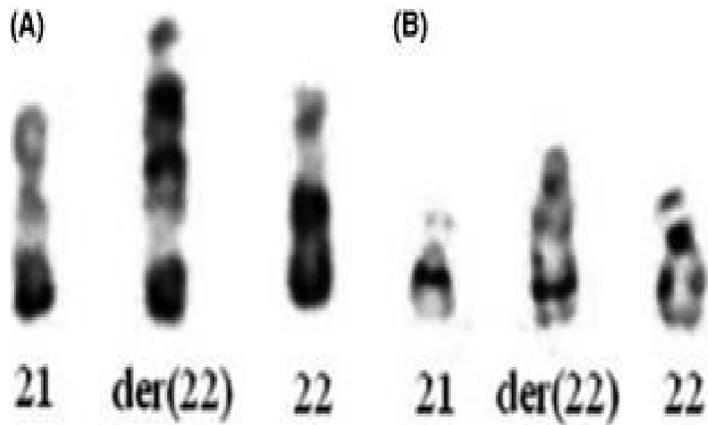
isochore. Q-banding and G- banding are similar in terms of preference to bind DNA regions that are heterochromatic (rich in A-T base pair) and therefore, Q-bands coincide with G-bands while R-banding creates the reverse banding pattern. That is, positively stained G and Q bands correspond to negatively stained R-bands and vice-versa.



Giemsa Banding

R (reverse)-banding: R-banding is just the reverse of G-banding pattern in that the lightly stained G-bands become darkly stained in R-banding and vice-versa. Here the chromosomes are incubated in phosphate (1M) buffer at 86-87⁰C at pH of 6.5 for 10 minutes, rinsed in water and stained with Giemsa stain. R band DNA is relatively G:C rich and contain more transcriptionally active genes.

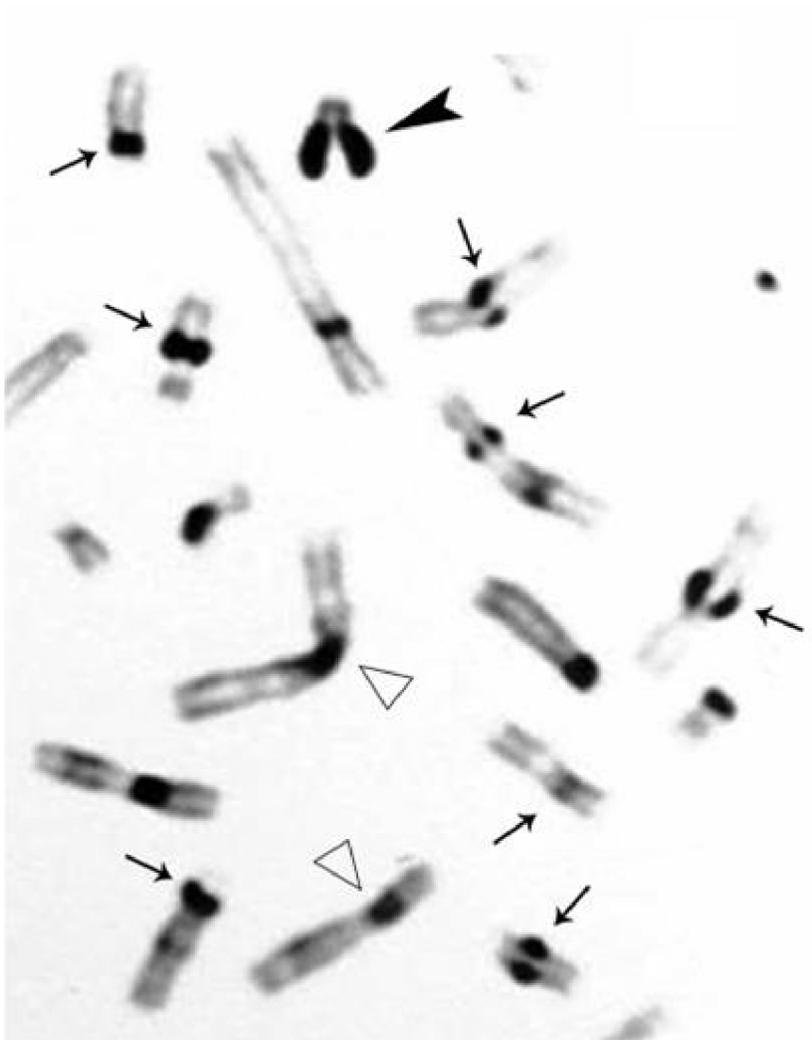
G-bands and Q-bands have less number of active genes due to less histone acetylation and are more heterochromatic than R-bands. R-bands have more transcriptionally active genes (euchromatic) due to histone acetylation.



R-banding (A) and G-banding (B) of chromosomes 21, 22, and the derivative chromosome 22

Constitutive heterochromatin banding (C-banding): a modification of in situ hybridization technique. It generally stains all constitutive heterochromatic regions such as centromere region, NOR region and Telomeres. Heterochromatic regions are condensed throughout cell cycle with genes remain in inactive state and contains highly repetitive DNA. In C-banding technique the

chromosomes are treated with hot alkali, or hot saline followed by staining with Geimsa.



C-banding of metaphase chromosomes. Arrows show selected chromosomes with premature centromere separation. Large arrowhead points to "splitting" of the Y [chromosome](#) heterochromatic region. Open arrows show selected chromosomes with normal C-banded regions.

N-banding: Unlike C-banding that stains all heterochromatic regions, N-banding stains specifically nucleolus organizers (NORs) in chromosomes. These nucleolus organizer region have rRNA genes.

Miscellaneous Bands

T-banding: Giemsa staining at higher temperatures such as 87°C and at pH of 6.7 will result in staining of terminal regions of chromosomes (that is, telomeres).