Genetic Improvement of Eucalypts; Tropical Hardwoods Breeding and Genetic Resources. **Tree Breeding, Principles**: Breeding Theory and Genetic Testing; Conifer Breeding Principles and Processes; Forest Genetics and Tree Breeding; Current and Future Signposts. **Tree Physiology**: A Whole Tree Perspective; Physiology and Silviculture. **Tropical Ecosystems**: Tropical Pine Ecosystems and Genetic Resources.

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Cytogenetics of Forest Tree Species

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Introduction

The discipline of cytogenetics was first defined by Sutton in 1903, as a field of investigation which developed from the separate sciences of genetics and cytology. It is concerned with studies on the correlation of genetic and cytological (especially chromosomal) features characterizing a particular genetic system under investigation. With respect to forest trees, cytogenetic studies have generally been limited to chromosome studies, on the number, appearance, and behavior of chromosomes during mitosis and meiosis, chromosomal and karyotypic evolution, and the role of chromosomes in the transmission and recombination of genes.

Plant breeding can be traced to the ancient Babylonians, but a clear understanding of genetics has its beginning in the nineteenth century with Mendel's hybridization experiments and their subsequent rediscovery by de Vries, Correns, and von Tschermack in 1900. Cytology required the invention of the microscope, and began when Robert Hook observed cork cells in 1665. Early scientists studied cell structure, organelles, and division. Nageli first described chromosomes as visual bodies during cell division in 1844, and Fleming in 1882 described the complete process of mitotic nuclear division. However, it was not until the independent observations of Sutton and Boveri that chromosomes were first linked with the emerging field of genetics.

Cytogenetic investigations of forest tree species were first conducted in the early 1900s, after cytological investigations in most crop plants and animals were well established. Leading discoveries were made in the research of insect cytogenetics, and then followed by maize (*Zea mays*) cytogenetics, especially from the standpoint of the applied methods and materials. Thomas Hunt Morgan and his group of students and scientists made fundamental discoveries in the early decades of the twentieth century, investigating giant chromosomes of fruit fly, *Drosophila melanogaster*. The fly's short life cycle and variant phenotypes/genotypes allowed rapid progress in understanding cell differentiation, cell divisions, and breeding results. In contrast, the relatively long time to reproductive maturity of many forest tree species, and logistical problems in sampling, make trees less desirable for cytogenetic research. However, papers written at the turn of the twentieth century pointed to the suitability of conifer species for cytological research.

The main interest in forest tree cytogenetics in the early 1900s was in discovering and interpreting the process of fertilization in pines. Ferguson conducted very detailed observations on the development of the egg cells, fertilization, and microsporogenesis in various pine species (*Pinus strobus*, *P. nigra*, *P. rigida*, *P. resinosa*, and *P. uncinata*). She determined the precise number of chromosomes in the haploid

state for these pine species ($n = 12$). Early embryological research, such as Ferguson's, also revealed that chromosomes of coniferous species are relatively large and easily investigated by techniques used at that time, including sectioning and chromosome smears. Working with large conifer chromosomes was made easier after 1921, when the squash technique was developed and camera lucidas utilized for illustrations.

The classic study of that era was by Sax and Sax in 1933, who pioneered karyological studies of 53 gymnosperm species and presented their results using rudimentary idiograms. They discussed the similarities and differences in karyotypes among species and advocated the use of female gametophyte tissue, which showed advantages in analyzing cells without thick cell walls and containing only the haploid number of chromosomes. Their paper was a prototype for further karyological research of coniferous species.

Karyological research of angiospermous (= hardwood) species in that era was hampered by chromosomes that were numerous and generally too small for detailed observations of morphology. Chromosome counts of many species, however, were registered and published in reference books of cumulative presentations of plant chromosome numbers, beginning with Darlington and Wylie's *Chromosome Atlas of Flowering Plants* in 1955, and followed by Moore in 1973, Fedorov in 1974, and by others.

Forest trees occur in a wide variety of taxonomic families and span different orders and classes. Correspondingly, there is a wide variation in the number and size of chromosomes among and even within different species (**Figure 1**). In some taxonomic groups, e.g., Pinaceae, there is great similarity in the chromosomes of different species, which makes identification and comparison difficult. Standardization in karyotype analysis was suggested by some authors, but was inconsistently adopted in later papers. This caused difficulties in comparing results of different cytological investigations. Additionally, differences in terminology, in statistical analysis procedures (if used), and in number of analyzed cells per species contributed confusion.

Forest tree cytogenetic research over much of the twentieth century was dominated by somatic studies on coniferous species, particularly in Pinaceae and Taxodiaceae, using conventional staining methodology. Studies by Saylor, Khosho, Mergen and Burley, Mehra, Hizume, Muratova and Kruklis, Stebbins, Schlarbaum and Tsuchiya, Borzan, and Toda and other representative studies were quoted by Schlarbaum, in his review of cytogenetic studies of forest trees. Many studies were botanical in nature, investigating inter- and intraspecific variation, cytotaxonomy, and phylogeny. In the latter part of the twentieth century, cytogenetic studies of trees exposed to air, heavy metal, and radioactive pollution were made under difficult conditions, and demonstrated the effects of pollution on the meiotic and mitotic processes.

Cell Division and Chromosomes

Cell division includes nuclear (karyokinesis) and cytoplasmic (cytokinesis) division. Simply, it is a cell reproduction process that enables growth (cell multiplication) and development (cell differentiation and growth) of an organism through mitotic division (mitosis), and parental transfer of hereditary determinants to their offspring through meiotic division (meiosis). The process of mitosis and meiosis is fundamentally similar in all organisms, but can differ in details among species.

Mitosis is a genetically controlled process that provides two identical daughter cells with chromosome numbers identical to their parental nucleus. It is followed by cytokinesis and gives rise to genetically equivalent cells in the growing somatic regions of eukaryotic species. This continuous process can be observed under the microscope and is usually described in five stages: prophase, prometaphase, metaphase, anaphase, and telophase. The period between division cycles is the interphase stage, when single-stranded chromosomes become double-stranded chromosomes due to DNA duplication prior to mitosis, with two identical chromatids attached to a common centromere (= primary constriction). Under a light microscope, the chromosomes appear as chromatin granules. As mitosis begins, each chromosome becomes visible as a distinct structure due to coiling, shortening, and thickening during the prophase stage. The spindle (= microtubules) is formed in prometaphase and becomes attached to the kinetochore within the centromere region of each chromosome. During prometaphase, the chromosomes migrate to the spindle equator. The metaphase chromosomes are most often analyzed for determining number and karyotype, as they are maximally condensed and appear as stretched or curved bars divided usually in long and short arms by centromere. In anaphase, the two sister chromatids of each chromosome are separated by movement to the opposite spindle poles. The "daughter chromosomes" of each chromosome arrive at each spindle pole, a cell wall is formed between the two new cells as the nucleus is reconstituted, and the cell proceeds into interphase.

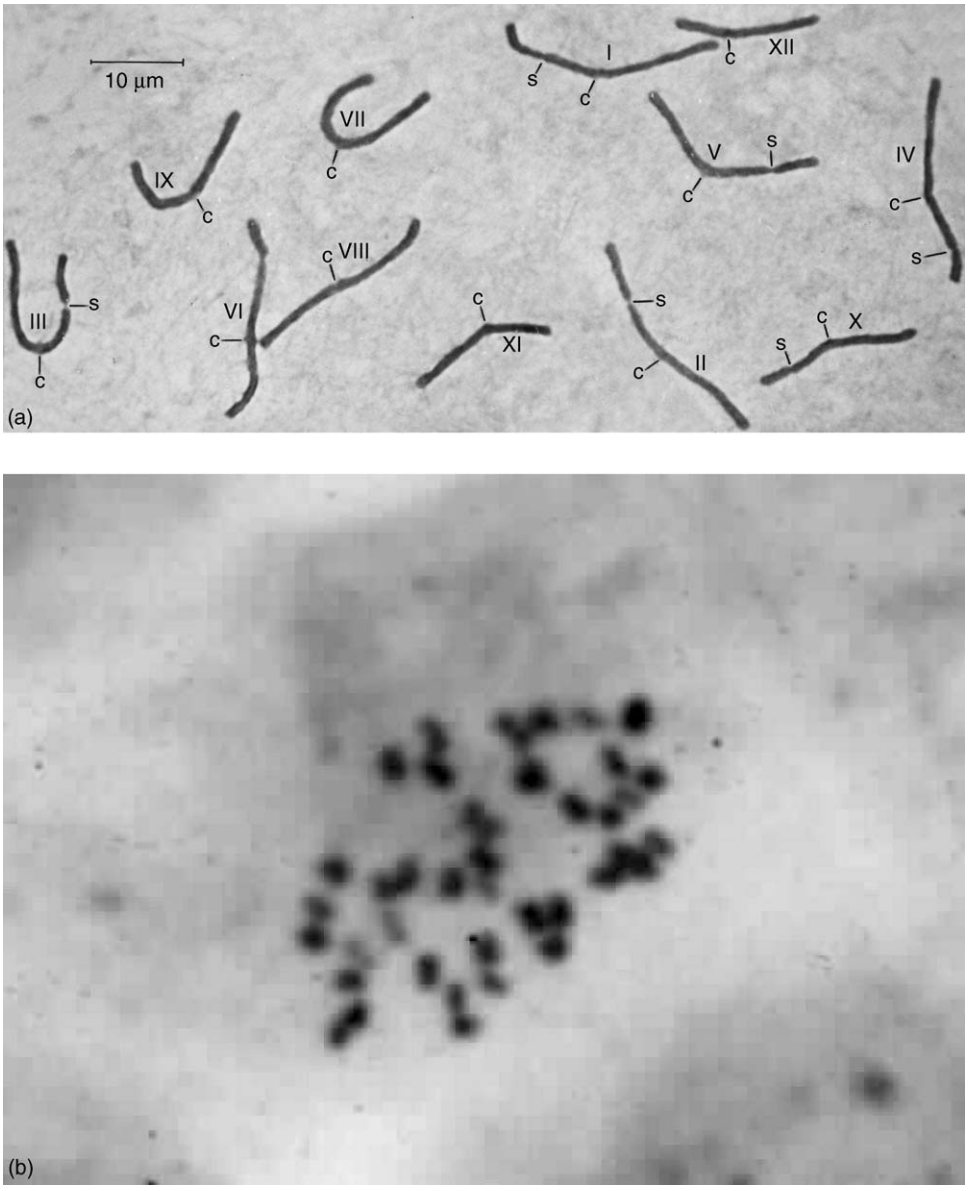

Figure 1 (a) Haploid chromosomes in the female gametophyte tissue of *Pinus nigra*; (b) Diploid chromosomes in somatic cells of root-tips in *Salix sitchensis* ($2n = 2x = 38$).

Meiosis differs from mitosis in having two successive nuclear divisions, with a reduction of chromosome number in the first division from the somatic (= sporophytic) state ($2n$) to the gametic (= gametophytic) state (n) in cells that will proceed to form gametes. Meiosis is a continuous process under genetic control and occurs over a number of stages in each division. The first meiotic division contains the stages leptotene, zygotene, pachytene, diplotene, diakinesis, prometaphase I, metaphase I, anaphase I, and telophase I. The chromosomes are loosely coiled in leptotene and become progressively more densely coiled through telophase I. Pairing of homologous chromosomes (= bivalents) begins in zygotene and is completed in pachytene, where genetic recombination can take place through reciprocal exchanges of genetic material that may occur between homologous nonsister chromatids (= crossing over). Chromosome contraction continues to occur in diplotene and diakinesis. During prometaphase I, the spindle fibers (= microtubules) are organized and become attached to the bivalent centromeres. In metaphase I and anaphase I, the chromatids do not divide as the homologous chromosomes are pulled to the opposite poles, thereby reducing the chromosome number to the haploid state.

The interkinesis stage between the first and second division of meiosis may or may not occur. During this

Figure 2 Asynapsis in *Cunninghamia lanceolata*.

stage, the chromosomes are partially uncoiled. Interkinesis is followed by the second meiotic division, which contains the stages prophase II, metaphase II, anaphase II, and telophase II. The second meiotic division is similar to mitosis, although prophase II does not occur in organisms where interkinesis is omitted. In telophase II, haploid (n) interphase nuclei are reconstituted, and cell walls are formed to separate the four cells, which in turn go through microsporogenesis (male) or megasporogenesis (female).

There can be many variations and anomalies in the meiotic process, particularly when polyploidy, instead of the typical diploidy, is involved. These variations and anomalies can be under genetic control, such as asynapsis where chromosome pairing fails completely among all chromosome pairs, or pairing is incomplete, where only certain homologous chromosomes fail to pair, and thus univalents are formed. Asynapsis can lead to fertility problems due to uneven distribution of the chromosomes in the gametes (**Figure 2**).

Variation in Chromosome Numbers

Generally, each species has a characteristic number of chromosomes in each cell (except for gametes) referred to as the somatic number ($=2n$), which is typically diploid. Most higher organisms have one species-specific set of homologous chromosomes donated by the male (pollen) ($=n$, the gametic number which is typically haploid), and the other set by the mother (egg). Through evolutionary processes, the number of chromosomes can increase by whole sets (polyploidy) and/or increase or decrease by individual chromosomes (aneuploidy).

Polyploidy can occur in different ways, spontaneously or induced. Autopolyploids are polyploids that have occurred through chromosome doubling (AA–$AAAA$). Allopolyploids are created when different species (AA and BB) hybridize and the chromosome number doubles or the hybridization involves unreduced gametes ($AABB$). A segmental allopolyploid occurs when the chromosome complements of

very closely related species or subspecies combine ($A_1A_1A_2A_2$).

As with polyploidy, aneuploidy can occur spontaneously or can be induced. There is a large body of terminology for individuals that have lost or gained individual chromosomes, e.g., nullosomic ($2n-2$; missing both homologs of a chromosome pair), monosomic ($2n-1$; missing one homologous chromosome), trisomic ($2n+1$; containing three homologous chromosomes).

In conifers, true polyploid coniferous species are rare, occurring only in Taxodiaceae (*Sequoia sempervirens*) and Cupressaceae (*Fitzroya cupressoides*), although individuals within Taxodiaceae, e.g., *Cryptomeria*, and Cupressaceae, e.g., *Juniperus*, are polyploid in nature. Aneuploidy is widespread in species of Podocarpaceae. In other coniferous families, however, polyploid and aneuploid individuals are generally stunted and not competitive in natural settings.

A review of Darlington and Wylie's *Chromosome Atlas of Flowering Plants* in 1955, coupled with a more recent overview by Schlarbaum in 1991, reveals a significant number of hardwood species where polyploid and aneuploid processes have been involved in the speciation process. Additionally, it is evident that there are species with polyploid races, e.g., *Fraxinus americana*, *Populus tremuloides*, and *P. tremula*. The chromosome nature of most hardwood species, however, is still unknown. While chromosome counts have been made on many species, those counts are often based on a single sample of individuals or a single individual.

Basic chromosome number (x) represents the smallest (monoploid) chromosome number in a taxon. Basic number can become variable as the taxon grouping becomes larger, e.g. *Cupressus* to Coniferales, etc. The basic chromosome number has evolutionary connotations, and there are many publications that speculate about the true basic number of different taxa, particularly those with high chromosome numbers.

Notation of chromosome number in the scientific literature is often incorrect, when the notation involves the somatic ($2n$) or gametic (n) number and basic number (x) of a species. For example, notation for somatic chromosome number of a diploid species with 24 chromosomes in somatic cells ($2n$), 12 chromosomes in haploid cells (n), and a basic number of 12 chromosomes, is written as: $2n=2x=24$. If there is a euploid (whole chromosome set) increase in chromosome number to 36, the notation would be $2n=3x=36$; not $3n=3x=36$. The notation for somatic number remains $2n$, despite the increase in chromosome number. With aneuploid changes in chromosome number, the $2n$ and $2x$ notation remains the same, but the number of chromosomes added or missing is noted, e.g., $2n-1=2x-1=23$.

Slide Preparation Methodology

Uniform Chromosome Staining

Until the mid-1970s, the majority of forestry cytogenetic studies were conducted to determine the chromosome number and karyotype by using a staining methodology that produced a uniform stain. Root-tip meristematic tissue and, to a lesser extent, terminal bud or young leaf tissues were used. Before fixing, the root tips are usually pretreated with a mitogen to inhibit spindle fiber formation in metaphase, resulting in slides with a large number of cells at the metaphase stage. In addition, the mitogens selected, e.g., colchicine, 8-hydroxyquinoline, were often used to shorten the extremely long chromosomes found in conifers, as well as to inhibit postmetaphase cell division. After fixation, usually in Farmer's or Carnoy's solutions, the sampled materials were hydrolyzed in different chemicals, e.g., $1\,\mathrm{mol\,l^{-1}}$ HCl, or later enzymes, e.g., pectinase, to secure the satisfactory separation of cells. Somatic investigations have used a variety of methods for slide preparation, including the smear and squash techniques.

Different cytological methods were described in detail by Darlington and La Cour in 1962, followed by various improvements made by many authors, depending on the species and tissue investigated. More recently, Fukui and Nakayama edited in 1996 an excellent laboratory manual describing methods for plant cytology investigations. An example of most commonly observed features of the prometaphase and metaphase chromosomes is shown in **Figure 3**.

Karyotype Analysis

The karyotype of an organism is a descriptive analysis of the chromosome complement. Each karyotype is defined numerically with statistical parameters of values based on the measurements of the chromosome's morphology. A graphic presentation is often used to give a better illustration of the chromosomes and their morphological features. Problems of comparison among studies can arise due to the lack of standardization in presenting a karyogram, graphically or numerically. An insight into this problem was published in *Forest Genetics* journal in 1996, with recommendations for standardized presentation of karyotypes for the species of the Pinaceae family. An example of the graphic presentation is shown in **Figure 4**.

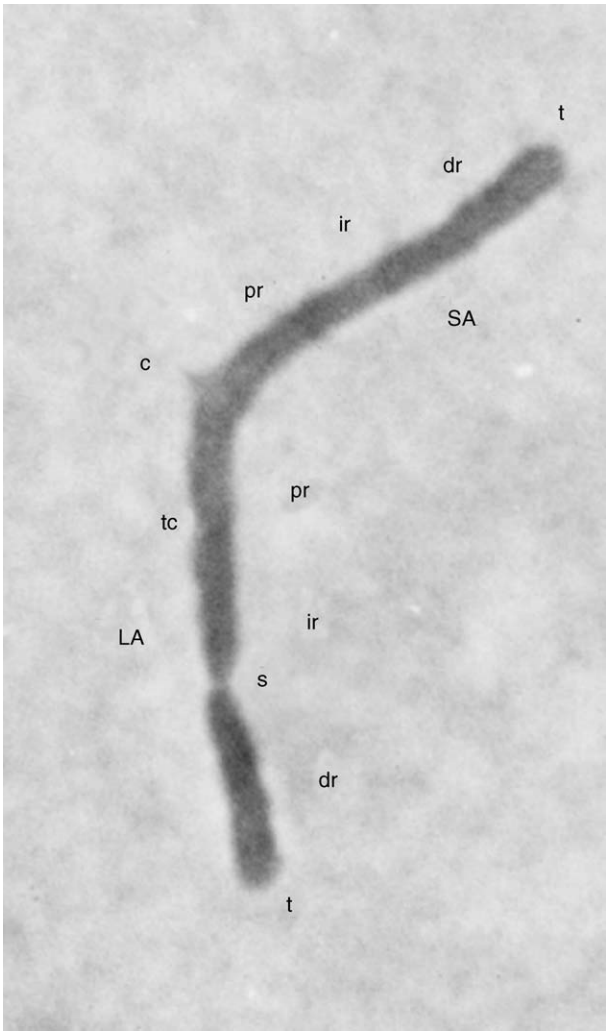

Figure 3 Chromosome terminology shown on the *Pinus nigra* metacentric chromosome V. SA, short arm; LA, long arm; t, telomere; dr, distal region; ir, interstitial region; pr, proximal region; c, centromere (primary constriction); tc, tertiary constriction; s, secondary constriction (nucleolar organization region or NOR).

Classification of chromosomes by centromere position is a basic feature of karyotype analysis. Depending on the centromere position, chromosomes can range from metacentric to telocentric. Centromeric nomenclature, however, can vary from study to study. In studies of *Pinus* species, the classification presented by Saylor's classic papers is most often used. Another classification system often cited is the nomenclature presented by Schlarbaum and Tsuchiya in 1984, which was developed according to protocols given by Levan and his coworkers in 1964. Recognizing the inconsistency in centromeric nomenclature in a wide range of studies and the need for a standard, Levan and his coworkers developed precise standards for nomenclature and devised a system for modifying the standards to allow for better distinction among chromosomes if needed. Other modifications can be used if warranted by chromosome morphology, but the modifications should be according to their protocols.

The ability for rapid communication among scientists through the internet presents an exciting possibility in sharing karyomorphological data of investigated species. An idea for consolidating data in a standardized manner in a centralized database that can be instantly analyzed and made available worldwide via the internet was presented during the Second IUFRO Cytogenetics Working Party S2.04.08 Symposium, held in Graz, in 1998.

Banding Methods

In the last quarter of the twentieth century, chromosome banding techniques began to be applied to forest tree species. These techniques allowed for better distinction between homologous chromosomes and among nonhomologous chromosomes of similar size and morphology. Chromosome banding

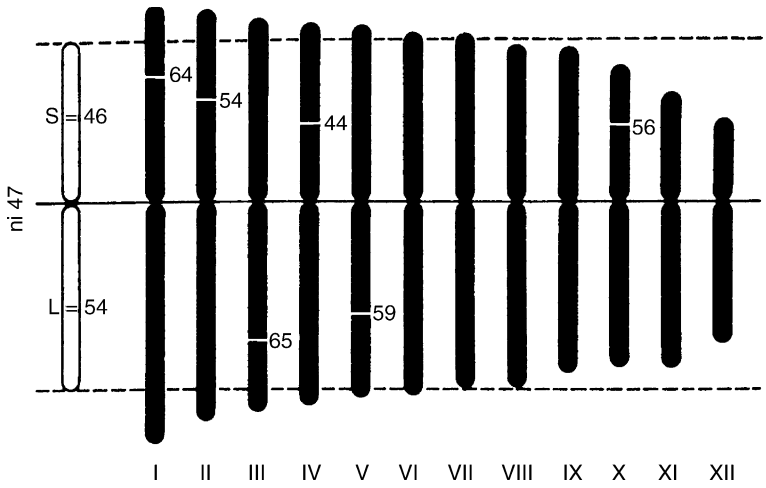

Figure 4 Graphic presentation of karyotypes shown by the idiogram of *Pinus nigra*.

is especially important in physical mapping of genes and can provide additional insight into the molecular organization of chromosomes.

Chromosome banding can be defined as a lengthwise variation in staining properties along a chromosome, induced by application of a variety of chromosome treatments by specific reagents, dyes, singly or in combination. It refers both to the process of producing banding patterns and to the patterns themselves. All of the many different banding methodologies have a common objective of accurately identifying chromosomes and parts of chromosomes. The use of banding methodology can also give insight into chromosome organization. Some banding methods have contributed greatly to both the molecular biology and cytogenetics, giving chromosome research a new and wider importance. However, successful attempts to band chromosomes of tree species using protocols for mammalian or plant species have been limited. Thus, banding of chromosomes of forest trees is currently still an enigma in terms of band numbers and/or consistency.

Important insights into chromosomal reactivity to applied reagents for revealing banding patterns was possible after Heitz in 1928 showed that certain specific chromosome segments, termed heterochromatic, do not decondense during the telophase. Constitutive heterochromatin is a permanent structural characteristic of a given chromosome pair, and is present in all cells at identical positions on both the homologous chromosomes, whereas facultative heterochromatin is heteropycnotic in special cell types or at special stages, and is related to differential gene activity, according to Brown in 1966. Constitutive heterochromatin is chromosome-specific and species-specific and can be used for chromosome identification; it is cold-sensitive, late-replicating, and genetically inert, and usually contains highly repetitive DNA sequences. After Pardue and Gall's paper in 1970 showed that Giemsa dye stained centromeres of mouse chromosomes more strongly than other chromatin, the Giemsa C-banding technique became the most widely used banding method for both animal and plant chromosomes. The first successful Giemsa C-banding of a forest tree species was on *Pinus nigra* chromosomes (**Figure 5**) by Borzan and Papeš in 1978 on haploid chromosomes in the female gametophytic tissue. Other scientists – Muratova, Tanaka and Hizume, Wochok and coworkers, and MacPherson and Filion – applied Giemsa banding to various coniferous species, mostly on root-tip meristematic tissue, and made further steps in that field. The use of Giemsa C-banding in hardwood species has been very limited. Generally, the small size of metaphase chromosomes in hardwoods limits the usefulness of this technique. An example of a Giemsa C-banding method applied to chromosomes from female gametophytic tissue of *Picea abies* is shown in **Figure 6**.

A review of Giemsa C-banding studies in conifers shows that this method successfully reveals bands of constitutive heterochromatin located in the region of the centromere, in secondary constrictions and, occasionally, in intercalary regions. In general, coniferous chromosomes contain a relatively small amount of constitutive heterochromatin. Owing to the lack of research on forest trees in this area, it is still not possible to formulate conclusions on heterochromatin distribution at the level of population, let alone of taxon.

Chromosome banding became more practical in the early 1970s, when staining protocols developed for banding chromosomes of one organism could be applied to other organisms with only minor modifications. As the use of banding protocols became more prevalent and more specific for certain chromatin or regions, classification of chromosome bands occurred as follows:

1. Heterochromatic bands, where constitutive heterochromatin (not facultative) is stained distinctively.
2. Bands occurring throughout the length of chromosome, which Sumner regarded provisionally as euchromatic bands.
3. Specific staining of the kinetochore structure.
4. Nucleolar organization region (NOR) bands.

Nomenclature of different banding methods is standardized and usually abbreviations are used to designate the method in use. In 1990 Sumner described banding nomenclature and reviewed C-banding and related methods, G-banding, R- and T-banding, Q-banding, banding with fluorochromes and methods for NOR and kinetochore staining. Fukui and Nakayama presented in 1996 banding plant chromosomes principles and detailed protocols for revealing C-bands, N-bands, fluorescent-bands 4′,6-diamidino-2-phenylindole (DAPI) for the detection of AT-rich and chromomycin A_3 (CMA) for the detection of CG-rich regions of constitutive heterochromatin in plant chromosomes, F-bands, Hy-bands, G-bands, RE-bands, and Ag-NOR-bands.

Fluorescence chromosome banding using CMA, Hoechst 33258 and DAPI has been successfully used in different coniferous species. Fluorescence *in situ* hybridization (FISH) is a technique for detecting a site of specific DNA sequences (rDNA, other classes

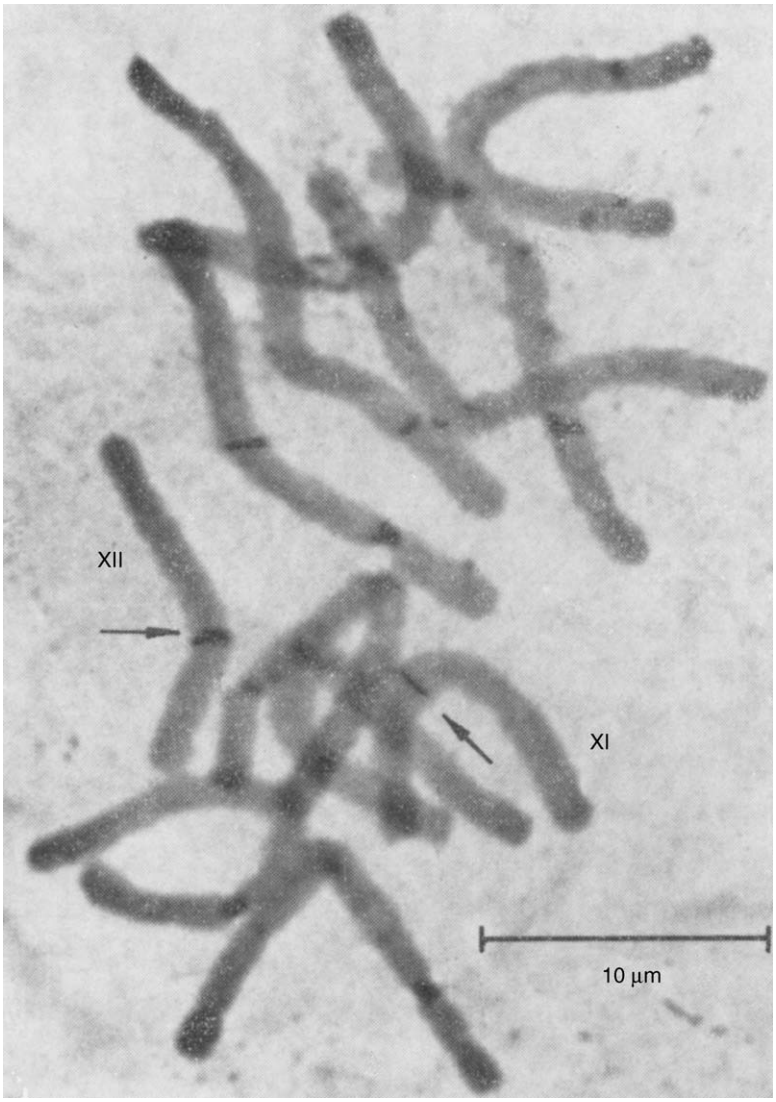

Figure 5 Giemsa C-banded chromosomes in the female gametophyte tissue of *Pinus nigra*. Arrows indicate centromeric bands on submetacentric chromosomes.

of repeated DNA, or single genes) in plant and animal chromosomes, thereby allowing physical mapping. CMA bands appearing at the secondary constrictions coincide with FISH signals when an 18S-5.8S-26S rDNA probe is used on chromosomes of coniferous and hardwood species, and in many other plant and animal species. **Figure 7** shows banded chromosomes from *Quercus pubescens* by the FISH technique, using 18S-5.8S-26S and 5S rDNA probes. Nakamura and Fukui applied in 1997 a laser dissection method to dissect specific regions of the chromosomes of giant sequoia (*Sequoiadendron giganteum*), showing that visible SAT-chromosome contains 18S rRNA genes and is the only location for those genes in the chromosome complement.

Applications of Cytogenetics to Basic Genetic Research in Forest Trees

Prior to the advent of molecular biology and *in-situ* hybridization of probes directly on chromosomes, physical gene mapping was essentially nonexistent in forest tree species. Agronomic and horticultural approaches that use chromosomal aberrations, e.g., translocations, or aneuploidy, such as monosomics or trisomics, in combination with breeding are generally not possible with coniferous species. Most conifers do not tolerate aberrations and aneuploid changes which usually affect growth and reproduction. With hardwood species, cytogenetic characterization of the different species was too limited to conduct mapping experiments. Long-term

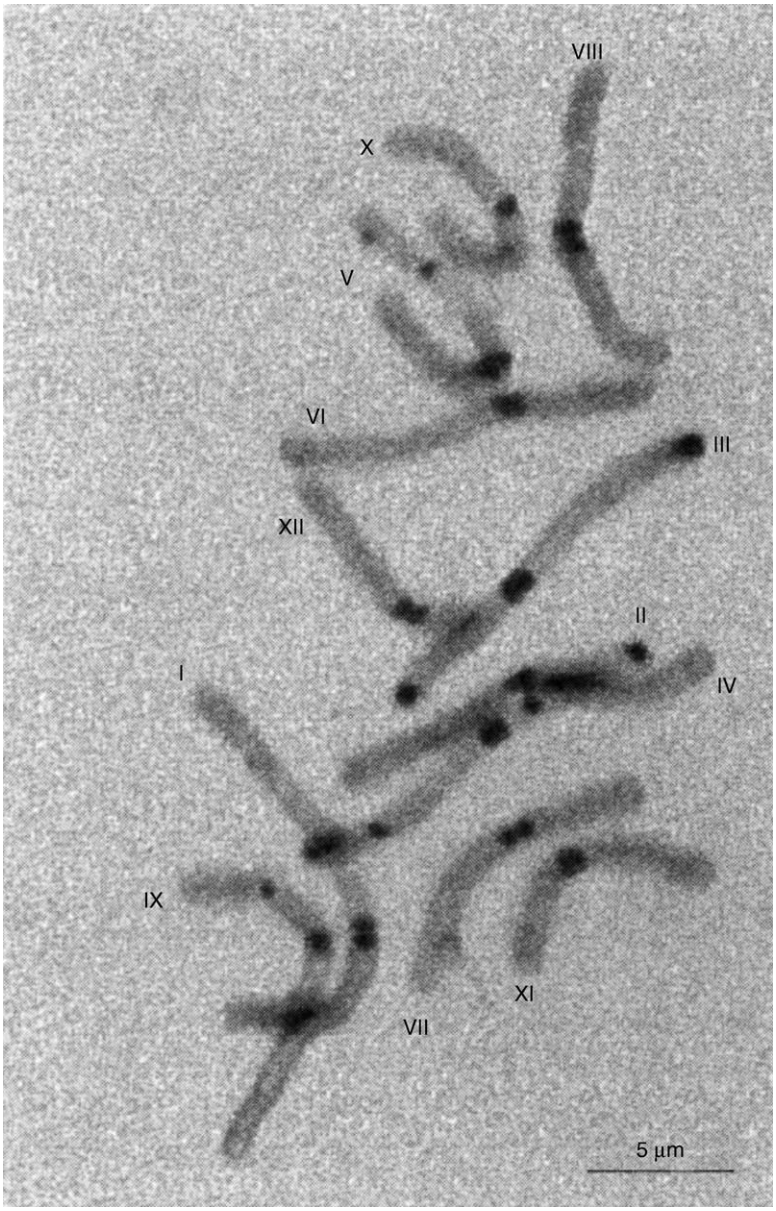

Figure 6 Giemsa C-banded chromosomes in the female gametophyte tissue of *Picea abies*.

reproductive cycles and, often, the physical size, contributed to difficulties in mapping forest tree species.

The application of chromosome banding techniques developed in the 1970s specifically to identify chromosomes was an initial step toward physical mapping. The development of chromosome imaging techniques for tree species by Fukui and by Guttenberger has also contributed to chromosome identification. *In-situ* hybridization with a variety of fluorescing probes has physically mapped gene sequences to chromosomes in a number of coniferous and some hardwood species. Nakamura and Fukui's laser microdissection of a SAT-chromosome in *Sequoiadendron* shows the potential for using a combination of cytogenetic and molecular techniques with instrumentation. Physical mapping efforts have been concentrated on coniferous species owing to their chromosome size, but advances in instrumentation from human genome projects may make studies on hardwood species more feasible.

Applications of Cytogenetics to Tree Improvement

Using a cytogenetic approach to improve a plant species usually involves breeding and/or euploid

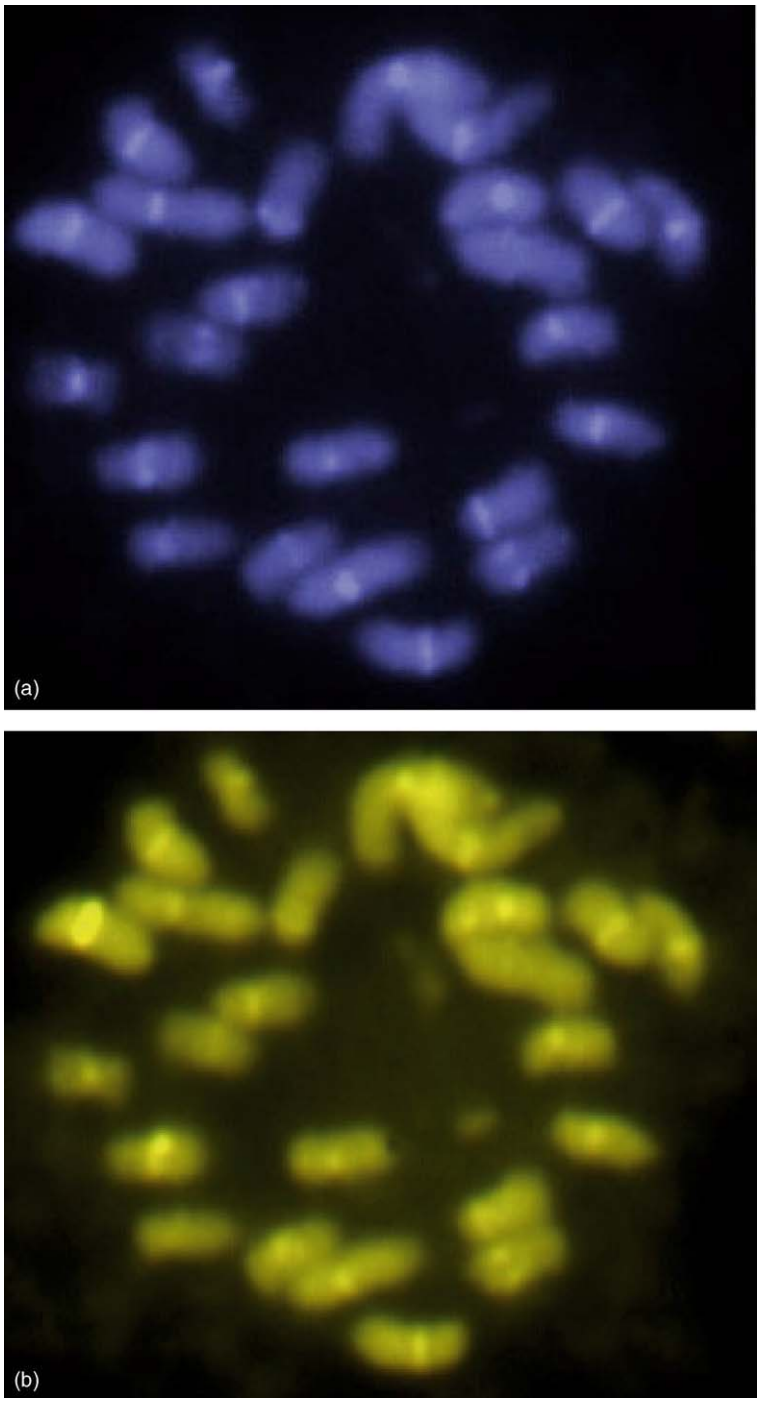

Figure 7 Banded chromosomes from the root-tip meristem tissue of *Quercus pubescens*. (a) Coloration with 4′,6-diamidino-2-phenylindole (DAPI) reveals fluorescent bands exclusively in centromeric regions of all 24 chromosomes of *Q. pubescens* complement. (b) Coloration with chromomycin A_3 (CMA) reveals fluorescent bands in all 24 chromosomes at the juxtaposition with those produced by use of DAPI. The most prominent CMA bands at the centromeric region of one metacentric pair correspond to 18S-26S rDNA sites. Note the same metaphase plate for both DAPI and CMA banding. Reproduced with permission from Zoldos V, Papes D, Cerbah M *et al.* (1999) Molecular-cytogenetic studies of ribosomal genes and heterochromatin reveal conserved genome organization among 11 *Quercus* species. *Theoretical and Applied Genetics* 99: 969–977.

increases in chromosome number. Unfortunately, the majority of cytogenetic studies of forest trees have been on coniferous species and little improvement has been made. Most species have a juvenile period that can be measured in years, which has precluded improvement via a cytogenetic approach when

breeding is involved. The delay in breeding may be circumvented by using accelerated breeding techniques that have been developed for some species. Shortening the breeding cycle, however, is only a partial solution. Chromosome changes in this group of trees are not well tolerated, with the exceptions of Taxodiaceae and Cupressaceae. It is only in *Cryptomeria japonica* that euploid changes from the normal diploid state have been exploited.

Cytogenetic improvement of hardwood species shows more promise than coniferous species. Some species have relatively short juvenile periods that would not greatly inhibit an integrated cytogenetic/breeding approach to improvement. Ploidy changes, either natural or induced, are not a problem in many species, and euploid changes from the diploid state have been shown to increase yield in some species. Studies have shown that triploidy is the optimal level for growth in *Populus* and could be for some *Quercus* species. In general, cytogenetic manipulation of hardwood species is a vast reservoir of potential waiting to be explored.

Conclusion

A general conclusion on the benefits from cytogenetic studies on forest trees is somewhat problematic. Studies on forest trees are able to follow successfully the methods applied in human, animal, and plant cytogenetic studies, but usually have not been pursued in depth, e.g., in an integrated long-term breeding and cytogenetic program with tangible objectives. During the era when cytogenetics was prevalent in science and resources were available, many studies concentrated on coniferous species in Pinaceae, in which chromosome aberrations and changes in chromosome number are usually disastrous. Early efforts in studying and developing triploid aspen were successful, but diminished in the 1960s. Despite the success of the triploid aspen program, interest in cytogenetic studies did not spread to other hardwood species. Although sporadic studies on ploidy changes in some hardwood species have shown promise for increasing timber yields, corresponding tree improvement programs have generally not had a cytogenetic component. Advances in instrumentation, e.g., chromosome imaging systems and laser microdissection, coupled with wise choices for experimental material can make cytogenetics an important component of basic and applied forestry research. Therefore, it can be concluded that the contribution of cytogenetics to the forestry profession and science in general has been small, but the potential for contribution still remains significant.

See also: **Ecology**: Reproductive Ecology of Forest Trees. **Genetics and Genetic Resources**: Genetic Systems of Forest Trees; Molecular Biology of Forest Trees. **Tree Breeding, Practices**: Southern Pine Breeding and Genetic Resources. **Tree Breeding, Principles**: A Historical Overview of Forest Tree Improvement; Forest Genetics and Tree Breeding; Current and Future Signposts.

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