HYBRIDIZATION BETWEEN *JUNIPERUS ERYTHROCARPA* CORY AND *JUNIPERUS PINCHOTII* SUDWORTH IN THE CHISOS MOUNTAINS, TEXAS

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**ABSTRACT**—Individuals from the Chisos Mountains, Texas, a population containing *Juniperus erythrocarpa* Cory, *Juniperus pinchotii* Sudw. and putative hybrids, were compared to *J. erythrocarpa* from west of Alpine, Texas, and *J. pinchotii* from northeast of Marathon, Texas, using morphological and terpenoid characters. Numerical analyses included weighted hybrid indices and principal coordinate analysis. These analyses were complementary in showing hybridization between *J. pinchotii* and *J. erythrocarpa* in the Chisos Basin population. The populations of *J. erythrocarpa* from west of Alpine and *J. pinchotii* northeast of Marathon were found to contain a few individuals intermediate in their chemistry and morphology, suggesting introgression.

During the past 40 years, the genus *Juniperus* has been the subject of numerous studies concerning gene flow between species. The early work by Fassett (1944, 1945a, 1945b) indicated hybridization between the closely related species *Juniperus horizontalis* Moench, *Juniperus scopulorum* Sarg., and *Juniperus virginiana* L. Portions of that work have been reexamined using terpenoids (von Rudloff, 1975a, 1975b; Flake et al., 1978; Comer et al., 1982; Adams, 1983), numerical analysis of morphological characters (van Haverbeke, 1968; Schurz, 1971), and combined morphological, isozyme, and terpenoid data (Palma-Octal et al., 1983). These studies were all in general agreement that gene flow either has occurred or is now occurring between the species studied.

On the other hand, the putative allopatic introgression between two very different species, *Juniperus ashei* Buch. and *Juniperus virginiana* (species in different subsections of the genus), has fared quite differently. The case was initially based (Hall, 1952a, 1952b) on the use of a few selected morphological characters and the scatter diagrams of the type devised by Anderson (1949). Later research utilized chemical characters that are not visible to the collector in the field and, thus, not subject to a non-random collection bias. These studies showed no evidence of gene flow between these rather different species (terpenoids, von Rudloff et al., 1967; and Flake et al., 1969, 1973; terpenoids and morphology, Adams and Turner, 1970; and Adams, 1975a, 1977).

Gene flow has also been suggested to occur between *J. ashei* and *Juniperus pinchotii* (Hall et al., 1962), between *J. pinchotii* and *Juniperus deppeana* Steud. (Hall et al., 1962) and between *J. pinchotii* and *Juniperus monosperma* (Engelm.) Sarg. (Hall and Carr, 1968; Correll and Johnston, 1970). Correll and Johnston (1970) included *Juniperus erythrocarpa* as part of *J. pinchotii*. They noted that the rose-fruited individuals (*J. erythrocarpa*) in trans-Pecos Texas were intermediate (which they are) between *J. monosperma* and *J. pinchotii* and concluded that hybridization was occurring. Hybridization between *J. pinchotii* and *J. monosperma* was not substantiated in studies using both morphology and terpenoids (Adams, 1972, 1975b; Zanoni and Adams, 1975, 1976), although one tree was found in Palo Duro Canyon that might show evidence of gene flow (Adams, 1972). In general, the studies of the 22 taxa of junipers in Mexico and Guatemala failed to show any evidence of hybridization except between infraspecific taxa or the most closely related species (Zanoni and Adams, 1975, 1976).

*Juniperus erythrocarpa* and *J. pinchotii* occupy rather separate habitats, with *J. erythrocarpa* found in the Bouteloua grasslands at elevations from 1,500 to 2,000 m in trans-Pecos Texas, southern New Mexico, and southern Arizona and southward into Mexico (see map in Adams and Zano-
ni, 1979). *Juniperus pinchotii* is found on eroded soils in west Texas and northern Coahuila (Fig. 1). Aside from a zone of sympathy in eastern Coahuila near Saltillo, the Basin area of the Chisos Mountains (Fig. 1) represents the most distinct zone of sympathy between *J. erythrocarpa* and *J. pinchotii*.

The zone of sympathy between *J. erythrocarpa* and *J. pinchotii* changed repeatedly during the Pleistocene pluvial periods. The last extensive zone of sympathy was about 10,000 to 15,000 YBP when the life zones around the Chisos Mountains descended 400 to 800 m (Wells, 1966). This led to an expansion of the range of *J. erythrocarpa* into the margins of the Chihuahuan Desert. Although *J. erythrocarpa* undoubtedly expanded westward, a discussion of its expansion eastward towards the range of *J. pinchotii* is more relevant for this paper. The area between the Chisos Mountains and Del Rio, Texas, was a pinyon-juniper woodland (Wells, 1966; Bryant, 1969; Adams, 1977) that probably contained *J. erythrocarpa*, *J. pinchotii*, *J. ashei*, and possibly *J. monosperma* (entering from the northwest). Elements of *J. pinchotii* still persist as far south as Saltillo, Coahuila, where they are sympatric with *J. erythrocarpa* (Adams, 1975b). Thus, as the Bou-

telova grasslands expanded, *J. erythrocarpa* expanded its range into these new sites, gradually replacing *J. pinchotii*, which persisted in rocky areas in the drier sites. This continual mixing during migrations gave ample opportunity for past gene exchange between these taxa.

**Materials and Methods**—Foliage samples consisted of eight to 10 terminal branches from each tree. The branches were placed in plastic bags and immediately frozen in a freezer in a field trailer. Herbarium and cone specimens were pressed and air-dried. Foliage for chemical analyses was kept at −20°C until steam-distilled (von Rudloff, 1967). All of the plants sampled in this study are voucheded by the deposition of a preserved herbarium specimen at the herbarium of Baylor University. Both voucher and sample numbers are listed by study area.

Twenty plants of *J. pinchotii* (Adams, 1975b) were sampled from northeast of Marathon, Texas (Adams 1381-1400; Fig. 1). Fourteen trees of *J. erythrocarpa* (Adams, 1975b) were sampled from a population just west of Alpine, Texas (Adams 1401-1414; Fig. 1). Eighty-four trees were sampled (Adams 1295-1378; Fig. 1) in the Basin of the Chisos Mountains (1,600 to 1,800 m elev.) where *J. erythrocarpa* and *J. pinchotii* grow, along with putative hybrids. Although the junipers growing on the alluvial fan just north of Green Gulch (1,200 to 1,500 m elev.) appeared to be, morphologically, fairly typical of *J. pinchotii*, 10 individuals were sampled (Adams 1285-1294; Fig. 1) for analyses of possible introgression. All samples were collected on 3 September 1975.

The terpenoids were analyzed by gas chromatography on a 60-m by 0.5-mm SS WCOT column coated with PEG 20 M (see Adams, 1975a, for details). The identification of the volatile leaf oils of *J. erythrocarpa* and *J. pinchotii* have been previously reported (Adams et al., 1981).

Initially, 26 morphological characters were scored using the pressed specimens and cones. Analysis of variance (ANOVA) tests were performed using two groups: *J. erythrocarpa* from west of Alpine and *J. pinchotii* from northeast of Marathon. Thirteen morphological characters were found to be more variable within species than between species. Thus, their F-ratios were < 1 (i.e., F = variance between/variance within). These characters should be eliminated because they contain no discriminatory information concerning differences between the groups under study (that is not to say they contain no information but just that they are not useful for classification of individuals into one or the other species). In the junipers, crown shape is an example of a character that is occasionally so influenced by shading and edaphic factors that the use of this character (e.g., to separate *J. ashei* and *J. virginiana* in Austin, Texas) is unreliable. In that zone, the two
TABLE 1—Morphological characters scored, their descriptions, and F-ratios from analysis of variance tests (d.f. = 2,33) between *Juniperus pinchoi*ii and *Juniperus erythrocarpa*.

<table>
<thead>
<tr>
<th>Character</th>
<th>F-ratio</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCO</td>
<td>47.97**</td>
<td>Cone color: 1 = tan, 2 = copper-red-tan, 3 = pink, 4 = purple-blue</td>
</tr>
<tr>
<td>BLM</td>
<td>205.55**</td>
<td>Cone glaucousness: 1 = none, 2 = some, 3 = most</td>
</tr>
<tr>
<td>MAT</td>
<td>7.11*</td>
<td>No. of mature cones/10 randomly chosen cones from voucher</td>
</tr>
<tr>
<td>SLN</td>
<td>1.80</td>
<td>Seed length, average of 5, in millimeters</td>
</tr>
<tr>
<td>SPF</td>
<td>3.39</td>
<td>Seeds per cone, average of 5</td>
</tr>
<tr>
<td>SCO</td>
<td>13.92**</td>
<td>Seed color: 1 = tan, 2 = mahogany</td>
</tr>
<tr>
<td>B/S</td>
<td>37.42**</td>
<td>Ratio of whip leaf length to whip leaf sheath length, average of 5</td>
</tr>
<tr>
<td>G/S</td>
<td>2.92</td>
<td>Ratio of whip leaf gland length to whip leaf sheath length, average of 5</td>
</tr>
<tr>
<td>SLL</td>
<td>8.96**</td>
<td>Scale leaf length, average of 5, in millimeters</td>
</tr>
<tr>
<td>L/B</td>
<td>16.31**</td>
<td>Ratio of scale leaf length to scale branch width, average of 5, in millimeters</td>
</tr>
<tr>
<td>BAN</td>
<td>3.14</td>
<td>Branching angle of ultimate branch, average of 5, to nearest 5 degrees</td>
</tr>
<tr>
<td>H/S</td>
<td>5.31*</td>
<td>Ratio of hilum scar length to seed length, average of 5 ratios</td>
</tr>
<tr>
<td>WGC</td>
<td>27.55**</td>
<td>Whip leaf tip glaucousness: 1 = none, 2 = some, 3 = most</td>
</tr>
</tbody>
</table>

* 0.01 < P ≤ 0.05.
** P ≤ 0.01.

taxa are sympatric and occur in intermediate habitat. As a consequence, one finds *J. ashei* that have a strong central axis (like typical *J. virginiana*) growing in dense forests and *J. virginiana* that are stunted with rounded crowns (typical of *J. ashei*) growing on limestone. However, analyses of numerous morphological and terpenoid characters showed no hybridization (von Rudloff et al., 1967; Adams and Turner, 1970). The interested reader is referred to Adams (1975c) for discussion of character weighting and Adams (1982) for the examination of multivariate methods for the analyses of hybridization using both artificial hybrids (sunfish) and natural hybrids (junipers).

Thus, due to several characters being more variable within species than between species, the following 13 characters were eliminated from further consideration: cone length, cone diameter, ratio of cone length to diameter, whip leaf length times width, ratio of whip leaf length to width, ratio of whip gland length to width, whip blades straight/recurred, scale leaves deciduous/persistent, color beneath branch bark, whip glands flat/protruding, and whip gland entire/ruptured. The remaining 13 morphological characters are shown in Table 1.

In like manner, ANOVA tests were performed on 134 volatile oil components found in either *J. erythrocarpa* (west of Alpine) or *J. pinchoi*ii (northeast of Marathon). Because the sensitivity of gas chromatographs is so great that even the smallest contamination (e.g., from plastic gloves) can be detected and quantified, we limited our mass spectrometry to components of approximately 0.2% of the total oil, and this value seems a reasonable threshold to use for the elimination of small components in the statistical analyses. After elimination of components that did not occur in concentrations >0.2% of the total oil in either *J. erythrocarpa* or *J. pinchoi*ii, ANOVA tests resulted in 40 terpenoids with F-ratios >1.

Various computer programs were written to generate scatter diagrams, along with the $F^{1/2} - 1$ and $F - 1$ (F from ANOVA) weighted hybrid indices. Similarity measures were computed using $F^{1/2} - 1$ (for morphological) and $F - 1$ (for terpenoid) character weights and mean character differences (MCD) as formulated by Adams (1975c). Principal coordinate analyses (PCOORD, Gower, 1966; Williams et al., 1971) were conducted on similarity matrices based on morphological (13) and terpenoid (40) characters.

The $F^{1/2} - 1$ weights were used for the morphological data because some of the characters had extreme F-values in ANOVA due to the meristic mode of variation. Cone glaucousness is a good example. Female cones of *J. erythrocarpa* generally have a glaucous bloom, and nearly all female cones would be scored as 3 (3 = most all glaucous). In contrast, female cones of *J. pinchoi*ii seldom have any glaucousness, so almost all female cones scored would be given a value of 1 (1 = not glaucous). Because there is almost no variance within the taxa (i.e., almost all cones are scored as either 3 for *J. erythrocarpa* and 1 for *J. pinchoi*ii), the F-ratio will approach infinity as the denominator approaches 0. We are then faced with a paradox. We have a very
good, discriminatory character, but we cannot quantify how good it is in relation to other characters. One solution is to give this character the same weight as the next most discriminatory character in the study (Zanoni and Adams, 1976). A second possibility is to add a small random variable to each observation to artificially generate some error variance. This can be done by using a random number table or generator on a computer. Numbers 0 to 4 can be scaled as $-0.01, -0.02, \ldots, -0.05$ and added to the corresponding data value. In like manner, numbers 5 to 9 can be scaled +0.01, \ldots, +0.05 and added to data values. If the random numbers are scaled by about 1% of the range of the data values, this will generate a small random error variance to keep the $F$-values from going to infinity.

One may still be faced with the problem encountered in the present study. Cone glaucousness accounts for 60% of the total sum of the $F$-ratios for the 13 morphological characters (Table 1). In preliminary analyses (J. R. Kistler, pers. obser.), this resulted in a very discontinuous classification of individuals, mostly into two groups. However, by taking the square root of $F$, the relative character weights are preserved, while limiting the contribution of meristic characters (see Adams, 1975c, for examples). In all cases, 1.0 was subtracted from the $F$-ratio or $F^{1/2}$ because a $F$ (or $F^{1/2}$) equal to 1.0 has no (i.e., zero) discriminatory information. It is only the $F$-ratios $>1.0$ that have any discriminatory value for that character. A previous study (Adams, 1982) demonstrated that $F - 1$ character weights are more powerful for the analyses of hybridization, but, unfortunately, the meristic nature of some of the morphological data precluded the use of $F - 1$ weights for the morphological characters in this study.

**RESULTS AND DISCUSSION**—In a study on the use of multivariate methods for the detection of hybridization, Adams (1982) found the $F - 1$ ($F$ from ANOVA of the parents) weighted hybrid index (WHI) and principal coordinate analysis based on similarity matrices using $F - 1$ weighted characters to be most useful. The morphological WHI shows (Fig. 2) $J. pinchotii$ and $J. erythrocarpa$ to be well resolved except for one intermediate $J. erythrocarpa$ individual (1401) from Alpine. There is also one outlier of $J. pinchotii$ (1382). Not all the individuals sampled in the study could be used in the morphological analyses because female cones were not present on some plants. This is a limitation of morphological data that does not apply to chemical data.

The Basin individuals (Fig. 2) have a bimodal distribution ranging from typical $J. pinchotii$ to typical $J. erythrocarpa$. Several of the Basin individuals appear to be intermediate. The Green Gulch individuals were ordinated about as expected from our field examination, fairly typical of $J. pinchotii$.

The WHI based on the terpenoids (Fig. 3)
revealed that the Marathon population of \textit{J. pinchotii} has one individual (1393) that clusters strongly with \textit{J. erythrocarpa} and three somewhat divergent (introgressed?) individuals. This probably accounts for the slight interdispersion found previously (population 14 in Adams, 1975b). The Basin individuals again show signs of bimodality, but the distribution is continuous except for a few individuals similar to typical \textit{J. pinchotii}. The Green Gulch individuals show a definite bimodality: some individuals are typical \textit{J. pinchotii}, while others have a greater similarity to \textit{J. erythrocarpa} but are not typical of that taxon.

The WHI based on terpenoids and based on morphology agreed in general but disagreed in many specific cases. The correlation between these indices was 0.80 (95% confidence interval = 0.72, 0.86). Several Basin individuals showed interdispersion in both indices. For example, if the WHI for chemical data = C and WHI for morphology = M; then C and M for \textit{J. pinchotii} = 1.0; C and M for \textit{J. erythrocarpa} = 0.0, then C = 0.56 and M = 0.62 for sample 1313, C = 0.45 and M = 0.55 for sample 1341, and C = 0.61 and M = 0.52 for sample 1346.

Principal coordinate analysis (PCOORD) has been shown to be superior to other multivariate methods for the detection of hybridization (Kistler, 1976; Adams, 1982). PCOORD using morphological data reveals (Fig. 4) the Basin (and Green Gulch) individuals cluster in a U or V shape with the first coordinate ordinating between \textit{J. pinchotii} and \textit{J. erythrocarpa} explaining 48.5% of the variation. The second axis ordinates between parental types and first filial generation (\textit{F}_1\textit{s}) and second filial generation (\textit{F}_2\textit{s}; see Kistler, 1976 and Adams, 1982, 1983). Thus, the hybrids should be found midway on axis 1 and low (-0.35) on axis 2 (the dashed line, Fig. 4); \textit{F}_2\textit{s} tend to scatter about the \textit{F}_1\textit{s} and backcrosses toward the recurrent parent (see Kistler, 1976, for computer-simulated data). Thus, samples 1346 and 1341 might be backcrosses (BC) to \textit{J. pinchotii}, and samples 1313 and 1323 might be BC\textsubscript{1} or BC\textsubscript{2} (see Figs. 4 and 5).

PCOORD using terpenoids shows a similar overall pattern (Fig. 5) to PCOORD with morphology in that a U- or V-shaped ordination is achieved with possible \textit{F}_1\textit{s}, \textit{F}_2\textit{s}, and backcrosses. As with the WHI, terpenoids show more of a continuous distribution than does morphology. Placement of specific individuals differs considerably in some cases. Individual 1313 looks like \textit{J. pinchotii} or an advanced generation backcross to \textit{J. pinchotii} morphologically but appears as an \textit{F}_1\textit{ or BC\textsubscript{1}} (to \textit{J. pinchotii}) using terpenes. The
morphyology of 1323 placed it close to Juniperus pinchotii or an advanced generation backcross (Fig. 4) whereas terpenoids show it to be intermediate. On the other hand, placement of 1346 is similar using either morphology (Fig. 4) or terpenoids (Fig. 5).

A detailed examination of individuals in the dashed line region of Figs. 4 and 5 revealed that very few were intermediate in both morphology and chemistry. This lack of intermediacy in both suites of characters lends support to the idea that few first generation hybrids are present. However, the lack of correspondence between morphological and chemical data when identifying hybrids has been noted by Levin (1968). In such cases, this probably indicates that the chemical characters segregate independently of the morphological characters.

In addition to the use of principal coordinate analysis, multiple step-wise discriminate function analysis (canonical variate analysis, CVA) of the terpenoids gave almost identical results to PCOORD (Kistler, 1976), except the groupings were not as well ordered in two dimensions. This is similar to studies with simulated data (Kistler, 1976) and sunfish hybrids (Adams, 1982). The CVA with morphological data also gave similar results as the PCOORD (Kistler, 1976), except no groups were apparent and the ordination was essentially only one-dimensional.

To have hybridization, several factors must occur: the taxa must be in close enough proximity to exchange pollen; their pollination times must overlap; there must be a suitable (intermediate) habitat (Anderson, 1949) for the F₁s to grow (of course, one could list numerous other factors involving genetic compatibility). The Chisos Basin population satisfies the proximity problem and provides considerable intermediate habitat. Juniperus pinchotii pollinates in September and October in the area and J. erythrocarpa pollinates in November and December (R. P. Adams, pers. obser.; C. Kruse, pers. obser.). However, variation in moisture and temperature can move these dates by several weeks, and, thus, considerable overlap in pollination times is to be expected.

Both morphological and terpenoid data indicated that hybridization is occurring between J. erythrocarpa and J. pinchotii in the Basin of the Chisos Mountains. The variation forms a continuum between the species suggesting considerable backcrossing. The two reference populations of J. erythrocarpa (west of Alpine) and J. pinchotii (northeast of Marathon) appear to contain a few introgressed individuals.

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