Course Name: PRINCIPLES OF CYTOGENETICS (2+1)
Course Code: MAGP 1102

Plant Cytogenetics: Rediscovery of Mendelian Laws of Inheritance generated interest in understanding chromosomal basis of inheritance. Therefore, experimental procedures and techniques were developed to elucidate structure of chromosomes and understand their behavior to underscore genetic principles. The hybrid discipline that combines cytology and genetics is termed as cytogenetics. Plant cytogenetics serves to integrate molecular biology, plant biotechnology and genetics with cytogenetics and also provide a conceptual foundation for modern genomics such as structural and functional genomics and comparative evolutionary genomics.

Course Objective: The course provides insight into the structure and number of chromosomes using microscopic analysis. For instance a decrease or an increase in the chromosomal number or translocation of one to another chromosome or even chromosome behavior during mitosis and meiosis. Haploidy and its applications in genetics and plant breeding are studied in detail. Additionally the course provides insight on chromosome mapping approaches in modern genomics, polyploidy and cytogenetic aspects of crop evolution.

Course Outcomes: Students gain knowledge on

CO-1: Evolution of various chromosomal aberrations (structural and numerical), their applications in alien gene transfer and hybrid seed development.

CO-2: pollen culture in haploid development and development of diploid inbreds or hybrids or doubled isogenic lines from haploids that has got important applications in plant breeding.

CO-3: With cytogenetic tools such as FISH and GISH (Genomic In Situ Hybridization) techniques, that rely on "painted chromosomes" approach, the behavior of individual genomes, individual chromosomes, or chromosomal fragments in natural and artificial hybrids (particularly allopolyploids) can be analyzed.

CO-4: Another important application of plant cytogenetics is in validation of physical maps and guiding efficient choice of bacterial artificial chromosomes for sequencing of genomes using chromosome walking and chromosome jumping.
UNIT I: Architecture of Prokaryotic Chromosomes: In many bacteria, the chromosome is a single covalently closed (circular) double-stranded DNA molecule that encodes the genetic information in a haploid form. The size of the DNA varies from 500,000 to several million base-pairs (bp) encoding from 500 to several thousand genes depending on the organism. The chromosomal DNA is present in cells in a highly condensed, organized form called nucleoid (nucleus-like), which is not encased by a nuclear membrane as in eukaryotic cells. The isolated nucleoid contains 80% DNA, 10% protein, and 10% RNA by weight. There are two essential aspects of nucleoid formation; condensation of a large DNA into a small cellular space and functional organization of DNA in a three-dimensional form. The haploid circular chromosome in *E. coli* consists of ~ 4.6 x 10⁶ bp. The essential aspect of nucleoid formation is the functional arrangement of DNA. Chromosomal DNA is not only condensed but The DNA in the chromosome appears to have a condition-dependent 3-D structure that is linked to gene expression so that the nucleoid architecture and gene transcription are tightly interdependent, influencing each other reciprocally. Almost five decades of research beginning in 1971, has shown that the final form of the nucleoid arises from a hierarchical organization of DNA. At the smallest scale (1-kb or less), nucleoid-associated DNA architectural proteins condense and organize DNA by bending, looping, bridging or wrapping DNA. At a larger scale (10-kb or larger), DNA forms plectonemic loops, a braided form of DNA induced by supercoiling. At the megabase scale, the plectonemic loops coalesce into six spatially organized domains (macrodromains), which are defined by more frequent physical interactions among DNA sites within the same macrodomain than between different macrodomains. Long- and short-range DNA-DNA connections formed within and between the macrodomains contribute to condensation and functional organization. Finally, the nucleoid is a helical ellipsoid with regions of highly condensed DNA at the longitudinal axis.

**Bacterial Chromosome Structure**

**A. Circular E. coli genome**

**B. Random coil of the DNA**

**C. Genome organization in vivo**

---

**Fig 1. Formation of the *Escherichia coli* nucleoid. A.** An illustration of an open conformation of the circular genome of *E. coli*. Arrows represent bi-directional DNA replication. The genetic position of the origin of bi-
directional DNA replication (oriC) and the site of chromosome decatenation (dif) in the replication termination region (ter) are marked. Colors represent specific segments of DNA as discussed in C. B. An illustration of a random coil form adopted by the pure circular DNA of E. coli at thermal equilibrium without supercoils and additional stabilizing factors [5, 6]. C. A cartoon of the chromosome of a newly born E. coli cell. The genomic DNA is not only condensed by 1000-fold compared to its pure random coil form but is also spatially organized. oriC and dif are localized in the mid-cell, and specific regions of the DNA indicated by colors in A organize into spatially distinct domains. Six spatial domains have been identified in E. coli. Four domains (Ori, Ter, Left, and Right) are structured and two (NS-right and NS-left) are non-structured (See section 4 of the main text for details). The condensed and organized form of the DNA together with its associated proteins and RNAs is called nucleoid. Drawings are not in scale with each other.

---

Eukaryotic Chromosome Structure

Eukaryotic cells contain their DNA within the nuclear membrane.

The Histone Code

- Methylation of histone or of DNA usually turns a gene off.
- Acetylation of histone usually turns a gene on.
- Phosphorylation — we're not sure what that does.

Nucleosome – solenoid model: This model was proposed by Kornberg and Thomas in 1974. Chromatin is composed of DNA, RNA, histones and other proteins. Histones are basic proteins rich in lysine and arginine and lacks tryptophan. The nucleosomes are basic sub units of chromatin and have bead like appearance. Each
nucleosome is a disc-shaped particle containing 2 copies of each type of histones–H2A, H2B, H3 and H4. This histone octamer forms a protein core [(H2A-H2B) (H3-H4)\textsubscript{2}(H2A-H2B)] around which the double-stranded DNA helix makes 1.7 turns left-handed superhelix that corresponds to 146 bp. Each nucleosome bead is separated from the next by a region of linker DNA which is generally 54 base pair long and contains single H1 histone protein molecule.

1. **The 10-nm fibre:** This is the first level of packing in chromatin is called nucleosome. The repeating nucleosomes with intervening "linker" DNA form a *10-nm-fiber*, described as "beads on a string", and have a packing ratio of about five to ten i.e., five to ten times more compact than free DNA.

2. **The 30-nm fibre (solenoid):** The second level of packing is called solenoid. In the second level of packing, histone H1 molecules are found responsible for packing of nucleosomes into the 30-nm thick chromatin fibre called solenoid. In the 30-nm fibre, nucleosomes are closely packed (about six nucleosomes per turn) with a packing ratio of \( \sim 50 \) and is known as solenoid. Hence, Solenoid is a compacted structure with a packing ratio of \( \sim 50 \) and whose formation is dependent on the presence of the H1 histone. This model is universally accepted as a model of chromatin fibre organization. The packaging of DNA into a 30-nm chromatin fibre leaves a human chromosome about 0.1 cm long, so there must be several higher orders of folding. Inside the nucleus, this 30 nm solenoid structure undergoes further coiling to produce a chromatin fibre of 300 nm thick. 300 nm thick chromatin filaments are condensed into the 700 nm thick chromatid and is seen at metaphase stage of cell division. During cell division a 1,400 nm thick metaphase chromosome is produced containing two chromatids, each chromatid being 700 nm.
Figure: The nucleosomes (10 nm each) are condensed into 30 nm solenoid fiber structure; 30 nm solenoids are condensed into 300 nm filament; 300 nm filaments are condensed into the 700 nm chromosome. During cell division, when the chromosomes duplicate, a 1,400 nm metaphase chromosome is produced containing two chromatids, each chromatid being 700 nm. The inset shows the position of individual histones with respect to each other as viewed from the top, and the left-handed wrapping of the DNA around each histone octamer core.

DNA → nucleosome → solenoid → chromatin fibre → chromatid → chromosome  
(2 nm dia)  (10 nm dia)  (30 nm diameter)  (300 nm diameter)  (700 nm diameter)  (1400 nm diameter)

At some places chromatin is densely packed to form darkly staining heterochromatin. At other places chromatin is loosely packed. It is called euchromatin. Euchromatin is lightly stained. It is transcriptionally active chromatin whereas heterochromatin is transcriptionally inactive and late replicating or heteropycnotic.
In mitosis, the chromosomes appear as the thick rod-shaped bodies which can be stained and visualized under light microscopy.

The modern way to visualize condensed chromosomes is by FISH -- fluorescence in situ hybridization. In this method, fluorescent antibody-tagged DNA probes hybridize to their complementary sequences in the chromosomes. By using FISH probes with different colored fluorophores, one can color each human chromosome independently, and thus identify all 23 chromosomes. This is called chromosome painting.

Architecture of Eukaryotic Chromosomes

E. Strasburger in 1875 first discovered thread-like structures which appeared during cell division. These thread like structures were called chromosomes due to their affinity for basic dyes. The term chromosome is derived from two Greek words; chrom = colour, soma=body. This term was first used by Waldeyer in 1888. Chromosomes contributed to the division of cells and they are of prime importance as they carry the genes which are the hereditary material.

**Chromosome number:** The number of chromosomes in a given species is generally constant. All the members of the species ordinarily have definite and generally a constant somatic and gametic chromosome number. Generally somatic cells contain two copies of each chromosome except the sex chromosomes. Both the copies are ordinarily identical in morphology, gene content and gene order and hence known as homologous chromosomes. Somatic chromosome number is the number of chromosomes found in somatic cells of a species and is represented by 2n. Gametic chromosome number is exactly half of somatic chromosome number and is represented by n. It denotes the number of chromosomes found in gametes of a species. The number of chromosomes varies greatly from $2n = 4$ ($n = 2$) in *Haplopappus gracilis* (Compositae) to $2n = > 1200$ in some pteridophytes. Among animals *Aulacantha* (a radiolarian)
the diploid chromosome number is 1600. For actual chromosome numbers, x should be used as follows: x, 2x, 3x, 4x, 5x, 6x represent chromosome number for haploids, diploids, and a variety of polyploids.

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Chromosome number (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>24</td>
</tr>
<tr>
<td>Tomato</td>
<td>24</td>
</tr>
<tr>
<td>Wheat</td>
<td>42</td>
</tr>
<tr>
<td>Onion</td>
<td>16</td>
</tr>
<tr>
<td>Maize</td>
<td>20</td>
</tr>
<tr>
<td>Garden pea</td>
<td>14</td>
</tr>
<tr>
<td>Upland cotton</td>
<td>52</td>
</tr>
<tr>
<td>Human beings</td>
<td>46</td>
</tr>
<tr>
<td>Evening primrose (Oenothera)</td>
<td>14</td>
</tr>
<tr>
<td>Drosophila</td>
<td>8</td>
</tr>
</tbody>
</table>

Size: The size of the chromosome shows a remarkable variation depending upon the stage of cell division. The chromosomes are the longest and thinnest during interphase (resting stage) and hence not visible under light microscope. Chromosomes are the smallest and thickest during mitotic metaphase. In general, plants have longer chromosomes than animals and species having lower chromosome number have longer chromosomes than those having a higher chromosome number. Among plants, dicots in general have shorter and higher number of chromosomes than monocots. Among the higher plants, the longest mitotic chromosomes are those of Trillium sps., which may reach 32 µ in size. In most fungi all chromosomes are extremely minute. Chromosome size is not proportional to the number of genes present on the chromosome.

Morphology: The outer covering or sheath of a chromosome is known as pellicle, which encloses the matrix. Within the matrix lies the chromatin. Flemming introduced the term chromatin in 1879. The term chromatin refers to the Feulgen positive materials observed in interphase nucleus and later during nuclear division. Chromatin readily stains with basic dyes especially Basic Fuchsin, which is specific for DNA which in turn is a major constituent of chromosomes. The chromosome morphology changes during cell division and mitotic metaphase is the most suitable stage for studies on chromosome morphology. In mitotic metaphase chromosomes, the following structural features can be seen under the light microscope.
1. **Chromatid:** Each metaphase chromosome appears to be longitudinally divided into two identical parts each of which is called chromatid. Both the chromatids of a chromosome appear to be joined together at a point known as centromere. The two chromatids of chromosome separate from each other during mitotic anaphase (and during anaphase II of meiosis) and move towards opposite poles. Since the two chromatids making up a chromosome are produced through replication of a single chromatid during synthesis (S) phase of interphase, they are referred to as sister chromatids. In contrast, the chromatids of homologous chromosomes are known as non-sister chromatids.

2. **Centromere:** Centromere and telomere are the most stable parts of chromosomes. The region where two sister chromatids appear to be joined during mitotic metaphase is known as centromere. It generally appears as constriction and hence called primary constriction. Centromere is a localized and easily detectable morphological region of the chromosomes which helps in the movement of the chromosomes to opposite poles during anaphase of cell division. The centromere divides the chromosomes into two transverse parts called arms. The centromere consists of two disk shaped bodies called kinetochores. The kinetochores do not form part of the chromatid but lie one on each side of the chromosome such that each chromatid is having its own kinetochore. One kinetochore is attached to the spindle fibres towards one pole and the other similarly towards the other pole. Depending on position of the centromeres, chromosomes can be grouped as:

   a) **Metacentric:** Centromere is located exactly at the centre of chromosome, i.e. both arms are equal in size. Such chromosomes assume ‘V’ shape at anaphase.

   b) **Submetacentric:** The centromere is located on one side of the centre point such that one arm is longer than the other. These chromosomes become ‘J’ or ‘L’ shaped at anaphase.

   c) **Acrocentric:** Centromere is located close to one end of the chromosome and thus giving a very short arm and a very long arm. These chromosomes acquire ‘ J’ shape or rod shape during anaphase.

   d) **Telocentric:** Centromere is located at one end of the chromosome so that the chromosome has only one arm. These chromosomes are ‘I” shaped or rod shaped.

Normally chromosomes are monocentric having one centromere each. Acentric (without centromere) and dicentric (with two centromeres) chromosomes, if produced due to chromosomal aberrations, cannot orient properly on the equatorial plate and lag behind other chromosomes during anaphase movements. In certain organisms, centromere does not occupy a specific position, but is diffused throughout the body of chromosome. Such chromosomes having diffused centromere, are found in **Luzula sps. and insects** belonging to the order Hemiptera.
3. **Telomere**: The two ends of chromosomes are known as telomeres. They are highly stable and do not fuse or unite with telomeres of other chromosomes due to polarity effect. The telomeres impart stability to the chromosome and hence confer identity and individuality to chromosomes through cell cycle and for many cell generations.

4. **Secondary constriction**: The constricted or narrow region other than that of centromere is called secondary constriction and the chromosomes having secondary constriction are known as satellite chromosomes or sat chromosomes. Chromosome may possess secondary constriction in one or both arms of it. Chromosomal end distal to the secondary constriction is known as satellite. Production of nucleolus is associated with secondary constriction and therefore it is also called nucleolus organizer region and satellite chromosomes are often referred to as nucleolus organizer chromosomes.

5. **Chromonema (plural chromonemata)** is the thread-like coiled filament are found in the chromosomes of early prophase. These extend the entire length of a chromosome and are the gene bearing portion of chromosomes. The term chromonema was coined by Vejdovsky in 1912.

6. **Chromomere**: The serially aligned beads or granules over the chromonema of a eukaryotic chromosome, resulting from tight coiling of DNA and appear as dark staining bands in the giant salivary gland chromosomes. These represent heterochromatic regions. It is visible on a chromosome during the prophase of meiosis (pachytene stage) and mitosis. These are invisible in mitotic metaphase chromosomes.

7. **Matrix**: The mass of acromatic material which surrounds the chromonemata is called matrix. The matrix is enclosed in a sheath which is known as pellicle. Both matrix and pellicle are non genetic materials and appear only at metaphase, when the nucleolus disappears.