GEOGRAPHIC VARIATION IN *JUNIPERUS DEPPEANA*

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ABSTRACT

Juniperus deppeana has numerous disjunct populations that include four taxonomic varieties and three forms. Disjunct and continuous populations of *Juniperus deppeana* from the southwest United States, Mexico and Guatemala were analyzed by DNA fingerprinting (RAPD) and the results compared with DNA sequence data from the literature. Some disjunct populations have maintained surprisingly strong genetic affinities despite having seemingly little or no gene flow at present. The expanded distribution of the Pinyon-Juniper woodland into the Chihuahuan desert during the Wisconsin maximal (70,000-13,000 ybp) was examined. The present disjunct *J. deppeana* populations may have been contiguous during the Pleistocene and as recent as the Wisconsin until the Holocene (13,000 ybp). Ancient gene flow seems to have contributed to the strong genetic affinities found among present day disjunct populations. Most varieties of *J. deppeana* have large, woody cones eaten mostly by mammals and are thus not amenable to long distance dispersal. However, *J. deppeana* var. *gamboana* of the highlands of Chiapas, MX and Guatemala, is unusual in having single-seeded, relative small (5-8 mm), soft, fleshy

female cones that are consumed by birds. Long distance bird dispersal seems to have been important in the establishment of *J. d.* var. *gamboana* across the isthmus of Tehuantepec from *J. deppeana's* center of diversity in north-central Mexico, to the highlands of Chiapas and Guatemala.

KEY WORDS: *Juniperus deppeana* varieties, *Cupressaceae*, DNA, RAPDs, systematics, geographic variation, speciation.

Juniperus deppeana Steudel has trunk bark that exfoliates in quadrangular plates and thus, the common name 'alligator' juniper. *Juniperus deppeana* is part of the serrate leaf margined *Juniperus* species of the western hemisphere (Adams, 2004). The serrate leaf junipers are characterized by having microscopic (visible at 40 X) serrations (teeth) on the scale and whip leaves and are generally xerophytic, occurring in the great North American deserts and arid mountains adjacent to the deserts. These junipers range from northern Guatemala, into Mexico, thence northward into the southwestern United States, as far north as Oregon (*J. occidentalis* Hook. var. *occidentalis* (Vasek) A.H. & N. H. Holmgr. and eastward on limestone outcrops in Arkansas (*J. ashei* Buch.). Axelrod (1958) proposed that *Juniperus* was a part of the Madro-Tertiary geoflora dating from pre-Eocene (55-33 mya). The Madro-Tertiary geoflora is thought to have arisen from elements of the northern, temperate, deciduous Arcto-Tertiary Geoflora mixing with elements of the southern, Neotropical-Tertiary Geoflora on the dry side of the Sierra Madre Oriental in central and northern Mexico and adjacent United States (Axelrod, 1958). Drying conditions created a different kind of habitat in the Chihuahuan desert and foothills that favored the evolution of sclerophyllous and microphyllous species. This is not to be confused with the geologically recent origin (2-3 mya, Thorne, 1986) of the Mojave and Colorado Deserts that resulted from the rain-shadow caused by the elevation of the southern Sierra Nevada Range. The pre-Eocene (<55 mya) date may be a little early for *Juniperus*, because the oldest known *Juniperus* fossil (*J. pauli* Z. Kvacek) has been radiometrically dated at 35.4 mya (Kvacek, 2002). *Juniperus pauli* is an entire leaf margined juniper discovered in the Czech Republic and appears to be related to the extant *J. excelsa* M-Bieb., a seemingly more recent member of

Juniperus. Kvacek (2002) reviewed and verified several *Juniperus* fossils from North America that included *J. creedensis* Axelrod (~24 mya, Creed geoflora, cf. *J. osteosperma*); *J. desatoyana* Axelrod, (w. Nevada ~22 mya, cf. *J. occidentalis*); *J. nevadensis* Axelrod, (w. North America, Neogene, cf. *J. osteosperma*). It is noteworthy that north-central Mexico has the largest number of *Juniperus* species in the western hemisphere and this is one of three centers of diversity for extant *Juniperus* species (Adams, 2004). The other two centers of diversity are the northern Mediterranean region and western China (Adams, 2004).

The first systematic treatment of the serrate leaf margined junipers was by Martinez (1963) who recognized *J. deppeana* Steudel. var. *deppeana* (checkered bark, (3)4-5(6) seeds/cone, *J. d*. var. *pachyphlaea* (Torrey) Mart. (checkered bark, (1)2-4(5) seeds/cone), *J. d*. var. *robusta* Mart. (checkered bark, (1)2-3(-6) seeds/cone), *J. d*. var. *zacatecensis* Mart. (checkered bark, 1-4(-7) seeds/cone), *J. patoniana* Mart. (laced bark, (1)2-3(-6) seeds/cone, and *J. gamboana* Mart. (checkered bark, 1(2) seeds/cone). Zanoni and Adams (1976), using morphology and essential oils, generally agreed with Martinez's treatment, except *J. patoniana* was reduced to *J. d.* var. *patoniana* (Mart.) Zanoni.

Recently, Adams and Schwarzbach (2006) recognized *J. gamboana* as *J. deppeana* var. *gamboana* (Mart.) R. P. Adams and *J. deppeana* var. *zacatecensis* as *J. deppeana* f. *zacatecensis* (Mart.) R. P. Adams. Figure 1 shows a partial phylogenetic tree (Schwarzbach, et al, 2007) that indicates a well-supported clade (95% bootstrap) composed of *J. d.* var. *gamboana* and *J. deppeana* var. *robusta* (both with quadrangular bark). In addition, all the *J. deppeana* taxa form a very distinct clade (Fig. 1) with 100% support, distinct from the nearest *Juniperus* (*J. saltillensis*). However, Schwarzbach et al. (2008) found that nrDNA and trnC-trnD sequence data did not resolve closely related species and varieties and this is apparent in figure 1, where there is little information concerning infra-specific relationships among the *J. deppeana* varieties.

Juniperus deppeana is one of the most widely distributed *Juniperus* species in Mexico (Fig. 2). The distribution of *J. deppeana* forms a discontinuous ring in the mountains above 2000 m (occasionally down to 1500 m) around the Chihuahuan desert in the

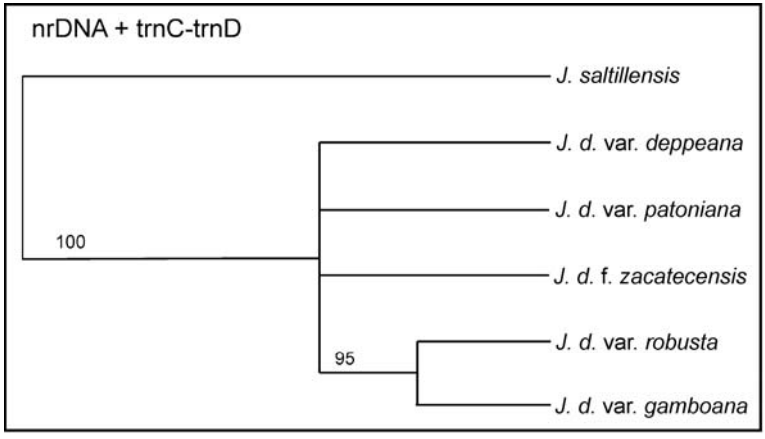

Figure 1. Phylogenetic tree based on nrDNA (ITS) and trnC-trnD sequences (from Schwarzbach, et al., 2007). *Juniperus saltillensis* is an outgroup. Note the bootstrap value of 95% support for the *J. d.* var. *gamboana*, *J. d*. var. *robusta* clade.

southwestern US and Mexico (Fig. 2), thence to the very southern-most part of Mexico and northern Guatemala at 1600 - 2200 m.

In this study, a very sensitive DNA technology (RAPDs, Random Amplified Polymorphic DNAs) was utilized to examine small differences between populations. Because there are sometimes problems in the gathering and analyses of RAPDs data in systematics, it seems appropriate to mention that over the past 15 years, we have applied RAPDs to a variety of systematic problems in *Juniperus*, *Brassica*, and *Vetiveria*. The juniper from the southwestern mountains of the Arabian peninsula has been called *J. excelsa* or *J. procera*. RAPDs and sesquiterpenoids analyses revealed that the Abha, Saudi Arabia plants were clearly the same as *J. procera* from Ethiopia (Adams, et al., 1993). In another study (Adams et al., 2003) of five *Juniperus* species comparing classifications based on nrDNA (ITS) sequences, RAPDs, ISSRs, and terpenoids, the highest correlation (0.95) was found between nrDNA sequence and RAPD classifications. This was surprising considering that some systematic information was lost by the lack of resolution of similar molecular weight bands on agarose. Non-homology of co-migrating bands has been shown to be

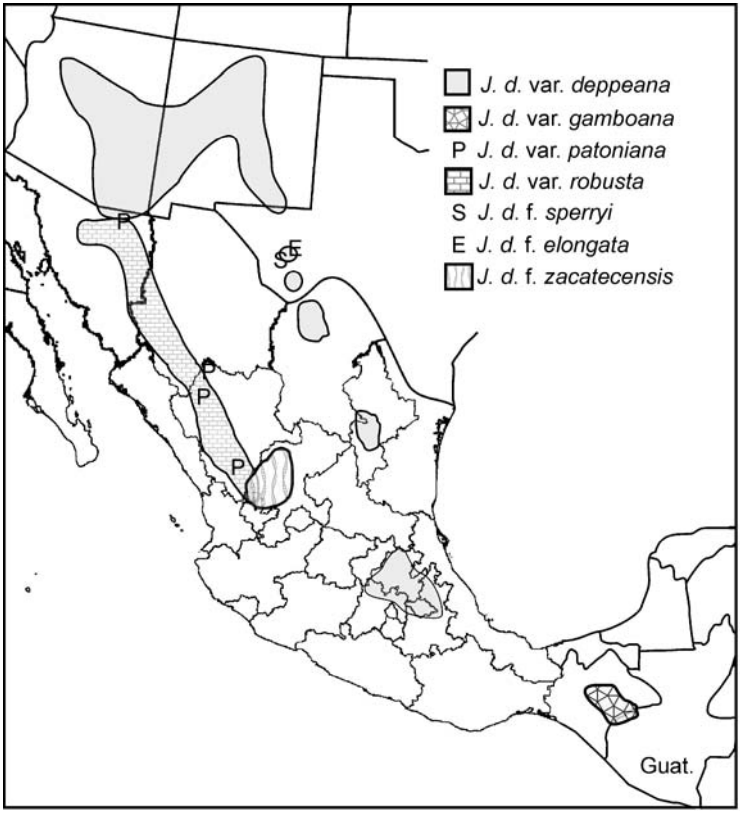

Figure 2. Distribution of *J. deppeana* and its varieties. The population of *J. d.* var. *patoniana* (P) in n. Sonora, MX has previously been called *J. d.* f. *sperryi*, but appears more likely to be *J. d.* var. *patoniana*. Populations sampled are in bold. A, B, C, D, F = *J. d.* var. *deppeana* from the USA, H, L = *J. d.* var. *deppeana*, Mexico, P = *J. d.* var. *patoniana*, R= *J. d.* var. *robusta*, Z = *J. d.* f. *zacatecensis.* G = *J. d.* var. *gamboana.*

9% between two *Helianthus* species (Rieseberg, 1996), ca. 10% in *Glycine (*Williams et al., 1993), and ca. 20% in a *Brassica* - *Raphanus* comparison (Thormann, et al., 1994). However, Adams and Rieseberg (1998) did a detailed computer simulation study using *Brassica* species

that form the classical U triangle (U, 1935; Demeke, et al., 1992). That study (Adams and Rieseberg,1998) showed that the use of similarity measures based on character differences coupled with multivariate methods such as principal coordinates analysis (PCO) effectively eliminated the non-homologous band problem even when up to 20% incorrectly scored bands were included in a PCO analysis. However, Adams and Rieseberg (1998) also point out that other numerical methods, as well as phylogenetic tree building programs, may be affected because they generally do not have any provision for accounting for error variance in the data.

So why did RAPDs work in the aforementioned systematic studies? We need to examine the molecular basis for RAPDs. RAPDs are actually inverted DNA repeats (IRs). Inverted repeats (IRs) in ssDNA form hairpin loops that are important for the control of gene transcription and subsequent protein processing (Brown, 2002). In addition, IRs are extremely important in determining the primary structure of RNA. Recently (Noller, 2005) published the structure of 16S rRNA and hairpin loops are the dominant features of 16S rRNA primary structure. Interestingly, most of these hairpins are secured by only 3 to 6 bp with a few 9 - 10 bp clamps. Figure 3 shows, that the

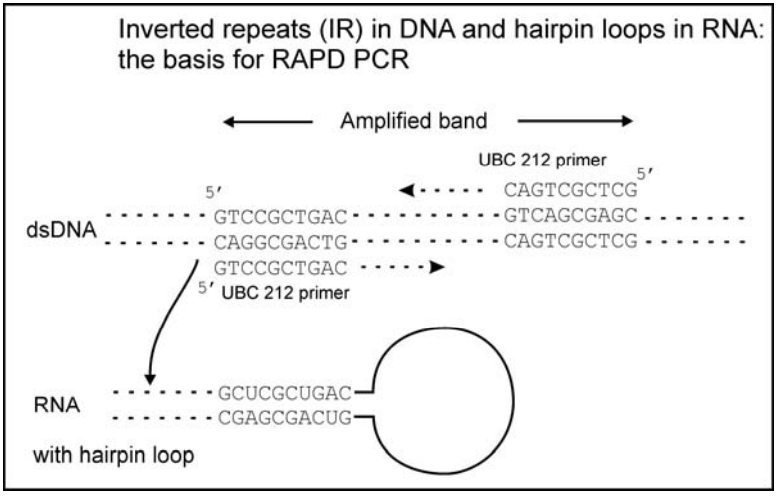

Figure 3. Diagrammatic representation of an inverted repeat PCR and the relationship of the inverted repeat to a RNA hairpin loop.

formation of hairpin loops in RNA. PCR using the single UBC (University of British Columbia) 212 primer results in an amplified band from this section of DNA (Fig. 3). The distance between the inverted repeats determines the size of the amplified band and also the size of the hairpin loop in the RNA (in this example). Of course, an additional priming site(s) may be present much further downstream (or even in an intron or in an inter-genic region) and would result in an additional, larger amplified band(s).

The use of single primers (inverted repeats) was co-discovered by Welsh and McClelland (1990) and Williams, et al. (1990). It is unfortunate that the terms 'random' and 'arbitrary' were used to describe the sequences of these primers, because we have discovered that the sequences are definitely neither 'random' nor 'arbitrary'. Beginning in 1990, we began to screen 10 bp RAPD and 17-21 bp ISSR primers available in kits from the University of British Columbia (UBC). We have evaluated 500 RAPD and 100 ISSR primers for their ability to: 1. amplify DNA (from various sources, both plants and animals); 2. obtain reproducible bands in replicate runs; 3. produce many bands, and 4. produce bands that are polymorphic between closely related species. These screenings discovered about 20 RAPD primers (4%) and 6 ISSR primers (6%) that met those criteria. It is now very apparent that only certain sequences of IRs are common in genomes (about 4% of the primers tested).

The focus of this study was to examine geographic variation among both contiguous and disjunct populations of *J. deppeana* by the use of a sensitive DNA technology (RAPDs) to investigate the effects of genetic isolation in relation to populational differentiation.

MATERIALS AND METHODS

Specimens used in this study: *J. deppeana* var. *deppeana*, Adams 10539-10541, El Chico National Park, Hidalgo, MX (H), Adams 10547-10549, Los Liros (El Tunal), Coahuila, MX (L); Adams 7632-34, Sacramento Mtns., New Mexico, USA (A); Adams 10616-10618, Chisos Mtns., TX, USA (C); Adams 10621-10623, Davis Mtns., TX, USA (D); Adams 10640-10642, Oak Creek Canyon-Flagstaff, AZ (F), USA; Adams 10645-10647, Bisbee, AZ, USA (B); *J. deppeana* var. *patoniana*, Adams 6837-6839, km 152, w. Durango (city), Durango, MX (P); *J. deppeana* var. *robusta*, Adams 6826-6828, Creel,

Chihuahua, MX (R); *J. deppeana* f.. *zacatecensis*, Adams 6840-6842, 18 km w. Sombrette, Zacatecas, MX (Z) *J. deppeana* var. *gamboana*, Adams 6863-67, Comitan, Chiapas, MX (G); *J. saltillensis*, Adams 6886-90, 14 km e. San Roberto Junction, Nuevo Leon, MX (S). Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20^{o} C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 268, AGG CCG CTT A; 338, CTG TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 431, CTG CGG GTC A; 478, CGA GCT GGT C.

PCR stock solutions (Taq, primer, buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams et al. 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 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. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams and Rieseberg 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation occurred in the heating/ cooling block. The thermal cycle used was: 94^{O}C (1.5 min) for initial strand separation, then 40 cycles of 40^{O}C (2 min), 72^{O}C (2 min), 91^{O}C (1 min). Two additional steps were used: 40^{O}C (2 min) and 72^{O}C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams and

Rieseberg 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed over UV light with Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (1) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational 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, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for by using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in a network that was superimposed on a geographic map (Adams, et al. 2003).

RESULTS AND DISCUSSION

Principal Coordinates analysis (PCO) of the similarity matrix of all samples yielded five eigenroots that accounted for 27.7, 14.4, 8.2, 6.7 and 6.0% of the total variance. The eigenroots appeared to asymptote after five roots and accounted for 63% of the variance. Ordination of the first three roots (Fig. 4) shows that the major trend (28%) to be the separation of *J. saltillensis* from *J. deppeana*. *Juniperus saltillensis* was included in the analysis because, although DNA sequence data indicates it to be one of the most closely related species to *J. deppeana* (Fig. 3), it is quite distinct and provides a relative comparison of speciation in this section. Just as seen in the nrDNA and trnC-trnD sequence data (Fig. 3), *J. d.* var. *gamboana* appears about as differentiated from other *J. deppeana* varieties as *J. deppeana* var. *robusta* is from other varieties (Fig. 4). This affirms the recognition of *J. d.* var. *gamboana* (Adams and Schwarzbach 2006) as

a part of the *J. deppeana* complex. It should be noted that *Juniperus d*. var. *gamboana* has checkered bark (exfoliation in squares or rectangles) as is characteristic of most *J. deppeana* varieties.

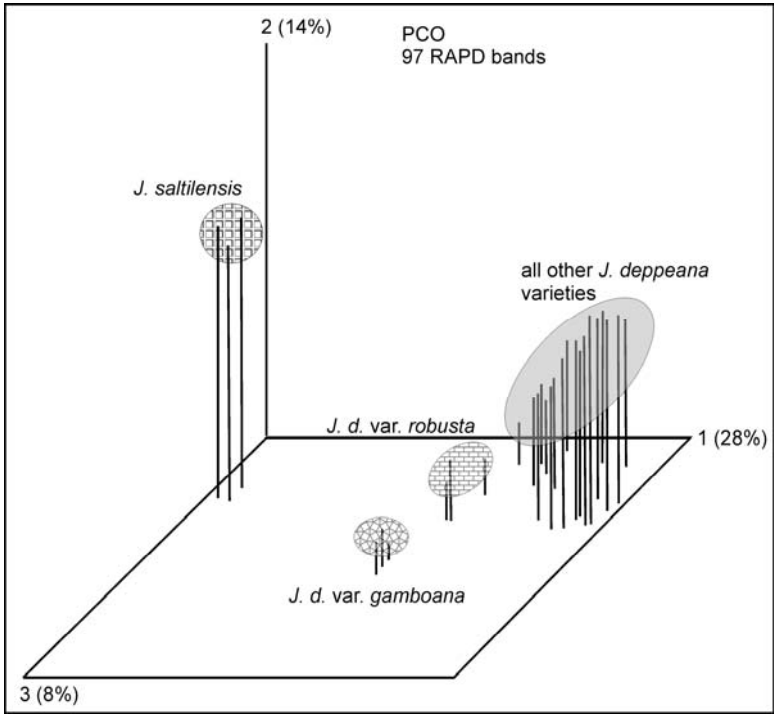

Figure 4. Principal Coordinate Ordination (PCO) for *J. saltillensis* and *J. deppeana* varieties.

Juniperus saltillensis was removed from the data set and a new PCO was performed to allow one to further examine the variation within *J. deppeana*. This PCO yielded five eigenroots that accounted for 58.35% of the variance (23.9, 11.04, 9.29, 8.13, 5.98%) before reaching an asymptote. PCO ordination (Fig. 5) shows that the major eigenroot (24%) tended to separate *J. d.* var. *gamboana* and *J. d.* var. *robusta* from the northern-most populations (C, D, A, B, F). In figure 5,

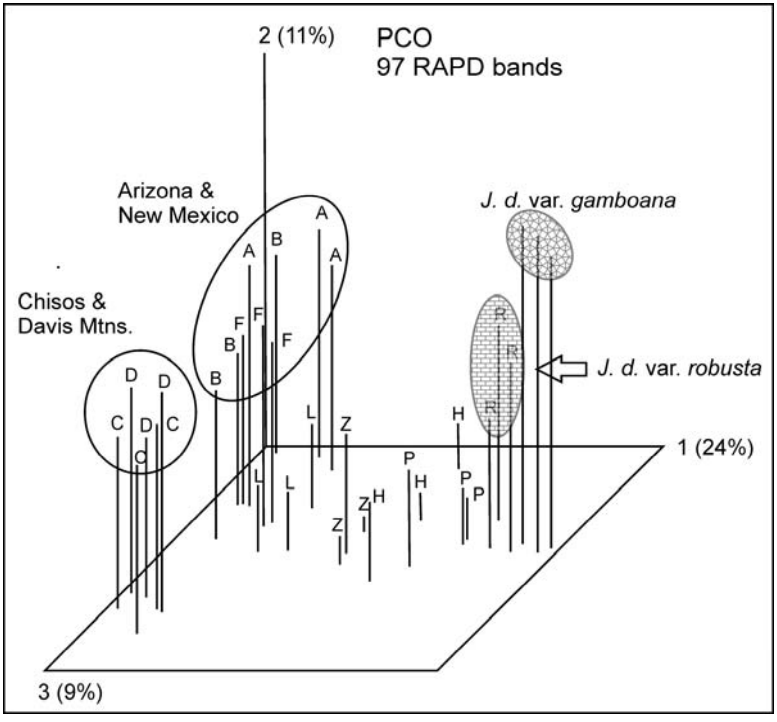

Figure 5. PCO of *J. deppeana* varieties. The first principal coordinate separates *J. d*. var. *gamboana* and *J. d*. var. *robusta* from the other *J. deppeana* varieties. Notice some separation between the Arizona - New Mexico and Chisos - Davis Mtns. populations.

one can see that *J. d*. var. *deppeana* (H, L), *J. d*. f. zacatecensis (Z) and *J. d.* var. *patoniana* (P) appear to be intermediate on these three axes. However, it should be noted that *J. deppeana* var. *patoniana* was well resolved on the fourth axis (8.13%) (data not shown). There appears to be some division between the Chisos - Davis Mtns. (C, D) populations and the Arizona - New Mexico (B, F, A) populations on the third axis (Fig. 5).

To visualize geographic variation, the order of populational clustering is shown on a contour map (Fig. 6). The most similar populations are the Chisos and Davis Mtns. (C, D, 0.932, Fig. 6). These

populations were probably contiguous during the Wisconsin if the vegetation descended 800 m (Wells, 1966). It appears that the Chisos and Davis Mtns. (and Sierra del Burro Mtns., in Mexico, just south of

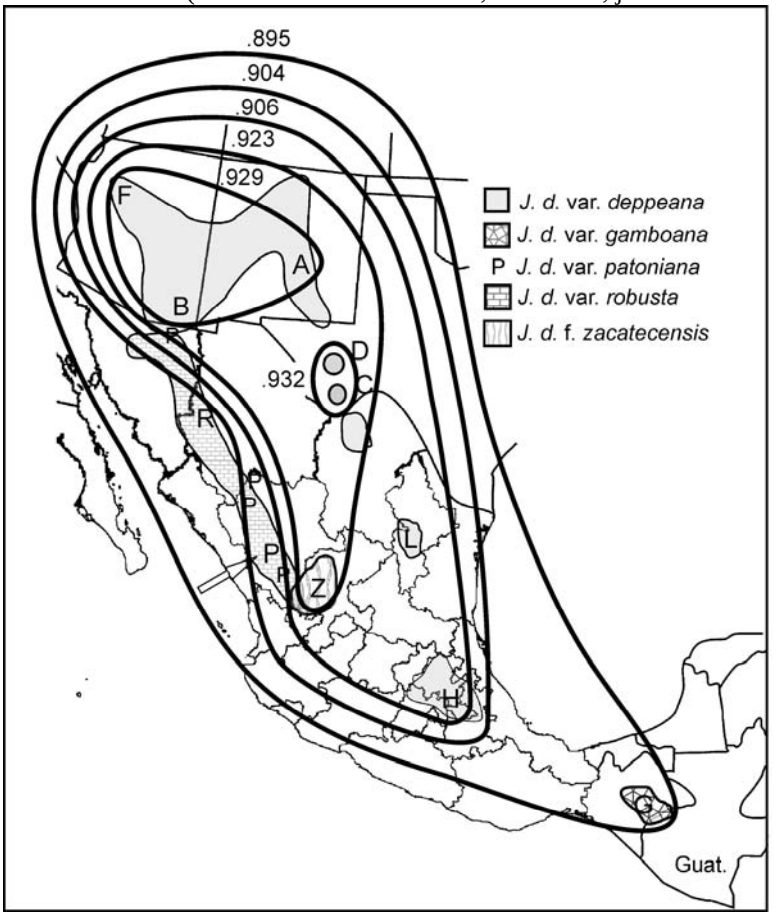

Figure. 6. Contoured groupings based on 97 RAPD bands. The open arrow indicates the location of *J. d.* var. *patoniana* (P) sampled. See text for discussion.

the Chisos) have only been 'island' populations for the past 10,000 y (Wells, 1966). A similar pattern is seen for the Arizona (B, F) and New Mexico (A) populations (0.929 cluster, Fig. 6). It is noteworthy that population A (Sacramento Mtns., NM) has greater affinities to Arizona (B, F) than to the Chisos - Davis Mtns. (C, D, Fig. 6). Both the Arizona - New Mexico (A, B, F) and the Chisos - Davis Mtns. (C, D) populations were displaced downward and southward during the Pleistocene (Wells, 1966). The uniformity of the Arizona - New Mexico populations suggests that the refugia for these *J. deppeana* populations may have been in the low mountains in the vicinity of Benson, Arizona (B), with recolonization proceeding towards northern Arizona (F) and central New Mexico (A).

Both the Chisos - Davis Mtns. *J. d.* var. *deppeana* (C, D) and the population of *J. d.* f. *zacatecensis* (Z) join the Arizona - New Mexico *J. d.* var. *deppeana* group at about the same level. Thus, although *J. d.* f. zac*atecensis* has distinctively large cones covered with copious amounts of glaucous wax (bloom), this taxon does seem to fit well within *J. d.* var. *deppeana*. The final two Mexican populations of *J. d.* var. *deppeana* are added (H, L, 0.906) giving *J. d.* var. *deppeana* a range from Flagstaff (F) to southern Mexico (Hidalgo, H, Fig. 6). The geographical clustering is completed by the addition of *J. d.* var. *patoniana* (P), *J. d.* var. *robusta* (R) from the Sierra Madre Occidental, and finally, *J. d.* var. *gamboana* (G) from Chiapas and Guatemala (Fig. 6).

To gain additional insight into the geographic dimension of the differentiation among these taxa, the populations were linked by a minimum spanning diagram (Fig. 7). Notice again that the populations in Arizona (B, F) and New Mexico (A) are closely linked (Fig. 7). However. *J. d.* var. *deppeana*, Flagstaff, AZ is a little more closely linked to *J. d.* f. zac*atecensis* (F-Z, 0.923) than to the Chisos Mtns. *J. d.* var. *deppeana* (F-C, 0.921, Fig. 7). The Los Liros population (L) links to Bisbee, AZ (B-L, 0.912, Fig. 7), rather than to the nearer Chisos - Davis Mtns. group (nearest L-C link was 0.894). The Hidalgo (H) population of *J. d.* var. *deppeana* in the Sierra Madre Oriental links to *J. d.* f. *zacatecensis* at a lower level (H-Z, 0.906). Martinez (1963) considered the Hidalgo population as typical for *J. d.* var. *deppeana* and recognized *J. deppeana* var. *pachyphlaea* (Torrey) Mart. in northern Mexico and southwestern United States. From figure 5, it is apparent that individuals from Hidalgo (H) do not form a uniform,

cohesive group but seem genetically diverse. At present, it seems prudent to treat both the northern populations in the USA and the eastern Mexico populations as *J. deppeana* var. *deppeana*. Morphologically, it is very difficult to separate the northern populations from the Hidalgo population. Sequencing additional genes and/or introns may help resolve these relationships.

The *J. d.* var. *gamboana* population (G) has the lowest linkage (G-R, 0.895 to *J. d.* var. *robusta*), followed by the linkage of *J. d.* var.

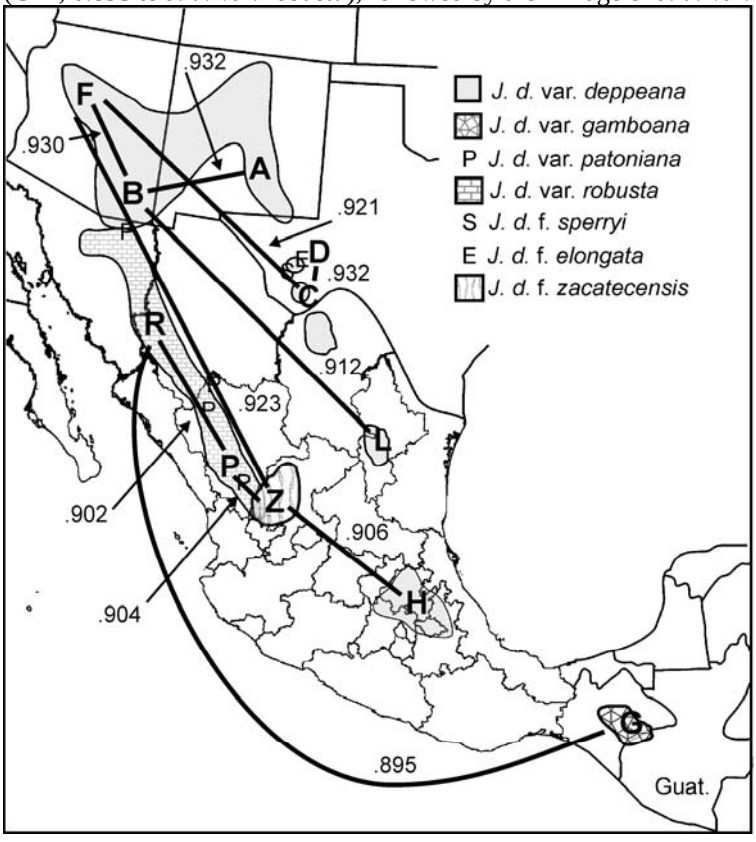

Figure 7. Minimum spanning diagram showing linkage between populations and taxa. Notice that *J. d.* f. *zacatecensis* is linked to *J. d.* var. *deppeana*, Flagstaff(F). See text for discussion.

patoniana to *J. d*. f. *zacatecensis* (P-Z, 0.904) and then *J. d*. var. *robusta* to *J. d.* var. *patoniana* (R-P, 0.902) (Fig. 7).

CONCLUSION

It is surprising to find high genetic similarities between disjunct populations (Fig. 7). For example, *Juniperus d.* var. *deppeana* (F, Flagstaff, Arizona) was a little more similar to *J. d*. f. *zacatecensis* (Z, Fig. 7) than to the Chisos - Davis Mtns. (C, D) *J. deppeana* populations. In addition, plants from the Sacramento Mtns. (A, Fig. 7) were more similar to Benson, AZ (B, Fig. 7), than to the Davis Mtns. (D, Fig. 7) population. The Los Liros population of *J. d*. var. *deppeana* was more similar to the Benson, Arizona population (B, Fig. 7) than to the nearer Hidalgo population (H, Fig. 7).

Although most *Juniperus* species' seeds are disseminated by birds (see discussion in Adams, 2004), some species are dispersed by small mammals (raccoons, opossums, squirrels). The female cones of most of the *J. deppeana* varieties are probably too large and woody to be a food choice of migratory birds. It would seem that cone dispersal for *J. deppeana* is chiefly by mammals, gravity and water. These kinds of dispersal makes the high genetic similarities between disjunct populations seem even more unusual. The disjunct population of *J. d*. var. *gamboana* in the mountains of Chiapas and Guatemala (1670-2200 m) would not likely to have ever been contiguous across the isthmus of Tehuantepec (Fig. 7) to the Sierra Madre del Sur and thence into central Mexico. It seems more reasonable that the establishment of *J. d*. var. *gamboana* in Chiapas, MX and Guatemala was by long distance dispersal by birds because it has one-seeded, relative small (5-8 mm), soft, fleshy female cones that birds consume.

The reason for the high genetic similarities between disjunct populations in central and northern Mexico and the southwest United States may be due to ancient climate and past distributions of *J. deppeana*. Wells (1966), using data from rat middens from the Big Bend of Trans-Pecos Texas region (C in Fig. 2), concluded that during the Wisconsin (70,000 - 13,000 ybp) life zones descended about 800 m leading to the formation of a pinyon-juniper woodland in the present Chihuahuan desert between the Big Bend of Trans-Pecos Texas and the city of Del Rio. Assuming that the effects of glaciation were mediated southward into Mexico so that life zones descended only a few hundred

meters in Hidalgo (E, Fig. 2), it appears that most of the now disjunct populations of *J. deppeana* may have been connected in a nearly continuous population of distribution around the Chihuahuan desert (Fig. 8). It is likely that desert peaks within the ring also supported stands of *J. deppeana*. These Wisconsin populations would have

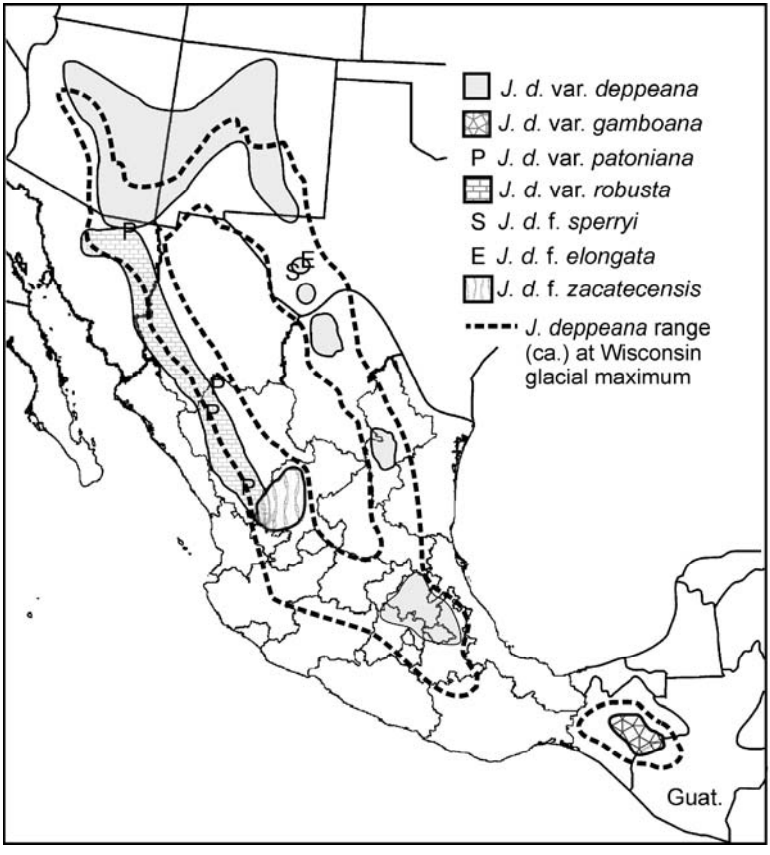

Figure 8. Possible range of *J. deppeana* during the Wisconsin glacial maximum (based on Wells, 1966). The present day disjunct populations were likely continuous in the foothills around the Chihuahuan desert during the Wisconsin.

become spatially separated as dryer, warmer climate developed during the Holocene (past 13,000 y). Of course, the Wisconsin was only the most recent of several pluvial events during the Pleistocene, spanning 1.8 my (Flint, 1971). It is likely that during any one (or several) of these pluvial events, *Juniperus deppeana* occupied lower elevation and more southward habitats, leading to more contiguous populations in Mexico and the southwest United States. If divergent populations (or varieties) became sympatric during the Wisconsin, this would have facilitated infra-specific crossing. This may account for the large genetic variation within some populations. In addition, the millennia of continuous populations could explain the lack of differentiation between the recently (Holocene) geographically isolated populations.

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