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Topic: Introduction to Taxonomic Evidences from Cytology

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CYTOLOGY IN RELATION TO TAXONOMY

[CYTOTAXONOMY]

[CYTOLOGY]

- Cytology refers to the study of cell and its organelles for their structure and function.
- Cytogenetics is the study of heredity and variation in terms of chromosome number, form, size and gross behaviour.
- The **chromosome number is considered as an important taxonomic character**. The individuals within a species usually have the same chromosome number.
- Study of **chromosomes at individual and population level has immensely contributed to our understanding on variation and evolution and as a phylogenetic character**.
- **Genome size is the total amount of nuclear DNA in one basic set of replicated chromosomes**.
- The knowledge of the total DNA amount within taxa can help suggest evolutionary relationships, especially in cases which involve chromosomal change.

Chromosomes ('chroma' means 'color' and 'soma' means 'body') contain the genetic material that is responsible for maintaining reproductive barriers as well as integrity of species and thus, play an important role as the source of comparative data in taxonomy.

Lewis (1957) pointed out that chromosomes derive their prominence as a tool in taxonomy due to their direct relation with genetic system, of which they are integral part.

Love (1960) opined that chromosomes are not just another character comparable to the superficial morphological characters which taxonomists are forced to use for the identification of herbarium material.

CYTOTAXONOMY is the integration of cytology and taxonomy in the attempt of better understanding to solve problems of plant affinities.

Cytological evidences are used for distinguishing taxa, to determine the origin of groups and to understand the evolutionary history of inter specific and intraspecific taxa.

Cytogenetics is of importance to taxonomy (cytotaxonomy) firstly because chromosome number, structure and behaviour often have taxonomic value in the same manner as any other morphological character. Secondly, as the chromosomes are the physical location of heredity material, they are direct source of evidence to the nature and origin of variation.

Each plant species has a characteristic number of pairs of chromosomes; this may vary among plants from two to >100 pairs in the nucleus of each cell.

The chromosomes determine the characters, whereas the characters are not determined by the chromosomes.

Concrete examples of cytology playing an important role in modern taxonomy has been proved by **Gundersen (1950)** in his publication, "**System of Phylogenetic Classification**". His system of classification was based on Basic Chromosome Number together with anatomical and morphological characters.

METHODS OF CHROMOSOME STUDY

Chromosomes give information about the biological basis of relationships among taxa. Different types of cytological data have been used in elucidation of taxonomic problems includes the study of various attributes chromosomes such as:

- i. Number, ii. Size and morphology, iii. The position of Centromere, iv. Behaviour of chromosomes at meiosis, v. Chromosomal aberration in reproduction, vi. Special banding patterns (C-banding, G-banding, Q-banding and Hy-banding), vii. Genes, viii. Noncoding sequence content, ix Behaviour during meiosis and x. Total DNA content**

HOW CYTOLOGY RELATES TO TAXONOMY

1. **Species identification:** Cytology can help in the identification of species by examining the cellular characteristics unique to different organisms. e.g., the number and structure of chromosomes can be used to distinguish between species.
2. **Reproductive Mechanisms:** Cytology can provide insights into the reproductive mechanisms of organisms, including how they reproduce and whether they can interbreed. This information is vital for species classification.
3. **Hybridization Studies:** When different species can interbreed, cytological techniques can be used to study hybridization events. This information is considered when determining the taxonomy of such hybrids.
4. **CRYPTIC SPECIES:** Cytology can reveal hidden diversity within species that may look similar externally. Differences in cellular characteristics may lead to the recognition of cryptic species, which can impact taxonomy.
5. **Phylogenetics:** Cytological data, such as chromosome number and structure, can be used to infer evolutionary relationships among species. This information contributes to the construction of phylogenetic trees and the classification of organisms based on their evolutionary history.

In summary, cytology provides valuable data that taxonomists can use to differentiate, classify, and understand the evolutionary relationships among living organisms. It complements other methods such as morphology, genetics, and ecology in the field of taxonomy.

CYTOLOGICAL TECHNIQUES COMMONLY USED FOR RESOLVING TAXONOMIC PROBLEMS

1. **Karyotyping:** This involves analysing an organism's chromosome number and structure. Differences in karyotypes can help distinguishing between species or subspecies.
2. **Flow Cytometer:** It's used to measure the DNA content in cells, which can reveal ploidy levels and variations between closely related species.
3. **Fluorescence in situ hybridization (FISH):** FISH helps detect and locate specific DNA sequences on chromosomes, aiding in the identification of genetic differences.
4. **Genomic Sequences:** Whole-genome sequencing and comparative genomics can uncover genetic variations and similarities among taxa.
5. **Microscopy:** High-resolution microscopy can reveal differences in cell and organelle structures, which may be taxonomically informative.
6. **Nuclear DNA Marker:** DNA barcoding using specific nuclear DNA marker like ITS (Internal Transcribed Spacer) regions can aid in species identification.
7. **G-banding and C-banding:** Staining techniques to reveal banding patterns on chromosomes, which can be used for comparative cytogenetics.

These techniques help taxonomists to classify and differentiate species accurately.

A. CHROMOSOME NUMBER

Of all the chromosomal characters used in taxonomic and evolutionary studies, Chromosome Number and Morphology have been the most popular. For more than 90 years, it has been an important element in evaluating relationships and deducing phylogenetic sequences in the angiosperms.

The chromosome number is considered as an important taxonomic character. The importance of chromosome number as a taxonomic character is that it is usually constant within a species. However, there are exceptions where chromosome numbers vary.

The chromosome number is usually constant in a species, hence considered as an important taxonomic character. The individuals within a species usually have the same chromosome number. **Similar chromosome numbers among species of the same genus may indicate close relationship.**

The lowest chromosome number in the somatic cells of a plant is its diploid number ($2n$). For example, in *Haplopappus gracilis* the **diploid number is 4 ($2n=4$) and the haploid number is 2**. Ferns show unusually high chromosome numbers. For example, *Ophioglossum reticulatum*, a pteridophyte, have **chromosome number ($2n=1260$)**.

CYTOTYPE

A species normally shows a single chromosome number. In some cases, the populations or infraspecific taxa (subspecies, variety, forma) may sometimes show a different chromosome number (or even different chromosomal morphology). Such populations or infraspecific taxa are known as cytotypes.

The population or infraspecific taxa showing different chromosome number or morphology are known as CYTOTYPES.

In higher plants there are three main different cytological situations, these are **DIPLOIDS, POLYPLOIDS** and **ANEUPLOIDS**, even though **HAPLOIDS** are reported in species like *Prunus persica* and *Theobroma cacao*.

The genus *Pinus* e.g., has got all its species with **$n=12$** .

In certain groups of vascular plants the chromosome number is constant throughout the whole group. The genus *Quercus* and most other members of the family Fagaceae have **$n=12$** . **In such cases chromosome number is not of any help in distinguishing various taxa within within the group.**

But in most cases, the numbers are quite variable and among angiosperms it ranges from $n=2$ in *Haplopappus gracilis* (Asteraceae) to $n=263-265$ in *Poa littorosa* (Poaceae). Ferns show usually high chromosome number ($2n=1260$).

The Number of Chromosomes in each cell of all organisms of a single species is constant. It is also established that the more closely related species are likely to have similar chromosome number while the more distantly related ones shall have different numbers. Due to this relative conservativeness, Chromosomes Numbers have become an important

International Association of Plant Taxonomy (IAPT) published an Index to Plant Chromosome Number in series of *Ragnumvgetabile* (1967-77) in 9 volumes. Diploid numbers are indicated as $2n$ and haploid as n . The lowest chromosome number in the somatic cells of a plant is its diploid number ($2n$).

Closely related plants such as different species of a genus, show chromosome numbers which reveal an arithmetic relation with one another, often in multiples of a base number, characteristic of the genus.

Solanum nigrum is a good example of the existence of a species complex, comprising diploid, tetraploid and hexaploidy forms.

Table : Chromosome polyploid series of some angiosperms

| Taxa | Chromosome series |
|------------------|---------------------------|
| <i>Brassica</i> | $n = 6, 7, 8, 9, 10$ |
| <i>Aster</i> | $n = 9, 18, 27$ |
| <i>Taraxacum</i> | $n = 8, 12, 14, 20, 24$ |
| <i>Vicia</i> | $n = 5, 6, 7, 12, 14$ |
| <i>Festuca</i> | $2n = 14, 28, 42, 56, 70$ |

In genus *Festuca*, different species have different chromosome numbers forming an arithmetic series (See above Table). We may assume that different species may have some common basis. If we assume that these chromosome numbers are based on a **common denominator or base number called x** ($x = 7$), then we can consider the different species to have multiples of this number. This denominator of base number ($x = 7$) can be considered as the **basic set of genetic information** carried by a plant and due to the multiplication of this basic genetic set, the evolution of different species have occurred. Such a series number of chromosomes in a diploid species (i.e., $x = n = 7$).

According to Cronquist (1981), $2n = 16$ is the characteristic of **Trimeniaceae** and $2n = 26$ of **Amborellaceae**.

Hutchinson (1973) placed *Thalictrum* and *Aquilegia* in two different families **Ranunculaceae** and **Helleboraceae** respectively. Both genera are distinct in having small chromosomes ($x = 7$), [most of the other members of the Ranunculaceae have $x = 8$] and as such have been placed together into a separate tribe.

On the basis of morphology, anatomy and embryological features the genus *Paeonia* was placed in the family Ranunculaceae. This genus has been transferred from the family Ranunculaceae to a separate family **Paeoniaceae** on the basis of chromosome data [very large chromosomes and $x = 5$, whereas other members of Ranunculaceae mostly have $x = 8$].

Two members of the Asparagaceae, *Drimiacoromandeliana* and *D. indica* have different gametic chromosomes, $n=20$ and $n=10$ respectively. Hence both accepted as two distinct species on the basis of Cytology.

| Major Subfamilies of Poaceae | Basic chromosome number |
|------------------------------|---------------------------------|
| Bambusoideae | 12 |
| Ehrhartoideae | 10, 11, 12, 15 |
| Pooideae | 2, 4, 5, 6, 7, 8, 9, 10, 13, 19 |
| Chloridoideae | 7, 8, 9, 10 |
| Panicoideae | 5, 6, 7, 8, 9, 10, 12 |

Thus, chromosome studies can give information about the numerical variability, structural variations among taxa and play a significant role in taxonomy.

Polyploidy has been utilized as a positive marker of the direction of evolution, to indicate primitive and derived groups. **It has been widely held that haploids are more primitive forms from which diploids arose.**

When in a polyploidy series a plant possesses an exact multiple of the basic number of chromosomes, it is called **EUPLOID**. Foreexample, in family Malvaceae, the basic numbers in various species range from 10, 15, 20, 25, till 40, from 12, 18, 24 to 30, from 14, 28, 42, 56 to 84 and so on .

On the other hand, when the chromosome numbers found within a group do not show simple numerical relationship to each other, it is said to be **ANEUPLOID**.

Another type of supernumerary chromosome found in plants are **B-CHROMOSOMES**. These chromosomes which are usually small and heterochromatic do not pair with normal **A-CHROMOSOMES** They reduce fertility or increase the vigour of plants and restricted to certain plants, e.g. Maize.

B. BASIC CHROMOSOME NUMBER [BASE NUMBER]

The basic chromosome number is an ancestral number (often the lowest detectable haploid number) within a group of related taxa. It is referred to as the **base number** and is represented as x . Duplication of chromosome numbers leading to polyploidy may prove to be of taxonomic significance. In diploid species as such $n = x$, whereas in polyploidy species n is in multiples of x . A hexaploid species with $2n = 42$ will thus have $n = 21$, $n = 3x$ and $2n = 6x$.

If in a genus the haploid numbers are $n = 8, 16, 24,$ and $32,$ $x=8$ would be regarded as base number.

Base number is the least gametic number of a genus, which has evolved and given rise to different numbers among species through multiplication (i.e., $2x, 3x, 4x,$ etc.).

- In an euploid series, the various members may share a common basic number (x) which is the gametic number of diploid species.
- As in family Malvaceae, basic number $x = 5$. The other species in the series are described as triploids ($3x$), tetraploids ($4x$), hexaploids ($6x$), and polyploids (nx).
- The basic number is usually constant for a genus or higher taxa and has proved useful in supraspecific studies.

Primary and Secondary basic numbers

- In many cases more than one basic number can be present in a group.
- For example, in the living species of *Chlorophytum* of the family Liliaceae, the chromosome numbers vary from 14 to 28, 42, 56, 84, etc. and also from 16 to 32. This indicates that *Chlorophytum* has two basic numbers $x = 7$ and $x = 8$.
- In such cases, the inferred base numbers ranging between 2 and 13 may be referred to as a Primary Basic Numbers in the absence of living diploid members. The remaining are termed Secondary Basic Numbers.
- However, in case of *Chlorophytum*, detailed analysis of the meiotic behaviour of chromosomes in one of the species, *C. laxum*, has shown that the two base numbers 7 and 8 should be considered as secondary, most probably derived from the primary basic number $x = 4$.

The chromosome in some taxa is in exact multiple of the base number. For example, the genus *Salix* has the base number of 19.

Chromosome number variation in *Salix* species

| Taxa | Chromosome number | Ploidy |
|------------------------|-------------------|------------|
| <i>Salix viminalis</i> | $2n = 38$ | Diploid |
| <i>S. atrocinerea</i> | $2n = 76$ | Tetraploid |
| <i>S. phylicifolia</i> | $2n = 114$ | Hexaploid |
| <i>S. myrsinites</i> | $2n = 152$ | Octaploid |

In the genus *Rubus*, the base chromosome number is 7. The apomicts and biotypes have the chromosome numbers $2n=14, 21, 28, 35, 42$ and 49 . Thus, chromosome numbers may provide a clue to reproductive irregularities as apomixis.

In the genus *Nervilia*, the base number is 18 and the species have different chromosome numbers.

| Taxa | Chromosome number |
|----------------------------------|-------------------|
| <i>Nervilia infundibulifolia</i> | $2n=52$ |
| <i>N. aragoana, N. discolor</i> | $2n=72$ |
| <i>N. plicata</i> | $2n=108$ |
| <i>N. monantha</i> | $2n=144$ |

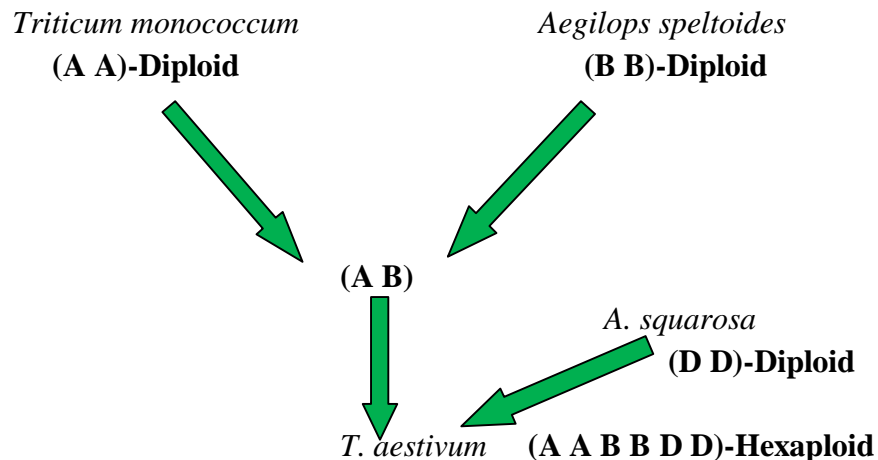
Almost 72% angiosperms are one or other type of polyploids and this has been a major feature in plant evolution.

In the genus *Festuca*, the $2n$ numbers are 14, 28, 42 and 70. These species are known as diploids, tetraploids, hexaploids, octoploids, and decaploids respectively. These numbers are based upon 7, the gametophytic chromosome number of the diploid species. This number is known as the base number or basic chromosome number (x).

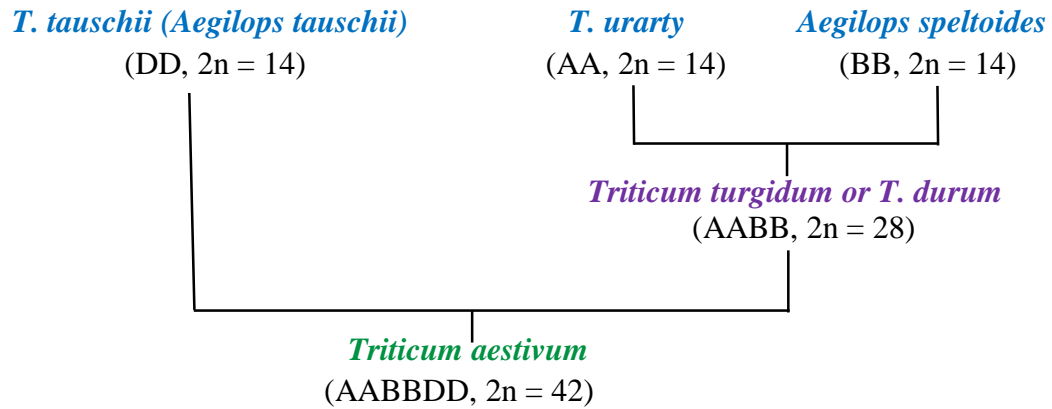
Major sub-divisions of the family Poaceae as recognised recently are characterized by the number and size of chromosomes. In Poaceae the subfamily **Poideae** has $x=7$ and **Bambusoideae** has $x=12$. Ploidy level plays a significant role in taxonomy, e.g. *Triticum* contains **diploid** ($2n=42$), **Triploid** ($2n=21$), and **Hexaploid** ($2n=42$) etc.

Bread wheat (*Triticum aestivum*) is an example of polyploidy. It has six chromosomes. In *Triticum*, $x=7$, and the chromosome number of bread wheat is therefore $2n=6x=42$.

Meiotic behaviour of chromosomes is helpful in comparing the genomes to detect degree of homology, e.g. *Triticum aestivum* is hexaploidy (AA BB DD) where 'A' is derived from *T. monococcum*(**diploid**), and 'B' from *Aegilops speltoides* and 'D' is from *Aegilops squarrosa*(**diploid**).



According to **Fedmenet *al.* 1995** and **Huang *et al.* 2002**, the origin of common bread wheat occurred as follows:



Recent detailed genome analysis have established that three diploid species have contributed to the evolution of this allo-hexaploid wheat by multiple hybridization. After hybridization between *T. urartu* (AA, $2n = 14$) and wild grass *Aegilops speltoides* (BB, $2n = 14$), *T. turgidum* (AABB, $2n = 28$) produced. At the next step, hybridization of tetraploid wheat with *Aegilops tauschii* (DD), resulted in the emergence of the hexaploidy bread wheat *T. aestivum* (AABBDD, $2n = 42$).

There are several examples where chromosome numbers support generic status.

The genus *Cistus* (Cistaceae) was previously included in the *Helianthemum*. The former has base chromosome number 8 and the latter 9. This supports the recognition of *Cistus* as a separate genus.

Cicendiafiliformis and *Microcalapusilla* (Gentianaceae) were earlier placed in the same genus *Cicendia*. They have base chromosome number 13 and 10, respectively. This thus support their generic separation.

A great deal of cytological variation occurs at specific and infra-specific level.

Monotropahypopitys (Monotropaceae) was formerly treated as a single species with two varieties, **var.hirsute** and **var.glabra**. On cytological examination, the former was found to be a hexaploidy, with $2n = 16$. The hexaploidy was retained as the species *M. hypopitys* and the var. *glabra* was raised to the species rank as *M. hypophagea*.

PRIMARY AND SECONDARY BASIC CHROMOSOME NUMBER

The Primary Basic Chromosome Number (PBCN) and the Secondary Basic Chromosome Number (SBCN) are concepts related to the ploidy of an organism's chromosome.

The PBCN represents the number of unique types of chromosomes in a basic set within an organism. It is the base number from which various ploidy levels are derived. PBCN often represented as "x". For example, if the PBCN of a plant species is 7, it means that the basic set of chromosomes in that species contains 7 unique types.

The SBCN is derived from the PBCN and represents the total number of chromosomes in a specific set in a cell. It is calculated by multiplying the PBCN by the ploidy level. e.g., if the PBCN is 7, and the organism is diploid ($2n$), the SBCN would be 14 ($7 \times 2 = 14$). The SBCN accounts for the total number of chromosomes, including both homologous and non-homologous chromosomes, in a cell.

C. KARYOTYPE AND CHROMOSOME MORPHOLOGY

Chromosome number, size and structural features together constitute the **karyotype**.

To study cytological parameters of a species, or more specifically the karyology of a species, the somatic chromosomes spread at metaphase is studied.

The appearance of somatic chromosomes at mitotic metaphase is termed as the KARYOTYPE.

KARYOTYPE

“the phenotypic appearance of the somatic chromosomes” – Levitsky, 1924.

OR

“the basic chromosome set of a somatic cell (under microscope) as seen under the light microscope”

- ❖ The first step in cytotaxonomy is sampling of a taxon and study of chromosomes of many populations within geographic races or species.
- ❖ Differences in chromosome number, their morphology and behaviour at meiosis usually indicates genetic differences of taxonomic significance.
- ❖ Succeeding step includes the determination of the ability of different populations to hybridise.
- ❖ This reveals the presence or absence of breeding barriers between groups and is of great taxonomic importance as this indicates the limits of the taxa.

Delaunay (1926) formulated the concept of karyotype for the first time as, “a group of species resembling each other in the number, size and form of their chromosomes”.

The structure of genome (Chromosome set) in a species is KARYOTYPE and its diagrammatic representation as ‘KARYOGRAM’ or ‘IDIOGRAM’. Karyotype studies are mainly concerned with morphological aspects like size and shape of the chromosomes.

Chromosomes differ not only in their length but also in the length of the two chromosomal arms. The location of the centromere (the arm-length ratio of each chromosome in the genome) determines whether the arms are more or less equal or unequal in length. The distinction between chromosomes can be made according to the location of the centromere. It may be **metacentric** (centromeres near the middle, also called ‘**V**’ types), **acrocentric** (centromeres near one end or ‘**J**’ type) or **telocentric** (truly terminal centromere, or ‘**I**’ types). [In each chromosome along with relative position of the centromere and length of arms, secondary constrictions can also provide valuable taxonomic information.]

In some taxa evolutionary trends can be worked out in these features, e.g. family Ranunculaceae

The Principal ways in which Karyotypes differ from each other in their

- ❖ Basic Chromosome Number;
- ❖ Form and relative Size of different chromosomes of the same set;
- ❖ Number and Size of the of satellites and Secondary constrictions;
- ❖ Euchromatin and Heterochromatin

Karyotype Symmetry :Chromosome size and Karyotype symmetry have served as a good taxonomic character.

It has been accepted that the absolute size of chromosome in a karyotype is fairly constant, **species specific character**.

Being inversely proportional to the ploidy level within a group, it has been often taken as an **EVOLUTIONARY MARKER**.

A Karyotype consisting of chromosomes all essentially similar to each other in size and with median or sub-median centromeres may be termed 'Symmetrical'.

'Asymmetrical' karyotypes possess many chromosomes with sub-terminal centromeres and are characterized by great difference in size between the largest and smallest chromosomes.

Asymmetric karyotypes shows the advancedness of the group, while Symmetric karyotype shows primitiveness of the group. In *Dianthus* and *Chrysanthemum* the polyploids have similar chromosomes than the diploids. In *Crepis* annual species have smaller chromosomes than the perennials.

The monocots have usually large chromosomes than dicots.

In general, woody plants have smaller chromosomes than their herbaceous relatives.

- Large chromosomes, low chromosome number and symmetrical karyotype represent a **PRIMITIVE STATUS**.
- Small chromosomes, high number and extreme asymmetry indicate **ADVANCEMENT**.

Hosta (Hostaceae), *Camassia* and *Chlorogatum* (Liliaceae) transferred to family Agavaceae on the basis of **Bimodal Karyotype**(a karyotype with two distinct sets of chromosomes).

The karyotype study can be very helpful in taxonomic evaluation of taxa at various taxonomic levels. On the basis of chromosome morphology, *Triphora* and *Nervilia* have been separated from *Pogonia*. The genus *Pogonia* has $2n=18$ have long chromosomes whereas *Triphora* and *Nervilia* have small and many chromosomes.

The genera *Agave* and *Yucca* were placed in two different families Amaryllidaceae and Liliaceae respectively. Both possess 5 long and 25 short chromosomes. The karyotype supports the placement of both the genera in a single family Agavaceae.

It is reported by Gohil *et al.* (1983) that *Fagopyrum esculentum* and *F. cymosum* from Western Himalaya have the longest and smallest chromosomes respectively. Most of the other species had median and sub-median chromosomes except *F. cymosum* which had two sub terminal chromosomes. This shows that chromosome can be of great taxonomic significance.

Karyological differences between related species have come about by gradual series of changes that have altered the visible appearance of the chromosomes. According to Darlington (1937) the breakage of the chromosomes is the first visible step in the speciation process.

D. BANDING PATTERNS

The chromosomes are microscopic and with use of appropriate **nuclear stains**, they are clearly visible during the two types of divisions in the nucleus, i.e., mitosis and meiosis. For the observation of banding patterns **Giemsa and Fluorochrome stains** are used. The investigation of banding pattern provides data for distinguishing the heterochromatic and euchromatic regions. **G-banding** and **C-banding** are staining techniques used to reveal banding patterns on chromosomes. It is also useful in indicating the position of centromeres in cases where they cannot be identified by conventional staining protocols. The technique of silver-staining has also been developed to highlight **Nucleolar Organizing Regions (NORs)** of chromosomes.

The information contained within karyotypes can be refined by the use of banding patterns on the chromosomes. For example: **Giemsa banding, Silver staining, Fluorochrome banding, FISH (fluorescence in situ hybridization)**, it helps to detect and locate specific DNA sequences on chromosomes; aiding in the identification of genetic differences), and **GISH (Genomic in situ hybridization)**.

E. BEHAVIOUR OF CHROMOSOMES AT MEIOSIS

Chromosome behaviour at meiosis provides some valuable information about the relationship of populations and species.

The **kind and degree of pairing** show whether hybridisation has occurred?

It also indicates **structural differences in the parental chromosomes**, and **explain causes of sterility**.

The **degree of chromosome homology in hybrids** is an indication of the degree of relationship of the parental species.

In sexually reproducing organisms, there are two major aspects for the behavioural pattern of chromosomes considered to be of phylogenetic significance:

- (a). Synapsis during the meiotic prophase; and
 - (b). Chiasma frequency.
-

Swertia chirayita is a medicinally important plant that is used extensively for its hypoglycemic and antimalarial activities. Fluorochrome banding and Genome size estimation was used by Than *et al.* (2017) to distinguish between critically endangered *S. chirayita* and its well known adulterants *S. nervosa* and *S. bimaculata*. The cytological characterization of these species showed distinct differences within congeneric species.

| <i>S. chirayita</i> | <i>S. nervosa</i> | <i>S. bimaculata</i> |
|---|--|--|
| Significant high length of total chromatin and nuclear DNA content. | Low length of total chromatin and low nuclear DNA content. | Low length of total chromatin and low nuclear DNA content. |
| <i>S. chirayita</i> | <i>S. nervosa</i> | |
| Type D, CMA banding pattern | Type E, CMA banding pattern | |

-*Aegilops aucheri* and *A. speltoides* differ with respect to apical spikelets and awns. In both species, karyotypes are similar except for differences in arm-ratio of satellite chromosomes. Based on the karyotypes, both have been considered as subspecies, e.g., *Aegilops speltoides* ssp. *speltoides* and *A. speltoides* ssp. *aucheri*.

-Hutchinson (1973) placed *Thalictrum* and *Aquilegia* in two different families Ranunculaceae and Helleboraceae respectively. Both genera are distinct in having small chromosomes ($x=7$), [most of the other members of the Ranunculaceae have $x=8$] and as such have been placed together into a separate tribe.

-On the basis of morphology, anatomy and embryological features the genus *Paeonia* was placed in the family Ranunculaceae. This genus has been transferred from the family Ranunculaceae to a separate family Paeoniaceae on the basis of Chromosome data [very large chromosomes and $x=5$, whereas other members of Ranunculaceae mostly have $x=8$].

-Two members of the family Asparagaceae, *Drimiakoromandeliana* and *D. indica* have different gametic chromosomes, $n=20$ and $n=10$ respectively. Hence both accepted as two distinct species on the basis of Cytology.

-Comparative analysis has shown that grasses are derived from $n=5$ to 12 ancestral karyotypes (known as **AGK for Ancestral Grass Karyotype**) and all modern grass genomes formed from these AGKs through **Whole Genome Duplication** (WGD) followed by ancestral chromosome fusion (CF).

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- ❖ Cytology has played an important role in resolving relationships in different plant groups. Raven (1975) provided a review of chromosome numbers at the family level in angiosperms and concluded that the original base-number for angiosperms is $x=7$.
 - ❖ **Interspecific variation in chromosome numbers has proved to be one of the richest source of cytological data of value to taxonomists.**
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It is usually seen that closely related plants, like the different species of a genus, shows chromosome numbers which reveal an **arithmetic mean** with one another. For example, the different species of *Piper* show chromosome numbers **in multiples of 26**, like $2n = 52$ in *P. nigrum*, $2n = 78$ in *P. betle*, and $2n = 104$ in wild species of *Piper* (Mathew, 1958).



Haplopappus gracilis



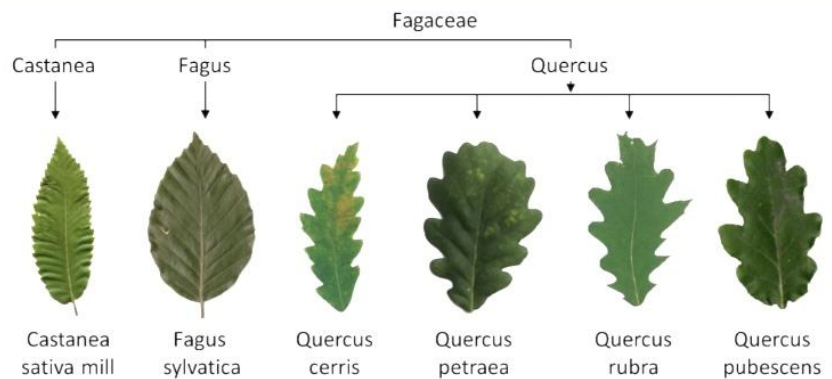
Chlorophytum



Ophioglossum

The chromosome number relationship with taxonomic groups can be broadly classified into the following three classes

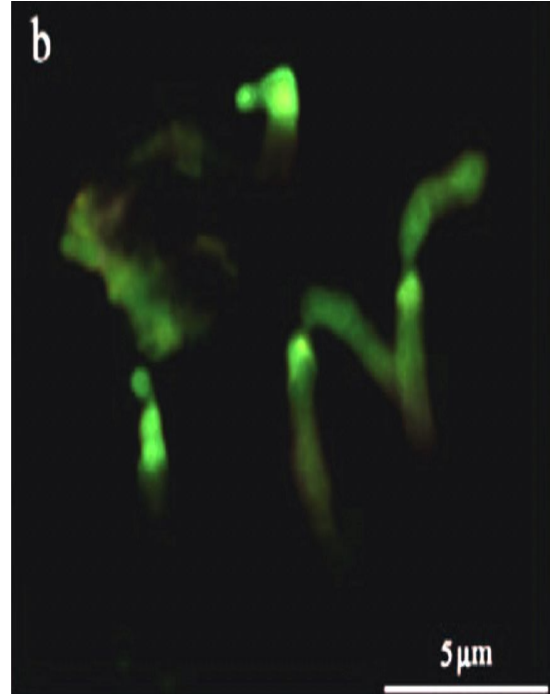
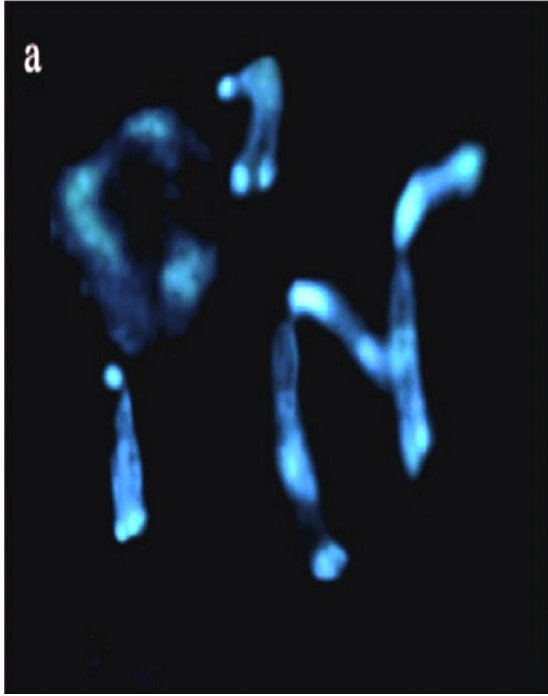
1. Constants number: in certain groups of vascular plants the chromosome number is constant throughout the whole group example *Quercus* and other member of the Fagaceae have the same basic number and is equal to 12. In such cases chromosome number is not of any help in distinguishing various taxa within the group.



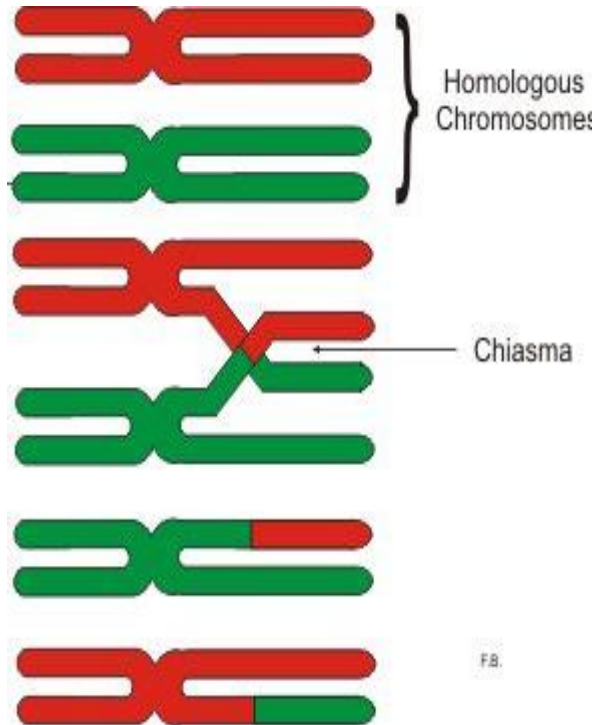
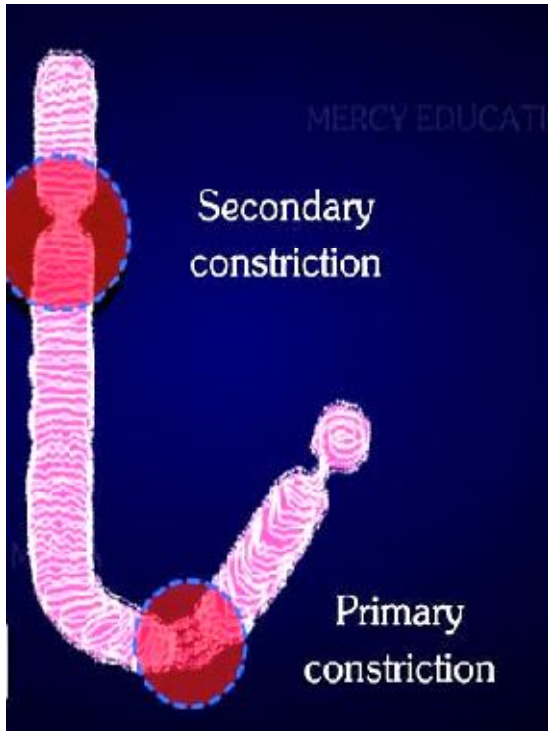
<https://www.researchgate.net/publication/325857944> Fine-Grained Hierarchical Classification of Plant Leaf Images using Fusion of Deep Models/figures

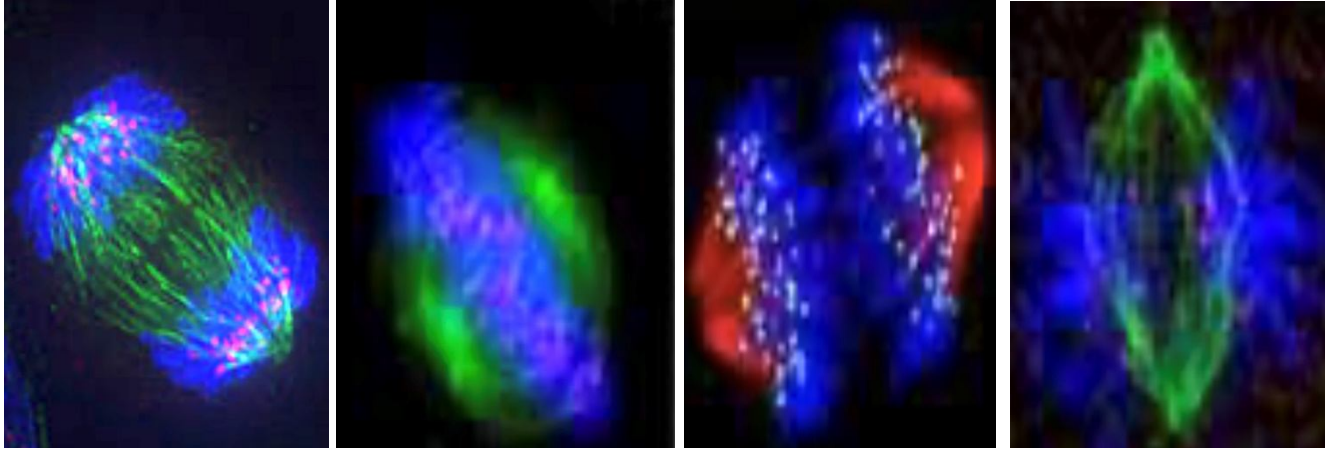
2. Euploidy: when the chromosome numbers in various members of a taxon are in the proportion of exact multiples, the series is described as euploidy. For example, in family Malvaceae, the basic numbers in various species range from 10, 15, 20, 25, till 40; from 12, 13, 24 to 30; from 14, 23, 42, 56 to 84 and so on.



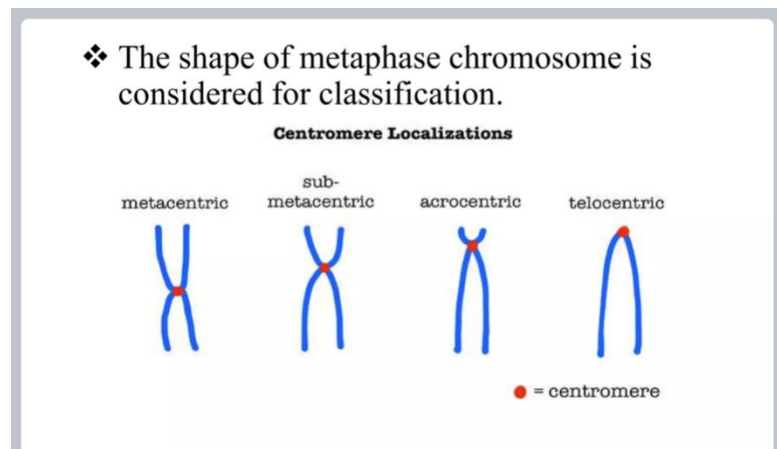


Haploppus gracilis metaphase plates after staining with DAPI (a) and CMA (b) fluorochrome





Chromosomes in different stages of Cell division



Declaration:

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THANX