INFRASPECIFIC TERPENOID VARIATION IN *JUNIPERUS SCOPULORUM*: PLEISTOCENE REFUGIA AND POST-PLEISTOCENE RECOLONIZATION

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ABSTRACT

The patterns of leaf terpenoid variation are examined from throughout the range of *Juniperus scopulorum*. The oils of all of the central Rocky Mountain populations are very uniform, suggesting that these populations were mostly displaced to lower elevation sites during the Pleistocene. Populations in Wallowa, Ore. and British Columbia (BC) (northern Rocky Mountains) are differentiated from the central Rocky Mountain populations. It is postulated that a refugium for this germplasm was in the Wallowa Mtns., Ore. and that the glaciated populations of BC were re-colonized by seeds from the Wallowa refugium. *Phytologia 93(1): 3-12 (April 1, 2011).*

KEY WORDS: *Juniperus scopulorum*, *J. blancoi, J. maritima, J. virginiana*, leaf terpenoids, geographic variation, Pleistocene refugia, recolonization, Wisconsin glaciation.

In 1983, I published an analysis of geographic variation in leaf oils of *J. scopulorum* (Adams, 1983). It was reported that the samples from Puget Sound and Vancouver Island were the most distinct of all populations and that their oils were actually more similar to *J. virginiana* than *J. scopulorum* (Fig. 13, Adams, 1983). Subsequently, Adams (2007) recognized the Puget Sound and Vancouver Island (including the Strait of Georgia) plants as *J. maritima* R. P. Adams based on the combined use of terpenoids, morphology and nrDNA sequence data. In addition, analyses of nrDNA from herbarium specimens of the juniper from Serranias del Burro, Mexico (Adams, in prep.) indicate that the latter is a relictual population of *J. virginiana*, not *J. scopulorum* as treated in Adams (1983, 2008). The inclusion of these two species (*J. maritima, J. virginiana*) in the computation of ANOVA and SNK (Adams, 1983) biased the character weighting (F-1 weights). With this new knowledge of speciation in *Juniperus* from DNA sequencing, it is appropriate to re-examine variation in *J. scopulorum* with the aforementioned taxa excluded from ANOVA and SNK analyses.

MATERIALS AND METHODS

Plant material: (species, population acronym, location, vouchers): *J. blancoi*: BO, El Oro, Mexico, 14 mi S of El Oro, 4.5 mi S of Carmona, 8460', *Adams 1486-1500*; BS, El Salto, Mexico, 3 mi S of El Salto on road to Guadalupe along stream, ca 8500', *Adams 1455, Zanoni 2766-2775*; *J. maritima*: PW, Bayview St. Park and Whidbey Island, WA, 3-100', *Adams 1740-1747;* VB, Vancouver Island, BC, Mill Bay and Cowichan Bay, seaside, 3-10', *Adams 2465-2477*; *J. scopulorum*: SI, 1 mi W of Soda Springs, ID on US30, 5779', *Adams 1662-1676*; TU, Thistle Utah, 5250', *Adams 1677-1688*; LN, 5.7 mi E of Lamoille, NV, 7500', *Adams 1708-1722*; WO, 3 mi W. of Wallowa, Ore., 2950', *Adams 1725-1739*; MB, Manning Park, BC, on Talus slope, on Canada Hwy 3, 7.4 mi E of Manning Park Lodge, 3500', *Adams 1749-1763*; TB, 3.6 mi E of Telkwa, BC on BC Hwy 16, 2100', *Adams 1765-1779*; WB, Williams Lake, BC, 3.7 mi S of William Lake on BC Hwy 97, 2100', *Adams 1780-1794*; DC, Dutch Creek, BC, 3.5 mi S of Fairmont, 2600', *Adams 1811-1825*; KM, Kalispell, MT, 4.5 mi. S of US 83 & MT 208 jct. on US 83, N of Lakeside, 2960', *Adams 1826-1840*; BM, Butte, MT, 3-4 mi. W of Butte, on I90, 5700', *Adams 1841-1855*; BR, Bridger, MT, 16.6 mi. N of Bridger on US310, 4100', with *J. osteosperma* and *J. horizontalis, Adams 1864-1877*; MM, Missouri River, MT, 3.4 mi. S of Missouri River Bridge on US191, 2700', *Adams 1881-1896*; AN, Amidon, ND, Burning Coal Seam Park, 12 mi NW of Amidon, 3200', *Adams 1902-1919*; NW, Newcastle, WY, on US 85, 0.9 mi N of jct of US 85 & WY 16, 4310', *Adams 1920-1934*; SC, Stove Prairie Landing, Poudre River, 15.8 mi. W of jct. US 287 and CO 14, 18 mi. W of Ft. Collins, CO, *Adams 1935-1949;* RN, Raton, NM, 2 mi. N of Raton, NM on I25, 6800', *Adams 1965-1979*; DC, Durango, CO, w of town on hill, 6600', *Adams 2010-2024*; NA, Nutrioso, AZ, on US 180, 2 mi. N of Nutrioso, 7900', *Adams 2052-*

2066; OA, Oak Creek Canyon, AZ, 16.2 mi. N of Sedona, on AZ 89A, 6500', *Adams 2097-2111*; CN, Charleston Peak, NV, at Ranger Station, 20 mi W of US 85 on NV 39, 7400', *Adams 2154-2167*; CU, Cedar City, UT, 7.5 mi E of Cedar City on UT 14, 6900', *Adams 2173-2187*; RD, Ruidoso, NM, between Ruidoso and NM 24 on US 70, 6600', *Adams 2233-2246*; CM, Colonia Pacheco, Mexico, 3 mi. E of Colonia Pacheco on Rio Piedras Verde, 6950', *Adams 2501-2515*; *J. virginiana/ scopulorum*: PT, Palo Duro Canyon, TX, 1 mi E of Tanglewood Lake dam on the John Currie Ranch in deep canyons along the Prairie Dog Fork of the Red River, ca. 3300', *Adams 2263-2277*; *J. virginiana*: AO, Altus, OK, Quartz Mtn. St. Park, Lake Altus, OK, *Adams 2323-2337*; WD, Washington, DC, 10 mi. E of Dulles Airport, *Adams 2409-2423*; SM, Serranias del Burro, Mexico, Arroyo de la Zorro Canyon, ~6000', *Adams 2424-2438*; Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU). The distribution of *J. scopulorum* (*sensu stricto*) and populations sampled are shown in figure 1.

Isolation of Oils - See Adams (1983). Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by ANOVA and SNK (Student-Newman-Keuls) analyses (after Steele and Torrie, 1960). Gower or Manhattan metric (Adams, 1975; Gower, 1971) were computed among all populations using character weighting of F-1 (F from ANOVA). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

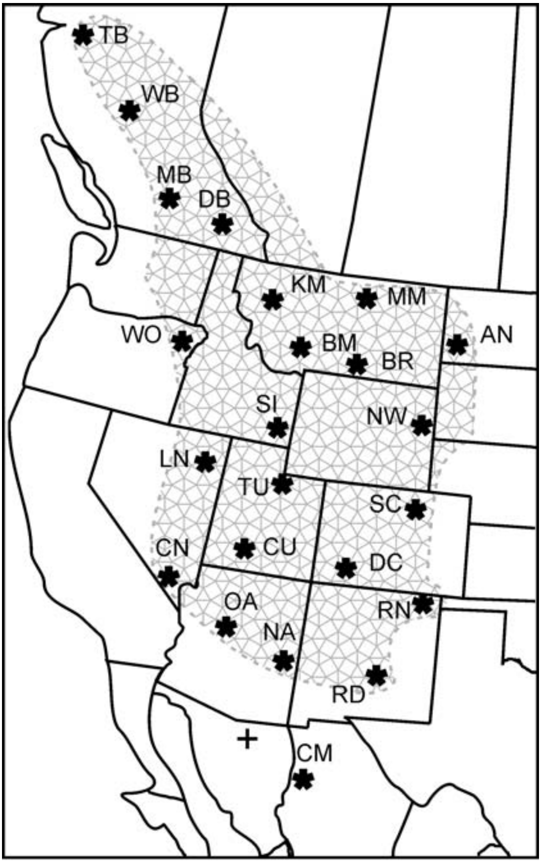

Figure 1. Distribution of *J. scopulorum* (*sensu stricto*) and populations sampled in this study. The + symbol in Sonora, Mexico is an outlier population.

RESULTS AND DISCUSSION

SNK analyses of 177 leaf terpenoids of *J. scopulorum* revealed 33 compounds with F ratios (from ANOVA) and the largest population value greater than 0.5% concentration. The similarity matrix factored by PCO (Principal Coordinate Analysis). Nine eigenroots accounted for 80% of the variance among populations. The eigenroots appeared to asymptote after the first five: 30.18, 15.57, 8.32, 6.43, 5.20%. Contoured similarities are shown in figure 2. The

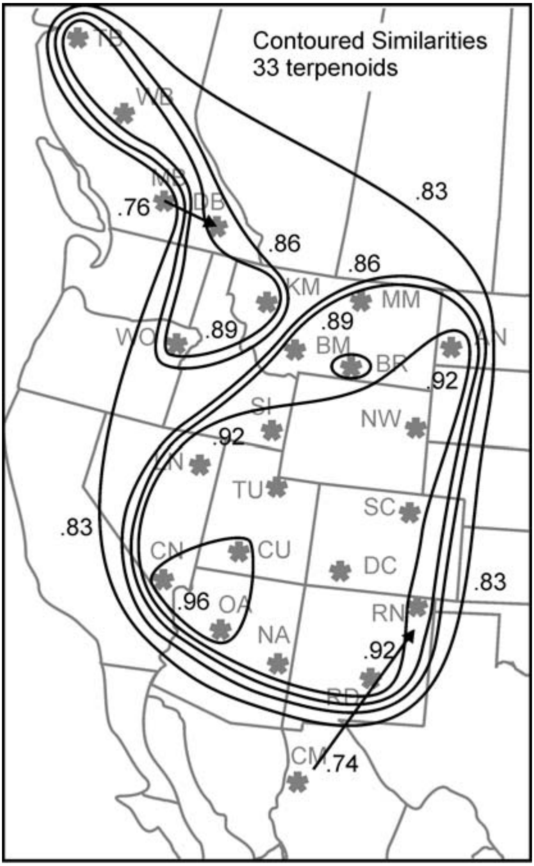

Figure 2. Contoured similarities among populations of *J. scopulorum* based on 33 terpenoids. See text for discussion.

oils from plants at Oak Creek, AZ (OA), Charleston Peak (CN) and Cedar City, UT (CU) were nearly identical (0.96 similarity, Fig. 2). The oils of all of the central Rocky Mountain populations were found to be very similar (0.89-.96, Fig. 2). However, the populations from the north-western Rocky Mountains form a separate group (Fig. 2, WO, KM, DB, WB, TB). The break between the two groups is quite distinct between Kalispell, MT (KM) and Butte, MT (Fig. 2).

The Colonia Pacheco, Mexico (CM) population was an outlier and had its highest similarity to the oil from Raton, NM (RN) with a similarity of 0.74 (Fig. 2). Because *J. blancoi* var. *mucronata* (R. P. Adams) Farjon grows near Yecora (about 140 air miles southwest of Colonia Pacheco), it is possible that the CM plants contain germplasm from *J. b. var. mucronata*. This question is beyond the scope of this paper and is currently being investigated (Adams, in prep.).

The oil of the Manning Park, BC population (MB) is another anomalus population that is joined to the nearby Dutch Creek, BC population (DB) by similarity of 0.76 (Fig. 2). There seems no apparent explanation for this divergence.

Pleistocene Patterns

The late Wisconsin maximum ice advance is shown in figure 3 (based on Flint, 1971 and Crandell, 1971). All of the Canadian *J. scopulorum* populations were glaciated. In addition, the Kalispell (KM), Missouri River (MM) and Amidon, ND (AN) populations were probably exterminated. Other populations (BM, BR and NW) were likely displaced by boreal forests and tundra (Flint, 1971; Porter, 1971). *Juniperus scopulorum* is a lower montane species, with the widespread lowering of vegetation zones, it likely moved to lower, drier habitats throughout most of the central Rocky Mountains. Adams (1983) reviewed the literature on packrat middens and pollen profiles; the study suggested that life zones descended 300 to 1100 m throughout the southwest and Great Basin from 13,500 to 10,000 ybp. The current separation of *J. scopulorum* and *J. virginiana* appears to have been bridged with the eastward expansion of *J. scopulorum* and the western expansion of *J. virginiana*. Trees of *J. scopulorum* are currently growing in ravines in northeastern New Mexico, while *J. virginiana* has now migrated westward into the Canadian River canyons in the Texas panhandle. The population of *J. scopulorum/ virginiana* in Palo Duro Canyon resembles both species and is likely a relictual stand of hybrid origin (Adams, 1983).

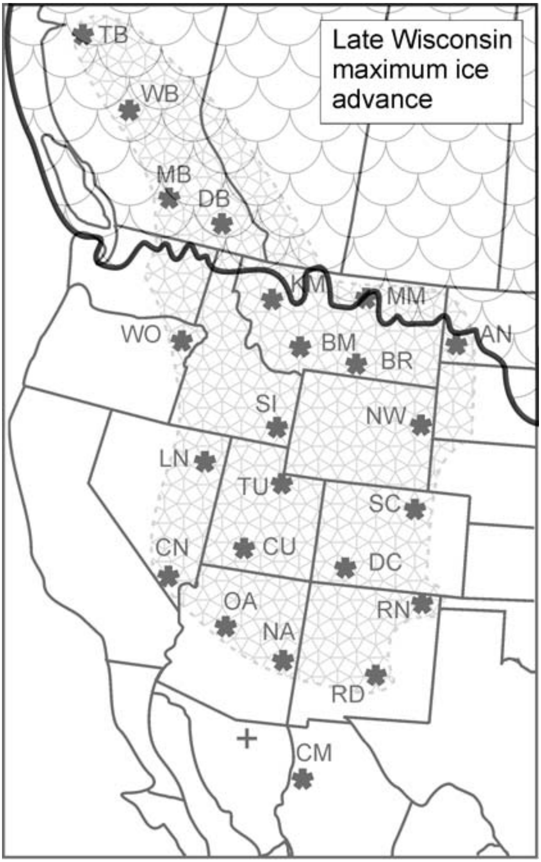

Figure 3. Maximum ice advance during the late Wisconsin (based on Flint, 1971; Crandell, 1971).

With the retreat of the Wisconsin glacial ice, and the subsequent altithermal period 9000 to 5000 ybp (Wells, 1970), *Juniperus* expanded into the drying, higher elevation habitats that it occupies today. Figure 4 shows the proposed post-Pleistocene re-colonization of the northern portion of the range of *J. scopulorum*. Based on the terpenoid data, the BC populations could have been recolonized by seed from the Wallowa Mtns. refugium (WA, Fig. 4) and thence northward to the present day northern-most population at Telkwa, BC (TB). At Telkwa, *J. scopulorum* is found on dry, southeast facing slopes (ca. 45º - 60º).

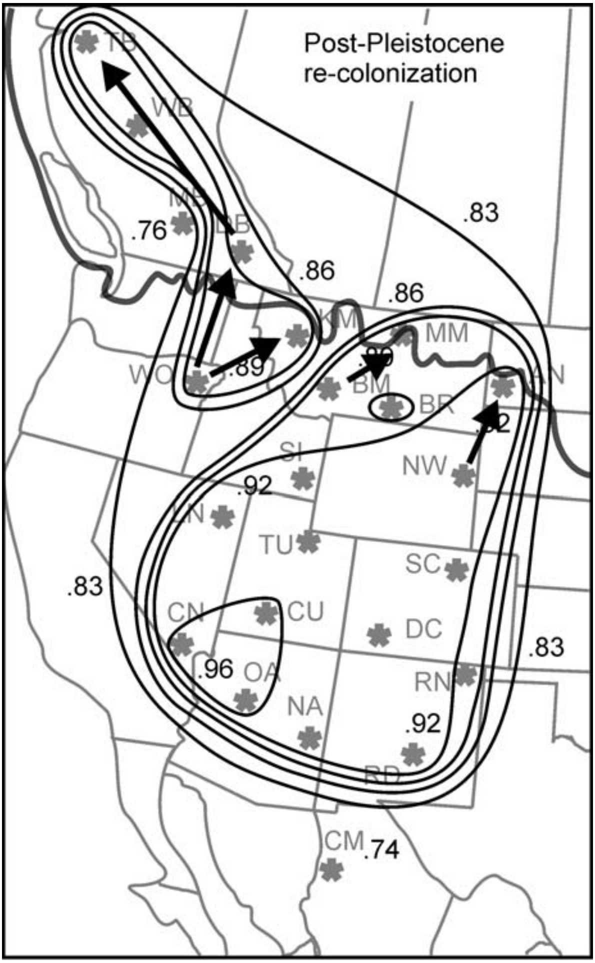

Figure 4. Proposed post-Pleistocene re-colonization of the northern range of *J. scopulorum* during the post-glacial period (9000 - 5000 ybp).

The Kalispell, MT (KM) population shares the divergent terpene nature with the BC and WA populations and is postulated to have arisen by seed from the Wallowa population. Of course, the Wallowa population may have been displaced lower, and perhaps a bit to the south during the Wisconsin. The Amidon, ND (AN) population is typical of the central Rocky Mountains and seems likely to have been derived by seed from the nearest *J. scopulorum* population (perhaps near Newcastle, WY, NW) or any of the scarp land *J. scopulorum* populations to the south.

There is not evidence from the terpene data for the extinction of the central Rocky Mtn. populations; it is likely they persisted near the present day locations at lower, drier elevation sites.

Juniperus scopulorum is presently found along running steams in the disjunct populations of northern Mexico. It is possible that the latter have been isolated since the Pliocene (Martin and Harrell, 1957).

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