CHEMOSYSTEMATICS—ANALYSES OF POPULATIONAL DIFFERENTIATION AND VARIABILITY OF ANCESTRAL AND RECENT POPULATIONS OF *JUNIPERUS ASHEI*[1]

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ABSTRACT

Three types of data were used to analyze 28 natural populations of *Juniperus ashei*: 16 morphological characters, 152 terpenoids, and 23 peroxidases. In this paper the peroxidase electromorphs were treated as ordinary qualitative chemical characters to examine the feasibility of using isozymes for taxonomic purposes and as indicators of populational variability. The data sets were subjected to various numerical analyses to examine regional trends, ancestral affinities, and variability within populations. Principal coordinate analysis was used to resolve the major coordinates of variation from the similarity matrix of each data set. Coordinate loadings were then contoured for the first three coordinates of each similarity matrix to aid the visualization of the regional trends. The terpenoids and morphology showed a series of uniform populations from central Texas into the Ozarks with divergent populations on the south and west portions of the range, extending into northern Mexico. No regional trends were apparent in the peroxidases and no corresponding modes of variation were seen between the peroxidases and the other two data sets. Pleistocene vegetation is reviewed and migration paths are speculated upon. Advanced and primitive character states are discussed. The uniform body of *J. ashei* populations from central Texas to the Ozarks appear to be advanced (recent), whereas the divergent populations seem to be more primitive (ancestral). A method called differential similarities is introduced to analyze the clinal gradation of *J. ashei* toward *J. saltillensis* in Mexico. Intrapopulational variability was analyzed by use of the average similarity within populations and the coefficient of phenetic variation (CPV). In general, the recent populations had high similarities and low variability, and the ancestral populations had lower average similarities and higher CPVs with both the morphological and terpenoid data. The pattern of variation in the peroxidases could not be generalized upon, but appeared to be mosaic. Peroxidases did not appear useful in this analysis when subjected to standard numerical analysis procedures. The evolution of *J. ashei* into its present distribution appears to have had at least two phases composed of very uniform, recent migrations and persistent, variable, relict populations perhaps extending close to the geographic origin of this taxon in northern Mexico.

The use of chemical characters has gained widespread acceptance during the past decade to the point that a graduate student thesis in systematics is now unusual if no chemical data are utilized. Because of the relative ease of use, flavonoids are widely utilized in systematic and evolutionary plant studies. The early works on *Asplenium* (Smith & Levin, 1963), *Lemnaceae* (McClure & Alston, 1966) and *Baptisia* (Alston & Turner, 1963) are classics, required reading for chemosystematic students. Likewise, classic is the work on betalains by Mabry and coworkers (summarized in this symposium). Whereas flavonoids and betalains have been extensively used above the species level (probably due to the qualitative nature of the methods), terpenoids, due to the quantitative nature of gas/liquid chromatography, have been more widely used at or below

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the species level. The gymnosperms have been the focus of many studies of populational differentiation which have uncovered clines (Flake et al., 1969, 1973), chemical races (Smith et al., 1969), hybridization (see von Rudloff, 1975 for an excellent review), and ancestral migrations (Adams, 1975a; Zavarin & Snajberk, 1973). In the angiosperms, work on the monterpenes of *Bursera* (Mooney & Emboden, 1968) demonstrated the use of these compounds in the detection of clinal variation. Of course, the work on the Australian *Eucalyptus* species has been of tremendous use in the classification of populations of these taxa and is well known.

Although studies using terpenoid characters to analyze populational differentiation are well known, the analysis of population variability in relation to important population biology questions such as the founder's effect, genetic drift, the effects of small versus large populations and central versus peripheral sites on variability have not been addressed. The relatively recent rise in the use of isoenzyme data has rekindled an interest in the examination of these questions. Gottlieb (at this symposium) has reviewed the literature on isozymes and their use in systematics. Nevertheless, it seems in order to mention that the "isozyme bandwagon" has become the current fad before we have developed a very thorough knowledge about the molecular basis of the electromorphs distinguished on gels.

Before the widespread use of isozymes, the study of variability within populations seems to have stagnated with the exception of the numerical taxonomic school (including morphometrics). Gilmartin (1969a, 1969b, 1974, 1976) has introduced a new idea called the coefficient of phentic variation (CPV) to examine the combined effects of many characters on variability. The CPV is merely the standard deviation of the mean similarity among a group of operational taxonomic units (OTUs) divided by that mean similarity. Whereas the mean similarity of a group tells about the average affinities, the CPV shows how homogeneous are the similarities of one group versus another group. Since the CPV is normalized by the mean similarity, different character sets can be compared as well as different levels of organization (i.e., population vs. species vs. genus). To my knowledge, the CPVs have not been used to study population variability with the exception of the studies by Gilmartin. The purpose of this paper is to examine population differentiation and variability in *Juniperus ashei* Buch. using three contrasting sets of characters: morphological characters, volatile terpenoids from leaves, and leaf peroxidases. The literature on *J. ashei* has been reviewed by Adams & Turner (1970).

Juniperus ashei is a taxon of a rather restricted range, occurring on limestone outcrops from northern Mexico to southern Missouri (Fig. 1). The Edwards Plateau region of central Texas supports dense populations covering thousands of acres, whereas the disjunct populations (Lubbock-Post, Texarkana, Arbuckle Mountains, Ozark Mountains, and northern Mexico) often have nearly pure stands of *J. ashei*, but seldom cover such large areas. Being a fairly conspicuous conifer tree, one can be relatively confident in the taxonomic distribution records which imply that there are few, if any, trees between the disjunct populations and the Edwards Plateau populations. Thus, this would appear to

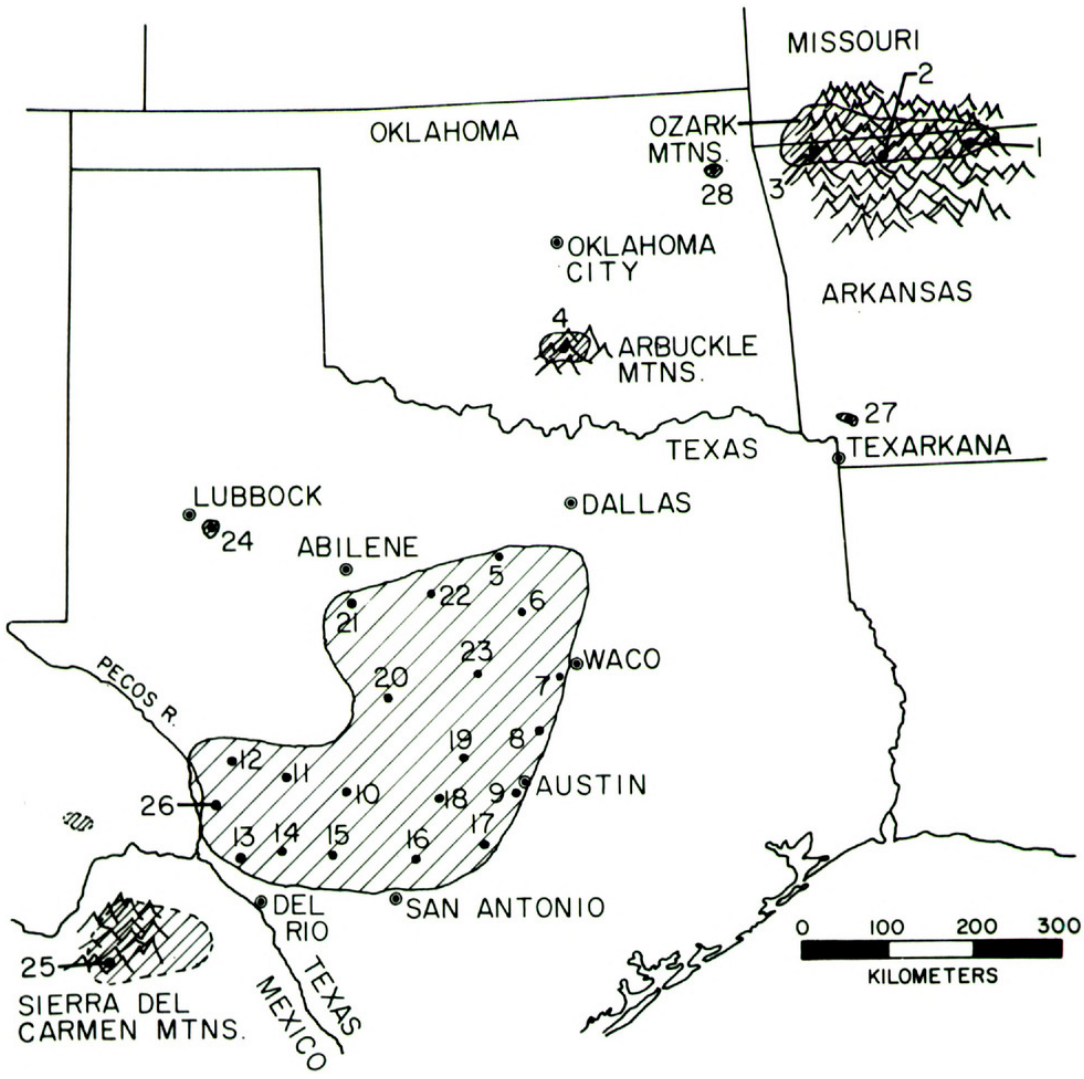


FIGURE 1. Distribution of *Juniperus ashei* showing the 28 populations sampled for this study. The exact distribution of *J. ashei* in northern Mexico is not known and is indicated generally by a dashed line.

be an excellent taxon to test some of the hypotheses advanced by Ehrlich & Raven (1969) in regard to gene flow versus selection in the maintenance of species.

Previous research (Adams & Turner, 1970; Adams, 1975a) has shown that the terpenoids of this taxon exhibit a remarkably high similarity between central Texas and the Ozarks (Fig. 2). However, many significant differences were found between populations 12, 13, and 17 and the other populations. One tree of *J. ashei* (number 116 in Fig. 2) was discovered in Mexico and found to cluster with the atypical populations (12, 13, 17). This, along with similar evidence in *J. pinchotti* populations (Adams, 1975b) seemed to imply that relicit migrations have been very important in the establishment of these patterns.

Evidence from rat middens and palynology in the southwestern United States is considerable (King, 1973; Mehringer et al., 1970; Van Devender & King, 1971; Wells, 1965, 1966, 1970; Wells & Berger, 1967; Whitehead, 1972; Wright, 1970)

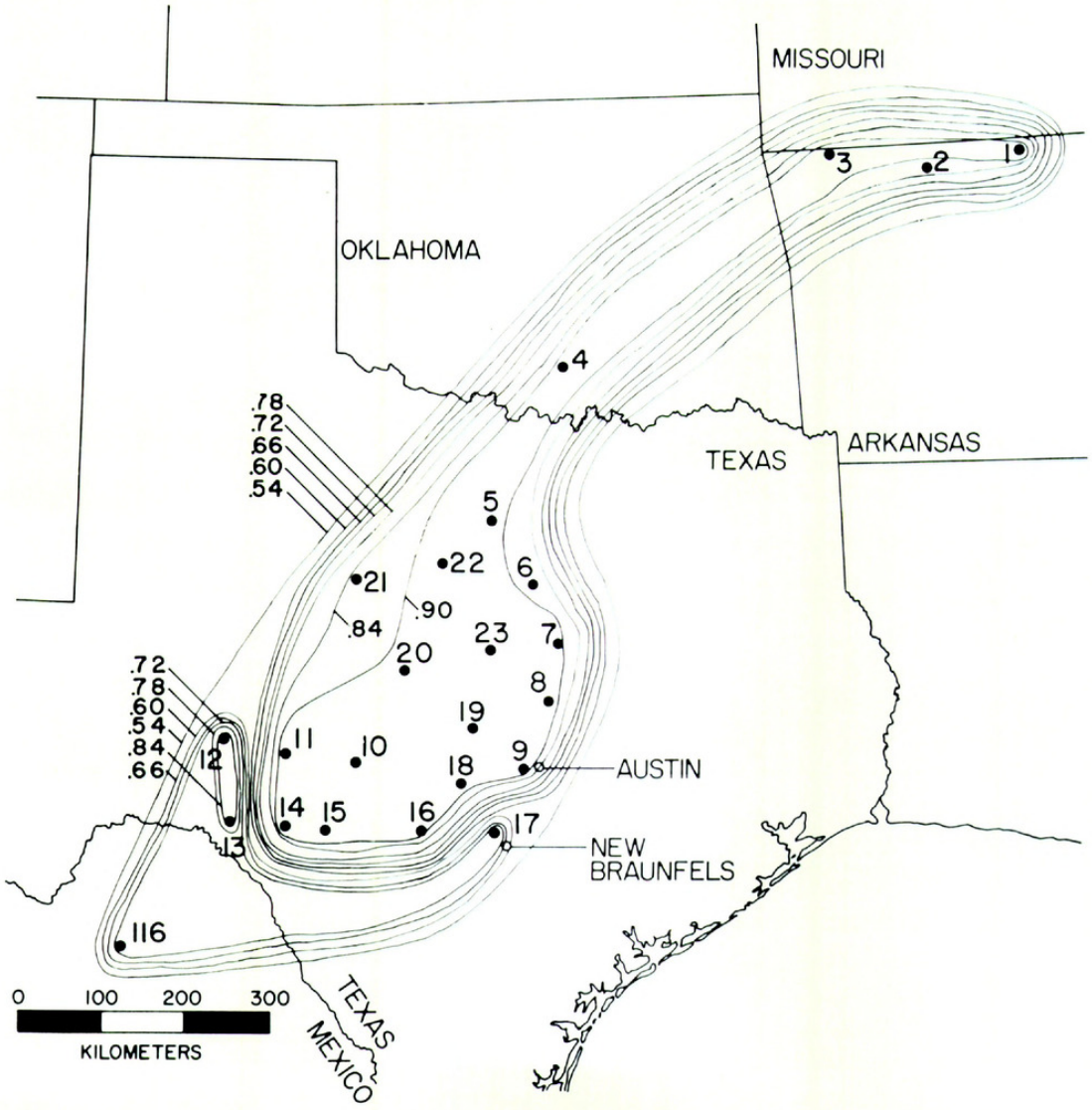


FIGURE 2. Contoured similarities based on 54 terpenoid characters, F-1 weighted. Notice the uniformity from central Texas to the Ozarks and the clustering of populations 12, 13 and 17 with tree 116 from northern Mexico (from Adams, 1975a).

that the Pleistocene ice advances pushed boreal and temperate species to lower elevations and southward. The northern Chihuahuan desert was certainly invaded by *Juniperus* (Wells, 1966) and crossed repeatedly. Even so, the data presented by Adams (1975a) for the close similarities of populations 12, 13 and 17 to northern Mexico *J. ashei* have remained somewhat tentative. This is due to the use of only 1 tree (number 116) from northern Mexico and the fact that no morphological data were used except in the largely preliminary study by Adams & Turner (1970).

In this study I will remedy these shortcomings by reporting on 15 trees of *J. ashei* from northern Mexico (population 25 in Fig. 1), as well as 4 additional populations: Post (near Lubbock), 24; Pandale, 26; Texarkana, 27; Saline Creek, Oklahoma, 28 (see Fig. 1). In addition, I report data on 16 morphological

TABLE 1. Sixteen morphological characters and states scored for 15 trees from each of the 28 populations of *J. ashei* sampled (Fig. 1). Missing data was coded by a –1.0 for a flag in statistical analysis.

Character	States (if applicable)
FDI	FEMALE CONE DIAMETER: avg. of up to 10 and not less than 4 (in mm).
FCO	FEMALE CONE COLOR: 1.0–4.0 (blue-yellow/brown).
SPF	SEEDS PER FEMALE CONE: avg. of up to 10 cones scored, not less than 4.
BLM	BLOOM ON CONE: 1.0–3.0 (none to very heavy coating).
SEA	SEED AREA: seed length × width, avg. of 10 seeds and not less than 4.
SER	SEED WIDTH/LENGTH: avg. of 10 seeds and not less than 4.
WGA	WHIP LEAF GLAND AREA: whip leaf gland width × length; avg. of 5 glands.
WGR	WHIP LEAF GLAND LENGTH/WIDTH: ratio, avg. of 5 glands.
WLM	WHIP LEAF MARGINS: 1.0–4.0 (smooth-heavy serration) avg. of 5 leaves.
WGP	WHIP GLANDS/PROTRUSION: 1.0–3.0 (sunken-smooth-protrudes), avg. of 5 glands.
WRP	WHIP GLANDS RUPTURED: 1.0–3.0 (none-some-almost all), avg. of 5 observations.
B/S	WHIP LEAF BLADE LENGTH/SHEATH LENGTH: avg. of 5 leaves.
G/S	WHIP LEAF GLAND LENGTH/SHEATH LENGTH: avg. of 5 leaves.
SLL	SCALE LEAF LENGTH: avg. of 5 leaves.
L/B	SCALE LEAF LENGTH/BRANCH WIDTH: Ratio of scale leaf length to the width of the branch (twig) where that scale leaf was borne. Avg. of 5 measurements.
BAN	BRANCHING ANGLE: Angle of branching of ultimate twig, avg. of 5 measurements (each to nearest 5 degrees).

characters, as well as peroxidases, from leaves. Finally, I compare these 3 sets of characters both in regard to their use in the analysis of populational differentiation and in the analysis of variability within populations.

MATERIALS AND METHODS

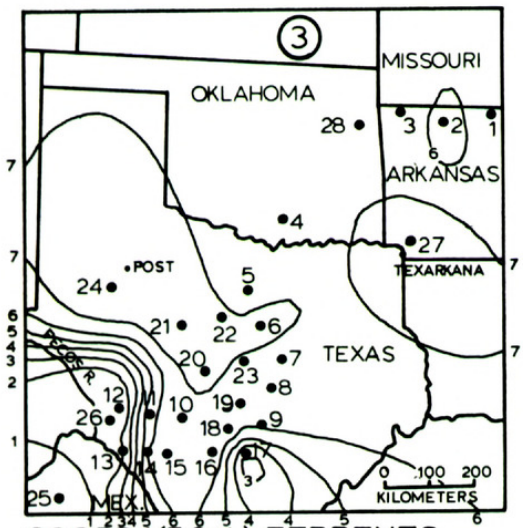
Twenty-eight populations of *J. ashei* were sampled throughout the natural range (Fig. 1). For the terpenoid and morphological characters, 15 trees were sampled from populations 1 through 23 in December, 1970, and 15 trees were sampled from populations 24 through 28 in December and January, 1974–1975, to complete the sampling. The sampling methods are given in Adams & Turner (1970), except that in 1974–1975, the foliage was generally frozen within a few hours in the freezer of our field trailer. Voucher specimens are on file at Colorado State University. All samples from each of the two sampling periods were placed in a random sequence for distillation as advocated by Adams (1975c). These procedures convert the temporal changes in foliage, oils, columns, etc. to random variables. Therefore population differentiation patterns can be readily separated from experimental procedural errors in the statistical analysis phase. The volatile terpenoids were steam distilled for 2 h as outlined by Adams (1970) and the extracts were kept at –20°C until analyzed by gas/liquid chromatography. Separation was made on a 200 ft × 0.02 in. capillary column (wall coated with PEG 20M) as described by Adams (1975a). The identities of the terpenoids of *J. ashei* are given in von Rudloff (1968) and Adams & Turner (1970). Individual peaks were quantified with an electronic digital integrator and automatically punched onto computer cards.

Sixteen morphological characters were scored as outlined in Table 1 for 15 specimens of 28 populations. Some fruit (female cones) and seed characters were not scored (and were thus set to –1.0 as a flag) since not all trees sampled had female cones.

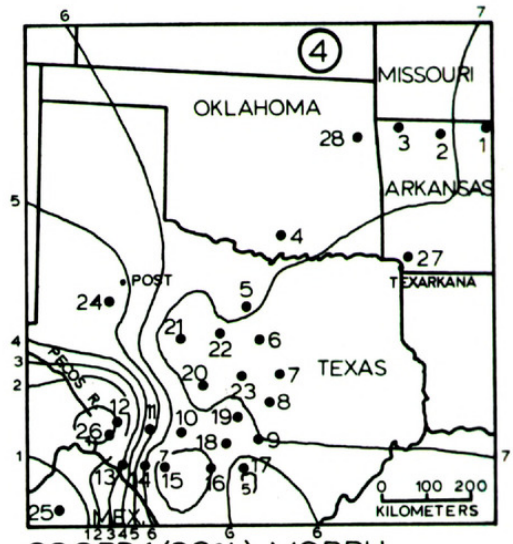
One hundred and forty-two terpenoids were subjected to analysis of variance (ANOVA) to determine which characters showed significant differences among populations. Fifty-nine terpenoids had F ratios greater than 1.0, a maximum population average greater than 0.1% and were used to compute F-1 weighted (Adams, 1975c) mean character differences (MCD or Manhattan metric) similarity measures between populations (see Adams, 1972, for exact formulation). This similarity matrix (28 × 28) was then used as input for principal coordinate analysis (Gower, 1966, 1967; Williams et al., 1971) to factor the similarity matrix into major coordinates of variation. The first 3 principal coordinates were used to contour map populations as they were ordinated on each of the orthogonal axes.

The 16 morphological characters were also analyzed by ANOVA and the Student-Newman-Keuls (SNK) multiple range test was applied ($P = 0.05$) to determine which populations were significantly different. Fifteen morphological characters (FEMALE CONE COLOR was omitted, $F = 0.88$) were used to compute a similarity matrix which was then factored by principal coordinates. The first 3 coordinates were contour mapped as outlined above.

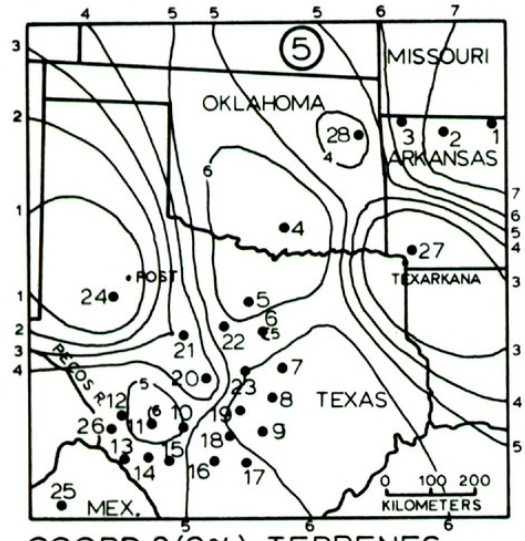
For the peroxidase work, foliage of 30 plants (occasionally less, see Kelley, 1976) were sampled from 15 populations in November–December, 1974, and frozen in the field trailer within a few hours. This foliage was kept frozen until extracted. The enzymes were extracted by grinding the foliage in liquid nitrogen with alumina then adding an extraction buffer of 0.10 M trismaleate, pH 7.00 containing: 0.02 M sodium tetraborate; 0.25 M sodium ascorbate; 0.02 M sodium meta-bisulfite; 0.02 M sodium diethldithiocarbamate (DIECA); 0.01 M germanium dioxide; 10% (v/v) dimethyl sulfoxide (DMSO) plus polyvinylpolypyrrolidone (PVPP), 10 gms/50 ml buffer. The complete instructions are lengthy and the interested reader is referred to Kelley (1976) and Kelley & Adams (1977a) for complete details. The peroxidases were concentrated and electrophoresed on acrylamide gels (discontinuous 4.5, 6, and 8% anodic, see Kelley, 1976) within 72 hours from the time of extraction. Although Kelley (1976) analyzed peroxidases, esterases, and an alcohol dehydrogenase, I am only using the peroxidase data since it showed much of the same pattern of variability as the other systems (Kelley, 1976). Peroxidases in *Juniperus* are little effected by seasonal differences (Kelley & Adams 1977a), and peroxidases are generally very stable (Kelley, 1976). Peroxidases were stained with o-tolidine/H_2O_2 (Denna & Alexander, 1975). An aggregate total of 23 peroxidase bands were found in the 15 populations of *J. ashei* sampled. In cases where bands were very close together on the gel, samples were corun to determine which electromorphs were different. These bands were each scored as 1.0 (present) or 0.0 (absent) for each plant and then subjected to ANOVA to obtain some estimate of F ratios for character weighting. Of course, ANOVA of qualitative data has a tendency to underestimate the F ratios, but this did provide a crude method to obtain relative character weights. Sixteen peroxidases had F greater than 1.0 and were



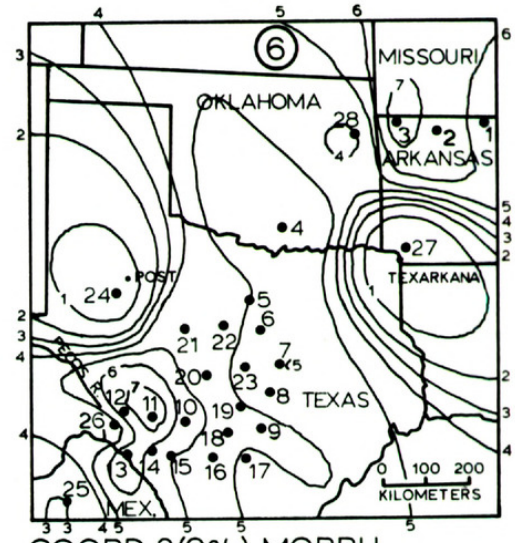
COORD. 1 (50%), TERPENES



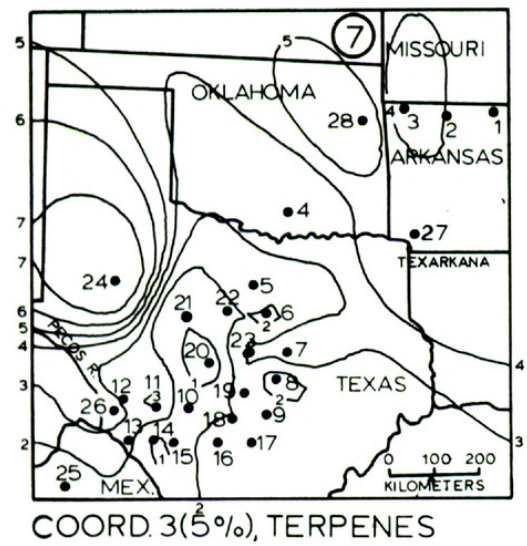
COORD. 1 (38%), MORPH.



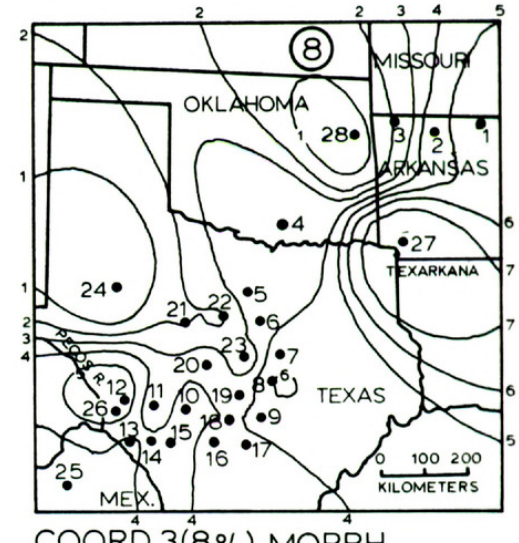
COORD. 2 (9%), TERPENES



COORD. 2 (9%), MORPH.



COORD. 3 (5%), TERPENES



COORD. 3 (8%), MORPH.

not uniformly unique to one population. Similarity measures were computed as outlined above, and the similarity matrix (15 × 15) was factored to obtain principal coordinates. The first 2 coordinates were contour mapped for comparison of regional trends.

For the analysis of within populational variability, 3 sets of similarity measures were calculated using all terpenoid, morphological, and peroxidase characters, equally weighted. It appears that F weighting is not desirable when examining intrapopulational variation. These analyses resulted in 3 kinds of similarity matrices (terpenoid, morphological, and peroxidase) for each population. The average similarity ($\overline{Sr}$) was then computed for each population along with the coefficient of phenetic variation ($CPV = Sd_{\overline{Sr}}/\overline{Sr}$). The $\overline{Sr}$'s and CPVs were then contour mapped to examine regional trends of intrapopulational variation.

Populational Differentiation

The principal trend in the terpenoid similarities is that of the differentiation of populations 25, 26, 12, 13 and 17 from the rest of the populations (Fig. 3). From these coordinate loadings one can see (Table 2) that 50% of the variation in the similarities is mostly due to the divergent nature of populations 25, 26, 12, 13 and 17. The high negative loading of population 17 onto coordinate one indicates that population 17 (New Braunfels, Texas) has considerable affinities with the west Texas and Mexico plants. It is interesting to compare the major trend of the terpenoids with that of the morphology (Fig. 3 vs. Fig. 4). This major trend in the morphology accounts for 38% of the variation in similarities and is practically identical to the major trend of the terpenoids. A couple of exceptions are that the Post population (24) seems more similar to the west Texas-Mexico populations in the morphology, while the New Braunfels population (17) is not quite as different from the central Texas populations in its morphology as in its terpenoids. In both cases, from central Texas to the Ozarks a picture of uniformity is presented. It might be noted that this compares very closely with the contoured terpenoid phenogram in Fig. 2 (from Adams, 1975a). It appears that the major coordinate of principal coordinates analysis is the dominant

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Figures 3–8.—3–4. Contoured loadings of principal coordinate 1 extracted from similarity measures among populations, (see Tables 2–3).—3. This pattern extracted 50% of the variation from the terpenoid similarity matrix. Notice that this pattern is the principal pattern previously shown (Fig. 2). Contours: 1 = –0.70; 7 = 0.18.—4. This pattern accounted for 38% of the variation from the morphological similarity matrix. The Post population (24) shares some affinities to the west Texas-Mexico populations. Population 17 seems a little less divergent in its morphology than its terpenoids (Fig. 3). Contours: 1 = –0.64; 7 = 0.13.—5–6. Contoured principal coordinate 2.—5. This trend (9%, terpenoids) seems to be due to the divergence of populations 24, 27, and 28, plus sampling differences (see text). Contours: 1 = –0.37; 7 = 0.16.—6. This trend (9%, morphological) seems to be due to procedural differences in scoring the morphological characters (see text). Contours: 1 = –0.32; 7 = 0.16.—7–8. Contoured principal coordinate 3.—7. Divergence of the Post (24) population is most evident along this coordinate (5%, terpenoids). Contours: 1 = –0.27; 7 = 0.28.—8. Note the strong divergence between populations 27 and 28 (8%, morphological). Contours: 1 = –0.22; 7 = 0.27.

TABLE 2. Principal coordinate analysis of similarity matrices using terpenoids, morphology, and peroxidase characters for the computations of similarity measures between populations of *J. ashei*. All eigenroots were extracted from each matrix until they failed to converge. It is thought that when eigenroots begin to level off in values, additional roots represent only random error variance.

TERPENOIDS (Srs based on 59 terpenoids, 28 × 28 matrix) 71% of variation extracted by 5 roots.										
Eigenroots	3.56	0.66	0.36	0.29	0.25					
% variation extracted	49.6	9.2	5.1	4.0	3.6					
MORPHOLOGY (Srs based on 15 morphological characters, 28 × 28 matrix) 72% of variation removed by 7 roots.										
Eigenroots	2.40	0.64	0.53	0.36	0.34	0.30	0.26			
% variation extracted	35.9	9.5	8.0	5.4	5.1	4.6	3.9			
PEROXIDASES (Srs based on 16 peroxidases, 15 × 15 matrix) 93% of variation extracted by 10 roots.										
Eigenroots	1.68	0.85	0.62	0.53	0.35	0.30	0.27	0.21	0.19	0.18
% variation extracted	30.1	15.2	11.2	9.5	6.3	5.4	4.8	3.8	3.5	3.2

theme of a single linkage phenogram (see Adams, 1975a). Thus, we see that the west Texas-Mexico type populations account for 50% and 38% of the variation in the terpenoid and morphological similarities, respectively.

The second coordinate extracted from the terpenoid similarity matrix largely separates the small island populations at Post (24), Texarkana (27), and Saline Creek (28) from the rest of *J. ashei* (Fig. 5). These populations, along with 25 and 26, were collected and analyzed 4 years later (1974) than the other populations (1970 collections). Therefore part of these differences may be due to sampling methods, seasonal variations, and different gas chromatographic conditions. However, populations 25 and 26 seem to cluster well with populations sampled in 1970, so this factor may be only a minor cause of this trend. It seems that this small amount of variation (9%) is chiefly accounted for by the divergence of these 3 small, isolated populations (24, 27, 28), along with a contribution resulting from different sampling and analysis times. The second coordinate of the morphological similarity matrix (Fig. 6) is clearly due to the fact that populations 24, 26, 26, 27, and 28 were sampled and analyzed in 1974 rather than with the other populations (sampled and analyzed in 1970). It is felt that most of these differences (approximately 9% of the variation in the similarity matrix) are due to the fact that a different technician measured the morphological characters of populations 24, 25, 26, 27, and 28 (1975) than the other populations (1970–1972). Even with close supervision and training, it is very difficult to get two people to score morphological characters in the same manner. My experience has been that comparisons between morphological data sets scored by completely different research projects is almost impossible. If we consider that the eigenroots of about 5% may be mostly random noise (see below), then the 9% of coordinate 2 is only about twice the experimental error but 25% the size of the major trend.

The third coordinate does not appear to be very significant in the terpenoid

similarity matrix since only 5.1% of the variation was extracted and the eigenroots have leveled off at this value (Table 2). Contouring of this coordinate (Fig. 7) shows that most of the variation along this axis is due to population 24 at Post. This population is on one of the most unusual sites that I have seen for *J. ashei*. It is in a deep ravine, cut into the Permian red clay, just east of the Llano Estacado. The stand is occasionally mixed with *J. pinchotii*, with *J. ashei* found in the more mesic spots. This trend could represent a response to microhabitat selection or environmentally induced plasticity. Transplant studies will probably be needed to answer this question. Another trend is that the northern-most (including Post) populations seem to be more heavily loaded onto this coordinate than those populations in the central and southwestern portion of the range.

The third coordinate of the morphological similarity matrix extracted 8.0% of the variation and might be significant as the 4th through 7th roots seem to have asymptoted to about 4 or 5%. The contour map of this coordinate (Fig. 8) shows a northwest-southeast trend across the populations, somewhat like that in Fig. 7, except there is a decided split between the Texarkana population (27) and those to the north and west. This population (27) is almost as atypical for *J. ashei* as the one at Post, Texas (24). At population 27, *J. ashei* is found on a small (few acres?) limestone outcrop that is gently sloping and very moist (1,143–1,270 mm of precipitation per year). It is a mixed stand with some *J. virginiana*. Whether this pattern represents some small microhabitat selections or environmentally induced plasticity in the morphology must await transplant studies for additional information.

In any case, it is obvious that the major trend in both the terpenoids and morphology is the differentiation of populations 25, 26, 13, 12, and 17 from the rest of the species.

Principal coordinate analysis of the similarity matrix based on peroxidases (Tables 2–3) yielded quite different results. A most notable difference being that 10 eigenroots were extracted from a 15 × 15 matrix, whereas only 5 and 7 roots accounted for most of the definable variation in the much larger (28 × 28) matrices of the terpenoids and morphology. This seems to indicate that the peroxidases are varying in many different directions, whereas the terpenoids and morphology seem to display much more directional or concurrent variation. Another interesting facet is that the eigenroots of the terpenoid and morphological similarity matrices quickly decreased to rather constant values after 2 and 3 roots, whereas the roots of the peroxidases seem to tail out much farther. This seems to imply a considerable amount of independence among the peroxidases. Examination of the first coordinate of the peroxidase similarity matrix (Fig. 9) reveals a northeast-southwest pattern (remember that only the 15 populations marked with an asterisk were analyzed for peroxidases). The Texarkana (27) and Junction (10) populations are most similar to each other, and the Ozark populations (1, 2) are most similar to the north Texas (5, 7) and west Texas-Mexico populations (12, 25). This trend is unlike any other seen in either the morphological or terpenoid data. The divergence of the Texarkana population (27) from the Ozark (1, 2) and north Texas (5, 7) populations would be easy to explain (if one ignores the morphological and terpenoid data) as genetic drift and/or

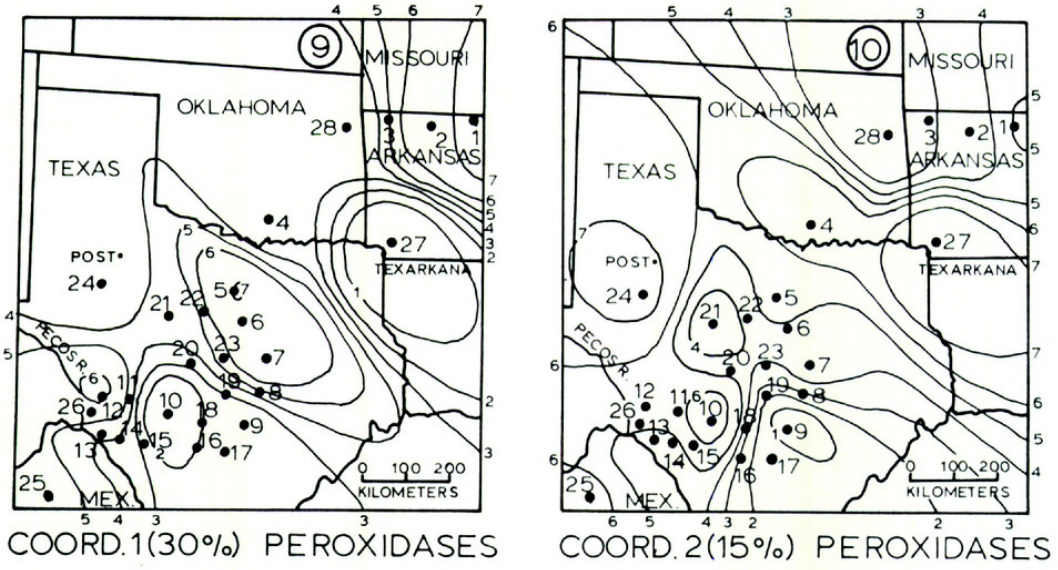
TABLE 3. Coordinate loadings (principal coordinates 1, 2, 3 in each case) for populations onto coordinates. These coordinate loadings were used for generating the contour maps in successive figures. Note the close correspondence between coordinate 1 for the terpenoids and morphology. Values in parenthesis indicate the amount of the variation in the Sr matrix accounted for by each of the coordinates.

	Terpenoids			Morphology			Peroxidases		
Population	C_1 (50%)	C_2 (9%)	C_3 (5%)	C_1 (38%)	C_2 (9%)	C_3 (8%)	C_1 (30%)	C_2 (15%)	C_3 (11%)
1	0.11	0.18	0.13	0.16	0.06	0.15	0.52	0.06	0.11
2	–0.02	0.20	0.08	0.13	0.13	0.10	0.32	–0.17	0.04
3	0.17	0.12	0.03	0.06	0.21	–0.15	—	—	—
4	0.15	0.11	0.04	0.13	0.06	–0.02	–0.16	0.21	–0.14
5	0.13	0.17	0.02	0.12	0.00	0.00	0.44	0.06	0.12
6	0.23	–0.08	–0.09	0.18	0.09	0.01	—	—	—
7	0.13	0.18	0.10	0.20	–0.02	0.11	0.38	–0.04	0.02
8	0.15	0.04	–0.14	0.20	0.09	0.15	—	—	—
9	0.11	0.18	0.01	0.17	–0.05	0.11	–0.27	–0.51	–0.05
10	0.21	–0.04	–0.14	0.13	0.03	0.10	–0.50	0.20	–0.20
11	0.11	0.15	0.06	–0.14	0.27	–0.14	—	—	—
12	–0.84	–0.05	–0.03	–0.71	0.28	0.20	0.31	0.16	–0.04
13	–0.78	–0.03	–0.03	–0.49	0.18	–0.02	–0.06	–0.06	0.04
14	0.21	–0.14	–0.20	0.20	0.06	0.04	—	—	—
15	0.20	–0.05	–0.10	0.26	–0.04	0.10	—	—	—
16	0.18	0.06	0.02	0.18	–0.05	–0.04	—	—	—
17	–0.67	0.02	–0.00	0.23	0.05	–0.06	–0.33	–0.34	0.10
18	0.18	0.03	–0.08	0.14	–0.05	0.05	—	—	—
19	0.10	0.16	–0.03	0.09	0.00	–0.07	—	—	—
20	0.22	–0.23	–0.18	0.17	–0.05	0.03	—	—	—
21	0.26	–0.21	–0.13	0.17	0.01	–0.15	–0.02	–0.11	–0.28
22	0.19	0.07	–0.07	0.13	–0.07	–0.18	—	—	—
23	0.18	0.08	0.07	0.14	0.03	–0.17	—	—	—
24	0.22	–0.42	0.32	–0.14	–0.34	–0.27	–0.11	0.30	–0.42
25	–0.78	–0.07	–0.07	–0.71	–0.16	–0.06	0.18	0.17	0.15
26	–0.71	–0.05	0.08	–0.72	–0.26	0.13	—	—	—
27	0.20	–0.26	0.11	0.16	–0.36	0.31	–0.58	0.34	0.49
28	0.16	–0.13	0.19	0.02	–0.09	–0.26	–0.14	–0.28	0.07

founder's effect, but the peroxidase similarity to the Junction population (10) rather stretches the point.

Coordinate two of the peroxidase similarity matrix shows (Fig. 10) high loadings of populations 27, 4, 24, 10, and 25. This coordinate seems to be a random assortment of populations distributed across the range of *J. ashei*. Similar variation (high similarities across disjunct populations and a random mosaic pattern) has been previously observed in nonsignificant variation of individual morphological characters (see Adams & Turner, 1970, for several contoured morphological characters). Coordinate three shows another pattern of mosaic variation and the interested reader is referred to Kelley & Adams (1977b) for more detailed maps of peroxidases, esterases, and alcohol dehydrogenases.

How can we interpret these conflicting results? One way to view geographical variation is to consider the number of gene differences needed to produce the observed changes. For morphological characters, Charles & Goodwin (1943) have shown that in *Solidago* many morphological characters used in taxonomy



FIGURES 9–10.—9. Contoured loadings of principal coordinate 1, extracted from the F-1 weighted peroxidase similarity measures among populations (see Tables 2–3). This coordinate extracted 30% of the variation from the matrix. Only those 15 populations marked with an asterisk were analyzed for the peroxidases. See text for discussion. Contours: 1 = −0.51; 7 = 0.43.—10. Contoured principal coordinate 2 (15% of the variation, peroxidase similarities). No regional trends were uncovered in this or any of the successive coordinates extracted. See text for discussion. Contours: 1 = 0.42; 7 = 0.28.

are controlled by a minimum of 4, 5, and 6 genes. Irving & Adams (1973) in a study of *Hedeoma* terpenoids found that those terpenoids were controlled by a minimum of 1, 2, and 3, but up to 7, genes which agrees with the work on *Pinus* by Hanover (1966) and others. The peroxidase electromorphs isolated on gels represent probably no *more* than 1 gene for each 2 bands in the composite. Suppose we assume that the 15 morphological characters are each controlled on the average by 5 genes, the 59 terpenoid characters each are controlled by 2 genes (average), and the 16 peroxidase bands are each controlled by 1 independent allele, with 2 alleles (simple codominance) per gene. This means that the pattern displayed by the morphological data sampled a *minimum* of 75 genes, with a *minimum* sample of 118 genes for the terpenoids, and a *maximum* sample of 8 genes for the peroxidases. Of course, we have ignored pleiotropy, epistatis, and linkage, but we have no *a priori* knowledge that these factors are of differential genetic importance in any of these 3 kinds of data. To obtain a random sample of the genome, one would have to favor the morphological and terpenoid data on the basis of sample size alone. Together the morphology and terpenoids (*minimum* of 193 genes) overshadow the peroxidase data (*maximum* of 8 genes). Even so it is striking that no logical regional trends emerged from the peroxidases (nor from the esterases or alcohol dehydrogenases, Kelley & Adams, 1977b). The problems of homology may account for much of this random similarity between widely, disjunct populations (e.g., population 10 and 27, Fig. 9). Homology between the morphology of these populations (Table 1) is practically assured. The terpenoid variation is almost totally quantitative in this taxon, and

the resolution obtained with capillary gas chromatography greatly increases the probability that peaks from different populations of a quantitatively varying species are in fact the same compound (although there is a small finite probability that different genes produce the same compound in different populations). On the other hand, the peroxidases were often found to be qualitatively varying between close, adjacent populations with a band being in very high frequencies in one population and totally missing from our sample in another population. The high similarities obtained in mosaic patterns (Figs. 9–10) are most readily explained by lack of homology between peroxidase bands, although parallel microselection could play an important role. As far as I know, there have been no cases showing that electrophoretic mobility, *per se*, is under selection (that is not to say that proteins bearing more positive or negative charges might not be selected due to substrate affinity, etc.).

Four hypotheses have been advanced (Adams & Turner, 1970) to explain the pattern of regional variation seen in the terpenoids and morphology of *J. ashei*. Two of these, sampling errors and parallel selection (in populations 17, 12, 13, 25, 26) have been pretty well disposed of by Adams (1975a). The other two, predominately southerly winds during pollination (December–January) and northward bird migration during the spring, and ancestral migration leaving relict populations, deserve additional discussion. The prevailing wind during the pollination period (December–January) is generally from the south on the Edwards Plateau (Arbingast et al., 1967). Thus, one might expect pollen to be generally blown northward. Coupled with the northward migration of Cedar waxwings and other birds that feed on *J. ashei* berries (female cones), this would tend to isolate population 17 from breeding with adjacent populations (9, 16, 18). This would also help explain the north-south line of differentiation between populations 12, 13 and 11, 14 (Figs. 3–4). Although these phenomena help explain the persistence of the pattern, they do little to explain the common patterns seen in populations 25, 26, 12, 13, and 17. Ancestral migrations leaving relict populations could help explain these patterns.

PLEISTOCENE PATTERNS

Although there is considerable evidence of a continuous band of sclerophyllous vegetation from central Texas into northern Mexico during the Tertiary (Axelrod, 1975), I would like to focus on events of the Pleistocene, particularly the last pluvial and interglacial periods. I have reconstructed parts of the vegetation during the Wisconsin pluvial, 10,000–20,000 B.P., in Fig. 11. According to King (1973) the western Missouri Ozarks were covered with boreal spruce forest from about 25,000 to at least 13,000 B.P., with pine parkland preceding the boreal spruce. Since the pine parkland and boreal spruce forest both appear to have been pushed southward from the north (Dillon, 1956), I have assumed that the area south of the Ozarks may have been pine woodland or parkland (also see Bryant, 1969). A pine-spruce woodland seems likely in the Llano Estacado of northwest Texas (staked plains) according to Hafsten (1961). Bryant (1969) suggested that based on pollen profiles, the present Chihuahuan desert area around Del Rio, Texas (430 m) was a pinyon woodland. Wells (1966),

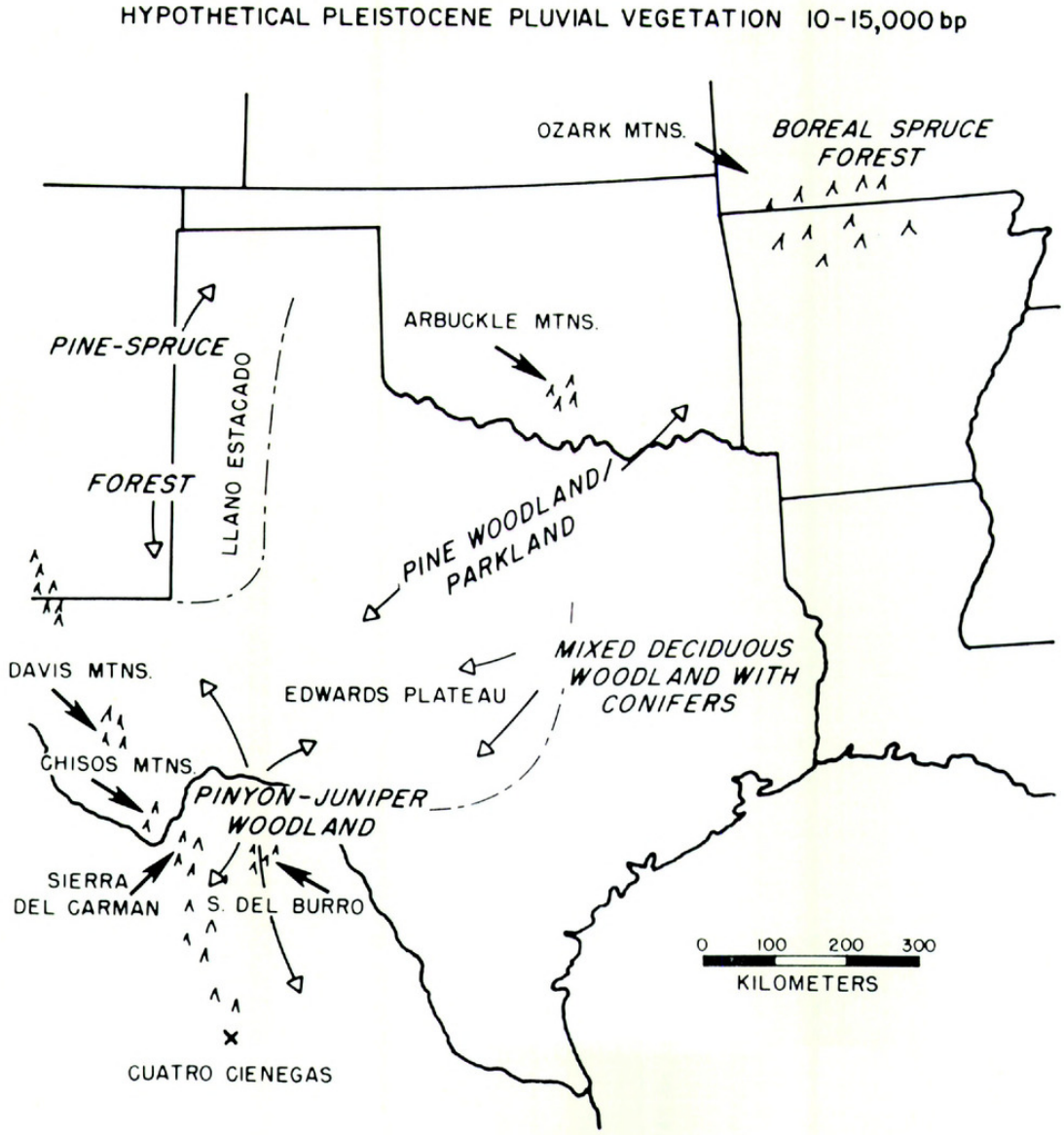


FIGURE 11. Hypothetical Pleistocene pluvial vegetation, 10,000–15,000 B.P. based on pollen profiles and rat midden data from the literature. See text for discussion.

using data obtained from rat middens from the Big Bend Texas region, concluded that the life zones descended about 800 m for pinyon-juniper (*J. pinchotii* in that case), allowing the advance of pinyon-juniper into most of the present desert region between Big Bend and Del Rio. Another important fact has been the recent discovery (D. H. Riskind, pers. comm.) of *J. ashei*, *J. pinchotii*, *J. flaccida*, and *J. scopulorum* from the Sierranas del Burro (Fig. 11). Since typical *J. pinchotii* has been found (Adams, 1975b) just south of the Sierra del Carman (growing with *J. ashei*), it appears that the Sierranas del Burro may have been an important refugium or island point in the pinyon-juniper woodland. A mixed deciduous woodland with conifers is postulated in central Texas (Bryant, 1969; based on a pollen profile).

TABLE 4. SNK tests for 15 morphological characters with F ratios greater than 1.0 in ANOVA. SNK tests were run at P = 0.05, $F_{0.05}$ = 1.58, $F_{0.01}$ = 1.90 (df = 27/380). Any two populations not underlined by a common line are significantly different. Populations are listed in decreasing order of their means from largest to smallest. Ranges refer to the maximum and minimum means over all populations for that character.

FEMALE CONE DIAMETER (FDI), F = 9.3, no obs. for populations 24, 27, range = (8.91–6.4 mm)
15 5 14 6 3 23 4 7 11 21 16 2 10 19 8 22 28 20 1 18 9 13 12 17 26 25

SEEDS PER FEMALE CONE (SPF), F = 9.7, no obs. for populations 24, 27, range = (1.69–1.01)
12 26 13 25 4 17 11 28 2 19 3 10 14 16 21 1 18 8 15 23 20 5 7 6 9 22

BLOOM ON FEMALE CONES (BLM), F = 1.2, no significant differences
SEED AREA (SEA), F = 9.8, no obs. for populations 24, 27, range (27.1–13.6 mm^2)
20 16 21 14 5 15 4 23 6 22 8 7 10 3 9 19 2 28 11 18 1 17 13 12 25 26

SEED WIDTH/LENGTH (SER), F = 1.3, no significant differences
WHIP GLAND AREA (WGA), F 9.2, range (0.93–0.31 mm^2)
12 25 11 13 3 26 23 17 5 6 28 14 7 20 16 21 4 18 1 22 24 15 19 10 8 2 9 27

WHIP LEAF GLAND LENGTH/WIDTH (WGR), F = 4.4, range (2.5–1.5)
13 25 17 26 5 12 11 4 1 19 8 2 7 14 3 27 22 16 18 20 6 9 10 23 15 28 24 21

WHIP LEAF MARGINS (WLM), F = 5.0, range (2.3–1.9)
24 28 25 22 23 26 16 13 21 27 5 17 9 6 19 18 20 14 4 3 15 10 7 1 12 8 11

WHIP LEAF GLANDS PROTRUSION (WGP), F = 2.6, range (3.00–2.87)
1 3 6 7 10 11 15 19 20 21 23 25 18 12 17 22 28 16 8 9 14 2 24 13 4 5 26 27

WHIP LEAF GLANDS RUPTURED (WRP), F = 2.7, range (1.08–1.00)
13 12 11 14 4 6 7 8 9 10 2 3 1 5 15 16 17 18 19 20 21 22 23 24 25 26 27 28

WHIP LEAF BLADE LENGTH/SHEATH LENGTH (B/S), F = 2.9, range (0.77–0.54)
24 25 2 26 9 12 11 1 27 28 14 15 16 10 6 5 13 19 22 20 21 4 7 3 8 18 23 17

WHIP LEAF GLAND LENGTH/SHEATH LENGTH (G/S), F = 16.7, range (0.41–0.22)
25 26 12 13 17 11 24 3 28 19 22 21 23 16 4 5 18 20 9 6 14 2 10 8 1 7 27 15

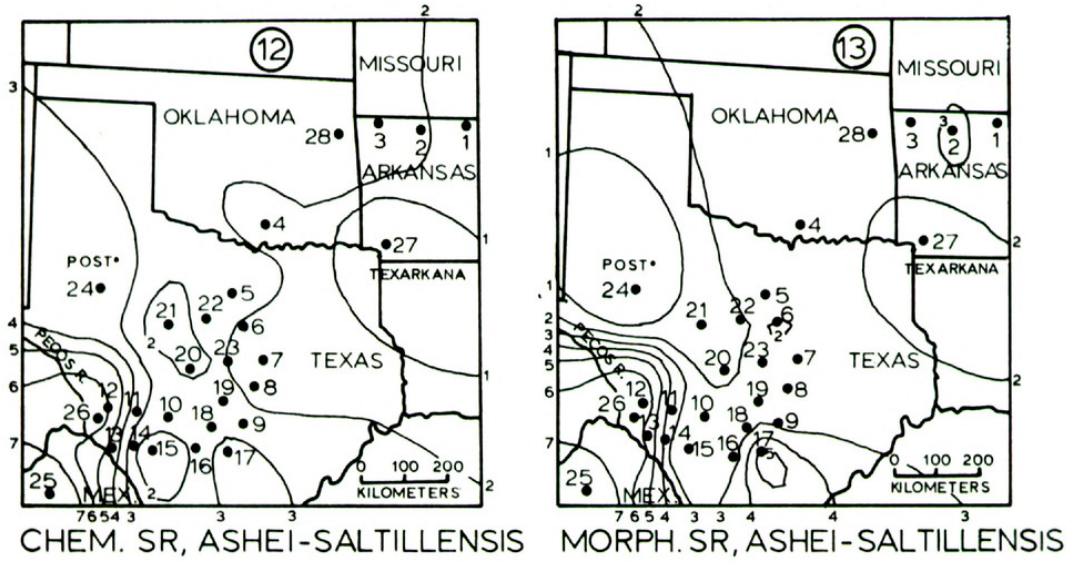
SCALE LEAF LENGTH (SLL), F = 4.1, range (1.74–1.43 mm)
28 27 24 25 19 7 18 16 1 22 15 26 14 21 20 4 5 23 11 13 9 17 12 3 2 8 6 10

SCALE LEAF LENGTH/BRANCH WIDTH (L/B), F = 4.1, range (1.43–1.15)
9 27 17 15 19 18 5 16 7 20 1 14 8 22 23 4 21 25 28 11 3 2 13 6 10 24 12 26

BRANCHING ANGLE (BAN), F = 9.5, range (55.2–39.9 degrees)
12 26 25 10 24 13 27 20 7 17 5 18 11 28 19 16 9 4 23 8 15 6 14 22 21 1 2 3

Life zones were pushed southward and compressed during the Wisconsin pluvial (Dillon, 1956), but how far they were extended into Mexico is not well known. Additional rat midden and pollen profiles are needed in northern Mexico and the Mexican plateau region. A study by Meyer (1973) in the Cuatro Cienegas basin (Fig. 11) revealed no changes in the pollen profiles during the past 30,000 years. He concluded that there was no evidence for pluvial nor hypsithermal (Deevey & Flint, 1957) periods at Cuatro Cienegas during the time sequence studied. This agrees with Dillon (1956: 174) who shows a considerable compression of life zones from Nebraska to south Texas but few differences past northern Mexico. It seems likely that any generalized Mexican refugium must have been in northern Mexico. One other point that seems relevant is that Wells (1966) mentioned that the pinyon found in the rat middens in the Big Bend area contained consistently 2-needled fasicles suggesting that the pine involved may not have been the predominately 3-needled *Pinus cembroides* Zucc, but perhaps *Pinus cembroides* var. *remota* E. L. Little. *Pinus cembroides* var. *remota* now persists on the Balcones escarpment of the Edwards Plateau (near population 14, Fig. 1) about 300 km to the east of the fossil site. I have recently examined a herbarium specimen of *J. ashei* from eastern Brewster County, Texas and have indicated this location in Fig. 1 (dashed lined population, about 150 km west of population 26). This new population is just north of Wells's (1966) Maravillas Canyon rat midden site. Perhaps his juniper twigs should be reexamined for the presence of *J. ashei.* In any case, this western-most disjunct population of *J. ashei* seems to be of the same relict nature (on preliminary morphological examination) as populations 12, 13, 25, and 26.

Although I have previously considered populations 12, 13, 25, 26, and 17 to be ancestral (Adams, 1975a), one might ask why these might not be advanced, with the central Texas-Ozark populations being ancestral. Examination of Table 4 reveals the significant morphological differences between populations 12, 13, 17, 25, 26, and the other populations. The following characters show significant differences: female cone diameter (smaller in 25, 26, etc.); seeds per cone (generally more in 25, 26, etc.); seed area (smaller in 25, 26, etc.); whip leaf gland area (larger in 25, 26, etc.); whip leaf gland length/width (more elongated in 25, 26, etc.); whip leaf gland length/sheath length (larger in 25, 26, etc.); and branching angle (larger in 25, 26, etc.). Reviewing the *Sabina* section of *Juniperus* in North America, it seems that some of these character states are rather unusual and are likely advanced (rather than primitive). Advanced character states (central Texas-Ozarks) are: larger female cones concurrent with fewer seeds (just the opposite found in most of the junipers); whip leaf gland area small (whip leaf gland area is generally large in junipers where the glands are visible); whip leaf gland length/width close to 1 or 1.5 (*J. ashei* is unique in the genus, so far as is known, in having raised, round glands), the more elongated glands (populations 25, 26, etc.,) are definitely the more primitive type; and whip leaf gland length/sheath length (almost always large in *Juniperus*, except the central Texas-Ozark *J. ashei*). Advanced and primitive states are not known for two characters: seed area and branching angle. Overall, the characters expressed in central Texas and the Ozarks are generally unusual in occurrence



FIGURES 12–13.—12. The contoured F-1 weighted morphological similarity of each population of *J. ashei* to *J. saltillensis*, collected near Saltillo, Mexico. *Juniperus saltillensis* is thought to be closely related to the ancestral stock of *J. ashei*. Notice the clinal differentiation from west Texas to the Mexico population (25). Contours: 1 = 0.16; 7 = 0.46.—13. The contoured F-1 weighted terpenoid similarity of each population of *J. ashei* to *J. saltillensis*. The clinal trend seen with the morphology (Fig. 12) is steeper in the terpenoids, and population 17 is obviously more closely related to the ancestral stock of *J. saltillensis-J. ashei*. Contours: 1 = 0.17; 7 = 0.41.

in *Juniperus* compared to the character states found in the southwest Texas-Mexico populations. Further evidence regarding the ancestral nature of populations can be obtained by comparison of each population of *J. ashei* with its presumed nearest ancestor (Zanoni & Adams, 1976), *J. saltillensis* Hall. Although *J. ashei* probably did not descent from *J. saltillensis*, that taxon appears to bear the closest morphological and terpenoid similarities to *J. ashei* of any in North America. In Fig. 11 I have constructed differential similarities of each population of *J. ashei* to a sample of 15 trees of *J. saltillensis* from near Saltillo, Mexico. ANOVA was performed on 29 data sets (28 *J. ashei* populations and 1 *J. saltillensis* population) to determine a set of F-1 weights. Similarity measures were calculated as outlined before, then each population of *J. ashei* was contour mapped showing the change (differential) in similarity to *J. saltillensis* (the geographical source of this taxon is not important for obtaining the similarities and is not shown on the maps). This method of "differential similarity" should prove very useful in the analysis of the interaction of two species across a geographical area. Figure 12 is based on 15 morphological characters (female cone color omitted, F = 0.88), F-1 weighted. Notice that the highest similarity to *J. saltillensis* is from the Mexico population (25), followed by populations 26, 12, 13, and 17. The knife edge break previously seen (Fig. 4) between populations 12, 13 and 11, 14 is quite widened in this analysis with a cline from populations 12 to 10. The Post population (24) bears some similarity, but part of this similarity may be due to environmental factors.

The terpenoids of *J. ashei* are interesting evolutionarily because there is a

greater shift toward the predominance of a single compound (camphor, see von Rudloff, 1968; Adams & Turner, 1970) than in any other member of the genus. In populations of central Texas camphor averages about 75% of the total oil (2 hr. extraction) whereas the divergent populations average about 60%. *Juniperus ashei* has by far the simplest oil mixture of the North American junipers, and this seems to be an advanced character state of specialization. The central Texas populations are particularly low in the sesquiterpene oxygenated compounds such as elemol, elemol-acetate, and α, β and γ-endesmols. Larger quantities of these compounds are the rule in the rest of the junipers and conifers in general (see von Rudloff, 1975).

Differential similarities, based on ANOVA (28 *J. ashei* populations plus 1 *J. saltillensis* population) and using 68 terpenoids F-1 weighted, reveal (Fig. 13) a pattern almost identical to the differential similarities for the morphological characters (Fig. 12). These similarities indicate that the divergent populations (25, 26, 12, 13, 17) bear a stronger affinity to *J. saltillensis* than the central Texas-Ozark populations (lest the reader be suspicious of mixed sampling in population 25, etc., I should note that these divergent populations clustered strongly with the central Texas type when an OTU of *J. saltillensis* was added to the matrix set, and intrapopulational cluster analysis of each of the 28 populations of *J. ashei* revealed no other taxa as would be the case in mixed species samples). Thus we see that in considering a fairly large set of characters (15 morphological and 68 terpenoids), the dominant theme is for the divergent populations to be progressively more similar to *J. saltillensis*. It should be noted that *J. saltillensis* is not conspecific with *J. ashei* (Zanoni & Adams, 1975, 1976). In fact, several characters found in *J. saltillensis* (curved terminal whips and beady scale leaves) have not been found, even in the relict populations, in *J. ashei*. Although relict hybridization could not be conclusively ruled out at present, it seems unlikely since we have no direct evidence that the two taxa have been sympatric, and several distinguishing characters of *J. saltillensis* have not been found in divergent *J. ashei* plants. It would appear that the most probable hypothesis at present is that *J. ashei* and *J. saltillensis* had a common ancestor (Tertiary?) in the Sierra Madre Oriental. *Juniperus ashei* differentiated and migrated northeastward to the exposed limestone outcrops (Edwards Plateau, Arbuckles, Ozarks, etc.), while *J. saltillensis* adapted to the drier, interior portion of the Sierra Madre Oriental.

During the Pleistocene ice advances, *J. ashei* may have become extinct in Missouri, Arkansas, Oklahoma, and most of central Texas as depicted in Fig. 14. During the same period, *J. ashei* probably expanded westward into the current Chihuahuan desert (Wells, 1966; Bryant, 1969), but not as far south as Cuatro Cienegas (Meyer, 1973). Migration west of the Sierra del Carman was also possible since the species is currently found at the top of a pass (La Cuesta) just south of the Sierra del Carman. Whether *J. ashei* could have crossed the high plateau around Alpine and Marfa (1,500 m) is not known, but suitable habitat was probably available for colonization in the Presidio area. With this model, populations of *J. ashei* would be forced to extinction in central Texas, Oklahoma, Arkansas, and Missouri. The subsequent recolonization could then take place

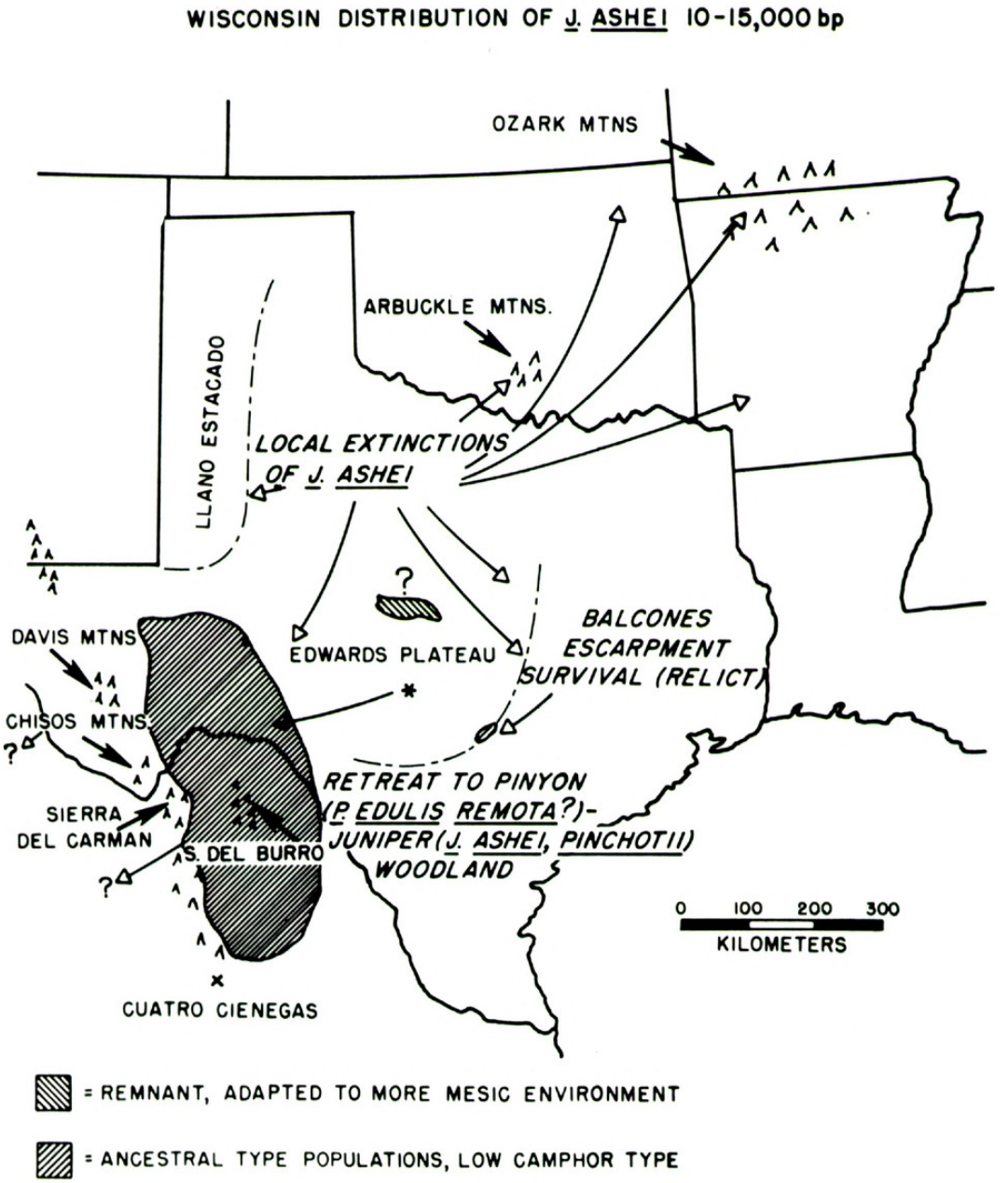


FIGURE 14. Possible Wisconsin distribution of *J. ashei*, 10,000–15,000 B.P. Following the advance of subalpine and montane species (Fig. 11), *J. ashei* populations may have gone extinct north of the Edwards Plateau. See text for discussion.

according to Fig. 15 over a very short period of time (hundreds of years?) from some population in central Texas that may have gone through a selection "bottleneck," perhaps coupled with genetic drift. This "relict" population would have had considerably more camphor in the oil (as a plant defense?), more roundish glands, larger female cones, fewer seeds (therefore a higher pulp to seed ratio for bird dispersal), and a more lax foliage (smaller branching angle) which seems to be associated with more mesic species. The rapid recolonization of limestone outcrops (Fig. 15) could then lead to a uniform taxon from central Texas through

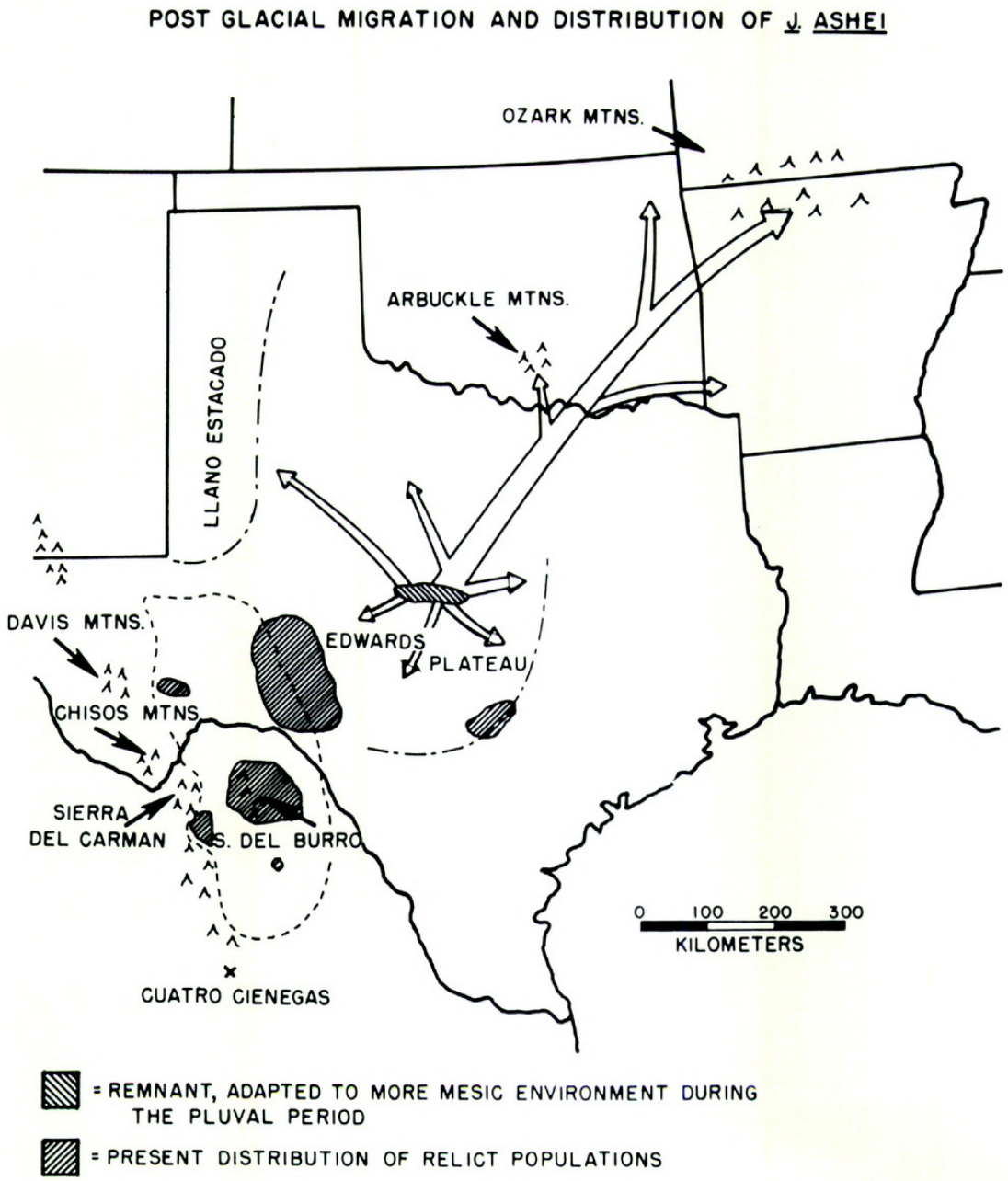
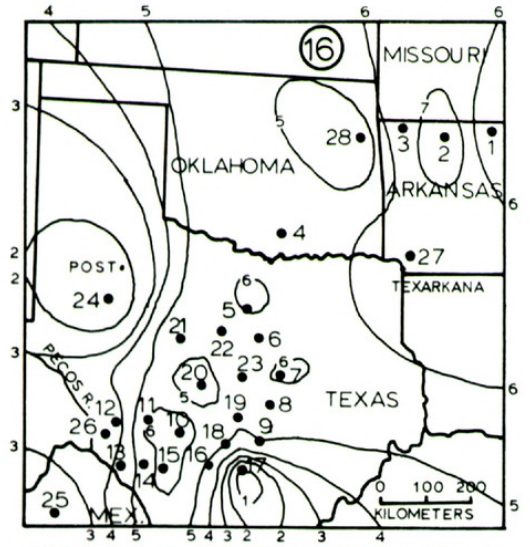
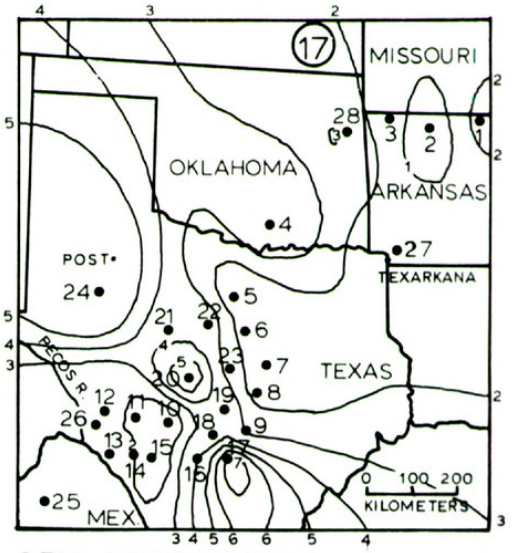


FIGURE 15. Possible post-glacial migration to attain the present distribution of *J. ashei*. The remnant (high camphor type) adapted to a more mesic environment may have quickly expanded during the hypsithermal to reach the present distribution (see Fig. 1). The dashed line shows the pluvial distribution of the ancestral type (lower camphor) populations (see Fig. 14).

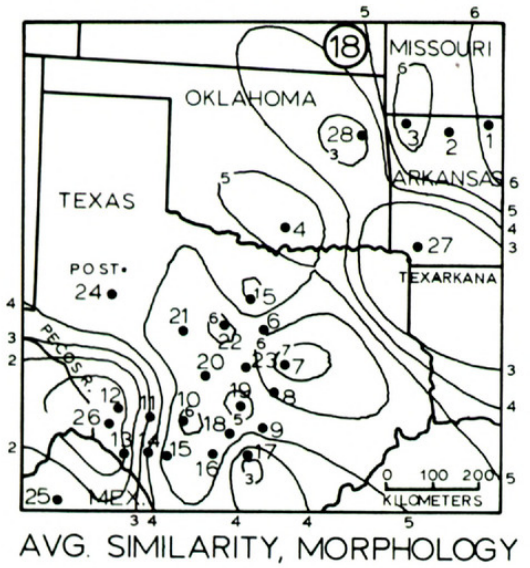
the Ozarks. Although this would explain the observed patterns, many uncertainties remain. For instance, Dillon (1957) argues that the boreal forest elements were merely mixed with the present floral components in the southern states. Graham (1973) feels that most central-southern communities incorporated boreal elements (e.g., spruce) but retained the general character of the original vegetation. If small pockets of *J. ashei* did persist during the full glacial,



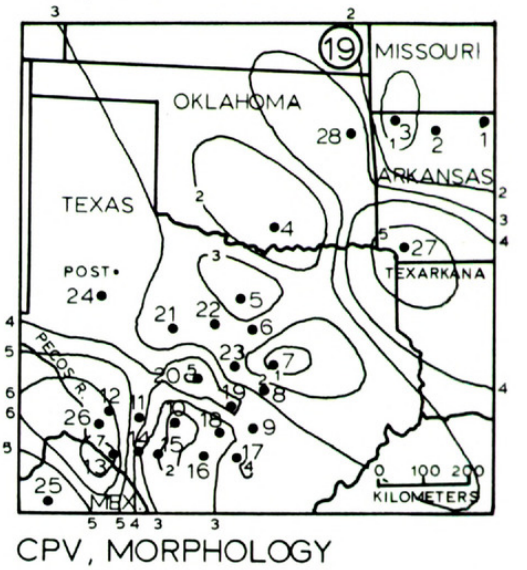
AVG. SIMILARITY, TERPENOIDS



CPV, TERPENOIDS



AVG. SIMILARITY, MORPHOLOGY



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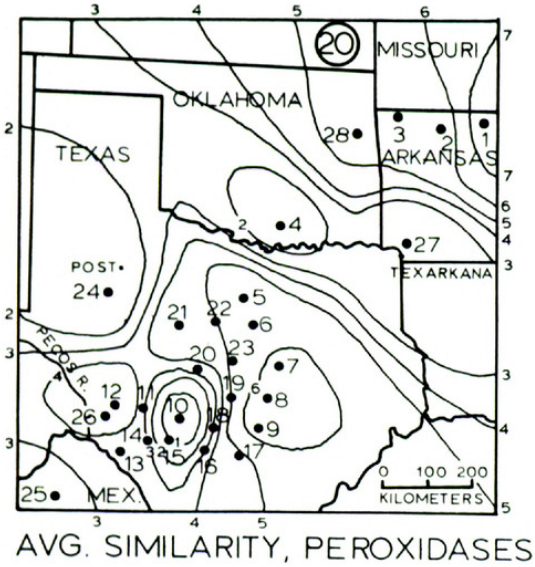
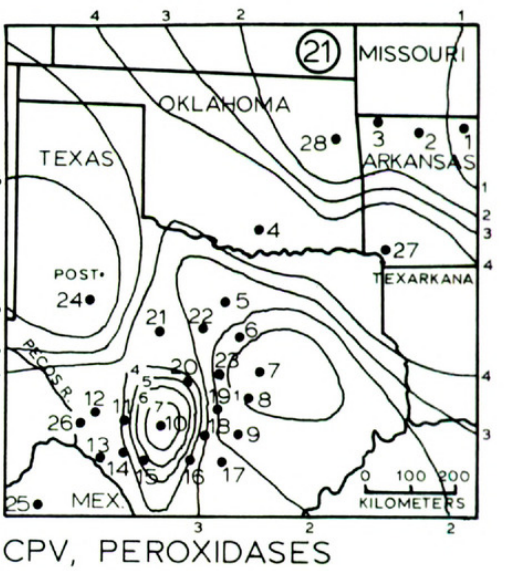


AVG. SIMILARITY, PEROXIDASES



CPV, PEROXIDASES

one might find some evidence of this based on intrapopulation variability, with the smaller Pleistocene relictual populations having less variability than larger (south-central Texas) populations.

Intrapopulational Variability

Mean similarity ($\overline{Sr}$) within each population (15 trees) for 152 terpenoid characters (W = 1) shows high average similarities in the Ozarks and central Texas (10, 11, 15) and low similarities at New Braunfels (17), Post (24), and Mexico (25). The divergent populations (12, 13, 17, 25, 26) tend to be a little less uniform, although Post (24) is also quite variable. Populations that showed the major trend of the terpenoids (Fig. 3) tended to be uniform. Examination of the homogeneity of the similarities was accomplished by computing the standard deviation of the mean similarity and dividing by the mean similarity of that population for normalization (CPV). The most homogeneous similarities are in the Ozarks (2, 3) and central Texas (10, 15, 7), whereas the least homogeneous are New Braunfels (17), Brady (20), and Post (24). The populations which showed the highest similarities are the most homogeneous except for population 20 (Brady). The low similarities and lack of homogeneity at New Braunfels seems to be due to the interaction between relict and modern genotypes. One might question if the population at Post (24) is hybridizing with sympatric *J. pinchotii* trees but notice the close ordination of 24 with the central Texas *J. ashei* (Fig. 3). Examination of the intrapopulation phenogram revealed no major groups within any population.

Analysis of 16 morphological characters (W = 1) shows the highest similarities in central Texas (7, 10, 18) and the Ozarks (1, 3), with lowest similarities in the relict populations (12, 13, 26, 17, 25) and at Texarkana (27). Two small island populations (27, 28) both show considerably lower similarities in their morphology than they did in their terpenoids, whereas Post (24) is more medial in its morphological similarities than with the terpenoids (Fig. 16). The CPV

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Figures 16–21.—16. Average similarity ($\overline{Sr}$) within each population (15 trees) based on 152 equally weighted terpenoids. Most populations had high internal similarities with the exception of the ancestral populations (12, 13, 17, 25, 26) and the Post population (24). Contours: 1 = 0.78; 7 = 0.87.—17. Contoured coefficient of phenetic variation (CPV) of the terpenoid similarities. In general the populations with high intrapopulation similarities were homogeneous (low CPVs) and vice versa, except for population 20 which had high similarities and was not so homogeneous (high CPV). Contours: 1 = 0.30; 7 = 0.91.—18. Average similarity within each population based on 16 equally weighted morphological characters. Note that the ancestral populations are of generally lower internal similarities compared to high similarities throughout central Texas. The small populations at Texarkana and northeastern Oklahoma are morphologically quite variable. Contours: 1 = 0.85; 7 = 0.91.—19. Contoured coefficient of phenetic variation (CPV) of the average morphological similarities. The CPV seems highly negatively correlated with the mean Sr except for populations 19 and 20 which are not very homogeneous. Contours: 1 = 0.28; 7 = 0.60.—20. Contoured average similarities of 23 equally weighted peroxidases. The Junction population (10) had the lowest similarities along with Post (24) and the Arbuckles (4). Contours: 1 = 0.54; 7 = 0.96.—21. Contoured coefficient of phenetic variation of the peroxidase similarities gives an almost identical pattern as seen in the average similarities (Fig. 20). Contours: 1 = 0.13; 7 = 0.32.

gives a fairly similar pattern of homogeneity except for populations 19 and 20, which, although very typical (Fig. 4) and of high average similarities, are not very homogeneous. This same phenomenon was seen with the terpenoids (Figs. 3, 16–17) for populations 19 and 20. The Post (24) population is somewhat more homogeneous in its morphology than its terpenoid's similarity. With the exception of populations 24 and 27, one notices that for each of these four statistics, the populations generally present some trend of variability which is correlated with either regional differentiation or proximity of one population to another (the case for 19 and 20).

All 23 peroxidase electromorphs were subjected to the computation of average similarities within population and CPVs, as with the morphology and terpenoids. Only the 15 populations marked with an asterisk in Figs. 20 and 21 have isoperoxidases analyzed. The average similarities within populations for these 23 isozymes show the Ozark population (Fig. 20) to be quite similar (0.97–0.80), while the Junction, Texas population (10) has the lowest average similarity (0.47). A surprising aspect of these average similarities is the low average similarity found in population 10 (Junction, Texas). It is interesting that 3 different peripheral populations (1, 27, 24) show the whole range of variation from little to large amounts to intermediate variability. The CPV (Fig. 21) of these 23 peroxidases reveals that those populations that are highly similar are generally most homogeneous and vice versa. A combined total of 43 isoenzymes has been analyzed by Kelley & Adams (1977b), and the results are comparable to those shown in Figs. 20 and 21. However, the addition of 14 alcohol dehydrogenases and 4 esterases to the analysis seemed to have produced a slightly less mosaic pattern in central Texas.

The pattern obtained from the isoenzymes is quite different from either the morphology or terpenoids for in both of those analyses, population 10 appeared to be quite uniform and homogeneous and the relict populations consistently displayed high to medium variability. It seems apparent that whatever variability the peroxidases are indicating, it is not directly related to variability in the morphology nor terpenoids. Of course, it is possible that the variation seen in peroxidases is below the level of selection and merely represents "random noise." Until more information is gathered on the selection value of various electromorphs, we can only speculate.

The patterns of variability seem to give us a few clues as to whether the disjunct populations are of recent origin or relicts of the advanced high camphor types. However, presently it is difficult to make generalizations about populational variability versus founder's effect, "bottlenecks," relictness, etc. since different character sets give somewhat (to vastly) different answers, and one could get the same observed pattern depending on time, microselection intensity, or site variability.

CONCLUSION

Treating peroxidase data as qualitative taxonomic chemical characters did not appear to be feasible. This is likely due to the lack of homology, intense microhabitat selection or random (neutral) variations in the electromorphs.

The use of these peroxidase electromorphs for the analysis of intrapopulation variability could not be readily evaluated due to the mosaic pattern produced. It appears that chemosystematists will need more detailed biochemical information about the nature of isoenzymes, and their genetic control in taxa to be studied.

The most probable center of origin for the modern (high camphor) populations of *J. ashei* seems to be in central Texas, perhaps near Brady (20) or Burnet (19). These populations showed considerable variability (high CPVs), yet these populations are quite similar to the rest of the modern *J. ashei* populations. Northward migrations of birds during the spring carrying juniper seeds could have (re)colonized limestone outcrops in Arkansas, Oklahoma, and Missouri in a span of a few hundred years. This could lead to the highly uniform pattern observed in the morphology and terpenoids from central Texas to the Ozarks. Predominately southerly winds during pollination may have been important in maintaining the north-south split in west Texas as well as the relict population at New Braunfels, Texas (17). However, it is possible that the populations persisted throughout the pluvial periods and failed to diverge due to either a lack of variability, the relatively short time span involved, or intense selection for the modern phenotype.

Whether the modern populations of this taxon invaded the limestone outcrops in the Tertiary or during the Pleistocene will probably not be known until some pollen or macrofossil (rat midden) data has been analyze in the disjunct populations of Arkansas, Missouri, and Oklahoma. The differential similarity of *J. ashei* population to *J. saltillensis* from Saltillo, Mexico shows a clear trend of past (Pleistocene) migration from northern Mexico. The northern Mexico Sierra Madre Oriental seems a likely site for the origin of both *J. ashei* and *J. saltillensis*, perhaps from a common ancestor.

This study presents additional evidence that selection may be more important than gene flow (Ehrlich & Raven, 1969) in the maintenance of species. In *J. ashei* we have found that populations with disjunctions of 200–300 km, and a trivial chance for gene exchange, were very similar to other populations covering 1,000 km of range (cf. Ozarks and central Texas populations). Yet populations which are in close (almost continuous) proximity have maintained either ancestral or modern patterns in spite of potentially large amounts of gene flow. (New Braunfels and populations to the north and west, and the relict/modern populations of west Texas).

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