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# Geographic Variation in the Volatile Terpenoids of *Juniperus monosperma* and *J. osteosperma*

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Key Word Index—*Juniperus monosperma*; *J. osteosperma*; terpenoids; geographic variation.

Abstract—The steam volatile leaf terpenoids of *Juniperus monosperma* and *J. osteosperma* were analyzed by principal coordinate analyses. *Juniperus monosperma* was found to have the least amount of populational variability of any juniper in North America analyzed to date. The major trend found was a east–west cline. Analyses of *J. osteosperma* populations revealed typical infra-specific variation with a major north–south trend. Some limited hybridization might be possible between the taxa south of Shiprock, New Mexico. In addition, several terpenoids previously tentatively identified or unidentified are now identified.

## Introduction

*Juniperus monosperma* (Engelm.) Sarg. var. *monosperma* is a one-seeded juniper distributed in the southwestern United States (see Adams and Zanoni, 1979 for distribution map). Although often reported from Mexico, it has not been confirmed using terpenoids (Zanoni and Adams, 1976; Adams *et al.*, 1981) or morphology (Zanoni and Adams, 1975). The variety *J. monosperma* var. *gracilis* Martinez, from Mexico, differs in having bark that exfoliates in rectangular plates, thin foliage, and smaller fruit (female cones). Re-examination of the relationship of var. *gracilis* to var. *monosperma* has recently resulted in the recognition of var. *gracilis* at the species level [Adams, in press; *J. angosturensis* (Mart.) R. P. Adams] and will not be further discussed in this paper.

Vasek and Scora (1967) reported two races of *Juniperus monosperma* ('A' and 'B') based on terpenoids. Upon reexamination, Adams and Zanoni (1979) found that 'A' was *J. erythrocarpa* Cory and 'B' was *J. monosperma*. The composition of the steam volatile leaf oil of *J. monosperma* has been reported (Adams *et al.*, 1983).

*Juniperus monosperma* and *J. pinchotii* Sarg. have been reported to hybridize (Hall and Carr, 1968; Correll and Johnston, 1970). But hybridization between *J. pinchotii* and *J. monosperma* has not been substantiated in studies using both morphology and terpenoids (Adams, 1972, 1975a). In the course of this study, some plants morphologically intermediate between *J. monosperma* and *J. osteosperma* (Torr.) Little were found near Shiprock, New Mexico and their analyses are included in this study.

*Juniperus osteosperma* appears to be closely related to *J. monosperma* and, without female cones, the taxa are difficult to distinguish (Adams, in press). *Juniperus osteosperma* is also distributed in the southwestern United States [from Montana to California, see Vasek (1966) for a distribution map]. It is the most common juniper in Utah and called the Utah juniper. The composition of the steam volatile leaf oil of *J. osteosperma* has been reported (Adams *et al.*, 1983).

## Materials and Methods

Foliage samples consisted of 8–10 terminal branches taken from each tree. The branches were placed in plastic bags and immediately placed in field-trailer freezer. Herbarium and cone specimens were pressed and air-dried. All of the plant sampled are vouchered at BAYLU. Vouchers (location, collection numbers, population acronym) *J. monosperma*: north of Walsenburg, Colorado, 1950–1964, WC; south of Tres Piedras, New Mexico, 1980–1994, PN; southeast of Belen, NM, 1995–2009, BN; south of Shiprock, NM, 2025–2031, SN; north

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of Gallup, NM 2037–2051, GN; north of Nutrioso, Arizona, 2067–2081, NA; east of Flagstaff, AZ, 2082–2096, FA; east of Peach Springs, AZ, 2138–2152, PA; east of Alamogordo, NM, 2218–2232, AN; northeast of Van Horn, Texas, 2248–2262, VT; Palo Duro Canyon rim, TX, 2278–2292, PT; Tucumcari, NM, 2293–2307, TN; west of Kenton, Oklahoma, 2308–2322, KO; south of Canadian, TX, 2338–2351, CT; *J. osteosperma*: Thistle, Utah, 1689–1699, 1701–1705, UT; south of Shiprock, NM, 2032–2036 NM; Charleston Mountains, Nevada, 2168–2172, NV; southeast of Bridger, Montana, 1856–1860, MT; and Oak Creek, Canyon, AZ, 2117–2121, AZ. Seven individuals of *J. monosperma* that appeared morphologically intermediate to *J. osteosperma* were also sampled: south of Shiprock, NM, 2025–2031, SN.

Foliage for chemical analyses was kept at −20°C until steam-distilled (von Rudloff, 1967). The terpenoids were analyzed by gas chromatography on a 60 m ×0.5 mm SS WCOT column coated with PEG 20 M (see Adams 1975b for details). Although identification of the volatile leaf oils of *J. erythrocarpa* and *J. pinchotii* have been previously reported (Adams *et al.*, 1981), additional analyses were performed on a Finnigan Ion Trap (ITD), model 800, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB5, 0.26 mm i.d.×30, 0.25 micron coating thickness, fused silica column (see Adams, 1989, for standard operating conditions). Identifications were made by use of our volatile oil library, LIBR(TP), using both Finnigan library searches and retention times.

Analysis of variance (ANOVA) tests were performed using various combinations of the data sets as needed to generate F-1 character weights. Similarity measures were computed using F-1 character weights and mean character differences (MCD) as formulated by Adams (1975c) divided by the maximum value for the character (encountered over all the individuals). Principal coordinate analyses (PCO, Gower, 1966) were performed on the resulting similarity matrix. The populations were ordinated in three-dimensions or, as appropriate, the population score on a principal coordinate was plotted to produce a contour map. Hybridization was examined using PCO and then plotting the first two coordinates (Adams, 1982).

## Results and Discussion

Several compounds, previously unidentified in *J. monosperma* and *J. osteosperma* oils (Adams *et al.*, 1983), have now been identified [RRTs from Adams *et al.* (1983)]: RRT 0.204 = verbenene; RRT 0.315 = α-pinene oxide; RRT 0.339 = α-camphenal; RRT 0.437 = *trans*-carveol; RRT 0.860 "Acetate II" = 8-α-acetoxyelemol.

ANOVA using 14 *J. monosperma* populations and five *J. osteosperma* populations resulted in 49 terpenoids with *F*-ratios greater than 1.0 and present at greater than the 0.5% level (total oil). The average *F*-ratio was 30.43 ($P_{0.01} = 5.69$; $P_{0.05} = 4.99$). Principal coordinate analysis (PCO) of the similarity matrix produced three eigenroots that were larger than the average diagonal element (Veldman, 1966). In addition, the eigenroots appeared to asymptote after the third eigenvalue (Pimentel, 1979) so only the first three eigenroots will be discussed. The first three eigenroots accounted for 72.2, 9.1 and 3.7% of the total variance among the populations.

The major factor (72% of the variance) in this data set separates *J. monosperma* populations from *J. osteosperma* (Fig. 1). Although these two species are morphologically very similar, they differ greatly in volatile leaf oils. Note also the extremely tight cluster of *J. monosperma* populations and the diversity among the *J. osteosperma* populations. The only diversity seen in *J. monosperma* is that the SN population (Shiprock, NM) is ordinated a little towards *J. osteosperma*, see below. *Juniperus monosperma* is the most uniform *Juniperus* species examined to date.

The second principal coordinate (9%) separates *J. osteosperma* (Fig. 1) but no obvious pattern is seen. The third axis (4%) appears to separate the southern populations (AZ, NM, NV) from the northern populations (MT, UT). Additional population samples will be needed to adequately examine infraspecific variation in *J. osteosperma.*

ANOVA using 13 populations of *J. monosperma* (SN, was omitted due to possible hybridization with *J. osteosperma*) resulted in 25 compounds with *F*-ratios greater than 1.0 and greater than 0.5% of the total oil. The average *F*-ratio was only 2.81. This is very low compared to the average *F*-ratio in infraspecific studies of other juniper species: *J. erythrocarpa*, 13.2 (Adams, 1993a); *J. deppeana*, 12.5 (Adams *et al.*, 1984); *J. osteosperma*, 6.9 (this study); and *J. virginiana*, 10.8 (Adams, 1986).

PCO of the similarity matrix (13 *J. monosperma* populations) yielded eigenroots accounting for 33.2, 13.1, 11.7, 10.1, and 7.2% of the variance. Thereafter, the eigenroots

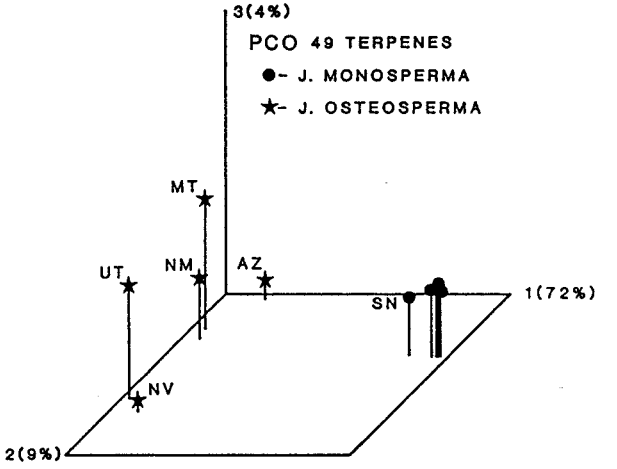


FIG. 1. PCO ORDINATION OF FIVE *J. OSTEOSPERMA* AND 14 *J. MONOSPERMA* POPULATIONS USING 49 TERPENES. Note the clear separation of the two species on the first axis.

appeared to asymptote. Only the first two eigenroots appeared significant by the Veldman test i.e. greater than the average value of the diagonal. Ordination reveals some populational differentiation with the eastern populations (KO, TN, CT, PT) somewhat differentiated from the western populations (Fig. 2). Notice also that the Gallup, NM (GN) population is separated on axis 3. Axis 2 chiefly separates the westernmost population (PA, Peach Springs, AZ).

A clearer picture can be seen by contouring the population scores on each axis onto a geographic map. Figure 3 shows fully 33% of the variation is due to an east–west trend. One factor that could explain the divergence of the easternmost

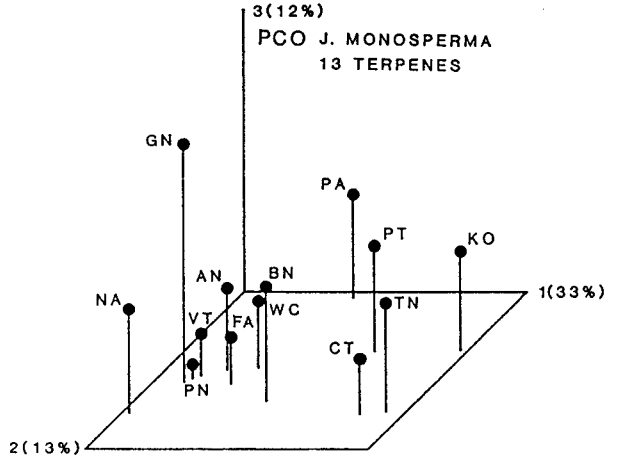


FIG. 2. PCO ORDINATION OF 13 *J. MONOSPERMA* POPULATIONS USING 13 TERPENES. The first axis tends to separate the easternmost populations (KO, TN, CT, PT). Notice the divergence of the Gallup, New Mexico (GN) and Peach Springs, Arizona (PA) populations.

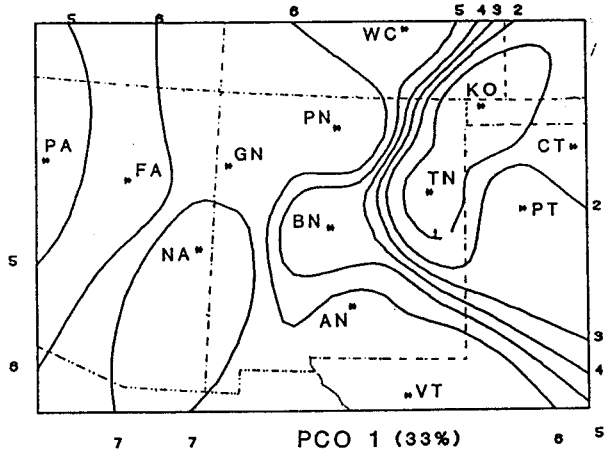


FIG. 3. CONTOURED PCO 1 SCORES FOR EACH POPULATION. The divergence of the eastern populations is clearly seen. These populations (CT, PT, KO, TN) are probably recent colonizations since the Pleistocene. Colonization is suggested from a refugium in central New Mexico (BN).

populations would be wide-spread introgression from *J. pinchotti.* An ANOVA was run using terpene data from *J. pinchotii* [Glen Rose, TX, Adams (1975a)] and 13 populations of *J. monosperma.* The resulting 34 compounds had an average *F*-ratio of 31.75. Similarities of *J. pinchotii* to *J. monosperma* populations were: WC (0.114); PN (0.100); BN (0.096); GN (0.112); NA (0.105); FA (0.123, highest); PA (0.104); AN (0.100); VT (0.99); PT (0.105); KO (0.094, lowest); CT (0.108); and TN (0.102). The average similarity between *J. pinchotti* and *J. monosperma* was 0.105, whereas the average similarity among *J. monosperma* populations was 0.965. The easternmost populations of *J. monosperma* (KO, CT, PT, TN) were no more similar to *J. pinchotii* than the westernmost populations (FA, PA, etc.). Clearly, if the divergence of the eastern populations was due to introgression from *J. pinchotti,* this would not be the case.

It would seem likely that the eastern New Mexico, northern Texas, and Oklahoma populations of *J. monosperma* originated since the Pleistocene because this area was covered with Pine and Spruce up to perhaps 12 000 ybp (Wells, 1970). These eastern populations may have been established by birds carrying seeds from central N. Mexico. The Walsenburg, Colorado population shows more affinity to the Tres Piedras, New Mexico (PN) population and may reflect a dispersal route from central, northern New Mexico to Colorado.

The second principal coordinate (13%) shows differentiation of the westernmost population (PA, Peach Springs, AZ) from the central region and some variation among the easternmost populations (Fig. 4). Analyses of the third axis (12%, not shown) primarily depicted the differentiation of the Gallup, NM (GN) population from all the other populations. No consistent geographic pattern was seen in the fourth axis, suggesting that it and additional axes are not significant.

As previously mentioned, field examination of plants in the area approximately 50 km south of Shiprock, New Mexico suggested that *J. monosperma* and *J. osteosperma* may be hybridizing in that area, where they are sympatric. Principal coordinate analysis (PCO) has been found to be an excellent tool for the analysis of putative hybrids (Adams, 1982; Adams and Kistler, 1991). Reference populations of *J. monosperma* (BN) and *J. osteosperma* (UT) were used in ANOVA to generate a set of

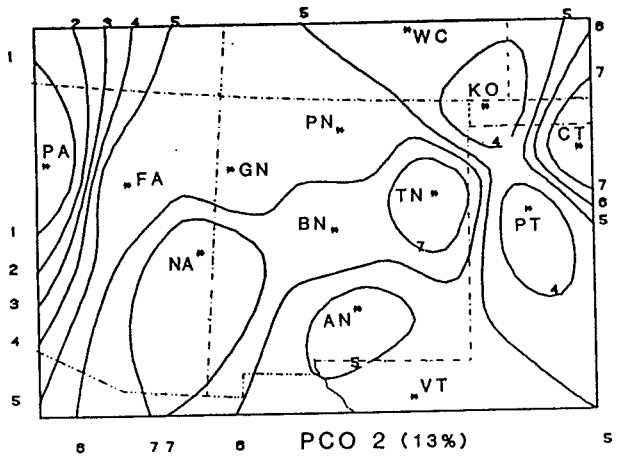


FIG. 4. CONTOURED PCO 2 SCORES FOR EACH POPULATION. Notice the divergence of the westernmost, Peach Springs, Arizona (PA) population and some diversity among the eastern populations.

character weights (F-1, *F* from ANOVA), then similarity measures were computed between individuals in both reference populations and the putative hybrids using the F-1 weighted Gower metric [absolute character difference divided by the maximum encountered in all plants; see Adams (1982) for discussion]. Previously, the range of each character has been used as the divisor, but if a character is greater in the putative hybrids than in either reference population, the dissimilarity measure can become greater than 1.0 and thus the similarity can be negative. Using the maximum observed value for the divisor eliminates this potential problem. PCO of *J. monosperma* (BN), *J. osteosperma* (UT) and the putative hybrids yielded two axes (63 and 4% respectively). The *J. osteosperma* population appears very uniform, whereas the *J. monosperma* (BN) population has considerable diversity (Fig. 5). The morphologically intermediate plants all ordinate with *J. monosperma*, except for one individual which appears closer to *J. osteosperma*. The fact that most of the morphologically intermediate plants are not chemically intermediate does not in itself disprove hybridization [see Adams and Kistler (1991) for examples]. Certainly this preliminary report is equivocal and a large random sample of plants in this area needs to be taken and analyzed.

As previously mentioned, a previous report of hybridization between *J. monosperma* and *J. pinchotii* (Hall and Carr, 1968) was not confirmed using both morphological and terpenoid data (Adams, 1972, 1975a). Furthermore, examination of pollen shedding times reveals that these two taxa are unlikely to hybridize, because *J. monosperma* sheds its pollen from February to April and *J. pinchotti* sheds pollen in September and October. However, *J. osteosperma* produces pollen in the spring, so hybridization with *J. monosperma* could be possible. *Juniperus erythrocarpa* sheds pollen from November to mid-January, but seasonal variation could result in overlapping dates with *J. monosperma*. The only known region of near-sympatry between *J. erythrocarpa* and *J. monosperma* is north of Sedona, Arizona. No evidence of hybridization has been found.

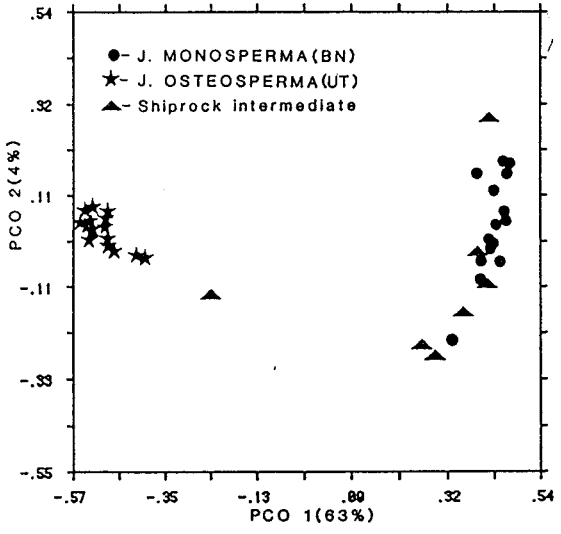


FIG. 5. PCO BASED ON TERPENE DATA OF *J. MONOSPERMA* (circles, individuals from near Belen, New Mexico), *J. OSTEOSPERMA* (stars, individuals from Thistle, Utah), AND SHIPROCK INTERMEDIATE INDIVIDUALS (triangles). One Shiprock individual actually appears somewhat intermediate.

## Conclusions

Overall, only a small amount of geographic variation was found in the terpenoids of *J. monosperma*, compared to previous juniper studies. The major geographic trend in *J. monosperma* was an east–west trend.

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