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CHEMOSYSTEMATIC AND NUMERICAL STUDIES OF NATURAL POPULATIONS OF JUNIPERUS PINCHOTII SUDW.

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Summary

Juniperus pinchotii Sudw. was analyzed for areas of population differentiation and possible hybridization with other species of *Juniperus*, utilizing both morphological and chemical (terpenoid) characters. The volatile terpenoids of the foliage were analyzed by gas chromatography. Numerical methods included: analysis of variance; SNK tests; contour mapping of individual characters; differential systematics; and numerical taxonomy. *Juniperus pinchotii* appears to be a somewhat variable taxon with some quite divergent populations in the trans-Pecos region of west Texas where it is morphologically similar to *J. monosperma* Sarg. No evidence of introgression was detected but some hybridization with *J. monosperma* in the Palo Duro Canyon of the Texas Panhandle was confirmed.

Introduction

The use of biochemical data in plant systematics has increased rapidly in the past ten years. Although many of the early studies served chiefly to validate the chemical approaches, such as Smith and Levin's (1963) reappraisal of the *Asplenium* complex (Wagner, 1954) and Brehm and Ownbey's (1965) re-examination of *Tragopogon* (Ownbey, 1950), studies such as McClure and Alston's (1966) work on the *Lemnaceae* and Alston and Turner's (1963) work on *Baptisia* have shown that the use of biochemical data can often be used to supplement classical morphological methods in resolving very difficult systematic problems.

The application of biochemical investigations to the analysis of infraspecific variation was delayed, for the most part, until the development of sensitive and repeatable analytical devices for quantification of individual components. The volatile terpenoids have been used quite successfully in several recent studies of infraspecific variation and hybridization (Mooney and Emboden, 1968; Flake, von Rudloff, and Turner, 1969; Adams and Turner, 1970).

The genus, *Juniperus*, is well suited for infraspecific studies since there has been a lengthy controversy as to the amount of hybridization and introgression among the North American species (see recent papers by Adams and Turner, 1970; and Flake, von Rudloff, and Turner, 1969). Figure 1 shows the distribution of *J. pinchotii* and 3 other partially sympatric species. Hall and Carr (1968) have reported *J. pinchotii* to be hybridizing with *J. monosperma* Sarg. in the Palo Duro Canyon of the Texas Panhandle (the area in fig. 1 where these two species meet in north Texas). In an earlier study Hall, McCormick, and Fogg (1962) concluded that *J. pinchotii* was undergoing widespread hybridization with *J. ashei* in

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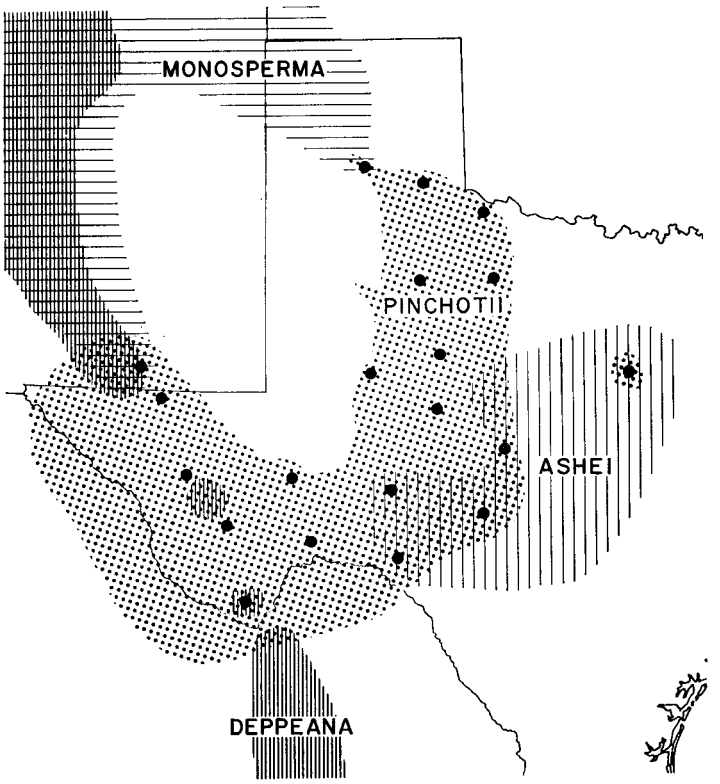

FIG. 1. Distribution of 4 of 6 species of *Juniperus* considered in this study. For the distribution of the other two species, *J. scopulorum* and *J. flaccida*, see text.

south central Texas. Introgression of *J. ashei* genes into *J. pinchotii* is reported to occur ". . . from the western Edward's Plateau becoming progressive lower in intensity to the western slopes of the Sacramento Mountains of New Mexico." (Hall, McCormick, and Fogg, 1962). Variation in *J. pinchotii* in the trans-Pecos region of west Texas is well known and has been described as *J. erythrocarpa* by Cory (1936) and *J. texensis* by van Melle (1952). Neither taxa have been recognized by Correll (1967) in his treatment of the *Cupressaceae* of Texas, although he does mention that in *J. monosperma* of the trans-Pecos ". . . very little material of this species (*monosperma*) fails to show what appears to be introgression with *J. pinchotii*."

The purpose of this study is to investigate the structure of natural populations of *J. pinchotii* and interactions with other sympatric species. Three of these species are shown in figure 1. Two other partially sympatric species, *J. flaccida* Schlecht. and *J. scopulorum* Sarg. are not shown but occur with *J. pinchotii* in the Big Bend (Chisos Mtns.) of the trans-Pecos, and in Palo Duro Canyon of the Texas Panhandle, respectively. *J. scopulorum* also occurs with *J. pinchotii* in the Guadalupe Mountains of southern New Mexico.

Materials and Methods

Figure 1 shows the 20 populations of *Juniperus pinchotii* sampled in December and January, 1967-68 (all trees were sampled within a 3 week period). Population samples consisted of terminal foliage from 5 or 6 trees at each site. Samples from individual trees consisted of 6 to 8 inches of fresh foliage from 4 or 5 branches. Preference was given to female trees since many of the morphological characters used for recognition purposes are associated with the female cones. In populations where hybridization was suspected, the most "hybrid-looking" individuals were selected for analysis. In populations of *J. pinchotii* in which *J. ashei*, *J. monosperma*, *J. deppeana*, *J. scopulorum*, or *J. flaccida* was present, 5 plants were sampled from each of the species present. The fresh foliage was sealed in plastic bags and kept as cool as possible until returned to Austin where the samples were frozen until they were steam distilled to remove the volatile terpenoids. Voucher material for each plant is deposited in the University of Texas herbarium, Austin, Texas.

Table 1 lists 19 morphological characters and their character states. If any character was not applicable (or unavailable), it was coded as a negative number and ignored in the statistical computations. Although some character states are indicated in table 1, most of the characters are

TABLE 1. Morphological characters and the states used.

Characters	States (if applicable)
M1.	GROWTH HABIT: 1. = depressed shrubby; 2. = shrubby; 3. = weak central axis; 4. = strong central axis.
M2.	RATIO OF BLADE/SHEATH OF WHIP LEAVES: Average of 5 measurements.
M3.	RATIO OF WHIP LEAF GLAND LENGTH/SHEATH LENGTH: Average of 5 measurements.
M4.	LENGTH OF WHIP LEAVES: Average of 5 measurements (in mm.).
M5.	RATIO OF WHIP LEAF GLAND WIDTH/GLAND LENGTH: Average of 5 measurements.
M6.	FEMALE CONE COLOR: 1. = blue; 2. = rose; 3. = red/brown; 4. = yellow/brown.
M7.	GLANDS (whip leaf): 1. = absent; 2. = faintly present; 3 = conspicuously present.
M8.	GLANDS (whip leaf): 1. = flat; 2. = intermediate; 3. = raised.
M9.	GLANDS (whip leaf): 1. = single; 2. = divided.
M10.	GLANDS (whip leaf): 1. = not ruptured; 2. = ruptured.
M11.	LEAF MARGINS: 1. = smooth; 2. = intermediate; 3. = serrate.
M12.	BLOOM ON FEMALE CONES: 1. = no; 2. = yes.
M13.	SEED COLOR: 1. = tan, 2 = light brown; 3. = dark brown.
M14.	HILUM SCAR ON SEED: 1. = less than 1/3 length of seed; 2. = less than 1/2, greater than 1/3 length of seed; 3. = greater than 1/2 length of seed.
M15.	GROOVES IN SEED: 1. = none; 2. = 1 to 5; 3. = more than 5.
M16.	NUMBER OF SEEDS/CONE: Average ratio for up to 10 cones/plant and not less than 5.
M17.	FEMALE CONE DIAMETER: Average of up to 10 cones and not less than 5 (in mm.).
M18.	RATIO OF SEED WIDTH/LENGTH: Average of 10 seeds.
M19.	SEED WIDTH X LENGTH: Average of 10 seeds.

TABLE 2. Composition of the oil of *J. pinchotii* based on 16 trees from populations 5, 17 and 18. Those compounds in parenthesis are tentatively identified.

Cpd. #	Identity	Percent
1	(tricyclene)	.38
2B	α-thujene	2.41
3	(camphene)	.44
4	(β-pinene)	trace
5	sabinene	24.88
6	(3-carene)	.36
7	myrcene	1.99
8	α-terpinene	1.21
9	limonene	2.98
10	(β-phellandrene)	trace
11	(γ-terpinene)	1.77
13	(ρ-cymene)	.21
14	terpinolene	.77
15B		trace
16		trace
18		trace
19		trace
19A		trace
21		trace
23	(alcohol)	1.59
25	citronellal	.98
26	camphor	32.18
26A		trace
27	(linalool)	1.79
28		.65
29		trace
30	(bornyl acetate)	
31	(camphene hydrate)	2.87
32	4-terpinenol	5.25
33	(trans-2-methyl-6-methylene-3,7-octadien-2-ol)	.33
34		.13
35		trace
35A		trace
36		trace
37A		trace
38		trace
39	borneol	1.15
41	(methyl vinyl anisole)	.36
42	(δ-cadiene)	trace
43	(carvone)	trace
44		.16
45		trace
46	citronellol	4.21
47		.10
48		trace
49		trace
50	(alcohol)	.21
51	(geraniol)	.11
52		trace
54	(alcohol)	trace
55		trace
56	(alcohol)	.39
57A		trace

Cpd. #	Identity	Percent
58		trace
59	(methyl eugenol)	.11
59A		trace
60		trace
61		.26
62	elemol	2.10
63	(elemol acetate)	.90
63A	(decomposition?)	trace
64		1.75
65		.78
66	(γ-eudesmol)	.75
67		.33
68		trace
69	(α-eudesmol)	.70
70	(β-eudesmol &/or elemicin)	
72		trace
72B		trace
72A		trace
72C		trace
73		trace
74	(C_{15} ester)	.34
74E		trace
74D		trace
74A		trace
74C		trace
75		.17
76	(acetate II)	trace
77		trace
78		trace

continuous. Those characters with states were often given intermediate values (i.e., Ml = 1.5).

All of the foliage samples were steam distilled to remove the volatile terpenoids (monoterpenes and sesquiterpenes) as outlined in Adams (1970a). The period from initial freezing to distillation ranged from 1 day to 3 weeks. After freezing, the foliage samples were not thawed or disturbed during the storage period. Oil samples were concentrated with a jet of nitrogen and stored in tightly capped vials at -20° C until analyzed by gas/liquid chromatography. For a detailed description of column conditions and methodology, see Adams (1970a). The major constituents of the volatile oil of *J. pinchotii* were identified by comparisons of their infrared spectra with known compounds (see table 2). The volatile terpenoids of *J. ashei*, and *J. scopulorum* have been identified by von Rudloff (1968) and von Rudloff and Couchman (1964). The major constituents of *J. monosperma* were identified by infrared analysis and will be presented in a later paper along with some analysis of *J. deppeana* and *J. flaccida.*

Each of the terpenoid components was assigned a unique code by superimposition of the chromatographs. Although some errors occur in this process since 2 different compounds may have the same retention times (even on very good columns), these few misidentifications are thought to only slightly affect comparisons between species (see Adams, 1971, for an analysis of these errors). The chances of a misidentification of peaks be-

tween individuals within a species is probably quite small (Adams, 1972). The relative percentage of each compound was determined by an electronic digital integrator with automatic printed output. In cases where peak shoulders were not resolved as separate peaks, they were approximated by comparison of the relative size of the main peak with the shoulder peak. Due to the difficulty of separating consistently peaks 30 and 31, they were treated as one entity, as were peaks 69 and 70.

Those components which were present in amounts less than 0.10% of the total oil were called traces and given an arbitrary value of 0.05% in order that they might be processed differently in subsequent analyses.

Figure 2 shows the gas/liquid chromatograms of the volatile terpenoids of *J. scopulorum, J. pinchotii,* and *J. monosperma.* The gas/liquid chromatograms of *J. flaccida, J. ashei,* and *J. deppeana* are shown in figure 3. It is obvious that numerous differences exist between *J. pinchotii* and these other 5 taxa. It would appear that widespread hybridization between *J. pinchotii* and any of these taxa should be easily detected by the presence of complimentary peaks in the chromatograms in the hybrid plant. Although it is not the purpose of this paper to examine the relationships among these taxa, it is apparent that the large number of characters (approx. 135 compounds) seriously limits any meaningful comparisons between these taxa. In analyses of populational differentiation, where the differences are mostly quantitative, numerical techniques must generally be relied upon.

The first step in the analysis of population differentiation in *J. pinchotii* was to analyze individual characters to determine the significance of each

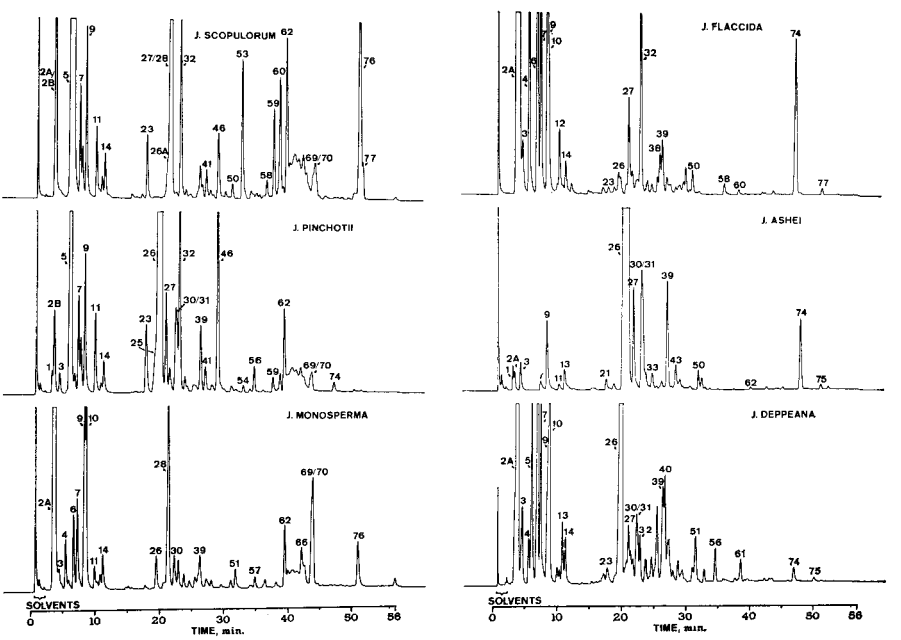

FIG. 2. Gas-liquid chromatograms of the volatile terpenoids of *J. schopulorum, J. pinchotii,* and *J. monosperma.*

FIG. 3. Gas-liquid chromatograms of the volatile terpenoids of *J. flaccida, J. ashei,* and *J. deppeana.*

character relative to the variation observed in *J. pinchotii* populations. Analysis of variance was performed on each of 19 morphological characters and 97 terpenoid characters to determine (by use of the F ratio of the variance among populations/variance (pooled) within populations) which characters exhibited statistically significant differences among populations.

The Student-Newman-Keuls (SNK) multiple range test was modified to accommodate unequal population samples (Steel & Torrie, 1960) and each character was analyzed to determine which population means were highly significantly different (at the .01 level).

In order to visualize the trends in populational differentiation in *J.pinchotii,* those characters with both highly significant F ratios and SNK tests were contour mapped by computer (Adams, 1970b). Since several characters often reflected similar trends and to conserve space, a representative contour map will be shown with the appropriate SNK test summaries below the contour map. The total trend of several characters

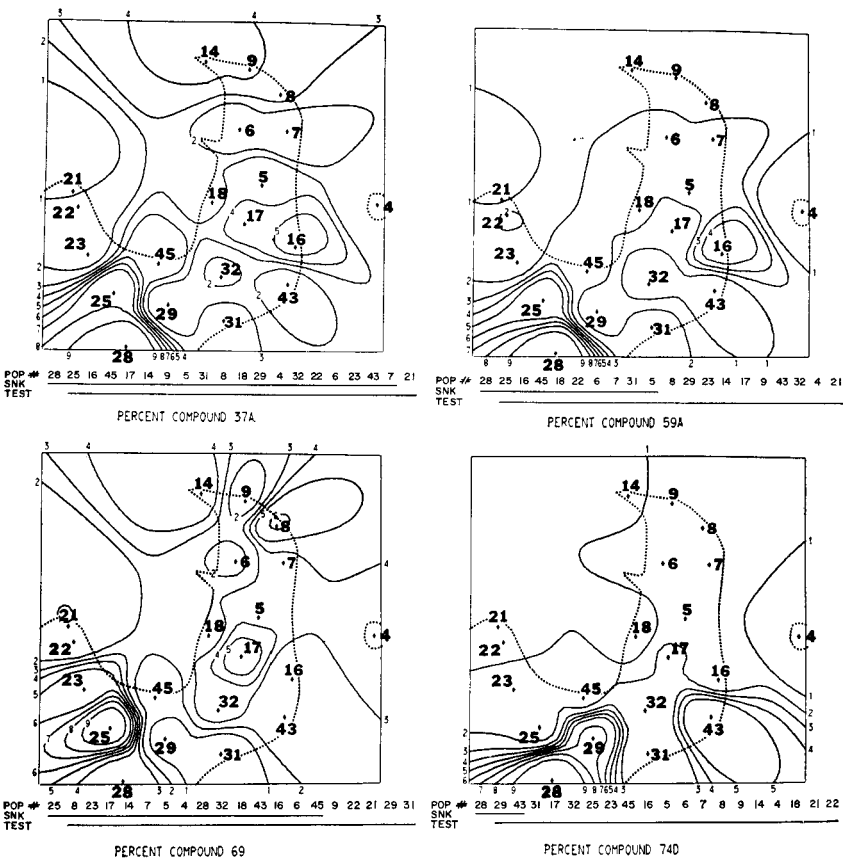

FIG. 4. Contour maps of 4 terpenoids found in *J. pinchotii.*
Upper left: Cpd. 37A, contour range = .09-.35%
Upper right: Cpd. 59A, contour range = .03-.17%
Lower left: Cpd. 69, contour range = .44-1.12%
Lower right: Cpd. 74D, contour range = 0.1-.22%

taken together was investigated by differential systematics (Womble, 1961; Adams, 1970b; Adams and Turner, 1970). This method involves summing the F weighted, absolute differentials of each character (with regard to distance) into a composite differential to delimit zones of rapid changes in several characters considered simultaneously.

Numerical taxonomy was used to determine similarities among populations of *J. pinchotii* and similarities between populations of *J. pinchotii* and various sympatric species of Juniperus. The similarity measure used was basically a matching coefficient described by Sokal and Sneath (1963) and is described in detail by Adams and Turner (1970). The character matches were weighted by use of the F ratios (variance among populations/variance within populations). The use of variance parameters for character weighting has been adequately discussed by Farris (1966), Flake and Turner (1968), and Adams and Turner (1970a).

The single linkage method of Sneath (1957) was used for cluster analysis (for further discussions of single linkage clustering see Jardine, *et al.* (1967), Jardine and Sibson (1968), Jardine (1969), Hall (1969), and Adams and Turner (1970).

Results

Terpenoid data. — Table 3 lists those compounds whose F ratio indicated that population differences were highly probable (P = 0.01). When SNK tests were run on each of the 97 terpenoids found in *J. pinchotii*, one com-

TABLE 3. Analysis of variance (ANOVA) for 22 compounds of *J. pinchotii* which had highly significant (P = .01) F tests implying that populational differences are present. $F_{.01} = 2.20$ (df $\doteq$ 19/84).

Terpenoid		F ratio	Species Avg. (% of total oil)
1	(tricyclene)	2.63	.34
3	(camphene)	3.71	.43
5	sabinene	2.34	24.32
7	myrcene	3.08	1.99
19A	unknown	4.14	.04
25	citronellal	7.74	1.09
26	camphor	3.52	32.67
28	unknown	4.60	.66
29	unknown	2.647	.06
34	unknown	3.68	.18
36	unknown	2.91	.20
37A	unknown	2.49	.17
54	(alcohol)	4.89	.16
55	unknown	5.23	.10
56	(alcohol)	4.94	.70
59A	unknown	2.38	.05
61	unknown	5.36	.50
66	(γ-eudesmol)	2.24	.70
69/70	(α & β-eudesmols &/or elemicin)	2.70	.63
72	unknown	3.30	.17
74D	unknown	4.48	.05
72	(acetate II)	16.55	.10

pound (≠ 33) had one highly significant difference by the SNK test but had a non-significant F ratio (at P = .01). Otherwise only those compounds which had highly significant F ratios in the analysis of variance had highly significant SNK tests. Two compounds, 5 (sabinene) and 66 (γ-eudesmol), had highly significant F ratios but no SNK differences. Thus, of the 22 terpenoids in table 3, 20 had both highly significant F ratios and SNK tests. These 20 compounds were used for contour mapping and differential systematics.

Figure 4 shows the contour maps for compounds 37A, 59A, 69, and 72D. Compounds 37A and 59A both show increased amounts in populations 25, 28, and to a lesser extent, population 16. Compound 69 shows an increased amount in population 25 with lesser amounts in populations 8, 23, and 17. The pattern for compound 74D shows divergence in populations 28, 29, and 43. Examination of other compounds revealed that several could be grouped into classes which show the same contour surface. Two such classes are shown in figure 5. Compound 55 is contoured on the left with the SNK tests for compounds 19A, 25, 72, and 76 shown below. All five of the compounds showed the same pattern of divergence of population 25 then 28 from the rest of the 20 populations. The contour map on the right in figure 5 is of compound 61. Here we see a reversal of the pattern in that population 28 is most divergent, with population 25 being somewhat intermediate. This trend is shared by compounds 29, 34, 36, 54, and 56, whose SNK tests are shown below that of compound 61.

Figure 6 shows 4 patterns which are somewhat different from each

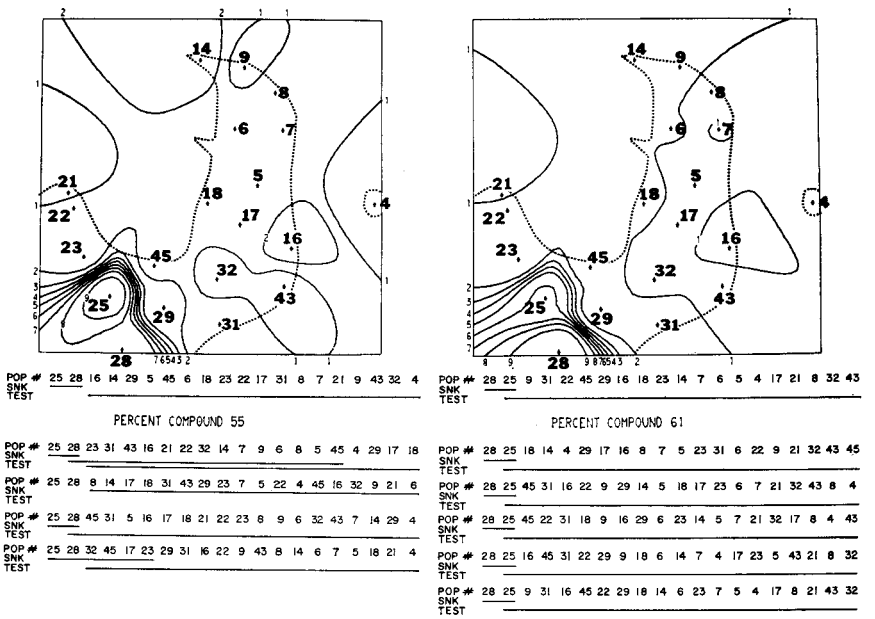

FIG. 5. Contour maps of 2 terpenoids found in *J. pinchotii* along with the SNK tests for several terpenoids which had the same patterns. See text for discussion.
Left: Cpd. 55, contour range = .07-.30%
Right: Cpd. 61, contour range = .29-2.34%

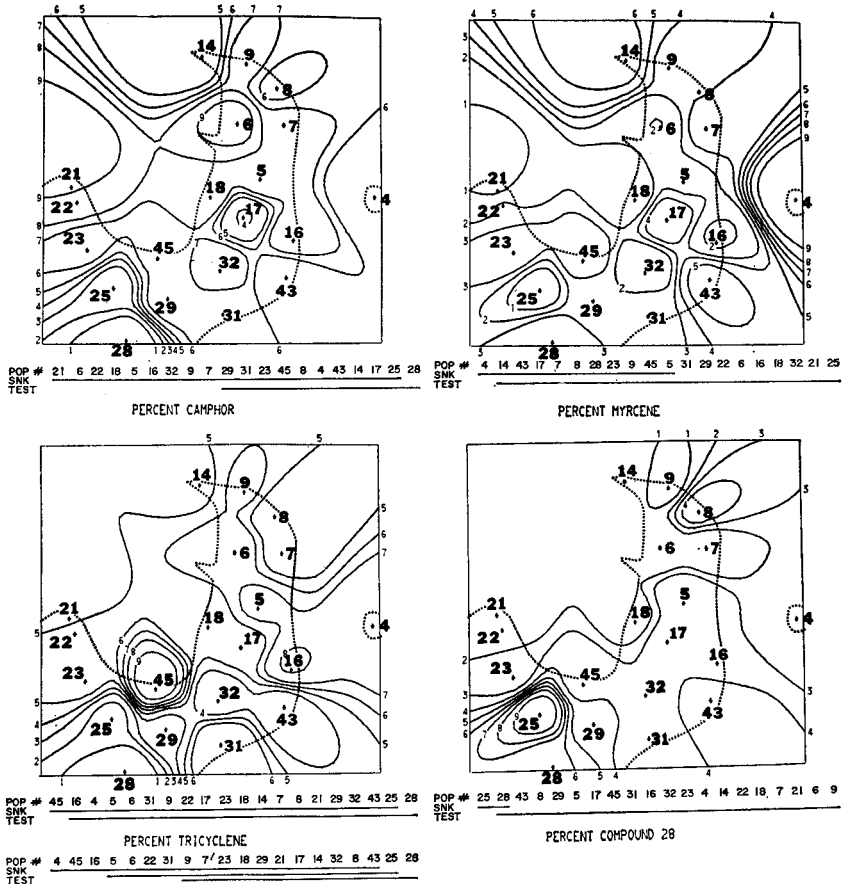

FIG. 6. Contour maps of 4 terpenoids found in *J. pinchotii.*
Upper left: Camphor, contour range = 11.11-43.14%
Upper right: Myrcene, contour range = 1.73-2.69%
Lower left: Tricyclene, contour range = .11-.55%
Lower right: Cpd. 28, contour range = .51-1.02%

of the preceeding ones. Camphor, myrcene, tricyclene, and compound 28 each have several differences. Camphene had practically the same pattern as tricyclene and its SNK test is shown beneath that of tricyclene.

It seems apparent that some differentiation has occurred between populations 25 (Alpine, Tex.) and 28 (Chisos Mtns.) and the rest of *J. pinchotii.* In order to summarize these differences, the composite differential was taken using all 20 compounds (Adams, 1970b). The high contour levels in figure 7 are the areas where the differentials were largely additive and represent areas of rapid changes between populations. The most rapid changes in these 20 characters are between population 25 and population 23 and 45, and between population 28 and 29. A smaller differential is seen between populations 25 and 28, with a very small differential between populations 16 and 43. It should be noted that the contribution of each character is proportional to its F ratio since the differentials were F weigh-

ted in taking the composite absolute differential.

In order to better visualize the divergence of populations 25 and 28, similarity measures were computed using 55 chemical characters whose F ratios were greater than 1.0 and were non-trace components in some population. The result of single linkage clustering is shown in figure 8. Notice that populations 25 (Alpine, Tex.) and 28 (Chisos Mtns.) are more similar to each other than to any other population. The rest of the populations cluster very close together and there is probably little significance to their

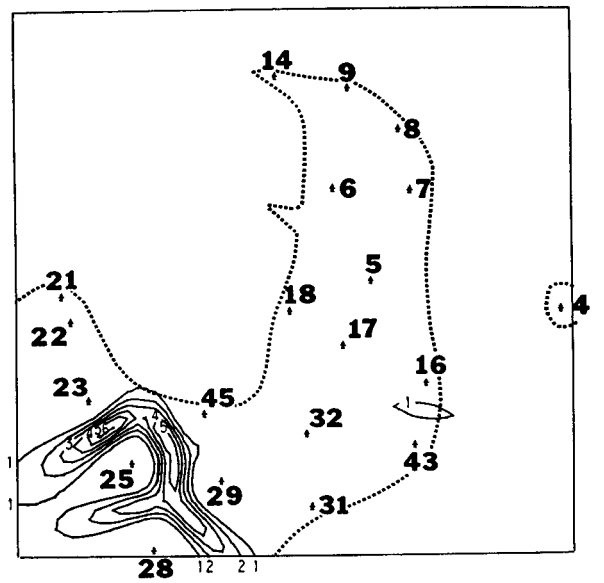

FIG. 7. The composite differential of 20 terpenoids of *J. pinchotii*. The dotted line shows the distribution of this taxon. The contour values are the average, F weighted, absolute differential of each of the 20 terpenoids. Contour symbols and values are: 1 = .07; 2 = .12; 3 = .17; 4 = .22; 5 = .27; 6 = .32.

ordering. The isolated population 4 (Bosque Co.) shows little difference (on terpenoid grounds) from the main body of *J. pinchotii*.

Morphological Data. — When analysis of variance was run on the 19 morphological characters examined (cf. table 1), seven were found to have highly significant F ratios implying that populational differences were present. These 7 characters and their F ratios are shown in table 4, along with female cone diameter. SNK tests revealed significant differences with growth habit, female cone color, bloom on cones, seed color, hilum scar size, and female cone diameter. One significant difference was found by the SNK test of female cone diameter, even though the F ratio was not significant (at P = .01).

Each of these 6 characters were contoured mapped and the results are shown in figures 9 and 10. Notice that populations 25 and 28 are tending toward rose colored female cones with bloom. Populations 7, 8, and 9 have generally larger hilum scars and population 31 had lighter colored seeds and larger female cones. Unfortunately, some of these characters may not be too reliable due to the subjective methods used in scoring them.

Nevertheless, 5 of these characters (omitting growth habit, which appears to be strongly influenced by shading) were used to obtain the composite differential shown in figure 11. One is immediately impressed with the increased variation in the morphological characters compared to the terpenoids (cf. figure 7). Populations 25 and 28 still show some divergence along with a high differential between populations 32 and 31. Numerous small differentials are also evident. It is interesting to note the small differential between population 21 (ca. 5500 ft., on Ca CO_3 in the Guadalupe

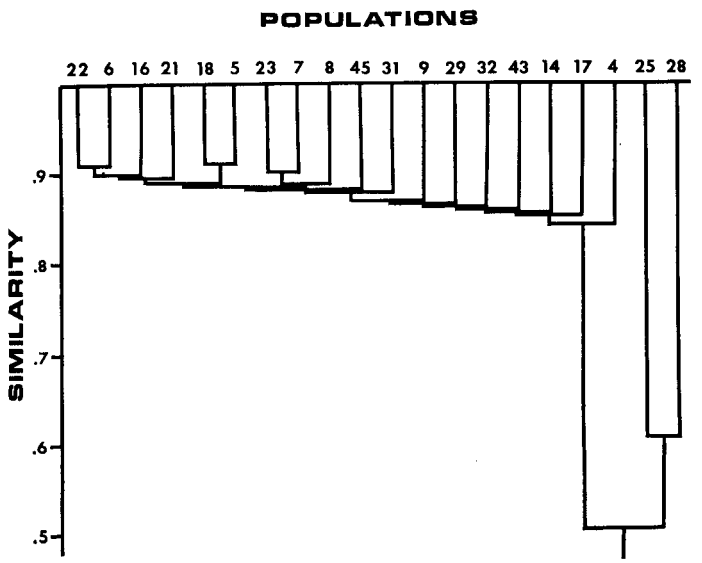

FIG. 8. Phenographic representation of single linkage clustering of 20 populations of *J. pinchotii*, using 55 terpenoid characters, F weighted.

Mtns.) and population 22 (ca. 3300 ft., on gypsum, $CaSO_4$). These two populations occupy quite different niches but their morphology and terpenes are remarkably similar (cf. figures 7 & 8).

Similarity measures were calculated using the 17 morphological characters with F ratios greater than 1.0 as previously outlined and a phenogram constructed by single linkage (figure 12). The increased diversity suggested by the composite differential is shown in this phenogram with several subclusters (23,4; 17,6; 7,9; 5,8) which were not evident with the terpenoid datum (figure 8). In addition, there is considerable "tailing out" of populations 29, 22, 21, and 31 from the central group. Nevertheless, we still see mutual divergence of populations 25 and 28 as with the terpenoid datum.

Composite Analysis. — The study of concordant variation in the terpenoids and morphology can best be seen by recalculation of the similarity measures using both the 55 terpenoids and the 17 morphological characters. Discordant variation tends to be lost with concordant variation amplified. When these F weighted similarity measures were computed and the phenogram constructed (figure 13) we see the divergence of populations 25 and 28 with the rest of the populations forming a rather tight group.

Discussion

Although hybridization and introgression of *J. pinchotii* with *J. ashei* had been reported by Hall, McCormick, and Fogg (1962) in the lower Devils River region (populations 31 and 32) as well as throughout most of west Texas where these two taxa are sympatric, I could find no evidence

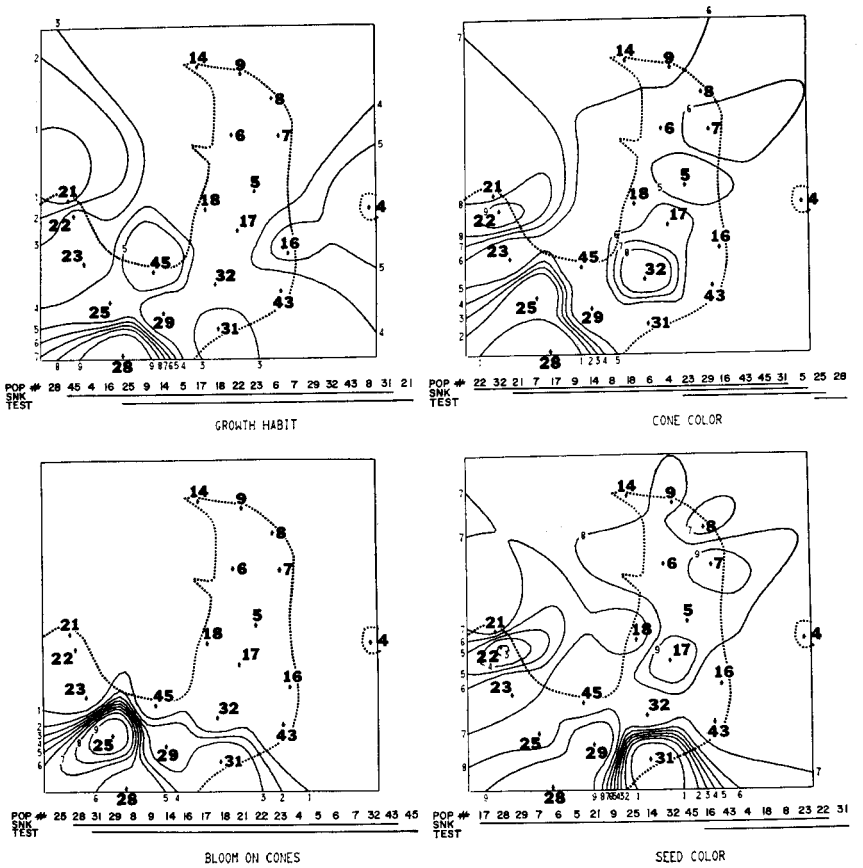

FIG. 9. Contour maps of 4 morphological characters of *J. pinchottii.*
Upper left: Growth habit, contour range = 1.68-2.69
Upper right: Cone color, contour range = 2.26-3.56
Lower left: Bloom on cones, contour range = 1.05-1.94
Lower right: Seed color, contour range = 1.93-2.94

for the existence of such hybrids nor did I detect introgression of *J. ashei* into *J. pinchotii* (likewise no evidence was found of introgression of *J. pinchotii* into *J. ashei*, see Adams and Turner, 1970). Indeed, upon examination of two of the key morphological characters, gland width/length and whip leaf length, used by Hall *et al.* (1962) to show hybridization and introgression between *J. pinchotii* and *J. ashei*, I found that individuals of *J. pinchotii* in sympatric populations had more oval shaped glands but

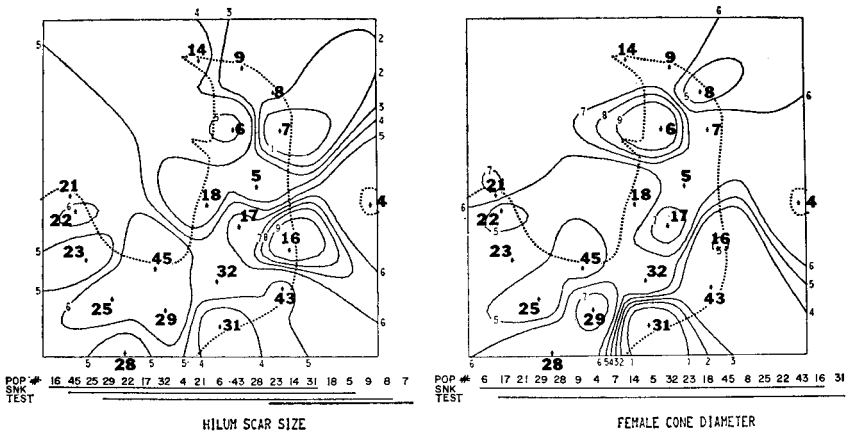

FIG. 10. Contour maps of 2 morphological characters of *J. pinchotii.*
Left: Hilum scar size, contour range = 1.17-2.70
Right: Female cone diameter, contour range = 5.41-7.52 mm

other populations geographically quite removed from *J. ashei* also had oval shaped glands. Hall *et al.* (1962) reasoned that these allopatric populations were introgressed from *J. ashei.* Yet populations exist between these 2 populations which show no tendency towards oval shaped glands. The other character, whip leaf length, was of intermediate length in sympatric populations and shortest (as in *J. ashei*) in some of the allopatric

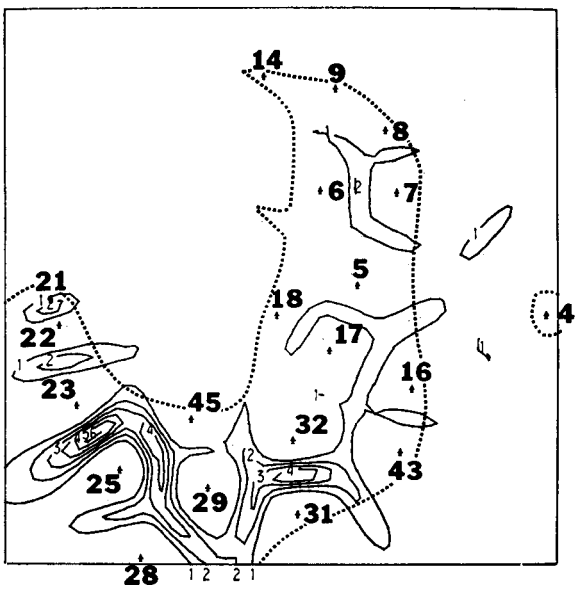

FIG. 11. The composite differential of 5 morphological characters of *J. pinchotii.* The contour values are the average, F weighted, absolute differential of each of the 5 morphological characters. Contour symbols and values are: 1 = .057; 2 = .095; 3 = .133; 4 = .171; 5 = .209; 6 = .248

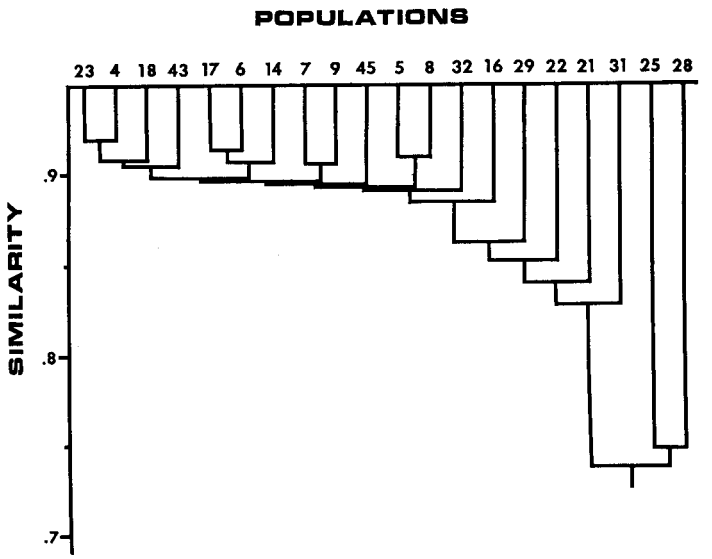

FIG. 12. Phenographic representation of single linkage clustering of 20 populations of *J. pinchotii*, using 17 morphological characters, F weighted.

populations of *J. pinchotii*. In addition no evidence of hybridization or introgression with *J. ashei* was found in the terpenoid characters and I conclude therefore that very little or no hybridization is occurring between *J. ashei* and *J. pinchotii*. If hybrids occur they are certainly difficult to detect (since I attempted to select for hybrid types in the field) and, if present, presumably are either sterile, or strongly selected against such that introgression from *J. ashei* into *J. pinchotii* is of little consequence.

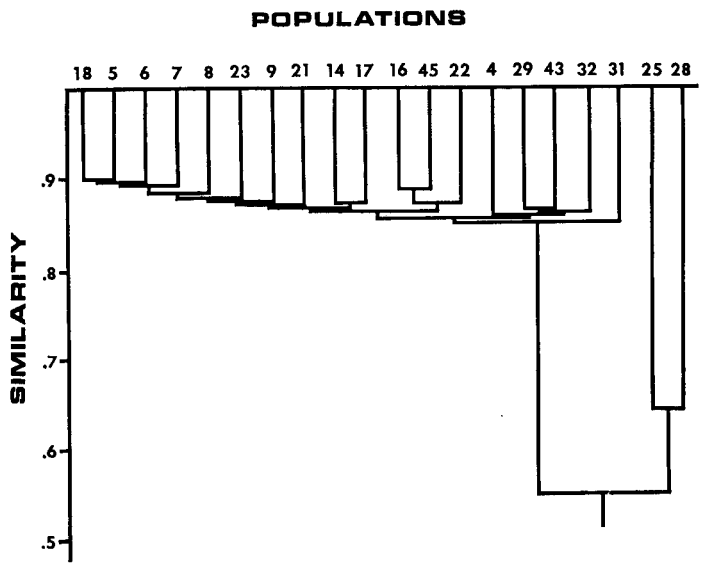

FIG. 13. Phenotypic representation of single linkage clustering of 20 populations of *J. pinchotii*, using 17 morphological and 55 terpenoid characters, F weighted.

The plants from population 14 were of special interest due to the report by Hall and Carr (1968) of widespread hybridization between *J. monosperma* and *J. pinchotii* in the Palo Duro Canyon of the Texas Panhandle. In my studies of this region I found that several trees collected as *J. monosperma*, contained unusual quantities of various terpenoids. Two trees, # 183 and # 191, contained several terpenoids not previously found in 3 populations of *J. monosperma*, but characteristically found in all of the *J. pinchotii* trees examined in this study. In addition, several of the *J. monosperma* trees contained terpenoids which had not been found in any other populations of *J. monosperma* or *J. pinchotii*. The presence of "extra" compounds in hybrid plants is well known (Alston and Turner, 1963; Alston *et al.*, 1965). Figure 14 shows the gas chromatograms of *J. monosperma* (population 12, at Folsom, New Mexico), tree # 191, and *J. pinchotii* (population 18 at Big Spring, Texas). It should be noted that several trees were collected from Palo Duro Canyon which were practically the same as those of the Big Spring population of *J. pinchotii* and the Folsom, New Mexico population of *J. monosperma*. That is to say, several *J. pinchotii* and *J. monosperma* trees were collected from Palo Duro Canyon which appeared to be quite representative of these taxa. In the gas chromatogram of tree # 191, those peaks indicated by a "p" are normally found in *J. pinchotii* and either are not normally found in *J. monosperma* or else are highly significantly different in plant 191. Those peaks not normally found in either *J. monosperma* or *J. pinchotii* are indicated by an "e". The peaks labeled with a "p" are compounds 34, 36, 41, 46, 54, 56, and 61 (compound 36 is a small peak that is present but unlabeled in figure 14). These compounds are highly intercorrelated (Adams, 1969) with the exception of compound 46, citronellol. Thus we find a group of compounds, in an otherwise *J. monosperma* plant, which are characteristically

TABLE 4. Analysis of variance for 8 morphological characters of *J. pinchotii*. $F_{.01} = 2.20$ (df = 19/84).

Character	F ratio
M1, growth habit	6.23
M3, whip leaf gland length/sheath length	2.82
M6, female cone color	7.04
M8, glands flat vs. raised	5.25
M12, bloom on cones	6.48
M13, seed color	3.13
M14, hilum scar size	6.17
M17, female cone diameter	1.64 n.s.

TABLE 5. Similarity values based on 80 terpenoid characters, F weighted. P18, P25, and P28 are populations 18, 25, and 28 of *J. pinchotii*; *Scop* = *J. scopulorum*; Depp = *J. deppeana*; Mono = J. *monosperma*; Flac = *J. flaccida*.

OTUs	P18	P25	P28	Scop	Depp	Mono	Flac
P18	1.00	.82	.80	.70	.61	.52	.40
P25	.82	1.00	.87	.74	.60	.52	.37
P28	.80	.87	1.00	.70	.59	.49	.36

highly intercorrelated in *J. pinchotii*. The most acceptable explanation for the coherence of these compounds in tree # 191 must be that it is due to introgression from *J. pinchotii*. None of the *J. pinchotii* trees from this site showed definite signs of introgression from *J. monosperma*. In September, 1968, a resample was taken from Palo Duro Canyon consisting of 24 "randomly chosen" trees plus tree # 191. Terpenoid analysis revealed tree # 191 to be essentially the same as that found in the sample examined in December, 1967. In addition, one tree appeared to be a *J. monosperma* plant with some introgression from *J. pinchotii*, and another appeared to be relatively pure *J. monosperma*. The other 22 trees were *J. pinchotii* with no evidence of introgression from *J. monosperma*.

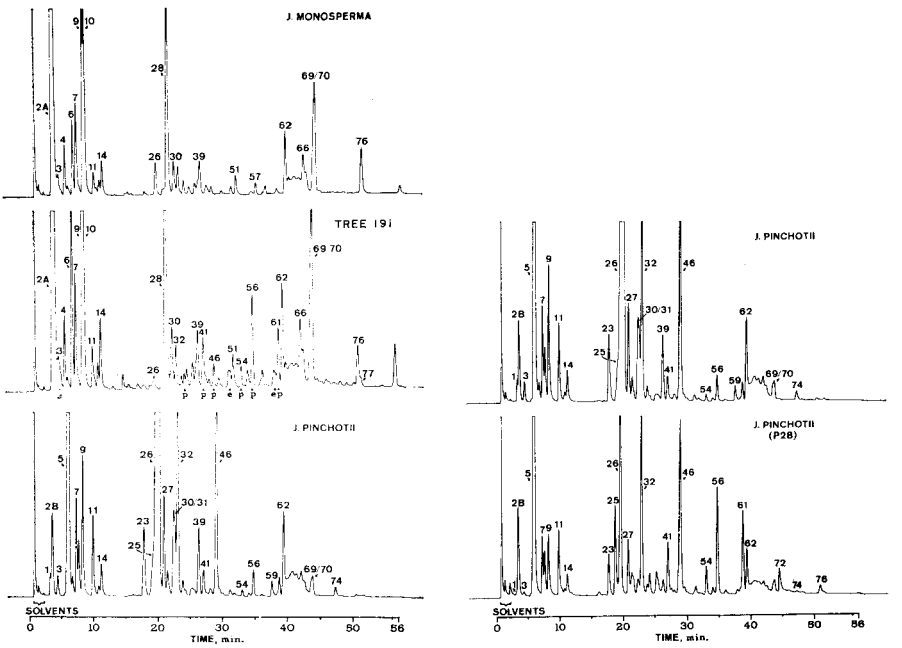

FIG. 14. Gas-liquid chromatograms of the volatile terpenoids of *J. monosperma*, tree 191 from Palo Duro Canyon of the Texas Panhandle, and *J. pinchotii*. Those peaks marked with a 'p' denote compounds normally found in *J. pinchotii*, those marked with an 'e' denote compounds not normally in either *J. monosperma* or *J. pinchotii*.
FIG. 15. Gas-liquid chromatograms of the volatile terpenoids of 'typical' *J. pinchotii* (P18), and a divergent population, P28, in the Big Bend region of trans-Pecos Texas.

Morphological characters failed to show population 14 of *J. pinchotii* to be tending toward *J. monosperma* by introgression in Palo Duro Canyon (refer to fig. 9 and note the contours for: bloom on cone, and female cone color, the two morphological often used to separate these taxa). Thus it is reasonable to conclude that hybridization between *J. monosperma* and *J. pinchotii* is occurring but introgression into *J. pinchotii* is not evident. Introgression of *J. pinchotii* into *J. monosperma* could not be adequately measured since only a small portion of the range of *J. monosperma* was examined.

Juniperus pinchotii is also sympatric with *J. monosperma* in the Guadalupe Mountains of southern New Mexico (population 21). If hybridization is occurring in this locality, I was unable to detect it. One possible reason for this is that "hybridized habitats" (Anderson, 1949) are not as available as they are in Palo Duro Canyon which has a range of different soils and moisture conditions which may vary over distances of only a few feet. In the Guadalupe Mountains, most of the *J. pinchotii* seems to be restricted to the rocky slopes up to ca. 6000 feet. *J. monosperma* generally occurs with *J. deppeana* in the more mesic areas above this level. Thus there are relatively few disturbed or intermediate habitats which might support such hybrids.

In the Chisos Mountains in the extreme southwest Texas, *J. pinchotii* is sympatric with *J. flaccida*. No evidence of hybridization of introgression was found between these two taxa.

Juniperus pinchotii occurs with *J. deppeana* in the Guadalupe Mountains (population 21), the Davis Mountains (just north of population 25), and in the Chisos Mountains (population 28). It is in this region that considerable controversy has arisen. Correll (1967) lists population locations for *J. monosperma* in the Chisos Mountains, Davis Mountains, and elsewhere in the trans-Pecos region of Texas. Yet, I have not found *J. monosperma* plants as revealed by gas chromatography in my several field trips to this region. Instead, several plants, thought to be *J. monosperma* from the Davis Mountains, the Chisos Mountains, and near Alpine, Texas (due to bloom on the female cones and the bluish color of the female cones) were in fact, chemically, very close to "pure" *J. pinchotii*. In figure 15 one can see some of the differences between "pure" *J. pinchotii* (popn. 18, Big Spring, Texas) and population 28 (Chisos Mountains). The most striking differences are the presence of compound 72, the decrease in camphor (cpd. 26) and the increase in citronellal (cpd. 25). In populations of *J. pinchotii* in the Davis Mountains and Chisos Mountains the female cone color varies from the characteristic reddish-brown with no bloom to rose with bloom, yet those with the typical cone color are somewhat diverged, chemically, from the populations of *J. pinchotii* of central and north Texas. As shown in the composite differential of 20 terpenoids, the Davis and Chisos Mountains populations appear quite divergent from the rest of *J. pinchotii*.

In order to determine if this divergence is due to introgression from some other taxon, similarity measures were calculated using trees from 3 populations of *J. pinchotii* (populations 18 at Big Spring, Texas; 25 at Alpine; and 28 in Chisos Mtns.), one population of *J. monosperma* (from the Guadalupe Mtns.), one population of *J. scopulorum* (from Folsom, N. M.), one population of *J. deppeana* (from Chisos Mtns.), and one population of *J. flaccida* (from Chisos Mtns.). Analysis of variance revealed 80 chemical characters and 17 morphological characters which had F ratios greater than 1.0. Table 5 shows the similarity values obtained with the 80 terpenoids characters, F weighted. It is readily apparent that, on chemical grounds, P25 and P28 are closely related (.87) and belong with *J. pinchotii* (P18). If these two divergent populations are introgressed, we would expect to find a higher similarity between some other taxon and population 25 or 28 than with population 18 (seemingly pure *J. pinchotii*). These similarity values reveal only one case where either P25 or P28 is more similar to another taxon than P18. This case is with *J. scopulorum* (Scop in table 5). Notice that P25 is slightly more similar to Scop (.74) than P18 is similar

TABLE 6. Similarity values based on 17 morphological characters, F weighted. P18, P25, and P28 are populations 18, 25, and 28 of *J. pinchotii*; Scop = *J. scopulorum*; Depp = *J. deppeana*; Mono = *J. monosperma;* Flac = *J. flaccida.*

OTUs	P18	P25	P28	Scop	Depp	Mono	Flac
P18	1.00	.88	.88	.77	.61	.78	.19
P25	.88	1.00	.96	.81	.56	.87	.14
P28	.88	.96	1.00	.82	.55	.87	.14

to Scop (.70). The strong linkage between *J. pinchotii* and *J. scopulorum* is certainly interesting in light of Hall and Carr (1968), who suggest that *J. pinchotii* may be of hybrid origin between *J. monosperma* and *J. deppeana.*

When similarity measures were calculated using the 17 morphological values (with F ratios greater than 1.0), the relationships are somewhat different (table 6). P25 and P28 are very similar (.96) with almost identical similarities to P18 (*J. pinchotii*) and *J. monosperma* (Mono). Notice that both P25 and P28 are more similar to *J. monosperma* than P18 (typical *J. pinchotii*) is similar to *J. monosperma.* Again we see that the trans-Pecos *J. pinchotii* (P25, P28) are closer to *J. scopulorum* than the typical *J. pinchotii* (P18) is to *J. scopulorum.* It is not surprising that many of the junipers in the Alpine and Big Bend region of the trans-Pecos have been called both *J. pinchotii* and *J. monosperma* as well as *J. erythrocarpa* and *J. texensis*!

In recent field work in Mexico in the areas of Saltillo, Coahuila, and Chihuahua, I have collected specimens of *J. erythrocarpa* Cory var. *coahuilensis* Martinez. This plant appears to be the same as found south of Alpine, Texas (P25). The morphology is very similar to *J. monosperma,* just as those plants of P25 and P28. Preliminary results indicate the terpenoids are very similar to those of P25 and P28 (as well as P18, *J. pinchotii*). Martinez (1963) was of the opinion that var. *coahuilensis* is the ancestral link between *J. monosperma* and *J. erythrocarpa* (*J. pinchotii*). If further study confirms that P25 and P28 are northern extensions of var. *coahuilensis,* then some nomenclatural changes will be needed. This problem is currently being investigated.

Conclusion

Juniperus pinchotii is a somewhat variable taxon throughout much of west-central Texas, with some quite divergent populations in the trans-Pecos region. The divergent trans-Pecos populations show morphological similarity to *J. monosperma* but some similarity to *J. scopulorum.* At present, I would favor maintaining these divergent forms in *J. pinchotii.* No hybridization or introgression of *J. pinchotii* and *J. ashei* was detected and hybridization is therefore presumed to be rare or non-existent. Some evidence of hybridization between *J. monosperma* and *J. pinchotii* was detected in Palo Duro Canyon of the Texas Panhandle, but there is no evidence of introgression of *J. monosperma* into *J. pinchotii.*

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HI-IAPT *Portraits of botanists*, no. 19. NICOLAUS JOSEPH JACQUIN. Lithograph by Lanzadilly after a painting by Kinninger, Hunt Institute.

Nicolaus Joseph [after 1806 Freiherr von] Jacquin; *b.* Leiden, Netherlands, 16 February 1727; *d.* Wien, Austria, 26 October 1817: Dutch born Austrian botanist, traveller in Central America 1755-1759, professor of chemistry at Schemnitz. Hungary 1763-1768, professor of botany and chemistry in Wien 1769-1797, author of numerous sumptuously illustrated works, e.g. *Selectarum stirpium americanarum historia* (1763). Biogr.: Raimann, Rede zur Gedächtnissfeyer Jacquin, 1818; Wurzbach, Biogr. Lex. Kaiserth. Oesterr. 10: 26-32. 1863; Kronfeld, Österr. Rundschau 3: 237-251. 1905; Garside, J. S. Afr. Bot. 8: 200-224. 1942; Stafleu, Tax. lit. 230-233. 1967; Stafleu, Jacquin and his American plants, in facs. ed. 1970 of *Selectarum stirpium*; Stafleu, Linnaeus and the Linnaeans 182-191, 375. 1971.