Origin and genetic nature of polyploidy in paleoendemic coast redwood (*Sequoia sempervirens* (D. Don) Endl.)

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Abstract

It is not known when the polyploid coast redwood (*Sequoia sempervirens*) evolved from its diploid ancestors, and what is its type of polyploidy. Whether close relatives of *Sequoia*, giant sequoia (*Sequoiadendron giganteum*) and dawn redwood (*Metasequoia glyptostroboides*), have possibly contributed to the ancestry of hexaploid of *Sequoia* remains an open question. The nature of hexaploidy in *Sequoia* has baffled biologists for more than a century. Based on the chromosome configurations in *Sequoia*, G. Ledyard Stebbins was the first geneticists who postulated in 1948 that *Sequoia* is an autoallohexaploid (AAAABB), and an ancient species of *Metasequoia* might have been one of the putative ancestors of *Sequoia*. After its chromosome number (2n=6x=66) was confirmed in hexaploid *Sequoia*, the type of polyploidy in *Sequoia* has been further investigated for the past 70 years by a number of investigators, using cytogenetic and genetic data. Although an autoallohexaploid (AAAABB) origin of *Sequoia* has remained one of the dominant hypotheses until recently, an alternative hypothesis, amongst other possible origins, was also put forth by Ahuja and Neale (2002), that *Sequoia* may be partially diploidized autohexaploid (AAAAAA), derived from some ancestral species of *Sequoia*, thus carrying a single ancestral genome. Cytogenetic, molecular genetics, and genome sequence data now support the hypothesis that *Sequoia* originated as an autohexaploid.

Keywords: *Coast redwood, Sequoia, Hexaploid, Type of polyploidy, Cytogenetics, Phylogenetics, Molecular genetics, Autohexaploid, Auto-allohexaploid*

Introduction

Polyploidy or Whole genome duplications (WGDs) are widespread in vascular plants. Several studies based on genomic data on a number of plant species have suggested that WGDs in land plants seem to have coincided with mass extinction event during the Cretaceous-Paleogene (K/Pg) boundary (~65 mya) and major episodes of global climate upheavals (Fawcett et al. 2009; Vanneste et al. 2014; Lohaus and Van de Peer, 2016; Cai et al. 2019; Van de Peer et al. 2021). Although this mass extinction event wiped out the dinosaurs and caused mass extinctions in the plant kingdom, the polyploids may have survived as a spandrel (byproduct) of selection for asexuality, but occasional sex, in plants (Freeling 2017). That might imply that vegetative propagation of polyploids had a better chance for survival compared to their diploid ancestors. Polyploids often thrive in harsh environments and changing climate due to buffering effects of their three or more genomes. And that global catastrophic event, in most likely-hood, might have provided a greater selective advantage for the survival and evolution of polyploid plants. Fossil studies have suggested that polyploidy in *Sequoia* might also have occurred during the Cretaceous Period (~65 mya) (Miller 1977; Ahuja 2005, 2009).

Recent estimates suggest that 35 to 70 % of all angiosperms are ancient polyploids (Otto and Whitton 2000; Wood et al. 2009; Ohri 2021). However, polyploidy is rather infrequent in the gymnosperms (Khoshoo 1959; Wright 1976; Ahuja 2001). The frequency of polyploidy in the gymnosperms may be close to 5 %, and around 1.5 % in the conifer (Khoshoo 1959; Ahuja 2005). Of the 8 families in conifers, natural polyploids so far predominantly occur only in 3 different genera of the family Cupressaceae. These include the genus *Juniperus*, which has around 75 species (Adams 2014), of which 11 are tetraploids and one hexaploid (Farhat et al. 2019, 2021); *Fitzroya cupressoides*, which is monospecific is a tetraploid (Hair, 1968, Premoli et al. 2000), and *Sequoia sempervirens*, also a monospecific genus is a hexaploid.

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The evolutionary history of coast redwood is unique and checkered, starting with its naming, its chromosome number, and its origin and nature of polyploidy in this enigmatic conifer. *Sequoia* is an ancient relict, which is member of the Arcto-Tertiary Geoflora (Axelrod 1967), that started its journey in the northern hemisphere millions of years ago, and due to global upheavals and climate change has moved to its current endemic location in California-Oregon, USA.

Coast redwood, the evergreen conifers, was discovered near Santa Cruz, California, in the 1790's by Archibald Menzies, a surgeon and botanist, leader of British Expedition. However, it was not classified until 1824 by David Don, who named it *Taxodium sempervirens*. In between coast redwood was also named as *Americus giganteus*. Coast redwood was later classified by Stephan Endlicher, an Austrian botanist, in 1847 as *Sequoia sempervirens* (O'Brien 2016). It has been speculated that Endlicher, who named the genus *Sequoia*, was paying homage to the Cherokee linguist Sequoyah (Lowe 2012).

Coast redwood (*Sequoia sempervirens*), a native forest tree of California, USA, is one of the tallest (116 m) and oldest (more than 2000 years old) trees (Sillett et al. 2021) in the world. Coast redwood formerly belonged to the family Taxodiaceae. Later it was transferred to Sequoioideae, a subfamily of the large family Cupressaceae. Sequoioideae also includes giant sequoia (*Sequoiadendron giganteum*) and dawn redwood (*Metasequoia glyptostroboides*), which are close relatives of *Sequoia sepmpervirens*. Almost two hundred years ago coast redwood forests dominated coastal California, before the European settlers starting logging these massive trees around 1850's, which is continuing to the present day. The current endemic distribution of coast redwood is restricted to the coastal summer fog belt of northern California and southeastern Oregon. The redwood fog belt is an irregular coastal strip of 725 km long and 8-56 km wide (Olson et al. 1990). Although coast redwood thrives in moist, cool places at elevations ranging from 30 m to 760 m, its range extends to 915 m above sea level (Olson et al. 1990). *Sequoia* is a fast-growing tree attaining a growth rate of 1 to 1.5 m per year, and the biggest trees reaching a height of more than 110 m. It is also a long-lived tree, ranging in age from 600 to 1500 years, some trees attaining a mature age of 2200 years (Barbour and Whitworth, 2001). The mean annual temperatures vary between 10° and 16° C, while mean annual maximum and minimum temperatures range between -12° to -1° C in the *Sequoia* region, and *Sequoia* forests remain frost-free from 6 to 11 months. The spatial pattern of *Sequoia* forests is mostly continuous, but in portions of their range they are fragmented. Most stands of *Sequoia* are found between 30 m to 760 m, and this region receives an annual precipitation between 640 to 3100 mm/year (Olson et al. 1990). *Sequoia* has undergone both expansions and contractions in its range in the past 10,000 years, and currently it appears that *Sequoia* is retreating from its southern range and expanding northwards (Sawyer et al. 2000).

The hexaploid *Sequoia* seems to have a relatively higher level of genetic diversity and variation as compared to other redwoods, *Metasequoia glyptostroboides*, *Sequoiadendron giganteum*, and *Fitzroya cupressoides* (Rogers 2000; Libby et al. 1996; Ahuja 2009). In addition, *Sequoia* reproduces by both sexual (seed) and vegetative methods (from basal sprouts and burls), and has good wood properties and is a fast growing tree. Because of these genetic traits *Sequoia* has been considered a prospective timber species for its potential adaptability out of its natural range in other countries with similar or environmentally different conditions in the face of climate change. *Sequoia* has been grown out of its endemic California region with varying degrees of success, depending upon the trial locations and climatic conditions, in different parts of the world, including France, Germany, Spain, Chile, Turkey, South Africa, and New Zealand (Kuser et al. 1995; Meason et al. 2016; Klápště et al. 2020; Breidenbach et al. 2020; Ahuja 2021). *Sequoia* is generally vulnerable to prolonged frost conditions. However, it has been introduced in Germany many years ago, and some of its genotypes have shown frost tolerance. These frost tolerant trees have been clonally propagated by tissue culture and are currently growing for the past 25 years in the arboretum of Institute of Forest Genetics, Grosshansdorf, Germany (Ahuja, 1996, 2017).

Sequoia has undergone expansions and contractions in its range in the past 10,000 years, and currently it appears that *Sequoia* is retreating from it southern range and expanding northwards (Sawyer et al. 2000), most likely due to global warming. Although extinction of endemic *Sequoia* seems unlikely in the foreseeable future because of human conservation intervention (state parks and reserves), it is still vulnerable in its native range. Therefore, strategies involving biotechnological and genetic approaches for the conservation of Sequoia germplasm would be valuable for the evolution and survival of this ancient conifer (Ahuja, 211, 2017). Recently, *de novo* transcriptome assembly of cold stressed clones of *Sequoia* was investigated to determine the mechanisms of frost tolerance in this species (Breidenbach et al. 2020).

More recently, genome-wide climate adaptation in the iconic coast redwood was investigated towards identifying genomic regions of adaptive significance to climate change (De La Torre, et al. 2021). Genome-wide association identified 78 new marker-trait markers for drought tolerance genes in *Sequoia* (De La Tore et al. 2021). These genes were involved in several metabolic and stress signaling pathways. The genome sequence analysis of *Sequoia*, has further revealed that, "compared to other conifers, *Sequoia* has an expansion of plethora of abiotic and biotic response genes, including those involved in fungal disease resistance, detoxification, and physical injury/structural remodeling and others supporting flavonoid biosynthesis" (Neale et at. 2022). All these genes provide an evolutionary adaptive potential for the survival of the coast redwood.

Fossil history of Sequoia

Fossils resembling *Sequoia* and other genera of the family Taxodiaceae have been discovered in different parts of the world (Miller, 1977; Chaney 1949; Ma et al. 2005; Zhang et al. 2015). The oldest *Sequoia* fossils (*S. jeholensis* Endo) were reported by Endo (1951) from the Jurassic deposits (~199-145 million years ago; mya) from Jehol, in south Manchuria. The foliage of fossil *S. jeholensis*, according to Endo, resembled the foliage

of extant *Sequoia sempervirens*, and had changed only slightly during the past millions of years. However, according to Miller (1977) the affinity of *S. jeholensis* foliage seems to be superficial with that of *Sequoia sempervirens* foliage.

Sequoia-like fossils occur widely in the northern hemisphere, and some of these have been dated to the Tertiary Period (Miller 1977). The ancient *Sequoia* species (*S. affinis*, *S. langsdorffii*, *S. abietina*) discovered around ~55 mys had dimorphic foliage (in two distinct forms). The foliage was scale-like, having overlapping scales, and occurring in two vertical rows. The foliage of fossil *Sequoia* from Paleocene through Oligocene (~40 mya) was more delicate in the extinct *Sequoia* compared to the extant *Sequoia* (Wolf 1996). By Miocene (~20 mya) the foliage of fossil *Sequoia abietina* (from USA) and *S. chinensis*, *S. maguanensis* (from China) was somewhat similar to that of the extant *Sequoia sempervirens* (Chaney 1949; Ma et al. 2005; Zhang et al. 2015). It seems that foliage of fossil *Sequoia* underwent distinct changes during Oligocene through Miocene, and differed only slightly from the living *Sequoia* (Fields, 1993). Until Miocene the polyploidy status of *Sequoia* was not known.

An investigation of guard cells from foliage of fossil *Sequoia* from Pliocene (~7 mya) deposits in Japan indicated that there was no difference between the guard cell from fossil *Sequoia* foliage and living *Sequoia* leaves (Miki and Hikita, 1951). If the size of guard cells is predictive of polyploidy, that there is an increase in guard cells with increase in ploidy level, then this suggest that Sequoia was already a polyploidy during Neogene (~23-2.6 mya), and it seems that increase in ploidy level occurred at an earlier geologic period. However, an increase in the size of guard cells alone may not necessarily indicate increase in ploidy level (particularly in allopolyploid or auto-allopolyploids), as additional phenotypic and genetic traits must also be considered.

It is not known when the hexaploid *Sequoia* evolved from its diploid ancestors, and which are its putative progenitors, living or extinct. Fossil studies suggest this event might have occurred during late Cretaceous and Tertiary periods (65 mya). Whether *Sequoia's* close relatives *Metasequoia* or *Sequoiadendron*, or other ancient species of Taxodiaceae, were the putative progenitors of hexaploid *Sequoia* has been debated for a long time.

Hybridization in Taxodiaceae

One 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, California, 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.

Another approach to resolve the ancestry of *Sequoia* would be to attempt in situ genomic hybridization (GISH) between the three genera to find homology between their genomes. We had employed GISH in *Sequoia*, but our preliminary study did not detect any homology between *Metasequoia* or *Sequoiadendron* genomes with that of the *Sequoia* genome (Ahuja, unpublished).

Types and origin of polyploidy in Sequoia

There are at least four possible hypotheses we can consider for the origin of polyploidy in *Sequoia*:

- An autohexaploid with a genomic connotation (AAAAAA), derived by one round (1R) of genome duplication (Fig. 1), or two rounds (2R) of genome duplication (Fig. 2) from an ancient species of *Sequoia*
- A segmental allohexaploid with genomic constitution (A_1A_1 $A_1A_1A_2A_2$), derived by either hybridization between geneti cally differentiated populations of an ancient species of *Sequoia*, or closely related ancient species of *Sequoia*, follo wed by two rounds (2R) of genome duplications (Fig. 3)
- An autoallohexaploid with a genome of (AAAABB) derived by hybridizations between two ancient species from the family Taxodiaceae, followed by two rounds (2R) of genome duplications (Fig. 4)
- An allohexaploid with a genome of (AABBCC) derived by hybridization between three ancient species of family Taxo diaceae, followed by two rounds (2R) of genome duplica tions (Fig. 5).

According to prevailing hypothesis (Fawcett et al. 2009; Vanneste et al. 2014; Lohaus and Van de Peer, 2016; Cai et al. 2019; Van de Peer et al. 2021), the burst of polyploidizations in land plants occurred at the P/Kg boundary (65 mya), and it is has been previously speculated that *Sequoia* possibly originated at that time during the early or late Cretaceous Period (Ahuja and Neale 2002; Ahuja 2005, 2009). Sequoia could have evolved as an autohehexaploid with one round of WGD (Fig. 2) at that time. However, if two rounds of WGDs occurred for the origin and evolution of *Sequoia*, whether as an autohexaploid (Fig. 3), or a segmental allohexaploid (Fig. 4) or an auto-allohexaploid (Fig 5), then that could have occurred either during the P/Kg boundary epoch, or in much later Tertiary geological periods. At the present time, there is no evidence to support the hypothesis that *Sequoia* originated as an allohexaploid (Fig. 6) involving three parental genomes. The evolution and survival of *Sequoia*, regardless of its origin, was accomplished by its capacity to reproduce both by sexual and vegetative means during the millions of years of its existence. That dual mode of reproduction was critical for its survival as an autohexaploid.

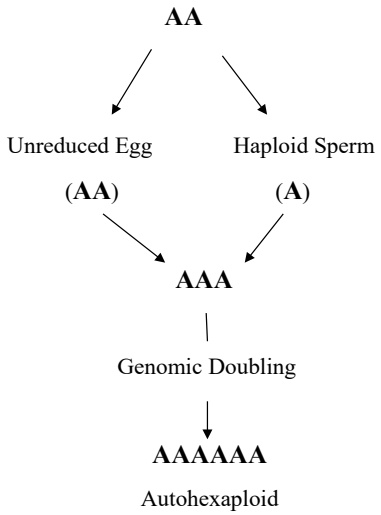


Fig. 1
Origin of an autohexaploid (AAAAAA) from a diploid ancestor (AA: 2n=22). This model assumes production of a triploid (AAA) by fusion of unreduced gamete (AA) from the female gametophyte and a reduced gamete (A) from the diploid (AA) ancestor. Following doubling of chromosomes in the triploiod (AAA), an autohexaploid (AAAAAA; 2n=6x=66) may be produced. Meiotic stabilization may occur over millions of years to increase the frequency of bivalents over multivalent to produce a **partially diploidized hexaploid *Sequoia***. This model requires only one round (1R) of genome duplication. Partial meiotic stabilization over long periods of time may produce a partially diploidized autohexaploid.

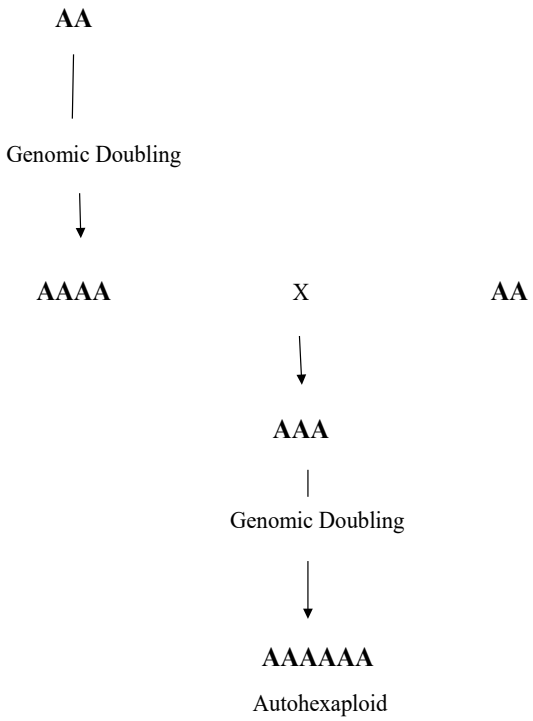


Fig.2
Origin of an autohexaploid (AAAAAA) by a slightly different route as shown in Fig. 1.In this model , the autotetraploid may develop by chromosome doubling in the diploid ancestor (AA: 2n=22). Hybridization between the autotetraplod (AAAA) and the same diploid ancestor (AA) would result in an autotriploid (AAA). By doubling of the chromosomes in the triploid (AAA), an autohehaxaploid (AAAAAA; 2n=6x=66) could be formed. This mode would require two (2R) genome duplications, compared the one in the model in Fig 1.

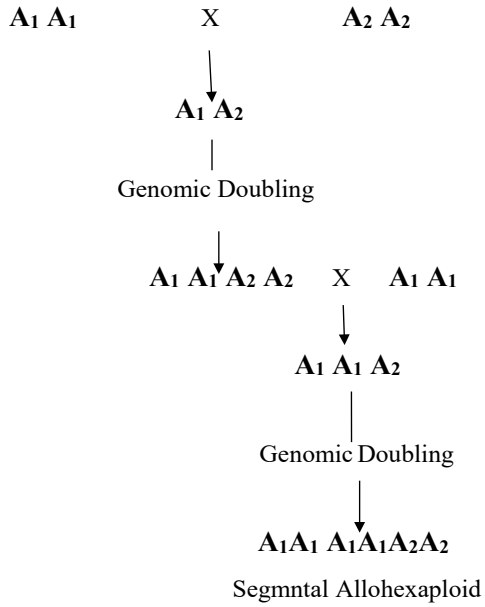


Fig. 3
Origin of a segmental allohexaploid (A_1A_1 $A_1A_1A_2A_2$) by hybridization between two related species (A_1A_1) and (A_2A_2) that have partially homologous chromosomes, both with the genetic constitution (2n=22). The F_1 hybrids between these two species would be A_1A_2. By doubling the chromosomes in the 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 ancestors (A_1A_1) would result in a segmental allotriploid ($A_1A_1A_2$). Doubling of chromosomes in this triploid would lead to the production of a segmental allohexaploid (A_1A_1 $A_1A_1A_2A_2$; 2n=6x=66). This mode also would require two rounds (2R) of genome duplications.

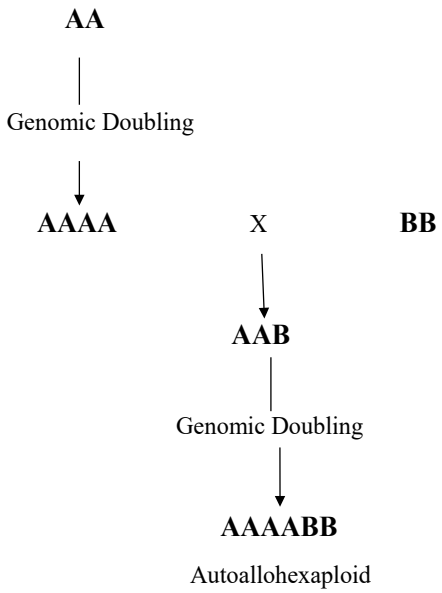


Fig. 4
Origin of an auto-allohexaploid (AAAABB) by hybridization between two distinct species (AA: 2n=22; and BB: 2n=22).In this model the first step the first step would be the production of an autotetraploid (AAAA) in one species (AA). Hybridization between this autotetraploid (AAAA) and the second species (BB) would result in an auto- allotriploid (AAB). Doubling of chromosomes in the triploid would lead to the formation of an auto-allohexaploid (AAAABB; 2n=6x=66). This mode also requires two rounds (2R) of genome duplications to produce a hexaploid.

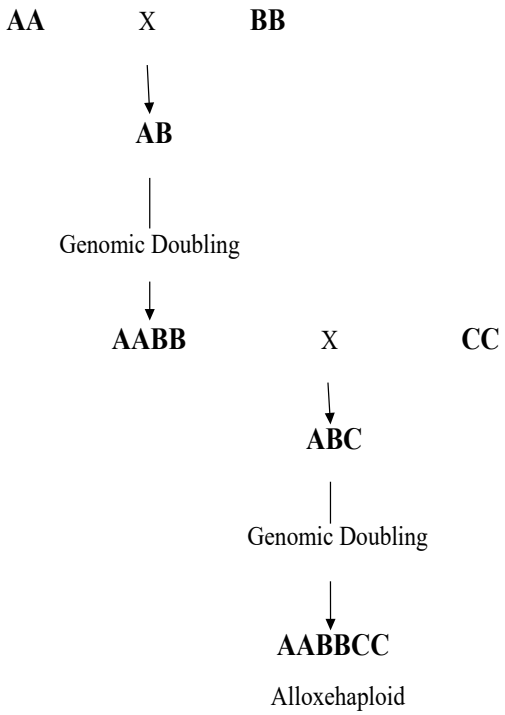


Fig. 5
Origin of an allohexaploid (AABBCC) by hybridization between three different species (AA, BB, and CC; all three with a chromosome number of 2n=22). Hybridization between two parents (AA and BB) would produce an F_1 (AB). Doubling of chromosomes in the F_1 would produce an allotetraploid (AABB). Hybridization between this allotetraploid (AABB) and the third species (CC) would result in an allotriploid (ABC). Doubling of chromosomes in this triploid would result in an allohexaploid (AABBCC; 2n=6x=66). This model also would require two rounds (2R) of genome duplications.

Chromosomal analysis of Sequoia

The chromosome number in coast redwood also changed over the years as well (Stebbins, 1948; Ahuja and Neale 2002). The haploid chromosome number (n=16) in *Sequoia* was reported in 1904 by Lawson; later revised to diploid number to 2n=50 by Dark (1932); further revised by Sax and Sax (1933) to 2n=40+; and later to 2n=44 by Buchholz in 1939. Until then the polyploidy nature of *Sequoia* was not known. Hirayoshi and Nakamura (1943) were the first to report that coast redwood had a somatic chromosome number of 66 and that it was a hexaploid (2n=6x=66). Subsequently, this chromosome number has been confirmed by Stebbins (1948) and many others (Saylor and Simons, 1970; Schlarbaum and Tsuchiya, 1984; Hizume et al. 1988; Ahuja and Neale, 2002).

After the establishment of the chromosome number in coast redwood, the next critical question revolved around the origin of polyploidy in this genus. It is not known when polyploidy in *Sequoia* evolved from its diploid ancestors, and, which are its putative progenitors, living or extinct. Based on meiotic configurations of *Sequoia* showing a few multivalent and majority of bivalents adding up to 2n=66, Stebbins (1948) suggested that *Sequoia* is a complex hexaploid, which is neither an autopolyploid nor a typical allopolyploid, but one of the two intermediate types. Stebbins postulated that coast redwood may be either an autoallopolyploid (AAAABB), or a segmental allopolyploid ($A_1A_1A_1A_1A_2A_2$) or (A_1A_1 $A_2A_2A_3A_3$). Both of these two types of hexaploids would require hybridizations between two, or perhaps three distinct ancestral species. Stebbins further examined similarities and differences between 27 morphological characteristics for example, arrangements of leaves, branches, bud scales, microsoporophylls, megasporophylls, etc, between several genera of Taxodiaceae. Stebbins found out that *Metasequoia* resembled *Sequoia* in the largest number of characters(18), and differed it in only 9; compared to 14 character similarities and 13 differences between *Sequoia* and *Taxodium*; and 11 similarities and 16 differences between *Sequoia* and *Sequoiadendron*. Based on these morphological traits, Stebbins postulated that, "There are grounds, therefore, on which to erect a working hypothesis that *S. sempervierns* originated as an allopolyploid from hybrids between an early Tertiary or Mesozoic species of *Metasequoia* and some probably extinct type of Taxodiaceaous plant not unlike three modern genera mentioned. This hypothesis has value as a speculation, because it can form the basis of certain predictions which will test it" (Stebbins 1948, p. 98). Stebbins (1948) hypothesis on the origin of *Sequoia* polyploidy remained uninvestigated for the next 22 years (till 1970), and the enigmatic ancestry of

Sequoia was further probed for the next 50 years for determining the true nature of the hexaploidy in *Sequoia*.

In order to further investigate the origin of polyploidy in *Sequoia*, it was necessary to provide detailed photographic images (not diagrams) of mitotic and meiotic chromosome morphology and structure in the *Sequoia* and other members of the family Taxodiaceae. Detailed description of *Sequoia* karyotype was reported by Saylor and Simons in 1970. Based on somatic chromosome of *Sequoia*, Saylor and Simons measured arm lengths of the 66 chromosomes and divided them into 33 triplets. Based on the arm length, each triplet was either derived from AAB genome or ABB genome. Saylor and Simons suggested that *Sequoia* was a possible auto-allopolyploid (AAAABB), as postulated by Stebbins in 1948. Further support for auto-allopolyploid nature of Sequoia was also provided by Schlarbaum and Tsuchiya (1984). However, Saylor and Simons (1970) opined that more detailed studies of *Metasequoia* chromosomes would shed light whether *Metasequoia* was one the ancestors of *Sequoia*, as suggested by Stebbins.

Li (1987) hypothesized that some ancient species of *Metasequoia* (2n=22) and *Sequoiadendron* (2n=22) may have contributed to the ancestry of *Sequoia* (2n=6x=66), based on similarities in metacentric chrromosomes of the three genera. Further, molecular phylogenetic studies also supported the idea that Sequoia originated by hybridization/recombination between *Metasequoia* and *Sequoiadendron* (Yang et al. 2012). Comparative studies have suggested that most of the chromosomes are metacentric (have medium centromeres) and that there is gradual transition in the size from long to short chromosomes in the three genera. However, there are some differences between the markers chromosomes, particularly satellite chromosomes in the three genera. Each of the three genera (*Sequoia*, *Metasequoia*, *Sequoiadendron*) has distinct Satellite chromosomes and phenotypic traits (Hizume 1989; Toda 1996; Ahuja, 2009), which suggests that they might have evolved independently of each other. In *Sequoia* the characteristic satellites are located at the distal portion of three pairs of chromosomes (Saylor and Simons, 1970; Hizume 1989; Toda 1996; Ahuja and Neale 2002). On the other hand, there is one pair of satellites located in the proximal region of the smallest chromosomes in *Sequoiadendron*, and three pairs of satellites located in the proximal regions of chromosomes in *Metasequoia* (Hizume 1989; Toda 1996; Ahuja, 2009). This is further substantiated by differences in fluorescent band patterns in *Sequoiadendron* having two heavy chromomycine A_3 (CMA) stained bands in proximal locations of a pair of chromosomes, *Metasequoia* with six CMA bands at centromeric locations, while *Sequoia* had three CMA bands at terminal position of three pairs of chromosomes (Hizume et al. 1988). Based on our somatic chromosome studies between *Metasequoia*, *Sequoidendron*, and *Sequoia* (Fig. 6); and meiotic chromosome studies (Fig. 7) that show that there are majority of bivalents and a couple of quadrivalents and hexavalents at the meiosis in pollen mother cells (Ahuja and Neale, 2002), we had proposed for the first time that, among other possibilities, that *Sequoia* presumably originated as **autohexaploid** (AAAAAA) from an ancient species of *Sequoia* in early Tertiary (Ahuja and Neale, 2002).

Diploidization in autopolyploids

Whole genome duplications (WGDs) have repeatedly occurred in the plant kingdom over millions of years. They have played an important role in the evolutions of plant species (Baduel et al. 2018). The immediate challenge for the genome duplicated autopolyploids from a single common ancestor was to maintain a certain degree of long-term chromosomal and genomic stability in the in the autopolyploids and their progeny. Although in the initial stages of WGD there were hit and run experiments in autopolyploids in nature. But in due course of time, some sort of genetic stability in the generative reproductive process caused by mutations in the autopolyploids for the production of viable seeds for future generations. In the absence of such mutations to restore at least partial generative reproductive stability, those autoplopyploids were doomed to become extinct, unless they had some mechanism for vegetative propagation in nature. The prevalence of autopolyploidy in plants has long been a subject of debate (Muntzing 1936), and according Clausen et al (1945) and Stebbins (1947), autopolyploids were not of common concurrence in plants. However, in recent years this situation has changed, and the rate occurrence of autopolyploids have found in nature may be more frequent than allopolyploids (Soltis et al. 2007; Parisod, Hoderegger, Bochmann 2010; Baker et al. 2016; Spoelhoh, Soltis and Soltis 2017; Li et al. 2021).

Relative frequency of meiotic multivalent and bivalent in artificially induced autopolyploid has been investigated in a number of plant species. In an autotetraploid strain (2n=4x=40) of maize quadrivalent and bivalents frequency was studied over ten year period, and at the end of this period there were fewer qauadrivalents and more bivalents at diakinesis stage in meiosis (Giles and Randolph, 1951), resulting in partial stability of the meiotic process. In another study the frequency of quadrivalents and bivalents was investigated in an artificially induced autotetraploid *Brassica compestris* var Toria (2n=4x=40) over 18 generations (Swaminathan and Sulbha, 1959). There was considerable reduction in the frequency of quadrivalents and increase in the frequency of bivalents after 18 generations, which resulted in increased seed fertility in the partially diploidized autotetraploid (Swaminathan and Sulbha 1959). The meiotic pairing in naturally occurring autohexaploid *Lathyrus palustris* (2n=6x=42) was invested was investigated. By using a mathematical model Sybenga (1995) has shown that there was reduced frequency of multivalents and increased frequency of bivalent pairing. Similar reduction in the multivalent frequency and increase in frequency of bivalents was reported in autotetraploid lines of *Arabidopsis thaliana* after 13 generations of selfing (Santos et al. 2003).

It would appear that autopolypolyploid plants undergo a process of diploidization for their survival and evolution. There is consensus that most of the land plants are ancient polyploids that have rediploidized (Li et al. 2021) in the course of their evolutionary history spanning millions of years (Wolfe 2001; Van de Peer, 2021; Zhang et al. 2020). The process of diploidization in the autopolyploid plants involve many changes in the genome organization that ultimately result in increased frequency of bivalents over multivalent that results in improved

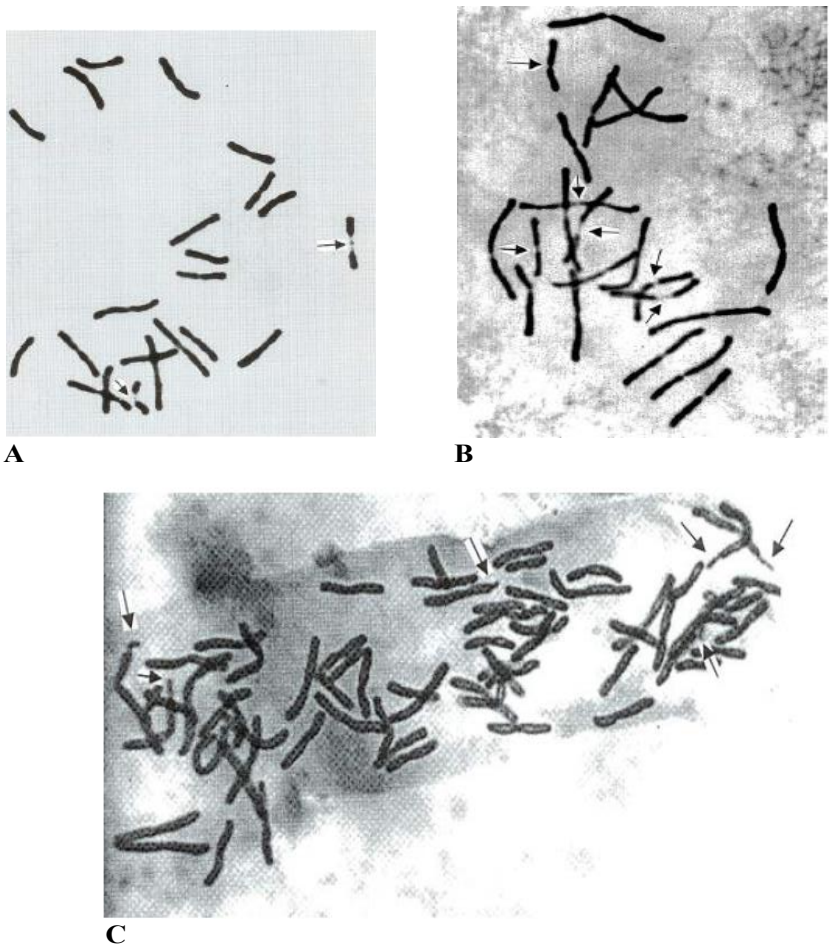


Fig. 6
Somatic chromosomes in *Sequoiadendron giganteum* (2n=22) (A), *Metasequoia glyptostroboides* (2n=22) (B), and *Sequoia sempervirens* (2n=6x=66) (C), showing distinct satellite chromosomes (arrows) in *Metasequoia* (A), *Sequoiadendron* (B) and *Sequoia* (C). (Figures reproduced from Ahuja, Euphytica, 165:5-19, 2009, Springer Nature)

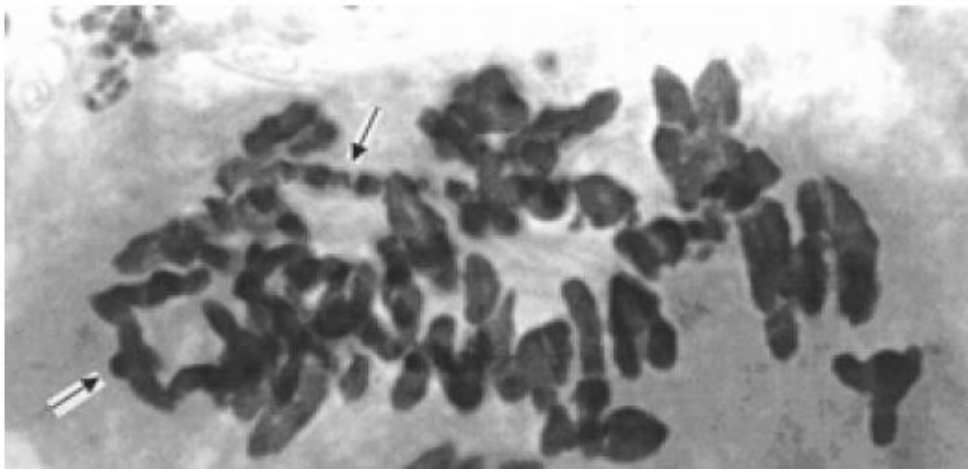


Fig. 7
Meiotic chromosome of *Sequoia sempervirens* at metaphase I (MI), showing majority of bivalents and a few quadrivalents and hexavalents (arrows). (Figure reproduced from Ahuja and Neale, 2002, Silvae Genetica, 51:93-100)

reproductive stability for seed production and inheritance in the progeny. Although diploidization occurs both in autopolyploids and allopolyploids, disomic polyploids (allopolyploids) major studies have been focused mainly on disomic segregating polyploids compared to polysomic polyploids (autopolyploids) (Hieu 2019). However currently this situation has changed and we are learning more about the presence of more copies of alleles and allelic interactions in the polysomic polyploids that can lead to unique gene regulation to adjust variation in phenotypic traits, metabolic products, and stress resistance in a changing environment (Spoelhof, Soltis and Soltis 2017; Hieu 2019).

Recent studies (Fawcett et al. 2009; Vanneste et al. 2014; Lohaus and Van de Peer, 2016; Cai et al. 2019; Van de Peer et al. 2021) have suggested that the burst of polyploidizations in land plants occurred at the P/Kg boundary (65 mya), and it is possible that *Sequoia* might have originated during the K/Pg period after one (Fig. 2) or two rounds of genome duplications (Fig. 3) in the original diploid genome. And probably had mostly multivalent and some bivalents due to discriminately pairing with some homologous chromosomes at meiosis. It would appear that it has taken millions of years to reach the current genomic state with majority of bivalents and a few multivalents at the meiotic stage to accomplish its present state. Ever since an auto-allohexaploid (AAAABB) origin of *Sequoia* has remained one of the dominant hypotheses.

An alternative hypothesis, amongst other possible hexaploid origins of Sequoia, was also put forth by Ahuja and Neale (2002, p. 99) that, "**Sequoia may be partially diploidized autohexaploid (AAAAAA), derived from some ancestral species of Sequoia, thus carrying a single ancestral genome**". Since its meiosis is still in a state of flux and it has not reached complete diploidization so far following whole genome duplications in an ancient species of *Sequoia*. In spite of the fact that *Sequoia* has low seed set due to irregular meiosis, it has survived for millions of years because of its unique capacity to vegetative propagate from its basal sprouts and burls in nature (Tredici 1999; O'Hara et al. 2017), and additionally, a large reservoir of genetic variability (Libby et al., 1996; Rogers, 1997, 2000; Ahuja 2009, 2017). In the light of several studies (Ahuja and Neale, 2002; Ahuja 2005, Hizume et al. 2014; Scott et al. 2016), it is highly likely that *Sequoia* has evolved as an autohexaploid from an ancient species of *Sequoia*.

Phylogenetic and molecular genetics of Sequoia

Comparative morphological (Stebbins 1948; Hida 1957; Takaso and Tomlinson 1992; Jagel and Dörken 2014), and molecular phylogeny studies (Brunsfeld et al. 1994; Gadek et al. 2000; Kusumi et al. 2000; Yang et al. 2012) have supported the notion that *Metasequoia* and *Sequoiadendron*, both diploids (2n=22) are more closely related to *Sequoia* (2n=6x=66) than other genera of Taxodiaceae. Recently, transcriptose data followed by phylogenetic analysis of single copy genes also strongly supported that *Sequoia* is more closely related to *Sequoiadendron* as compared to *Metasequoia* (Scott et al. 2016). However, the phylogenetic data based on single copy genes did not exclude the possibility of hybridization between *Sequoia* and *Sequoiadendron* clad to produce an auto-allohexaploid. Nevertheless, support for autohexaploidy came from the orthogroups or homologs of *Sequoia*, where duplicate genes showed more than expected concordance of sequences (Scott et al. 2016). Furthermore, according to Scott et al (2016) "hexaploidy in *Sequoia* is best explained by autopolyploidy or, if there was allopolyploidy, it happened within the California redwood clade". Their data suggests that if one uses one set of markers (single genes), the results could be interpreted that *Sequoia* originated as an autoallopolyploid (AAAABB) involving *Sequioadendron* as one of putative the parents; while using duplicate genes results can be explained by postulating that *Sequoia* originated as an autopolyploid (AAAAAA). In order to resolve the conundrum of polyploidy in *Sequoia*, it would be critical to sequence its genome to determine whether haploid *Sequoia* has three similar (AAA), or two similar and one different (AAB), or all three different (ABC) genomes.

Inheritance of isozymes in control crosses of coast redwood has indicated hexasomic segregation in the megagametophytes, and precludes strictly disomic segregation of allozyme markers in *Sequoia* (Rogers 1997). Further, microsatellite markers also detected up to six alleles in each individual consistent with the polyploidy nature in *Sequoia*, and suggesting that the parental genomes in *Sequoia* may be at least partially homologous (Douhovnikoff and Dodd, 2011). Both isozyme and microsatellite data are also compatible with the autopolyploid origin of *Sequoia*.

Sequoia has a complex genome, and its abominable ancestry and nature of polyploidy has been investigated by a number of investigators (Table 1). Whether *Sequoia* is an allohexaploid (AABBCC) seems to be less likely, as most studies so far have suggested that Sequoia is either an autoallohexaploid (AAAABB) derived by hybridization between ancient *Metasequoia*-like and *Sequoiadendron*-like species, or other species of Taxodiaceae; or a segmental allohexaploid ($A_1A_1A_1A_1A_2A_2$) originating from hybridization between related ancient species of *Sequoia* (Stebbins, 1948; Saylor and Simons, 1970; Schlarbaum and Tsuchiya, 1984; Li, 1987; Rogers, 1997).

On the other hand, cytogenetic studies showing distinct somatic chromosomes and meiotic configurations (Ahuja and Neale, 2002; 2009; Hizume 1989; 2014), along with isozyme studies indicating hexasomic segregation (Rogers 1997), and microsatellite studies showing six alleles per locus (Douhovnikoff and Dodd, 2011) in *Sequoia*, further support the hypothesis that Sequoia originated an autohehaxaploid. A segmental allopolyploid, and possibly an auto-allopolyploid, may also form majority of bivalents and some multivalent at meiosis due to pairing between homeologous chromosomes from $A_1A_2A_3$ or ABB genomes derived from different ancient species of *Sequoia* (Stebbins, 1948).

More recently, the genome size of *Sequoia* has been estimated at 26.5 Gbp (Gigabase pairs) by genomic sequencing techniques (Neale et al. 2022), which is lower than the one based on flow cytometry (31.5 Gbp) reported an earlier study (Hizume et al. 2001). However, the new estimate seems close to the size of euchromatin, but excludes the non-centromeric

Table 1
Studies on nature of polyploidy in *Sequoia sempervirens*

Type of Polyploidy	Genomic Connotation	Method used	Reference
Auto- allohexaploid or	AAAABB	Meiotic chromosomes & Morphological traits	Stebbins 1948
Segmental allohexaploid	$A_1A_1A_1A_1A_2A_2$	Meiotic chromosomes & Morphological traits	
Autoallohexaploid	AAAABB	Somatic chromosomes	Saylor and Simons, 1970 Schlarbaum and Tsuchiya 1984; Li 1987
Autoallohexaploid	AAAABB	Isozymes	Rogers, 1997
Autoallohexaploid	AAAABB	Molecular phylogenetics	Yang et al. 2012
Autohexaploid	AAAAAA	Somatic and meiotic Chromosomes	Ahuja and Neale, 2002 Ahuja, 2005, 2009
Autohexaploid	AAAAAA	Microsatellites	Douhovnikoff and Dodd, 2011
Autohexaploid	AAAAAA	Meiotic chromosomes	Hizume et al. 2014
Autohexaploid	AAAAAA	Transcriptose and Molecular phylogenetics	Scott et al. 2016
Autohexaploid	AAAAAA	Genome sequence	Neale et al. 2022

DNA (Neale et al. 2022). **Genome Sequencing data** on the hexaploid *Sequoia*, which has one of the **mega genomes** in conifers, has revealed that multiple genes that exist in triplicate in *Sequoia* and only once in the diploid *Sequoiadendron*, a close relative of *Sequoia* (Neale et al. 2022). However, according to Neale et al (2022, p. 10), "We hoped that full genome sequencing in coast redwood and giant sequoia (Scott et al. 2020) might have resolved the issue once for all". But in reality, it did not. They further state that even though their genomic data on *Sequoia* and *Sequoidendron* was not conclusive, but mostly they supported the autopolyploidy hypothesis that was previously proposed (Ahuja and Neale 2002; Ahuja 2005, 2009; Scott et al. 2016). Further Neale at al. (2022) have suggested that highly contiguous genome sequences in the three extant and extinct redwood species could possibly shed more light on the time of origin and nature of hexaploidy in coast redwood.

After more than 70 years since Stebbins (1948) proposed the hypothesis on the nature of polyploidy in *Sequoia*, we are still at the crossroads of resolving the origin and genetic nature of polyploidy in this complex hexaploid species. Let me reiterate. Based on morphological and chromosomal analyses, an autoallopolyploid (AAAABB) origin of *Sequoia* was fist postulated by Stebbins (1948), involving *Metasequoia* as a possible ancestral species, that was later supported by Saylor and Simons (1970). On the other hand, recent studies involving transcriptose assembly analysis (Scott et al. 2016, p. 190, "when analyzed on the basis of single gene basis"), and genome sequence data (Neale et al. 2022, "p. 6, Fig. 1. "hexaploid nature of the *Sequoia* genome is identical in two of three of the subgenomes") have suggested the possibility of involving *Sequoiadendron*, and not *Metasequoia*, as a possible putative ancestor of hexaploid *Sequoia*. However, studies based on cytogenetics, isozymes, microsatellites, and duplicate genes data have favored an autopolyploid (AAAAAA) origin of *Sequoia* (Ahuja and Neale 2002; Ahuja 2005, 2009; Douhovnikoff and Dodd 2011; Hizume et al. 2014; Scott et al. 2016). In order to further resolve the genetic nature of polyploidy in *Sequoia*, whether it is an autoallopolyploid or an autopolyploid, I would propose genome sequencing, in addition to ancient species of Metasequoia and Sequoiadendron, also from the extinct species of *Sequoia* (*S. abietina*, *S. affinis*, and *S. langsdorffii*) to resolve the conundrum of hexaploidy in the extant complex and enigmatic *Sequoia*.

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