



Cytotaxonomy in Plants: Chromosomal Insights into Classification and Evolution

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Cytotaxonomy, a fusion of cytology and taxonomy, utilizes chromosomal characteristics to classify and identify plant species, offering significant insights into their evolutionary relationships, genetic diversity, and systematics. By analyzing chromosome number, structure, and behavior, cytotaxonomy reveals distinctions and similarities that are often undetectable through morphological studies alone. Chromosomal variations, including polyploidy, aneuploidy, and structural changes such as inversions and translocations, play a critical role in determining species identity and their evolutionary connections. The early 20th century saw the emergence of cytotaxonomy, driven by advancements in chromosome staining, karyotyping, and microscopy, which allowed for more precise analysis of chromosomal features. The discovery of polyploidy, particularly in economically important plant families like Poaceae, Rosaceae, and Brassicaceae, underscored the significance of cytotaxonomy in understanding plant evolution and classification. Modern techniques, such as fluorescence in situ hybridization (FISH) and comparative genomics, have further refined cytotaxonomic methods, enabling detailed analysis of chromosomal rearrangements and broader genetic relationships. Cytotaxonomy also plays a pivotal role in understanding the evolutionary mechanisms underlying plant diversity, including speciation and the origins of polyploidy. Beyond its theoretical contributions, cytotaxonomy has practical applications in plant breeding, where it aids in predicting hybridization outcomes and developing new varieties, and in conservation, where it helps identify and protect distinct genetic lineages. As technology continues to advance, cytotaxonomy remains an essential tool for unraveling the complexities of plant classification and evolution.

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Introduction

Cytotaxonomy is a specialized area within taxonomy that focuses on using the characteristics of chromosomes to classify and identify organisms, particularly plants. This field merges cytology, the study of cell structures, with taxonomy, the science of classification. By analyzing various aspects of chromosomes, such as their number, structure, and behavior, cytotaxonomy provides important insights into the evolutionary relationships, genetic diversity, and systematic classification of plant species. Chromosomes, which carry an organism's genetic information, play a vital role in determining a species' identity and its relationships with other species. In plants, chromosome number and structure can vary significantly, even among species that are closely related. Such variation may arise from processes like polyploidy, which is the presence of multiple sets of chromosomes, aneuploidy, which involves an abnormal number of chromosomes, and structural changes

like inversions, translocations, or fusions [1]. Studying these chromosomal differences helps taxonomists identify distinctions and similarities that might not be apparent through morphological analysis alone. For instance, two plants that look similar morphologically may have different chromosome numbers or structures, indicating they are separate species. Conversely, plants with differing appearances might share similar chromosomal characteristics, suggesting a closer evolutionary connection than previously recognized. Cytotaxonomy began to take shape in the early 20th century when cytologists started to realize the importance of chromosome numbers and structures in classifying plants. Pioneering work by scientists such as K. Sax and J. B. S. Haldane laid the groundwork for this field by showing that chromosomal traits could be used to distinguish between species and genera. As methods for chromosome staining, karyotyping, and microscopy improved, researchers gained the ability to analyze chromosomal details with greater precision [2].

The discovery of polyploidy in plants marked a significant advancement in cytotaxonomy. Polyploidy, where an organism possesses more than two complete sets of chromosomes, is a common occurrence in plants and is often linked to speciation, adaptation, and evolutionary success. Understanding polyploidy and its effects has been crucial for classifying and understanding many plant groups, especially those in economically significant families like Poaceae (grasses), Rosaceae (roses), and Brassicaceae (mustards). In contemporary times, cytotaxonomy has benefited from a range of advanced techniques that enable more accurate and detailed chromosomal analysis. For example, fluorescence in situ hybridization (FISH) uses fluorescent probes that bind to specific DNA sequences, allowing for the visualization of particular chromosomes or chromosomal regions. This method has been instrumental in identifying chromosomal rearrangements and comparing chromosomal structures across different species. Another valuable tool in modern cytotaxonomy is comparative genomics, which involves comparing the entire genome sequences of different species. While traditional cytotaxonomy largely depended on observable chromosomal features, comparative genomics offers a broader understanding of genetic relationships, including the identification of conserved and divergent genomic regions. This approach has been particularly useful in resolving complex taxonomic challenges where traditional methods have proven inadequate. Cytotaxonomy is crucial for understanding the evolutionary processes that contribute to and sustain plant diversity. By studying chromosomal changes, cytotaxonomists can infer the mechanisms of speciation, the origins of polyploidy, and the evolutionary history of plant lineages. For example, research into chromosomal variation in certain plant groups has illuminated the roles of hybridization and polyploidy in their evolution. Furthermore, cytotaxonomy has significant practical applications in plant breeding and conservation. In plant breeding, knowledge of the cytogenetic makeup of parent species is essential for predicting the outcomes of hybridization and developing new varieties with desirable traits. In conservation, cytotaxonomy can help identify distinct genetic lineages that may require protection or special management strategies.

Cytology and Taxonomy: Cytotaxonomy and Karyosystematics

Chromosomal studies are valuable at two levels: They assist in comparing closely related species, including sibling species, and they are instrumental in determining phylogenetic relationships among various organisms. Most chromosomal events are unique to the ancestral populations where they first appeared, characterizing all subsequent descendants. Among the different branches of omega taxonomy, cytotaxonomy is the most fundamental. This is because species are real entities with specific genetic continuity, and chromosomes serve as the blueprint for ancestry, relationships, and the current and future trends of the species involved [1], [3].

Principles of Cytotaxonomy

The core principle of cytotaxonomy is that species with closer relationships exhibit more similar karyotypes compared to those less related. However, phylogenetic studies have shown that some karyotype features recur more often than previously thought, suggesting that similarity alone is not sufficient. Additionally, there are two other criticisms of cytotaxonomic interpretation: (i) intraspecific variations were traditionally overlooked, and (ii) chromosomal aberrations were illustrated based solely on classical structural changes without sufficient inspection.

Variables of Cytotaxonomy

These are chromosome characteristics that hold taxonomic value, including (1) chromosome number, (2) chromosome size, (3) chromosome morphology, and (4) chromosome behavior during meiosis (figure 1). Chromosome number and nuclear DNA content are the most practical and reliable features for cytotaxonomical studies covering a large number of species. Conversely, chromosome mapping with molecular probes is typically recommended for a limited number of species.

(i) Chromosome Number: Among various karyotypic features, chromosome number is the most commonly used in cytotaxonomical studies. Variations in chromosome number may include base number, aneuploidy, paleopolyploidy, and aneopolyploidy. These concepts have been interpreted in different ways by various authors and are crucial in cytotaxonomy and karyotype evolution. Variation in chromosome numbers, along with their relative stability within populations and species, provides an important tool for taxonomic grouping of many plants, with some exceptions. These changes generally occur during chromosome division and may affect gene sequences, gene numbers, or even result in chromosome loss. The continuation of this slow process leads to the evolution of new chromosomal races. The *Ophioglossum* species (a type of pteridophyte) holds the highest chromosome count in the plant kingdom ($2n = 1240$), while the smallest count is found in *Haplopappus gracilis* ($n=2$).

(ii) Chromosome Size: In most plants, chromosome length ranges from 0.5 to 30 μm . Individual chromosomes within some taxa exhibit significant differences in shape and size during the mitotic metaphase stage. Chromosome size varies widely among different families and even among members of the same family. Generally, monocots tend to have larger chromosomes than dicots, while woody plants typically have smaller chromosomes compared to their herbaceous counterparts. Within monocots, for example, Zingiberaceae have small chromosomes, Iridaceae possess small to medium-sized chromosomes, and Amaryllidaceae have larger chromosomes. According to Stebbins (1938), chromosome size does not correlate with the overall phylogeny of angiosperms but is specific to certain groups and families.

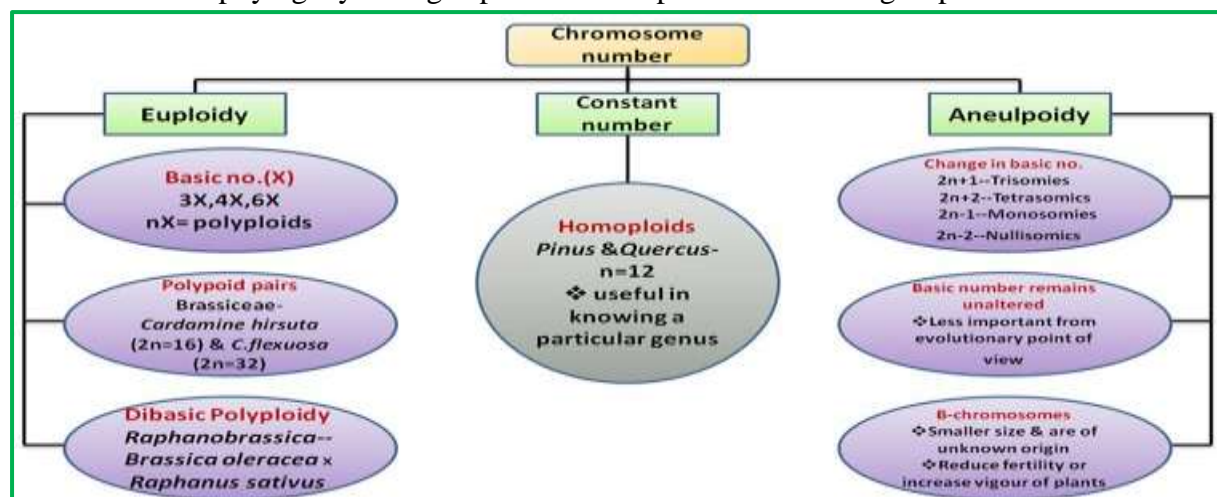


Fig. 1 Chromosome number in relation to taxonomy

(iii) **Chromosome Morphology:** In addition to chromosome number and size, flowering plants also show noticeable differences in chromosome karyotype appearance, even among species with the same chromosome number. Chromosomes are most easily observed during the mitotic metaphase. The karyotype of chromosomes can be distinguished based on the following criteria: a. The relative length of chromosome arms; b. The position of the centromere; and c. The presence of satellite structures.

(iv) **Chromosome Behavior at Meiosis:** Chromosome pairing behavior during meiosis is primarily determined by chromosome number and homology. Studying chromosome behavior during meiosis can provide valuable taxonomic information.

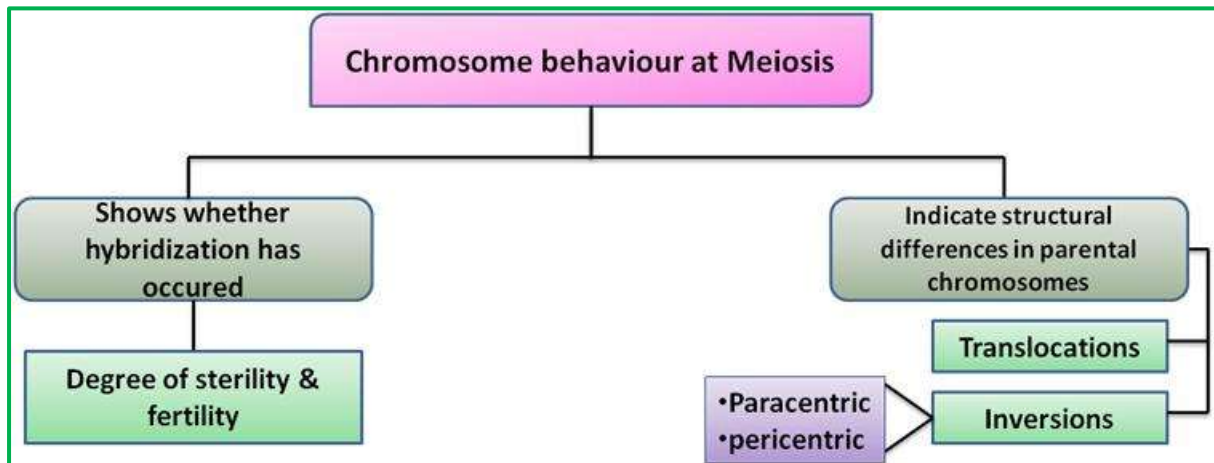


Fig. 2 Chromosome behavior at meiosis in relation to taxonomy

All these features together make of cytological data an excellent and irreplaceable source of information for taxonomical and evolutionary studies.

Systematic value of cytological studies

Cytological data, encompassing various features such as chromosome size, number, morphology, and behavior during meiosis, is an invaluable and irreplaceable resource for taxonomical and evolutionary studies. According to Jackson (1971), chromosome characteristics are useful across all taxonomic levels. For example, members of the Cyperaceae and Juncaceae families, which possess chromosomes with diffuse or non-localized centromeres and inverted meiosis, demonstrate a close relationship between these two families. In Ranunculaceae, cytological data has led to the reorganization of genera, grouping those with $n = 7, 8,$ and $9,$ as well as those with long and short chromosomes, into the tribes Anemoneae and Helleboreae. Due to karyological similarities, genera like *Thalictrum* and *Anemone* (Anemoneae) and *Isopyrum* and *Aquilegia* (Helleboreae), all with small chromosomes and a base number of $n = 7,$ have been reclassified into the tribe Thalictreae. Similarly, *Coptis* and *Zanthorhiza* of the Helleboreae tribe, with $n = 9$ and very small chromosomes, have been grouped under Coptideae (6). *Yucca* and *Agave*, traditionally classified under Liliaceae and Amaryllidaceae respectively, have been reclassified into a single family by Hutchinson due to their shared bimodal karyotype with 25 small and 5 large chromosomes. The hexaploid wheat (*Triticum aestivum*) has a genome constitution of AABBDD, with the A genome contributed by *Triticum monococcum/urartu*, the B genome by *Aegilops speltoides*, and the D genome by *Aegilops squarrosa*, illustrating how meiosis studies can trace the parentage of polyploid taxa. Stebbins (1971) distinguished grasses based on chromosome size and number, noting that bambusoid grasses have many small chromosomes, chloridoid grasses have few small chromosomes, festucoid grasses have large chromosomes, and panicoid grasses have medium-sized chromosomes [1], [5]. In the genus *Oenothera* (family Onagraceae), all species are diploid with $2n = 14,$ and during meiosis,

species can be identified by their distinct chromosome pairing patterns, such as *O. biennis* with two rings (one of six chromosomes and one of eight), *O. erythrosepala* with a ring of 12 chromosomes and a bivalent, and *O. strigosa* with a single ring of 14 chromosomes [4].

Advancement in cytotaxonomy

Over the last 20 years, the emergence of molecular cytogenetics, phylogenetics, and genomics has revitalized plant cytotaxonomy and studies on chromosome evolution, providing deeper insights into chromosome structure and the mechanisms driving chromosomal changes (Lysak et al. 2006; Abrouk et al. 2010; Wu & Tanksley 2010; Heslop-Harrison & Schwarzacher 2011). Studies that combine analyses of chromosomal changes with phylogenetic trees (as reviewed by Murray 2002) have revealed that certain chromosome characteristics are more recurrent than previously believed, emphasizing the need for more precise cytological evidence to support the interpretation of chromosome data (Dobigny et al. 2004) [6].

Advantages of Cytotaxonomy

Comparative karyotype analyses are essential for both taxonomy and understanding chromosome evolution. They enable the comparison of species that are closely related, including those that are sibling species. Cytotaxonomy holds greater significance than physiological taxonomy because it focuses on comparing chromosomes, which helps identify subtle differences among individuals. This approach classifies organisms based on cellular structure characteristics. Unique chromosomal events often define all descendants of ancestral populations where these patterns first emerged. Among the various branches of taxonomy, cytotaxonomy is fundamental because it provides insights into the genetic continuity of species, with chromosomes serving as a blueprint for their ancestry, relationships, and future developments [1], [2], [5].

Limitations of cytotaxonomy

Phylogenetic research has revealed that certain karyotype features recur more frequently than previously believed, suggesting that similarity alone is not enough. Intraspecific variation was often overlooked in the past. Relying solely on classical structural changes to interpret chromosomal alterations is a labor-intensive process that requires skilled personnel. Cytotaxonomy, while valuable, is an expensive approach and is not always suitable for defining taxa at every level. Accurate taxonomic delineation often requires integrating evidence from multiple disciplines to ensure proper identification and classification of organisms.

Future perspectives in cytotaxonomy

There is a pressing need to build a substantial group of scientists skilled in cytology, cytological techniques, computational biology, molecular biology, and taxonomy. Developing strong connections and collaborations with the user community is essential to benefit from and access the latest advances in cytotaxonomy. Adequate funding for gene banks is crucial to support effective cytotaxonomic research. Additionally, maintaining a comprehensive national cytotaxonomic database would enhance access to information associated with gene-bank collections stored ex-situ. Cytotaxonomic efforts should also focus on unexplored plant areas, particularly underutilized, minor, and neglected (orphan) crops, as these can significantly contribute to food and nutritional security in the future.

Conclusion

Karyotype analyses, comparative genomics, and DNA-based phylogenies offer various methods to explore genetic divergence between species at different levels, each with its own strengths and limitations. Insights gained from combining these approaches have advanced

our understanding of cytotaxonomy and chromosome evolution. However, cytotaxonomy alone cannot delineate taxa across all levels. Effective taxonomic classification often requires integrating evidence from multiple disciplines to achieve accurate identification and classification of organisms.

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