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Using Random Amplification of Polymorphic DNA for a Taxonomic Reevaluation of Pfitzer Junipers

Alice Le Duc[1], Robert P. Adams[2], and Ming Zhong[3]
Department of Horticulture, Forestry and Recreation Resources, 2021 Throckmorton Plant Science Center, Kansas State University, Manhattan, KS 66506-5506

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Abstract. **Van Melle (1947) proposed that juniper cultivars of the Pfitzer Group were of hybrid origin and ascribed the name *Juniperus ×media* Melle. This purported hybrid of *J. chinensis* L. × *J. sabina* L. has not been accepted unanimously by the horticultural community. Random amplified polymorphic DNAs (RAPDs) were used to analyze and establish new evidence for the hybrid origin of the Pfitzer Group, using both parents and seven cultivars of the Pfitzer Group. Principal coordinate analysis (PCO) of 122 RAPD bands demonstrated that samples of *J. chinensis* cluster tightly together, as do the *J. sabina* samples. Cultivars of the Pfitzer Group lacked affinity with either species, but stood apart as a distinct cluster. The data support Van Melle's conclusion that the Pfitzer Group is separate from *J. chinensis* and indicate hybrid origin from parents *J. chinensis* and *J. sabina.* We recognize *Juniperus ×pfitzeriana* (Späth) Schmidt [Pfitzer Group] as the correct name for cultivars of Pfitzer junipers. *Juniperus ×media,* proposed by Van Melle, was rendered illegitimate because of the earlier name *J. media* V.D. Dmitriev.**

Historically, cultivars of the Pfitzer Group have been ascribed to *Juniperus chinensis* (*Cupressaceae* Bartling). Van Melle (1947) studied an extensive number of both preserved and living specimens of both cultivated and noncultivated plants. He concluded that *J. chinensis* 'Pfitzeriana' was of hybrid origin (*J. chinensis* × *J. sabina*), and proposed the name *Juniperus ×media* for the purported hybrid. He recognized var. *pfitzeriana* (male), var. *globosa* (male), var. *arbuscula* (female), and var. *plumosa* (female). The probable origin of the Pfitzer Group was seed sent back to France in the 1860s by Armand David from Ho Lan Shan of "Inner Mongolia." Plants from the seed were grown extensively by French and Belgian nurserymen by the 1870s (Van Melle, 1947). 'Pfitzeriana' was selected by Spath Nursery in 1890s and has become one of the most commonly planted cultivars of juniper (Krüssmann, 1991).

Welch (1966) was one of the first proponents of the new nomenclature. Some universities were incorporating the new nomenclature into woody plant courses by the 1970s, but the horticultural community has been slow to adopt Van Melle's classification. Currently, some authors (Dirr, 1998; Flint, 1998) await proof before adopting *J. ×media* as the correct name for the Pfitzer Group. However, at least 14 important horticultural references have adopted Van Melle's treatment (Lewis, 1995). Krüssmann (1991), although adopting the new classification, qualified his position by stating that "Detailed cytological investigation could determine if it (Pfitzeriana) is a hybrid or a form of *J. sabina* ...". He recognized 28 cultivars in the Pfitzer Group.

Van Melle's name, *J. ×media,* is rendered illegitimate under Article 64.1 of the *International Code of Botanical Nomenclature* (Greuter, 1994), for it is a homonym of *J. media* V.D. Dmitriev (Czerepanov, 1973). Therefore, Dimitriev's use of the name *J. media* has priority. Lewis (1995) argued for the conservation of the name *J. ×media* because of its historical use, dating from 1947. The request was rejected and the name proposed by Schmidt (1983) of *J. ×pfitzeriana* has been accepted. The original Pfitzer plant, still alive at the Späth Arboretum, Berlin, Germany, has been assigned the denomination *Juniperus ×pfitzeriana* (Späth) Schmidt 'Wilhelm Pfitzer'.

A comparison of volatile leaf essential oils by Fournier et al. (1991) showed that *J.* 'Pfitzeriana' and several of its cultivars contained significant percentages of both bornyl acetate and sabinyl acetate, while *J. chinensis* contained only bornyl acetate and *J. sabina* only sabinyl acetate. This chemical evidence supported the argument for the putative hybrid origin of *J.* 'Pfitzeriana'. Adams and Demeke (1993) found that systematic relationships in *Juniperus* could be established based on random amplified polymorphic DNAs (RAPDs). The objective of this study was to use RAPDs as a tool to establish additional evidence for the hybrid origin, resolve some of the questionable relationships of the complex, and to determine the correct name for Pfitzer junipers.

Materials and Methods

Samples of *J. chinensis*, *J.* 'Wilhelm Pfitzer', *J.* 'Pfitzeriana Aurea', *J.* 'Pfitzeriana Glauca', *J.* 'Hetzii', *J.* 'Fruitlandii', *J.* 'Gold Coast', *J.* 'Kalley's Compact', *J. sabina*, and *J. sabina* 'Tamariscifolia' were collected for analysis. Voucher specimens have been deposited in Herbaria. Adams' collections are with the Gruver Science Research Center Herbarium (SRCG) and Le Duc's collections are at the Kansas State Univ. Herbarium (KSC) (Table 1).

Leaves were desiccated in silica gel (Demeke et al., 1992) in the field. DNA was extracted using the hot cationic hexadecyl trimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1987) with the addition of 1% (w/v) polyvinyl pyrrolidone (PVP) and Pronase E (150 µg). Polymerase chain reaction (PCR) was performed in a volume of 15 µL containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01% gelatine, and 0.1% Triton X-100, 0.2 mM of each dNTP, 0.36 µM Primers, 0.3 ng genomic DNA, and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, except genomic DNA, was run with each primer to check for contamination.

A MJ Programmable Thermal Cycler (MJ Research) was used for DNA amplification. The thermal cycle was: 94 °C (1.5 min) for initial strand separation, then 40 cycles of 38 °C (2 min), 72 °C (2 min), 91 °C (1 min). Two additional steps were used for final extension: 38 °C (2 min) and 72 °C (5 min). Amplification products were analyzed by electrophoresis on 1.5% agarose gels and detected by staining with ethidium bromide. The molecular weight marker was pGEM DNA (Promega) and the gels were photographed under UV light with Polaroid® film 667. Fourteen-mer primers (Table 2) gave several bright bands, had no false bands (in the controls), and yielded reproducible results in replicated analyses. The RAPD bands were scored by molecular weight and assigned a code based on primer number prefix and molecular weight category. In addition, the RAPD band intensity was scored as: 0 = no band; 4 = faint; 5 = medium; 6 = bright band, in reference to a gray tone standard. Nonlinear scoring was used to accentuate presence or absence. Simple presence or absence was found to be less sensitive than this semiquantitative scoring.

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[1]Assistant Professor; current address: 10180 Santa Ana Rd., Atascadero, CA 93422.
[2]Plant Biotechnology Center, Baylor Univ., Box 669 Gruver, TX 79040.
[3]Agronomy Dept., August 1st Agricultural College, Urumqi, Xinjiang, China.

Table 1. Vouchers for the specimens studied.

Species	Cultivar	Sample designation	Specimen origin	Collection identification
J. chinensis	Pyramidalis	C1	China	*Adams* 6764
		C2	China	*Adams* 6765
		C3	China	*Adams* 6766
J. ×pfitzeriana	Fruitlandii	FR	Kansas State Univ., tag on plant	*Le Duc* 353
	Gold Coast	GC	Nursery stock, Jenco Nursery	*Le Duc* 356
	Hetzii	HZ	University, typical Hetzii	*Le Duc* 350
	Kallay's Compact	KC	Nursery stock, Jenco Nursery	*Le Duc* 363
	Wilhem Pfitzer	PZ	Kansas State Univ., typical Pfitzer	*Le Duc* 352
	Pfitzeriana Aurea	AR	Nursery plant purchased at WalMart store	*Adams* 8237
	Pfitzeriana Glauca	G1	Nursery stock, Jenco Nursery	*Le Duc* 359
	Pfitzeriana Glauca	G2	Nursery plant purchased at WalMart store	*Adams* 8238
J. sabina		S1	Switzerland	*Adams* 7611
		S2	Switzerland	*Adams* 7612
		S3	Switzerland	*Adams* 7614
	Tamarisicifolia	TM	Nursery plant purchased at WalMart store	*Adams* 8236

Table 2. List of the primers used in this study for the random amplification of polymorphic DNA (RAPD) by PCR.

Code	Sequence (5´-3´)	Code	Sequence (5´-3´)
134	AAC ACA CGA G	249	GCA TCT ACC G
153	GAG TCA CGA G	250	CGA CAG TCC C
184	CAA ACG GAC C	265	CAG CTG TTC A
212	GCT GCG TGA C	268	AGG CCG CTT A
218	CTC AGC CCA G	327	ATA CGG CGT C
234	TCC ACG GAC G	346	TAG GCG AAC G
244	CAG CCA ACC G	347	TTG CTT GGC G

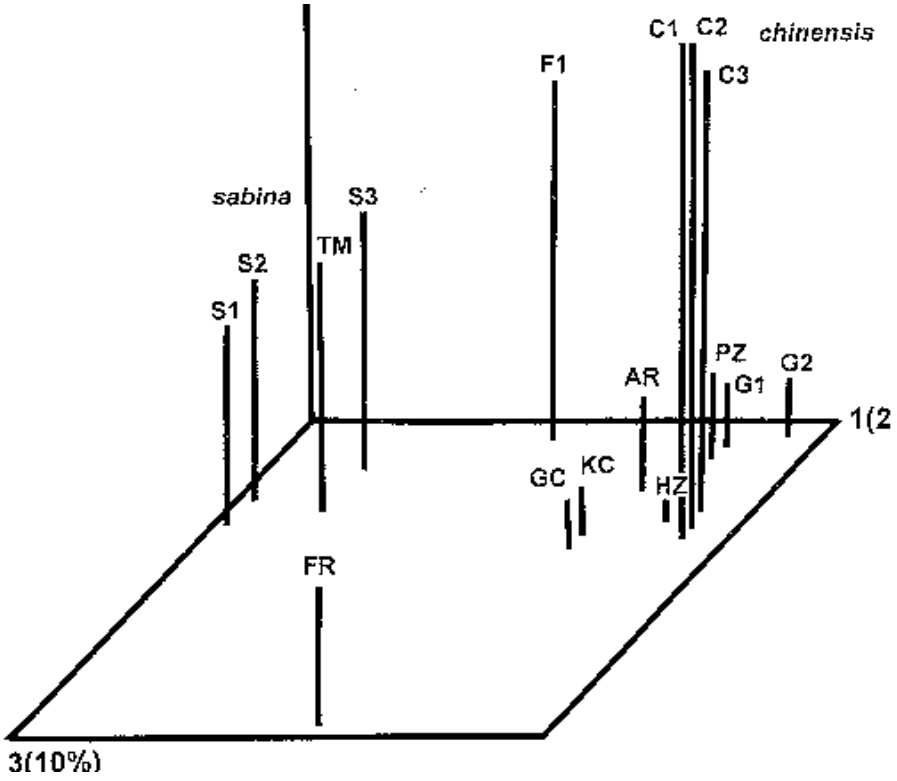

Fig. 1. PCO ordination of individuals of *J. chinensis* (C1, C2, C3), *J. sabina* (S1, S2, S3) and selected cultivars of the Pfitzer Group AR = 'Pfitzeriana Aurea', HZ = 'Hetzii', FR = 'Fruitlandii', GC = 'Gold Coast', G1 and G2 = 'Pfitzeriana Glauca', KC = 'Kallay's Compact' and PZ = 'Wilhelm Pfitzer' based on 122 RAPD bands.

Data were coded into a matrix by character values. Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric; Adams, 1975). Division by the character state range was tried, but was less informative than using the maximum observed character value (i.e., including zero in the range). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966).

Results and Discussion

The 14 primers gave a total of 122 usable RAPD bands. Computation and subsequent PCO ordination using the first three coordinate axes revealed several patterns (Fig. 1). The first three principal coordinates extracted 23%, 20%, and 10%, respectively, of the variance among the 15 operational taxonomic units (OTUs). All samples of *Juniperus chinensis* clustered tightly together. A similar grouping appeared among the *J. sabina* samples, with the *J. sabina* 'Tamariscifolia' nesting within the cluster. The purported *J. ×media* cultivars exhibited no affinity with either *J. sabina* or *J. chinensis*, but stood apart as a distinct cluster, with the exception of 'Fruitlandii' (FR). Principal coordinate 3 accounted for 10% of the variation and chiefly separated 'Fruitlandii' (FR) from the other junipers (Fig. 1). Coordinate 4 (7.64%) (not shown) separated 'Kallay's Compact' (KC) and 'Gold Coast' (GC) from the other junipers. No pattern was evident in any of the other coordinates.

Adams (1982) demonstrated that the most effective way to visualize both artificial and natural hybridization was to plot the first two axes that separate the putative parents. However, samples of *J. chinensis* and *J. sabina* from the Ho Lan Shan area were not available for comparison, and the *J. chinensis* and *J. sabina* samples are presented as proxies. Therefore, a synthetic F_1 hybrid was created for the purpose of this analysis by adding together all the bands that were present in either *J. sabina* or *J. chinensis*, because RAPD markers are inherited as simple dominants (Tingey and Tufo, 1993). The synthetic F_1 appeared midway between *J. chinensis* and *J. sabina* (Fig. 2). Nevertheless, ordination was similar to that obtained by Adams (1982) for both synthetic and natural hybridization. Adams (1982) noted that principal coordinates separate groups, and a group of hybrids arising from several different parents can form a group. Thus, whether intermediate plants constituted a hybrid or a third "intermediate" taxon is difficult to prove. The F_1 (Fig. 2) appeared midway between the clusters formed by the samples of *J. sabina* and *J. chinensis*, but was separated from the Pfitzer cluster. Although FR was somewhat removed from the other Pfitzer cultivars, it was still within the hybrid distance. These cultivars showed a strong cluster group. Note that the original purported hybrid was derived from a collection of seeds germinated, grown, and dispersed throughout the nurseries of Europe.

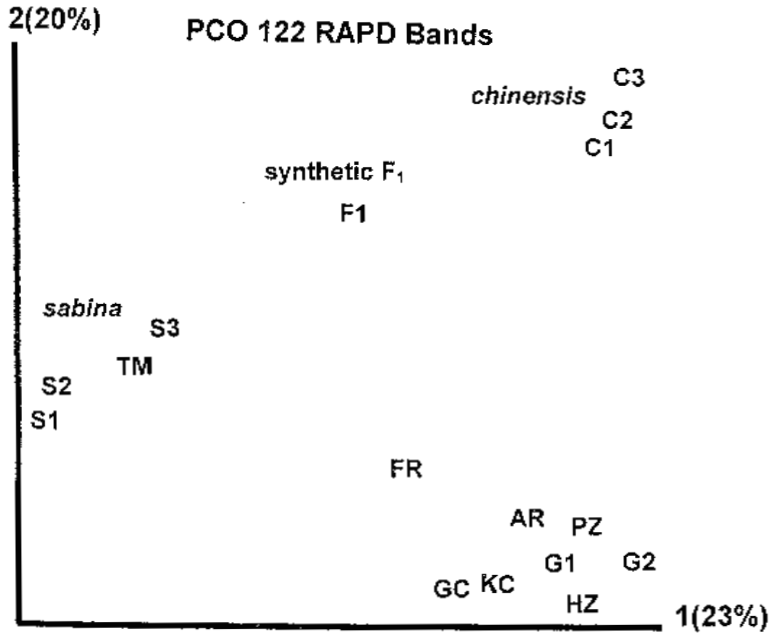

Fig. 2. Plot of the first two axes of PCO ordination showing the artificial F_1, the putative parents *J. chinensis* (C1, C2, C3), *J. sabina* (S1, S2, S3) and the natural hybridizations AR = 'Pfitzeriana Aurea', HZ = 'Hetzii', FR = 'Fruitlandii', GC = 'Gold Coast', G1 and G2 = 'Pfitzeriana Glauca', KC = 'Kallay's Compact' and PZ = 'Wilhelm Pfitzer'.

Ownbey (1950) listed three criteria in the classical treatment of the taxonomic status for hybrids of *Tragopogon* L. (*Asteraceae* Dumort.). These criteria were rephrased as questions: 1) Are the taxa natural groups, characterized by a combination of distinctive morphological features (*and/or DNA differences our addition*)?; 2) Are the taxa reproducing themselves under natural conditions?; 3) Is there free gene exchange between taxa? In the present case, criterion 1 is fulfilled because the DNA data support Van Melle's premise that the Pfitzer group of junipers is distinct from *J. chinensis*. Unfortunately, we lack field knowledge that would enable us to evaluate criteria 2 and 3.

Conclusion

The data provide additional molecular evidence that supports Van Melle's conclusion that the Pfitzer Group is separate from *J. chinensis* and indicates hybrid origin, the parents being *J. chinensis* and *J. sabina*. Based on the decision of the International Commission for the Nomenclature of Cultivated Plants, the correct name is *Juniperus* ×*pfitzeriana* (Späth) Schmidt [Pfitzer Group] and includes 'Fruitlandii', 'Gold Coast', 'Hetzii', 'Kallay's Compact', 'Wilhelm Pfitzer', 'Pfitzeriana Aurea', and 'Pfitzeriana Glauca'. Additional studies are needed to analyze the relationships of the remaining purported cultivars of the "Pfitzer Group."

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