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Geographic Variation and Systematics of Monospermous *Juniperus* (Cupressaceae) from the Chihuahua Desert Based on RAPDs and Terpenes

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Key Word Index—*Juniperus pinchotii*; *J. coahuilensis*; *J. angosturana*; *J. monosperma*; Cupressaceae; RAPDs; terpenes; systematics.

Abstract—Random amplified polymorphic DNAs (RAPDs) and the steam volatile leaf terpenoids of *Juniperus erythrocarpa, J. monosperma* var. *monosperma, J. m.* var. *gracilis* and *J. pinchotii* were analyzed by principal coordinate analyses and contour mapping. *Juniperus monosperma* var. *gracilis* was elevated to specific rank as *J. angosturana* R. P. Adams *nom. nov.; J. erythrocarpa* var. *erythrocarpa* was treated as a segregate of *J. pinchotii*; and *J. erythrocarpa* var. *coahuilensis* was accepted as a distinct species, *J. coahuilensis* (M. Martinez) Gaussen ex. R. P. Adams. Geographic and chemical analyses of *J. coahuilensis* suggests that hybridization and introgression with *J. angosturana* has been factor in the variation of this taxon. RAPDs and terpene data supported the recognition of Arizona–New Mexico populations of *J. coahuilensis* as a new variety, *J. c.* var. *arizonica* R. P. Adams, var. *nov.* In addition, several terpenoids previously reported as unknown are now identified.

Introduction

The single-seeded, elongated gland junipers of Mexico and the southwestern United States are comprised of *J. erythrocarpa* Cory, *Juniperus monosperma* (Engelm.) Sarg. var. *monosperma, J. m.* var. *gracilis* Mart., and *J. pinchotii* Sudw. (Adams, 1975a; Adams *et al.*, 1981; Adams and Zanoni, 1979). These junipers occupy mostly allopatric sites distributed in northern Mexico and the southwestern United States (see Adams and Zanoni, 1979 for distribution map).

Vasek and Scora (1967) reported two races of *Juniperus monosperma* ('A' and 'B') based on terpenoids, but upon re-examination, Adams *et al.* (1979) found that 'A' was *J. erythrocarpa* and 'B' was *J. monosperma. Juniperus monosperma* var. *monosperma*, although often reported from Mexico, 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*, from Mexico differs in having bark that exfoliates in rectangular plates, thin foliage, and smaller fruits (female cones). The steam volatile leaf oils of all these junipers have been reported (Adams *et al.*, 1981; Adams, 1993).

Juniperus erythrocarpa and *J. pinchotii* have recently been shown to hybridize in the basin of the Chisos Mountains, Texas (Adams and Kistler, 1991). *Juniperus monosperma* var. *gracilis* appears to intergrade into *J. erythrocarpa* in Coahuila, Mexico. Furthermore, *J. monosperma* var. *gracilis* is more similar in its morphology and terpenes to *J. erythrocarpa* than to *J. monosperma* (Adams *et al.*, 1981). In addition, *J. erythrocarpa* appears to have two chemical populational subsets, one in west Texas and Mexico, and other in Arizona and New Mexico (Adams *et al.*, 1981).

Recently, DNA polymorphisms have been detected by Random Amplified Polymorphic DNAs (RAPDs): Williams *et al.*, 1990; Hu and Quiros, 1991; Demeke *et al.*, 1992; Heusden and Bachmann, 1992.

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One of the earliest taxonomic use of RAPDs was for the analysis of the classical *Brassica* U triangle relationships (Demeke *et al.*, 1992). They found that using about 100 RAPD bands gave very good agreement with the classical *Brassica* relationships. Heusden and Bachmann (1992) used RAPDs for systematics studies in *Microseris elegans* and found RAPDs to be complementary to isozymes and morphology.

Adams and Demeke (1993) found RAPDs to be useful in *Juniperus* at taxonomic levels ranging from the sub-generic to the varietal. RAPDs appear ideal for taxonomic use because analyses are very quick, inexpensive and randomly distributed over the entire genome.

The present study focuses on systematic and geographical variation in the monospermous juniper-complex of the Chihuahuan Desert utilizing RAPDs and terpenoid data.

Materials and Methods

Foliage samples consisting of eight–10 terminal branches were taken from each tree. All of the plants sampled are vouchered at BAYLU! Vouchers (location, *R. P. Adams* collection numbers, population acronym) *J. erythrocarpa*; west of the city of Chihuahua, MX, *63-77,* CM; south of Saltillo, MX, *78-81, 87-97,* SM; Sierra Blanca, TX, *387-401,* ST; southwest of Marfa, TX, *417–431,* MT; south of Alpine, TX, *432-446,* AT, Trancas, MX, *1471-1485,* TM; Dr. Arroyo, MX, *1501-1515,* DR; Galeana, MX, *1516-1530,* GM; Alvaro Obregon, MX *1531-1573,* OM; north of Matehuala, MX, *1547-1561,* MM; Sedona, AZ, *2122-2119,* SA; west of Benson, AZ, *2188-2202,* BA; Rock Hound Park, Deming, NM, *2203-2217,* RN; La Ascension (northeast of Casas Grandes) MX, *2518-2532,* LM; Huecho Tanks Park, TX, *2533-2544,* HT; *J. monosperma*: north of Walsenburg, CO, *1950-1964,* WC and Santa Rosa, NM, *5027-5036,* MON; *J. monosperma* var. *gracilis*: Angostura, MX, *1546-1470, 6881-6885,* AG; *J. pinchotii*: east of Ft. Stockton, TX, *4997-5001,* PIN.

The RAPDs analyses follow that of Adams and Demeke (1993). Leaves were either transported fresh and frozen upon arrival or desiccated in silica gel (Adams *et al.*, 1992). DNA was extracted from juniper leaves by the SDS protocol (Dellaporta *et al.*, 1983) with 1% (w/v) PVP added to the extraction buffer (CTAB extraction was not as effective as SDS in obtaining DNA that could be amplified). Ten-mer primers (116-244) were purchased from the University of British Columbia and one 10-mer primer (RC42) was synthesized at the Plant Biotechnology Institute, NRC, Canada (Table 1). PCR was performed in a volume of 25 µl containing 50 mM Tris–HCl (pH 9), 1.5 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each DNTPs, 0.36 µM primers, 0.5 ng genomic DNA, and 1 unit of *Taq* DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. Inhibition of PCR amplification was observed for some taxa when the amount of genomic DNA exceeded 1.0 ng per PCR tube. Reducing the amount of DNA to 0.5 or 0.2 ng per PCR tube resulted in amplification of all DNA samples (apparently by diluting the polysaccharides, see Demeke and Adams, 1992). DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 37°C (2 min), 72°C (2 min), 94°C (1 min). Two additional steps were used: 37°C (2 min) and 72°C (5 min) for final extension. See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

TABLE 1. LIST OF THE PRIMERS USED IN THIS STUDY FOR THE RANDOM AMPLIFICATION OF POLYMORPHIC DNAs (RAPDs) BY PCR

Code	Sequence (5′–3′)	Code	Sequence (5′–3′)
116	TAC GAT GAC G	218	CTC AGC CCA G
131	GAA ACA GCG T	227	CTA GAG GTC C
134	AAC ACA CGA G	232	CGG TGA CAT C
143	TCG CAG AAC G	234	TCC ACG GAC G
153	GAG TCA CGA G	237	CGA CCA GAG C
184	CAA ACG GAC C	239	CTG AAG CGG A
212	GCT GCG TGA C	244	CAG CCA ACC G
RC42	GCA AGT AGC T		

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, Gower, 1971; Adams, 1975b). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966). Program *PCO3D* is available for MS-DOS IBM compatibles with a hard disk and math coprocessor (correspond to RPA for distribution details).

The terpenoids were extracted by steam distillation (von Rudloff, 1967) and analyzed by gas chromatography (Adams, 1975a). Additional terpenoid identifications were made using a Finnigan Ion Trap (ITD) mass spectrometer (Adams, 1989). Anova, similarities and PCO were computed as above.

Results and Discussion

Several compounds, previously unidentified (Adams *et al.*, 1981), have now been identified (*RRT*s from Adams *et al.*, 1981): *RRT* 0.204 = verbenene; *RRT* 0.294 = *trans*-Sabinene hydrate; *RRT* 0.315 = *cis*-pinene hydrate; (β-terpineol) = *cis*-Sabinene hydrate; ((3,7,7)-trimethyl-bicyclo-(3,1,1)-2-heptanone)) = (*Z*)-Decenal; *RRT* 0.557 = (dihydroeugenol); *RRT* 0.640 = β-cadinene; *RRT* 0.673 = epi-Cubenol; (4,10-dimethyl-4-isopropyl-bicyclo-(4,4,0)-decadiene) = cadina-1,4-diene; *RRT* 0.750 = 1-epi-Cubenol; *RRT* 0.760 = epi-α-cadinol (= τ-cadinol); Acetate II, *RRT* 0.860 = 8-α-acetoxyelemol. In addition, trace amounts of thujopsene and cedrol were found in the oil of *J. monosperma* var. *gracilis*. These wood oil components are very rare in *Juniperus* leaf oils in the western hemisphere, but fairly common in leaf oils of junipers of the eastern hemisphere (Adams, 1991).

Because the type specimen of *J. erythrocarpa* came from the laguna (basin) at the west base of Mt. Emery in the Chisos Mountains of western Texas and recently significant hybridization has been reported between *J. erythrocarpa* and *J. pinchotii* at that very site (Adams and Kistler, 1991), an examination of the type specimen for *J. erythrocarpa* was initiated. Adams and Kistler (1991) found a range of fruit colors, varying from copper-reddish (typical of *J. pinchotii*) to very rose (almost purple) colored fruits in the Chisos basin. Analyses based on both morphology and terpenoids revealed essentially a continuum of individuals including hybrids, and backcrosses to both species. A number of 'bright red' fruited individuals in the basin were found to be similar to *J. pinchotii* in all other characteristics. Cory (1936) clearly described *J. erythrocarpa* Cory as having 'bright red' fruits and the type itself is clearly a segregate of *J. pinchotii*. It is therefore treated as a synonym of *J. pinchotii*, along with another 'red' fruited taxon, *J. texensis* van Melle.

As presently interpreted, the nomenclature involved for this taxon are as follows:

Juniperus pinchotii Sudworth, Forest & Irrig. 10:204. 1905;

J. erythrocarpa Cory;

J. texensis van Melle;

J. monosperma var. *pinchotii* (Sudw.) van Melle;

Sabina pinchotii (Sudw.) I. M. Lewis;

J. pinchotii var. *erythrocarpa* (Cory) Silba.

The description for *J. erythrocarpa* var. *coahuilensis* Martinez reveals this to be a rose-fruited taxon, such fruits being typical of the taxon throughout its range in Mexico and Texas. Martinez (1946) mentions in detail that his var. *coahuilensis*, had a blue-gray waxy bloom on the fruits. *Juniperus erythrocarpa sensu* Cory lacks bloom on its fruit. These facts led Adams (1993) to recognize *J. erythrocarpa* var. *coahuilensis* at the specific level. Unfortunately, Gaussen (1968) failed to properly cite the basionym, so his publication was invalid. However, the name was recently validated (Adams, 1993) as:

Juniperus coahuilensis (M. Martinez) Gaussen ex. R. P. Adams, *Phytologia* **74**, 450 (1993).

J. erythrocarpa var. *coahuilensis* M. Martinez.

With the foregoing discussion in mind, I shall henceforth refer to *J. pinchotii* as the taxon having copper to reddish fruits without bloom on the fruits (or beneath the leaves) and *J. coahuilensis* as having pink to rose-colored fruits with bloom on both fruit and beneath the leaves.

The PCO from the similarity matrix based on 136 RAPDs from *J. coahuilensis, J. monosperma* var. *monosperma, J. m.* var *gracilis* and *J. pinchotii* yielded four

eigenroots of fairly similar sizes (36; 25; 22; 18% variance). Figure 1 shows the most similar taxa as *J. coahuilensis* [COA(AT), Texas] and *J. monosperma* var. *gracilis* (AG, Angostura, Mexico, 0.7 similarity). *Juniperus monosperma* var. *monosperma* (MON), *J. pinchotii* (PIN) and *J. coahuilensis* [COA(RN), New Mexico] are each distinct. The fourth principal coordinate (18% variance) contains significant biological information. Axis four (Fig. 2) separates *J. coahuilensis* [COA(AT), Texas] from *J. monosperma* var. *gracilis* (AG). Note also (Figs 1 and 2) that *J. coahuilensis* from Alpine, TX [COA(AT)]

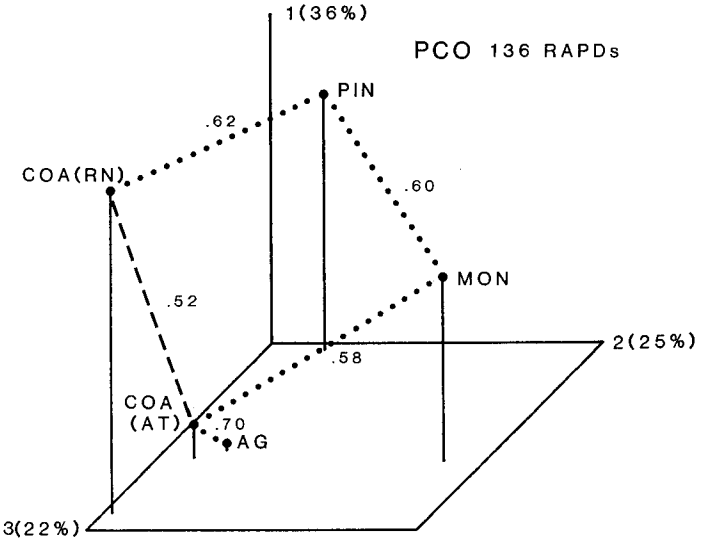

FIG. 1. PCO ORDINATION USING 136 RAPDs (BANDS). Note the clear separation of *J. monosperma* (MON), *J. pinchotii* (PIN), and the two populations of *J. coahuilensis*, COA(RN), Rock Hound, NM and COA(AT), Alpine, TX. *Juniperus monosperma* var. *gracilis* (AG) is most similar to *J. coahuilensis* from Alpine, TX, COA(AT). Dashed line indicates the similarity between the *J. coahuilensis* populations.

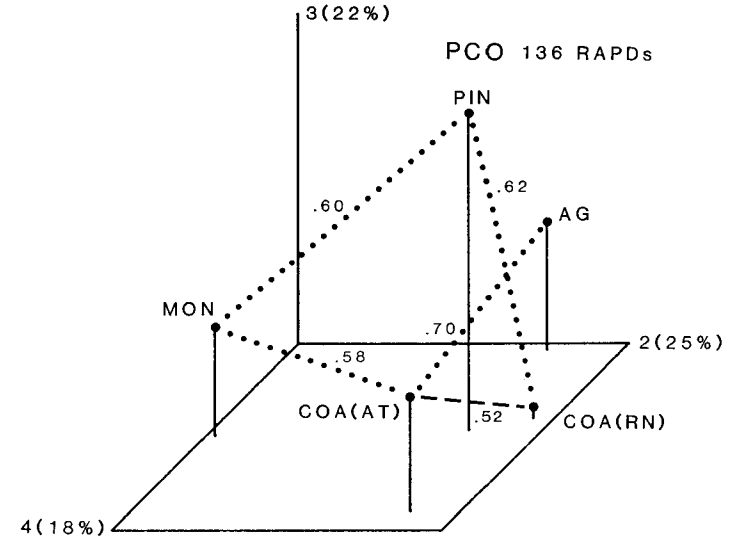

FIG. 2. PCO, 136 RAPDs, USING AXES 2, 3, 4. Axis 4 clearly separates *J. coahuilensis*, COA(AT) and *J. monosperma* var. *gracilis* (AG). Dashed line indicates the similarity between the *J. coahuilensis* populations.

has a higher similarity (0.62) to *J. pinchotii* (PIN) than its similarity (0.52) to *J. coahuilensis* from Rock Hound Park, NM [COA(RN)].

These levels of similarities are lower than found between the serrate leaf-margined juniper species of section *Sabina* (Adams and Demeke, 1993). Clearly the RAPDs data separate these samples into five distinct taxa.

Analyses of the volatile leaf oils provides another view of the relationships. ANOVA of the same five taxa yielded 50 terpenoids larger than 0.5% (of the total oil) and with F ratios greater than 1.0. The average F ratio was 48.67 ($P_{0.01} = 5.69$; $P_{0.05} = 4.99$). Because the eigenroots did not appear to asymptote (Pimentel, 1979), all four eigenroots likely contain biological information. The four eigenroots accounted for: 50.0, 25.5, 16.0 and 8.5% of the total variance among the taxa.

Figure 3 shows the three-dimensional ordination based on 50 terpenes. Of immediate interest is the high similarity (0.84) between the oils of *J. pinchotii* (PIN) and *J. coahuilensis* [COA(AT)] from Alpine, TX. *Juniperus coahuilensis* from Rock Hound, NM [COA(RN)], *J. m. monosperma* (MON) and *J. monosperma* var. *gracilis* (AG) are each quite distinct. *Juniperus monosperma* var. *gracilis* is only slightly more similar (0.52) to the var. *monosperma* than to *J. coahuilensis* (0.51). Axis four (8,5%, not shown) separated *J. coahuilensis* [COA(AT)] from *J. pinchotii.*

Thus, the RAPDs and terpenes both agree on several factors: *J. monosperma* var. *gracilis* is no more similar to the var. *monosperma* than to *J. coahuilensis*; the two populations of *J. coahuilensis* are approximately as dissimilar as most juniper species; although *J. pinchotii* is quite distinct in the RAPDs, its terpenes are very similar to *J. coahuilensis* from Alpine, TX.

Several taxonomic questions immediately arise: should *J. monosperma* var. *gracilis* be recognized as a species? Should *J. coahuilensis* be treated as a variety of *J. pinchotii*? Should the divergent populations of *J. coahuilensis* be recognized as varieties?

In considering the recognition of species status, Ownbey (1950) encountered a similar situation in *Tragopogon* and listed three criteria (which I shall rephrase as questions): (1) are the taxa natural groups, characterized by a combination of

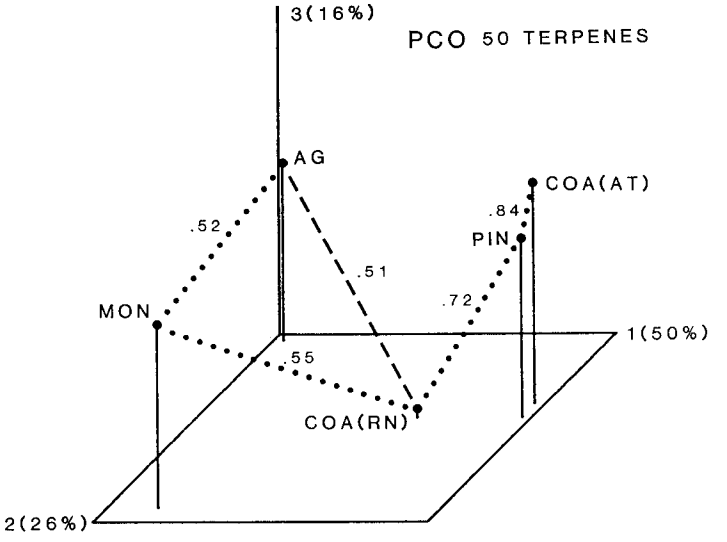

FIG. 3. PCO, 50 TERPENES. Four groups are apparent: MON, *J. monosperma*; AG, *J. monosperma* var. *gracilis*; COA(RN), *J. coahuilensis*, Rock Hound, NM; and PIN, *J. pinchotii* and COA(AT), *J. coahuilensis*, Alpine, TX. The dashed line indicates that *J. monosperma* var. *gracilis* (AG) is next most similar to *J. coahuilensis*, from New Mexico. The fourth axis (not shown) clearly separates *J. pinchotii*, PIN from *J. coahuilensis*, COA(AT).

distinctive morphological features (and/or chemical features, my addition)?; (2) are the taxa reproducing themselves under natural conditions?; (3) is there free gene exchange between the taxa?

Juniperus monosperma var. *monosperma* and the var. *gracilis* certainly differ in their RAPDs and terpenes. In addition, var. *gracilis* has bark that exfoliates in quadrangular plates, slender, lax foliage and small female cones (4–5 mm) compared to *J. monosperma*, which has bark that exfoliates in strips, coarse, erect foliage, and medium-sized female cones (6–8 mm diameter).

Both taxa are reproducing themselves under natural conditions. The var. *monosperma* is a wide spread, weedy, uniform taxon in Arizona, New Mexico, west Texas, western Oklahoma and southern Colorado (Adams, 1993), whereas the var. *gracilis* is a geographically restricted taxon that appears to hybridize with *J. coahuilensis* (see below) and is only known, at present, in its typical form, from the type locality, Angostura, San Luis Potosi, Mexico. During the last visit by the author to the type locality (December, 1991), trees were in full fruit and some young individuals were found.

The two taxa are separated by a distance of some 1150 km. In addition, their pollen shedding times do not overlap (var. *monosperma*: February–April; var. *gracilis*: November–December).

Based on the aforementioned data, var. *gracilis* should be recognized as a distinct species. Unfortunately, the specific epithet *gracilis* has already been applied to what is now a synonym of *J. flaccida* Schlecht. (=*J. gracilis* Endl.; =*J. gracilis* Hort. in Roezl.; Zanoni and Adams, 1979). Consequently, I propose the following new name and brief description:

Juniperus angosturana R. P. Adams, *nom. nov.,* slender one-seeded juniper, cedro.

Based upon *Juniperus monosperma* var. *gracilis* M. Martinez, *Anal. Inst. Biol. Mexico* **17**, 111–112 (1946). *Type:* Mexico; San Luis Potosi; Hacienda de Angostura, *Pringle 3771* (*Holotype*: not at MEXU; *Iso-types*: VT, ARIZ, F, GH, MO, NY, UC).

Ultimate twigs, 6–12 mm long, 1.0–1.3 mm diameter, the foliage lax and slender; branching angle of ultimate twigs, about 60–70°; at least some of the bark exfoliating from the trunk in quadrangular plates; female cones 4–5 mm in diameter.

Distribution: Previously reported (Zanoni and Adams, 1979) in grasslands from east Coahuila, south Nuevo Leon, southeast Tamaulipas, northeast Queretaro and north Hidalgo, Mexico. But the range is probably more restricted (see discussion below).

Should *Juniperus coahuilensis* be treated as a variety of *J. pinchotii*? McVaugh (1992) presents a key to *J. erythrocarpa, J. pinchotii* and *J. martinezii*, then he suggests that the three taxa might be considered a single species. No mention is made of the chemical data previously published on *J. erythrocarpa* and *J. pinchotii* (Adams *et al.*, 1981; Adams and Kistler, 1991). Also ignored are the field and chemical analyses (Adams *et al.*, 1990) of *J. martinezii* that clearly confirmed it to be a variety of *J. flaccida* [*J. f.* var. *martinezii* (Perez de la Rosa) Silba].

Silba (1984), citing no data whatsoever, reduced *J. erythrocarpa* to a variety of *J. pinchotii*, and cited *J. e.* var. *coahuilensis* as a synonym of *J. pinchotii* var. *erythrocarpa* (Cory) Silba.

In spite of the aforementioned opinions, examination of the RAPDs data (Figs 1 and 2) clearly shows *J. coahuilensis* to be distinct. This taxon is also morphologically distinct (Adams and Zanoni, 1979). So the convergence (or lack of divergence) in the terpene character set cannot be an overriding factor. In addition, the taxa occupy different habitats with *J. pinchotii* found at lower elevations (300–1000 m, rarely at 1500 m) in largely disturbed sites while *J. coahuilensis* occurs at higher elevations (1600 m or higher) in *Boutelouia* grasslands. *Juniperus coahuilensis* is very unusual in that it will vigorously sprout when cut, perhaps an adaptation to grazing in the

grasslands. Both taxa are reproducing themselves under natural conditions. There is generally not free gene exchange between *J. coahuilensis* and *J. pinchotii.* The Basin of the Chisos Mountains and south of Saltillo are the only significant areas of sympatry. In the Basin, *J. coahuilensis* sheds pollen in November–December while *J. pinchotii* sheds pollen in September–October. A detailed study using morphology and terpenoids (Adams and Kistler, 1991) revealed some hybridization and backcrossing in the Basin population. Aside from the trans-Pecos and Saltillo areas there does not appear to be significant gene transfer between the taxa. Therefore, *J. coahuilensis* and *J. pinchotii* are maintained as separate species.

A previous study on the terpenes of *J. erythrocarpa, J. monosperma* var. *gracilis* and *J. pinchotii* (Zanoni and Adams, 1976) utilized samples from Nuevo Leon, Mexico (Dr. Arroyo population). However, additional examination of this population as well as junipers from the type locality of var. *gracilis* (*J. angosturana*), revealed that the junipers at Dr. Arroyo are, morphologically quite distinct (but highly variable) from *J. angosturana.* Zanoni and Adams (1976) indicated that only a more detailed populational study would resolve the classification of the junipers in the Sierra Oriental of Mexico. Therefore, these population were recollected, additional populations were sampled, including plants from the type locality of *J. angosturana.*

ANOVA of the terpenoids from 14 populations of *J. coahuilensis* plus *J. angosturana* from the type locality resulted in 59 compounds with an average *F* ratio of 13.31. PCO of the similarity matrix yielded four eigenroots (33.5, 21.6, 10.4, 6.9% variance) that appeared to asymptote after these four roots. Ordination of these taxa (Fig. 4) reveals several interesting facts. *Juniperus agosturana* is clearly separated on the second axis, as expected. But the major trend (34%) was the separation of the *J. coahuilensis* populations from Arizona and New Mexico (BA, SA, RN) from all the other populations. Three populations (HT, LM, ST) appear somewhat intermediate between the Arizona–New Mexico, *J. coahuilensis* and the rest of *J. coahuilensis* populations. In addition, the population at Alvaro Obregon (OM), appears intermediate between *J. angosturana* and *J. coahuilensis.*

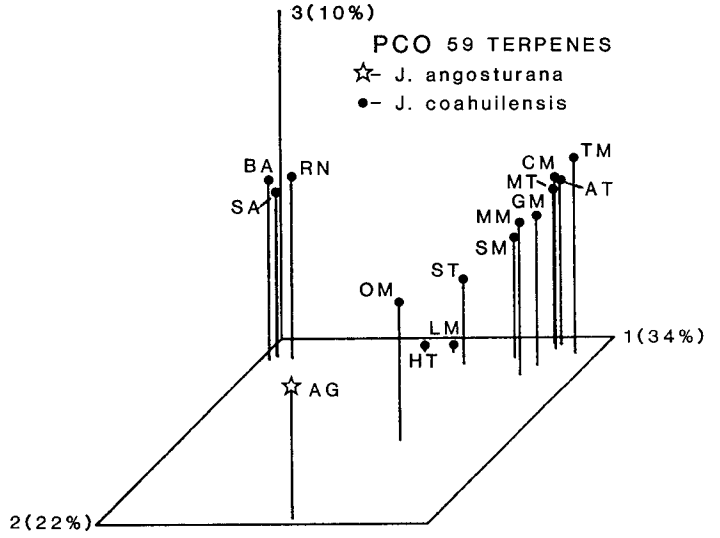

FIG. 4. PCO, 59 TERPENES, FOR *J. ANGOSTURANA* AND POPULATIONS OF *J. COAHUILENSIS.* The first axis (34%) clearly separates the Arizona–New Mexico (BA, SA, RN) *J. coahuilensis* populations. Note also the intermediate position of the OM (Alvaro Obregon, MX) population between *J. angosturana* (AG) and the *J. coahuilensis* populations and some divergence of the HT and LM populations.

The most difficult problem in this complex is the evaluation of the taxonomic status of the divergent *J. coahuilensis* populations in Arizona and New Mexico. To study this, ANOVA was performed on the terpenoids of 14 populations of *J. coahuilensis* (the Alvaro Obregon population was included despite affinity to *J. angosturana*, Fig. 4). ANOVA resulted in 57 terpenoids with an average *F* ratio of 10.47. PCO of the similarity matrix yielded eigenroots of 42.6, 13.4, 11.2 and 6.6% variance before they began to asymptote. Contour mapping of the coordinate score for each population was used to examine geographic variation. The first principal coordinate (42.6%) clearly shows (Fig. 5) the divergence of the Arizona and New Mexico populations (SA, BA, RN), with the HT, LM and ST populations being intermediate. Almost all of the populations in Mexico appear fairly uniform on this coordinate. Thus, almost half of the variation among the *J. coahuilensis* populations (42.6%) is due the divergent Arizona and New Mexico populations. A large population of *J. coahuilensis* also occurs from south of the Chihuahua City (CM) population, to just north of Durango. Recently a few individuals were collected (La Zarca, Adams *6829–6831*; just south of Aguascalientes, San Juan de los Lagos, Adams, *5955–5957*). The oils of the junipers from La Zarca and San Juan de los Lagos, were both similar to the oil from Chihuahua rather than the oils from Arizona and New Mexico.

One possible reason for the divergence of the Arizona and New Mexico population might be introgression from *Juniperus monosperma.* The terpenes from all 14 populations of *J. coahuilensis* plus one population of *J. monosperma* (Walsenburg, CO), where *J. monosperma* is the only juniper, were analyzed by ANOVA and

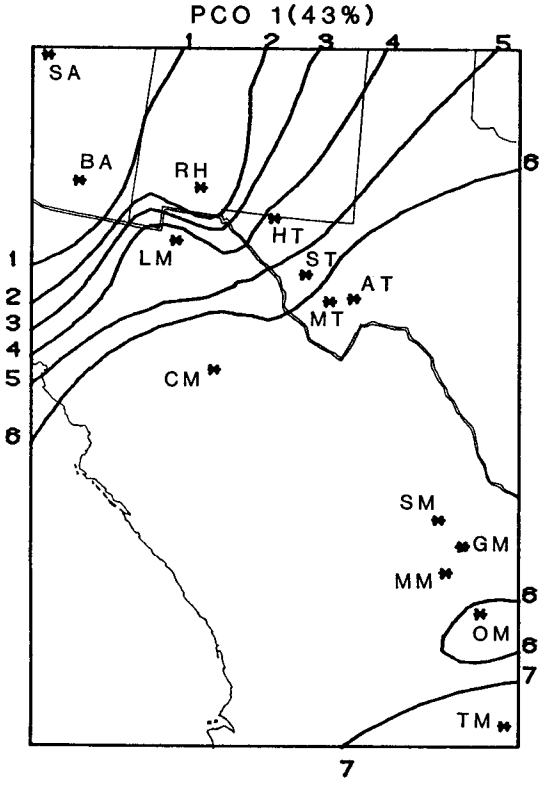

FIG. 5. CONTOUR MAP OF *J. COAHUILENSIS* BASED ON PRINCIPAL COORDINATE-1 SCORES USING 59 TERPENES. This major trend (43% of the variation) is the separation of the Arizona–New Mexico populations (BA, SA, RN). There appears to be clinal intergradation in northern Mexico (LM) and west Texas (HT, ST).

similarities were computed within this set. The similarities of *J. monosperma* to *J. coahuilensis* populations were (largest to smallest): OM 0.62; RN 0.52; SM 0.51; ST 0.51; GM 0.50; SA 0.48; BA 0.48; MT 0.47; MM 0.46; AT 0.45; LM 0.45; CM 0.44; HT 0.43; TM 0.42. Thus, we do not see larger similarities to *J. monosperma* in the RN, SA and BA populations as would be expected if introgression were causative. In fact, aside from OM, the *J. coahuilensis* populations all have approximately the same level of similarity to *J. monosperma.*

The second principal coordinate (13%) depicts an interesting pattern in which the HT and LM populations show a co-divergent trend. Notice that this trend includes, to a lesser extent, the Sierra Blanca (ST) population. All three of these populations are small and isolated. It appears likely that these populations could have been established by migratory birds or they may merely represent intermediate populations (Fig. 4) as a part of the transition from the *J. coahuilensis* of Mexico to the Arizona–New Mexico populations.

The uniqueness of the Alvaro Obregon (OM) population is seen in Fig. 7 [note: *J. angosturana* (AG, star) only indicates its geographically near proximity to population OM, data from AG was not included in this analysis]. The TM population is not similar in this pattern to OM. One should also note that the MM population (north of Matehuala) shows some affinity to the trend as does the GM and SM populations. Possible hybridization and introgression between *J. angosturana* and *J. coahuilensis* will be examined below.

The fourth principal coordinate (6.6%, not shown) largely revealed differences between the HT and ST populations and some divergence of the TM population.

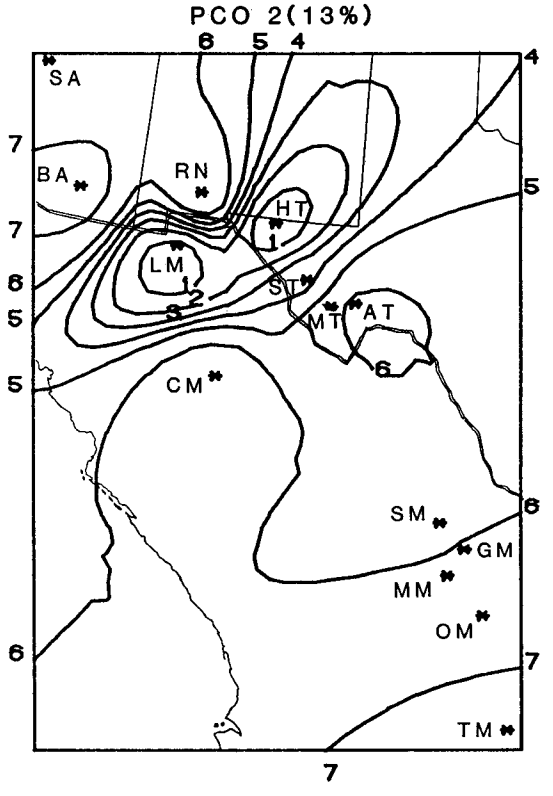

FIG. 6. CONTOUR MAP OF *J. COAHUILENSIS* BASED ON PRINCIPAL COORDINATE-2 SCORES USING 59 TERPENES. This trend (13% of the variance) shows some divergence of the HT and LM populations.

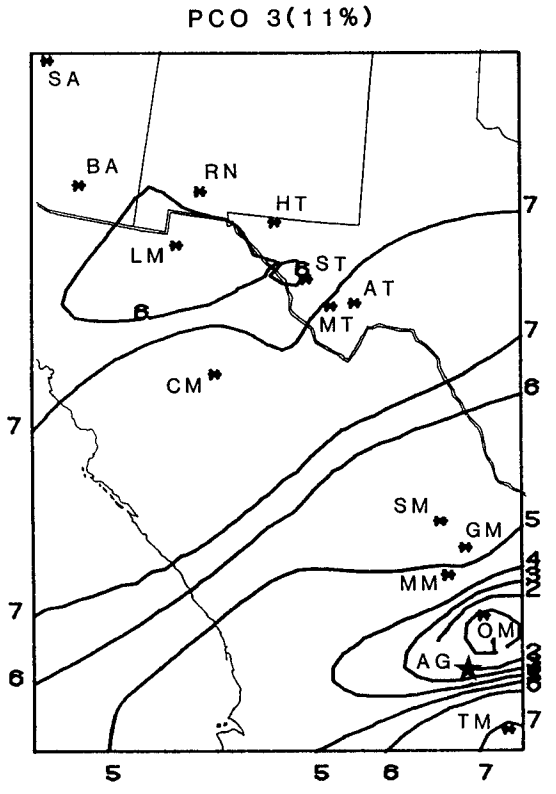

FIG. 7. CONTOUR MAP OF *J. COAHUILENSIS* BASED ON PRINCIPAL COORDINATE-3 SCORES USING 59 TERPENES. This trend (11%) is due to differences in the OM population. The location of the type locality of *J. angosturana* (AG) is indicated by a star. Note that the AG data was not used. Some co-divergence with the OM population is seen to the north in populations MM, GM and SM.

As we review the total pattern in *J. coahuilensis*, using RAPDs (Figs 1 and 2), and terpenoids (Figs 4 and 5), there emerges a consistent pattern that the species is composed of two population groups or taxa: one from most of Mexico and west Texas and another in Arizona and New Mexico (BA, SA, RN). Morphologically the taxa are almost identical. Sixteen morphological characters were measured (whip-gland area, whip-leaf gland length/width, ratio of gland-length/sheath length, whip-glands protruding, whip-glands ruptured, whip-blades recurved, whip-leaves glaucous beneath, scale-leaf length, scale-leaf length/branchlet width, branching angle of ultimate branchlets, fruit diameter, fruit color, fruit glaucousness, seeds per fruit, seed length $\times$ width, ratio of seed width/length, ratio of hilum scar length/seed length, number of seed grooves. Only the ratio of whip-gland length/whip-leaf sheath length clearly separated the two groups ($F = 14.33^{**}$, $P_{0.01} = 4.82$). The plants from Mexico and west Texas had whip-leaf glands about two-thirds as long as the sheath. The junipers from Arizona and New Mexico had whip-leaf glands about half as long as the sheath.

On the combined basis of RAPDs, terpenes, morphology and distributions, it seems appropriate to recognize the Arizona and New Mexico populations as a distinct taxon, as follows:

Juniperus coahuilensis (M. Martinez) Gaussen ex. R. P. Adams var. *arizonica* R. P. Adams, var. *nov.*

Juniperus coahuilensis (M. Martinez) Gaussen ex. R. P. Adams *similis sed glandibus breviorbus foliorum flagelliformium. Glans folii flagelliformis vaginum consociatum dimidio brevior distinctus. Type*: United States, Arizona, Yavapai, Co.: 72 km south of Flagstaff, 1160 m, *R. P. Adams 2132* (*Holotype* BAYLU!), Arizona juniper.

Similar to *Juniperus coahuilensis* but distinguished by shorter whip-leaf glands. Whip-leaf glands half as long as the associated sheath. Distribution: Arizona, South of the Mogollon Rim; and in southwestern New Mexico.

As previously mentioned, *Juniperus angosturana* and *J. coahuilensis* in northern Mexico show considerable morphological variation. For example, in the region of Dr. Arroyo a wide array of fruit colors and foliage characters are found. Two reference populations (AG, Angostura, *J. angosturana*; CM, w. Chihuahua City, *J. coahuilensis*) were selected for study in this region and ANOVA was performed on their terpenes to generate a *F*-1 weighted character set for analysis of hybridization (Adams, 1982).

Figure 8 shows the ordination on the first two coordinate axes. *Juniperus angosturana* plants cluster very tightly as do the *J. coahuilensis* plants from Chihuahua. Junipers from Galeana (GM) are mostly like CM, but some appear to be hybrids and backcrosses (see Adams and Kistler, 1991, for discussion of backcross and hybrid ordination). The junipers north of Matehuala (MM) mostly cluster with *J. coahuilensis*, but several appear to be backcrosses (Fig. 8). Plants from Dr. Arroyo (DM) are mostly intermediate with one individual clustering well with *J. coahuilensis*, and one individual tending towards *J. angosturana* (Fig. 8). The Alvaro Obregon (OM) population is decidely intermediate between *J. angosturana* and *J. coahuilensis* (Fig. 8), with only two individuals appearing to be backcrosses to *J. coahuilensis.*

Introgression, if occurring, appears to be from *J. angosturana* into *J. coahuilensis.* If so, this would favor the survival of *J. angosturana* genes, at least at the type locality.

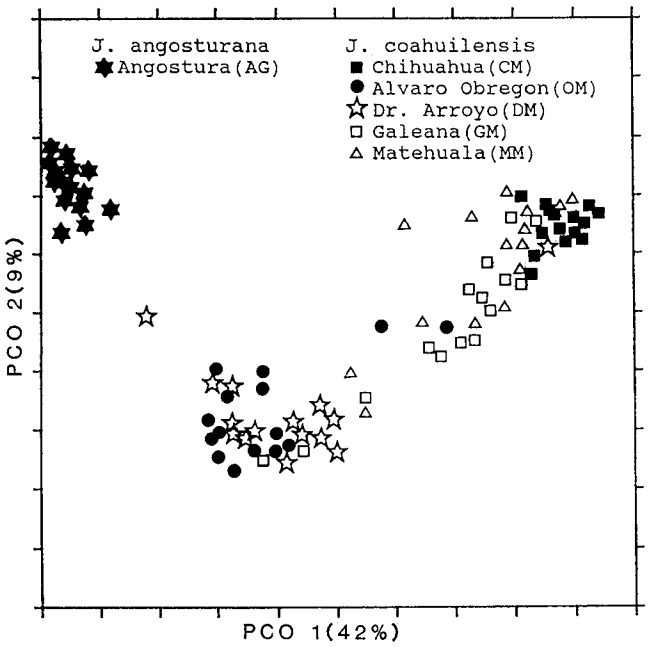

FIG. 8. EXAMINATION OF POSSIBLE HYBRIDIZATION USING PCO OF TERPENES AND ORDINATION OF INDIVIDUALS FROM VARIOUS POPULATIONS BASED ON REFERENCE POPULATIONS OF *J. ANGOSTURANA* (AG) AND *J. COAHUILENSIS* (CM). See text for discussion.

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