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# Origins of Polyploidy in Coast Redwood (*Sequoia sempervirens* (D. DON) ENDL.) and Relationship of Coast Redwood to other Genera of Taxodiaceae

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## Abstract

Coast redwood (*Sequoia sempervirens* (D. DON) ENDL.) is the only conifer which is a hexaploid. It is not known when the polyploid *Sequoia* evolved from its diploid ancestors and which are its putative progenitors, living or extinct. The polyploid nature of the coast redwood, whether autopolyploid, autoallopolyploid, segmental allopolyploid, or allopolyploid also remains an unresolved problem.

In this paper, we present models for the possible origins of polyploidy in coast redwood, and speculate whether progenitors of *Sequoia* had experienced one or more episodes of polyploidization during their genomic evolution to hexaploidy. We draw on studies from fossil history, morphology, embryology, cytology, somatic and meiotic chromosome analyses, taxonomy and molecular phylogeny that are relevant to understanding the origin of hexaploid coast redwood and its relationship to other genera in Taxodiaceae.

**Key words:** *Sequoia*, coast redwood, polyploidy, fossil history, cytology, molecular phylogeny, ancestry, Taxodiaceae

## Introduction

Polyploidy is widespread in land plants. Recent estimates suggest that 50% to 70% of all angiosperms may have experienced one or more episodes of polyploidization during their evolution (MASTERSON, 1994; SOLTIS and SOLTIS, 1999; WENDEL, 2000). The frequency of polyploidy may be close to 95% in pteridophytes (GRANT, 1981). Although polyploidy is relatively common among gymnosperm trees, it is rather infrequent among gymnosperms (WRIGHT, 1976; AHUJA, 2001). There are only a few naturally occurring polyploids among conifers (KHOSHOO, 1959; DELEVORYAS, 1980). These include hexaploid coast redwood (*Sequoia sempervirens*,  $2n=6x=66$ ), tetraploid pfitzer juniper (*Juniperus chinensis pfitzeriana*,  $2n=4x=44$ ), and tetraploid larch (*Larix decidua*,  $2n=4x=48$ ).

Coast redwood (*Sequoia sempervirens* (D. DON) ENDL.) belongs to the family Taxodiaceae, which includes nine genera and 13 species (Table 1). Amongst the members of Taxodiaceae, coast redwood is the only hexaploid ( $2n=6x=66$ ), while all other genera are diploids ( $2n=22$ ). It is not known how the hexaploid coast redwood evolved from its diploid ancestors. The phylogenetic relationship of coast redwood to other genera of Taxodiaceae has been addressed by fossil history, comparative morphology, embryology, secondary compounds, cytology, karyotype analysis, taxonomy and molecular analysis. In this review, we shall examine the origins and nature of polyploidy and evolutionary relationship of coast redwood to its likely relatives and ancestors.

## Fossil history

Mesozoic Era fossils resembling *Sequoia* and other genera of Taxodiaceae have been discovered from different parts of the world (MILLER, 1977). The oldest fossil *Sequoia* (*Sequoia jeholensis* ENDO) was recorded by ENDO (1951) from the Jurassic deposits (~195 to 141 mya; million years ago) of South Manchuria. According to ENDO (1951), the foliage of fossil

*Sequoia* resembled the foliage of the living coast redwood, suggesting that *Sequoia* foliage has changed only slightly during the past 100 million plus years. However, affinity with coast redwood was based on only superficial resemblance of the fossil foliage (MILLER, 1977).

*Sequoia*-like fossils occur widely in the Northern hemisphere, and some of these fossils have been dated from the late Paleocene (~55 mya) (WOLFE, 1996). The ancient *Sequoia* species referred to as *S. affinis*, *S. langsdorfii*, *S. abietina*, or *S. couttsiae* had foliage typically in two distinct forms (dimorphic). The foliage was scale-like, having overlapping scales (imbricated), and occurring in two vertical rows (distichous). The foliage of *Sequoia* from Paleocene to Oligocene (~38 mya) is more delicate than in coast redwood. However, the cuticular detail is somewhat similar to that of extant *Sequoia* and *Sequoiadendron*. It appears that fossil *Sequoia* collected from North America from Eocene and Oligocene (~54-25 mya) differs from the Miocene (~25-10 mya) *Sequoia*, which in turn differs only slightly from the living coast redwood (FIELDS, 1993, and

Table 1. – A new infrafamilial classification of Taxodiaceae (as considered part of the family Cupressaceae; after GADEK et al. 2000).

Subfamily/Genus	Species	Common name
<b>Cunninghamioideae</b>		
<i>Cunninghamia</i>	<i>lanceolata</i>	Chinese-fir
<i>Cunninghamia</i>	<i>konishii</i>	Formosan China-fir
<b>Taiwanioidae</b>		
<i>Taiwania</i>	<i>cryptomeriodes</i>	Taiwan-cryptomeria
<b>Athrotaxidoideae</b>		
<i>Athrotaxis</i>	<i>cupressoides</i>	Tasmanian pencil-pine
<i>Athrotaxis</i>	<i>laxifolia</i>	summit-cedar
<i>Athrotaxis</i>	<i>selaginoides</i>	King William-pine
<b>Sequoioideae</b>		
<i>Sequoia</i>	<i>sempervirens</i>	coast redwood
<i>Metasequoia</i>	<i>glyptostrobooides</i>	dawn-redwood
<i>Sequoiadendron</i>	<i>giganteum</i>	giant sequoia
<b>Taxodloideae</b>		
<i>Taxodium</i>	<i>distichum</i>	bald-cypress
<i>Taxodium</i>	<i>mucronatum</i>	Montezume bald-cypress
<i>Cryptomeria</i>	<i>japonica</i>	Japanese-cedar
<i>Glyptostrobus</i>	<i>pensilis</i>	Chinese swamp-cypress

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pers comm.). The earlier lineages from Eocene-Oligocene bear narrow appressed leaves and small cones, while the Miocene material has comparatively longer leaves and larger cones, and is much more modern appearing.

A study of guard cells of fossil *Sequoia* from Pliocene (~7 mya) deposits of Japan indicated that there were no significant differences in the size of guard cells between fossil *Sequoia* and extant *Sequoia* (MIKI and HIKITA, 1951). If guard cell size is predictive of polyploidy, that is, there is an increase in guard cell size with increase in ploidy level, (MASTERTON, 1994), then this would suggest that *Sequoia* was already a hexaploid during Neogene, and that increase in ploidy level occurred at an earlier geologic period. A study of guard cells of stomata from fossil *Sequoia* from earlier geologic periods might shed some light on the time of origin of hexaploidy in coast redwood. However, an increase in guard cell size alone may not necessarily indicate increase in ploidy level (particularly in allopolyploids or auto-allopolyploids), as additional phenotypic traits must also be considered.

What about fossil remains of other members of the family Taxodiaceae? Did some of them also occur at the same time periods along with *Sequoia*, or did they occur at different time periods? Several paleogeographic patterns are apparent in the northwestern United States (FIELDS, 1991). *Athrotaxis*, *Cryptomeria*, *Taiwania* have no apparent fossil record in the Neogene of the western USA. *Glyptostrobus* and *Cunninghamia* occurred sporadically on a few sites. *Taxodium* is the most widespread, and occurred in a number of northern sites. On the other hand, *Sequoiadendron* was found alone mostly in southern sites, whereas *Sequoia*, *Metasequoia*, and *Taxodium*, and *Glyptostrobus* co-occurred in a number of common locations.

Although *Sequoia* fossils have been described mainly from the northern hemisphere, there are at least two reports from the southern hemisphere describing new genera resembling modern *Sequoia* and *Sequoiadendron*. A new genus/species within Taxodiaceae, *Austrosequoia wintonensis*, was described from mid-Cretaceous fossil cones that were discovered in Queensland, Australia (PETERS and CHRISTOPHEL, 1978). The cone morphology of this new species showed strong similarities to that of *Sequoia sempervirens*, but only limited similarities with the Australian endemic *Athrotaxis*. In a recent study, POLE (1995) has added a new genus/species of Taxodiaceae, *Otakauia lanceolata*, from the late Cretaceous Period in New Zealand. In addition, he reported fossil *Sequoia* or *Sequoiadendron* foliage from the same area. A personal communication with MIKE POLE clarified that the fossil foliage cuticle is more like that of *Sequoiadendron*, or a related genus. These reports would suggest that *Sequoia/Sequoiadendron* lineages might have also occurred in Australia-New Zealand axis in the past. In that event, a fresh look at the ancestry of coast redwood, and relationship of coast redwood to the members of Taxodiaceae from the southern hemisphere would be merited.

Thus, it appears that Taxodiaceae lineages may have evolved by the Jurassic. The present day coast redwood may have originated during late Cretaceous and Tertiary Periods by hybridizations between *Metasequoia*-like genera, and presumably other Taxodiaceous genera like *Sequoiadendron*, *Taiwania*, or *Athrotaxis*. Although it is not known when *Sequoia*-like species evolved to higher polyploidy levels, but it is conceivable that a step-wise evolution from a diploid to hexaploid state may have occurred over a period of time.

#### Morphology, embryology and secondary compounds

**Morphology.** A first attempt to understand relationships between *Sequoia* and other members of Taxodiaceae was made by

STEBBINS (1948). Based on morphology (27 characters, including leaf arrangement, leaf shape, cotyledon number, arrangement of pollen-bearing and ovulate cone, seed wing, and deciduousness), STEBBINS concluded that *Sequoia* resembled *Metasequoia* by 18 characters and differed from it only by nine characters. The next closest taxon to *Sequoia* was *Sequoiadendron* (10.5 differences), suggesting these three genera are more closely related to each other than other genera of Taxodiaceae. Based on morphology, STEBBINS (1948) had postulated that *Metasequoia* might be one of the two likely ancestors of *Sequoia*. He was uncertain, if the second ancestor could be *Sequoiadendron*-like.

Comparative studies on development of the cone scales (HIDA, 1957) and cone and ovule development (TAKASO and TOMLINSON, 1992) in Taxodiaceae also support systematic affinity between *Sequoia*, *Metasequoia*, and *Sequoiadendron*, although there are also distinct differences between the three genera in terms of morphology and embryology (Table 2).

Table 2. — Comparison of some morphological and embryological characters in *Metasequoia*, *Sequoia*, and *Sequoiadendron*

Character	<i>Metasequoia</i>	<i>Sequoia</i>	<i>Sequoiadendron</i>
Leaf arrangement	opposite or decussate	alternate or spiral	alternate or or spiral
Leaf shape	linear	needle and scale	scale
Deciduousness	yes	no	no
Number of microsporangia	3	3	3
Seed scale	wide	wide	wide
Seed maturation	first year	first year	second year
Cotyledon number	2	2	4-6
Ovule orientation	anatropous	anatropous	anatropous
Number of free nuclear divisions in proembryo	not studied	0	8
Cleavage polyembryony	yes	yes	yes

**Embryology.** Detailed embryological studies have been carried out in Taxodiaceae (LAWSON, 1904; LOOBY and DOYLE, 1937; BUCHHOLZ, 1939; DOGRA, 1966). According to DOGRA (1966), Taxodiaceae shows three types of embryology: (1) Taxodiant-type, (2) *Sequoia sempervirens*-type, and (3) *Athrotaxis*-type. These three types differ from each other in the presence or absence of free-nuclear proembryo, the time of first nuclear division, and subsequent development of embryos. These studies have revealed that a free-nuclear pro-embryonal stage precedes cell wall formation in all the genera, except in *Sequoia* (Table 2). The Taxodiant-type includes *Taxodium*, *Cyptomera*, *Cunninghamia*, *Glyptostrobus*, *Taiwania* and *Sequoiadendron*, and in these genera a free-nuclear pro-embryonal stage (up to 8 nuclei) precedes cell wall formation. In the *Sequoia*-type, cell division follows immediately after fertilization, and there is no free-nuclear pro-embryo. In the *Athrotaxis*-type cell wall formation begins after the 2-nucleate pro-embryo. The embryology of *Metasequoia* has not been studied.

**Secondary compounds.** A study on biflavone content (secondary compounds) in Taxodiaceae showed that there are no unique patterns that characterizes any relationship between *Sequoia*, *Metasequoia*, and *Sequoiadendron*, or other genera

(GADEK and QUINN, 1989). The biflavonoid data provide evidence of the heterogeneous nature of the secondary compounds in the genera within the family Taxodiaceae.

### Cytology - stomata and pollen

**Stomata.** The structure and arrangement of stomata have been investigated in *Sequoia*, *Sequoiadendron*, *Taxodium*, *Cryptomeria*, *Cunninghamia*, and *Metasequoia* (FLORIN, 1931; YANG, 1999). Differences in structure, size and frequency of stomata have been observed within Taxodiaceae. The frequency of stomata was the lowest in *Sequoia* (15/mm<sup>2</sup>) and considerably higher in *Taxodium* and *Taiwania* (24-25/mm<sup>2</sup>) (CHATURVEDI, 1993). We have estimated the size (length) of stomatal cells from peels of leaves/needles from different species of Taxodiaceae. The size (length) of the stomatal guard cells in *Sequoia* are significantly larger than those of *Metasequoia*, *Sequoiadendron*, *Cunninghamia*, *Cryptomeria*, and *Taxodium* (Table 3).

Table 3. - Average Size of stomata and pollen grains in coast redwood and some other species of Taxodiaceae.

Species	Length of guard cells <sup>a</sup> (μ)	Diameter of Pollen <sup>b</sup> (μ)	Reference
<i>Sequoia sempervirens</i>	60	-	Miki & Hikita, 1951
	61	30	present study
<i>Metasequoia glyptostroboides</i>	42	-	Miki & Hikita, 1951
	50	25	present study
<i>Sequoiadendron giganteum</i>	54	-	present study.
<i>Cunninghamia lanceolata</i>	54	-	present study.
<i>Cryptomeria japonica</i>	48	-	present study
<i>Taxodium distichum</i>	40	-	present study.

- = not reported.

<sup>a</sup>The length of the guard cells of stomata was determined from unstained leaf/needle peels under the microscope, using an ocular micrometer.

<sup>b</sup>The size of pollen was determined from acetocarmine-stained pollen grains under the microscope using an ocular micrometer.

**Pollen.** We have examined the size of pollen in *Sequoia* and *Metasequoia* collected from trees at the Institute of Forest Genetics, Placerville. The pollen from *Sequoia* is about 20% larger in diameter than that of *Metasequoia* (Table 3). We did not have access to pollen of *Sequoiadendron* and other members of Taxodiaceae.

### Karyotype analysis

Different chromosome numbers have been reported in *Sequoia* during the early years of the last century. These range from a haploid number of n=16 to a diploid number of 66 (Table 4). Although there is consensus of 2n=6x=66, our cytogenetic studies based on the root tip squashes and staining of chromosomes with feulgen have also confirmed 2n=6x=66 in coast redwood (Figure 1). In addition to 66 chromosomes in the somatic cells, extra (accessory) chromosomes have been

occasionally observed in *Sequoia* (FOZDAR and LIBBY, 1968; SAYLOR and SIMONS, 1970). The origin of these accessory chromosomes in *Sequoia* is unknown.

Table 4. - Chromosome numbers reported by different investigators in coast redwood.

Reference	Chromosome number	
	Haploid (n)	Diploid (2n)
Lawson, (1904)	~16	
Dark (1932)		~50
Sax and Sax (1933)		40+
Buchholz (1939)		44
Hirayoshi and Nakamura (1943)	33	66
Stebbins (1948)	33	66
Miki and Hikita (1951)		66
Fozdar and Libby (1968)		66 plus extra chromosomes
Saylor and Simons (1970)		66 plus extra chromosome
Schlarbaum and Tsuchiya (1984)		66
Hizume et al (1988)		66
Toda (1996)		66
present study		66
Hizume (pers. Comm.)		66 plus extra chromosomes



Fig. 1. - Somatic chromosomes of coast redwood (2n=66) stained with feulgen. Three pairs of satellite (SAT) chromosomes are clearly discernable (arrows). X 975

Comparative karyotype analyses have revealed that most chromosomes are metacentric (have median centromeres) and that there is a gradual transition in the size from short to long chromosomes in *Sequoia* (SAYLOR and SIMONS, 1970; SCHLARBAUM and TSUCHIYA, 1984a; TODA, 1996; present study) and other members of Taxodiaceae (SCHLARBAUM and TSUCHIYA, 1984b; TODA, 1996; present study). This suggests evolution of the karyotype in Taxodiaceae from a common ancestral progenitor.

LI (1987) hypothesized that some ancient species of *Metasequoia* and *Sequoiadendron* may have contributed to the ancestry of *Sequoia*, based on similarities in karyotype between *Sequoia*, *Metasequoia*, and *Sequoiadendron*. However, there are some differences between the marker chromosomes, particularly satellite (SAT) chromosomes, and the number of nucleoli in *Sequoia*, *Metasequoia* and *Sequoiadendron*. (Tables 5 and 6). *Sequoia* karyotype shows three pairs of distinct SAT chromosomes (Figure 1) that are not observed in *Metasequoia* and/or *Sequoiadendron* (SCHLARBAUM and TSUCHIYA, 1984a, 1984b; TODA, 1996; present study).

Table 5. — Number of chromosomes, secondary constrictions and nucleoli in Taxodiaceae

Species	Chromosome number (2n)	Number of secondary constrictions	Number of nucleoli
<i>Athrotaxis selaginoides</i>	22	2	2
<i>Cryptomeria japonica</i>	22	2-4	2-4
<i>Cunninghamia lanceolata</i>	22	2	2
<i>Glyptostobus pensilis</i>	22	2	2
<i>Metasequoia glyptostroboides</i>	22	6	6
<i>Sequoia sempervirens</i>	66	6	6-8
<i>Sequoiadendron giganteum</i>	22	2	2
<i>Taiwania cryptomerioides</i>	22	2	2
<i>Taxodium distichum</i>	22	2	2

Table 6. — Number of satellite (SAT) chromosomes and nucleoli in coast redwood

Reference	No. of SAT Chromosomes	Maximum No. of nucleoli
Saylor and Simons (1970)	6	6
Schlarbaum and Tsuchiya (1984)	4	not reported
Hizume et al. (1988)	6	8
Toda (1996)	6	6
present study	6	8

Generally, the number of SAT chromosomes correspond to the number of nucleoli, and is characteristic of a species. The origin of three distinct pairs of SAT chromosomes and their relationship to polyploidy in *Sequoia* is unknown. The presence of three pairs of characteristic SAT chromosomes might suggest that possibly different diploid species have contributed to the ancestry of hexaploid coast redwood. Alternatively, evolution of the SAT chromosomes presumably occurred by rearrangements involving reciprocal translocations/inversions between certain chromosomes in the ancestral *Sequoia* genome during polyploidization.

### Nature of hexaploidy in *Sequoia* based on meiosis

In diploid organisms (AA) meiosis occurs in the reproductive cells to form haploid gametes carrying a single genome (A). During meiosis, the homologous chromosomes derived from maternal and paternal parents pair to form bivalents. Polyploids, however, may exhibit variation from bivalent chromosome associations at meiosis. For example, in an autotetraploid (AAAA), instead of bivalents, mostly quadrivalents are formed. Allotetraploids (AABB) mostly form bivalents during meiosis, since pairing occurs between homologous chromosomes of AA or BB genomes, but not normally between chromosomes of distinct genomes. Since coast redwood is a hexaploid, meiotic chromosome associations are informative for determining the nature of hexaploidy in coast redwood. Earlier meiotic studies revealed the presence of a few to several multivalents, in addition to a majority of bivalent configurations at meiosis in coast redwood (HIRAYOSHI and NAKAMURA, 1943; STEBBINS, 1948). Based on meiotic configurations, STEBBINS (1948) postulated that coast redwood is a complex hexaploid, which may be either an autoallopolyploid with a genomic formula AAAABB or a segmental allopolyploid with either  $A_1A_1A_1A_2A_2$  or  $A_1A_1A_2A_2A_3A_3$ . A segmental allopolyploid may form some multivalents due to pairing between partially homologous chromosomes from different ( $A_1$ ,  $A_2$  or  $A_3$ ) genomes. In other words, a segmental allohexaploid, like the autoallohexaploid may exhibit multivalent formation, even though the origins of the two types of polyploids are different. The hexaploid level in coast redwood was probably achieved by hybridization between two, or possibly three ancestral species.

Our preliminary meiotic studies on *Sequoia* also revealed the presence of mostly bivalents, in addition to a few multivalents (Figure 2). However, current and previous studies are based on only a small number of meiotic cells from a few trees. *Sequoia* exhibits a high level of genetic and phenotypic variability (LIBBY et al. 1996; ROGERS, 2000). It is possible that the frequency of multivalents may vary among coast redwood populations. In addition, some populations may show nearly all



Fig. 2. — Two meiotic chromosome configurations at metaphase I (MI) of coast redwood. The MI plate on top shows a majority of bivalents and at least two hexavalents (arrows). The lower MI plate is typically observed at meiosis, where it is difficult to determine the number of bivalents and multivalents. X 850

bivalents. This might suggest that perhaps coast redwood may not be a single entity, but a complex species having distinct genotypes and different type of hexaploids. It is not inconceivable that during the evolution of *Sequoia* there could have been more than one episodes of polyploidization, a phenomenon not uncommon in plants (see SOLTIS and SOLTIS, 1993, 1999; LEITCH and BENNETT, 1997; OTTO and WHITTON, 2000; WENDEL, 2000). The multiple origin hypothesis of polyploids, may provide insight into whether *Sequoia* consists of one type of polyploid or different types of polyploids, namely autopolyploid, autoallopolyploid, segmental allopolyploid, or allohexaploid. The study of meiosis, combined with molecular techniques, particularly genomic in situ hybridization (GISH) and comparative genome sequencing, may reveal the origin and nature of polyploidy in coast redwood.

The conceptual origins of different types of hexaploids, that is, autohexaploid (AAAAAA), autoallohexaploid (AABBBB or AAAABB), segmental allohexaploid ( $A_1A_1A_1A_2A_2A_2$ ,  $A_1A_1A_2A_2A_2A_2$ , or  $A_1A_1A_2A_2A_3A_3$ ) and allohexaploid (AABBCC) are presented in Figures 3 to 6. The nature of hexaploidy in coast redwood, although still unresolved, would be dependent on the presence of one, two, or three similar or different genomes. If meiotic configurations are indicators of the type of polyploidy, coast redwood may be either a partially diploidized autohexaploid (Figures 3a, 3b), an autoallohexaploid (Figures 4a, 4b), or a segmental allohexaploid (Figures 5a, 5b), but less likely an allohexaploid (Figure 6).

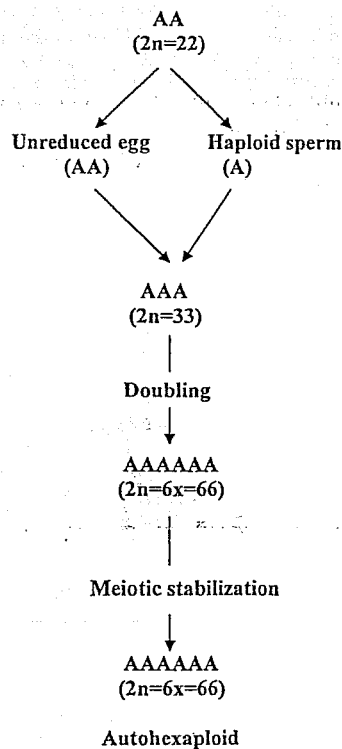


Fig. 3a - Origin of an autohexaploid (AAAAAA) from a diploid ancestor (AA;  $2n=22$ ). This model assumes production of a triploid (AAA) by fusion of an unreduced (AA) and a reduced gamete (A) in a diploid (AA). Following doubling of chromosomes in the triploid (AAA) an autohexaploid (AAAAAA) may be produced. Such an autohexaploid would form mostly multivalents. However, meiotic stabilization may occur over a long period of time (million of years) and increase the frequency of bivalents over multivalents to form some functional gametes in this autohexaploid (AAAAAA). Partial meiotic stabilization (diploidization) has been observed in artificially induced autotetraploid in maize over a period of 10 years (GILES and RANDOLPH, 1951), *Brassica campestris* after 19 generations of inbreeding (SWAMINATHAN and SULBHA, 1959) and some other autopolyploids (see SYBENGA, 1996).

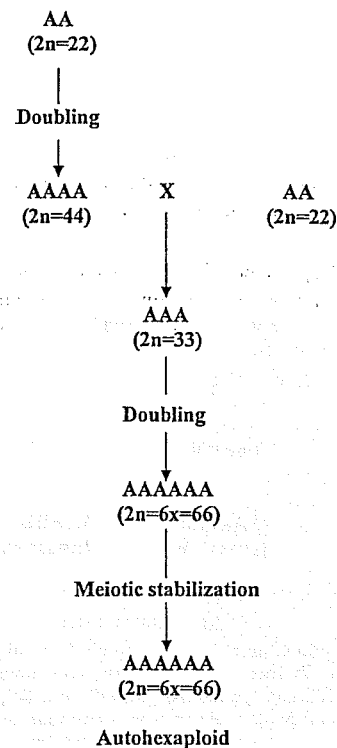


Fig. 3b. - Origin of an autohexaploid (AAAAAA) by a slightly different route as in Figure 3a. In this model, an autotetraploid (AAAA) is formed by chromosome doubling in the diploid progenitor (AA;  $2n=22$ ). Hybridization between the autotetraploid (AAAA) and the diploid ancestor (AA) would result in a triploid (AAA). By doubling of chromosomes in the triploid (AAA), a hexaploid state (AAAAAA) could be achieved. Meiotic stabilization may occur over a long period of time to produce some functional gametes as in the model in Figure 3a.

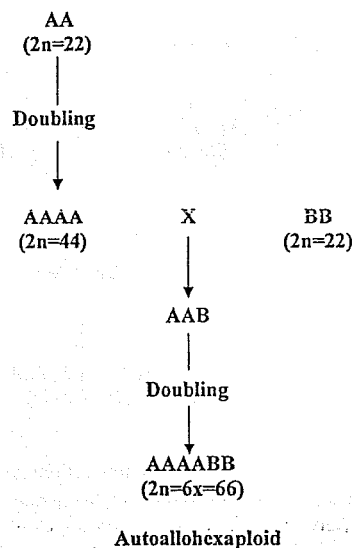
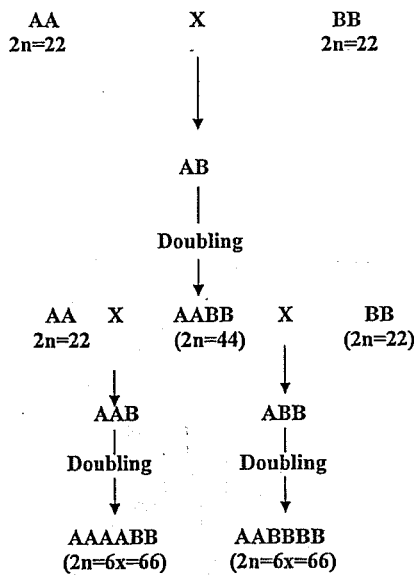
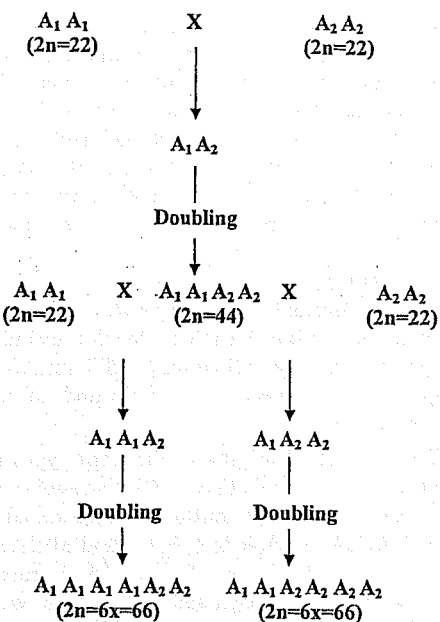


Fig. 4a. - Origin of an autoallohexaploid (AAAABB) by hybridization between two distinct species (AA,  $2n=22$ ; and BB,  $2n=22$ ). In this model, the first step is the production of an autotetraploid (AAAA) in one species (AA). Hybridization between this tetraploid (AAAA) and the second species (BB) would produce a triploid (AAB). Doubling of the chromosome in the triploid (AAB) would lead to the formation of an autoallohexaploid (AAAABB). Because of the genomic constitution, the autoallohexaploid would produce bivalents and multivalents.



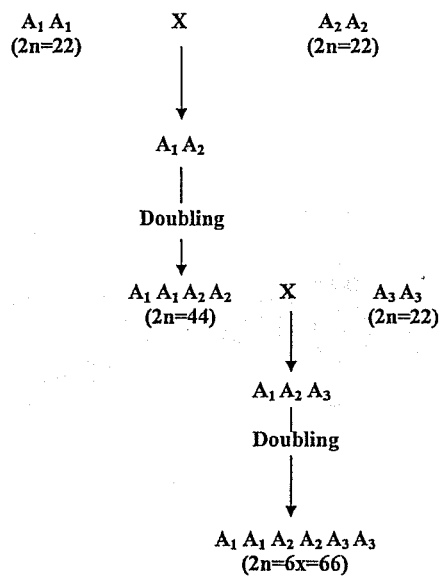
#### Autoallohexaploid

Fig. 4b. — Origin of an autoallohexaploid by a slightly different route as in Figure 4a. In this model, the first step involves hybridization between two different species (AA,  $2n=22$ ; and BB,  $2n=22$ ) to produce an  $F_1$  (AB). Doubling the chromosome number or fusion of unreduced gametes in the  $F_1$  would result in an allotetraploid (AABB). Hybridization between this allotetraploid (AABB) and either one of the diploid ancestors (AA or BB) would produce triploids (AAB or ABB). Doubling of chromosomes in the triploid would lead to the production of an autoallohexaploid (AAAABB or AABBBB). Both types of autoallohexaploids would produce multivalents as well as bivalents.



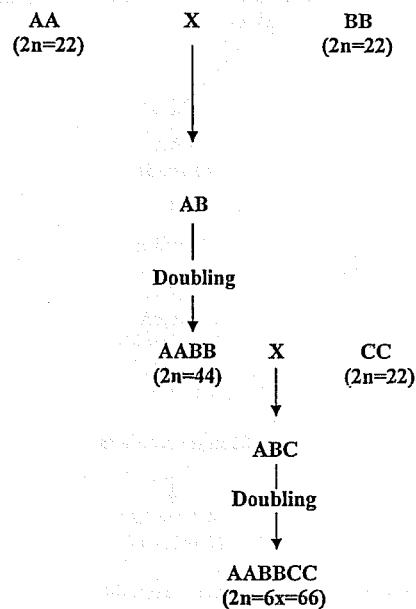
#### Segmental Allohexaploid

Fig. 5a. — Origin of a segmental allohexaploid by hybridization between two related species that have partially homologous chromosomes ( $A_1A_1$  and  $A_2A_2$ ; both with  $2n=22$ ). Hybridization between these species would produce an  $F_1$  ( $A_1A_2$ ). By doubling of the chromosomes in this  $F_1$  would result in a segmental allotetraploid ( $A_1A_1A_2A_2$ ). Hybridization between this segmental allotetraploid ( $A_1A_1A_2A_2$ ) and one of the diploid parents ( $A_1A_1$  or  $A_2A_2$ ) would produce triploids ( $A_1A_1A_2$  or  $A_1A_2A_2$ ). Doubling of chromosomes in the triploids would lead to the formation of segmental allohexaploids ( $A_1A_1A_1A_1A_2A_2$  or  $A_1A_1A_2A_2A_2A_2$ ). The segmental allohexaploid, like in auto allohexaploid, would also produce both bivalents and multivalents because of partial homology between the chromosomes of the two ancestral diploids.



#### Segmental Allohexaploid

Fig. 5b. — Origin of a segmental allohexaploid ( $A_1A_1A_2A_2A_3A_3$ ) by hybridization between three parental species ( $A_1A_1$ ,  $A_2A_2$  and  $A_3A_3$ ; all three with  $2n=22$ ) that share partial homology between their chromosomes. Hybridization between two parents would produce an  $F_1$  ( $A_1A_2$ ). Chromosome doubling would lead to a segmental allotetraploid ( $A_1A_1A_2A_2$ ). Hybridization between this segmental allotetraploid ( $A_1A_1A_2A_2$ ) and the third parental species ( $A_3A_3$ ) would produce a triploid ( $A_1A_2A_3$ ). Doubling of chromosomes in the triploid would lead to the production of segmental allohexaploid ( $A_1A_1A_2A_2A_3A_3$ ). Meiotic behavior would be same as in the model in Figure 5a.



#### Allohexaploid

Fig. 6. — Origin of an allohexaploid (AABBCC) by hybridization between three different species (AA, BB and CC; all three with  $2n=22$ ). A cross between two parents (AA and BB) would produce an  $F_1$  (AB). Doubling of chromosomes in the  $F_1$  would produce an allotetraploid (AABB). Hybridization between this allotetraploid (AABB) and the third diploid parent (CC) would result in a triploid (ABC). By doubling chromosome number in the triploid (ABC) would lead to the production of an allohexaploid (AABBCC). An allohexaploid with the genomic formula AABBCC would ostensibly form only bivalents, and no multivalents.

## Hybridization in Taxodiaceae

Another approach to determine relationship among Taxodiaceae and ancestry of *Sequoia* is by studying hybridization among the putative ancestors. Hybridization, accompanied by cytogenetics, has been useful in determining the ancestry of polyploids such as wheat, cotton, tobacco, and other plant species (see STEBBINS, 1951, 1971).

Hybridizations between *Sequoia*, *Sequoiadendron*, *Taxodium*, and *Cryptomeria* were reported in Russia by YABLOKOV (1960). However, it is not clear whether these crosses were successful and if any of the hybrid seed germinated/survived, as no details are available.

Intergeneric hybridization was also performed by LEROY JOHNSON and ROGER STUTTS at the Institute of Forest Genetics, Placerville, in 1974, using pollen from *Sequoia* to pollinate the *Metasequoia* female strobili. However, there was no germination of hybrid seeds tested in the nursery in 1975 (LEROY JOHNSON and ROGER STUTTS, pers. communication).

One way to overcome sexual barriers in intergeneric crosses would be to employ biotechnological techniques involving protoplast fusions between *Sequoia*, *Metasequoia* and *Sequoiadendron* to generate parasexual hybrids.

## Taxonomy and molecular phylogeny

Taxodiaceae and Cupressaceae have long been recognized as separate families (PILGER, 1926); however, in recent years this distinction has been questioned on the basis of morphology (ECKENWALDER, 1976), immunological data (PRICE and LOWENSTEIN, 1989), and molecular analysis based on chloroplast genes (BRUNSFELD et al. 1994; TSUMURA et al. 1995; GADEK et al. 2000; KUSUMI et al. 2000). These studies support the hypothesis that Taxodiaceae and Cupressaceae form a monophyletic group, and that Taxodiaceae should be treated as part of the family Cupressaceae (Table 1). *Sciadopitys*, previously included in Taxodiaceae, represents a distinct genus in a separate family Sciadopityaceae. Molecular phylogeny data suggest that: 1) *Cunninghamia* is the basal lineage of Taxodiaceae, which diverged first, then *Taiwania*, followed by *Athrotaxis*; 2) *Metasequoia*, *Sequoia*, and *Sequoiadendron* form a group; and 3) *Taxodium*, *Glyptostrobus*, and *Cryptomeria* form a group. These data are consistent with the previous morphological groupings. The molecular phylogeny studies also suggest that *Sequoia*, *Metasequoia*, and *Sequoiadendron* rank closely in the triplet clade of the phylogenetic tree in the family and seem to be more closely related to each other than other genera of Taxodiaceae.

## Relationship and ancestry of *Sequoia*

Comparative morphological, karyotype and molecular phylogeny studies support the notion that *Metasequoia* and *Sequoiadendron* are more closely related to *Sequoia* than to other genera in Taxodiaceae. Although origins and the nature of polyploidy in *Sequoia* are still unresolved, we speculate on the putative ancestors of *Sequoia* and when it became a hexaploid:

- *Sequoia* may either be a partially diploidized autohexaploid (AAAAAA) derived from some ancestral species of *Sequoia*, thus carrying a single ancestral genome; or

- *Sequoia* may be an autoallohexaploid (AABBBB or AAAABB) thus containing two ancestral genome. Although we are not certain about the two ancestral species, *Metasequoia*-like and *Sequoiadendron*-like species may have contributed to the ancestry of coast redwood; or

- *Sequoia* may be a segmental allohexaploid ( $A_1A_1A_1A_1A_2A_2$ ,  $A_1A_1A_2A_2A_2A_2$ , or  $A_1A_1A_2A_2A_3A_3$ ), presumably derived by hy-

bridization between two or three closely related ancestral species. We are not sure if these ancestral species belong to ancient *Metasequoia*, *Sequoiadendron*, and *Cryptomeria*, or they were different species of *Sequoia* that existed in the pre-historic times.

- Although it is not known when *Sequoia* became a hexaploid, but this event may have occurred in early to late Cretaceous Period.

Molecular analysis involving comparative genome sequencing and the number of copies of specific genes may provide clues to the number of similar or different genomes in hexaploid *Sequoia*. In addition, fossil guard cell data from earlier geologic periods may shed light on when *Sequoia* became a hexaploid. Whether recurrent polyploidy formation has contributed to the genome evolution in *Sequoia* remains to be investigated.

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## Cytological Investigations in Some Important Tree Species of Rajasthan I. Karyomorphological Studies in some Species of *Anogeissus* (DC.) Guill., Perr. & A. Rich.

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### Summary

Karyomorphological studies have been done in ten different accessions belonging to three different species of *Anogeissus* (*A. pendula*, *A. latifolia* and *A. sericea*) an important hardwood tree of Rajasthan. The somatic chromosome number of  $2n=24$  has been observed in all the species/accessions with distinct interspecific variation in the arm ratio of respective homologous pairs of chromosomes. Nucleolar chromosomes are reported in two taxa (*A. pendula* BSJO 19564 and *A. sericea* var. *sericea* BSJO 19568) and heteromorphism is recorded in *A. pendula* BSJO 19563, BSJO 19564, *A. latifolia* BSJO 19570 and *A. sericea* var. *sericea* BSJO 19569. The karyotypes of all the species/accessions were more or less symmetrical. The role of karyotypic variation in speciation and evolution of the genus *Anogeissus* has been discussed in detail.

**Key words:** *Anogeissus*, species, hardwood tree, karyotype analysis, nucleolar chromosomes, symmetry.

### Introduction

The data on chromosome numbers and comparative karyology is fundamental to overall understanding of genome in different species or in morphologically diverse populations within a species (STACE, 2000). Karyological studies in hardwoods are hampered by the difficulty in obtaining well spread metaphase plates and optimum staining thereby obscuring the morphological details. These problems have earlier been pointed out by GILL and SINGHAL (1998a and b); DAS et al. (1995). Hence very little information is available on tropical hardwood species especially of arid and semiarid regions. The present study therefore, deals with karyological details of some taxa of the genus *Anogeissus* (Combretaceae) which is important as timber, fuel wood and fodder tree in the arid ecosystem of western Rajasthan, India.

### Material and Methods

Extensive surveys were conducted in different areas in the state of Rajasthan to locate various populations of *Anogeissus*.

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