Nuclear and chloroplast DNAs reveal diverse origins and mis-identifications of *Juniperus chinensis* cultivars from Windsor Gardens, UK. Part 2 of 3.

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ABSTRACT

Ploidy was determined for twenty four (24) plants labeled as *Juniperus chinensis* cultivars at the Windsor Gardens, UK and revealed 16 were tetraploids (2*n*=4*x*=44), 7 diploids (2*n*=2*x*=22), and one triploid (2*n*=3*x*=33). nrDNA (ITS) and cp DNA sequencing found one of the diploids was actually a cypress; *J. chinensis* cv Savill Sentinel was *Cupressus gigantea*. A second diploid, cv 'Spartan' was *J. virginiana*. Only three of the remaining 22 '*chinensis* cultivars' had both nrDNA and chloroplasts (cp) of *J. chinensis*: Iowa (=Globosa), Obelisk and Plumosa Aurea and these, having homozygous nrDNA, appear to be autotetraploids. Four other cultivars had *J. chinensis* nrDNA but cp of *J. tsukusiensis*. Two cultivars, Richeson and Fruitlandii, were determined to be *J. xpfitzeriana*. Two cultivars, Japonica and Japonica Variegata, had nrDNA and cp of *J. chinensis* var. *sargentii*. The remaining ten '*J. chinensis* cultivars' had *J. chinensis* hybrid nrDNA. But, these 10 cultivars had 3 kinds of cp DNA: 7 had *J. chinensis* var. *sargentii* cp; 2 with *J. sabina* var. *balkanensis* cp; and one, Kek, had *J. chinensis* cp. The amount of hybridization among the parents of cultivars in botanic gardens makes it very difficult to identify cultivated junipers. In this sample of 24 '*J. chinensis*' cultivars, only 3 plants were 'pure, autotetraploid '*J. chinensis*' by DNA. A DNA barcode system, if utilized, would greatly aid botanic gardens to screen current and incoming accessions to assign taxonomic names to junipers. Published on-line www.phytologia.org *Phytologia 102(3): 106-115 (Sept 21, 2020).* ISSN 030319430.
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It has now been shown that genome size assessment using flow cytometry (FC) can be successfully used as a proxy for ploidy level in *Juniperus* (Farhat et al. 2019a, b) from both fresh and silica gel dried leaves of *Juniperus*. Thus, the ploidy of Juniper hybrids can now be determined by FC. This is very important because it is known that several *J. chinensis* cultivars are triploid or tetraploid (Hall, et al. 1979). With the confluence of both DNA methodology and FC ploidy determination, this presents us with a great opportunity to examine the origin of *J. chinensis* cultivars.

As a first step in this work, we recently analyzed *Juniperus* x*pfitzeriana* cultivars, one of the most commonly cultivated junipers in the world (Adams, et al. 2019). The origin of *J.* x*pfitzeriana* is thought to be a hybrid of *J. chinensis* x *J. sabina*. Nuclear DNA (nrDNA, ITS) and 4 chloroplast gene regions were sequenced from 14 *J.* x*pfitzeriana* cultivars from Windsor Gardens, UK, and compared with all *Juniperus*, sect. *Sabina*, smooth leaf margin species. All of the 14 cultivars were identical in their chloroplast DNA and their cp DNA was identical to that of *J. sabina* var. *balkanensis* (Table 1). In addition, 13 *J.* x*pfitzeriana* cultivars were allo-tetraploids with heterozygous bases at 5 to7 sites that distinguish *J. chinensis* and *J. sabina* var. *balkanensis*. These cultivars had identical nrDNA. Two of the 14 cultivars, 'Old Gold' and 'Sea Green', showed a slightly different nrDNA pattern, being homozygous at sites 410 and 1139, as found in *J. s.* var. *balkanensis*. The origin of *J.* x*pfitzeriana* is from a cross of a male, tetraploid *J. sabina* var. *balkanensis* and a female, tetraploid, *J. chinensis*, resulting in an allo-tetraploid, dioecious, *J.* x*pfitzeriana* (Spath) Schmidt.

Table 1. nrDNA (ITS) variable sites in *J. chinensis* cultivars. (Windsor Gardens), *J. chinensis*, and *J. sabina*. K=G/T; S=C/G; Y=C/T; M=A/C; W=A/T; R=A/G. chloroplast types: *balkanensis* = *J. sabina* var. *balkanensis*/ *J. thurifera*; *sabina* = *J. sabina* var. *sabina*; and *chinensis* = *J. chinensis*. Modified from Adams et al. (2019). Site numbers modified to correspond with site numbers in Table 3 of this report.

taxa: *J.* x*pfitzeriana* (=x*media*), unless noted otherwise	ploidy	212[a] K	410 S	665 Y	986 Y	996 M	1034 K	1073 W	1137 R	ITS classification hybrid?	chloroplast, ex. pollen from:
Most probable male (pollen) parent	4*x*	G	C	T	T	A	T	T	G	*J. sabina* var. *balkanensis*	*J. sabina* var. *balkanensis*
Most probable female parent genotype	4*x*	T	G	C	C	C	G	A	A	*chinensis*	*chinensis*
15442 Arctic	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15454 Armstrongii	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15418 Aurea, Paris-sud	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15474 Aurea	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15423 Saybrook Gold	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15425 Carberry Gold	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15463 Carberry Gold	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15443 Gold Star	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15462 Golden Saucer	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15482 Goldenkissen	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15430 pfitzeriana prostate	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15435 Wilhelm Pfitzer	4*x*	G/T	C/G	C/T	C/T	A/C	G/T	A/T	A/G	chin x sab	*balkanensis*
15453 Old Gold	4*x*	G/T	**C**	C/T	C/T	A/C	G/T	A/T	**G**	chin x sab*	*balkanensis*
15436 Sea Green, Windsor	3x	G/T	**C**	**T**	C/T	A/C	G/T	A/T	**G**	chin x sab*	*balkanensis*
15604 Sea Green Home Depot, Inc.	3x	G/T	**C**	**T**	C/T	A/C	G/T	A/T	**G**	chin x sab*	*balkanensis?*

[a]Variable sites located at: 212, xGGCCAAGC; 410, xGTTGAGAT; 665, xTCTTCGTC; 986, xGCCCTCCC; 996, xGCGAGGAG; 1034, xGCGGTCGG; 1073, xCGCGACGA; 1137, xGAACTTTG.

The purpose of the present research is to present new DNA sequencing utilizing both chloroplast and nuclear DNA in the determination of the origin of *J. chinensis* cultivars.

METHODS

Plant materials:

Samples: Leaf samples were collected in Windsor Gardens, Windsor Great Park, Windsor, *SL4 2HT* UK from 24 *J. chinensis* cultivar accessions (see Table 2) and immediately placed in activated silica gel for DNA sequencing and Flow Cytometry - ploidy determination.

Table 2. Windsor 24 *Juniperus chinensis* cv and origin table < = earlier than (before).

taxon *Juniperus chinensis* '	Adams #	Windsor acc. #	ploidy this study	Chrom number, 2*n*, litr.	Origin: based on Den Oden and Boom 1965; Krussmann 1991; Welch 2012, Lewis 1998, Auders & Spicer 2012.
J. chinensis 'Savill Sentinel'	15426	2003-153	2x		1999, cutting ex *J. chinensis* (1999-6117), Windsor Card
J. chinensis "Shepherdii'	15471	1999-757	4x		China (Robert Fortune) 1855 but named in 1867
J. chinensis ' 'Belvedere' ,= 'Armstrongii'	15427	2000-271	3x	(44)	'Belvedere' Austria 1973; 'Armstrongii' Canada 1932
J. chinensis ' 'Keteleerii'	15432	1999-5819	4x	(44)	Belgium <1910
J. chinensis ' 'Japonica'	15433	2001-465	4x		1855 Carriere
J. chinensis 'Japonica Variegata'	15439	1999-5816	4x	(44)	1867 Carriere
J. chinensis 'Kuriwao Mist'	15441	1999-5821	4x		New Zealand < 1993?
J. chinensis 'Kuriwao Sunbeam'	15446	1999-5822	2x		New Zealand <1993
J. chinensis 'Richeson' = x pfitzer	15451	1999-5832	4x		= x pfitzer USA 1941, pfitzer sport
J. chinensis 'sargentii 'Glauca'	15452	1999-5996	2x	(22)	UK 1855
J. chinensis 'Lombarts'	15458	2000-1334	4x		Windsor Great Park <1998?
J. chinensis 'Aurea' = 'Alba'.	15461	1999-5805	4x	(44)	'Aurea' 1855 UK; 'Alba' = 'Plumosa Albovariegata'
J. chinensis 'Spartan'	15464	1999-5838	2x		USA 1950s
J. chinensis 'Jacobiana'	15466	1999-6183	4x	(33)	< 1887 = 'Hetzii'
J. chinensis Pfitzer Gp. 'Blaauw'	15466	1999-6078	2x	(44)	Japan, Introduced by Blaauw & Co., 1924, Netherlands
J. chinensis 'Robusta Glauca'	15467	1999-5833	4x		unknown
J. chinensis 'Obelisk'	15469	1999-5829	4x	(44)	Japan seed germinated in Holland 1930
J. chinensis 'Iowa' = 'Globosa'	15470	1999-5814	4x	(44) (22?)	USA 1930
J. chinensis s 'Fruitlandii'	15472	1999-5812	4x	(33)	x media =x pfitzer USA 1977
J. chinensis Pfitzer Gp. 'Shimpaku'	15473	1999-6111	2x		= x pfitzer, Japan <1966
J. chinensis Pfitzer Gp. 'Globosa Cinerea'	15477	1999-6083	2x	(44) ?	Japan <1930
J. chinensis Pfitzer Gp. 'Plumosa Aurea'	15478	1999-6105	4x		<1884
J. chinensis 'Kek'	15484	1999-5818	4x		Windsor Great Park 1992?
J. chinensis 'Mathot'	15488	1999-5826	4x		Holland <1947

Reference Species: *Juniperus chinensis*, *J. sabina* var. *sabina*, *J. s*. var. *balkanensis* see Adams et al. (2018a) for collection details.

DNA extraction and sequencing

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Chromatograms analyzed by use of Chromas 2.31 (Technelysium Pty Ltd.).

Flow cytometric analyses for ploidy level determination

Nuclear DNA amount was assessed by flow cytometry (FC) based on the technique of Bourge et al. (2018) on silica dried leaves of *Juniperus* samples and fresh leaves of *Hordeum vulgare* L. 'Sultan'[2C= 9.81 pg in Garnatje et al. (2004)] used as an internal standard. Approximately, 30 mg of leaves of both the internal standard and *Juniperus* were simultaneously chopped using a razor blade in a plastic Petri dish with 500 µl of cold Gif nuclear-isolation buffer-GNB (Bourge et al. 2018): 30 mM sodium citrate, 45 mM $MgCl_2$, 60 mM MOPS (4-morpholine propane sulphonate, pH 7), and 1% (w/v) polyvinylpyrrolidone 10,000, pH 7.2 containing 0.1% (w/v) Triton X–100, supplemented with 10 mM sodium metabisulphite and RNase (2.5 U/ml). The nuclei suspension was filtered through 50 µm nylon mesh. The nuclei were stained with 100 µg/ml propidium iodide (PI), a specific DNA fluorochrome intercalating dye, and kept at 4°C for 5 min. DNA content of about 3,000 stained nuclei was determined for each sample using the cytometer CytoFLEX S (Beckman Coulter- Life Science United States. Excitation 488 nm, 26 mW; emission through a 610/20 nm band-pass filter). Measurements of each sample were repeated twice. The software CytExpert was used for histogram analyses. The total 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of the species and the internal standard, according to the following formula:

2C DNA sample (pg) = (Sample 2C peak mean / Standard 2C peak mean) x Standard 2C DNA (pg).

RESULTS AND DISCUSSION

Ploidy was determined for twenty four (24, only 23 were *Juniperus*, see below) plants labeled as *J. chinensis* cultivars at the Windsor Gardens, UK and analyses revealed (Tables 2, 3) that of the 23 juniper plants, 16 were tetraploids (44), 6 diploids (22), and one triploid (33). Farhat et al. (2019a) discovered that about 15% of *Juniperus* taxa were tetraploids and one, *J. foetidissima*, was a hexaploid, based on analysis of samples from junipers that were naturally occurring not cultivated. In this study, we found most of these cultivated plants were tetraploids. It is worthwhile to review an interesting study by Zinnai and Chiba (1951) who in a survey *Cryptomeria japonica* in seedling nurseries (2 and 3-year old seedlings) found 4 seedlings with twisted needles that were thick and bent at the tip-end. In addition, the stomatal bands tended to be larger. Chromosome counts on these plants confirmed they were tetraploids. Chiba (1951), later, selected 39 (putative) polyploid seedlings with twisted needles from the germination beds and found 18 were diploids, 3 triploids and 18 tetraploids. The polyploids randomly occurred in beds at a rate of 5×10^{-6} frequency (e.g., 0.0005%). Normally in a forest seedling nursery, abnormal appearing seedlings (such as these with twisted needles) are removed by gardeners to maintain robust seedlings for out-planting. Ahuja (2005) noted that "sporadic polyploids and aneuploids occur at a very low frequency in nurseries in conifers, but most of them show growth abnormalities, remain dwarf, and may not reach maturity".

Ploidy shown in Table 2 is compared with literature reports of chromosome number (Hall, et al. 1979). Note that several literature reports differ from the flow cytometry ploidy determination: Belvedere, litr. = tetraploid (44) vs. triploid (33); Jacobiana, litr = triploid (33) vs. tetraploid (44); Blaauw, litr = tetraploid (44) vs. diploid (22), Fruitlandii, litr. = triploid (33) vs tetraploid (44); Globosa Cinerea, litr = tetraploid (44?) vs. diploid (22). It is very likely that there have been labeling errors over the decades in transferring plants among botanic gardens and nurseries. It nearly impossible to obtain samples from the original plants for which the names originated.

Analysis of nrDNA (ITS) revealed 12- 14 polymorphic sites among the 24 'J. chinensis cv' studied (Table 3). Analysis of 3 chloroplast (cp) genes: petN-psbM, trnS-trnG and trnL-trnF revealed that petN-psbM (hereafter petN), as the most informative in distinguishing *J. chinensis*, *J. sabina*, and related species, thus, trnS-trnG and trnL-trnF were not further utilized. petN sequence utilized to reveal the chloroplast source (e.g., pollen, paternal) for the *J. chinensis* cultivars studied.

The 24 '*J. chinensis* cultivars' were found to be in 8 groups (Table 3). The first group (yellow) included 'Richeson' and 'Fruitlandii, both tetraploids, which have *J. sabina* var. *balkanensis* cp, and *J. xpfitzeriana* ITS, as seen in the Wilhelm Pfitzer (*xpfitzeriana*) sample (from Adams et al. 2019). So, both of these are *xpfitzeriana*, not *J. chinensis*.

Japonica, and Japonica Variegata (2nd group, blue), tetraploids, are part of *J. chinensis* var. *sargentii* (Table 3) with *J. c*. var. *sargentii* cp and ITS.

Kuriwao Sunbeam is in the 3rd (purple) group and is very unusual being a diploid with *J. sabina* var. *balkanensis* cp and *J. chinensis* var. *procumbens* ITS, because both of these taxa are tetraploid (Farhat et al. 2019a).

The 4th and 5th groups are closely related with all 7 cultivars having *J. chinensis* ITS DNA, but the red group 4, contains Obelisk, Iowa (=Globosa), and Plumosa Aurea which are tetraploids with *J. chinensis* cp. In contrast, group 5 (salmon) contains 4 diploids (*chinensis* var. *sargentii* Glauca, Pfitzer Blaauw, Pfitzer Shimpaku, and Pfitzer Globose Cinerea), all have *J. chinensis* ITS, but each has *J. tsukusiensis* (sometimes treated as *J. chinensis* var. *tsukusiensis*, Adams 2014) chloroplasts. The use of Pfitzer as part of the cultivar name is confusing, as *xpfitzeriana* is tetraploid and of hybrid origin from *J. sabina* x *J. chinensis*, see Adams et al. 2019).

The 6th group (green) is the largest with 9 tetraploids and one triploid, all have *J. chinensis* hybrid ITS DNA (Table 3). Seven (Aurea, Jacobiana, Shepherdii, Keteleerii, Robusta Glauce, Lombards, Belvedere) have *J. chinensis* var. *sargentii* cp. Two (Kuriwao Mist, Mathot) have *J. sabina* var. *balkanensis* cp and one (Kek) is the only plant in these analyses with *J. chinensis* cp. The tremendous diversity in the hybrid nature of nrDNA (ITS) in this group indicating that the maternal parent arose by hybridization with a variety of junipers.

The 9th group was most surprising to find that 'Savill Sentinel' was not a juniper, but a cypress, *Cupressus gigantea* by ITS DNA (Table 3). Interestingly, this plant is of hybrid origin (note the heterozygous ITS sites, Table 3), with a male *Cupressus gigantea* parent chloroplast. We not able to identify the maternal parent of the hybrid at this point. Even with 3 botanists collecting samples, none of us noted that it was a cypress. Perhaps we were too focused on the mechanics of collecting and accurately labeling the samples to observe the plant.

Group 10 produced the second surprise in that 'Spartan' had ITS and cp DNA of *J. virginiana* (Table 3). *Juniperus chinensis* and *J. virginiana* look very similar, especially if juvenile (decurrent) leaves are present on *J. virginiana*, so it is not surprising that Spartan was labeled *J. chinensis* as some time in history.

Five diploid cultivars have cp parents that differ from their homozygous maternal parents nrDNA: Kuriwao Sunbeam (*J. sabina* var. *balkanensis*, cp, *J. chinensis* var. *procumbens*, nrDNA); Glauca, Blaauw, Shimpaku, and Globosa Cinerea (all 4 with *J. tsukusiensis* cp and *J. chinensis*, nrDNA). These 5 cultivars with conflicting cp and nrDNA seem likely to have experienced a chloroplasts capture event as has been found often in natural populations of *Juniperus* (Adams et al. 2017 a,b; Adams et al. 2018 a,b; Adams et al. 2020; Farhat et al. 2019 a,b; Hojjati et al. 2019).

It is interesting that some of the aforementioned diversity was discovered Le Duc et al. (1999) by the use of RAPDs (Random Amplified Polymorphic DNAs). Figure 1 shows a PCO based on 122 RAPD bands of *J. chinensis*, *J. sabina* and 9 cultivars. Notice the Pfitzer cultivars group are near the base of *J. chinensis*, but intermediate on axis 2, to *J. sabina*, giving an evidence that they are *chinensis* x *sabina* hybrids, although the synthetic (computer generated) hybrid is precisely intermediate. Fruitlandii (a

xpfitzeriana, Table 3) is intermediate on axis 3. Kallay's Compact, Gold Coast and Hetzii form a group near the Pfitzers (yellow oval).

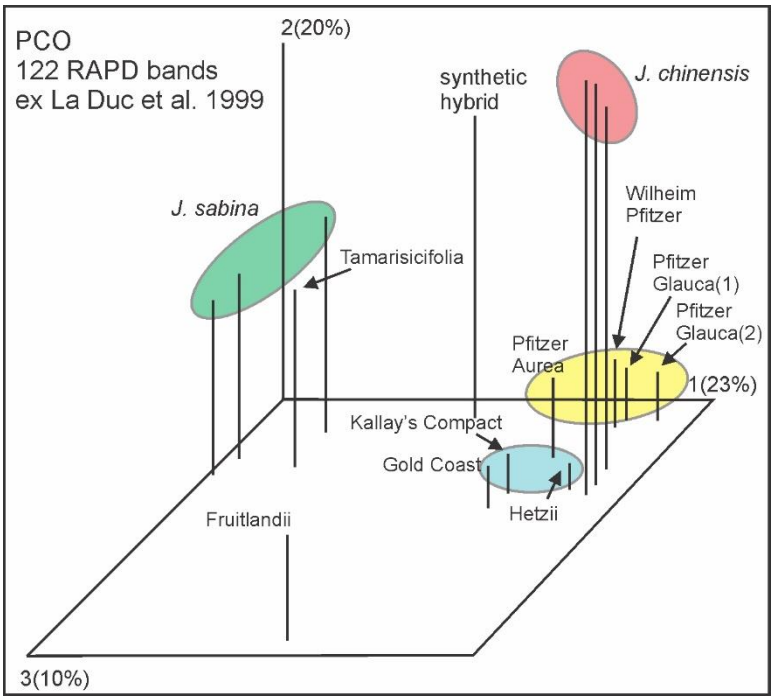

Figure 1. PCO using 122 RAPD bands of *J. chinensis* (natural, Japan), *J. sabina* (natural, Switzerland) and 9 cultivars.

Possible ploidy levels of putative parents of '*J. chinensis*' cultivars in this study

It is very interesting that the ploidy of all the male parents of the '*J. chinensis*' cultivars as well as the female parents have been reported (Farhat et al. 2019a) as tetraploids (4x), (Table 4: *J. sabina* var. *balkanensis*, *J. chinensis*, *J. c.* var. *sargentii*, *J. c.* var. *procumbens*, *J. tsukusiensis*, and *J. xpfitzeriana*). However, Kuriwao Sunbeam is diploid (2x, Table 4) suggesting that haploid (1x) gametes of *J. s.* var. *balkanensis* and *J. c.* var. *procumbens* united to form the diploid. The four male *tsukusiensis* x female *chinensis* parentages resulted in diploid (2x) *chinensis* var. *sargentii* 'Glauca', and 3 Pfitzer 'Blaauw', 'Shimpaku', and 'Globosa Cinerea' (Table 4). Although Farhat et al. (2019a) found their natural *tsukusiensis* to be 4x, it is very possible there are cultivars of *tsukusiensis* that are diploid. And, it is certainly possible that putative 'chinensis' female parents were diploids. Unfortunately, we know very little about variation in ploidy of *J. chinensis* in the wild. In a recent study of nearly all *Juniperus* species, Farhat et al. (2019a) reported that *J. chinensis*, *J. c.* var. *procumbens*, and *J. c.* var. *sargentii* were tetraploids in nature. However, only one plant each of *J. chinensis*, *J. c.* var. *procumbens*, and *J. c.* var. *sargentii* were analyzed. Nagano et al. (2000, 2007) analyzed *J. chinensis* varieties from Japan and reported that *J. chinensis* var. *chinensis*, *J. c.* var. *kaizuka*, *J. c.* var. *jacobiana* were tetraploids (2n=44), but *J. c.* var. *sargentii* was a diploid (2n=22). In Nagano et al. (2007), they report that their *J. c.* var. *sargentii* was obtained from Mt. Shiroiwa, Miyazaki Prefecture. Farhat et al. (2019a) obtained their *J. c.* var. *sargentii* from Mt. Kirigishi, Furano-Ashibetsu Natural Park, Hokkaido. However, Nagano et al. (2007) strongly felt the chromosome karyomorphological differences between their *J. chinensis* var. *chinensis* and *J. c.* var. *sargentii* warranted the recognition of *J. sargentii* at the specific level. In contrast, Adams and Schwarzbach (2013) and Adams et al. (2011) found that their *J. c.* var. *sargentii* (4x) material was in a well-supported clade with *J. chinensis*, supporting its recognition as *J. c.* var. *sargentii*. The confusion may rest on the fact that *J. c.* var. *chinensis* and *J. c.* var. *sargentii* are difficult identify when collecting.

The final unusual case is that of Belvedere, a triploid with male *chinensis* v. *sargentii* (4x, Farhat et al. 2019a; or 2x, Nagano et al. 2007) and female *chinensis* hybrid (4x) (Table 4). If the var. *sargentii* was 2x and the female chinensis hybrid was tetraploid, then the triploid follows simply (2x + 1x = 3x). If the male parent was a tetraploid, then the explanation of triploid hybrid would be more difficult.

Table 4. Analyses of ploidy of putative parents' ploidy and ploidy of the cultivars at Windsor Garden.

Paternal (male) parent cp source	Farhat et al. 2019 ploidy	Maternal (female) parent nrDNA (nuclear) ITS classification	Farhat et al. 2019 ploidy	Windsor Garden accessions grouped by DNA aff. (affiliation): **2 accessions identical to *xpfitzeriana***	Actual ploidy of hybrid
J. sab. v. *balkanensis*	4x	*Juniperus xpfitzeriana*	4x	*15435* Wilhelm Pfitzer *xpfitzeriana*, 4x	4*x*
J. sab. v. *balkanensis*	4x	*Juniperus xpfitzeriana*	4x	*15451* chinensis 'Richeson' allo-tetraploid = *J. xpfitzeriana*	4*x*
J. sab. v. *balkanensis*	4x	*Juniperus xpfitzeriana*	4x	*15472* chinensis 'Fruitlandii' allo-tetraploid = *J. xpfitzeriana*	4*x*
Male parent (cp)	Farhat ploidy	Female parent	Farhat ploidy	***aff: J. chinensis var. sargentii***	Actual ploidy
chinensis/ sargentii[1]	4x	*chin. v. sargentii*	4x	*15433* chin 'Japonica'	4*x*
chinensis/ sargentii[1]	4x	*chin.* v. *sargentii*	4x	***15439*** chin 'Japonica Variegata'	4*x*
J. sab. v. *balkanensis*	4x	*chin.* v. *procumbens*	4x	*15446* chin 'Kuriwao Sunbeam'	2*x*
Male parent (cp)	Farhat ploidy	Female parent	Farhat ploidy	***aff. J. chinensis hybrids***	Actual ploidy
chinensis	4x	*chinensis*	4x	*15469* chin 'Obelisk'	4*x*
chinensis	4x	*chinensis*	4x	*15470* chin 'Iowa' 'Globosa'	4*x*
chinensis	4x	*chinensis*	4x	*15478* chin Pfitzer 'Plumosa Aurea'	4*x*
Male parent (cp)	likely ploidy	Female parent	likely ploidy	***aff. J. chinensis x J. tsukusiensis hybrids***	Actual ploidy
tsukusiensis Farhat 4x	2x	*chinensis*, cultivar?	2x	*15452* chin sargentii 'Glauca'	2*x*
tsukusiensis Farhat 4x	2x	*chinensis*, cultivar?	2x	*15466* chin Pfitzer 'Blaauw'	2*x*
tsukusiensis Farhat 4x	2x	*chinensis*, cultivar?	2x	*15473* chin Pfitzer 'Shimpaku'	2*x*
tsukusiensis Farhat 4x	2x	*chinensis*, cultivar?	2x	*15477* chin Pfitzer 'Globosa Cinerea'	2*x*
Male parent (cp)	Farhat ploidy	Female parent	likely ploidy	***aff. J. chin.* var. *sargentii* x *chin* hybrid**	Actual ploidy
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15461* chin 'Aurea'	4*x*
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15465* chin 'Jacobiana'	4*x*
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15471* chin 'Shepherdii'	4*x*
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15432* chin 'Keteleerii' ~= 'Kuriwao Mist'	4*x*
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15467* chin 'Robusta Glauca'	4*x*
chinensis v. *sargentii*	4x	*chinensis* hybrid	4x	*15458* chin 'Lombarts'	4*x*
chinensis v. *sargentii*	4x,2x	*chinensis* hybrid	4x	*15427* chin 'Belvedere'	3*x*
J. sab. v. *balkanensis*	4x	*chinensis* hybrid	4x	*15441* chin 'Kuriwao Mist'	4*x*
J. sab. v. *balkanensis*	4x	*chinensis* hybrid	4x	*15488* chin 'Mathot'	4*x*
chinensis	4x	*chinensis* hybrid	4x	*15484* chin 'Kek'	4*x*
Male parent (cp)	Farhat ploidy	Female parent	Farhat ploidy	**Mis-identified taxa**	Actual ploidy
Cupressus gigantea	2x	*Cupressus gigantea*	2x	*15426 chin* 'Savill Sentinel **ID = *Cupressus gigantea* (hybrid)**	2*x*
J. virginiana	2x	*J. virginiana*	2x	*15464* chin 'Spartan' **ID = *Juniperus virginiana***	2*x*

In this study, we found tremendous variation among nrDNA and cp parentage. The development and implementation of a DNA barcode system would greatly aid botanic gardens to screen current and incoming accessions to assign taxonomic names to junipers and other conifers.

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Table 3. Analyses of cp (chloroplast) source and nrDNA (ITS) variable sites in *J. chinensis* cultivars (Windsor Gardens), K=G/T; S=C/G; Y=C/T; M=A/C; W=A/T; R=A/G.

Juniperus x pfitzeriana	ploidy	cp source	ITS classif.	Heterozygous sites												
15435 Wilhelm Pfitzer *xpfitzeriana* (ex. Adams et al. 2019)	4*x*	*J. sabina* v. *balkanensis*	*Juniperus xpfitzeriana*	212K	410S	665Y	986Y	996M	1034K	1073W	1137R	Note: nrDNA (ITS) shows it is hybrid *J. sabina* v. *balkanensis* x *J. chinensis*				
15451 chinensis 'Richeson' allo-tetraploid = *J. xpfitzeriana*	4*x*	*J. sabina* v. *balkanensis*	*Juniperus xpfitzeriana*	212K	410S	665Y	986Y	996M	1034K	1073W	1137R					
15472 chinensis 'Fruitlandii' allo-tetraploid = *J.x pfitzeriana*	4*x*	*J. sabina* v. *balkanensis*	*Juniperus xpfitzeriana*	212K	410S	665Y	986Y	996M	1034K	1073W	1137R					
aff: J. chinensis var. sargentii	ploidy	cp source	ITS classif.	Heterozygous sites												
15433 chin 'Japonica'	4*x*	*chinensis/ sargentii*[1]	*chinensis v. sargentii*	301R	638R		[1] ITS ~= var. *chinensis* and var. *sargentii*.									
15439 chin '**Japonica 'Variegata'**	4*x*	*chinensis/ sargentii*[1]	*chinensis* v. *sargentii*	301R	638R											
15446 chin 'Kuriwao Sunbeam'	2*x*	*J. sabina* v. *balkanensis*	*chinensis* v. *procumbens*[2]	212K			[2]ITS = (100%) to *J. chinensis* var. *procumbens.*									
Juniperus chinensis/ tsukusiensis	ploidy	cp source	ITS classif.	Heterozygous sites												
15469 chin 'Obelisk'	4*x*	*chinensis*	*chinensis*	212R		392S										
15470 chin 'Iowa' 'Globosa'	4*x*	*chinensis*	*chinensis*													
15478 chin Pfitzer 'Plumosa Aurea'	4*x*	*chinensis*	*chinensis*						960Y							
15452 chin sargentii 'Glauca'	2*x*	*tsukusiensis*	*chinensis*		315K											
15466 chin Pfitzer 'Blaauw'	2*x*	*tsukusiensis*	*chinensis*					533K								
15473 chin Pfitzer 'Shimpaku'	2*x*	*tsukusiensis*	*chinensis*		315K					1026R						
15477 chin Pfitzer 'Globosa Cinerea'	2*x*	*tsukusiensis*	*chinensis*				396Y					,				
J. chinensis hybrids	ploidy	cp source	ITS classif.	Heterozygous sites												
15461 chin 'Aurea'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid		212K		350R		431R					1093Y		11489Y
15465 chin 'Jacobiana'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid				351Y							1094S	1137R	1149Y
15471 chin 'Shepherdii'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid	201K 202R		301R										
15432 chin 'Keteleerii' ~= 'Kuriwao Mist'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid	179Y	212K	238Y 230M	351Y 361R	389S	428Y	614Y 615S	883Y	986Y	1073W		1145R	1149W
15467 chin 'Robusta Glauca'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid	201K	212K		351Y	389S	531S	545K 552R	639Y	784Y	986Y	1034R	1035K	1192M
15458 chin 'Lombarts'	4*x*	*chinensis* v. *sargentii*	*chinensis* hybrid	201K 202R	212K		351Y	389S		545K 552R	639Y	784Y	986Y		1035K	1192M
15427 chin 'Belvedere'	3*x*	*chinensis* v. *sargentii*	*chinensis* hybrid		212K		351Y	389S		606M	665Y	784Y	986Y		1073M	1149W
15441 chin 'Kuriwao Mist'	4*x*	*J. sabina* v. *balkanensis*	*chinensis* hybrid	179Y	212K	238Y	351Y 361R		428Y			986Y	1073W	1137R	1145R	1149W
15488 chin 'Mathot'	4*x*	*J. sabina* v. *balkanensis*	*chinensis* hybrid		212K							986Y	996M	1035K	1073W	
15484 chin 'Kek'	4*x*	*chinensis*	*chinensis* hybrid	201K 202R	212K		350R 351Y	389M				986Y			1073W	
15426 chin 'Savill Sentinel **ID = *Cupressus gigantea* (hybrid)**	2*x*	*Cupressus gigantea x ?*	*Cupressus gigantea*	289K	344Y	387R	467R	794K	977M	979R	1179Y	794K				
15464 chin 'Spartan' **ID = *Juniperus virginiana***	2*x*	*J. virginiana*	*J. virginiana*	742K	1019K											