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SURVEY OF *JUNIPERUS COMMUNIS* (CUPRESSACEAE) L. VARIETIES FROM THE WESTERN UNITED STATES USING RAPD FINGERPRINTS

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ABSTRACT

RAPD fingerprints were generated from seven wild populations of *Juniperus communis* L. to assess whether molecular data support subdivision into vars. *saxatilis, jackii* and *sibirica,* members of California Floristic Province, and *depressa,* a component of the Great Basin Floristic Province. Results from UPGMA and Neighbor Joining cluster analyses showed little correspondence between RAPD-derived distances and varietal boundaries. *Juniperus communis* var. *jackii,* in particular, was highly heterogeneous, lending support to the hypothesis that the characteristic growth habit of this serpentine dweller (elongated, sparsely branched lateral branches) is environmentally induced. In contrast to the RAPD results, nucleotide sequences of the ITS1 region of nuclear ribosomal DNA were identical in four of five var. *jackii* individuals sequenced, and the fifth exhibited three base substitutions.

Juniperus communis L. is a circumboreal species of juniper (Franco 1962) characterized by acicular leaves. Two varieties of *J. communis* (Cronquist et al. 1972; Adams 1993) are encountered in the western United States. *Juniperus communis* var. *depressa* Pursh is native to the Great Basin Floristic Province, extends northward into Alaska and eastward across much of Canada and the Great Lakes region, arching south along the east coast to North Carolina. *Juniperus communis* var. *saxatilis* Pallas occurs from British Columbia southward into California in the Cascade Ranges, North Coast Ranges, and Sierra Nevada, but also has a circumboreal distribution outside North America (Adams 1993).

The two varieties differ primarily in habit, leaf size and shape and width of the glaucous stomatal band on the adaxial leaf surface. Although both are low-growing, variety *depressa* develops a somewhat erect main stem whereas variety *saxatilis* is entirely prostrate. Leaf dimensions are ca. 1.0–1.6 mm broad × (6) 10–18 mm long (*depressa*) and (1.2) 1.5–1.8 mm broad × 5–10 (12) mm long (*saxatilis*) (Cronquist et al. 1972), and the glaucous stomatal band is as broad as, or narrower, than each green margin (*depressa*) or 2–3 times as broad as each green margin (*saxatilis*; Franco 1962).

In California, two other varieties are occasionally distinguished. *Juniperus communis* var. *jackii* Rehder (Rehder 1940) differs from var. *saxatilis* by having longer, more sparsely branched lateral branches and is encountered on serpentinite substrates of inland coastal areas in northern California and Oregon. *Juniperus communis* var. *sibirica* Rydb. is described as a very prostrate, almost matlike, form found on coastal bluffs and in the extreme northwest of California and southwestern Oregon, and as a disjunct population at Ebbett's Pass in the Sierra Nevada. According to Roof (1973), this variety is characterized by leaves that are more incurved, making it less prickly to the touch than *J. communis* vars. *jackii* or *saxatilis.* Adams (1993) and Cronquist et al. (1972) placed varieties *jackii* and *sibirica* in synonymy under *J. communis* var. *montana,* a name recently placed in synonymy under var. *saxatilis* (Farjon 1998). Our previous paper (Ashworth et al. 1999) used the older varietal epithet.

The purpose of this study was to make a preliminary assessment of genetic variability among the four varieties of *J. communis* in the Western United States and to examine whether molecular data favors one of the taxonomic schemes over another. Specifically, do the data support a subdivision into vars. *depressa* and *saxatilis,* and/or is there evidence supporting the recognition of varieties *sibirica* and *jackii*? A second goal was to ascertain whether the mats formed by these creeping junipers are genetically uniform (i.e., clonal) or harbor distinct genotypes. RAPD analysis was chosen as a quick and relatively inexpensive means of getting a fingerprint of the genome of plants from each of the native populations. This technique has been applied successfully to interspecific studies in *Juniperus* (Adams and Demeke 1993). Additionally, sequences of the ITS1 spacer region of the nuclear ribosomal DNA were generated for a subset of seven samples.

METHODS

Plant material. Plant material was gathered from seven wild *Juniperus communis* populations representing vars. *saxatilis* (*sa*A–*sa*C, *sa*G) and *depressa* (*de*D–*de*F). Under the alternative taxonomic scheme, populations *sa*A and *sa*G correspond to *J.*

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TABLE 1. COLLECTION LOCALITIES AND ALTERNATIVE VARIETAL DELIMITATIONS, WITH *JUNIPERUS COMMUNIS* SEGREGATED INTO TWO VARIETIES (*DEPRESSA* AND *SAXATILIS*, AS IN FLORA OF NORTH AMERICA COMMITTEE (1993) AND CRONQUIST ET AL. (1972); A) OR FOUR VARIETIES (*DEPRESSA* AND *SAXATILIS*, AS WELL AS *JACKII SENSU* REHDER AND "*SIBIRICA*" *SENSU* RYDBERG; B).

Sample designation	Varietal delimitation A	Varietal delimitation B	Collection locality
*sa*A1–*sa*A7	*saxatilis*	*sibirica*	OR, Curry County: Cape Sebastian
*sa*B1–*sa*B6	*saxatilis*	*jackii*	CA, Del Norte County: Gasquet Toll Road; two sites ca. 1 mile apart
*sa*C1–*sa*C2	*saxatilis*	*jackii*	CA, Humboldt County: Onion Mountain/Onion Lake intersection
*sa*G1–*sa*G3	*saxatilis*	*sibirica*	CA, Alpine County: Ebbett's Pass, Sierra Nevada
CV2	*saxatilis*	*saxatilis*	OR, Hood River County, Mt. Hood
CV5	*saxatilis*	*sibirica*	OR, Curry County, 'Gold Beach'
CV11	*saxatilis*	*sibirica*	CA, Del Norte County, 'Point St. George'
*de*D	*depressa*	*depressa*	UT, Iron County: between Cedar Breaks National Monument and Pan guitch
*de*E	*depressa*	*depressa*	UT, Iron County: Cedar Breaks National Monument
*de*F	*depressa*	*depressa*	NV, White Pine County: Wheeler Mtn., Great Basin National Park

communis var. *sibirica* and populations *sa*B and *sa*C to *J. communis* var. *jackii*. Table 1 summarizes collection details and taxonomic designations of each of the native populations (see Ashworth et al. 1999 for more complete information), as well as for three cultivated accessions originating from Mount Hood, OR (CV2), Gold Beach, OR (CV5), and Point St. George, CA (CV11), that were included in this study. These three plants grow at Rancho Santa Ana Botanic Garden but were established from cuttings collected in the wild. In a previous study that included both native and non-native *Juniperus* species (Ashworth et al. 1999) they clustered with the native *J. communis* varieties. CV2 represents *J. communis* var. *saxatilis* under all taxonomic systems presented here. CV5 and CV11 are var. *saxatilis* sensu Adams (1993) and Cronquist et al. (1972) and var. *sibirica* sensu Rydberg.

DNA analysis. Information on DNA extraction method, PCR reaction conditions and RAPD primer sequences are detailed in Ashworth et al. 1999. Bands were scored as present or absent by the first and last author. Average taxonomic distances generated from these binary scores were analyzed using the clustering algorithm UPGMA (Unweighted pair group method with arithmetic averages; Sneath and Sokal 1973) and Neighbor-Joining (NJ; Saitou and Nei 1987) available on PAUP* version 4.0 β1 (Swofford 1998). Effects of alternative measures of distance/similarity on clustering were explored using NTSYS version 2.0 (Rohlf 1993). Jaccard coefficients of similarity were calculated using the NTSYS 'SIMQUAL' module, and the cophenetic correlation coefficient was determined via the COPH and MXCOMP modules.

Sequences of the ITS1 spacer region were generated using the forward primer ITS5 (GGAAGTAAAAGTCGTAACAAGG) and reverse primer ITS4 (TCCTCCGCTTATTGATATGC; both primers from White et al. 1990). Amplification conditions consisted of 40 cycles, each with three successive phases of (1) 97°C for 1 min, (2) 48°C for 1 min, and (3) 72°C for 2 min, followed by a final extension time of 7 min at 72°C. Double-stranded template was purified by precipitation in polyethylene glycol/2.5 M NaCl (Morgan and Soltis 1993; Johnson and Soltis 1995) with a 70% and 95% EtOH wash. Single-stranded DNA template was generated by cycle sequencing with incorporation of dye terminators (PRISM™ Dye Terminator Cycle Sequencing Kit with AmpliTaq®; Perkin-Elmer, CT). Settings were 25 cycles of 0.5 min at 95°C, 0.25 min at 50°C and 1 min at 60°C. The resulting product was purified by ethanol precipitation (Sambrook et al. 1989) and electrophoresed on a 6% polyacrylamide gel (Sequagel®) in an Applied Biosystems Model 373A Automated Sequencer. Sequences were proofed and assembled using Sequencher 3.0 (Gene Codes Corporation, Inc., Ann Arbor, MI).

RESULTS

Of 65 primers screened for RAPD analysis, five primers showing scorable and reproducible banding patterns were entered into the final analysis. Scorable bands per primer ranged from one (UBC-329) to nine (UBC-244), with a total of 27 bands scored for 24 individuals. Identical banding patterns were found for *sa*A1, *sa*A3 and *sa*A4, with *sa*A2 differing by a single band.

Figure 1 shows the UPGMA and NJ phenograms generated from distance matrices derived from the RAPD scores. The UPGMA phenogram reveals six main clusters (#1–6), ranging in average within-cluster distance from 0.065 (cluster 4) to 0.273 (cluster 2). CV2 is the most distant accession. Clusters 6 and 5 are linked at a distance of 0.244, with cluster 4 attaching next (0.319), then cluster 3 (0.357), cluster 2 (0.370) and cluster 1 (0.395). Cluster 6 comprises mostly var. *sibirica* (*sa*A1–4, plus CV5, CV11) but also *sa*B4, cluster 5 includes the remaining two members of population *sa*A

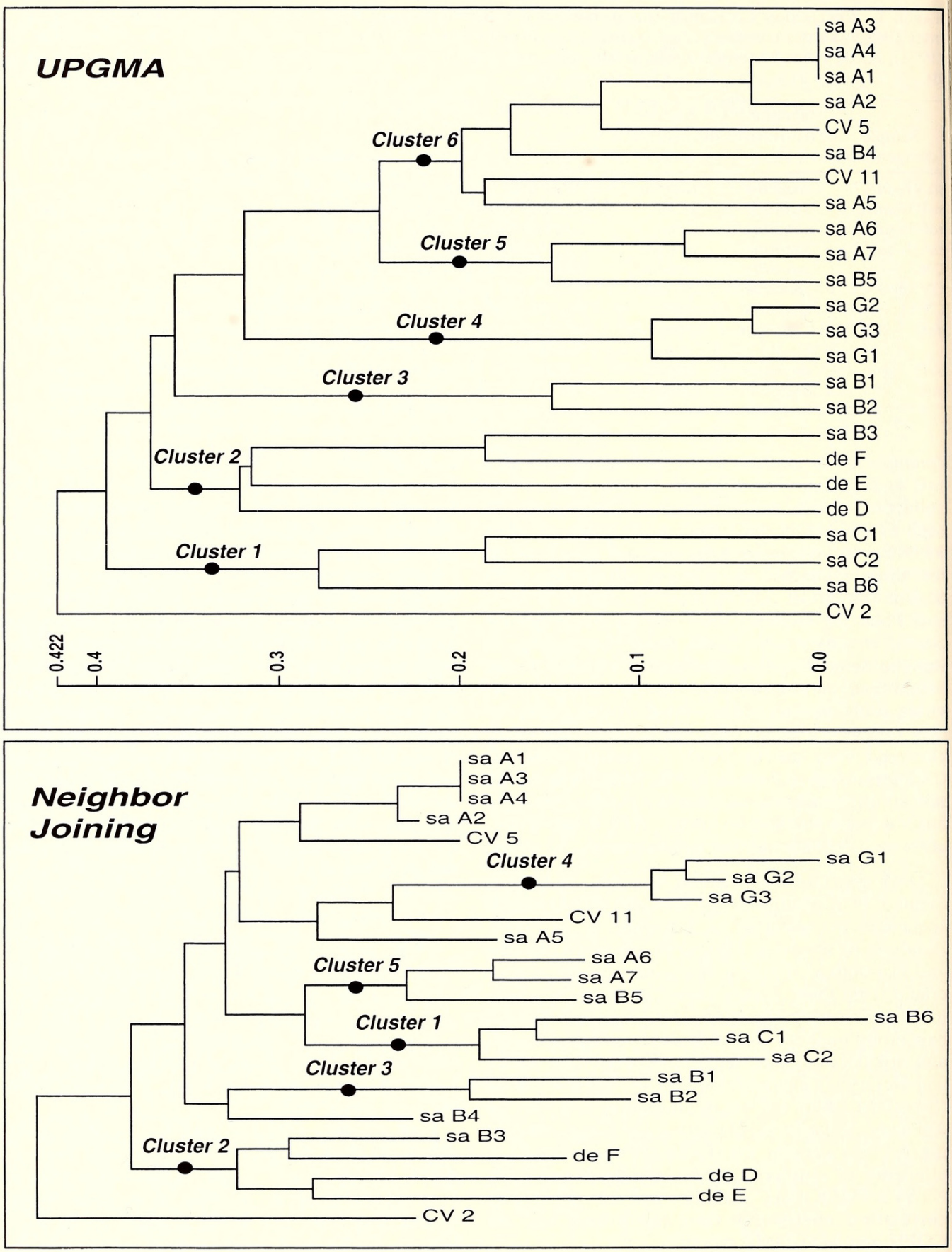

FIG. 1. UPGMA and Neighbor Joining phenograms generated from distances derived from RAPD data of 24 *Juniperus communis* accessions from the western United States.

(*sa*A6, *sa*A7) and also *sa*B5. Cluster 4 comprises population *sa*G, the Sierra Nevadan representatives of var. *sibirica.* Members of population *sa*B (var. *jackii*) appear in five of six clusters, including cluster 2 (*sa*B3) which contains all three accessions of var. *depressa.* Only clusters 1 and 3 contain exclusively var. *jackii.*

UPGMA clustering based on Jaccard coefficients of similarity resulted in identical cluster composition but an altered cluster hierarchy: cluster 3 is the most distant (0.36), followed in order of increasing similarity by CV2, cluster 4, cluster 1, cluster 2, cluster 5 and cluster 6. The matrix correlation coefficient indicates a good fit of distances derived from the phenogram to the original distance matrix.

Five of six clusters present in the UPGMA phenograms are also identified by the NJ algorithm. Three main differences emerge from a comparison of the UPGMA and NJ phenograms: (1) *sa*B4 resides in cluster 6 (predominantly var. *sibirica*) in the UPGMA tree but near cluster 3 (var. *jackii)* in the NJ tree; (2) population *sa*G, which forms a separate cluster below the bifurcation of clusters 5 and 6 in the UPGMA tree, inserts within cluster 6 in the NJ tree; and (3) clusters 1 and 5 are closest to each other in NJ but placed most distantly in the UPGMA analysis.

Of the seven ITS 1 sequences, identical sequences were found for *sa*B1, *sa*B3–5, and *sa*G1. Only CV2 and *sa*B6 each exhibited three autapomorphic base substitutions, and CV2 additionally had an insertion of three nucleotides.

Discussion and Conclusion

Regardless of the clustering algorithm or distance measure used, our RAPD fingerprint data are unable to clarify relationships among the four *J. communis* varieties *depressa, jackii, saxatilis* or *sibirica.* This is a consequence primarily of the markedly heterogeneous population *sa*B (*saxatilis/jackii*), which suggests that the *jackii* morphology (sparsely branched, elongated branches) is an environmentally induced growth form. Our data thus support Adams (1993) and Cronquist et al. (1972) who place the variety in synonymy under var. *saxatilis* on the grounds that the *jackii* habit disappears under common garden conditions (p. 15, Adams (1993)). Kruckeberg (1967) cites *J. communis* as an example of a substrate-indifferent ("bodenvag" *sensu* Unger 1836) serpentine dweller but makes no mention of morphological differences between serpentine and non-serpentine plants. It is well documented that the serpentine environment has a major impact on plant growth and adaptation, although the soil substrate is no longer seen as the only factor responsible. Instead, indirect effects, such as greater light availability, also exert a strong selective force (Baskin and Baskin 1988; Gankin and Major 1964). The elongated, sparsely branched habit of var. *jackii* may thus be the result of reduced competition from other vegetation and plentiful light.

The integrity of var. *saxatilis* is contradicted by the fact that the *saxatilis* accessions in this study are never united in a single cluster distinct from var. *depressa.* The proximity of clusters 4, 5 and 6 in the UPGMA analysis lends some support to var. *sibirica,* although this is weakened by the presence of *sa*B4 and *sa*B5. In the NJ analysis cluster 5, with its two *sibirica* representatives *sa*A6 and *sa*A7, is more similar to non-*sibirica* cluster 1 than to the other *sibirica* accessions.

Interestingly, the NJ analysis causes population G to cluster with *sibirica* representatives, consistent with its *sibirica*-like growth habit and in contrast to its geographic origin (Sierra Nevada). Although geographically close to the Great Basin variety *depressa,* none of the analyses presented here show a close association between var. *depressa* and population *sa*G.

Nucleotide substitutions and an insertion in the ITS1 region were revealed only in *sa*B6 and CV2, corroborating their basal placement on the UPGMA phenogram. By contrast, *sa*G1 and four members of the heterogeneous *sa*B population (*sa*B1 and *sa*B2–4) exhibited identical sequences, showing a lack of concordance between RAPD-derived distances and ITS1 sequence divergence.

The absence of support from our RAPD data for a distinction between vars. *saxatilis* and *depressa* is surprising but may be a function of relatively few markers in relation to the number of genotypes studied. A higher marker to genotype ratio and a greater sampling density might clarify some of the variation encountered.

Although our data are unable to provide answers to our taxonomic questions, they nonetheless give insight into the genetic composition of juniper mats. Individuals of var. *saxatilis* population A originated from various positions around the periphery of a large mat. The identical fingerprints of individuals *sa*A1, *sa*A3 and *sa*A4 suggest that this part of the mat is clonal (*sa*A2 differs only by a single band), but individuals *sa*A5–*sa*A7 have distinct fingerprints. This mat is therefore a combination of clonally-spread and seed-derived individuals. Population B was collected from two nearby mats. This makes the great diversity of distinct fingerprints even more surprising and we speculate whether individuals from this population constitute a hybrid swarm.

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