Democratic and Popular Algerian Republic
Ministry of Higher Education and Scientific Research
A. Mira University - Bejaia

جامعة بجاية
Tasdawit n Bgayet
Université de Béjaïa

Faculty of Natural and Life Sciences
Department of Environmental Biological Sciences

Cytogenetics Course

Prepared by: DJAFRI-BOUALLAG Linda

Intended for students in:

- Second year of the Master's in Biodiversity and Food Safety
- Second year of the Master's in Biotechnology and Plant Valorization

Academic Year 2025-2026

PREAMBLE

Target audience: This course is intended for students in:

- Second year of the Master's in Biodiversity and Food Safety
- Second year of the Master's in Biotechnology and Plant Valorization
- Any student whose curriculum includes cytogenetics (genetics, medicine, and related fields)

Total hours: 45 hours of lectures and 22 hours of practical work

Prerequisites: Basic knowledge of general genetics, cell biology (mitosis, meiosis) and molecular biology (DNA structure, replication). Familiarity with basic microscopy techniques is recommended.

Course objectives:

- Upon completion of this course, the student will be able to:
- Master the main chromosomal analysis techniques (karyotyping, banding, FISH)
- Identify different types of chromosomal aberrations and their consequences
- Understand the role of chromosomal rearrangements in the evolution and diversification of plant species

COURSE CONTENT

Introduction

Chapter I: Chromosome Structure and Ultrastructure

Chapter II: Methods for Studying Chromosomes

- 2.1. Mitosis (Karyotype, Karyogram)
- 2.2. Meiosis (Behaviour and Meiotic Irregularities)
- 2.3. Chromosome Banding (Euchromatin, Heterochromatin, C, R, G Bands)
- 2.4. Flow Cytometry
- 2.5. Molecular Hybridisation (Probes and Labelling, FISH and GISH Techniques)

Chapter III: Chromosomal Variations

- 3.1. Chromosome Number
  - Euploidy (Autopolyploidy, Allopolyploidy)
  - Aneuploidy (Polysomy, Monosomy, etc.)
- 3.2. Chromosome Morphology
  - Isochromosomes, Ring Chromosomes, Robertsonian Translocations, Breakage-Fusion-Bridge Cycle
- 3.3. Chromosome Size
- 3.4. Chromosome Structure (Translocations, Inversions, Deletions, Duplications)

Chapter IV: Cytogenetics and Evolution

- 4.1. Variation in DNA Quantity, Gene Duplication, and Transposable Elements
- 4.2. Chromosomal Restructuring and Evolution
- 4.3. Polyploidy and Evolution

Introduction

Cytogenetic is a discipline at the interface of cell biology and genetics, which studies the structure, behavior, and abnormalities of chromosomes within cells. It enables us to understand their role in heredity, genetic diseases, crop improvement, and, on a fundamental level, the evolution of species. Founded at the beginning of the 20th century by Walter Sutton and Theodor Boveri, it combines molecular and microscopy techniques to analyze chromosomes. There are two main approaches in cytogenetic. Conventional cytogenetic, also known as classical cytogenetic, analyses the karyotype (the number and morphology of chromosomes) using banding techniques (G-bands, C-bands, N-bands) which identify specific chromosomal regions. Molecular cytogenetic uses more advanced techniques such as *FISH* (Fluorescence *In Situ* Hybridization) and *GISH* (Genomic *In Situ* Hybridization) to locate specific DNA sequences or study hybrid genomes.

Areas of Application

Medical Field: Cytogenetic techniques are involved in diagnosing chromosomal abnormalities such as aneuploidies (Down's syndrome, Turner syndrome) and structural alterations (translocations, e.g., the Philadelphia chromosome in CML). They are also used in oncology to identify tumour markers. In reproduction, they are used for the analysis of spermatozoa in cases of male infertility and in prenatal diagnosis (amniocentesis, NIPT).

Crop Improvement (Agronomy): These techniques use polyploidization to create resistant varieties (wheat, banana), interspecific hybridization (triticale, a hybrid of wheat and rye) and genetic mapping to locate genes of interest using FISH.

Evolutionary Biology: In the study of speciation mechanisms, they are used to determine the origin of polyploids (autopolyploidy vs. allopolyploidy), to demonstrate the impact of chromosomal rearrangements (inversions, translocations), and to trace the evolutionary history of species (phylogeny).

Species Conservation: They are used for detecting inbreeding and managing threatened populations (analysis of gene flow).

Fundamental Research: They are used to study errors in meiosis (non-disjunction) and the structure of chromatin (the role of heterochromatin in genetic regulation).

Chapter I: Structure and Ultrastructure of Chromosomes

1. What is a chromosome?

The term "chromosome", from the Greek *khroma* (colour) and *soma* (body), was introduced by W. Waldeyer in 1888. He used it to describe the rod-shaped structures that become visible in the cell after the disappearance of the nuclear envelope during cell division, and which stain intensely.

Originally, the term "chromosome" was reserved for eukaryotic cells. After the Second World War, with advances in molecular biology, its use was extended to describe the genetic material of bacteria and even viruses.

The structure and number of chromosomes vary considerably between prokaryotes and eukaryotes. In prokaryotes (bacteria and archaea), the genome consists of only a single, circular chromosome, free in the cytoplasm. In contrast, eukaryotic cells possess several linear chromosomes, always contained within the nucleus. The number is characteristic of each species; thus, humans have 46, the fruit fly has 8, and rice has 24.

Functionally, a chromosome is a unit of the genome, which carries a set of genes arranged in a specific, linear order (a linkage group). Each of these gene groups is passed on intact from the mother cell to the daughter cells during cell division.

2. Chromosome morphology in light microscopy

During the life of a cell, the genetic material changes appearance remarkably, passing through two distinct states according to cellular needs.

During growth and functional phases (interphase), the genome exists in a decondensed form, called chromatin, which allows the cell to express its genes and carry out its activities.

During cell division, the chromatin adopts a compact and condensed form, forming chromosomes that become visible under the microscope. This temporary transformation ensures the precise distribution of genetic material between the two daughter cells. It is at the metaphase stage that the chromosome reaches its maximum condensation.

The metaphase chromosome consists of four main structural elements (**Fig. 1**):

- **Two sister chromatids:** Each consists of a condensed DNA molecule. They are identical, having been produced by the replication of DNA in the S phase. They are joined together at the centromere.
- **The centromere (or primary constriction):** This is the region where the two sister chromatids are joined. It is also the site of attachment for the spindle fibres, essential for chromosome segregation. The centromere divides the chromosome into two arms: a short arm (p) (by convention situated above the centromere) and a long arm (q) (below the centromere).
- **The telomeres:** These are specialised structures located at the end of each chromosomal arm (one on the p arm, one on the q arm). Their function is to protect the end of the DNA and prevent the degradation of the chromosome.
- **The secondary constriction and the satellite:** Present only on certain chromosomes (such as acrocentric chromosomes), this is a narrowing located on a chromosomal arm. The region beyond it forms a small mass called a satellite. This area is often the site of the nucleolar organizers, where the genes coding for ribosomal RNA are located.

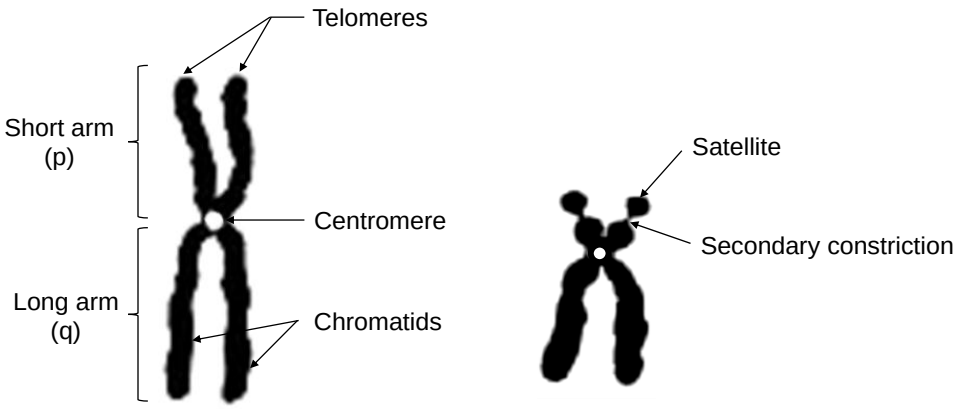

Fig. 1: Morphology of a metaphase chromosome

3. Structure of the Metaphase Chromosome

Chromosomes are composed of DNA, histone proteins, and non-histone proteins in roughly equivalent proportions. In vivo, DNA is not free in the nucleus but is associated with various proteins to form chromatin. This chromatin is organized into secondary and tertiary structures, resulting in varying degrees of compaction during the cell cycle – less compact during interphase to allow for gene transcription, and maximal during mitosis to facilitate the separation of chromatids into the daughter cells.

- **First level of organization: The 11 nm nucleofilament**

The basic structure of chromatin is the nucleosome. A nucleosome consists of a protein core formed by a histone octamer (two each of H2A, H2B, H3, and H4). A segment of DNA, approximately 146 base pairs long, wraps around this core, forming about 1.65 turns. This structure is then stabilized and locked by a linker histone, H1, which binds to the outside (**Fig. 2**). Nucleosomes are connected to each other by linker DNA segments of variable length (approximately 200 base pairs in mammals).

Observable under electron microscopy, this repetitive organization resembles a "string of beads" (regularly spaced nucleosomes, like beads threaded on a string of DNA). This chromatin fiber has a characteristic diameter of 11 nm and represents the first level of DNA compaction (**Fig. 3**).

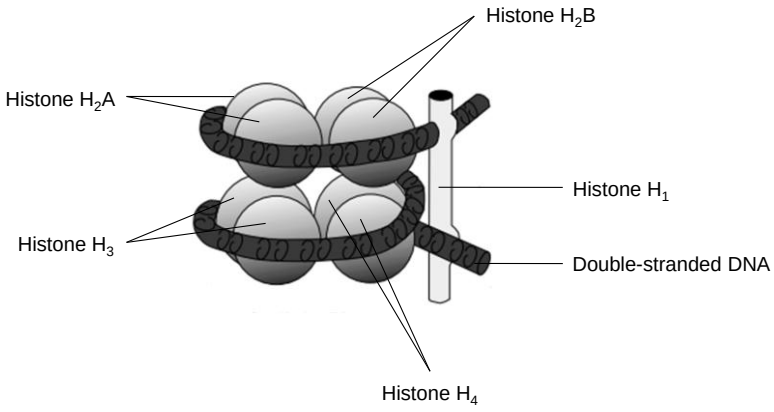

Fig. 2: Structure of a nucleosome (Griffiths et al. 2002)

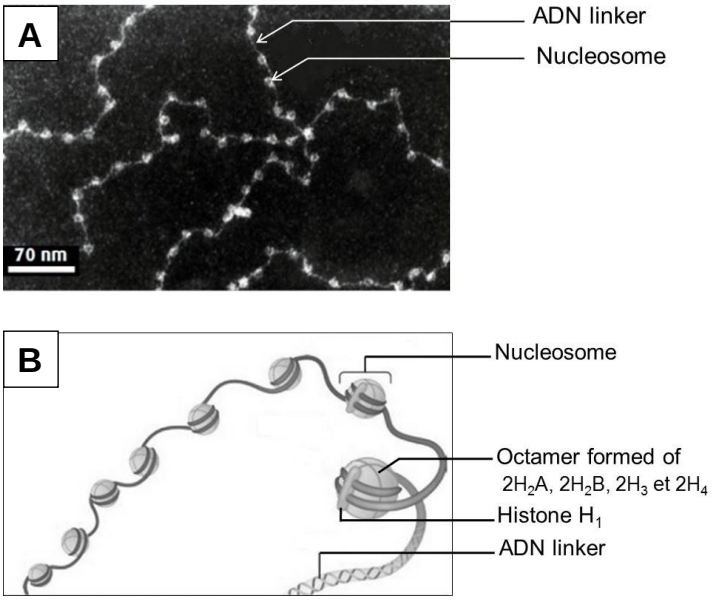

Fig. 3: The basic structure of a chromatin fiber;

A- View under a transmission electron microscope (TEM), B- Diagram. (Griffiths et al. 2002)

- **Second Level of Organization: The 30 nm Fiber**

The next level of chromatin compaction is the 30 nm fiber, which results from the coiling of the nucleosomal "string of beads". Two main models are proposed to describe its structure: the solenoid model (a tight helix) and the zigzag model (a looser helix).

➢ **The Solenoid Model:**

This model describes the 30 nm fiber as a tight, regular helix, within which successive nucleosomes coil around a central virtual axis, forming approximately six nucleosomes per helical turn. This compact architecture is stabilized by the linker histone H1 (**Fig. 4, 6**). It represents a high level of chromatin compaction, characteristic of the condensed, transcriptionally inactive regions found in heterochromatin.

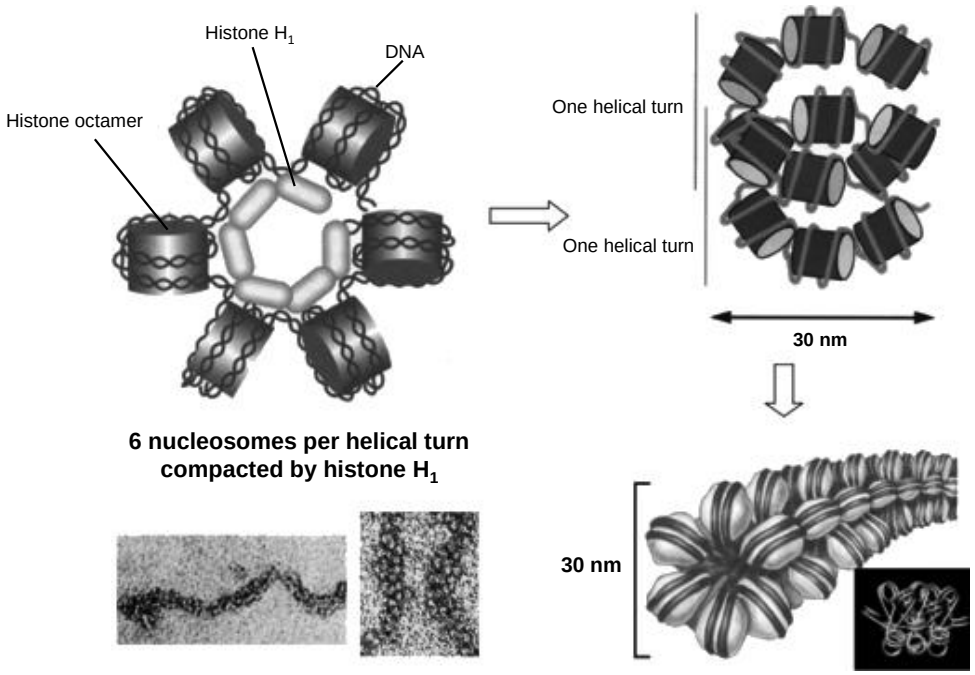

Fig. 4: The Solenoid Model (Griffiths et al. 2002)

- **The Zigzag Model:**

In contrast to the solenoid model, which proposes a tight, regular coil, the zigzag model (or "irregular helix") describes a looser, more open structure. Its main characteristic is that the linker DNA crosses the central axis of the fiber's coil between each successive nucleosome (**Fig. 5**). This creates an angular path where nucleosomes alternate on either side of the axis, forming a "zigzag" structure rather than a tight helix.

This organisation results from interactions between nucleosomes that are not adjacent on the DNA sequence (for example, nucleosome No. 1 interacts with No. 3 and so on). The length and flexibility of the linker DNA are therefore crucial. This model is predominantly supported by high-resolution structural data (crystallography, biophysics).

Unlike the compact solenoid, which is locked by histone H_1, the zigzag model represents a dynamic and flexible state of compaction. It is precisely this more open structure that characterises euchromatin, where DNA must remain accessible for gene transcription. It perfectly illustrates how chromatin structure (zigzag vs. solenoid) is directly linked to its function (gene expression vs. gene silencing).

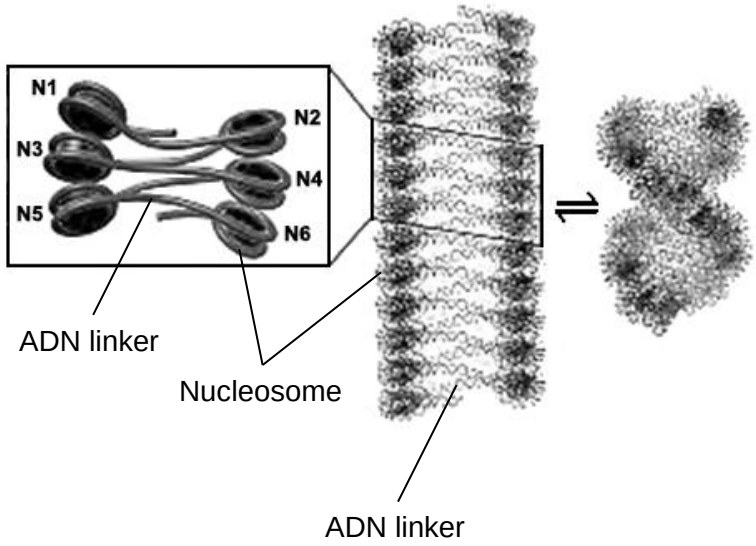

Fig. 5: The Zigzag Model (Robinson and Rhodes 2006)

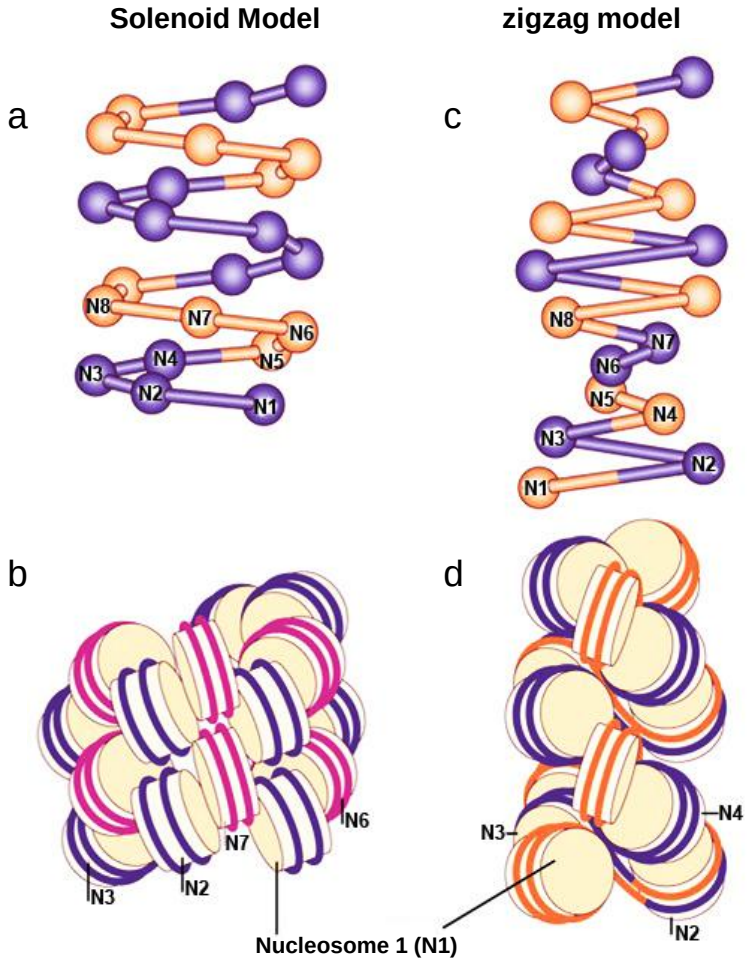

Fig. 6: Organization of chromatin into a 30 nm diameter fiber according to the solenoid model (a-b) in heterochromatin and the zigzag model (c-d) in euchromatin, adapted from Robinson and Rhodes (2006).

- **Third level of organization: The 300 nm fiber**

The 300 nm fiber represents the third level of chromatin compaction. It is formed by the folding of the 30 nm fiber into large DNA loops of 50,000 to 100,000 base pairs, anchored at their base to a central protein scaffold. This anchoring is mediated by specific sequences called SARs (Scaffold Attachment Regions) (**Fig. 7**).

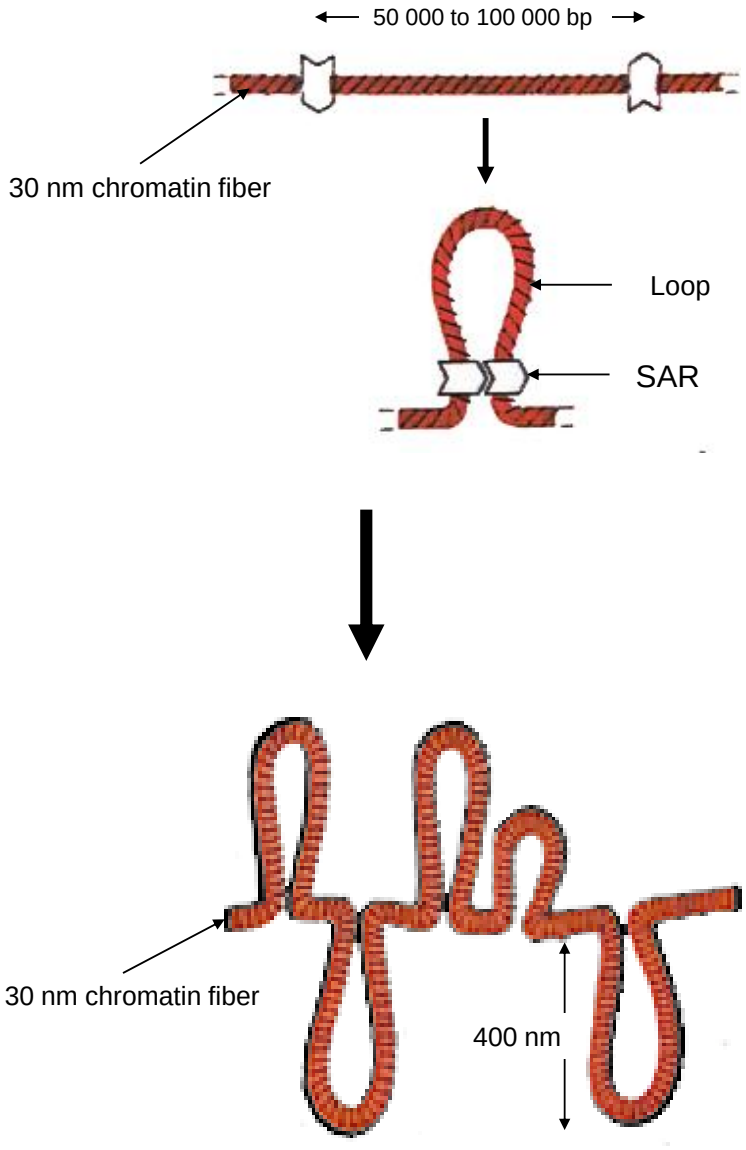

Fig. 7: Folding of a region of the 30 nm chromatin fiber to form a loop
SAR: Scaffold Attachment Regions (Alberts et al. 1986)

Architectural proteins such as condensins actively compress and stabilize these loops. This organization allows for extreme compaction while maintaining functional modularity: each loop forms an independent genetic domain, facilitating the targeted regulation of gene expression. This structure is a key step towards the formation of the highly condensed chromosomes observed during mitosis.

These loops do not function independently but are organized into rosettes, where several loops (typically 6 to 8) converge towards a common anchoring point on the nuclear matrix. The rosettes then cluster together to form larger chromosomal domains, which can reach up to 1 mega base in size (Fig. 8).

This hierarchical organization—from loops to rosettes and from rosettes to domains—is stabilized by architectural proteins like condensins, which actively compress and maintain the structure. This configuration enables extreme compaction while preserving functional modularity: each domain generally corresponds to a coherent genetic regulatory unit, where genes can be co-expressed or subjected to the same epigenetic regulation. Thus, the 300 nm fiber represents a crucial step towards the formation of metaphase chromosomes, while also allowing for the fine regulation of gene expression through its characteristic spatial organization.

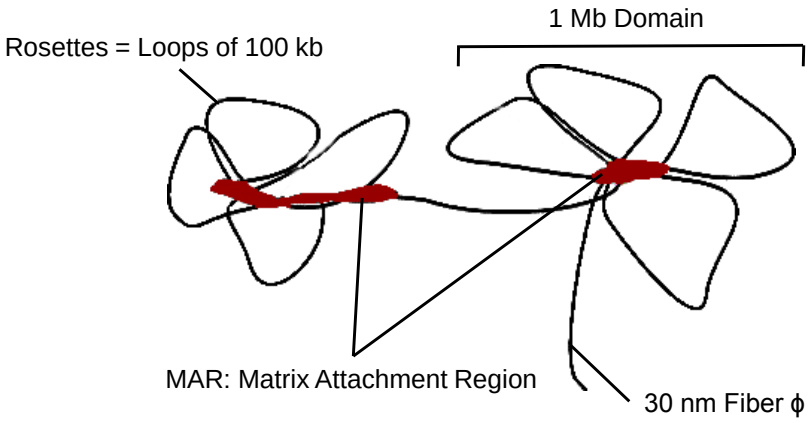

Fig. 8: The third level of chromatin organization

- **Fourth level of organization: The 700 nm chromatid**

The 700 nm chromatid represents the ultimate level of compaction before the formation of metaphase chromosomes. It results from the helical folding of the 300 nm fiber, which coils around a central protein axis to form a characteristic spiral structure. This central axis is composed mainly of topoisomerase II and SMC (Structural Maintenance of Chromosomes) proteins, which form a rigid scaffold allowing for the spatial organization of the chromatin.

This organization presents several remarkable structural features: the 300 nm fiber generates regular coils of approximately 700 nm in diameter around this central axis. The two sister chromatids display mirror symmetry with opposite coiling directions. This architecture is stabilized by specialized structural proteins, notably condensins (which compress the chromatin) and cohesins (which maintain the cohesion of the sister chromatids) (**Fig. 9**).

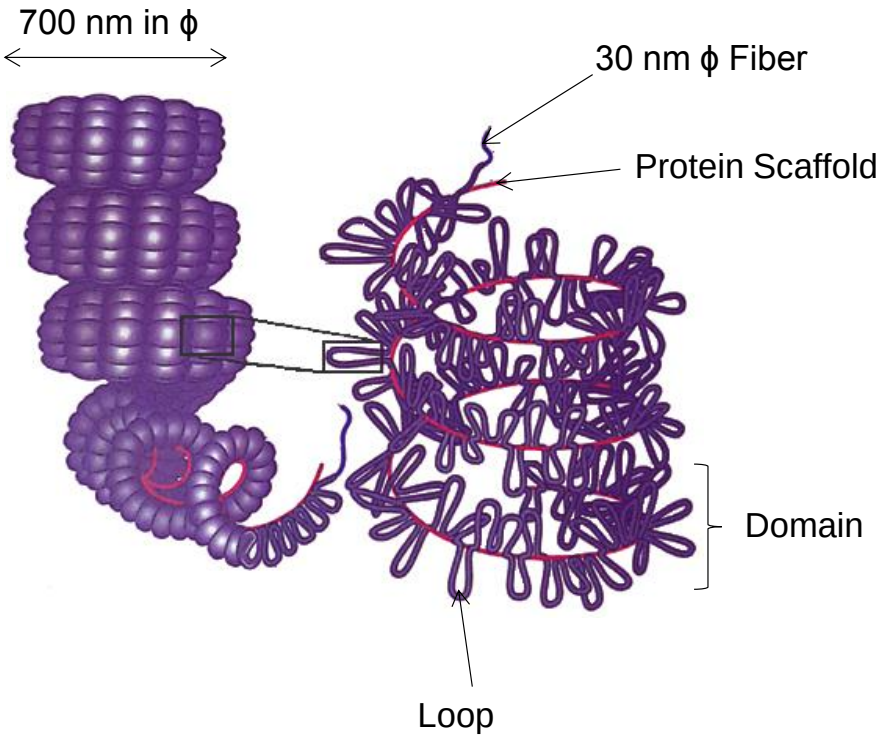

Fig. 9: The fourth level of chromatin organization (Griffiths et al. 2002)

From a functional perspective, this organization enables extreme compaction, reducing the chromosomal volume by a factor of 10,000 compared to decondensed DNA. It also ensures an organization into distinct chromosomal bands, where each coil roughly corresponds to a band observable in metaphase. Finally, this highly ordered structure is essential for ensuring the faithful segregation of chromatids during anaphase, thus representing the culmination of the compaction process necessary for cell division.

Figure 10 summarizes the process of chromatin fiber compaction.

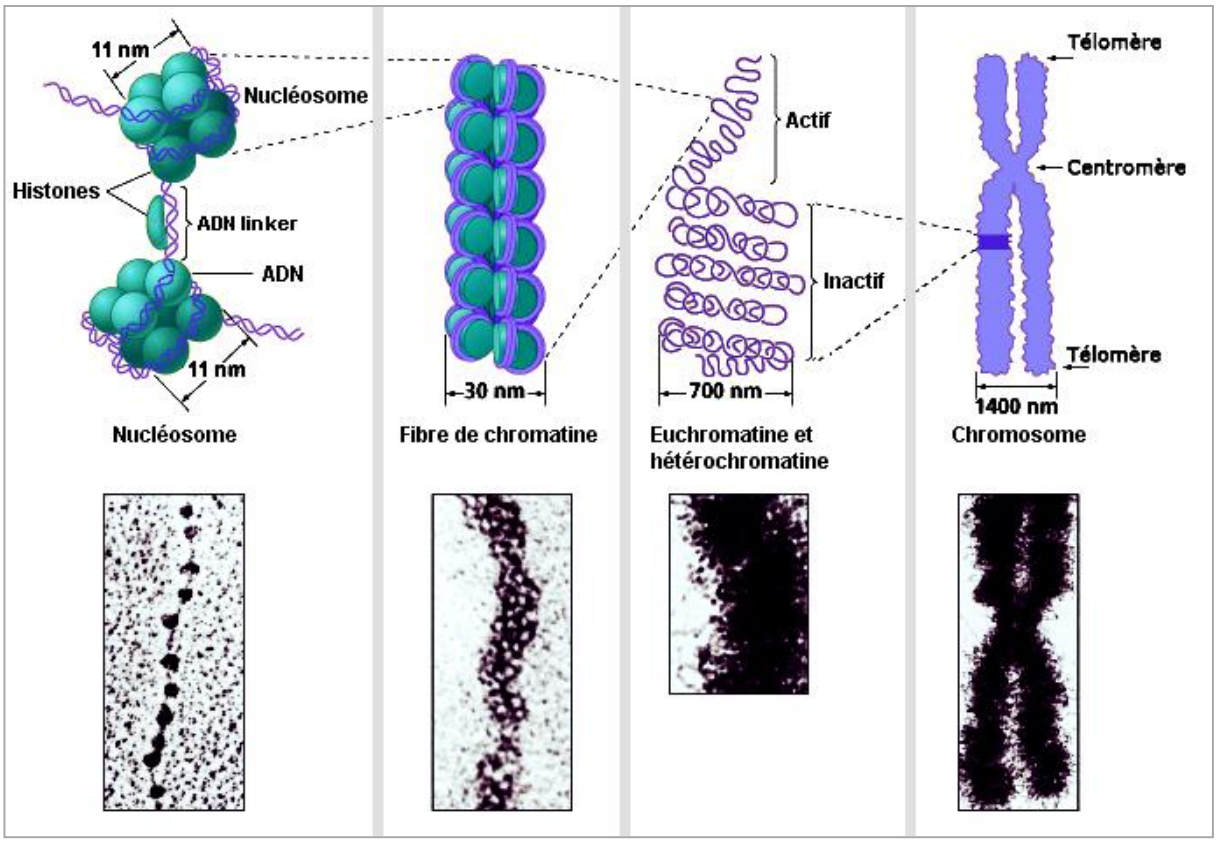

Fig. 10: The compaction of chromatin into a metaphase chromosome (Campbell and Reece 2004)

Chapter II: Methods for Studying Chromosomes

Chromosomes are only visible under a light microscope during cell division, when they are in their condensed state. Their study therefore necessarily relies on the analysis of dividing cells, primarily in mitosis or meiosis.

A set of complementary techniques has been developed for their analysis:

- Classical techniques allow for the observation of chromosomes in their entirety (number, shape, size, etc.).
- Molecular techniques allow for the study of specific regions of the DNA.

These methods are fundamental for:

- Establishing and characterizing an individual's karyotype and creating a karyogram for a population.
- Revealing chromosomal variations (number, structure, etc.).
- Analyzing chromosomal behavior during meiosis and identifying meiotic irregularities.
- Performing the physical mapping of genes on chromosomes.

1. Mitosis (Karyotype, Karyogram):

All multicellular organisms develop from a single initial cell, the fertilized egg (or zygote). The multiplication of this cell to form a complex organism is ensured by a strictly controlled process: the cell cycle. The purpose of this cycle is to produce, from one mother cell, two genetically identical daughter cells.

The cell cycle is divided into two main phases: Interphase, a period of preparation and growth, and the M Phase (Mitosis), a period of active division.

- **Interphase: The Preparation Phase**

Interphase, which represents the majority of the cell's life, is a phase of intense growth and preparation (**Fig. 13A**). It is itself divided into three stages (**Fig. 11**):

- **G1 Phase (Gap 1):** This is a phase of cell growth, protein synthesis, and intense metabolic activity. The cell carries out its specific function.
- **S Phase (Synthesis):** This is the key stage of replication. Each DNA molecule (each chromosome) is duplicated. By the end of this phase, each chromosome consists of two identical copies, called sister chromatids, attached at a centromere (**Fig. 12**).
- **G2 Phase (Gap 2):** The cell continues its growth and synthesizes the structures necessary for division, such as the proteins of the mitotic spindle. It also checks that DNA replication has occurred without errors.
- **G0 Phase: Exiting the Cycle**

Cells can leave the cell cycle and enter the G0 phase, a state of quiescence where they are metabolically active but not proliferating. The fate of these cells depends on their degree of differentiation: poorly differentiated cells, such as hepatocytes or plant parenchyma cells, can usually re-enter the cell cycle, whereas highly specialized cells, such as neurons or sclerenchyma cells in plants, remain in G0 permanently. Furthermore, a cell committed to the cycle that has passed the restriction point in late G1 phase must absolutely complete the S, G2, and M stages before it can potentially enter quiescence again.

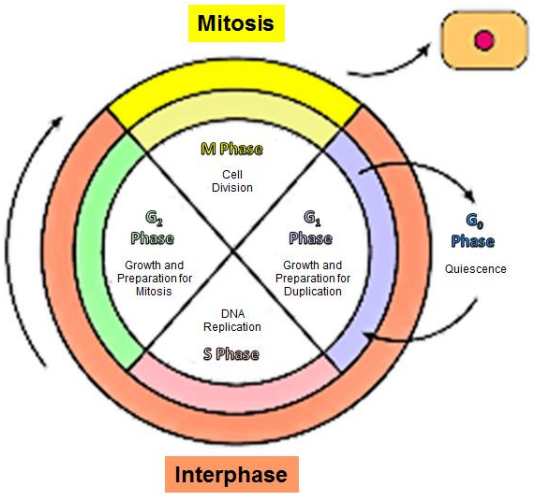

Fig. 11: The phases of the cell cycle and the G_0 phase

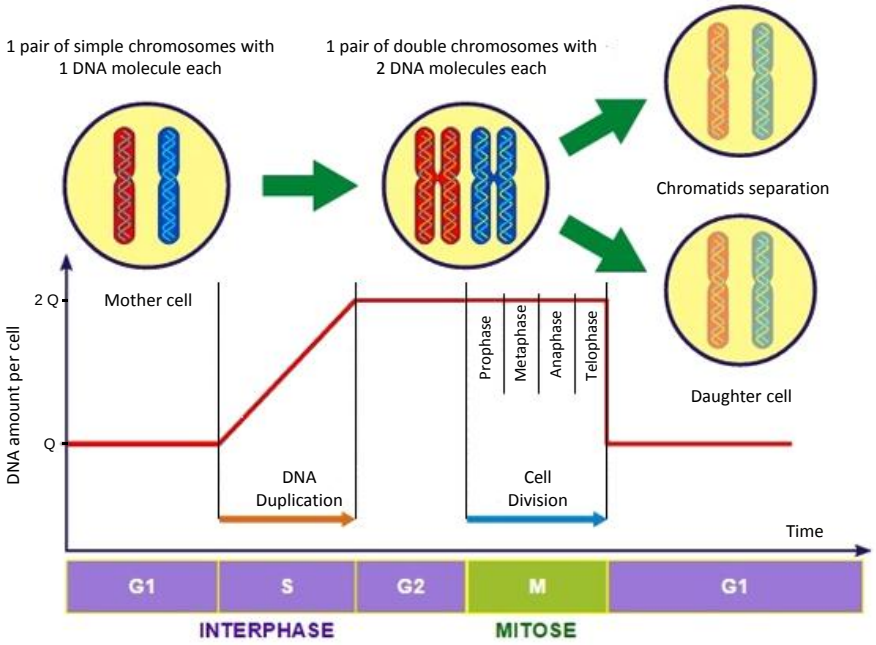

Fig. 12: Evolution of DNA quantity and the state of chromosomes during the cell cycle

The M Phase or Mitosis:

Mitosis is the process that ensures the equal distribution of duplicated chromosomes into the two daughter cells. It is the shortest phase of the cycle and occurs in four continuous stages:

- **Prophase:**

Prophase (**Fig. 13B**) marks the beginning of nuclear division (karyokinesis) and is characterised by three major, simultaneous events:

Chromatin Condensation: The chromatin, the diffuse form of DNA during interphase, undergoes a process of extreme compaction. It coils and supercoils to form distinct chromosomes, each composed of two sister chromatids joined at a centromere.

Formation of the Mitotic Apparatus (Spindle): This is the structure that guides the separation of the chromosomes. Its formation differs between animals and plants:

In Animal Cells: Centrosomes (containing centrioles) direct the operation. They duplicate and migrate to opposite poles, where they serve as "anchor points" for building the spindle.

In Plant Cells: In the absence of centrosomes and centrioles, microtubules self-organize to form the spindle.

Although the strategies are distinct, the result is identical: the formation of a functional spindle capable of ensuring the equal distribution of genetic material.

Disintegration of the Nuclear Envelope: The nuclear membrane fragments into vesicles, effectively mixing the nucleoplasm and cytoplasm. This disassembly is essential as it allows the spindle microtubules to access and attach to the chromosomes.

- **Metaphase:**

During metaphase (**Fig. 13C**), the spindle microtubules, attached to the kinetochores on either side of the chromosome, exert opposing pulling forces on the sister chromatids. These antagonistic forces bring and align each chromosome in the equatorial plane of the cell, forming the metaphase plate. The chromosomes are held in balance in this position by the symmetrical tension from the microtubules originating from the opposite poles of the spindle.

- **Anaphase:**

During anaphase (**Fig. 13D**), the sister chromatids separate at their centromere and migrate towards the opposite poles of the cell. Each chromatid is actively pulled towards a pole by the spindle microtubules, which are attached to its centromere via the kinetochore. This pole ward pulling causes the chromatid arms to trail passively in the cytosol, giving the chromatids a characteristic shape during their movement: metacentric chromosomes (centromere in the center) form a "V", sub metacentric ones (centromere off-center) form a "J", and telocentric chromosomes (centromere in a terminal position) form a "rod or I" shape.

- **Telophase:**

During telophase (**Fig. 13E**), the daughter chromosomes (former chromatids) are gathered into two genetically identical sets at each pole of the cell. They then begin to decondense back into the functional chromatin state characteristic of interphase. The mitotic spindle depolymerizes, nuclear membranes reform around each set of chromosomes, and the cytoplasm divides during cytokinesis. In animals, this final step is achieved by a constriction of the plasma membrane, which cleaves the mother cell in two. In plants, cytokinesis begins with the formation of a central structure, the cell plate, which develops into the middle lamella; the primary walls of the daughter cells are then synthesized on either side of this lamella.

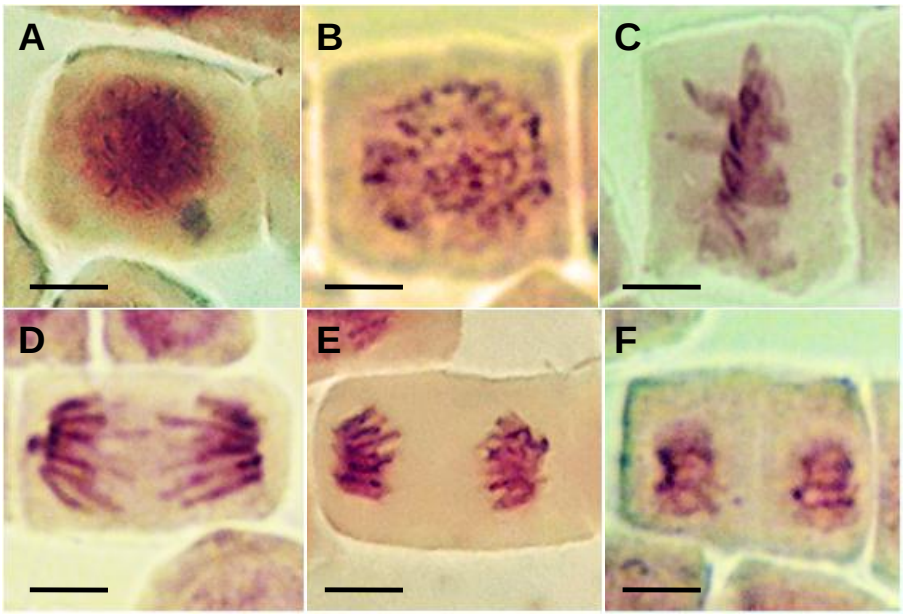

Fig. 13: Different stages of mitotic division in the root meristems of onion Allium cepa (acetic carmine staining). A- Interphase; B- Prophase; C- Metaphase; D- Anaphase; E- Telophase; F- Two daughter cells. (Photos Djafri-Bouallag L.)

- The Karyotype and the Karyogram:

- ✓ **The Karyotype:**

A karyotype is a standardized and organized representation of the chromosomes from a cell in mitotic metaphase (**Fig. 14 a-b**). It allows for the analysis of the number, shape, size, centromere position, and presence of secondary constrictions for each chromosome. To establish it, a cell is used whose chromosomes are at the stage of their maximum contraction and are perfectly individualized, typically in late prophase or metaphase, and arranged on the same plane to allow for accurate photography and analysis.

Metaphase chromosomes consist of a short arm (denoted "p") and a long arm (denoted "q"), connected by the centromere. In a karyotype, homologous chromosomes are paired and classified according to precise criteria:

- Decreasing size: By convention, the chromosome pairs are classified from the largest to the smallest.

- The position of the centromere: Defined by the centromeric index (CI = length of arm p / total length of the chromosome). According to the nomenclature of Levan et al. (1964), this index allows for the distinction of four main morphological types:
  - Metacentric chromosomes (CI = 0.50 - 0.37)
  - Submetacentric chromosomes (CI = 0.37 - 0.25)
  - Acrocentric chromosomes (CI = 0.25 - 0.12)
  - Telocentric chromosomes (CI ≈ 0.00)
- The Presence of Secondary Constrictions: Beyond the centromere (primary constriction), some chromosomes present constant secondary narrowing's. These regions, often associated with the nucleolar organizers (areas containing the ribosomal RNA genes), are important identification markers for specific chromosomes.
- The banding pattern: Characteristic of each chromosome pair, obtained by specific staining techniques.

✓ **The Karyogram:**

The karyogram (or ideogram) is a schematic and standardized representation of the chromosomes of a population (species), constituting its reference genome (Fig. 14 c). Unlike the karyotype, which is a photograph of an individual's chromosomes, the karyogram is created by synthesizing data observed from a large number of different karyotypes. This generalization allows for the establishment of a consensus map for each chromosome, indicating its characteristic size, the average position of its centromere, and its specific banding pattern. It is important to note that the karyogram represents the haploid genome, meaning one copy of each pair of homologous chromosomes, thus providing an essential overview of the set of chromosomal structures characteristic of the species.

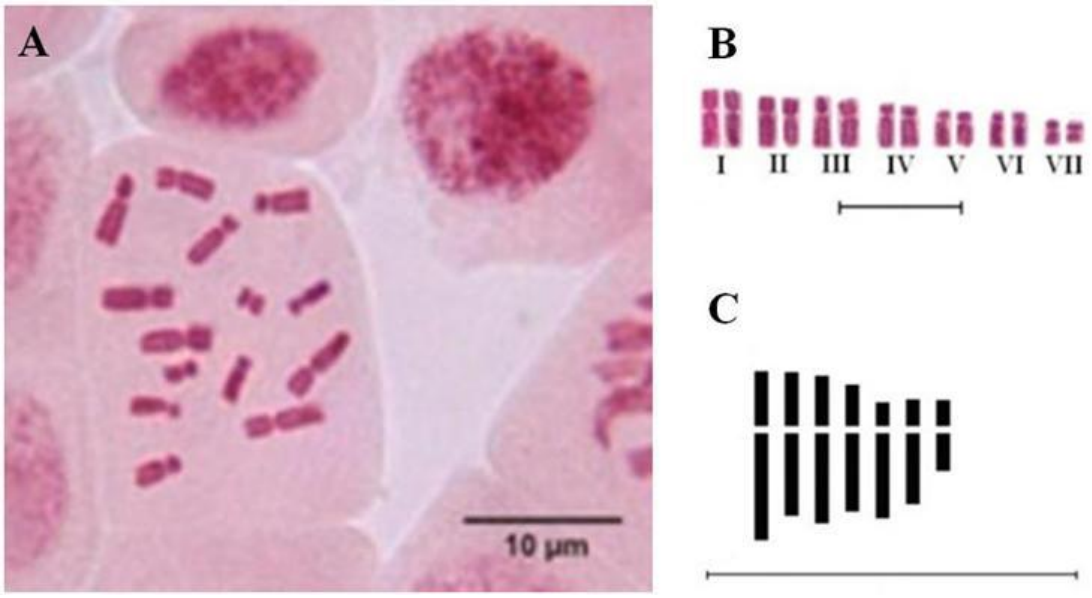

Fig. 14: Somatic metaphase, karyotype, and karyogram of Ornithogalum montanum ($2n = 2x = 14$). A- Metaphase plate; B- Karyotype; C- Karyogram (Öztürk et al. 2014).

3.2. Meiosis (Behavior and Meiotic Irregularities)

Meiosis is a specialized cell division process, consisting of two successive divisions, which reduces the chromosome number of the mother cell. Thus, a diploid cell (2n) gives rise to four haploid daughter cells (n). This mechanism is fundamental during gametogenesis (or sporogenesis in plants), as it produces gametes containing only a single copy of each pair of homologous chromosomes. This reduction is essential for maintaining the species' constant chromosome number after fertilization.

The meiotic cycle is characterized by a single DNA replication, which occurs during the interphase preceding the first division, followed by two cytoplasmic divisions. Meiosis I, known as the reductional division, separates the homologous chromosomes. It thus produces two haploid daughter cells, each possessing one chromosome from each pair, still consisting of two sister chromatids. Meiosis II, known as the equational division, is similar to mitosis and serves to separate the sister chromatids in each of these two haploid cells.

Each of these divisions occurs in four main stages: for Meiosis I, these are prophase I, metaphase I, anaphase I, and telophase I; for Meiosis II, prophase II, metaphase II, anaphase II, and telophase II. It is crucial to note that no DNA replication occurs between these two divisions.

3.2.1. Meiosis I: Reductional Division

- **Prophase I:** Phase of genetic recombination, divided into 5 stages:

- *Leptotene (thin thread stage):* Beginning of chromosome condensation (**Fig. 15 A and A'**).

This first stage of prophase I marks the visible beginning of meiosis. The chromosomes, already duplicated, begin to condense and gradually become discernible under the microscope as long, thin filaments. Each chromosome already consists of two sister chromatids, but these are not yet distinguishable. Another key event of this stage is the anchoring of the chromosome ends to the nuclear envelope, which prepares and organizes the nucleus for the pairing of homologous chromosomes that will occur in the next stage.

- *Zygotene (joined thread stage):* Pairing of homologous chromosomes (synapsis) forming bivalents, via the synaptonemal complex (**Fig. 15 B and B'**).

The central event of this stage is the initiation of synapsis, a perfect and precise pairing of homologous chromosomes (maternal and paternal). This alignment is mediated by the formation of a complex protein structure, the synaptonemal complex, which acts as a molecular ladder between the two homologues to keep them tightly associated. Each paired pair, now called a bivalent, consists of four chromatids (two per homologous chromosome). This stage lays the essential physical groundwork for the crossing-over that will follow.

- *Pachytene (thick thread stage):* Crossing-over between non-sister chromatids: exchange of DNA segments materialized by chiasmata (**Figs. 15 C and C'**).

This stage is crucial for genetic shuffling. Synapsis is completed and crossing-over occurs: non-sister chromatids (each belonging to one homologous chromosome) break and exchange segments of DNA reciprocally. These exchange points, visible under the microscope, are materialized by chiasmata. This recombination process creates new combinations of alleles on the chromosomes, which is a major source of genetic diversity among gametes.

- *Diplotene (double thread stage):* Homologues begin to repel each other but remain linked by chiasmata (**Figs. 15 D and D'**).

The synaptonemal complex disassembles, leading to a partial separation of the homologous chromosomes. They begin to repel each other, but they remain firmly attached at one or more points: the chiasmata. These structures, which materialise the sites of crossing-over from the previous stage, are now clearly visible and maintain the integrity of the bivalents. This physical tension ensures the correct orientation of the chromosomes for their future segregation in metaphase I.

- *Diakinesis* (*double movement stage*): (**Figs. 15 E and E'**).

At this stage, the chromosomes reach their maximum degree of condensation. The nucleolus and nuclear envelope disappear, while the mitotic apparatus (or achromatic spindle) begins to form.

- **Metaphase I:** Random alignment of bivalents at the cell's equator (**Figs. 15 F and F'**).

At this stage, the bivalents (pairs of homologous chromosomes) migrate and align randomly on the equatorial plate of the cell. Each bivalent is positioned independently, with the orientation of each pair (maternal chromosome towards one pole, paternal towards the other) being random. This independent assortment is a second fundamental mechanism of genetic shuffling, as it allows the production of gametes with novel combinations of maternal and paternal chromosomes. The chromosomes are held in place by the tension from the spindle fibers attached to their centromeres.

- **Anaphase I:** Separation and migration of homologous chromosomes (each with two chromatids) towards the poles (**Figs. 15 G and G'**).

This stage marks the definitive separation of homologous chromosomes. Under the effect of the shortening spindle microtubules, the homologous chromosomes of each bivalent are pulled and migrate towards the opposite poles of the cell. This is the key event of the reductional division: each pole receives a haploid set of chromosomes, each still consisting of two sister chromatids. This segregation of homologues halves the chromosome number and ensures the random distribution of chromosomes of maternal and paternal origin, continuing the genetic shuffling initiated in prophase I.

- **Telophase I and Cytokinesis:** Partial reformation of nuclei and cytoplasmic division giving rise to 2 haploid cells (**Figs. 15 H and H'**).

At this stage, the homologous chromosomes having reached the cellular poles begin to partially decondense, while a nuclear envelope temporarily reforms around each haploid set. Cytokinesis (division of the cytoplasm) follows immediately, splitting the mother cell into two haploid daughter cells. In plants, this division occurs through the formation of a cell plate in the center of the cell, which will develop into the middle lamella. Each daughter cell resulting from this first meiotic division now contains a haploid number of chromosomes, but each chromosome is still duplicated (composed of two sister chromatids), thus preparing the ground for Meiosis II.

3.2.2. Meiosis II: Equational Division

Separates the sister chromatids, without prior DNA replication.

- **Prophase II:** Condensation of chromosomes and formation of the spindle (**Figs. 15 I and I'**).

This phase marks the beginning of the second meiotic division. In each of the two haploid daughter cells from Meiosis I, the chromosomes (still consisting of two sister chromatids) re-condense

after the partial decondensation of telophase I. The nuclear envelope, if it had reformed, disintegrates again. Simultaneously, a new mitotic spindle forms, perpendicular to the spindle of the first division, to prepare for the separation of the sister chromatids. No DNA replication having occurred since Meiosis I, the genetic material is now ready for equational separation.

- **Metaphase II:** Alignment of chromosomes (with two chromatids) on the equatorial plate (**Fig. 15 J and J'**).

At this stage, the chromosomes, each composed of two sister chromatids, migrate and align individually on the equatorial plate of each haploid daughter cell. Their centromeres, each bearing two kinetochores, orient themselves so that spindle microtubules from opposite poles can attach to them. This alignment in metaphase II is similar to that of a mitotic metaphase, but it occurs in already haploid cells. The arrangement of chromosomes on the equatorial plate is random, which can contribute one last time to a slight increase in genetic diversity.

- **Anaphase II:** Separation of sister chromatids and migration towards the poles (**Fig. 15 K and K'**).

This crucial step involves the definitive separation of the sister chromatids. The centromeres of each chromosome divide, thus releasing the two chromatids, which then become independent chromosomes. Under the action of the shortening spindle microtubules, these daughter chromosomes are pulled and migrate towards the opposite poles of the cell. This separation mechanism is identical to that of mitotic anaphase, but its result is particular: it culminates in the distribution of a haploid set of single chromosomes into each future daughter cell.

- **Telophase II and Cytokinesis:** Decondensation and formation of four haploid nuclei giving rise to 4 genetically unique daughter cells (**Fig. 15 L-M and L'-M'**).

This final phase concludes meiosis. The daughter chromosomes, having reached the cellular poles, decondense to return to their functional chromatin state. A nuclear envelope reforms around each chromosomal set, thus creating four distinct haploid nuclei. Cytokinesis, occurring simultaneously, separates the cytoplasm of each cell in two. The result is the production of four haploid daughter cells. Thanks to the events of genetic shuffling (crossing-over and independent assortment), each of these cells possesses a unique chromosomal combination, guaranteeing the genetic diversity essential for sexual reproduction.

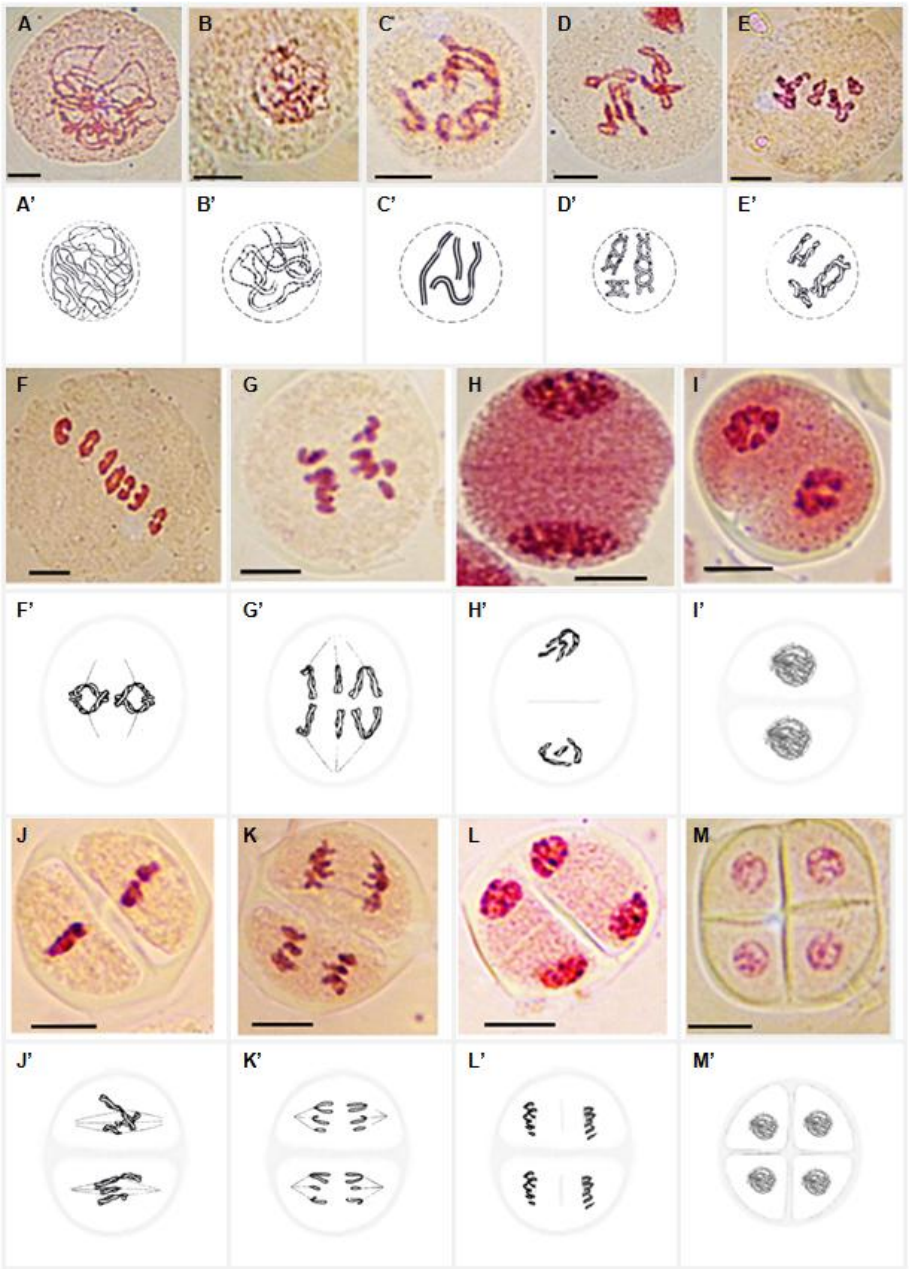

Fig. 15: Different stages of meiotic division in Hordeum bulbosum L.. Meiosis I (A-I): Prophase I (A-E): A-Leptotene, B-Zygotene, C-Pachytene, D-Diplotene, E-Diakinesis; F-Metaphase I; G-Anaphase I; H-Telophase I; Meiosis II (J-M): I-Prophase II, J-Metaphase II; K-Anaphase II; L-Telophase II (Photos Sedki W. 2016).

- **Meiotic Behavior and Irregularities**

Meiosis is the key process enabling genetic exchange between parental genomes. The analysis of the behaviour and associations of homologous chromosomes provides crucial information on the rate of genetic recombination, as well as the identity and origin of each genome.

The observation of meiotic behaviour is generally carried out on dividing pollen mother cells, more specifically at the metaphase I stage where the pairs of homologous chromosomes are perfectly aligned on the equatorial plate. The other stages of meiosis are also of interest for detecting potential anomalies in the division, such as the formation of chromatic bridges, the presence of lagging chromosomes, asynchronous divisions, etc. (**Fig. 16**).

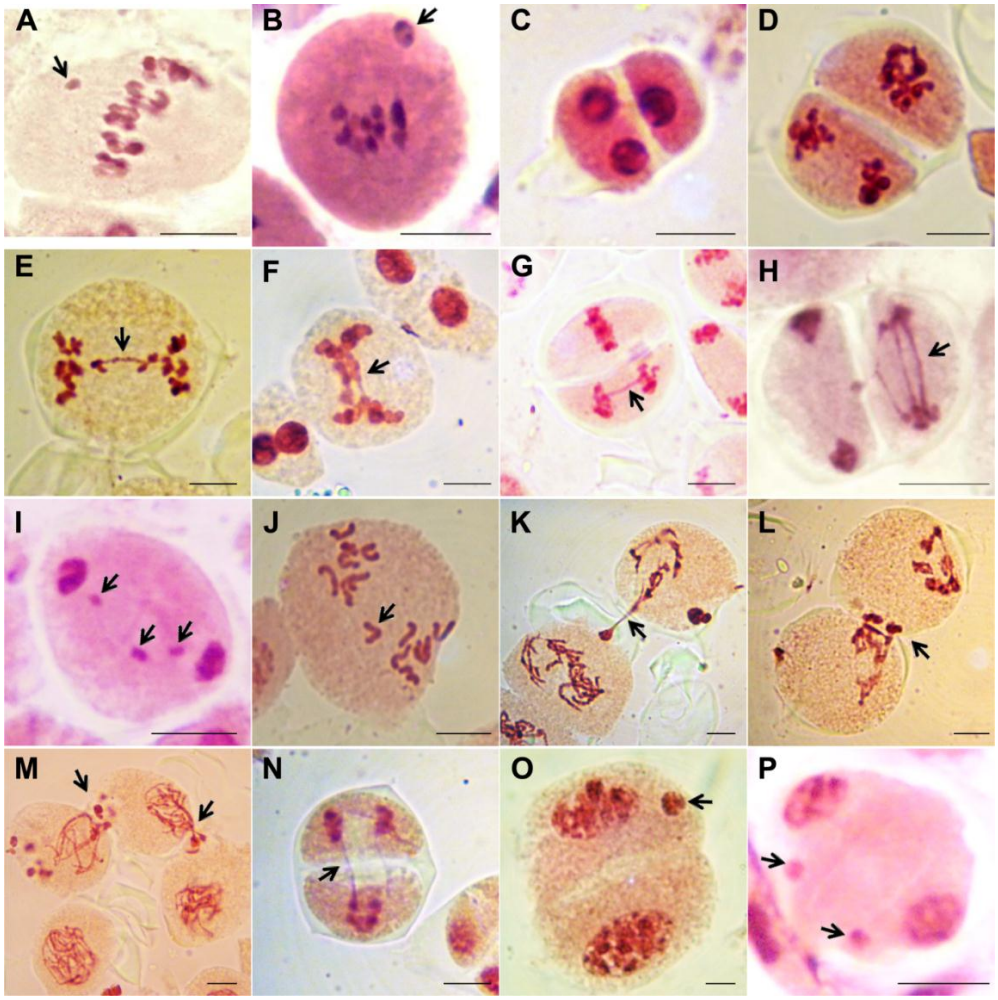

Fig. 16. Abnormal microsporocytes in Algerian populations of *Hordeum bulbosum* L. A- Syncyte. B- Metaphase I showing an unoriented bivalent (arrow). C- Triad. D- Asynchronous divisions in a dyad. E- Anaphase I showing a chromatin bridge (arrow). F- Anaphase I showing two chromatin bridges (arrow). G- Anaphase II showing a chromatin bridge (arrow). H- Anaphase II showing three chromatin bridges (arrow). I- Telophase I showing three lagging chromosomes (arrows). J- Telophase I showing one lagging chromosome (arrow). K- Prophase I showing cytomixis between two cells (arrow). L- Metaphase I showing cytomixis between two cells. M- Cytomixis among several cells. N- Cytomixis between two cells of a dyad. O- Dyad with a micronucleus (arrow). P- Telophase I with two micronuclei (arrow). Scale = 10 µm. (Djafri-Bouallag *et al.* 2025).

3.3. Chromosome Banding (Euchromatin, Heterochromatin, C, R, G Bands)

The study of chromosomes (karyotype) reveals a great diversity between species, not only in their number, but also in their size, shape, and fine structure. Modern staining techniques have made it possible to visualize this structure and precisely identify the different constituents of chromosomes (DNA, histones, non-histone proteins, ions, etc.).

- **Euchromatin and Heterochromatin**

Within chromosomes, two main states of DNA compaction are distinguished, which form a dynamic equilibrium essential for cellular function:

- **Euchromatin** represents the functional and active form of chromatin. Characterised by a less compacted state, its relaxed chromatin structure allows easy access to the DNA for transcription factors and enzymatic complexes. This is why it is the main site of gene transcription, where about 90% of eukaryotic genes are transcribed. Mainly located in the central regions of the nucleus during interphase, it is rich in unique genes and single-copy sequences, often presenting a higher proportion of Guanine-Cytosine base pairs (high GC%). In cytogenetic analyses, it corresponds to the light bands (G-negative bands) observed on chromosomes. Finally, it is a dynamic structure, capable of condensing and decondensing according to the cell's transcriptional needs.
- **Heterochromatin** constitutes the condensed and mostly inactive form of chromatin. Its highly compacted structure forms a dense environment that considerably limits access to the DNA, making it a genetically silent region, poor in transcribed genes. It is often found at the nuclear periphery or around the nucleolus. Its composition is mainly constituted of repetitive DNA, such as satellite sequences, and it is generally richer in Adenine-Thymine base pairs (high AT%). With conventional staining techniques, it appears as dark bands (G-positive bands). Two types of heterochromatin are distinguished:
  - ✓ **Constitutive heterochromatin,** which is constantly condensed and inactive in all cells (such as in centromeric and telomeric regions, revealed by C-banding);
  - ✓ **Facultative heterochromatin**, whose state is reversible and can vary according to cell type or developmental stage, the most well known example being the facultative inactivation of one of the two X chromosomes in female mammals.

3.3.1. The Principle of Chromosome Banding

Chromosome banding is a fundamental cytogenetic technique that produces a characteristic pattern of light and dark bands along metaphase chromosomes. This technique revolutionized chromosomal analysis by enabling:

- The precise identification of each chromosome
- The detection of structural chromosomal abnormalities
- The establishment of precise and reliable (reproducible) karyotypes
- The study of chromosomal evolution between species

The principle relies on structural and chemical differences along the chromosomes:

- The bands correspond to distinct chromatin domains
- Each band type has a specific base composition (AT/GC)
- The differential binding of dyes depends on the compaction and composition of the DNA

3.3.2. The Main Types of Bands

There are six staining techniques, each highlighting specific regions:

- **G-bands (Giemsa bands):** This is the most common technique. They are obtained by an enzymatic digestion (with trypsin) of the chromosomes, followed by staining with Giemsa. The chromosomes then show a characteristic succession of light and dark bands.
  - **Dark bands (G+):** Rich in adenine-thymine (AT), they correspond to condensed, late-replicating DNA, often associated with heterochromatin.
  - **Light bands (G-):** Rich in guanine-cytosine (GC), they correspond to decondensed, early-replicating DNA, often associated with euchromatin and active genes.
- **R-bands (Reverse bands):** This technique gives a pattern inverse to that of G-bands. They are obtained by thermal denaturation of the chromosomes followed by staining with Giemsa. The G- bands (light) then appear intensely stained (dark), while the G+ bands (dark) become light.
  - Dark R-bands (R+) are rich in GC sequences and correspond to active euchromatin.
  - Light R-bands (R-) are rich in AT and correspond to heterochromatin.
- **C-bands (Centromeric bands):** This technique allows for the differential staining of constitutive heterochromatin, which consists of highly repetitive DNA sequences. The protocol, based on DNA denaturation-renaturation (by treatment with acids, bases, or at high

temperature followed by incubation in a sodium citrate solution), leads to intense staining of heterochromatic regions rich in repetitive DNA.

- **Q-bands (Quinacrine bands):** This technique uses a fluorochrome, quinacrine. After staining, the chromosomes observed under fluorescence microscopy show a characteristic pattern of Q+ (fluorescent) bands. These bright bands correspond to regions rich in adenine-thymine (AT) base pairs and offer a pattern similar to that of G-bands.
- **T-bands (Telomeric bands):** This technique is based on the use of a fluorochrome, acridine orange, after a denaturation-renaturation treatment of the chromosomal DNA. Under ultraviolet light, the telomeric regions appear specifically stained, thus allowing the visualisation of the chromosome ends.
- **N-bands (NOR, for Nuclear Organizer Regions):** This technique, also called staining of the nucleolar organisers, uses silver nitrate to reveal the chromosomal regions active in nucleolus formation. It highlights the sites of ribosomal RNA gene transcription on the chromosomes.

3.4. Flow Cytometry

Flow cytometry is a quantitative cell analysis technique that allows for the high-speed characterisation and sorting of individual cell populations. To do this, cells in suspension are first specifically labelled using fluorochromes that bind to their components of interest, such as DNA or proteins. They are then aligned by a fluid stream and exposed one by one to a laser beam. The excitation of the fluorochromes then generates fluorescence and light scattering signals, which are captured and then quantified. This multiparameter analysis makes it possible to accurately reveal essential characteristics of the cells, including their size, internal complexity, and the expression of specific molecular markers.

Flow cytometry finds a powerful application in genome analysis via DNA quantification and the generation of flow karyotypes, offering a complementary and quantitative approach to classical cytogenetic techniques.

- DNA Quantification by Flow Cytometry relies on a proportional labelling principle. After isolation of cell nuclei by centrifugation, they are stained with a fluorochrome, such as propidium iodide, which has a stoichiometric affinity for DNA. This essential property guarantees that the amount of bound dye is directly proportional to the DNA mass present in each nucleus. During individual passage in front of the laser, each nucleus thus emits a fluorescent signal whose intensity accurately reflects its DNA content. The collected data are visualised as a DNA distribution histogram, where the characteristic peaks correspond to the different phases of the cell cycle: the G0/G1 peak represents cells with normal diploid content (2n), the G2/M peak represents cells in the pre-division phase (4n), while the intermediate S region corresponds to the active DNA synthesis phase. This approach allows for the rapid

detection of chromosomal abnormalities such as aneuploidies, which manifest as a significant shift of the main diploid peak, indicating an abnormal gain or loss of genetic material.

- Flow cytometry also allows for the generation of flow karyotypes, a quantitative approach that complements conventional cytogenetic analysis. For this, intact metaphase chromosomes are first isolated from dividing cells, then stained with one or more fluorochromes whose binding is proportional to the DNA mass or specific to certain nucleotide sequences. Each chromosome then individually passes through the laser beam, where two main parameters are measured: fluorescence intensity, which informs about its DNA content, and the light scattering angle, which reflects its relative length. These data make it possible to distinguish the different chromosomes not by their morphological banding, but by their unique biophysical profile. The result is presented as a distribution histogram, or flow karyotype, where each peak corresponds to a chromosome pair classified by size and genomic content. This technique offers particularly fine resolution for detecting and quantifying complex chromosomal aberrations – such as translocations, deletions, or additions of genetic material – that might escape classical visual analysis, by providing an objective and highly reproducible measurement of the entire genome.

3.5. Molecular Hybridization (Probes and Their Labelling, *FISH* and *GISH* Techniques)

Molecular hybridisation is a fundamental technique in biology that allows for the detection and localisation of specific nucleic acid sequences (DNA or RNA) in a complex sample. The fundamental principle of molecular hybridization is simple, it is based on:

- The complementarity of nitrogenous bases: A pairs with T/U, G pairs with C
- The physicochemical properties (denaturation: renaturation) of the DNA molecule:
  - Denaturation: The two strands of the target DNA (in a chromosome, a tissue, etc.) and the probe are separated by a treatment (heat, alkaline pH).
  - Hybridization: The labelled probe is brought into contact with the target under controlled conditions (temperature, salinity). If the probe sequence is complementary to a sequence in the sample, the two strands will pair to form a stable duplex.
  - Washing: Probes that have not bound specifically are removed (to reduce background noise).
  - Detection: The signal emitted by the probe that has hybridized to its target is detected.

- **Nucleic Acid Probes and Their Labelling**

What is a probe?

A probe is a fragment of DNA, RNA, or a synthetic oligonucleotide whose sequence is complementary to the target sequence one wishes to detect. It carries a marker that allows its detection.

Types of Probes

- Double-stranded probes: Often genomic clones (BAC, YAC, cosmids).
- Single-stranded probes: Produced by in vitro transcription (riboprobes) or chemical synthesis.
- Oligonucleotides: Short fragments (20-50 bases) synthesized chemically, very specific.

Probe Labelling Methods

Labelling can be radioactive or non-radioactive.

- ✓ **Radioactive Labelling:**
  - Isotopes : ^{32}P, ^{35}S, ^{33}P, ^{125}I.
  - Advantage: Very high sensitivity.
  - Disadvantages: Limited radioactive half-life, health hazard, and special waste.
- ✓ **Non-radioactive Labelling (most used currently):**

Principle: Incorporation of nucleotides coupled to a hapten.

- Common markers:
  - Biotin (detected by Streptavidin coupled to an enzyme or a fluorochrome).
  - Digoxigenin (DIG) (detected by an anti-DIG antibody conjugate).
- Advantages: Stability, safety, no radioactive waste.
- Detection:
  - Colorimetric: Enzyme (Alkaline Phosphatase, Peroxidase) that produces a coloured precipitate.
  - Fluorimetric (for FISH): Fluorochrome (FITC, TRITC, Cy3, Cy5) that emits light at a specific wavelength.
  - Chemiluminescent: The enzyme produces light.

Fluorescence In Situ Hybridization (*FISH*)

FISH allows the visualisation of the precise location of a DNA or RNA sequence directly on cytological preparations (metaphases, interphase nuclei, spread chromosomes).

✓ Typical Protocol

- Sample Preparation: Fixation of cells or spreading of chromosomes on a slide.
- Denaturation: Treatment of the slide with an alkaline solution or heat to separate the DNA strands of the target.
- Hybridisation: Application of the probe labelled with a fluorochrome onto the slide. Incubation (from a few hours to overnight) to allow pairing.
- Washes: Removal of non-hybridised probes.
- Counterstaining: Addition of a dye such as DAPI which stains all nuclear DNA blue, allowing visualisation of the overall architecture.
- Visualisation: Observation under a fluorescence microscope equipped with specific filters.

✓ **Applications of FISH**

- Detection of chromosomal abnormalities: Deletions, duplications, translocations.
- Genomic mapping: Physical localisation of a gene on a chromosome.
- Chromosome enumeration: Counting the number of specific chromosomes (e.g., chromosomes 13, 18, 21, X, Y) in interphase nuclei for non-invasive prenatal diagnosis.
- Study of nuclear organisation: Position of genes in the nucleus.
- Detection of RNA (RNA-FISH): Visualise the expression and localisation of messenger RNAs.

✓ **FISH Variants**

- mFISH (multicolour FISH): Uses probes for all chromosomes, each labelled with a different colour, allowing the karyotype to be "painted".
- SKY (Spectral Karyotyping): Similar to mFISH but uses a spectrometry system to distinguish more colours.
- Comparative Genomic Hybridisation (CGH): Allows detection of genomic imbalances (gains/losses of DNA) in a test genome compared to a reference genome.

Genomic *In Situ* Hybridization (*GISH*)

GISH is a variant of *FISH* specialized for the study of hybrid genomes or related species.

Principle

Total genomic DNA (unpurified, mixture of all sequences) from a species "A" is used as a probe, labelled, and hybridised to the chromosomes of a hybrid species "A x B" or a species "B". In the presence of "blocking" DNA (unlabelled) from species "A" or "B", the parental genomes can be discriminated.

Applications of GISH

- Identification of the parental origin of chromosomes in interspecific hybrids or allopolyploids (e.g., wheat, cotton).
- Detection of chromosomal introgression (gene transfer between species).
- Study of genome evolution and phylogenetic relationships between species.
- Detection of contaminants or species mixtures.

Chapter III: Chromosomal Variations

Chromosomal variations refer to any modification affecting the number, architecture, shape, or dimensions of chromosomes. These modifications can occur spontaneously (errors during meiosis or mitosis) or be induced by mutagenic agents.

Four main types of anomalies are distinguished:

- Numerical variations (aneuploidies, polyploidies)
- Structural modifications (translocations, deletions, duplications)
- Morphological alterations (dicentric chromosomes, ring chromosomes)
- Size changes (gain or loss of genetic material)

These anomalies result from various mechanisms such as meiotic non-disjunction, DNA breaks or aberrant chromosomal rearrangements.

Their impacts are observed at different scales:

- At the individual level: appearance of genetic pathologies (trisomies), fertility disorders, or, exceptionally, conferring selective advantages.
- In populations: alteration of genetic diversity and influence on evolutionary dynamics.
- For species: possibility of speciation (as in cases of plant polyploidy) or, conversely, a decrease in survival and reproductive capacity.

1. Variation in Chromosome Number

The study of numerical chromosomal variations relies on precise terminology, particularly crucial when addressing polyploid organisms. To avoid any confusion between the different "levels" of ploidy, it is imperative to distinguish two fundamental concepts:

- The basic chromosome number (x) represents the minimal number of chromosomes constituting a complete and non-redundant chromosome set. It defines the fundamental genomic identity of a species. This characteristic is a constant for the species, independent of the ploidy level of the individuals.

- The haploid chromosome number (n) designates the quantity of chromosomes contained in a gamete (whether it is a spermatozoon or an egg cell). This number n, characteristic of the species, results from meiosis, a cell division process that halves the chromosomal stock of somatic cells.

Numerical chromosomal variations correspond to any modification of the normal number of chromosomes in the nucleus of a cell.

They can concern the entire genome (variation in the number of complete chromosome sets) or one or more isolated chromosomes.

Two main types are therefore distinguished: euploidy and aneuploidy.

1.1. Euploidy

Euploidy refers to individuals or cells that possess a whole number of complete chromosomal sets, that is, a multiple of the basic chromosome number (x).

Example: x = 7, then a diploid individual will have 2n = 2x = 14, a triploid 2n = 3x = 21, a tetraploid 2n = 4x = 28, etc.*

Two forms are distinguished:

- **Autopolyploidy**

It results from the duplication of the same genome within a species, meaning that all the additional chromosome sets come from a single, same genetic background. This doubling can occur mainly in two ways:

- Either by non-disjunction of chromosomes during meiosis or mitosis,
- Or by the fusion of unreduced gametes (2x).

Typical examples of autopolyploidy are found in several cultivated plants, notably the potato (Solanum tuberosum, tetraploid, 4x), alfalfa, and certain varieties of wheat.

Autopolyploidy often leads to an increase in cell size, vegetative vigour, and organ size, but can also cause partial sterility due to meiotic imbalance.

- **Allopolyploidy**

It results from hybridisation between two different species, followed by chromosomal duplication. The genomes involved are therefore of distinct origin, for example A and B, then the duplication gives an AABB set. This chromosomal duplication allows fertility to be restored, as it makes the formation of homologous pairs possible during meiosis. This phenomenon is at the origin of many important cultivated species. Among the most well known examples are common wheat (Triticum aestivum, $2n = 6x = 42$), resulting from

successive hybridisations between diploid and tetraploid species, as well as cotton (Gossypium hirsutum, 2n = 4x = 52).

1.2. Aneuploidy

Aneuploidy corresponds to a variation in the number of one or a few chromosomes, not the entire set. It results from non-disjunction during meiosis or mitosis. The cells therefore do not possess an exact multiple of the basic number.

The main types of aneuploidy are:

Monosomy (2n = 2x – 1)

It results from the loss of one chromosome. *Example: Monosomy X (Turner syndrome: 2n = 46 – 1 = 45) in humans.*

Polysomy (2n = 2x + 1, 2n=2x + 2, etc.)

It results from the gain of one or more chromosomes. This includes: trisomy (2n = 2x + 1), tetrasomy (2n = 2n + 2), double trisomy (2n = 2x + 1+ 1). Example: In humans: trisomy 21 (Down's syndrome), trisomy 18, etc.

Nullisomy (2n = 2x – 2)

It results from the loss of an entire pair of homologous chromosomes.

2. Variations in Chromosome Morphology

2.1. The Isochromosome

This is an abnormal chromosome that has two identical arms (same loci). It forms during cell division following an abnormal division of the centromere. Instead of splitting longitudinally to separate the sister chromatids, the centromere divides transversely. This error leads to the separation of the short arms and the long arms, generating a new chromosome composed exclusively of two long arms (or two short arms) which fuse. The complementary arm is generally lost.

An isochromosome can be monocentric or dicentric depending on its formation mechanism (**Fig. 17**).

Note: The genetic status of the arms depends on the formation mechanism. During mitosis, the sister chromatids being identical, the resulting isochromosome will be homozygous. During meiosis, if crossing-over has occurred, the fusion of two homologous arms can create a heterozygous isochromosome in the recombined segments. In the absence of crossing-over, it will remain homozygous.

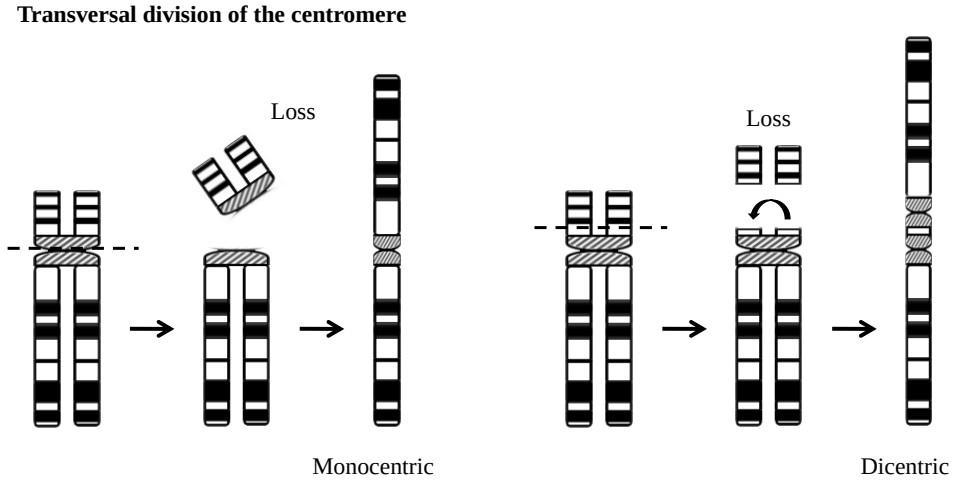

Fig. 17: The formation mechanism of an isochromosome

2.2. The Ring Chromosome

A ring chromosome (or annular chromosome) is an abnormal chromosome whose usual linear structure (rod-shaped) has closed in on itself to form a circle or ring.

The formation of a ring chromosome results from a two-step process. Firstly, double-strand breaks occur simultaneously on both arms of the chromosome, on either side of the centromere. Secondly, instead of faithful repair, an abnormal repair mechanism "glues" these broken ends together, leading to the loss of telomeres and adjacent genetic fragments, and the joining of the DNA strands into a closed loop. This closure into a loop thus permanently transforms the original linear structure into a ring, deprived of its natural protective ends (**Fig. 18**).

The formation of a ring chromosome has three major consequences. Firstly, it causes a loss of genetic material at the chromosomal ends, deleting essential genes and causing phenotypic abnormalities. Secondly, during cell divisions, the ring chromosome exhibits mitotic instability: the sister chromatids become entangled and fuse, creating abnormal structures that disrupt chromosomal segregation and generate permanent genomic instability.

Finally, the absence of telomeres leaves the chromosome unprotected, favouring further fusions and potentially triggering cell death. Together, these effects severely compromise cellular function and survival.

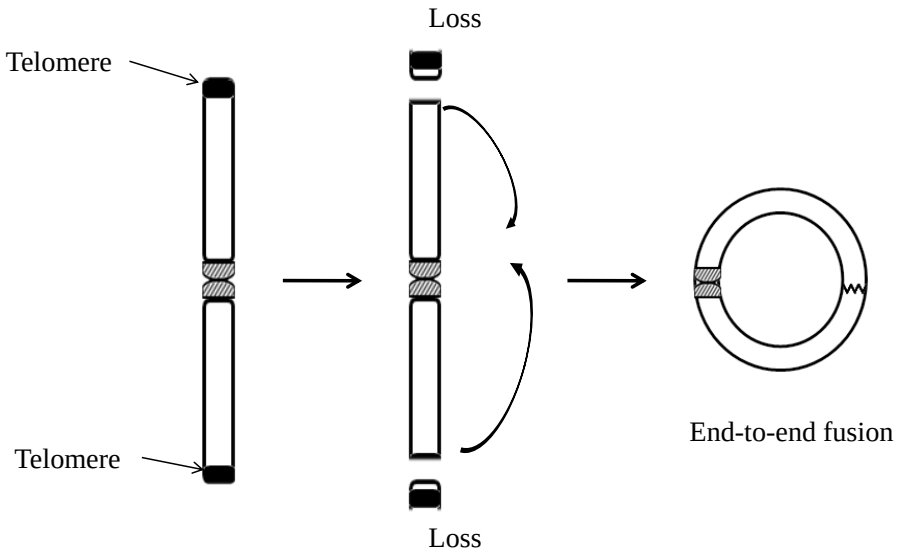

Fig. 18: The formation mechanism of a ring chromosome

2.3. Robertsonian Translocations (Centric Fusion)

A Robertsonian translocation is a structural chromosomal rearrangement where two chromosomes fuse at their centromeric region to form a single one. This type of translocation specifically involves so-called "acrocentric" chromosomes. The result of this fusion is the creation of a single, large "derivative" chromosome.

The Robertsonian translocation forms in three key steps. First, two acrocentric chromosomes undergo breaks at their short arms. Next, the fragments of the short arms are eliminated, which has no major consequence as these regions contain few essential genes. Finally, the remaining segments – carrying the centromeres and the long arms – fuse to create a new, stable derivative chromosome, which retains most of the genetic material from the two original chromosomes (**Fig. 19**).

The consequences of a Robertsonian translocation present two distinct aspects. In the balanced carrier, who has one chromosome fewer but all the essential genetic material, the translocation is generally without effect on health. However, during reproduction, this anomaly becomes significant: meiosis can produce gametes with an unbalanced chromosomal

content. Depending on the case, this transmission can lead either to non-viability of the embryo or to the development of chromosomal abnormalities (trisomies or monosomies) in the offspring, manifesting as malformations and potentially serious developmental deficiencies.

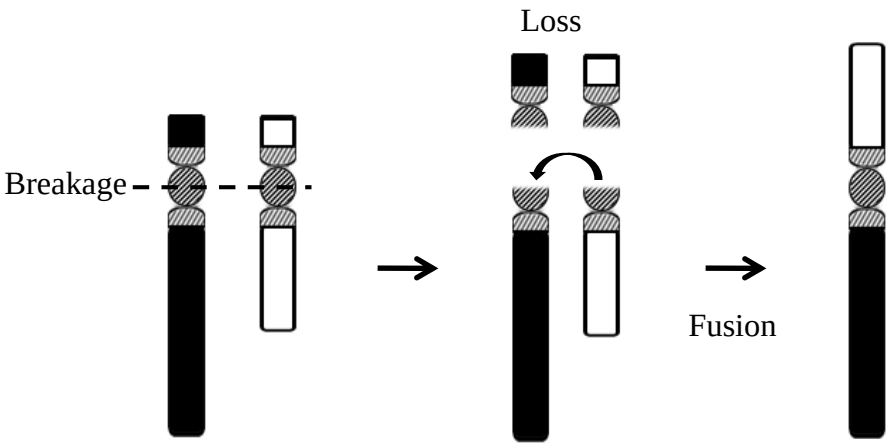

Fig. 19: The formation mechanism of a Robertsonian translocation

2.4. The Breakage-Fusion-Bridge Cycle

The Breakage-Fusion-Bridge cycle (also called the BFB cycle) is a self-perpetuating mechanism of chromosomal instability where a chromosome undergoes a succession of damage with each cell division. This vicious circle effectively transforms the chromosome's shape with each cell generation and can produce mosaic tissues exhibiting variegation – irregular patches of abnormal phenotype on a normal tissue background.

The cycle is initiated when a chromosome has an initial break. During replication, the broken ends of the sister chromatid, deemed "sticky", are fused by DNA repair mechanisms. At anaphase, when the chromatids migrate towards opposite poles, this abnormal fusion creates a characteristic chromosomal bridge. This bridge then breaks randomly along its length, generating new sticky ends that perpetuate the cycle in the next division (**Fig. 20**). The size of the abnormal tissues thus generated follows an inverse relationship with developmental timing: the earlier the initial break appears, the larger the area of affected tissue will be.

The consequences are threefold. The cycle produces massive gene amplification and deletion since each random break redistributes genetic material differently to the daughter

cells. It generates permanent genomic instability, creating a diversity of aberrant karyotypes within the cell population. Finally, it can lead either to cell death through the loss of essential genes, or to the emergence of advantaged cellular clones, thereby promoting the appearance of cancers. The resulting tissue mosaics manifest this cellular heterogeneity through their characteristic variegation.

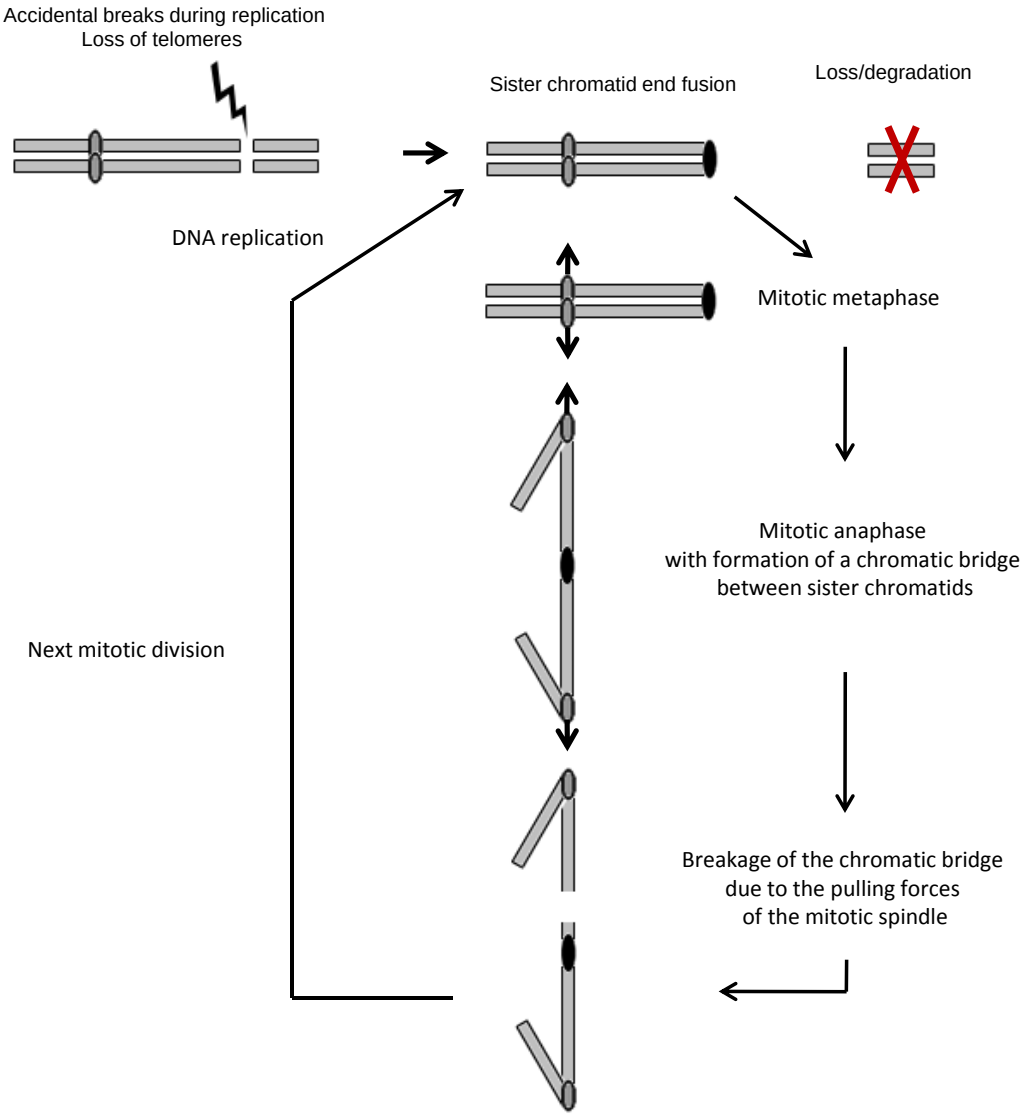

Fig. 20: The mechanism of formation of the breakage-fusion-bridge (BFB) cycle

3. Variation in Chromosome Size

Chromosome size variation refers to the observable differences in the dimensions of chromosomes, mainly in length, between species or within a single genome. These differences reflect disparities in the quantity of DNA and in the structural organisation of the chromosomes.

Chromosome size varies primarily under the influence of three interdependent mechanisms. Firstly, changes in DNA quantity occur through duplication (amplification of segments), deletion (loss of genetic material), or insertion of new sequences. Secondly, variation in repetitive sequences contributes significantly through the expansion or contraction of repetitive DNA regions, notably satellite sequences and intergenic DNA. Thirdly, major chromosomal rearrangements such as fusions (Robertsonian translocations), fissions, or the formation of giant chromosomes through amplification radically modify chromosome architecture and dimensions.

Chromosome size variations present a dual evolutionary and functional impact. Although they do not affect the complexity of organisms, they favour the adaptation and diversification of species. Functionally, they constitute a source of genetic diversity but can also lead to imbalances and diseases by disrupting gene regulation.

- **Example: Polytene Chromosomes (Fig. 21)**

Polytene chromosomes are giant chromosomal structures that form through a process of endoreduplication, where multiple cycles of DNA replication occur in succession without cell division, leading to the perfect alignment of hundreds of sister chromatids. Primarily observed in the salivary glands of dipterans like Drosophila, these chromosomes display a characteristic banded structure corresponding to different levels of DNA condensation. The transcriptional "puffs", visible areas of decondensation under microscopy, testify to intense gene activity. These exceptional chromosomes allow for massive protein production and offer a unique model for studying gene organisation and expression *in situ*.

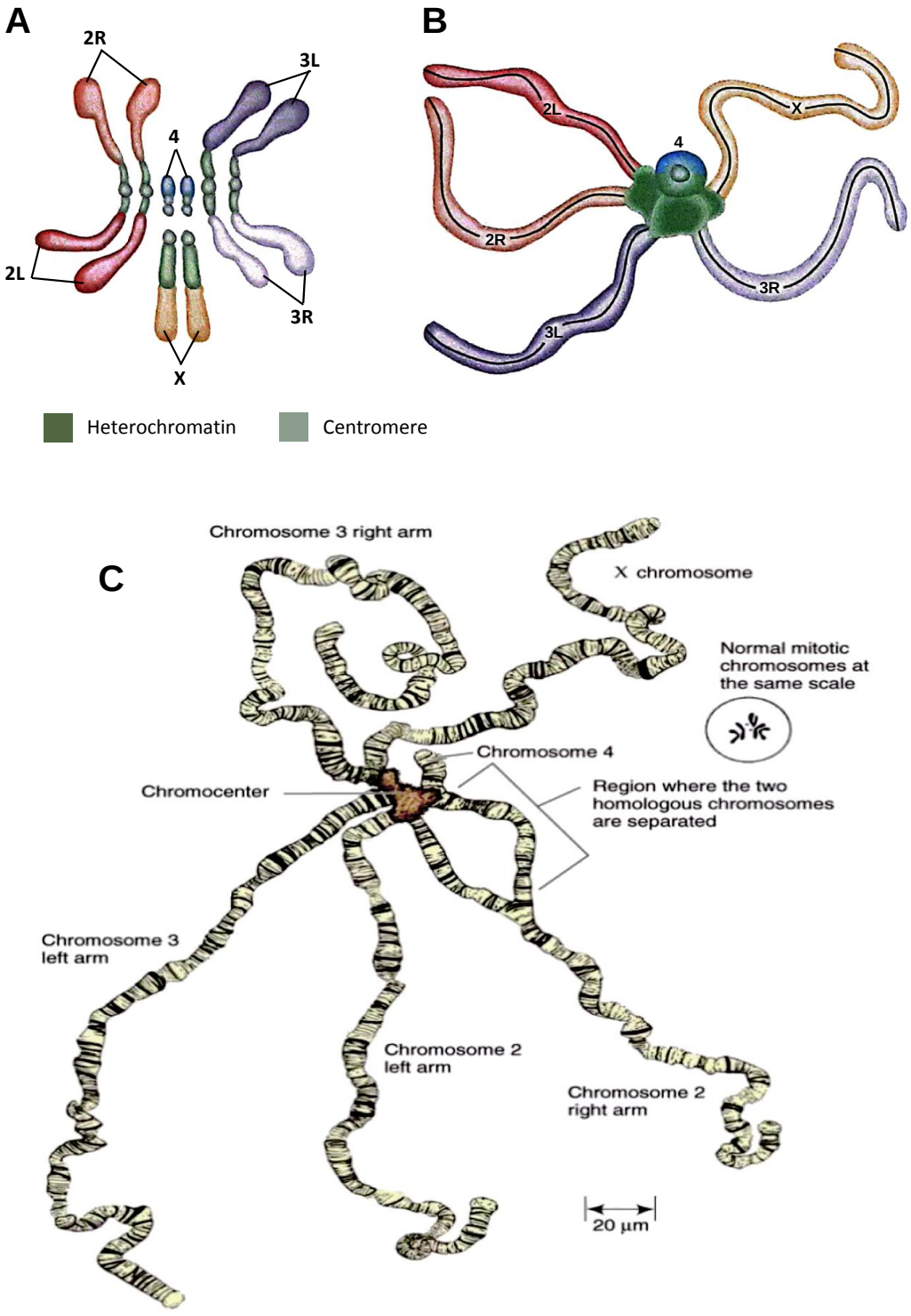

Fig. 21: Polytene chromosomes: A- chromosomes in mitotic metaphase, B- heterochromatin fuses to form the chromocenter, C- photograph of polytene chromosomes (Griffiths et al. 2002).

4. Structural Variations of Chromosomes

Structural abnormalities are the result of chromosomal breaks followed by abnormal repair mechanisms on one or more chromosomes. Although these events are random, certain genomic sites are more fragile, leading to recurrent anomalies. Most of these rearrangements result in an abnormal phenotype and reduced fertility in affected individuals. However, on an evolutionary scale, they represent an important source of genetic diversity and contribute to variation within species.

4.1. Deletion

A deletion is an unbalanced structural chromosomal abnormality, characterised by the loss of a segment of DNA. This loss, which can involve a few base pairs (microdeletion) or several genes (macrodeletion), results in partial monosomy for the affected region.

Two main forms are distinguished:

- Terminal deletion, caused by a single break leading to the loss of a chromosomal end (**Fig. 22a**).
- Interstitial deletion, resulting from two breaks on the same arm, followed by the loss of the intermediate segment and the fusion of the remaining parts (**Fig. 22b**).

The severity of the consequences of a deletion depends on the size of the lost region and the functional importance of the genes involved. While this anomaly is often a source of pathologies, it also participates in genome evolution by eliminating genetic sequences.

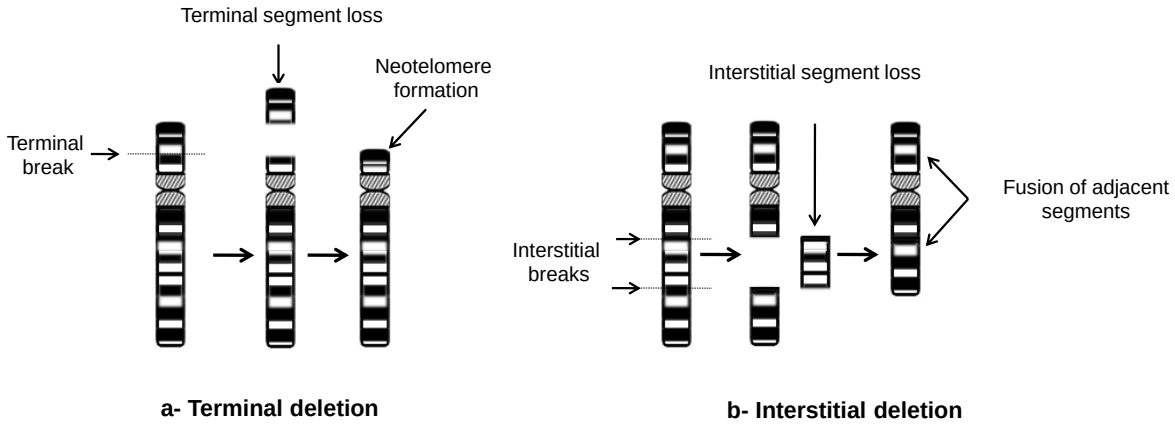

Fig. 22: The formation mechanism of deletions

4.2. Inversion

An inversion is a structural chromosomal rearrangement in which a segment of a chromosome is rotated 180 degrees relative to its normal orientation. It is a balanced abnormality, meaning there is no loss or gain of genetic material, only a change in the order of the genes.

The formation of an inversion follows a two-step process:

- Double Break: Two breaks occur simultaneously on the same chromosome.
- Re-insertion: The detached fragment reinserts itself into the chromosome after undergoing a 180-degree rotation.

Two types of inversions are distinguished:

- Paracentric Inversion: The inverted segment does not include the centromere and is located on a single chromosomal arm (**Fig. 23a**).
- Pericentric Inversion: The inverted segment includes the centromere (**Fig. 23b**).

Although generally without effect for the carrier, chromosomal inversions have major consequences during reproduction. During gamete formation, the presence of an inverted segment disrupts chromosome pairing and can generate unbalanced recombinants. These meiotic anomalies often lead to reduced fertility and an increased rate of spontaneous

abortions. On an evolutionary level, inversions play a crucial role in maintaining advantageous genetic combinations and promoting reproductive isolation, thereby contributing to the emergence of new species.

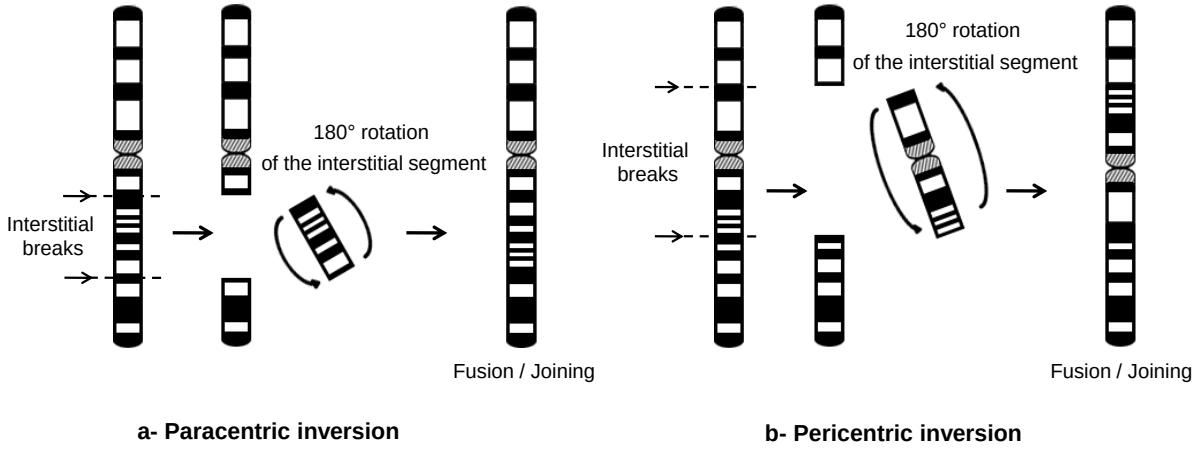

Fig. 23: The formation mechanism of inversions

4.3. Duplication

A duplication is a structural chromosomal abnormality characterised by the presence of a chromosomal segment in duplicate on the same chromosome. It involves a gain of genetic material which can affect a few genes or large chromosomal regions.

The formation of duplications results primarily from three mechanisms. Unequal crossing-over occurs due to misalignment of chromosomes during meiosis, leading to an unequal transfer of genetic material. Aberrant replication happens when the DNA polymerase "slips" and copies the same segment multiple times. Finally, errors in repair following chromosomal breakage can insert additional segments. These processes thus generate duplicated regions which become a source of evolutionary variation.

Two types of organisation for the duplicated segment are distinguished:

- Tandem duplication, where the segment is repeated in the same orientation as the original sequence (**Fig. 24a**)..
- Reverse tandem duplication, where the segment is repeated in the reverse orientation relative to the original sequence (**Fig. 24b**).

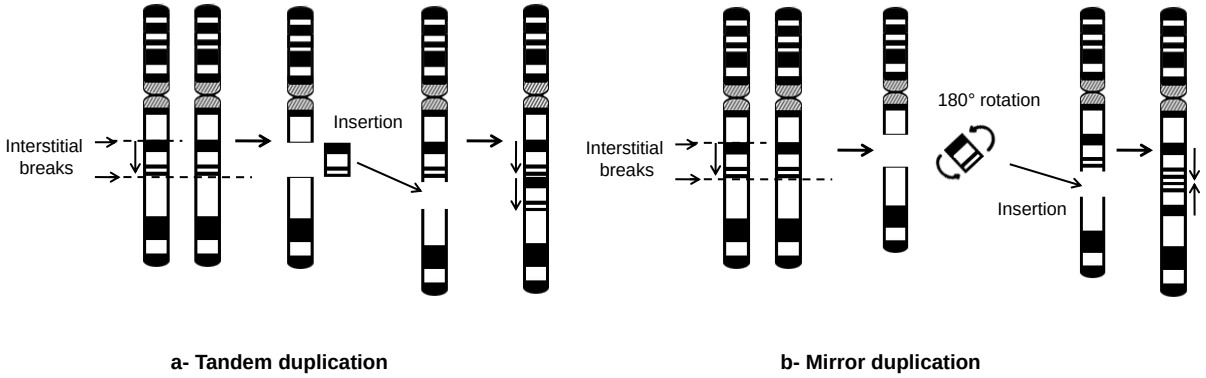

Fig. 24: The formation mechanism of a duplication

4.4. Translocations

A translocation is a chromosomal rearrangement characterised by the transfer of a segment of DNA from one chromosome to another, following one or more breaks. It is most often a balanced abnormality, involving neither loss nor gain of genetic material. It usually involves two chromosomes but can be more complex, with a significant risk of producing offspring carrying unbalanced rearrangements.

Two main types are distinguished:

- **Reciprocal Translocation**

This involves a mutual exchange of chromosomal fragments between two non-homologous chromosomes (**Fig. 25**). In its balanced form, this exchange results in no loss or gain of genetic material, and the carrier is typically phenotypically normal. However, if the exchange is accompanied by a deletion or an additional rearrangement, the translocation becomes unbalanced and can lead to phenotypic abnormalities.

During meiosis, the rearranged chromosomes present pairing difficulties:

In an individual homozygous for the translocation, the chromosomes pair normally, forming bivalents.

In a heterozygous individual, the partially altered homologous chromosomes form a complex pairing structure, often a quadrivalent (or tetravalent) (**Fig. 26**), which disrupts chromosomal segregation. This faulty meiotic segregation can produce gametes carrying unbalanced (and often non-viable) rearrangements.

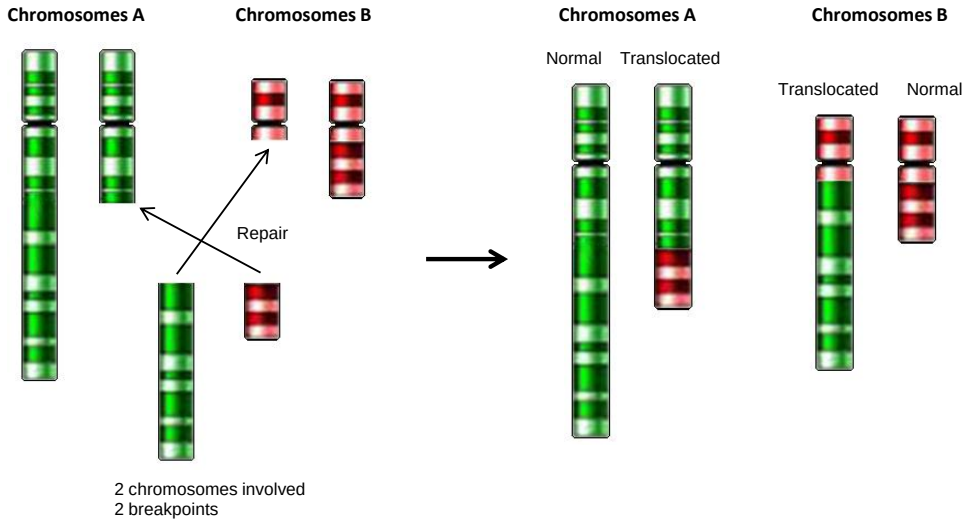

Fig. 25: The formation mechanism of a reciprocal translocation between the short arm of one chromosome and the long arm of another chromosome.

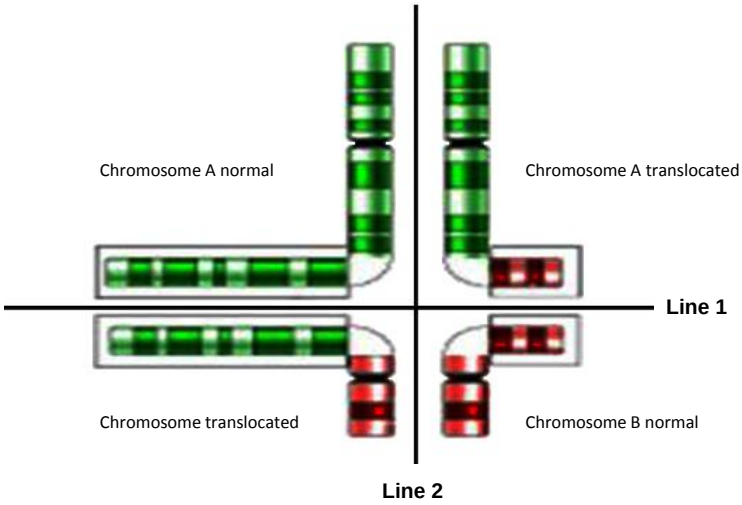

Fig. 26: Example of tetravalent formation during meiosis I in the case of a heterozygous reciprocal translocation

- Insertion

This is a special case of translocation, involving the transfer of an intercalary chromosomal segment into another chromosome. This anomaly requires three breakpoints: one on the recipient chromosome and two on the donor chromosome, which releases the intercalary fragment.

The donor chromosome can also be the recipient chromosome. In this case, it involves the displacement of a chromosomal fragment from one site to another on the same chromosome. The fragment can retain the same orientation (referred to as a direct insertion) or have a reverse orientation (inverted insertion).

Insertion is a genetically balanced rearrangement, but it is very unstable during meiosis.

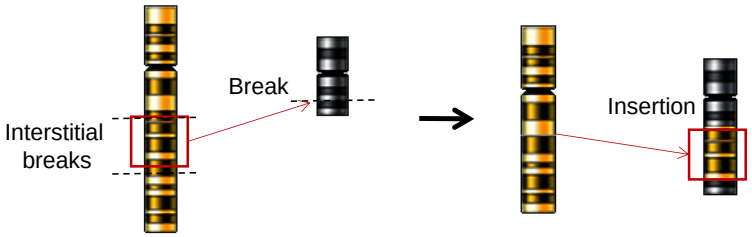

Fig. 27: The formation mechanism of an insertion.

Chapter IV: Cytogenetics and Evolution

The evolution of species results from genetic and chromosomal modifications that transform gene structure and genome organisation. Cytogenetics studies these variations to understand how they favour the adaptation and diversification of organisms.

These mechanisms include:

- Variation in DNA quantity, gene duplication, and transposable elements, which are sources of genetic innovation.
- Chromosomal rearrangements (inversions, translocations, deletions) which modify chromosome structure and influence the transmission of traits.
- Polyploidy, which increases the number of chromosome sets and stimulates diversification, especially in plants.

1. Variation in DNA Quantity, Gene Duplication, and Transposable Elements

Genome evolution relies on mechanisms that modify its size and organisation. Among them, variation in DNA quantity, gene duplication, and the action of transposable elements play a key role in organism adaptation and species diversification.

1.1. Variation in DNA Quantity

The amount of DNA in a cell can vary considerably from one species to another, and even within the same evolutionary lineage. This variation is influenced by several factors:

The accumulation of non-coding DNA, which can increase genome size without necessarily altering the number of genes.

The duplication of chromosomal segments, which leads to the addition of new copies of genes or regulatory sequences.

The elimination of DNA sequences, through recombination or deletion, allowing the genome size to be stabilised and redundant or unnecessary sequences to be removed.

These fluctuations directly influence genetic complexity and can play a role in species adaptation by modifying gene expression and genomic diversity.

1.2. Gene Duplication

Gene duplication is one of the major mechanisms of genome diversification. It occurs when additional copies of a gene appear due to:

Segmental duplications, which involve portions of chromosomes containing several genes.

Polyploidy events, where the entire genome is duplicated, especially in plants.

Retrotranspositions, where a messenger RNA is reverse transcribed into DNA and integrated elsewhere in the genome.

Newly formed gene copies can follow different evolutionary trajectories:

Conservation: if the duplicated gene remains functional and continues to perform its initial role.

Specialisation (Neofunctionalisation): one of the copies acquires a new, advantageous function for the organism.

Degeneration (Pseudogenisation): the duplicated gene accumulates mutations and becomes non-functional.

This phenomenon is at the origin of the evolution of many gene families involved in essential functions, such as immunity, photosynthesis, or development. Example: the emergence of trichromatic vision in primates.

1.3. Transposable Elements

Transposable elements, or transposons, are DNA sequences capable of moving from one location to another in the genome. They represent a significant portion of the genome of many organisms and can have varied effects:

Increase in genome size by inserting at different locations.

Modification of gene expression by influencing genetic regulation.

Creation of new regulatory sequences, favouring the emergence of new gene expression networks.

Chromosomal rearrangements, which can generate genetic diversity but also deleterious mutations.

Despite their disruptive potential, these elements have played a key role in evolution by contributing to the emergence of new genetic functions and favouring evolutionary innovation.

2. Chromosomal Restructuring and Evolution

Chromosomal restructuring refers to all modifications affecting the structure or organisation of chromosomes. These rearrangements, which change the order, number, or position of genes, play an essential role in species evolution.

They can influence genome stability, fertility, genetic diversity, and even contribute to speciation (the formation of new species).

These changes do not concern the gene sequences themselves, but the way these genes are organised in the genome, which can have considerable effects on their expression and transmission.

Chromosomal restructuring is divided into two broad categories:

- Intrachromosomal rearrangements (within the same chromosome),
- Interchromosomal rearrangements (between different chromosomes).

The main types are:

2.1. Deletions

A deletion corresponds to the loss of a chromosomal fragment containing one or more genes. It can be interstitial (in the middle of the chromosome) or terminal (at the end). This loss can have serious effects if it concerns essential genes.

Examples:

- Deletion on the short arm of chromosome 5 in humans causes Cri-du-chat syndrome.*
- In plants, small deletions can also favour local adaptations by eliminating genes that have become unnecessary in a given environment.

2.2. Duplications

A duplication is the repetition of a chromosome fragment. It increases the amount of DNA and can provide raw material for evolution, as a gene copy can evolve towards a new function (see the point on gene duplication).

Example: In the wheat genome, chromosomal duplications led to the appearance of new storage proteins, improving grain quality.

2.3. Inversions

An inversion occurs when a chromosomal fragment detaches, rotates 180°, and then reattaches to the chromosome.

If the centromere is not included: paracentric inversion.

If the centromere is included: pericentric inversion.

Inversions modify the order of genes, which can influence their expression and limit recombination during meiosis, thus favouring genetic divergence between populations.

Example: Inversions are involved in speciation in some flies of the genus Drosophila and in adaptation to variable climates in Arabidopsis thaliana.

2.4. Translocations

A translocation results from an exchange of fragments between two non-homologous chromosomes.

If the exchange is balanced, there is no loss of genetic material, but the offspring can be genetically unstable.

If it is unbalanced, there is a loss or gain of genes, often deleterious.

Translocations play a major role in chromosomal differentiation and the formation of new species.

Example: Certain species of wheat and rye (Triticum × Secale) result from natural translocations stabilised over the course of evolution.

2.5. Chromosomal Fusions and Fissions

A fusion corresponds to the union of two distinct chromosomes into one (reduction in chromosome number). A fission is the fragmentation of one chromosome into two (increase in chromosome number).

These phenomena modify the karyotype (number and shape of chromosomes) and can contribute to reproductive isolation between species.

Example: The fusion of two ancestral chromosomes gave rise to human chromosome 2, which is absent in other primates.

In plants, fusions and fissions are frequent and participate in genomic diversification. Example in the genus Medicago: the genome of M. murex *(2n=2x=14) originated from that of* M. lesinsii *(2n=2x=16) through the fusion of chromosomes 3 and 8, followed by reproductive isolation between these two species.*

Evolutionary Effects of Chromosomal Restructuring

Chromosomal restructuring can have neutral, beneficial, or deleterious effects, depending on their nature and scale. Their evolutionary impact manifests at several levels:

- **Generation of Genetic Diversity**

Rearrangements modify gene position, creating new genetic combinations and thus variability. This favours adaptation to different environments and can contribute to the evolutionary plasticity of species.

- **Modulation of Gene Expression**

The displacement of a gene into a new chromosomal region can expose it to different regulatory regions (position effect), modifying its expression level. Thus, duplicated or translocated genes can acquire new functions or be activated in other tissues.

- **Reproductive Isolation and Speciation**

Individuals carrying different chromosomal rearrangements can produce abnormal gametes, leading to reduced fertility. This phenomenon limits crossbreeding between populations, thus favouring the formation of new species (chromosomal speciation).

Example of karyotype evolution through chromosomal restructuring in the mouse *Perognathus goldmani* in northern Mexico.

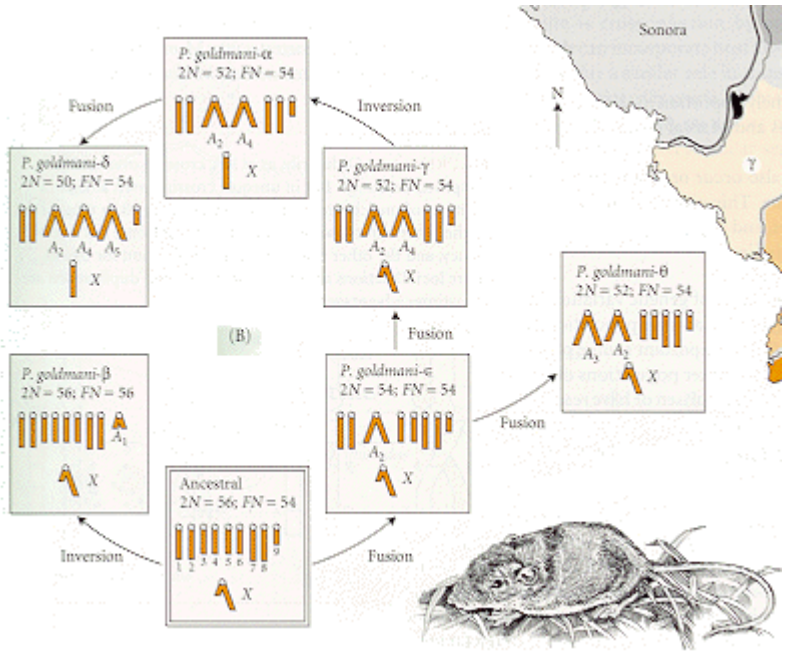

Fig. 28: Karyotype evolution through chromosomal restructuring in the mouse *Perognathus goldmani* in northern Mexico

- Stabilisation and Adaptation of the Genome

Certain restructurings can stabilise the genome by reducing undesirable recombinations or by adapting the chromosomal structure to the metabolic and ecological needs of the species.

3. Polyploidy and Evolution

Polyploidy refers to the presence of more than two complete sets of chromosomes in the cells of an organism. In eukaryotes, the genome is generally diploid (2n=2x), meaning it consists of two sets of homologous chromosomes, one of maternal and the other of paternal origin. However, some organisms possess 2n= 3x (triploid), 2n= 4x (tetraploid) or more chromosomal sets 6x, 8x … (hexaploid, octoploid, etc.).

The Different Types of Polyploidy

- **Autopolyploidy**
- It results from the duplication of the genome of a single species.
- All chromosome sets come from the same ancestral genome.
- This phenomenon can occur through non-disjunction of chromosomes during meiosis or mitosis, leading to the formation of unreduced gametes (2n).
- When these gametes fuse, the resulting organism becomes autotetraploid (4n).

Consequences:

- Cell and organ size are often increased (pollen grains, seeds, leaves).
- Reduced fertility, because homologous chromosomes pair with difficulty during meiosis.

Example: certain varieties of onions, potatoes, and alfalfa.

- **Allopolyploidy**

It originates from the fusion of genomes belonging to two different species, followed by a complete duplication of the hybrid genome.

The resulting organism combines the genetic characteristics of the two parent species.

This type of polyploidy is frequent in plants and is often the origin of new, stable, and fertile species.

Examples:

- Common wheat (*Triticum aestivum*, 2n = 6x = 42), resulting from the hybridisation between three ancestral grass species.*

- Rapeseed (*Brassica napus*, 2n = 38), born from the hybridisation between *Brassica rapa* and *Brassica oleracea*.

Mechanisms of Polyploidy Appearance

Polyploidy can result from several biological processes:

Chromosomal non-disjunction: Failure of homologous chromosome separation during meiosis or mitosis, generating cells with an abnormally high number of chromosomes.

Fertilisation of an unreduced gamete (n=2x) by a normal gamete (n=x), producing a triploid (2n=3x).

Spontaneous chromosomal doubling in a hybrid zygote, which restores fertility by re-establishing homologous pairs.

Experimental treatments (for example with colchicine), used in agriculture to induce polyploidy and create new, higher-performing varieties.

Biological and Evolutionary Consequences of Polyploidy

- Morphological and Physiological Effects

Polyploid cells are often larger and contain more DNA, leading to an increase in organ size (seeds, fruits, flowers, leaves).

Polyploidy also influences metabolism, stress resistance (cold, drought, salinity) and hybrid vigour.

- Genetic Effects

Genome duplication creates genetic redundancy: some gene copies can specialise, acquire new functions (neofunctionalisation) or become deactivated (pseudogenisation).

This flexibility favours evolutionary innovation and adaptation to new environments.

- Effects on Reproduction and Speciation

Polyploids are often reproductively isolated from their diploid forms, as crosses yield sterile hybrids.

This mechanism favours the rapid formation of new species (instantaneous speciation).

In other words, polyploidy acts as an accelerator of diversification in plant evolution.

- Evolutionary Role of Polyploidy

Polyploidy plays a major role in evolution, particularly in plants. It favours:

Adaptation to varied environments thanks to gene redundancy, allowing tolerance to mutations and extreme environmental conditions.

The appearance of new species through rapid reproductive isolation from diploid forms.

Increased genetic variability, offering a reservoir of genes that can be modified, specialised, or silenced over time.

It therefore contributes to evolutionary diversification and the ecological expansion of polyploid groups.

- **Distribution of Polyploidy in the Plant Kingdom**

The frequency of polyploidy varies strongly according to taxonomic groups:

- According to Löve (1964), polyploids represent about 90% of Pteridophytes, 50% of Monocotyledons, and 30% of Dicotyledons.
- Stebbins (1966, 1971) reports that they constitute 30 to 35% of Angiosperms, compared to only 4.5% in Gymnosperms.
- John and Lewis (1968) confirm that polyploidy is much more widespread in plants than in animals.

Bibliographic references:

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. (1986). Biologie moléculaire de la cellule (2e éd.). Flammarion Médecine-Sciences, Paris.

Campbell Neil A., Reece Jane B. (2004). Biologie ; ed. ERPI Editions du renouveau pedagogique Inc.

Djafri-Bouallag L., Ourari M., Sedki W., Fedoul D., Taleb-Toudert K. (2025). Microsporogenesis and reduced fertility in Algerian populations of *Hordeum bulbosum* L. (Poaceae). Cytologia 90 (4), 245-253.

Elrod Susan, Stansfield William D. (2003) Génétique; Ed. Ediscience.

Gorenflot, R. and Raicu, P. (1980). Cytogénétique et évolution. Masson, Paris.

Griffiths A. J. F., Miller J. H., Suzuk D. T., Lewontin, R. C. and Gelbart W. M. (2002). Introduction à l'analyse génétique Ed. De Boeck.

Löve, A. and Löve, D. (1975). Plant chromosomes. Cramer, Vaduz.

Öztürk, D., Koyuncu, O., Koray Yaylacı, Ö., Özgişi, K., Sezer, O., & Tokur, S. (2014). Karyological studies on the four Ornithogalum L. (Asparagaceae) taxa from Eskişehir (Central Anatolia, Turkey). Caryologia, 67(1), 79–85.

Robinson P.J. and Rhodes D. (2006) Structure of the ‘30 nm’ chromatin fibre: a key role for the linker histone. Curr Opin Struct Biol 16:336–343

Sedki W. (2016). Contribution à l’étude cytogénétique d’une population de *Hordeum bulbosum* L. d’Ighzer Amokrane (Béjaïa). Mémoire de Master en Biologie Option : Génétique et Amélioration végétale. Université Mouloud Mammeri Tizi Ouzou.