Polyploidy in Gymnosperms-A Reappraisal

Deepak Ohri

Research Cell, Amity University Uttar Pradesh, Lucknow Campus, Malhaur, (Near Railway Station), P.O. Chinhath, Lucknow-226028, U.P., India, E-mail: ohri_deepak@rediffmail.com

Abstract

Recent polyploidy in gymnosperms is unusually scarce being present in only 9.80 % of the 714 taxa studied cytologically. Polyploid forms are represented by sporadic seedlings and individual trees, intraspecific polyploidy in cultivation or in wild and entirely polyploid species and genera. Polyploidy shows a non-random distribution in different genera being mostly prevalent in *Ephedra* and *Juniperus*, besides the classic examples of *Sequoia* and *Fitzroya*. Remarkably, both *Ephedra* and *Juniperus* show adaptive radiation by interspecific hybridization followed by polyploidy while in *Ginkgo* viable polyploid cytotypes are found in cultivation. Induced polyploidy has not provided any tangible results in the past but recent attempts on certain genera of Cupressaceae hold some promise of producing cultivars for horticulture trade. Lastly, various evidences derived from cytological analysis, fossil pollen, guard cells and comparative genomic studies indicating the occurrence of paleopolyploidy have been discussed.

Keywords: *gymnosperms, polyploidy, Sequoia, Fitzroya, Juniperus, Ephedra, interspecific hybridization, allopolyploidy, diploidization, induced polyploidy, paleopolyploidy*

Introduction

Rarity of recent cases of polyploidy in gymnosperms has been a long standing subject of inquiry (Khoshoo 1959, Delevoryas 1980, Ahuja 2005). However, polyploidy has been a frequent phenomenon in angiosperms and its incidence has been estimated between 30-70 % of the extant species (Masterson 1994, Bratagnolle and Thompson 1995, Ramsey and Schemske 1998, Otto and Whitton 2000, Adams and Wendel 2005, Weiss-Schneeweiss et al. 2013, Carta et al. 2020) and is implicated in 15 % of the speciation events (Wood et al. 2009). Interestingly a recent study by Rice et al (2019) based on extensive spatial data has shown a highly positive correlation of polyploid frequency with higher latitudes. Moreover, many angiosperm lineages have been shown to have undergone

paleopolyploidies in the geological past (Bowers et al., 2003, Blanc and Wolfe 2004, Cui et al., 2006, Fawcett et al. 2009, Paterson et al. 2009, Soltis et al. 2009, International Brachypodium Initiative 2010, Jiao et al. 2011, 2014, Amborella Genome Project 2013, Van der Peer et al. 2017, Leebens-Mack et al. 2019, Wu et al. 2020) resulting in a burst of adaptive radiation and high level of biodiversity represented by estimated 3,52,000 species (The Angiosperm Phylogeny Group 2009). Furthermore, a large number of crop and ornamental species are of polyploid origin which again underlines the significance of polyploidy in their evolution and domestication (Reney-Byfield and Wendel 2014, Khoshoo 1979, Ohri 2013, Salman-Minkov et al 2016).

Gymnosperms on the other hand have very low species diversity with 1104 accepted species (The Plant List) therefore showing a huge difference as compared to angiosperms. Commensurate with this restricted biodiversity, the incidence of polyploidy is also very low being represented in only 9.80 % of the 714 taxa studied (Rastogi and Ohri 2020b, present data). Since the last review (Ahuja 2005) on this topic was written about 15 years back there is a need to make a reassessment of the incidence and consequences of polyploidy in this important group of plants. The present account makes an assessment of the occurrence of polyploid taxa in the form of stray seedlings, individual trees, intraspecific polyploidy in cultivation or in wild and entirely polyploid species and genera in each of the five orders (Christenhusz et al. 2011), the types of polyploidy in various taxa, the possibility of genetic improvement by induced polyploidy and the evidence of any ancient polyploidy.

Polyploidy in Gymnosperms Cycadales

Encephalartos hildebrandtii

Among the 10 genera included in this order only one instance of triploidy ($2n=27$) in *Encephalartos hildebrandtii* is known (Table 1). The chromosome matching, based on size and morphology, revealed the presence of a group of nine homologous pairs, and a haploid group of nine chromosomes. Therefore, an allotriploid origin of this individual has been suggested, resulting from fertilization between an unreduced and a reduced gamete of two related species (Abraham and Mathew 1966).

Table 1
Sporadic polyploidy

Sr. No.	Taxon name	2n=	Ploidy	Reference
1.	<i>Encephalartos hildebrandtii</i> A. Braun & Bouché	27	3x	Abraham & Mathew 1966
2.	<i>Welwitschia mirabilis</i> Hook.f.	84	4x	Fernandes 1936
3.	<i>Pinus densiflora</i> Siebold & Zucc.	48	4x	Zinnai 1952
4.	<i>Pinus elliottii</i> Engelm.	24, 36, 48	Mixoploid seedlings with 2x, 3x, 4x tissues	Mergen 1958
5.	<i>Pinus sylvestris</i> L.	36, 48	3x, 4x	Muratova 1997, Sedelnikova & Muratova 1999, 2001, Muratova et al. 2001
		48	4x	Pimenov & Sedelnikova 2002
6.	<i>Pinus thunbergii</i> Parl.	48	4x	Nishimura 1960, Toda & Sotoyama 1972
7.	<i>Picea abies</i> (L.) H. Karst.	36, 48, 24, 28-30, 30-36, 36,37,48,60-70	3x, 4x Mixoploids	Kiellander 1950 Illies 1953, 1958
8.	<i>Picea glauca</i> (Moench) Voss	36, 48, 96	3x, 4x, 8x	Winton 1964
9.	<i>Picea mariana</i> (Mill.) Britton, Sterns & Poggenb.	48	4x	Winton 1964
		38	hypertriploid	Tremblay et al. 1999
		24, 27, 36; 30, 39, 40, 55	Mixoploids	Tremblay et al. 1999
10.	<i>Larix decidua</i> Mill.	48	4x	Christiansen 1950
11.	<i>Larix kaempferi</i> (Lamb.) Carrière	48	4x	Chiba & Watanabe 1952
12.	<i>Larix gmelinii</i> (Rupr.) Kuzen.	36	3x	Muratova 1995
13.	<i>Larix sibirica</i> Ledeb.	36, 48	3x, 4x	Pimenov & Sedelnikova 2002
14.	<i>Larix decidua</i> X <i>L. occidentalis</i>	36	3x	Syrach-Larsen & Westergaard, 1938
15.	<i>Abies firma</i> Siebold & Zucc.	48	4x	Kanezawa 1949a
16.	<i>Abies sibirica</i> Ledeb.	36, 48	3x, 4x	Sedelnikova & Pimenov 2003
17.	<i>Cunninghamia lanceolata</i> (Lamb.) Hook.	33	3x	Zonneveld 2012
18.	<i>Taiwania cryptomerioides</i> Hayata	33	3x	Hizume 1989
19.	<i>Cryptomeria japonica</i> (Thunb. ex L.f.) D.Don	33	3x	Matsuda & Miyajima 1977, Matsuda 1980, Somego et al. 1981, Sasaki 1982, Kondo et al. 1985, Kondo 1988, Suyama et al. 1996, Kondo & Hizume 2000, Chiba 1951, Zinnai & Chiba 1951,
		33, 44	3x, 4x	
20.	<i>Chamaecyparis obtusa</i> (Siebold & Zucc.) Endl.	33	3x	Sasaki 1982
21.	<i>Glyptostrobus pensilis</i> (Staunton ex D.Don) K. Koch	33	3x	Price et al. 1973
22.	<i>Sequoiadendron giganteum</i> (Lindl.) J. Buchholz	24	2x+2 aneuploid	Hizume 1989

Ginkgoales

Ginkgo biloba

Ginkgo biloba has been known as a diploid species with $2n=24$ (Hizume 1997, Liu et al. 2017). However, recent investigations show the existence of spontaneous viable polyploids in artificial plantations (Table 2). A normal growing supposedly polyploid sapling was screened among the progeny of three female trees grown in the Botanic Garden of Faculty of Science, Masaryk University in Brno (Czech Republic). It was confirmed as a tetraploid with double (37.4 Gbp) the 2C value of a diploid (18.4 Gbp) as also from the larger dimensions of stomatal size ($60\pm6\mu\text{m}$) compared with that of diploid ($39\pm5\mu\text{m}$) (Smarda et al. 2016). Later an extensive screening was done in the 1533 seedlings obtained from the same maternal trees growing in University of Brno, various other samples cultivated by the growers and most importantly in 371 plants of about 200 named cultivars which together made up more than 2200 individuals. Their ploidy level was confirmed by the measurement of genome size and stomatal parameters which increase/decrease proportionately. Some triploid or tetraploid saplings or trees were found in growers' samples but the most substantial evidence of the spontaneous origin of polyploidy and its sustainability in cultivated condition was obtained from the screening of 200 commercial cultivars. Remarkably, out of these 200 cultivars, 13 were haploid ($2C=10.16\text{Gbp}$) three triploid ($2C=29.19\text{Gbp}$), eight tetraploid ($2C=38.12\text{Gbp}$) and rest diploid ($2C=19.53\text{Gbp}$). The individuals representing these ploidy levels show normal vegetative growth with characteristic morphological features as haploids show smaller leaves and dwarf or upright growth; triploids have relatively larger and bilobed leaves, while tetraploids are distinguished by larger, thicker leaves with lacinate margins. The haploid cultivars however, show a tendency to revert back to diploid level as indicated by some of the branches showing larger leaves. The regular spontaneous origin of these individuals with different ploidy levels with a reasonable frequency, and their survival and perpetuation under cultivation shows that there is no genomic constraint in the origin of polyploids in *Ginkgo* (Smarda et al. 2018).

Gnetales

Gnetum

Four species of *Gnetum* studied (*G. gnemon*, *G. montanum*, *G. ula*, *G. costatum*) show a high chromosome number of $2n=44$ (Fagerlind 1941, Mehra and Rai 1957, Ohri and Khoshoo 1986, Mehra 1988, Hizume et al 1993, Leitch et al 2001, Mathew et al. 2014b, Wan et al 2018). There is a strong possibility of polyploid derivation of this high basic number of $x=22$ and in fact Fagerlind (1941) suggested allopolyploidy from the markedly distinct 11 larger and 11 smaller bivalents. Allopolyploidy is further corroborated by the constant presence of high levels of ITS polymorphism as observed in 16 *Gnetum* species (Won and Renner 2005). This aspect needs to be studied further to have a proper understanding of *Gnetum* genome.

Ephedra

Out of the 70 recognized species in the genus 51 species comprising 55 taxa have been studied cytologically out of which 13

are diploid, 24 taxa are exclusively polyploid while 18 show intraspecific polyploid cytotypes and the ploidy ranges from 4x to 8x (Tables 2 and 3, Fig.1). The genus therefore shows a high incidence (76.36 %) of polyploidy. Among the species with both diploid and polyploid cytotypes, those with 2x/4x combination are most frequent (16 taxa) while more than two cytotypes are observed in *E. gerardiana* (2x, 4x, 8x) and *E. fasciculata* (2x, 4x, 5x, 6x) (Table 2). The exclusively polyploid species are most frequently 4x (19 taxa) followed by *E. aphylla*, *E. sarcocarpa* (6x), *E. funerea* (4x, 8x), *E. californica* (6x, 8x), and *E. antisiphilitica* (8x) (Table 3).

The nature of polyploidy can now be discussed in some species. Early karyotype studies revealed allopolyploidy in *E. altissima*, *E. intermedia*, *E. likiangensis*, *E. saxatilis*, and *E. sinica* mainly because two sets of 14 chromosomes could be identified depending on number and morphology of nucleolar organizers (Mehra 1946a) as also some of the species studied for their meiosis show mainly bivalent pairing (Mehra 1946b).

Recently, a study on the *Ephedra* species distributed in the Qinghai-Tibetan Plateau (QTP) has revealed a high frequency of the occurrence of allopolyploidy. Out of the 13 species studied, *E. equisetina*, *E. minuta*, *E. monosperma* and *E. rhytidosperrma* are diploid, *E. gerardiana*, *E. przewalskii* and *E. regeliana* have both 2x and 4x cytotypes while six taxa i.e., *E. likiangensis*, *E. glauca*, *E. intermedia*, *E. saxatilis*, *E. saxatilis* var. *mairei* and *E. sinica* are exclusively tetraploid (Wu 2016). The nature of polyploidy has been established based on phylogenetic analysis of two single copy nuclear genes i.e. *LFY* and *DDB2*, while cpDNA has been used to identify the possible maternal parents. In the gene trees based on nuclear genes each of the six polyploid taxa reveal two types of sequences distributed in different major clades. Subsequently, based on the similarity of chlorotypes, three tetraploid species *E. glauca*, *E. intermedia* and *E. sinica* have been shown to have some species closely related to *E. przewalskii* as the maternal parent and the diploids related to *E. equisetina*, *E. minuta* and *E. monosperma* as paternal parents. Similarly *E. saxatilis* might have *E. gerardiana* as the maternal parent, while that of *E. likiangensis* belongs to *E. equisetina*, *E. minuta* and *E. monosperma* lineage, and *E. saxatilis* var. *mairei* might be deriving its maternal parentage from two different lineages therefore indicating multiple origins (Wu et al. 2016). Furthermore, autotetraploidy has been proposed for the 4x cytotype of *E. przewalskii* and allopolyploidy for the 4x cytotypes of *E. regeliana* and *E. gerardiana* (Wu et al. 2016).

What underlying factors have led to this high frequency of polyploidy (Tables 2 and 3) and allotetraploid speciation in Asian species (Wu et al. 2016). These include frequent unreduced gamete formation (Mehra 1946a) substantiated also by pollen dimorphism reported in some species (Beug 1956, Chaturvedi 1978, Ickert-Bond et al. 2003), propensity for natural hybridization (Wendt 1993, Kitani et al. 2011) and low basic chromosome number (Leitch and Leitch 2012). Further establishment and survival of polyploids in nature can be related to the peculiar habit and reproduction of *Ephedra* species which are perennial shrubs, vines or small trees with underground rhizomes in contrast to conifers with large trees and lacking any vegetative mode of reproduction. The extensively long rhizomes of

Table 2
Intraspecific polyploid taxa

Sr. No.	Taxon name	n=	2n=	Ploidy	Reference
1.	<i>Ginkgo biloba</i> L.	12 (Haploid)	24,36,48	1x, 2x, 3x, 4x	Smarda et al. 2016, 2018
2.	<i>Ephedra americana</i> Humb. & Bonpl. ex Willd.		14	2x	Florin 1932, Resende 1937, Hunziker 1955, Nakata & Oginuma 1989,
			28	4x	Chouhdry 1984, Leitch et al. 2001
3.	<i>Ephedra chilensis</i> C.Presl		14	2x	Resende 1937, Hunziker 1953, 1955, Hizume & Tominaga 2016
			28	4x	Hunziker 1953, 1955, Chouhdary 1984, Ickert Bond et al. 2014
4.	<i>Ephedra distachya</i> L.		14	2x	Ickert Bond et al. 2020
			28	4x	Florin 1932, Resende 1937, Kawatani 1959, Bianco et al. 1988, Murin & Májovský 1979, Chouhdry 1984, Muratova et al. 2001, Leitch et al. 2001, Sedelnikova et al. 2011 Kozhevnikova & Kozhevnikov 2012, Ickert Bond et al. 2014,
			36		Tarnavarschi & Lungeanu 1970a, b
		14		4x	Terasaka 1982
5.	<i>Ephedra equisetina</i> Bunge		14	2x	Florin 1932, Wu et al 2009, Ickert Bond et al. 2014, Wu et al. 2016
			28	4x	Kawatani et al. 1959
6.	<i>Ephedra fasciculata</i> A.Nelson		14	2x	Ickert Bond et al. 2020
			28	4x	Ickert Bond et al. 2020
			35	5x	Ickert Bond et al. 2020
			42	6x	Ickert Bond et al. 2020
7.	<i>Ephedra foeminea</i> Forssk.		14	2x	Chouhdry 1984, Bianco et al. 1987, Ickert Bond et al. 2014, 2020
			28	4x	Kawatani et al. 1959
8.	<i>Ephedra fragilis</i> Desf.		14	2x	Chouhdry 1984
			28	4x	Chouhdry 1984, Colombo & Marceno 1990, Leitch et al. 2001, Ickert Bond et al. 2020
9.	<i>Ephedra gerardiana</i> Wall. ex Stapf		14	2x	Mehra 1988, Wu et al. 2016
			28	4x	Chouhdry 1984, Mehra 1988, Leitch et al. 2001, Wu et al. 2016
			56	8x	Kawatani et al. 1958
		7		2x	Mehra 1946a, 1988
10.	<i>Ephedra intermedia</i> Schrenk & C.A.Mey.		14	2x	Chouhdry & Tanaka R 1981, Chouhdry 1984
			28	4x	Mehra 1946a, 1988, Wu et al. 2009, Wu et al. 2016, Ickert Bond et al. 2020
11.	<i>Ephedra major</i> subsp. <i>procera</i> (C.A.Mey.) Bornm.		14	2x	Florin 1932, Ickert Bond et al. 2020
			28	4x	Ickert Bond et al. 2020
12.	<i>Ephedra minuta</i> Florin		14	2x	Chouhdry 1984, Wu et al. 2016,
			28	2x	Ickert Bond et al. 2020
13.	<i>Ephedra monosperma</i> J.G.Gmel. ex C.A.Mey.		14	2x	Wu et al. 2016, Ickert Bond et al. 2020
			28	4x	Leitch et al., 2001, Ickert Bond et al. 2020
14.	<i>Ephedra multiflora</i> Phil. ex Stapf		14	2x	Krapovikas 1954, Hunziker 1955
			28	4x	Ickert Bond et al. 2020
		7		2x	Hunziker 1955
15.	<i>Ephedra nevadensis</i> S.Watson		14	2x	Price et al. 1974
			28	4x	Chouhdry 1984, Ickert Bond et al. 2020
16.	<i>Ephedra przewalskii</i> Stapf		14	2x	Kong et al 2001, Wu et al. 2009, Wu et al. 2016
			28	4x	Ickert Bond et al. 2014, 2020, Wu et al. 2016
17.	<i>Ephedra regeliana</i> Florin		14	2x	Wu et al. 2016, Ickert Bond et al. 2020
			28	4x	Wu et al. 2016

Table 2: continued

Sr. No.	Taxon name	n=	2n=	Ploidy	Reference
18.	<i>Ephedra trifurca</i> Torr. ex S. Watson		14	2x	Ickert Bond et al. 2014
			28	4x	Ickert Bond et al. 2014, 2020
19.	<i>Cupressus dupreziana</i> A. Camus		22	2x	Goldblatt 1984
			44	4x	Goldblatt 1984
20.	<i>Cupressus macrocarpa</i> Hartw.		22	2x	Mukherjee & Hall 1979, Ohri & Khoshoo 1986, Hizume & Fuziwara 2016, Li & Fu 1996
			44	4x	Mathew et al. 2014a
21.	<i>Juniperus chinensis</i> L.		22	2x	Hall et al. 1973, 1979,
			33	3x	Evans & Rasmussen 1971, Hall et al. 1979
		11		2x	Sax & Sax 1933
			44	4x	Hall et al. 1973, 1979, Nagano et al. 2000, Farhat et al. 2019a
22.	<i>Juniperus chinensis</i> var. <i>sargentii</i> A. Henry		22	2x	Gurzenkov 1973, Nagano et al. 2000, Nagano et al. 2007
			44	4x	Farhat et al. 2019a
23.	<i>Juniperus deppeana</i> var. <i>gamboana</i> (Martínez) R. P. Adams		22	2x	Farhat et al. 2019a
			44	4x	Goldblatt 1984
24.	<i>Juniperus foetidissima</i> Willd.		22	2x	Zonneveld 2012
			66	6x	Farhat et al. 2019a
25.	<i>Juniperus phoenicea</i> L.		22	2x	Romo et al. 2013, Valles et al. 2015, Farhat et al. 2019a
			66	6x	Zonneveld 2012
		11		2x	Mehra & Khoshoo 1956 a
26.	<i>Juniperus pingii</i> W.C. Cheng ex Ferré		22	2x	Farhat et al. 2019a
			44	4x	Zonneveld 2012
27.	<i>Juniperus polycarpus</i> var. <i>seravschanica</i> (B. Fedtsch.) R.P. Adams		22	2x	Mehra 1988,
			44	4x	Farhat et al. 2019a
28.	<i>Juniperus sabina</i> L.		22	2x	Evans & Rasmussen 1971, Hall et al. 1979, Romo et al. 2013, Valles et al. 2015, Farhat et al. 2019b
			44	4x	Hall et al. 1979, Zonneveld 2012
29.	<i>Juniperus squamata</i> Buch. - Ham. ex D. Don		22	2x	Zonneveld 2012
			44	4x	Hall et al. 1979, Farhat et al. 2019a
30.	<i>Juniperus squamata</i> f. <i>wilsonii</i> Rehder		22	2x	Farhat et al. 2019a
			44	4x	Hall et al. 1973
31.	<i>Juniperus virginiana</i> L.		22	2x	Stiff 1951, Hall et al. 1973, 1979, Hizume et al. 2001, Zonneveld 2012, Farhat et al. 2019a
			33	3x	Stiff 1951, Hall et al. 1979
		11		2x	Sax & Sax 1933

Table 3
Polyploid taxa in gymnosperms

Sr. No.	Taxon name	n=	2n=	Ploidy	Reference
1.	<i>Ephedra alata</i> Decne.		42	6x	Ickert Bond et al. 2020
	<i>Ephedra altissima</i> Desf.		28	4x	Resende 1937, Mehra 1946a, Kawatani et al. 1959, Chouhdry 1984, Mehra 1988, Ickert Bond et al. 2014, 2020
2.	<i>Ephedra antisiphilitica</i> Berland. ex C.A.Mey.		56	8x	Ickert Bond et al. 2014, 2020
3.	<i>Ephedra aphylla</i> Forssk.		42	6x	Ickert Bond et al. 2014, 2020
4.	<i>Ephedra aspera</i> Engelm. Ex S.Watson		28	4x	Ickert Bond et al. 2014, 2020
5.	<i>Ephedra boelckeii</i> F.A.Roig		28	4x	Ickert Bond et al. 2014, 2020
6.	<i>Ephedra californica</i> S.Watson		42	6x	Ickert Bond et al. 2014, 2020
			56	8x	Ickert Bond et al. 2014, 2020
7.	<i>Ephedra coryi</i> E.L.Reed		28	4x	Ickert Bond et al. 2014, 2020
8.	<i>Ephedra cutleri</i> Peebles		28	4x	Ickert Bond et al. 2014, 2020
9.	<i>Ephedra distachya</i> subsp. <i>helvetica</i> (C. A. Mey.) Asch. & Graebn.		28	4x	Leitch et al. 2001, Ickert Bond et al. 2020
10.	<i>Ephedra funerea</i> Coville & C.V.Morton		28	4x	Ickert Bond et al. 2020
			56+B	8x	Ickert Bond et al. 2014
11.	<i>Ephedra gerardiana</i> var. <i>sikkimensis</i> Stapf		28	4x	Mehra 1988, Wu et al. 2016, Ickert Bond et al. 2020
		14		4x	Mehra 1946a, 1988
12.	<i>Ephedra glauca</i> Regel		28	4x	Wu et al. 2016, Ickert Bond et al. 2020
13.	<i>Ephedra</i> × <i>intermixta</i> Cutler		28	4x	Ickert Bond et al. 2020
14.	<i>Ephedra likiangensis</i> f. <i>mairei</i> (Florin) C.Y.Cheng		28	4x	Ickert Bond et al. 2014, Wu et al. 2016
15.	<i>Ephedra likiangensis</i> Florin		28	4x	Leitch et al. 2001, Wu et al. 2016
		14		4x	Mehra 1946a, 1988
16.	<i>Ephedra lomatolepis</i> Schrenk		28	4x	Ickert Bond et al. 2014
17.	<i>Ephedra pedunculata</i> Engelm. ex S.Watson		28	4x	Ickert Bond et al. 2020
18.	<i>Ephedra pseudodistachya</i> Pachom.		28	4x	Ickert Bond et al. 2020
19.	<i>Ephedra sarcocarpa</i> Aitch. & Hemsl.		42	6x	Ickert Bond et al. 2014, 2020
20.	<i>Ephedra sinica</i> Stapf		28	4x	Resende 1937, Chouhdry 1984, Kong et al. 2001, Wu et al. 2009, Ickert Bond et al. 2014, 2020, Wu et al. 2016
		14		4x	Mehra 1946a, 1988, Resende 1937
21.	<i>Ephedra strobilacea</i> Bunge		28	4x	Ickert Bond et al. 2014
22.	<i>Ephedra torreyana</i> S. Watson		28	4x	Ickert Bond et al. 2020
23.	<i>Ephedra transitoria</i> Riedl		28	4x	Ickert Bond et al. 2014
24.	<i>Ephedra viridis</i> Coville		28	4x	Chouhdary 1984, Hunziker, 1955b, Leitch et al. 2001, Ickert Bond et al. 2014, 2020
25.	<i>Sequoia sempervirens</i> (D. Don) Endl.		66	6x	Hirayoshi & Nakamura 1943, Stebbins 1948, Fozdar & Libby 1968, Saylor & Simons 1970, Sclarbaum & Tsuchiya 1984a, b, Sclarbaum et al. 1984, Hizume et al. 1988, 2001, Hizume 1989, Toda 1996, Ahuja & Neale 2002, Ahuja 2005, 2009, Scott et al. 2016
		33		6x	Hirayoshi & Nakamura 1943, Stebbins 1948, Terasaka 1982, Sclarbaum et al. 1984, Hizume et al. 2014
26.	<i>Fitzroya cupressoides</i> (Molina) I. M. Johnst.		44	4x	Hair 1968, Price et al. 1973, Ahuja 2009, Zonneveld 2012
27.	<i>Cupressus guadalupensis</i> var. <i>forbesii</i> (Jeps.) Little		44	4x	Goldblatt 1984
28.	<i>Juniperus</i> × <i>pfitzeriana</i> (Späth) P.A.Schmidt		33,44	3x, 4x	Zonneveld 2012
29.	<i>Juniperus coxii</i> A.B.Jacks		44	4x	Farhat et al. 2019a
30.	<i>Juniperus indica</i> Bertol		44	4x	Mehra 1976, 1988, Farhat et al. 2019a
31.	<i>Juniperus morrisonicola</i> Hayata		44	4x	Farhat et al. 2019a
32.	<i>Juniperus procumbens</i> (Siebold) Miq		44	4x	Xu et al. 1992, Nagano et al. 2000, 2007, Zonneveld 2012, Farhat et al. 2019a
33.	<i>Juniperus przewalskii</i> Kom.		44	4x	Farhat et al. 2019a
34.	<i>Juniperus recurva</i> Buch. - Ham. ex D.Don		44	4x	Farhat et al. 2019a
35.	<i>Juniperus rushforthiana</i> R.P. Adams		44	4x	Farhat et al. 2019a
36.	<i>Juniperus sabina</i> var. <i>balkanensis</i> R.P. Adams and A. Tashev		44	4x	Farhat et al. 2019b
37.	<i>Juniperus thurifera</i> L.		44	4x	Valles et al. 2015, Romo et al. 2013, Farhat et al. 2019a
38.	<i>Juniperus thurifera</i> subsp. <i>africana</i> (Maire) Romo & Boratynski stat. nov.		44	4x	Romo et al. 2013, Farhat et al. 2019a
39.	<i>Juniperus tibetica</i> Kom.		44	4x	Farhat et al. 2019a

Fig. 1
Gymnosperm phylogeny based on Bowe et al. (2000) and Chaw et al. (2000) showing the occurrence of ancient (black circles) and recent (grey squares) incidences of polyploidy in five orders of gymnosperms.

Ephedra (Pearson 1929) greatly facilitate vegetative reproduction and perpetuation of polyploids (Land 1913, Cutler 1939, Wu et al. 2016). A positive association between polyploidy and clonal reproduction has also been shown in angiosperms (Van Drunen and Husband 2019).

Another constantly observed feature is the absence of any genome downsizing in *Ephedra* allotetraploids as the genome size of these allopolyploids are nearly equal to the sum of the genome size of their putative parents (Ickert-Bond et al 2020, Wu et al. 2020). This is also shown by max./min. ratio of 2C (4.73) and 1Cx (1.37) observed in 49 diploid and polyploid species (Ohri unpublished) which underlines a highly conserved karyotype stability and a slow rate of diploidization (Ickert-Bond et al 2020, Wu et al. 2020). Furthermore, the transcriptome sequencing of two allotetraploid species *E. sinica* and *E. intermedia* and their putative diploid progenitors shows an unbiased subgenome evolution as equal number of homeologs are expressed

and similar percent show dominant expression from the two subgenomes (Wu et al. 2020).

Pinales **Sporadic polyploidy**

This order comprises 11 genera of which *Cedrus*, *Pinus*, *Cathaya*, *Picea*, *Pseudotsuga*, *Tsuga*, *Larix*, *Nothotsuga*, *Keteleeria* and *Abies* have somatic number of $2n=24$ based on $x=12$ (Ohri and Rastogi unpublished). It may be clarified here that two aberrant numbers as seen in *Pseudotsuga menziesii* ($2n=26$) and *Pseudolarix amabilis* ($2n=44$) have been actually derived by the centric fission in a pair of median chromosomes leading to the formation of 4 telocentrics in the former and in 20 median chromosomes leading to 40 telocentrics in the latter therefore they do not represent true aneuploidy/polyploidy as the total number of chromosome arms does not change (Ohri and Rastogi unpublished). In the rest of the genera there are sporadic reports of polyploid or mixoploid individuals (Table 1). All these cases lack normal growth and are therefore unsuccessful polyploids not being able to compete and survive in nature. These polyploid individuals are noticed in nurseries and in plantations where they grow under protection and have a low survival rate. Spontaneous polyploids include the triploid produced in hybrids between *Larix decidua* x *L. occidentalis* (Syrach-Larsen and Westergaard 1938), tetraploid in twin seedlings of *Abies firma* (Kanezawa 1949) and *Pinus thunbergii* (Nishimura 1960), the triploid and tetraploid of *Picea abies* (Kielander 1950), tetraploid of *Larix decidua* (Christiansen 1950), *L. kaempferi* (Chiba and Watanabe 1952) and *Pinus densiflora* (Zinnai 1952), mixoploids in *Pinus elliotii* (Mergen 1958) and *Picea abies* (Illies 1953, 1958), etc. (Table 1).

In *Picea glauca* and *P. mariana*, tetraploids were found with the frequency of 0.008 % and 0.004 % respectively which show stunted growth, longer internodes and shorter and thicker leaves (Winten 1964). Similarly in the plants regenerated from somatic embryogenesis in *Picea mariana* some dwarf plants with thicker leaves and low viability were found in low frequency with chimeral tissues having aneuploid cells (Trembley et al. 1999) (Table 1). Very exceptionally, the tetraploid of *Larix decidua* survived till maturity but had a very low fertility because of highly irregular meiosis (Christiansen 1950).

Cupressales **Sporadic polyploidy**

Triploids occur spontaneously in *Cunninghamia lanceolata* (Zonneveld 2012) *Taiwania cryptomerioides* (Hizume 1989), and *Chamaecyparis obtusa* (Sasaki 1982). Spontaneous tetraploids and triploids of *Cryptomeria japonica* have also been reported (Zinnai and Chiba 1951, Chiba 1951). In *Cryptomeria japonica* triploids have been identified cytologically among plus tree (trees with superior phenotype for growth and form) cultivars (Somego et al. 1981, Sasaki 1982). Kondo (1988) found 35 triploids (1.3 %) among 2743 plus trees by microdensitometry. The triploids in *C. japonica* survive well and are being maintained as triploid-plus tree clones (Matsuda and Miyajima 1977, Matsuda 1980, Kondo et al. 1985, Kondo 1988, Suyama et al. 1996, Kondo & Hizume 2000). The seed germination

percentage of seeds obtained from triploids is quite low being around 0.5 % and the progeny seedlings are mostly diploid besides some trisomics and rarely a tetraploid (Suyama et al. 1996, Kondo and Hizume 2000).

Polyploid genera and species ***Sequoia sempervirens***

The hexaploid ($2n=66$) genomic constitution of *S. sempervirens* (Table 3, Fig.1) and its mode of origin has always been inexplicable. The species has a close relationship with two other monotypic relict diploid ($2n=22$) species of Cupressaceae i.e. *Metasequoia glyptostroboides* and *Sequoiadendron giganteum* (Yang et al. 2012). The karyotype studies show that the chromosomes are median or submedian with gradually decreasing size, though the smallest six chromosomes are distinctly smaller, and with characteristic three pairs of satellite chromosomes (Saylor and Simon 1970, Schlarbaum and Tsuchiya 1984a, b, Hizume et al. 1988, Hizume 1989, Ahuja and Neal 2002, Toda 1996). On the basis of the karyotype features it has been conjectured that *Sequoia* is either segmental allopolyploid (A1A1A1A1AA) or autoallopolyploid (AAAABB) (Saylor and Simon 1970, Schlarbaum and Tsuchiya 1984a, b) or even a partially diploidized autohexaploid (AAAAAA) (Ahuja 2009), while not altogether discounting allohexaploidy (Toda 1996). Meiotic configurations in *Sequoia* further depict an overwhelmingly large numbers of bivalents and some multivalents including hexavalents indicating a partially diploidized autohexaploid, autoallohexaploid or a segmental hexaploid genome (Hirayoshi and Nakamura 1943, Stebbins 1948, Ahuja and Neale 2002, Hizume et al. 2014, Ahuja 2009). In any case, the complex hexaploid genome of *Sequoia* must have arisen by at least two rounds of polyploidy involving some parent genomes. However, the comparison between its karyotype features with those of its closest relatives e.g. *Metasequoia* and *Sequoiadendron*, shows distinct differences especially with respect to the satellite chromosomes (Schlarbaum and Tsuchiya 1975, 1984a, b, Schlarbaum et al. 1984, Ahuja 2005, 2009). This is further substantiated by differences in fluorescent band patterns as *Sequoiadendron* has heavy CMA bands at proximal position of a pair of chromosomes, *Metasequoia* shows bands at proximal position of three pairs of chromosomes and dots at centromeric positions in rest of the chromosomes while *Sequoia* has bands at terminal position of three pairs of chromosomes (Hizume et al. 1988). Furthermore, the inheritance pattern of allozymes in the megagametophytes show hexasomic instead of disomic segregation (Rogers 1997) as also the microsatellite markers which show a maximum of six alleles per individual for three loci studied (Duhovnikoff and Dodd 2011), therefore implicating autopolyploidy. Recently, transcriptome data followed by phylogenetic analysis of single-copy genes strongly supported *Sequoiadendron* rather than *Metasequoia* as the closest relative of *Sequoia* thereby discounting any genomic contribution from *Metasequoia* in the genome of *Sequoia*. Nevertheless, the phylogenetic relationships based on single-copy genes do not exclude hybridization within *Sequoiadendron-Sequoia* clade. Finally the evidence for autopolyploidy came from orthogroups or homeologs of *Sequoia*, where duplicate genes

showed more than expected similarity of sequences (Scott et al. 2016) which strongly suggest *Sequoia* as an undiploidized autohexaploid having its origin in early Tertiary (~65 mya) (Miller 1977). Consequently, with irregular meiosis leading to low seed viability (Olson 1990) *Sequoia* would not have survived in nature, but for its unique capacity (unlike most conifers) of vegetative multiplication by stem sprouts from lignotubers or burls which form at the base of trees (O'Hara et al. 2017).

Fitzroya cupressoides

This monotypic genus is represented by *F. cupressoides* which is endemic to the temperate forests of southwestern South America, the main distribution being in coastal and Andean Chile while some disjunct populations exist on the eastern slopes of Andes in Argentina, where it is capable of natural regeneration (Veblen et al. 1995). It is a long lived tetraploid with somatic number of $2n=44$ (Table 3, Fig.1), the complement shows only one pair of chromosomes with secondary constriction therefore indicating some diploidization, but it was not possible to explain the nature of polyploidy in the absence of meiotic data (Hair 1968). However, the tetrasomic inheritance observed in isozyme banding patterns along with the absence of fixed heterozygosity in any of the enzymes studied reject the possibility of allopolyploidy and provide strong support for autotetraploid origin of *Fitzroya* (Premoli et al. 2000).

Juniperus

This is a most diverse genus of evergreen trees or shrubs in Cupressaceae comprising 115 taxa (75 species and 40 varieties) and shows a wide distribution in Northern Hemisphere except for *J. procera* from Southern Hemisphere (Adams 2014). The species have a wide ecological amplitude being present from sea level to high altitudes in forests and deserts (Farjon 2005, Adams 2014). Studies done till now on 97 taxa show that polyploidy occurs in 22.30 % of the total taxa, out of which 11.6 % are exclusively polyploid, 10.7 % show intraspecific polyploid cytotypes and one species *J. foetidissima* is a confirmed hexaploid (Tables 2 & 3, Fig.1). Species showing intraspecific polyploid series are *J. chinensis* (2x, 3x, 4x), *J. chinensis* var. *sargentii*, *J. deppeana* var. *gamboana*, *J. pingii*, *J. polycarpus* var. *seravschianna*, *J. sabina*, *J. squamata*, *J. squamata* f. *wilsonii* (2x,4x), *J. foetidissima*, *J. phoenicea* (2x, 6x), and *J. virginiana* (2x, 3x), while exclusively polyploid species are *J. x pfitzeriana* (3x, 4x), *J. coxii*, *J. indica*, *J. morrisonicola*, *J. procumbens*, *J. przewalskii*, *J. recurva*, *J. rushforthiana*, *J. sabina* var. *balkanensis*, *J. thurifera*, *J. thurifera* subsp. *africana*, and *J. tibetica* (4x) (Tables 2 & 3, Fig.1).

The nature of polyploidy in some taxa can now be discussed in some detail. Two cytotypes 3x and 4x have been reported for *J. x pfitzeriana* based on genome size (Zonneveld 2012). The meiotic studies by Sax and Sax (1933) showed 22 bivalents and about 6 % pollen sterility which according to Khoshoo (1959) indicates allotetraploidy. Its hybrid origin has been suggested by the cumulative presence in *J. x pfitzeriana* of bornyl acetate and sabiny acetate present in the volatile leaf oil of *J. chinensis* and *J. sabina* respectively (Fournier et al. 1991). De Luc et al. (1999) further supported this parentage by using RAPD markers. However, the comparison of nrDNA (ITS) and

four chloroplast gene regions of 14 *J. xpfiteriana* cultivars with those of all *Juniperus* sect. *sabina* established *J. sabina* var. *balkanensis* and *J. chinensis* as paternal and maternal parents respectively (Adams et al. 2019).

Another exclusively tetraploid ($2n=44$) species *Juniperus thurifera* shows 2C values ranging from 39.90 to 42.65 pg in its 19 populations including three populations of *J. thurifera* subsp. *africana* (Romo et al. 2013). The authors have surmised that since all the populations studied are tetraploid the polyploidy must have originated early in the evolution of this species (Romo et al. 2013). Recently, study on the genome sizes of 111 out of 115 taxa of *Juniperus* covering 96.52 % of the total diversity has brought out extensive polyploidy in the genus. This study showed nine more exclusively tetraploid species i.e. *J. coxii*, *J. indica*, *J. morrisonicola*, *J. polycarpos* var. *seravaschiana*, *J. przewalskii*, *J. recurva*, *J. rushforthiana*, *J. sabina* var. *balkanensis* and *J. tibetica* besides a hexaploid *J. foetidissima* (Farhat et al. 2019a). Mehra (1976) reported tetraploidy ($2n=44$) in *J. indica* (= *J. wallichiana*) from eastern Nepal and its further confirmation in three other populations from Nepal indicates tetraploid nature of this species (Farhat et al. 2019a). Similarly two samples of *J. procumbens* (= *J. chinensis* var. *procumbens*) from Japan show tetraploidy (Nagano et al 2007, Farhat 2019a), interestingly this species shows exact doubling of 45S rDNA and 5S rDNA loci located at the same position of their respective chromosomes as in the diploid *J. chinensis* var. *sargentii* and *J. lutchuensis* (Nagano et al. 2007). Three samples of *J. foetidissima* from Greece, Lebanon and Turkey show 2C values ranging from 69.71 to 71.32 pg (Farhat et al. 2019a) which are roughly three-fold more than the range (19.10–29.11 pg) for diploid species and 1.5-fold of the range (39.61–50.20 pg) of the tetraploid species. Its hexaploid level has been confirmed from the somatic chromosome number of $2n=66$ which makes it second hexaploid species among conifers (Farhat et al. 2019a). The authors have discussed various pathways by which this hexaploidy might have been achieved but its genomic constitution and type of polyploidy remains a matter of conjecture.

An allotetraploid variety *J. sabina* var. *balkanensis*, showing morphological similarity with *J. sabina* var. *sabina*, has been described based on molecular data (Adams et al. 2016). This variety in fact is closely allied to *J. thurifera* as inferred from phylogenetic analysis of four cpDNA regions (petN-psbM, trnSG, trnDT, and trnLF) which resulted in 3114 bp of data, the indels within this sequence showed that while *J. sabina* var. *balkanensis* differs from *J. thurifera* by 6–8 mutations it differs from *J. sabina* by 36 mutations. Therefore, since *J. thurifera* is nested within *J. sabina* var. *balkanensis*, the cpDNA of the latter might have come from chloroplast from some ancestor of *J. thurifera* as the extant *J. thurifera* is nested within *J. sabina* var. *balkanensis* and not vice versa (Adams et al. 2016). On the other hand the phylogenetic tree based on nrDNA ITS sequences shows *J. sabina* var. *balkanensis* forming a clade with *J. sabina* var. *sabina* (Adams et al. 2016). This provides a strong support for the origin of *J. sabina* var. *balkanensis* from interspecific hybridization of *J. sabina* var. *sabina* and *J. thurifera* in the ancient past. Further, the genome size of 29 populations of both the varieties of *J. sabina* shows that *J. sabina* var. *sabina* is

diploid with 2C values ranging from 22.09 to 25.03 pg in its 13 populations while the 16 populations of *J. sabina* var. *balkanensis* studied are tetraploid with 2C values showing a range of 41.99 to 51.33 pg (Farhat 2019b). Farhat et al. (2019b) have further suggested different pathways either through triploid bridge or by the formation of unreduced gametes in *J. sabina* var. *sabina* leading to the allotetraploids with a *J. sabina*-like morphology and genome composition. Recently, in fact triploid hybrids between *J. thurifera* (4x) and *J. sabina* (2x) have also been discovered in the area of their sympatry (Farhat et al 2020a). Three such putative hybrid individuals have been confirmed based on genome size, ITS and cpDNA sequences and AFLP markers (Farhat et al 2020a). Later studies have also confirmed gene flow between sympatric populations of *J. sabina* var. *sabina* (2x) and *J. thurifera* (4x) resulting in triploid hybrids and between allopatric populations of *J. sabina* var. *balkanensis* (4x) and *J. thurifera* (4x) resulting in tetraploid hybrids (Farhat et al. 2020b). This amply shows that natural hybridization is possible both at intra and interploidal levels.

Induced polyploidy

Many attempts have been made in the past to induce polyploidy in various genera of conifers but without any tangible results from forestry point of view (Table 4). Studies done in this regard have been described in detail by Ahuja (2005). Earlier attempts in producing colchipooids in conifers resulted mainly in the production of mixoploids with irregular meiosis (Table 4). Johnson (1975) produced C_0 individuals in *Pinus sylvestris*, *P. contorta*, *Picea abies* and *Larix sibirica* and the tetraploids were maintained for 30 years till flowering. However, no triploid progeny could be produced because of abnormal pollen grains (Table 4). Recently attempts have been made to induce polyploidy in some members of Cupressaceae. In *Cryptomeria japonica* treatment of the seedlings with 150 μ M Oryzoline+0.1 % SilEnergy for 30 days resulted in 83.1 % success in the induction of tetraploidy. These plants are easily identifiable because of their thickened and broader leaves (Contreras et al. 2010). However, these plants need to be evaluated over a longer period of time at different sites for their potential as ornamentals. Later the same technique was applied to induce tetraploidy in *Platycladus orientalis*, *Thuja plicata* and *T. occidentalis* (Contreras 2012). The optimal duration of treatment differed in each species and the recovery of tetraploids ranged from 1.5 % to 18.3 % in different treatments in the three species (Contreras 2012).

Paleopolyploidy

The widespread occurrence of ancient whole genome duplications (WGD) is common in many plant and animal groups (Dehal and Boore 2005, Cui et al. 2006). Recent polyploidy in gymnosperms is very scarce and distributed non-randomly among various orders. Besides the classic cases of *Sequoia sempervirens* and *Fitzroya cupressoides* which are autopolyploids some adaptive radiation by hybridization and allopolyploidy is seen in *Ephedra* and *Juniperus*. Now the question arises whether any ancient rounds of polyploidy occurred in the past history of gymnosperms.

Table 4
Induced polyploidy

Sr. No.	Taxon	2n=	Ploidy	Reference
1.	<i>Pinus ponderosa</i> Douglas ex C.Lawson	24, 36, 48	Mixoploid	Hyun, 1953
2.	<i>Pinus attenuata</i> X <i>radiata</i>	24, 36, 48	Mixoploid	Hyun, 1953
3.	<i>Pinus jeffreyi</i> A.Murray bis	24, 48	Mixoploid	Hyun, 1953
4.	<i>Picea abies</i> (L.) H.Karst.	24-48	Mixoploids	Illies, 1951
5.	<i>Larix decidua</i> Mill.		Co crossed with untreated diploids resulted in mixoploids	Illies, 1951, 1957, 1966a, 1966b, 1969
6.	<i>Larix leptolepis</i> (Siebold & Zucc.) Gordon		Co crossed with untreated diploids resulted in mixoploids	Illies, 1951, 1957, 1966a, 1966b, 1969
7.	<i>Sequoiadendron giganteum</i> (Lindl.) J.Buchholz	48	4x	Jensen and Levan, 1941
8.	<i>Pinus sylvestris</i> L.	48	4x	Johnsson, 1975
9.	<i>Pinus contorta</i> Douglas ex Loudon	48	4x	Johnsson, 1975
10.	<i>Picea abies</i> (L.) H.Karst.	48	4x	Johnsson, 1975
11.	<i>Larix sibirica</i> Ledeb.	48	4x	Johnsson, 1975
12.	<i>Chamaecyparis obtusa</i> (Siebold & Zucc.) Endl.	48	4x	Kanezawa, 1951
13.	<i>Cryptomeria japonica</i> Thunb. ex L.f.) D.Don	48	4x	Contreras et al. 2010
14.	<i>Platycladus orientalis</i> (L.) Franco	48	4x	Contreras, 2012
15.	<i>Thuja occidentalis</i> L.	48	4x	Contreras, 2012
16.	<i>Thuja plicata</i> Donn ex D.Don	48	4x	Contreras, 2012

An equivocal indication of duplications within the complement has been provided by chromosome banding. The identification of each of the 12 chromosomes by G and Q banding in *Pinus resinosa* showed identical position of secondary constrictions and banding pattern among many non-homologous chromosomes which as direct cytological evidence, gives an indication of the presence of a duplicated complement (Drewry, 1988).

An exclusively tetraploid species *Juniperus thurifera* (2n=44) shows a strong indication of diploidization in its complement during the time elapsing from the origin of tetraploidy in the ancient past. Its tetraploid complement shows colocalization of CMA bands and 45S rDNA loci on only one pair of chromosomes clearly suggesting the loss of GC-rich chromatin and inactivation of the other pair of NORs (Valles et al. 2015). Conversely, the tetraploid eastern Asian species *J. chinensis* var. *procumbens* show four 45S rDNA loci proportionate to its ploidy level indicating a recent origin of polyploidy vis a vis *J. thurifera* (Nagano et al. 2007).

Another line of evidence comes from the formation of unreduced pollen, a mechanism widespread in angiosperms (Ohri and Zadoo 1986, Ramsey and Schemske 1998, Brownfield and Kohler 2011). In extant conifers unreduced pollen have been reported only in *Cupressus dupreziana* (Pichot and El Maataoui 2000, El Maataoui and Pichot 2001). However, there is possibility that this phenomenon was common among conifers in the geological past. The pollen size analysis of the fossil *Classopolis* pollen of the Cheirolepidiaceae, a family related to Cupressaceae or Araucariaceae, shows the evidence of WGD events (Kurschner et al. 2013). The distinct size difference in pollen size as well as the presence of aberrant tetrads, triads and diads strongly indicate increased levels of unreduced

gamete formation in Cheirolepidaceae about 200 Ma at the Triassic–Jurassic transition, corresponding to the fourth of the five major extinction events (Kurschner et al. 2013). Earlier, abnormal gymnosperm pollen has also been reported from Permian-Triassic transition corresponding to the third of the five major extinction events (Foster and Afonin 2005). A similar possibility of ancient tetraploidy is also suggested by McElwain and Steinthorsdottir (2017) in the fossil taxon *Sphenobaiera spectabilis* (Ginkgoales) based on 2C DNA amount extrapolated from guard cell length in two samples (~47.1 and 46.9 Gbp) which exceed that of extant *Ginkgo biloba* tetraploid cytotype (38.1–39.4 Gbp, Smarda et al. 2018).

Recent comparative genomic studies assisted by sequencing technology have shown that various plant groups have undergone recurrent rounds of polyploidization in the geological past. In a phylogenomic analysis involving 800 gene trees Jiao et al. (2011) showed the presence of two groups of duplications one occurring in the common ancestor of seed plants and the other in the common ancestor of angiosperms while providing no evidence for any ancient polyploidy in gymnosperms (Fig. 1). These findings were strongly refuted as the bimodal pattern of age distribution of gene duplications as observed by Jiao et al. (2011) was not supported on technical and methodological grounds (Ruprecht et al. 2017). Similarly, Zwaenepoel and Van der Peer (2019) also did not find any evidence of ancient polyploidy in Pinaceae using whole genome data of *Ginkgo biloba*, *Picea abies* and *Pinus taeda*. Earlier, Nystedt et al. (2013) also did not find any evidence of polyploidy by studying genome sequencing of *Picea abies*. However, Li et al. 2015, on the other hand showed that polyploidy indeed contributed to the evolution of conifers and other gymnosperms. Therefore, based on phylogenomic analysis of transcriptomes

from 24 gymnosperm species and three outgroups they demonstrated the incidence of two whole genome duplications in the ancestry of major clades of conifers i.e. Pinaceae and Cupressophytes and the third in *Welwitschia* (Gnetales) (Fig. 1). An equivocal evidence for ancient polyploidy in *Welwitschia mirabilis* was also shown in an earlier study (Cui et al. 2006). Since *Gnetum* and *Ephedra* show no evidence of an ancient WGD and only some recent episodes of polyploidy are seen in *Ephedra*, therefore the ancient WGD event in *Welwitschia* is supposed to have occurred after the divergence of *Gnetum* and *Ephedra* (Li et al. 2015, Wan et al. 2018). Recently, over 1000 plant (1KP) transcriptomes have been sequenced across green plants (Viridiplantae) (Leebens-Mack et al. 2019) which provide a unique opportunity to study the occurrence and distribution of ancient WGDs (Li and Barker 2019). The analysis of this dataset further provided the support for two rounds of duplications in the ancestry of Pinaceae as evidenced by two peaks of duplication consistently seen in *Pinus*, *Pseudotsuga* and *Cedrus* (Li and Barker 2019). Consequently, Li and Barker (2019) have attributed the lack of evidence for any Pinaceae WGD as deduced by Zwaenepoel and Van der Peer (2019), to the quality of gene assembly and annotation, and limited sampling of conifer species.

Gorelick and Olson (2011) attributed the relatively restricted diversity in cycads to the lack of polyploidy. Cycad chromosome numbers are conservative except for some variation occurring by chromosomal fission/fusion as in *Zamia* (Rastogi and Ohri 2020a). However, Roodt et al. (2017) using transcriptome assembly and paralog age distributions have shown that *Encephalartos natalensis* and *Ginkgo biloba* indeed share an ancient WGD which predates their divergence about 300 million years ago (Fig. 1). In another study of *Ginkgo* draft genome two different peaks have been demonstrated in the *Ks* distribution of paralogs (Guan et al. 2016). One of these occurred between 515 and 735 mya and the other between 74 and 177 mya, while the former peaks agrees with the earlier reports (Cui et al. 2006, Jiao et al. 2011, Li et al. 2015) the latter peak which occurred much later than the divergence of *Ginkgo* and conifers suggests an independent WGD event occurring after the origin of *Ginkgo* by at least 170 mya (Zhou 2009). Nevertheless the age of especially the older duplication event occurring between 515 and 735 mya thus predating the origin of land plants has been questioned by Roodt et al. (2017) as the one suggested by them occurred 300 mya just predating the divergence of cycads and *Ginkgo*. This is also substantiated by the fact that an older duplication would have also been shared by the Gnetales which show no evidence of any WGD (Wan et al. 2018) except *Welwitschia* which has undergone a WGD after the divergence of its lineage from the one leading to *Ephedra* (Li et al. 2015). However, there is all the possibility that the absence of evidence of any WGD in Gnetophytes might be the result of their faster rates of gene evolution than the rest of gymnosperms (Hajibabaei et al. 2006, De La Torre et al. 2017) thus erasing all the traces of more than 300 mya old WGD (Wan et al. 2018).

Conclusions

The above account shows that the recent cases of polyploidy are not only rare (being present in only 9.80 % of the taxa studied) but are distributed in a non-random manner among different orders of gymnosperms i.e. Ephedrales and Cupressales. Remarkably, besides the classic examples of *Sequoia sempervirens* and *Fitzroya cupressoides*, a very high incidence of polyploidy has been reported in *Ephedra* (76.0 %) and *Juniperus* (22.3 %) as also the recent discovery of spontaneous production and sustenance of various polyploid forms under cultivation in *Ginkgo* (Smarda et al. 2018). In sharp contrast to this angiosperm hardwoods not only show high basic numbers resulting from paleopolyploidy but also have well-developed polyploid series and complex dysploid number variation across various families and genera (Ohri 2015). Dysploidy is also observed in some gymnosperm taxa e.g. *Zamia* (2n=16-28), *Pseudotsuga menziesii* (2n=26), *Pseudolarix* (2n=44) and *Podocarpus* (2n=20-38) primarily caused by centric fusion or fission which changes the chromosome number while maintaining the arm number (Rastogi and Ohri 2020a, Ohri and Rastogi unpublished). A number of hypotheses have been put forth for the rarity of polyploidy in gymnosperms (Khoshoo 1959, Ahuja 2005). However, the recent studies depict a high propensity for interspecific hybridization followed by allopolyploidy in both *Ephedra* and *Juniperus* (Wu et al. 2016, 2020, Farhat et al. 2020a, b). It has therefore been suggested that a combination of high frequency of sympatry between the species leading to gene flow, production of unreduced gametes and capacity for vegetative reproduction has been responsible for the prevalence of polyploidy in these two genera (Wesche et al. 2005, Farhat et al. 2020a, b, Wu et al. 2016). Nevertheless, both *Ephedra* and *Juniperus* species show highly conserved karyotypes as the genome size increase in both the genera is primarily due to polyploidy as depicted by max./min. ratio of 2C (4.73) and 1Cx (1.37) in 49 diploid and polyploid species of *Ephedra* and 2C (3.36) and 1Cx (1.36) in 67 diploid and polyploid species of *Juniperus* (Ohri unpublished). The allopolyploids in both *Ephedra* and *Juniperus* have genome sizes equal to the sum of their respective parents and therefore show limited genome downsizing and slow diploidization (Farhat et al. 2019b, Ickert-Bond et al. 2020). Moreover, allotetraploid species of *Ephedra* show unbiased subgenome evolution (Wu et al. 2020). It therefore follows that if structural changes are rare in both the genera then how the meiotic fidelity leading to disomic inheritance is constituted immediately after the formation of a polyploid (Comai 2005, Madlung 2013). It may be surmised here that some inherent molecular mechanism similar to the Ph1 cyclin-dependent kinase (CDK)-like genes in wheat is controlling strict homologous pairing and therefore perpetuating these allopolyploids in nature (see Yousafzai et al. 2010, Mercier et al. 2015). Natural hybridization has also been observed in pine species because of weak interspecific crossability barriers (Critchfield 1975, 1986, Willyard et al. 2009; Menon et al. 2018, 2020, Buck et al. 2020). Furthermore, while homoploid hybrid speciation has been reported e.g. *Pinus densata* (Wang et al. 2011), *P. funabris*

and *P. takahasii* (Ren et al. 2012), allopolyploidy is completely lacking in pines. There are till now no reports of unreduced gamete formation and vegetative reproduction in pines, two critical pre-requisites for the formation and survival of initial polyploids. Besides, in addition to the unreduced gametes polyspermy has also been shown to result in triploid progeny in wheat, maize and orchids and also in experimentally produced triploid rice from polyspermic zygotes (see Toda and Okamoto 2016). However, while polyspermy might be one of the pathways for the production of triploids in angiosperms, selective karyogamy as a polyspermy barrier has been observed in *Pinus nigra* and *Picea glauca* where only one sperm migrates towards egg and fuses with it to produce a diploid zygote (Williams 2009). Furthermore, endopolyploidy which is prevalent in angiosperms is nearly absent or very rare in gymnosperms and woody angiosperms (Barow and Meister, 2003, Leitch, and Dodsworth 2017). Interestingly it is observed in Cupressaceae and *Ginkgo biloba* (Pichot and El Maataoui, 1997; El Maataoui and Pichot, 1999, Avanzi and Cionini, 1971) which also show amenability to polyploidy. There is, however no data on endopolyploidy available for *Ephedra*. Therefore it would be interesting to study correlation between polyploidy and endopolyploidy in this genus not only with high incidence of polyploidy (76.36 %) but also with intraspecific polyploid cytotypes. Lastly, the lack of polyploidy especially in Pinaceae may just be the result of nucleotypic effects caused by abrupt doubling of genome size of already massive genomes which might particularly adversely disturb the optimal ratio of tracheid lumen radius to cell wall thickness (Wakamiya et al. 1996). To sum up, the reasons for the rarity of polyploidy in gymnosperms can be many-fold yet there are examples of autopolyploidy in monotypic genera like *Sequoia* and *Fitzroya* and natural hybridization followed by allopolyploidy in *Juniperus* and *Ephedra*. In Pinaceae though the literature is replete with examples of natural interspecific hybridization and homoploid hybrid speciation yet allopolyploidy is missing because of the lack of certain factors required for the establishment and survival of initial polyploids. Some other groups like Podocarpaceae and *Zamia* among cycads have followed dysploidy through centric fission for adaptive radiation and speciation.

Induction of polyploidy for genetic improvement has been tried on conifers without any tangible results (Table 4). In this regard the success in the induction of polyploidy in some members of Cupressaceae (Contreras et al. 2010, Contreras 2012) provide some hope for the future. Here it needs to be mentioned that in *Cryptomeria japonica* spontaneous triploids survive as plus tree clones. Furthermore the spontaneous origin and viability of different polyploidy types of *Ginkgo* in cultivation opens up the possibilities of using this diverse germplasm for producing ornamental forms for horticulture trade (Smarda et al. 2018).

Now the question is that if the recent cases of polyploidy are rare in large majority of the gymnosperms, is there any evidence of ancient polyploidy which has been masked by extensive sequence divergence leading to diploidization. Preliminary evidence for this has been provided by the similarity of chromosome banding in non-homologous chromosomes in

Pinus, diploidization of 45S rDNA loci in a tetraploid *Juniperus* species and the finding of unreduced fossil *Classopolis* pollen and guard cells in *Sphenobaiera spectabilis*. However, recently comparative genomic studies assisted by sequencing technology have shown the possibility of at least one round of ancient duplication in the ancestry of gymnosperms. Clearly, further studies are required to unravel the occurrence and role of any ancient duplications in the evolution of different groups of gymnosperms.

Acknowledgements

This article is dedicated to my respected teacher Late Dr T.N. Khoshoo who introduced me to this subject. The author is also thankful to the anonymous reviewers for their suggestions.

References

- Abraham A, Mathew PM (1966) Cytology of *Encephalartos hildebrandtii* A. Br. & Bouche. Annals of Botany 30: 239–241.
<https://doi.org/10.1093/oxfordjournals.aob.a084071>
- Adams R (2014) Junipers of the World: The Genus *Juniperus*. Bloomington, IN: Trafford Publishing.
- Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Current Opinion in Plant Biology 8: 135–141.
<https://doi.org/10.1016/j.pbi.2005.01.001>
- Adams RP, Schwarzbach AE, Tashev AN (2016) Chloroplast capture by a new variety, *Juniperus sabina* var. *balkanensis* RP Adams and AN Tashev, from the Balkan Peninsula: A putative stabilized relictual hybrid between *J. sabina* and ancestral *J. thurifera*. Phytologia 98: 100–111.
- Adams RP, Johnson ST, Anderson J, Rushforth K, Farhat P, Valentin N, Siljak-Yakovlev S (2019) The origin of *Juniperus xpfitzeriana*, an allotetraploid hybrid of *J. chinensis* X *J. sabina*. Phytologia 101: 164–174.
- Ahuja MR (2005) Polyploidy in gymnosperms revisited. Silvae Genetica 54: 59–69. <https://doi.org/10.1515/sg-2005-0010>
- Ahuja MR (2009) Genetic constitution and diversity in four narrow endemic redwoods from the family Cupressaceae. Euphytica 165: 5–19.
<https://doi.org/10.1007/s10681-008-9813-3>
- Ahuja MR, Neale DB (2002) Origins of polyploidy in coast redwood (*Sequoia sempervirens*) (D. Don) Endl. and relationship of coast redwood to other genera of Taxodiaceae. Silvae Genetica 51: 93–100.
- Amborella Genome Project (2013) The *Amborella* genome and the evolution of flowering plants. Science 342: 1241089.
- Avanzi S, Cionini PJ (1971) A DNA cytometric investigation on the development of the female gametophyte of *Ginkgo biloba*. Caryologia 24: 105–116.
<https://doi.org/10.1080/00087114.1971.10796418>
- Barow M, Meister A (2003) Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. Plant, Cell and Environment 26: 571–584. <https://doi.org/10.1046/j.1365-3040.2003.00988.x>
- Beug HJ (1956) Pollendimorphismus bei *Ephedra*. Naturwissenschaften 43: 332–333. <https://doi.org/10.1007/bf00629402>
- Bianco P, Medagli P, D'Emmerico S (1988) Numeri cromosomici per la flora italiana: 1136–1138. Inform. Bot. Ital., 19: 319–321.

- Blanc G, Wolfe KH (2004) Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *Plant Cell* 16:1667–1678. <https://doi.org/10.1105/tpc.021345>
- Bowe LM, Coat G, de Pamphilis CW (2000) Phylogeny of seed plants based on all three genomic compartments: Extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. *Proc Natl Acad Sciences (USA)*. 97: 4092–4097. <https://doi.org/10.1073/pnas.97.8.4092>
- Bowers JE, Chapman BA, Rong JK, Paterson AH (2003) Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* 422:433–438. <https://doi.org/10.1038/nature01521>
- Bretagnolle F, Thompson JD (1995) Tansley Review No. 78. Gametes with the stomatic chromosome number: Mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol*. 129: 1–22. <https://doi.org/10.1111/j.1469-8137.1995.tb03005.x>
- Brownfield L, Kohler C (2011) Unreduced gamete formation in plants: mechanism and prospects. *Journal of Experimental Botany* 62: 1659–1668. <https://doi.org/10.1093/jxb/erq371>
- Buck R, Hyasat S, Hossfeld A, Flores-Renteria L (2020) Patterns of hybridization and cryptic introgression among one- and four-needled pinyon pines. *Annals of Botany* 126: 401–411. <https://doi.org/10.1093/aob/mcaa045>
- Carta A, Bedini G, Peruzzi L (2020) A deep dive into the ancestral chromosome number and genome size of flowering plants. *New Phytologist* 228:1097–1106. <https://doi.org/10.1111/nph.16668>
- Chaturvedi M (1978) Pollen grains in *Ephedra helvetica* C.A.Mey. *Current Science* 47: 66.
- Chaw SM, Parkinson CL, Cheng Y, Vincent TM, Palmer JD (2000) Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proceedings of the National Academy of Sciences (USA)*. 97: 4086–4091. <https://doi.org/10.1073/pnas.97.8.4086>
- Chiba S (1951) Triploids and tetraploids of sugi (*Cryptomeria japonica* D.Don.) selected in forest nursery. *Bull. Govt. For. Expt. Sta. No. 49*, 99–108.
- Chiba S, Watanabe M (1952) Tetraploids of *Larix kaempferi* in the Nurseries. *Bull Gov For Exp Sta. No. 57*, Tokyo, 187–199.
- Choudhry AS (1984) Karyomorphological and cytological studies in *Ephedra*. *J. Sci. Hiroshima Univ., Ser. B*, 19: 57–109.
- Choudhry AS, Tanaka R (1981) Diploid form of *Ephedra intermedia* var. *tibetica*. *Chromosome Inf. Serv.* 31:3–4.
- Christiansen H (1950) A tetraploid of *Larix decidua* Miller. *Det.Kgl. Danske Videnk. Selsk.* 18: 1–9.
- Christenhusz MJM, Reveal JL, Farjon A, Gardner MF, Mill RR, Chase MW (2011) A new classification and linear sequence of extant gymnosperms. *Phytotaxa* 19: 55–70. <https://doi.org/10.11646/phytotaxa.19.1.3>
- Critchfield WB (1975) Interspecific hybridization in *Pinus*: a summary review. In: Fowler DP, Yeatman CY eds. *Symposium on Interspecific and Interprovenance Hybridization in Forest Trees*. *Proceedings of the 14th Meeting of the Canadian Tree Improvement Association, Part II*, 99–105.
- Critchfield WB (1986) Hybridization and classification of the white pines (*Pinus* section *Strobus*). *Taxon* 35: 647–656. <https://doi.org/10.2307/1221606>
- Colombo P, Marceno C (1990) Número cromosomáticos de plantas occidentales. 539–550. *Anales Jard. Bot. Madrid* 47: 167–174.
- Comai L (2005) The advantages and disadvantages of being a polyploid. *Nature Review Genetics*. <https://doi.org/10.1038/nrg1711>
- Contreras RN (2012) A simple chromosome doubling technique is effective for three species of Cupressaceae. *HortScience* 47: 712–714. <https://doi.org/10.21273/hortsci.47.6.712>
- Contreras RN, Ruter JM, Schwartz BM (2010) Oryzalin induced tetraploidy in *Cryptomeria japonica* (Cupressaceae). *HortScience* 45: 316–319. <https://doi.org/10.21273/hortsci.45.2.316>
- Cui L, Wall PK, Leebens-Mack JH et al. (2006) Widespread genome duplications throughout the history of flowering plants. *Genome Research* 16: 738–749. <https://doi.org/10.1101/gr.4825606>
- Cutler HC (1939) Monograph of the North American species of the genus *Ephedra*. *Annals of Missouri Botanic Garden* 26: 373–428. <https://doi.org/10.2307/2394299>
- De La Torre AR, Li Z, Van de Peer Y, Ingvarsson PK (2017) Contrasting rates of molecular evolution and patterns of selection among gymnosperms and flowering plants. *Mol. Biol. Evol.* 34:1363–1377. <https://doi.org/10.1093/molbev/msx069>
- De Luc A, Adams RA, Zhong M (1999) Using random amplification of polymorphic DNA for a taxonomic reevaluation of Pfitzer *Juniperus*. *Hort-Science* 34:1123–1125. <https://doi.org/10.21273/hortsci.34.6.1123>
- Dehal P, Boore JL (2005) Two rounds of whole genome duplication in the ancestral vertebrate. *Plos Biology* 3: e314. <https://doi.org/10.1371/journal.pbio.0030314>
- Delevoryas T (1980) Polyploidy in gymnosperms. In: *Polyploidy-Biological Relevance*. Lewis W.H. (Ed.). Plenum Press, New York, pp. 215–218. https://doi.org/10.1007/978-1-4613-3069-1_12
- Douhovnikoff V, Dodd RS (2011) Lineage Divergence in Coast Redwood (*Sequoia sempervirens*), detected by a New Set of Nuclear Microsatellite Loci. *Am. Midl. Nat.* 165:22–37. <https://doi.org/10.1674/0003-0031-165.1.22>
- Drewry A (1988) G banded karyotype in *Pinus resinosa* Ait. *Silvae Genetica* 37: 218–221.
- El Maataoui M, Pichot C (1999) Nuclear and cell fusion cause polyploidy in the megagametophyte of common cypress, *Cupressus sempervirens* L. *Planta* 208: 345–351. <https://doi.org/10.1007/s004250050568>
- El Maataoui M, Pichot C (2001) Microsporogenesis in endangered species *Cupressus dupreziana* A. Camus: evidence for meiotic defects yielding unreduced and abortive pollen. *Planta* 213: 543–549. <https://doi.org/10.1007/s004250100531>
- Evans GE, Rasmussen HP (1971) Chromosome counts in three cultivars of *Juniperus* L. *Botanical Gazette* 132:259–262. <https://doi.org/10.1086/336589>
- Fagerlind F (1941) Bau und Entwicklung der *Gnetum*-Gametophyten. *Kongl. Svenska Vetensk. Akad. Handl.* 19, 1–55.
- Farhat P, Hidalgo O, Robert T, Siljak-Yakovlev S, Leitch I, Adams RP, Daghar Kharat MB (2019a) Polyploidy in the genus *Juniperus*: and unexpectedly high rate. *Frontiers in Plant Science* 10: Article 676. <https://doi.org/10.3389/fpls.2019.00676>
- Farhat P, Siljak-Yakovlev S, Adams RP, Daghar Kharat MB, Robert T (2019b) Genome size variation and polyploidy in the geographical range of *Juniperus sabina* L. (Cupressaceae). *Botany Letters*. <https://doi.org/10.1080/23818107.2019.1613262>
- Farhat P, Takvorian N, Avramidou M, Garraud L, Adams RP, Siljak-Yakovlev S, Daghar Kharat MB, Robert T (2020a) First evidence for allotriploid hybrids between *Juniperus thurifera* and *J. sabina* in a sympatric area in the French Alps. *Annals of Forest Science* 77: 93. <https://doi.org/10.1007/s13595-020-00969-7>
- Farhat P, Siljak-Yakovlev S, Valentin N, Fabregat C, Lopez-Udias S, Salazar-Mendiáz C, Altarejos J, Adams RP (2020b) Gene flow between diploid and tetraploid junipers—two contrasting evolutionary pathways in two *Juniperus* populations. *BMC Evolutionary Biology* 20: 148. <https://doi.org/10.1186/s12862-020-01688-3>
- Farjon A (2005) A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew, Kew.
- Fawcett JA, Maere S, VandePeer Y (2009) Plants with double genomes might have had a better chance to survive the Cretaceous Tertiary extinction event. *Proceedings National Academy of Sciences USA* 106: 5737–5742. <https://doi.org/10.1073/pnas.090906106>
- Fernandes A (1936) Sur la caryologie de *Welwitschia mirabilis* Hook. *Biol.Soc. Broteriana* 11: 267–282.
- Florin R (1932) Die Chromosomenzahlen bei *Welwitschia* und einigen *Ephedra*. *Arten.Svensk Bot.Tidsk* 26: 205–214.
- Foster CB, Afonin SA (2005) Abnormal pollen grains: an outcome of deteriorating atmospheric conditions around the Permian–Triassic boundary. *Journal of the Geological Society* 162, 653–659. <https://doi.org/10.1144/0016-764904-047>
- Fournier G, Pages N, Fournier C, Callan G (1991) Comparisons of volatile leaf essential oils of various *Juniper pfitzeriana*. *Pharmacie Acta Helvetica* 66: 74–75.
- Fozdar BS, Libby WJ (1968) Chromosomes of *Sequoia sempervirens*; 8-hydroxy-quinoline-castor oil pretreatment for improving preparation. *Stain Technol.* 43: 97–100. <https://doi.org/10.3109/10520296809115050>
- Goldblatt P (1984) Index to Plant Chromosome Numbers, 1979–1981. *Monogr. Syst. Bot. Missouri Bot. Gard.* 8: 1–427.
- Gorelick R, Olson K (2011) Is lack of cycad (Cycadales) diversity a result of a lack of polyploidy? *Botanical J. Linn.Soc.* 165:156–167. <https://doi.org/10.1111/j.1095-8339.2010.01103.x>

- Guan R, Zhao Y, Zhang H, Fan G, Liu X et al. (2016) Draft genome of the living fossil *Ginkgo biloba*. *GigaScience* 5:49. <https://doi.org/10.1186/s13742-016-0154-1>
- Gurzenkov NN (1973) Studies of chromosome numbers in plants from the south of the Soviet far east. *Komarov Lectures* 20: 47-61 (in Russian).
- Hair JB (1968) The chromosomes of the Cupressaceae. 1. Tetraclineae and Actinostrobae (Callitroideae). *New Zealand Journal of Botany* 6: 272-284. <https://doi.org/10.1080/0028825x.1968.10428813>
- Hall M, Mukherjee A, Webster R (1973) Chromosome counts in cultivated Junipers. *Journal of Arnold Arboretum* 54: 369-376.
- Hall M, Mukherjee A, Crowley WR (1979) Chromosome numbers of cultivated junipers. *Botanical Gazette* 140: 364-370. <https://doi.org/10.1086/337098>
- Hajibabaei M, Xia J, Drouin G (2006) Seed plant phylogeny: gnetophytes are derived conifers and a sister group to Pinaceae. *Molecular Phylogenetics and Evolution* 40: 208-217. <https://doi.org/10.1016/j.ympev.2006.03.006>
- Hirayoshi I, Nakamura Y (1943) Chromosome number in *Sequoia sempervirens*. *Plant & Animal* 1: 73-75 (in Japanese).
- Hizume M (1989) Karyomorphological studies in twelve species in the Taxodiaceae with special reference to cytotaxonomical position of *Sciadopitys verticillata*. *Mem. Fac. Educ. Ehime Univ., Ser. 3, Nat. Sci.* 9: 7-32.
- Hizume M (1997) Chromosomes of *Ginkgo biloba* In: *Ginkgo biloba* a global treasure from biology to medicine, Hori T, Ridge RW, Tulecke PD, Tredici J, Tremouillaux-Guiller J, Tobe H eds., Springer Verlag Tokyo pp 109-119.
- Hizume M, Fujiwara M (2016) Fluorescent chromosome banding patterns of several species in the Cupressaceae *sensu stricto*. *Chromosome Botany* 11: 1-8. <https://doi.org/10.3199/iscb.11.1>
- Hizume M, Tominaga K (2016) Fluorescent band pattern of chromosomes in *Ephedra americana* var. *andina*, Ephedraceae. *Chromosome Botany* 11: 27-30. <https://doi.org/10.3199/iscb.11.27>
- Hizume M, Abe KK, Tanaka A (1988) Fluorescent chromosome bandings in the Taxodiaceae. *La Kromosomo* 11-50: 1609-1619.
- Hizume M, Kaneko K, Miyake T (2014) A method for the preparation of meiotic chromosomes of conifers and its applications. *Chromosome Botany* 9: 83-88. <https://doi.org/10.3199/iscb.9.83>
- Hizume M, Gu Z, Yue Z, Kondo K (1993) Karyomorphological studies on *Gnetum montana* collected in Yunnan China *Chromosome Information Service* 54:23-25.
- Hizume M, Kondo T, Shibata F, Ishizuka R (2001) Flow cytometric determination of genome size in the Taxodiaceae, Cupressaceae *sensu stricto* and Sciadopityaceae. *Cytologia* 66: 307-311. <https://doi.org/10.1508/cytologia.66.307>
- Hunziker JH (1953) Numero de cromosomas de varias especies sudamericanas de *Ephedra*. *Rev. Argent. Agronom* 20: 141-143.
- Hunziker JH (1955) Morfológicromosómica de nuevespecies argentinasdel-genero "Ephedra". *Rev. Invest. Agr.* 9: 201-209.
- Hyun SK (1953) Induction of polyploidy in pines by means of colchicine treatment. *Z. Forstgenetik u. Forstpflanzenzüchtung* 3: 25-33.
- Ickert Bond SM, Rydin C, Renner SS (2003) Pollen dimorphism in *Ephedra* L. (Ephedraceae). *Review of Palaeobotany and Palynology* 124: 325-334. [https://doi.org/10.1016/s0034-6667\(03\)00002-2](https://doi.org/10.1016/s0034-6667(03)00002-2)
- Ickert-Bond SM, Sousa A, Ya M, Pellicer J, Leitch I (2014) The evolution of genome size in gymnosperm genus *Ephedra*: Flow cytometry and new chromosome counts support high levels of polyploidy. *Botany New Frontiers in Botany, The Boise Centre-Boise Idaho* July 26-30, 2014.
- Ickert-Bond SM, Sousa A, Ya M, Loera I, Metzgar J, Pellicer J, Hidalgo O, Leitch I (2020) Polyploidy in gymnosperms- Insights into the genomic and evolutionary consequences of polyploidy in *Ephedra*. *Molecular Phylogenetics and Evolution* 147: 106786. <https://doi.org/10.1016/j.ympev.2020.106786>
- Illies ZM (1951) Colchicineversuche an *Larix decidua* Miller und *Picea abies* Karst. *Z. Forstgenetik u. Forstpflanzenzüchtung* 1: 36-39.
- Illies ZM (1953) Keimlingsabnormalitäten bei *Picea abies* (L.) Karst. *Z. Forstgenetik u. Forstpflanzenzüchtung* 2: 28-32.
- Illies ZM (1957) Zytologische Beobachtungenaneiner 7 jährigen Co-Generation von Lärche. *Silvae Genetica* 6: 151-152.
- Illies ZM (1958) Polysomatie in Meristem von Einzelbaumsarten bei *Picea abies*. *Silvae Genetica* 7: 94-97
- Illies ZM (1966a) Die Variation unbalanzierter Chromosomenzahlen im Knospenmeristem fünf aufeinanderfolgender Astjahrgänge bei aneuploiden C1-Lärchen. *Silvae Genetica* 15: 133-135.
- Illies ZM (1966b) The development of an euploidy in somatic cells of experimentally produced triploid larches *Heredity* 21: 379-385. <https://doi.org/10.1038/hdy.1966.39>
- Illies ZM (1969) Two aneuploid generations of larch hybrids deriving from colchicines induced *Larix* sp. *Proc. Second World Consultation on Forest Tree Breeding*, 5p.
- International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* 463: 763-768. <https://doi.org/10.1038/nature08747>
- Jensen H, Levan A (1941) Colchicine induced tetraploidy in *Sequoiadendron giganteum*. *Hereditas* 27: 220-224. <https://doi.org/10.1111/j.1601-5223.1941.tb03257.x>
- Jiao Y, Li J, Tang H, Paterson AH (2014) Integrated syntenic and phylogenomic analyses reveal an ancient Genome Duplication in Monocots. *The Plant Cell* 26: 2792-2802. <https://doi.org/10.1105/tpc.114.127597>
- Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE et al. (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature* 473: 97-100. <https://doi.org/10.1038/nature09916>
- Johnsson H (1975) Observations on induced polyploidy in some conifers (*Pinus silvestris*, *P. contorta*, *Picea abies*, and *Larix siberica*. *Silvae Genetica* 24: 62-68
- Kanezawa R (1949) Polyploids appeared in twin seedling of forest trees. *Journal of Japanese Forest Society* 31: 22-24 (in Japanese).
- Kanezawa R (1951) Induced tetraploidy in Japanese cypress (*Chamaecyparis obtusa* Endl.). *Bul. of the Tokyo Univ. For.* 39:21-30.
- Kawatani T, Fujita S, Ohno T, Kuboki N, Hoshizaki K (1959) On the alkaloidal content of ephedras cultivated at Kasukabe. *J. Pharmaceutical Soc. Jap.* 79: 392-393. https://doi.org/10.1248/yakushi1947.79.3_392
- Khoshoo TN (1959) Polyploidy in gymnosperms. *Evolution* 13: 24-39. <https://doi.org/10.1111/j.1558-5646.1959.tb02991.x>
- Khoshoo TN (1979) Cytogenetics in relation to plant evolution and improvement. In: Khoshoo TN, Nair PKK, eds. *Progress in Plant Research*. Vol. 2. Silver Jubilee Publication, National Botanical Research Institute; 1979. pp. 1-74.
- Kiellander CL (1950) Polyploidy in *Picea abies*. *Hereditas* 36: 513-516.
- Kitani Y, Zhu S, Batkhuu J, Sanchir C, Komatsu K (2011) Genetic diversity of *Ephedra* plants in Mongolia inferred from internal transcribed spacer sequence of nuclear ribosomal DNA. *Biol. Pharm. Bull.* 34:717-726. <https://doi.org/10.1248/bpb.34.717>
- Kondo T (1988) Variation of karyotype in plus tree clones of Japanese cedar, *Cryptomeria japonica*. *Acta Radiobotanica et Genetica* 7: 1-48
- Kondo T, Hizume M (2000) Chromosomal variation and its application for genome study in Japanese cedar, *Cryptomeria japonica*. In: *Cytogenetic studies and outlook on the future*, eds: Guttenberger H, Borzan Z, Schlarbaum S E, Hartman P V. Arbora Publishers Zvole, Slovakia, pp. 89-93.
- Kondo T, Hizume M, Kubota R (1985) Variation of fluorescent chromosome bands of *Cryptomeria japonica*. *J. Jap. Forest. Soc.* 67: 184-189.
- Kong H, Chen Q, Ma J (2001) A study on karyotypes of two species in *Ephedra*. *J. Lanzhou Univ., Nat. Sci.* 37: 100-103.
- Kozhevnikova ZV, Kozhevnikov AE (2012) IAPT/IOPB Chromosome Data 13. *Taxon* 61: 895-897. <https://doi.org/10.1002/tax.614023>
- Krapovikas AMF De (1954) Complemento cromosómico de especies austro americanas de *Ephedra*. *Rev. Argent. Agron.* 21: 43-45.
- Kurschner WM, Batenburg SJ, Mander L (2013) Aberrant *Classopolis* pollen reveals evidence for unreduced (2n) pollen during the Triassic-Jurassic transition. *Proceedings Royal Society B* 280: 20131708. <https://doi.org/10.1098/rspb.2013.1708>
- Land WGJ (1913) Vegetative reproduction in an *Ephedra*. *Botanical Gazette* 55: 439-445. <https://doi.org/10.1086/331086>
- Leebens-Mack JH, Barker MS, Carpenter EJ, Deyholos MK, Gitzendanner MA et al. (2019) One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* 574: 679-685. <https://doi.org/10.1038/s41586-019-1693-2>
- Leitch AR, Leitch IJ (2012) Ecological and genetic factors linked to contrasting genome dynamics in seed plants. *New Phytologist* 194: 629-646. <https://doi.org/10.1111/j.1469-8137.2012.04105.x>
- Leitch IJ, Hanson L, Winfield M, Parker J, Bennett MD (2001) Nuclear DNA C-values complete familial representation in gymnosperms *Annals of Botany* 88: 843-849. <https://doi.org/10.1006/anbo.2001.1521>
- Leitch IJ, Dodsworth S (2017) Endopolyploidy in Plants. In: eLS. John Wiley & Sons, Ltd: Chichester. <https://doi.org/10.1002/9780470015902.a0020097.pub2>

- Li LC, Fu YX (1996) Studies on the karyotypes and the cytogeography of *Cupressus* (Cupressaceae). Acta Phytotax. Sin. 34: 117–123.
- Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS (2015) Early genome duplications in conifers and other seed plants. Science Advances 1: e1501084. <https://doi.org/10.1126/sciadv.1501084>
- Li Z, Barker MS (2019) Inferring putative ancient whole genome duplications in the 1000 plants (1KP) initiative: access to gene family phylogenies and age distributions. Giga Science:9:1–11. <https://doi.org/10.1093/gigascience/giaa004>
- Liu H, Cao F, Yin T, Chen Y (2017) A highly dense genetic map for *Ginkgo biloba* constructed using sequence based markers. Frontiers in Plant Science. <https://doi.org/10.3389/fpls.2017.01041>
- Madlung A (2013) Polyploidy and its effect on evolutionary success: old question revisited with new tools. Heredity 110: 99–104. <https://doi.org/10.1038/hdy.2012.79>
- Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421–424. <https://doi.org/10.1126/science.264.5157.421>
- Mathew PM, Mathew J, Christopher C, Haridas P (2014a) Karyomorphological studies in conifers. Journal of Cytology & Genetics 15 (NS): 107–121
- Mathew PM, Mathew J, Christopher C, Haridas P (2014b) Cytological studies with reference to karyomorphology of *Gnetum* and *Ephedra*. Journal of Cytology & Genetics 15 (NS): 123–134
- Matsuda K (1980) Triploids and meiosis in *Cryptomeria japonica*. Forest Tree Breeding 116: 23–24.
- Matsuda K, Miyajima H (1977) On the triploid of *Cryptomeria japonica* D. Don. Journal of Japanese Forest Society 59: 148–150.
- McElwain JC, Steinthorsdottir M (2017) Paleoeccology ploid paleoatmospheric composition and developmental biology: A review of the multiple uses of fossil stomata. Plant Physiology 174: 650–664. <https://doi.org/10.1104/pp.17.00204>
- Mehra PN (1946a) A study of the karyotype and the occurrence of diploid male gametophyte in some species of the genus *Ephedra*. Proceedings of National Academy of Sciences India 16: 259–291
- Mehra PN (1946b) Method of formation of diploid pollen and development of male gametophyte in *Ephedra saxatilis* Royle. Jour. Indian Bot. Soc. (Iyengar Comm. Vol.) pp. 121–132.
- Mehra PN (1976) Conifers of the Himalayas with particular reference to the *Abies* and *Juniperus* complexes. Nucleus 19:123–129.
- Mehra PN (1988) Indian Conifers, Gnetophytes and Phylogeny of Gymnosperms. Panjab University, Chandigarh
- Mehra PN, Khoshoo TN (1956a) Cytology of conifers I. Journal of Genetics 54:165–180. <https://doi.org/10.1007/bf02981708>
- Mehra PN, Rai KS (1957) Cytology of *Gnetum ula*. Journal of Genetics 55:394–396. <https://doi.org/10.1007/bf02981652>
- Menon M, Bagley JC, Friedline CJ, et al. (2018) The role of hybridization during ecological divergence of southwestern white pine (*Pinus strobus*) and limber pine (*P. flexilis*). Molecular Ecology 27:1245–1260. <https://doi.org/10.1111/mec.14505>
- Menon M, Landguth E, Leal-Saenz A, et al. (2020) Tracing the footprints of a moving hybrid zone under a demographic history of speciation with gene flow. Evolutionary Applications 13: 195–209. <https://doi.org/10.1111/eva.12795>
- Mercier R, Mezard C, Jenczewski E, Macaisne N, Grelon M (2015) The molecular biology of meiosis in plants. Annual Review of Plant Biology 66:297–327. <https://doi.org/10.1146/annurev-arplant-050213-035923>
- Mergen F (1958) Natural polyploidy in slash pine. Forest Science 4: 283–295.
- Miller CN (1977) Mesozoic conifers. Botanical Review 43: 217–280. <https://doi.org/10.1007/bf02860718>
- Mukherjee A, Hall M T (1979) In IOPB chromosome number reports LXIII. Taxon 28:269–270.
- Muratova EN (1995) Chromosome numbers in some species of the Pinaceae family. Bot. Zhurn. (Moscow & Leningrad) 80: 115.
- Muratova NE (1997) Studies on nucleolar chromosomes in representatives of Pinaceae Lindl. In: Cytogenetic studies of forest trees and shrub species ed. Borzan Z & Schlarbaum SE, Proceedings of the first IUFRO cytogenetics Working Party S2.04-08 Symposium Sept. 8–11 1993 pp 45–72.
- Muratova EN, Vladimirova OS, Sedelnikova TS (2001) Chromosome numbers of some gymnosperms. Bot. Zhurn. (Moscow & Leningrad) 86: 143–144.
- Murin A, Májovský J (1979) Karyological Study of slovakian flora I. - Acta Fac. Rerum Nat. Univ. Comen., Bot. 27:127–133., IAPT/IOPB Chromosome Data 13
- Nagano K, Umeda T, Toda Y (2000) Karyomorphological study of *Juniperus* In: Cytogenetics of forest trees and shrubs, Review, Present Status and Outlook for future. Ed. Guttenberger H, Borzan Z, Schlarbaum SE, Hartman TPV, Spl issue of the Forest Genetics Arbora Publishers, Zvolen, Slovakia, pp. 143–159.
- Nagano K, Matoba H, Yonemura K, Matsuda Y, Murata T, Hoshi Y (2007) Karyotype analysis of three *Juniperus* Species using fluorescence in situ hybridization (FISH) with two Ribosomal RNA Genes. Cytologia 72: 37–42. <https://doi.org/10.1508/cytologia.72.37>
- Nakata M, Oginuma K (1989) Cytological studies on phanerogams in southern Peru, II. Chromosomes of *Tetraglochin strictum* (Rosaceae) and *Ephedra americana* (Ephedraceae). Bull. Natl. Sci. Mus., Tokyo, B 15: 63–66.
- Nishimura S (1960) Chromosome numbers of polyembryonic seedlings of *Pinus thunbergii* Parl. J. Jap.For. Sci. 42:263–264.
- Nystedt B, Street NR, Wetterborn A, Zuccolo A, Lin WC et al. (2013) The Norway spruce genome sequence and conifer genome evolution. Nature 497:579–584.
- O'Hara KL, Cox LE, Nikolaeva S, Bauer JJ, Hedges R (2017) Regeneration dynamics of coast redwood, a sprouting conifer species: a review with implications for management and restoration. Forests 8: 144. <https://doi.org/10.3390/f8050144>
- Ohri D (2013) Cytogenetics of domestication and improvement of garden gladiolus and bougainvillea. Nucleus 56: 149–153. <https://doi.org/10.1007/s13237-013-0091-7>
- Ohri D (2015) How small and constrained is the genome size of angiosperm woody species. Silvae Genetica 64: 20–32. <https://doi.org/10.1515/sg-2015-0002>
- Ohri D, Khoshoo TN (1986) Genome size in gymnosperms. Plant Systematics and Evolution 153: 119–132. <https://doi.org/10.1007/bf00989421>
- Ohri D, Zadoo SN (1986) Cytogenetics of cultivated bougainvilleas IX. Precocious centromere division and origin of polyploidy taxa. Plant Breed. 97:227–31. <https://doi.org/10.1111/j.1439-0523.1986.tb01057.x>
- Olson DFJ, Roy DF, Walters GA (1990) *Sequoia sempervirens* (D. Don) Endl. Redwood (Agriculture handbook 654). In Silvics of North America, Volume 1. Conifers; USDA Forest Service: Washington, DC, USA, pp. 541–551.
- Otto SP, Whitton J (2000) Polyploidy incidence and evolution. Annual Review of Genetics 34:401–437. <https://doi.org/10.1146/annurev.genet.34.1.401>
- Paterson AH, et al. (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457: 551–556. <https://doi.org/10.1038/nature07723>
- Pearson HHW (1929) Gnetales. Cambridge: Cambridge University Press.
- Pichot C, Maataoui M (2000) Unreduced diploid nuclei in *Cupressus dupreziana* Z. Camus pollen. Theoretical and Applied Genetics 101: 574–579. <https://doi.org/10.1007/s001220051518>
- Pimenov AV, Sedelnikova TS (2002) Chromosome numbers of some Pinaceae from western and middle Siberia. Bot. Zhurn. (Moscow & Leningrad) 87: 136–137.
- Premoli AC, Kitzberger T, Veblen TT (2000) Conservation genetics of the endangered conifer *Fitzroya cupressoides* in Chile and Argentina. Conservation Genetics 1: 57–66. <https://doi.org/10.1023/a:1010181603374>
- Price H, Sparrow A, Nauman A (1973) Evolutionary and developmental considerations of the variability of nuclear parameters in higher plants. I. Genome volume, interphase chromosome volume, and estimated DNA content of 236 gymnosperms. Brookhaven Symposia in Biology 25: 390–421.
- Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploidy formation in flowering plants. Annual Review of Ecology and Systematics 29: 467–501. <https://doi.org/10.1146/annurev.ecolsys.29.1.467>
- Rastogi S, Ohri D (2020a) Karyotype evolution in cycads. Nucleus 63: 131–141. <https://doi.org/10.1007/s13237-019-00302-2>
- Rastogi S, Ohri D (2020b) Chromosome numbers in gymnosperms—an update. Silvae Genetica 69: 13–19. <https://doi.org/10.2478/sg-2020-0003>
- Renny-Byfield S, Wendel JF (2014) Doubling down on genomes: Polyploidy and crop plants. American Journal of Botany 101: 1711–1725. <https://doi.org/10.3732/ajb.1400119>
- Ren GP, Abbott RJ, Zhou YF, Zhang LR, Peng YL, Liu JQ (2012) Genetic divergence, range expansion and possible homoploid hybrid speciation among pine species in Northeast China. Heredity (2012) 108, 552–562. <https://doi.org/10.1038/hdy.2011.123>

- Resende F (1937) *Planta* 26: 757-807. Vide Darlington and Wylie (1955).
- Rice A, Smarda P, Novosolov M, Drori M, Glick L, Sabath N, Meiri S, Belmaker J, Mayrose I (2019) The global biogeography of polyploidy plants. *Nature Ecology & Evolution* 3: 265-273. <https://doi.org/10.1038/s41559-018-0787-9>
- Rogers DL (1997) Inheritance of allozymes from seed tissues of hexaploid gymnosperm, *Sequoia sempervirens* (D.Don) Endl. (coast redwood). *Heredity* 78: 166-175. <https://doi.org/10.1038/hdy.1997.24>
- Romo A, Hidalgo O, Boratynski A, obierajska K, Jasinka A, Vallès J, Garnatje T (2013) Genome size and ploidy levels in highly fragmented habitats: the case of western Mediterranean *Juniperus* (Cupressaceae) with special emphasis on *J. thurifera* L. *Tree Genetics and Genomes* 9: 587-599. <https://doi.org/10.1007/s11295-012-0581-9>
- Roodt D, Lohaus R, Sterck L, Swanepoel R, Van der Peer Y, Mizrahi E (2017) Evidence for an ancient whole genome duplication in the cycad lineage. *Plos One* 12: e184454. <https://doi.org/10.1371/journal.pone.0184454>
- Ruprecht C, Lohaus R, Vanneste K, Mutwil M, Nikoloski Z, Van der Peer Y, Persson S (2017) Revisiting ancestral polyploidy in plants. *Science Advances* 3: e1603195. <https://doi.org/10.1126/sciadv.1603195>
- Sasaki Y (1982) Triploids in plus trees in *Cryptomeria japonica* and *Chamaecyparis obtusa*. *Res.Rep. Oita Pref. For. Res. Inst.* 5: 5-13.
- Sax K, Sax HJ (1933) Chromosome number and morphology in the conifers. *Journal of Arnold Arboretum* 14: 356-375. <https://doi.org/10.5962/bhl.part.9959>
- Salman-Minkov A, Sabath N, Mayrose I (2016) Whole-genome duplication as a key factor in crop domestication. *Nature Plants* 2: 16115. <https://doi.org/10.1038/nplants.2016.115>
- Saylor, LC, Simons HA (1970) Karyology of *Sequoia sempervirens*: karyotype and accessory chromosomes. *Cytologia* 35: 294-303. <https://doi.org/10.1508/cytologia.35.294>
- Schlarbaum SE, Tsuchiya T (1975) The chromosome study of giant sequoia, *Sequoiadendron giganteum*. *Silvae Genetica* 24: 23-26.
- Schlarbaum SE, Tsuchiya T (1984a) A chromosome study of coast redwood, *Sequoia sempervirens* D. (Don) Endl. *Silvae Genetica* 33: 56-62
- Schlarbaum SE, Tsuchiya T (1984b) Cytotaxonomy and phylogeny in certain species of Taxodiaceae. *Plant Systematics & Evolution* 147: 29-54. <https://doi.org/10.1007/bf00984578>
- Schlarbaum SE, Tsuchiya T, Johnson LC (1984) The chromosomes and relationship of *Metasequoia* and *Sequoia* (Taxodiaceae): an update. *Journal of Arnold Arboretum* 65: 251-254.
- Scott AD, Stenz NWM, Ingvarsson PK, Baum DA (2016) Whole genome duplication in coast redwood (*Sequoia sempervirens*) and its implications for explaining the rarity of polyploidy in conifers. *New Phytologist* 211: 186-193. <https://doi.org/10.1111/nph.13930>
- Sedelnikova TS, Muratova EN (1999) Cytological study of „witches'-broom" type of *Pinus sylvestris* (Pinaceae) from a bog. *Citologija* 41: 1082.
- Sedelnikova TS, Muratova EN (2001) Karyological studies of „witches'-broom" type of *Pinus sylvestris* (Pinaceae) from a bog. *Bot. Zhurn. (Moscow & Leningrad)* 86: 50-59.
- Sedelnikova TS, Pimenov AV (2003) Chromosome mutations in marshland and dry valley populations of *Abies sibirica* Ledeb. *Citologija* 45: 515-520.
- Sedelnikova TS, Muratova EN, Pimenov AV (2011) Variability of chromosome numbers in gymnosperms. *Biology Bulletin Reviews* 1: 100-109. <https://doi.org/10.1134/s2079086411020083>
- Smarda P, Vesely P, Smarda Y, Bures P, Knappek O, Chytra M (2016) Polyploidy in a living fossil *Ginkgo biloba*. *New Phytologist* 212:11-14. <https://doi.org/10.1111/nph.14062>
- Smarda P, Horova L, Knappek O, Dieck H, Dieck M, Razna K, Hrubik P, Orloci L, Papp L, Vesela K, Vesely P, Bures P (2018) Multiple haploids triploids and tetraploids found in modern day 'living fossil' *Ginkgo biloba*. *Horticulture Research* 5:55. <https://doi.org/10.1038/s41438-018-0055-9>
- Soltis DE, Albert VA, Leebens-Mack J, Soltis PS (2009) Polyploidy and angiosperm diversification. *American Journal of Botany* 96:336-348. <https://doi.org/10.3732/ajb.0800079>
- Somego M, Ito S, Kanezawa T (1981) Natural triploid plants found in plus tree of sugi (*Cryptomeria japonica* D.Don.). *Trans 33rd Mtg. Kanto Branch Jp. For. Soc.* 81-82.
- Stebbins GL (1948) The chromosomes and relationship of *Metasequoia* and *Sequoia*. *Science* 108: 95-98. <https://doi.org/10.1126/science.108.2796.95>
- Stiff ML (1951) A naturally occurring triploid juniper. *Va. J. Sci.* 2: 317.
- Suyama Y, Mukai Y, Kondo T (1996) Assignment of RFLP linkage groups to their respective chromosomes in sugi (*Cryptomeria japonica*). *Theoretical & Applied Genetics* 92: 292-296. <https://doi.org/10.1007/bf00223671>
- Syrach-Larsen C, Westergaard M (1938) A triploid hybrid between *Larix decidua* Mill. and *Larix occidenatlis* Nutt. *J. Genet.* 36: 523-530.
- Tarnavarschi IT, Lungeanu I (1970a) Chromosomenzahlen von einigen in Rumänien wildwachsenden Anthophyten. *Rev. Roum. Biol., Bot.* 15: 381-383.
- Tarnavarschi IT, Lungeanu R (1970b) In IOPB chromosome number reports XX-VIII. *Taxon* 19: 608-610. <https://doi.org/10.1002/j.1996-8175.1970.tb03062.x>
- Terasaka O (1982) Nuclear differentiation of male gametophytes in Gymnosperms. *Cytologia* 47: 27-46. https://doi.org/10.1508/cytologia.47.1_27
- The Angiosperm Phylogeny Group (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot. J. Linn. Soc.* 161: 105-121. <https://doi.org/10.1111/j.1095-8339.2009.00996.x>
- The Plant List (2010) Version 1 Published on the Internet. <http://www.theplantlist.org/>
- Toda E, Okamoto T (2016) Formation of triploid plants via possible polyspermy. *Plant Signaling & Behavior* 2016, VOL.11, NO.9, e1218107 (5pages). <http://dx.doi.org/10.1080/15592324.2016.1218107>
- Toda Y (1996) Karyomorphological studies of the Taxodiaceae. *Forest Science* 3: 175-180.
- Toda Y, Sotoyama S (1972) Variants of woods. *Iden* 26: 50-54.
- Tremblay L, Levasseur C, Tremblay FM (1999) Frequency of somaclonal variation in plants of black spruce (*Picea mariana*, Pinaceae) and white spruce (*P. glauca*, Pinaceae) from somatic embryogenesis and identification of some factors involved in genetic instability. *American Journal of Botany* 86: 1373-1381. <https://doi.org/10.2307/2656920>
- Vallès V, Garnatje T, Robin O, Siljak-Yakovlev S (2015) Molecular cytogenetic studies in western Mediterranean *Juniperus* (Cupressaceae): a constant model of GC-rich chromosomal regions and rDNA loci with evidences for paleopolyploidy. *Tree Genetics & Genomes* 11:43. <https://doi.org/10.1007/s11295-015-0860-3>
- Van de Peer Y, Mizrahi E, Marchal K (2017) The evolutionary significance of polyploidy. *Nat. Rev. Genet.* 18:411-424. <https://doi.org/10.1038/nrg.2017.26>
- Van Druenen WE, Husband BC (2019) Evolutionary associations between polyploidy, clonal reproduction, and perenniality in the angiosperms. *New Phytologist* (2019) 224: 1266-1277. <https://doi.org/10.1111/nph.15999>
- Veblen TT, Burns BR, Kitzberger T, Lara A, Villalba R (1995) The ecology of the conifers of southern South America. In: *Ecology of Southern Conifers* (eds. Enright NJ, Hill RS), pp. 120-155. Melbourne University Press, Carlton, Victoria, Australia.
- Wakamiya I, Price HJ, Messina MG, Newton RJ (1996) Pine genome diversity and water relations. *Physiologia Plantarum* 96: 13-20. <https://doi.org/10.1034/j.1399-3054.1996.960103.x>
- Wan T, Liu ZM, Li LF, Leitch AR, Leitch IJ, Lohaus R et al. (2018) A genome for gnetophytes and early evolution of seed plants *Nature Plants* 4: 82-89. <https://doi.org/10.1038/s41477-017-0097-2>
- Wang BS, Mao JF, Gao J, Zhao W, Wang XR (2011) Colonization of the Tibetan Plateau by the homoploid hybrid pine *Pinus densata*. *Mol Ecol* 20: 3796-3811. <https://doi.org/10.1111/j.1365-294x.2011.05157.x>
- Wendt T (1993) A new variety of *Ephedra torreyana* (Ephedraceae) from West Texas and Chihuahua, with notes on hybridization in the *E. torreyana* complex. *Phytologia* 74: 141-150.
- Wesche K, Ronnenberg K, Hensen I (2005) Lack of sexual reproduction within mountain steppe populations of the clonal shrub *Juniperus sabina* L. in semi arid southern Mongolia. *Journal of Arid Environment* 63: 390-405. <https://doi.org/10.1016/j.jaridenv.2005.03.014>
- Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Evolution* 140: 137-150. <https://doi.org/10.1159/000351727>
- Williams CG (2009) *Conifer reproductive biology*. Springer, Dordrecht, Heidelberg, London, New York.
- Willyard A, Cronn R, Liston A (2009) Reticulate evolution and incomplete lineage sorting among the ponderosa pines. *Molecular Phylogenetics and Evolution* 52: 498-511. <https://doi.org/10.1016/j.ympev.2009.02.011>

- Winton LL (1964) Natural polyploidy in juvenile white and black spruce. Minnesota Forestry Notes Published as Sci. Jour. Ser. Paper No. 5452 of the Minn. Agri. Exp. Stn.
- Won H, Renner SS (2005) The internal transcribed spacer of nuclear ribosomal DNA in the gymnosperm *Gnetum*. *Molecular Phylogenetics and Evolution* 36: 581-597. <https://doi.org/10.1016/j.ympev.2005.03.011>
- Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH (2009) The frequency of polyploidy speciation in vascular plants. *Proceedings National Academy of Sciences USA*. 106:13875–13879. <https://doi.org/10.1073/pnas.0811575106>
- Wu H, Ma Z, Wang M M, Qin A L, Ran J H, Wang XQ (2016) A high frequency of allopolyploid speciation in the gymnospermous genus *Ephedra* and its possible association with some biological and ecological features. *Molecular Ecology* 25: 1192-1210. <https://doi.org/10.1111/mec.13538>
- Wu H, Yu Q, Ran J-H, Wang X-Q (2020) Unbiased subgenome evolution in allotetraploid species of *Ephedra* and its implications for the evolution of large genomes in gymnosperms. *Genome Biology and Evolution* evaa 236. <https://doi.org/10.1093/gbe/evaa236>
- Wu JL, Li S, Jiang H, Gao YH, Niu JY (2009) Slide preparing technique of mitotic chromosomes and application in karyotype technique of mitotic chromosomes and application in karyotype determination of *Ephedra*. *China Journal of Chinese Materia Medica* 34: 2725-2729.
- Wu S, Han B, Jiao Y (2020) Genetic contribution of paleopolyploidy to adaptive evolution in angiosperms. *Molecular Plant* 13: 59-71. <https://doi.org/10.1016/j.molp.2019.10.012>
- Xu BS, Weng RF, Zhang MZ (1992) Chromosome numbers of Shanghai plants I. *Invest. Stud. Nat.* 12: 48–65.
- Yang ZY, Ran JH, Wang XQ (2012) Three genome-based phylogeny of Cupressaceae s.l.: further evidence for the evolution of gymnosperms and Southern Hemisphere biogeography. *Molecular Phylogenetics and Evolution* 64: 452–470. <https://doi.org/10.1016/j.ympev.2012.05.004>
- Yousafzai FK, Kaff AI, Moore G (2010) The molecular features of chromosome pairing at meiosis: the polyploidy challenge using wheat as a reference. *Functional & Integrative Genomics* 10:147-156. <https://doi.org/10.1007/s10142-010-0171-6>
- Zhou Z-Y (2009) An overview of fossil Ginkgoales. *Palaeoworld* 18(1-22): 206. <https://doi.org/10.1016/j.palwor.2009.01.001>
- Zinnai I, Chiba S (1951) (Naturally occurring tetraploids of *Cryptomeria japonica*) *Ikushugaku Zasshi*. *Japanese Journal of Breeding* 1:43-46.
- Zinnai L (1952) Tetraploid plants in Japanese red pine (*Pinus densiflora* Sieb. Et Zucc.) discovered in transplant beds. *J. Jap. For. Soc.* 34: 185-187.
- Zonneveld BJM (2012) Conifer genome sizes of 172 species, covering 64 out of the 67 genera, range from 8 to 72 picogram. *Nordic Journal of Botany* 30: 490-502.
- Zwaenepoel A, Van der Peer Y (2019) Inference of ancient whole genome duplications and the evolution of the gene duplication and loss rate. *Mol. Biol. Evol.* 36: 1384-1404. <https://doi.org/10.1093/molbev/msz088>