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CHEMOSYSTEMATIC AND NUMERICAL STUDIES OF NATURAL POPULATIONS OF *JUNIPERUS ASHEI* BUCH. †

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Summary

Juniperus ashei Buch. was analyzed throughout its Texas distribution for areas of population differentiation and possible hybridization with *J. virginiana* L. and *J. pinchotii* Sudw. 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. Some peripheral populations of *J. ashei* show divergence of both terpenoid and morphological characters. This divergence is not, apparently, due to hybridization with *J. virginiana* or *J. pinchotii*. No evidence of hybridization was detected between *J. ashei* and *J. virginiana* or *J. pinchotii*. Differential systematics and numerical taxonomy were found to be of considerable value in the analysis of infraspecific variation.

Introduction

Several of the North American species of *Juniperus* have been studied in considerable detail, especially their possible involvement in hybridization and introgression (Fassett, 1944, 1945; Ross and Duncan, 1949; Hall, 1952, 1955; Hall, McCormick, and Fogg, 1962; Hall and Carr, 1962; etc.). Most of these studies were approached using measurements taken from exomorphic characters as expressed in natural populations. Such data received relatively simple statistical treatments or else these were presented in the form of pictoralized scatter diagrams or bar graphs. Numerical procedures were not sufficiently developed at the time of these studies to permit more refined analyses, but their data did suggest that at least some of the species, notably *J. horizontalis*, *J. virginiana*, and *J. ashei* were involved to some considerable extent in situations involving hybridization and introgression, often over considerable distances. For example, Hall (1952) states that "... *Juniperus ashei* influences *J. virginiana* by introgression throughout the Ozark Plateau and probably as far east as the Tennessee River in the vicinity of the 36th parallel."

Indeed, Hall's studies (1952; 1955) of introgression between *Juniperus ashei* and *J. virginiana* have been hailed as "one of the most detailed studies of allopatric introgression..." (Davis and Heywood, 1963). Nevertheless, von Rudloff, Irving, and Turner (1968; unpubl.), and Flake, von Rudloff, and Turner (1969), using chemical data, were unable to substantiate the validity of Hall's studies. In fact, the former authors could find no evidence of hybridization between these two species, even when sampling some of the same populations examined by Hall.

In addition to these controversies, several other factors influenced the selection of species of *Juniperus* for detailed populational study. These are the following: 1. The plants are widespread, conspicuous, usually weedy trees. This makes possible

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the collection of population samples at systematic intervals throughout the ranges of the taxa.
2. The populations are relatively stable; therefore they can be resampled for confirmatory studies. In addition this permits the study of seasonal variations in the terpenoids (Adams, 1969b; Flake, von Rudloff, and Turner, 1969).
3. The species are wind pollinated and mostly dioecious. This would tend to make for relatively uniform populations except where strong differential selection or hybridization occurs.
4. Species of *Juniperus* are known to be rich in terpenoids and the major monoterpenes and sesquiterpenes of several taxa have been identified (Fahey and Kurth, 1955; Couchman and von Rudloff, 1964; von Rudloff and Couchman, 1964; Vasek and Scora, 1967; von Rudloff, 1968; Vinutha and von Rudloff, 1969).

In recent years the chemosystematic approach has been used to resolve systematic problems which were very difficult to resolve using classical morphological methods (Alston and Turner, 1963; Turner, 1967). Terpenoids are especially well-suited for such studies since they can be quantitated with considerable precision using an automatic digital integrator attached to a gas/liquid chromatograph. With the advances in better columns, and more sensitive and repeatable detection devices, several chemosystematic studies have been made using terpenoid data (Mooney and Emboden, 1968; Vasek and Scora, 1967; von Rudloff, 1967; Flake, von Rudloff, and Turner, 1969). Thus a method is available whereby one can analyze large samples relatively quickly, accurately, and with no preselection of characters, or bias in their measurements, by the investigator.

Until recently evaluation of the voluminous data obtainable from gas/liquid chromatographic analysis would have proved formidable, even to those systematists whose analyses might consist of only a few simple statistics such as the mean, range, and standard deviation. Such calculations were usually done by hand or with a calculator. The results were often expressed as mere tabulations which effectively obscured most trends that might have been detected.

With the advent of electronic computers the more sophisticated methods of discriminate functions (Hill, 1959; Hatheway, 1962; Johnson, 1962), multivariate analysis and factor analysis (Rayment, 1963; Vandermeer, 1965; Marcus and Vandermeer, 1966), distribution mapping by computer (Perring and Walters, 1962; Soper, 1964), surface trend analysis (Krumbein, 1962; Fisher, 1968), and numerical taxonomy (Sokal and Sneath, 1963; Estabrook and Rogers, 1966; Fisher, 1968; Crovello, 1968a; Flake and Turner, 1968; etc.) were developed. These techniques, with rare exceptions, have been applied using morphological characters but are equally suitable for bio-chemical data.

The present populational study utilizes both morphological and terpenoid characters. With these data we have attempted to contribute the following:

1. Development of suitable computer technique for the analysis of natural populations.
2. Determination of the structure of natural populations of *J. ashei* in central Texas.

Materials and Methods

Figure 1 shows the distributions of *Juniperus ashei, J. pinchotii,* and *J. virginiana* (as pertains to the study area), and the populations of *J. ashei* sampled in January, 1968. Population samples consisted of terminal branches from 5 trees at each site (except population 30 in which only 4 trees were sampled). Samples from individual

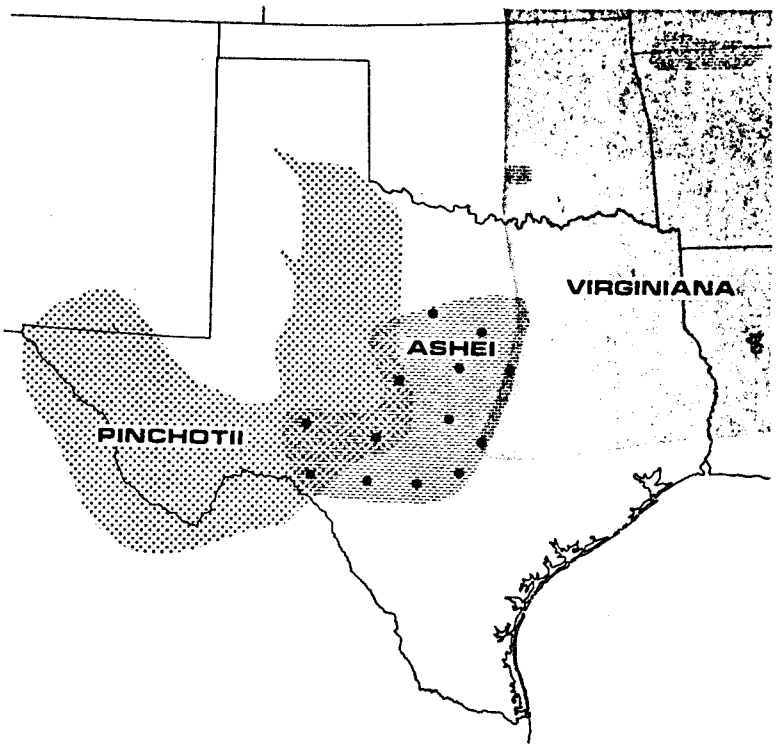

Figure 1. The distributions of *Juniperus ashei*, *J. pinchotii*, and *J. virginiana*. The populations sampled are indicated by dots and include each of the 3 species which was present in that area.

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. ashei* in which *J. pinchotii* or *J. virginiana* was present, 5 plants were sampled from each of the species concerned. The fresh foliage was sealed in plastic bags in the field 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 sampled is deposited in the University of Texas Herbarium, Austin, Texas.

Table 1 lists 19 morphological characters and their character states. In addition to these characters, 8 characters (tree color, height, bark exfoliation pattern, bark color, stiffness of terminal whips, terminal whip lengths, amount of fungus on the trunk and limbs, and soil habitat) were subjectively scored in the field and subsequently discarded.

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 continuous and were often given intermediate values (i.e., M1 = 1.5).

All of the foliage samples were steam distilled to remove the volatile terpenoids (monoterpenes and sesquiterpenes) as outlined in Adams (1969b). The period from

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 × LENGTH:	Average of 10 seeds.

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 the methodology and column conditions, see Adams (1969b). The volatile terpenoids of the foliage of *Juniperus ashei* and *J. virginiana* have been identified by von Rudloff (1968) and Vinutha and von Rudloff (1969). The major constituents of the volatile oil of *J. pinchotii* were identified by comparisons of their infrared spectra with known compounds. The identities of terpenoids found in these three taxa are listed in Table 2. Those compounds in parenthesis have been tentatively identified based on retention times.

Each of the terpenoid components was assigned a unique number by superimposition of the chromatograms. Obviously some errors may arise at this stage since 2 different compounds may have the same retention times even on the best available columns. Since virtually nothing is known about the synthesis of monoterpenes and sesquiterpenes from geraniol pyrophosphate onward, the actual identity of an individual component is not of overwhelming importance in this study. The

TABLE 2. A composite list of 135 terpenoids found in *J. ashei*, *J. virginiana*, and/or *J. pinchotii*. Terpenoids enclosed in parenthesis were tentatively identified by retention times.

Cpd. #	*Identity*	Cpd. #	*Identity*	Cpd. #	*Identity*
1	tricyclene	29		57A	
2A	α-pinene	30	bornyl acetate	58	
2B	α-thujene	31	camphene hydrate	58A	
2C		32	4-terpinenol	58B	
3	camphene	32A		59	methyl eugenol
3A		33	(trans-2-methyl-6-	59A	
3B			mythylene-3,7-	59B	
3C			octadien-2-ol)	60	
4	β-pinene	34		61	
5	sabinene	34A		62	elemol
5A		35		63	(elemol acetate)
6	3-carene	35A		63A	(decomposition?)
6A		36		64	
7	myrcene	36A		65	
8	α-terpinene	37	estragole	65A	
9	limonene	37A		66	(γ-eudesmol)
10	β-phellandrene	38		66A	
10A		39	borneol	67	
11	γ-terpinene	40		68	
12		40A		69	(α-eudesmol)
13	ϱ-cymene	41	(methyl vinyl anisole)	70	(β-eudesmol &/or
14	terpinolene	42	(δ-cadinene)		elemicin)
14A		43	carvone	72	
15		44		72A	
15A		45		72B	
15B		45A		72C	
15C		46	citronellol	73	
16		46A		73A	
16A		47		73C	
17		48		74	(C_{15} ester)
18		48A		74A	
19		49		74B	
19A		50	(alcohol)	74C	
20		50A		74D	
21		51	(geraniol)	74E	
22		52		74G	
23	(isothujone?)	52A		75	
24		53	safrole	75B	
25	citronellal	54	(alcohol)	76	(acetate II)
25A		54A		77	
26	camphor	54B		78	
26A		55		79	
26B		55A			
27	linalool	56	(alcohol)		
28	(methyl citronellate + ?)	57			

relative percentage of each compound was determined by an electronic digital integrator with automatic printed output. In cases where peak shoulders were not integrated as separate peaks, they were approximated by comparison of the relative size of the main peak with the shoulder peak. Occasionally, upon rechromatogramming, a shoulder would be sufficiently resolved to permit integration. This provided a very reliable check and confirmed the above approximation. Nevertheless, due to the difficulty in 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 analysis.

Figure 2 shows the gas/liquid chromatograms of the volatile terpenoids of *J. virginiana*, *J. pinchotii*, and *J. ashei*. These taxa are so different in terpenoid composition that hybridization should be easily detected by the presence of complementary peaks in the chromatograms from hybrid plants.

Even a cursory examination of the chromatograms in Fig. 2 reveals numerous differences between these taxa. Yet the sheer number of characters interferes with one's grasp of their total similarities. In the analysis of populational differentiation at the infraspecific level much more sensitive methods of analysis must be relied upon.

In the first step of the analysis of populational differentiation with *J. ashei*, one must first find which of the 19 morphological and 88 chemical characters show populational differentiation. Analysis of variance (ANOVA) was performed on each of the morphological and terpenoid characters to determine (by use of the F test of the variance between populations/variance within populations) which characters exhibited statistically significant differences between populations (formulation from Steel and Torrie, 1960).

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

Having reduced the character set to those characters which had both highly significant F tests and SNK tests, the next step was to attempt to visualize the trends of these characters between populations. As was recently mentioned (Adams, 1970) contour mapping of population means is quite effective in showing the trend of a character over a geographic surface. Therefore, each of the characters of the reduced set was contour mapped as outlined by Adams (1970), with the corresponding SNK test below each of the maps. Since no real statistical significance can be attached to the individual contour lines, the SNK test summary serves as a reference to the real significance of the differences between the population means depicted on the contour map.

Although one may easily correlate the contour maps with each other and/or other factors, the total trend of several characters taken together is not readily apparent. To facilitate this synthesis a method called Differential Systematics (Womble, 1951) was used. 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. Areas of high differential may then be correlated with geological discontinuities, changes in climatic variables, etc. It is across these zones that one would expect to find incipient speciation processes (at least those most clearly defined) as well as varietal and ecotypic

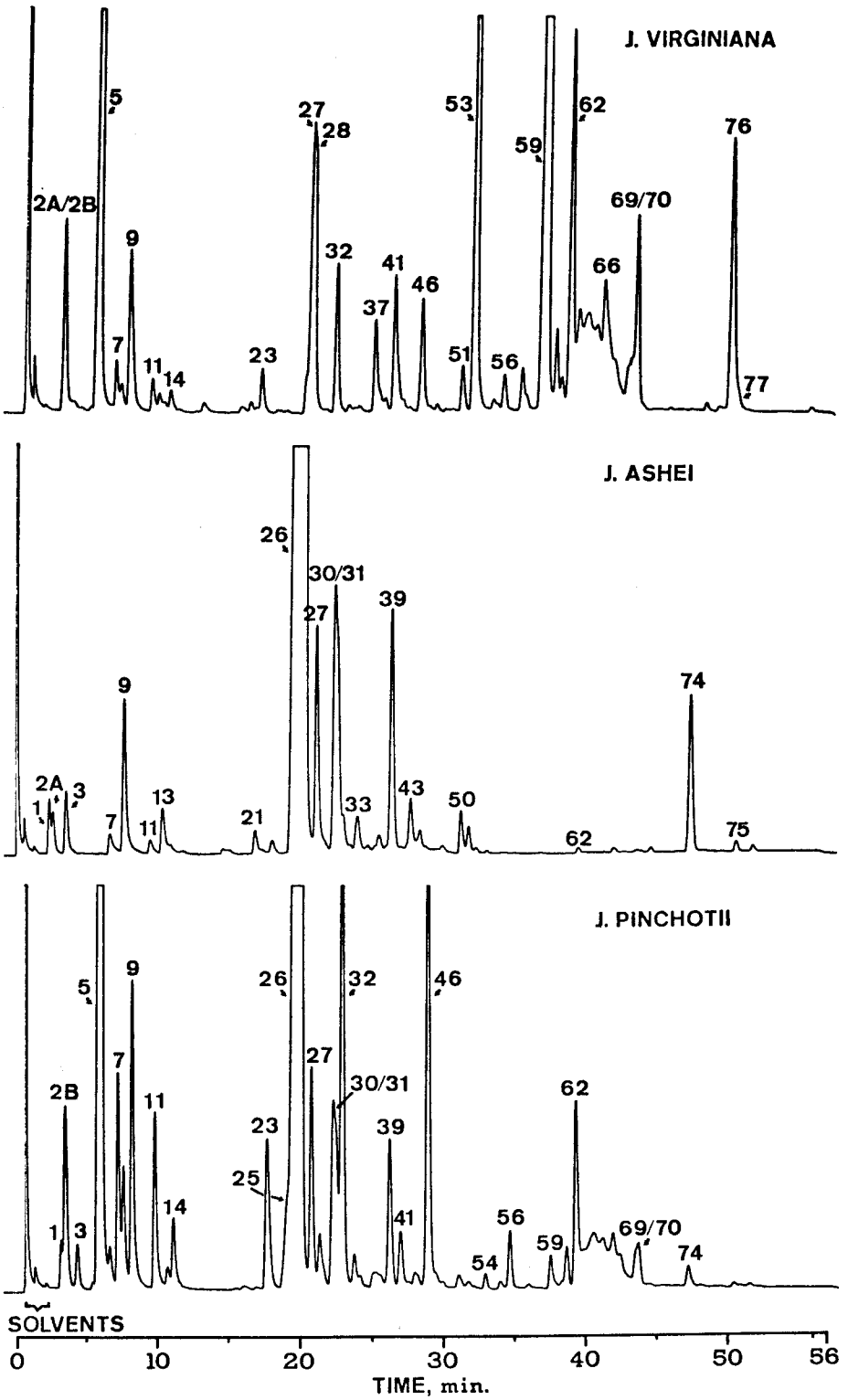

Figure 2. The gas/liquid chromatograms of the volatile terpenoids of *J. ashei*, *J. pinchotii*, and *J. virginiana*. See table 2 for the identity of individual components.

differentiation. A computerized approach to Differential Systematics as well as a more detailed discussion is given by Adams (1970).

The final method considered in this populational analysis of *J. ashei* is that of numerical taxonomy. Although numerical taxonomy is most often applied at the specific level and above, there is no reason why this approach can not be applied at the infraspecific population level. Certainly populations do differ from each other at the infraspecific level and it seems meaningful to devise methods for their analysis. Studies by Flake, von Rudloff, and Turner (1969) have recently shown numerical taxonomy to be of considerable value in determining the infraspecific relationship of natural populations in *Juniperus virginiana.*

The similarity measure used in the present study is basically a matching coefficient described by Sokal and Sneath (1963) with some similarity to the "condensation" method of Crovello (1968).

Let: x_i = value of character i in OTU (population) x.

y_i = value of character i in OTU (population) y.

Rd_{xy} = relative dissimilarity between OTU's x and y.

Sr_{xy} = relative similarity between OTU's x and y = $1 - Rd_{xy}$.

F_i = F test for character i (variance between pop./variance within pop.).

Rg_i = range of character i encountered in all population averages.

NC = number of legitimate comparisons between OTU's x and y (characters which were inapplicable were skipped as were negative matches).

Then:
$$Rd_{xy} = \frac{\sum_{i}^{NC} F_i \left| x_i - y_i \right| / Rg_i}{\sum_{i}^{NC} F_i}$$

$$Sr_{xy} = 1 - Rd_{xy}; \quad 0 < Sr_{xy} < 1$$

The use of variance parameters for weighting characters has been proposed by Farris (1966) and Flake and Turner (1968). The effect of the use of correlated characters has been discussed by Rohlf (1967) and others, but apparently no clear consensus has been reached. A detailed examination of the effect of the use of correlated characters is beyond the scope of this paper but will be considered in a later paper.

The clustering method used is the so-called single linkage method of Sneath (1957). Although single linkage methods have been generally regarded unfavorable since 1963, recently, Jardine, *et al.* (1967), Jardine and Sibson (1968), and Jardine (1969) have shown that certain theoretical constraints can only be satisfied by single linkage clustering (in comparison with most of the currently accepted methods). Hall (1969) and others have repeatedly stated that much information is lost by single linkage clustering. Yet, is not most of the information lost when one tries to present the results in the form of a dendrogram or almost any of the currently accepted formats? The single linkage method is the only clustering method (known to the authors) which can assure that *an OTU of a cluster will be more similar to some OTU in that cluster than to some OTU outside the cluster.* None of the average linkage methods can guarantee that this condition will always be satisfied.

Results

Terpenoid data

Table 3 shows the results of ANOVA of the terpenoid datum for those compounds whose F tests indicated population differences that were highly probable (P = 0.01). To determine which of the population means were highly significantly different, SNK tests were applied at the 1% confidence level. No significant differences were detected by the SNK tests for any compounds other than those whose analysis of variance indicated that populational differences were present, although the converse was not true. That is, 3 of the 17 compounds which showed highly significant differences via the F tests failed to show highly significant differences by the SNK tests. This points out the robustness of the SNK tests. Compounds 2A (alpha pinene), 44 (unknown), and 62 (elemol) failed to show highly significant differences by the SNK tests and were removed from consideration in the subsequent contour mapping and differential systematics analysis.

Table 3. Analysis of variance (ANOVA) for 17 terpenoids which had highly significant (P = 0.01) F tests implying that populational differences are present. $F_{.01} = 2.61$ (df = 12/51).

Terpenoid		F test	Species Avg. (% of total oil)
2A	α-pinene	3.536	1.12
7	myrcene	14.327	.58
9	limonene	10.112	2.39
11	γ-terpinene	8.985	.25
13	ϱ-cymene	4.554	.56
14	terpinolene	5.032	.11
21	unknown	4.201	.21
31/31	bornyl acetate & camphene hydrate	5.497	6.43
37A	unknown	3.481	.12
43	carvone	14.254	.70
44	unknown	2.79	.29
50	(alcohol)	5.450	.44
51	(gernaiol)	5.878	.20
62	elemol	2.852	.11
66	(γ-eudesmol)	5.479	.11
69/70	(α-eudesmol, β-eudesmol &/or elemicin	5.140	.06
75	unknown	4.268	.15

Each of the 14 compounds was contour mapped as shown in figures 3–6 by computer as previously outlined. The dotted line indicates the relationship of the contour map with the distribution of *J. ashei* within the state of Texas. At the bottom of each contour map is a summary of the SNK test indicating those population means which are highly significantly different. These contour maps are designed

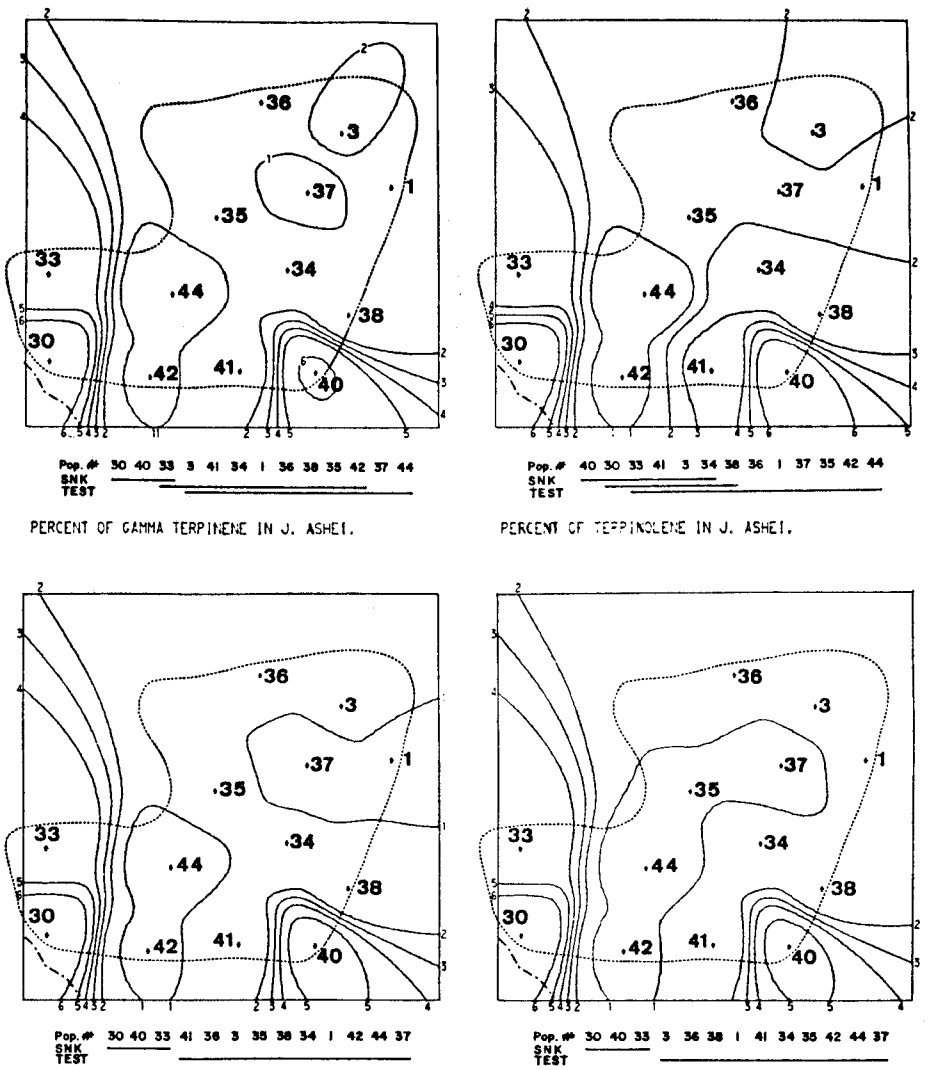

Figure 3. Contour maps of 4 terpenoids found in *J. ashei*.
Upper left: gamma terpinene (cpd. 11), contour range = .19–.41%.
Upper right: terpinolene (cpd. 14), contour range = .07–.19%.
Lower left: myrcene (cpd. 7), contour range = .40–1.28%.
Lower right: limonene (cpd. 9), contour range = 1.91–4.03%.
The dotted line indicates the Texas distribution of *J. ashei* (cf. fig. 1 and text).

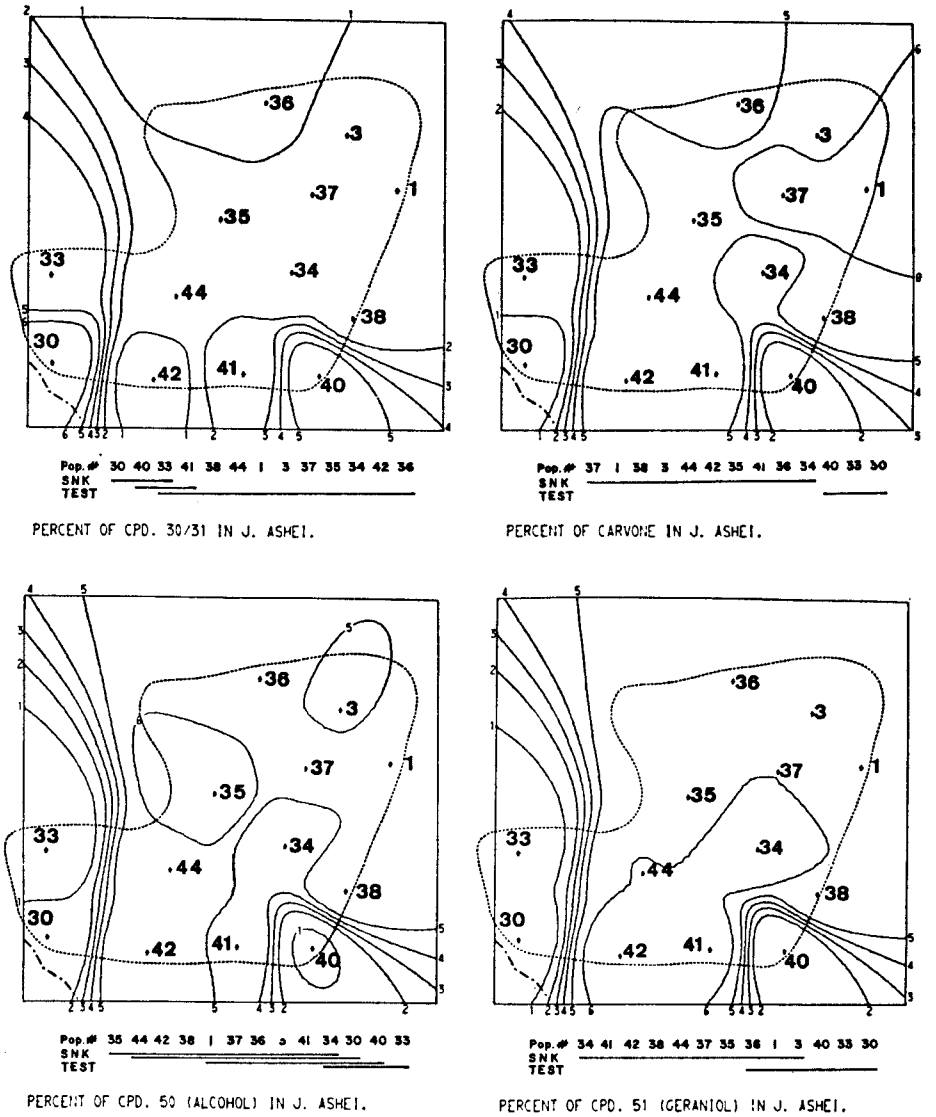

Figure 4. Contour maps of 4 terpenoids found in *J. ashei.*
Upper left: Cpd. 30/31, contour range = 4.72–11.60%.
Upper right: carvone (cpd. 43), contour range = .09–.93%.
Lower left: Cpd. 50, contour range = .20–.55%.
Lower right: Cpd. 51, contour range = .06–.26%.

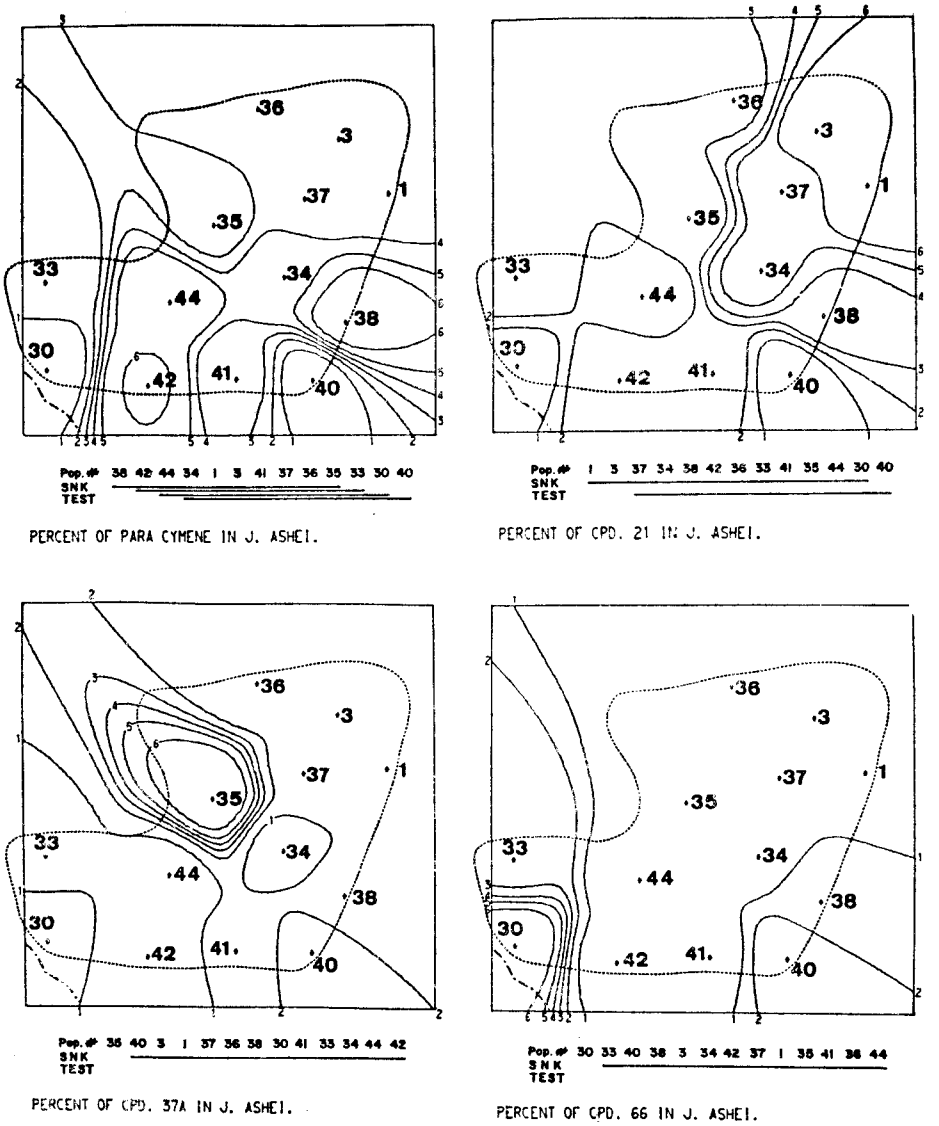

Figure 5. Contour maps of 4 terpenoids found in *J. ashei.*
Upper left: para cymene (cpd. 13), contour range = .35–.78%.
Upper right: Cpd. 21, contour range = .07–.37%.
Lower left: Cpd. 37A, contour range = .07–.42%.
Lower right: Cpd. 66, contour range = .08–.40%.

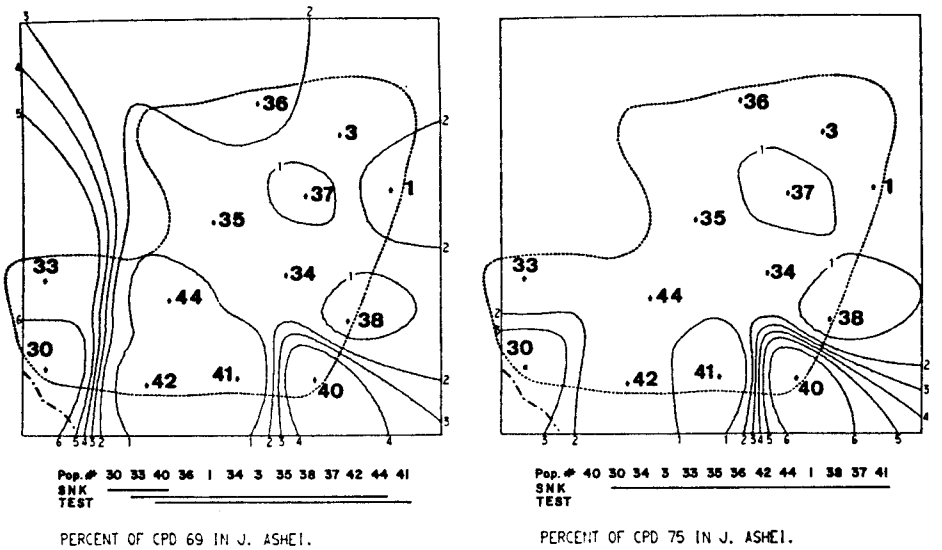

Figure 6. Contour maps of 2 terpenoids found in *J. ashei.*
Left: Cpd. 69, contour range = .02–.15%.
Right: Cpd. 75, contour range = .09–.45%.

to show, graphically, major trends in the population means of the characters investigated. For example, in the topographic surface of gamma terpinene (cpd. 11) one should pay scant attention to the differences indicated between populations 38, 35, 34, 37, etc., since none of these differences is significant. It should be merely noted that all of the populations (except 40, 30, and 33) are fairly uniform in the concentration of gamma terpinene, whereas populations 40 and 30 have a high concentration, with population 33 being somewhat intermediate in its concentration. It might be concluded that populations 40 and 30 are in a situation which seems to be favoring the increased production of gamma terpinene. Population 33 appears to be in a transition zone with respect to this compound.

Examination of figure 3 reveals that each of the four compounds shows basically the same surface. Several different patterns are revealed in the 14 contour maps of figures 3 through 6. Although some of the compounds appear to be varying together or in the same direction, it is not completely clear where most of the differentiation is occurring.

The composite differential of these 14 compounds was taken and is shown in figure 7. It is obvious that rapid changes are occurring between population 40 and populations 38, 34, and 41. Population 40 appears to be quite different from those populations immediately to the north and west. Likewise, population 30 (and to a lesser extent 33) is somewhat different from the populations immediately to the east and northeast. A region of less rapid change is also present between populations 35 and 34. One should bear in mind that, even when the contours are set at regular intervals between two populations, this does not imply that the zone of differentiation is at the midpoint (or mountain top) of the contours between the populations. For example, the differentials indicate a ridge of high differentiation between populations 40 and 38 (New Braunfels and Austin, Texas respectively). This does not necessarily mean that a new population sample taken halfway between these sites would be

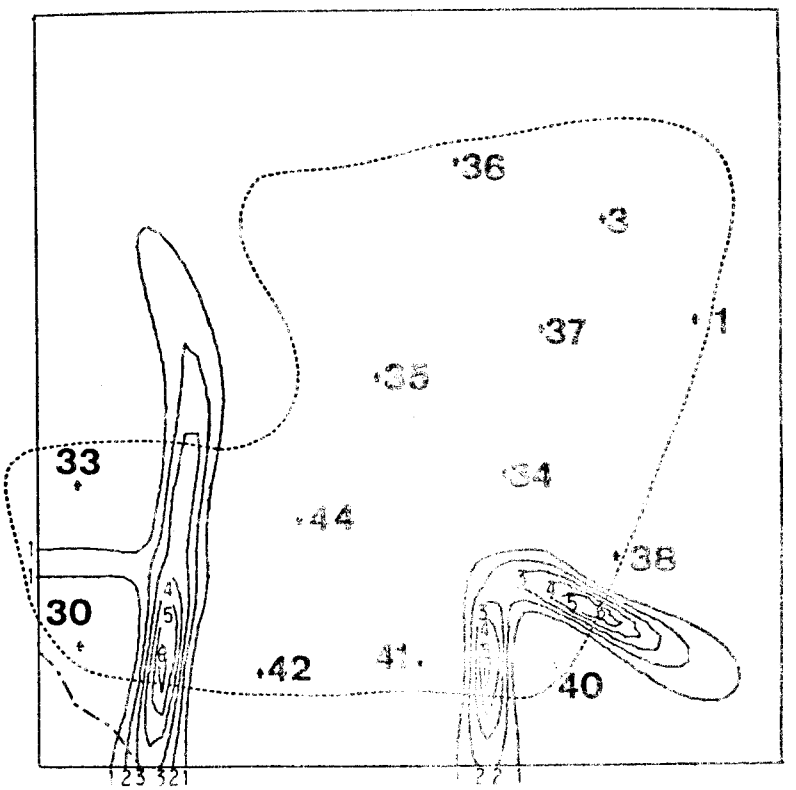

Figure 7. The composite differential of 34 terpenoids of *J. ashei*. The contour values are the average F weighted absolute differential of each of the 34 terpenoids. Contour symbols and values are: 1 = .081; 2 = .136; 3 = .190; 4 = .244; 5 = .298; 6 = .353.

intermediate. Indeed, it might be exactly like the Austin (or New Braunfels) population. That is to say, if a discontinuity exists between Austin and New Braunfels, we have no information to predict whether it is near Austin or New Braunfels. One can only say that a change occurs somewhere between these populations. The simplest assumption is that it is uniformly changing between the two (or any two) populations. At least, that is the assumption made in this study.

Perhaps the most interesting questions at this point are: 1. Which populations are the most similar? and 2. Are populations 43, 30, and 33 mutually similar (i.e., diverging as a group) or diverging from each other as well as from the other populations?

To answer these questions, similarity measures were calculated, as previously described, using 39 terpenoids which were larger than trace amounts in some population(s) of *J. ashei* and had F tests greater than 1.0.

Clustering was performed using the single linkage method. Figure 8 shows, graphically, the results of this clustering. Notice that populations 1 and 3, in the northeast extremity of the Texas distribution, show the strongest affinity. There is probably little significance to the order of entrance of the first 9 populations. The gap between population 35 and the preceding populations is larger than expected on the basis of the contour maps and composite differential, but one must remember

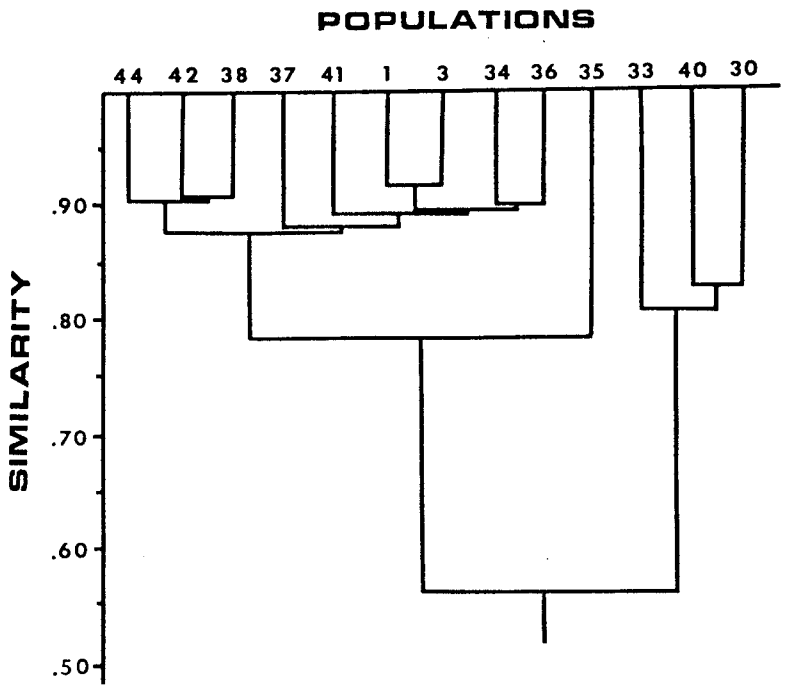

Figure 8. Dendrographic presentation of single linkage clustering of 13 populations of *J. ashei* using 39 terpenoid characters, F weighted.

that only 14 compounds with highly significant F tests were used in the composite differential. Thus, several of the remaining 25 terpenoids must have revealed this divergence. The co-divergence of populations 33, 40, and 30 from the rest of the populations of *J. ashei* is quite evident.

Thus, on the basis of the terpenoid datum, *J. ashei* appears to be fairly uniform across its distribution in Texas, with the exception of the peripheral populations 35, 33, 30, and 40.

Morphological data

Analysis of variance was performed on the 19 morphological characters which were considered "reliable." Six characters had highly significant F tests and are shown in table 4. In order to find which population means were different, the SNK tests were run on all 19 morphological characters. Only those 6 which had highly significant F tests in the ANOVA had highly significant SNK tests.

Each of the 6 highly significant morphological characters was contour mapped by computer. Figures 9 and 10 show the contour maps for each of these characters. In general, populations 40, 30, and 33 are characterized by: a larger ratio of whip leaf gland/sheath length, whip leaf glands which are more elongate, more seeds per cone, smaller female cones and smaller seeds. The 4 morphological traits contoured in figure 9 confirm the trends shown by the terpenoids. It is interesting to note that population 33 (Ozona, Texas) was shown (figure 10) to be the most divergent with respect to the number of seeds/cone.

Growth habit shows the most irregular trend as might be expected from the varied

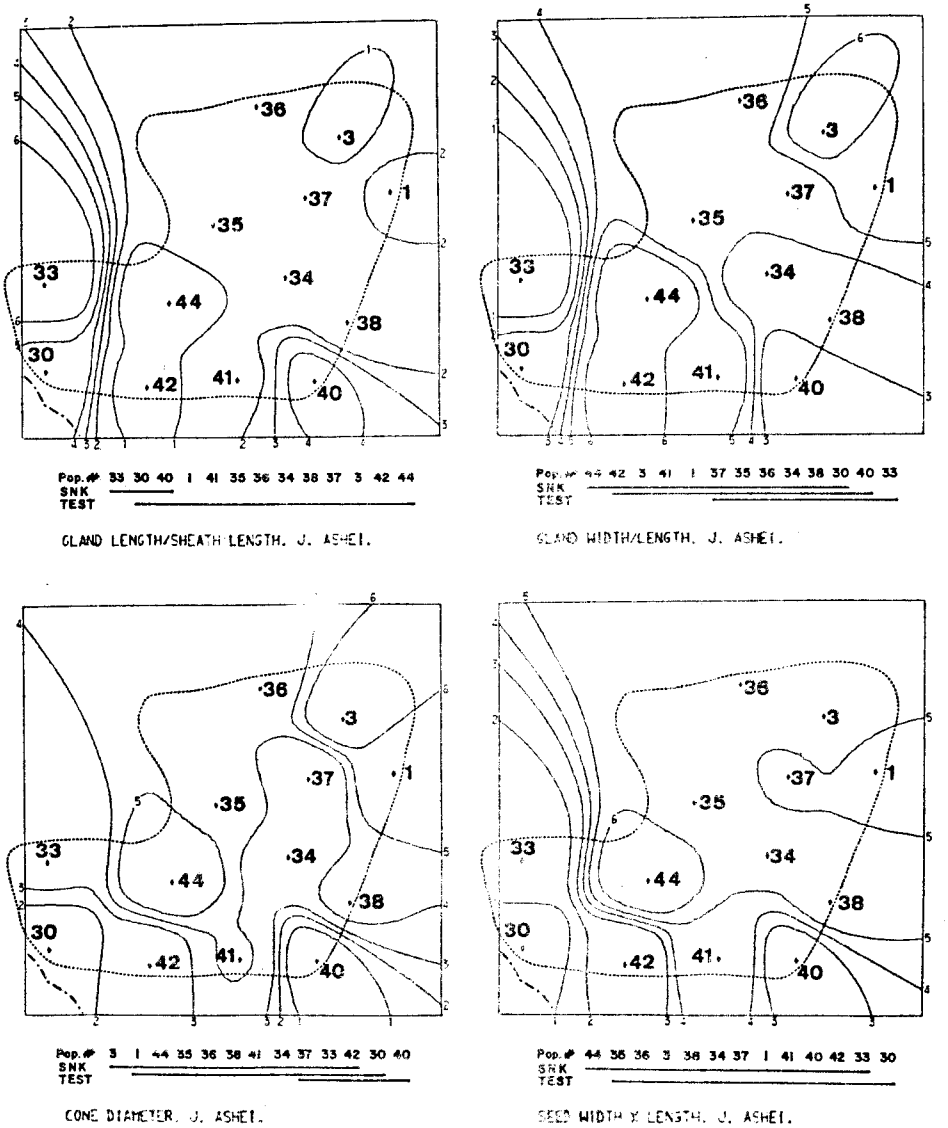

Figure 9. Contour maps of 4 morphological characters of *J. ashei*.
Upper left: gland length/sheath length, contour range = .28–.53.
Upper right: gland width/length, contour range = .33–.75.
Lower left: female cone diameter, contour range = 5.35–7.14.
Lower right: Seed width x length, contour range = 10.2–19.0.

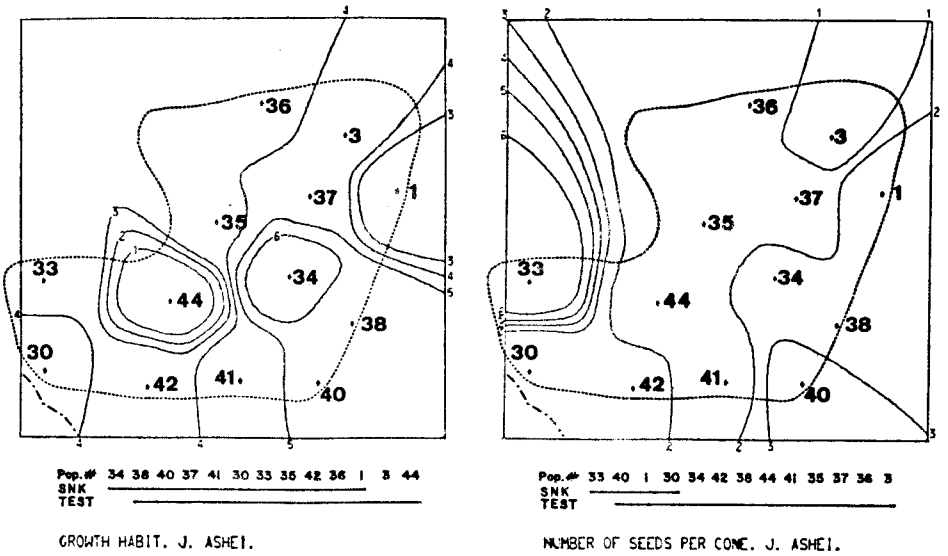

Figure 10. Contour maps of 2 morphological characters of *J. ashei.*
Left: growth habit, contour range = 2.11–3.28.
Right: number of seeds per cone, contour range = .96–1.61.

habitats of the different populations. This character was not used in the computation of the composite differential since growth habit appeared to vary in the field according to the ecological site occupied (e.g., in shady sites plants had a better developed central axis; in sunny sites plants were more shrubby; etc.).

The composite differential of 5 of the morphological characters (excluding growth habit) is shown in figure 11. The differential of these characters is very similar to the differential of the 14 terpenoids (Figure 7). One exception appears to be a zone of rapid change around population 3 (Bosque Co.) and population 1 (McLennan Co.). Also, population 33 appears to be slightly more divergent than population 30, whereas the converse seemed true with respect to their terpenoids. Small differences between populations 41, 42, 44, 34, and 35 are also apparent.

TABLE 4. Analysis of variance (ANOVA) for 6 morphological characters which had highly significant F tests. $F_{.01.} = 2.61$ (df = 12/51).

Character	F test
M1, growth habit	3.50
M3, whip leaf gland length/sheath length	4.60
M5, whip leaf gland width/length	5.27
M16, number of seeds per cone	3.05
M17, female cone diameter	6.00
M19, seed width x length	4.68

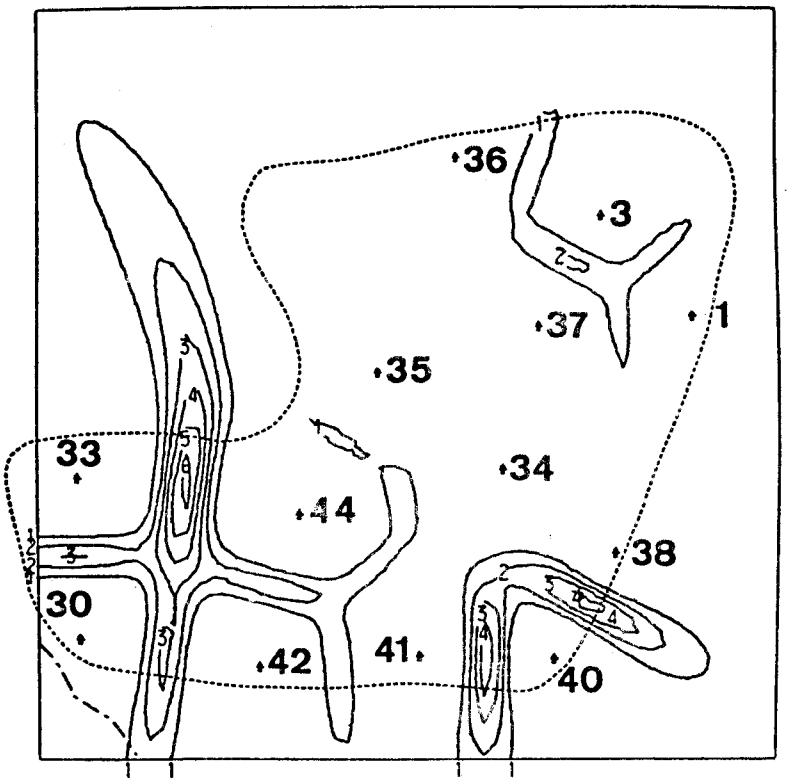

Figure 11. The composite differential of 5 morphological characters of *J. ashei*. The contour values are the average F weighted absolute differential of each of the 5 morphological characters. Contour symbols and values are: 1 = .067; 2 = .112; 3 = .157; 4 = .201; 5 = .246; 6 = .291.

Before computing similarity measures based on the morphological characters, 8 characters with F tests smaller than 1.0 were discarded. The remaining 11 morphological characters and F tests were: growth habit, 3.5; ratio of whip leaf gland length/sheath length, 4.6; length of whip leaves, 1.9; ratio of whip leaf gland width/length, 5.3; glands raised, 1.5; seed color, 1.1; hilum scar on seed, 2.6; grooves in seed, 1.25; number of seeds/cone, 3.0; female cone diameter, 6.0; seed width x length, 4.7. Growth habit was included in the computation of the similarity measures but, as above, there are good reasons for not including it.

Similarity measures of the 13 populations of *J. ashei* were computed utilizing the aforementioned 11 morphological characters using the F tests as character weights. It is interesting to note that the average similarity ratio, using the morphological datum, is almost identical with the average similarity for the terpenoid datum (0.6697 vs. 0.6677).

Populations 30 and 40 were more similar to each other than to any other population and population 33 was more similar to population 30 and then to 40. This is slightly different from the similarities based upon the terpenoid characters.

Illustration of such similarities is shown in a dendrogram (Fig. 12). In comparison with the dendrogram based on terpenoid datum, the morphological datum indicates

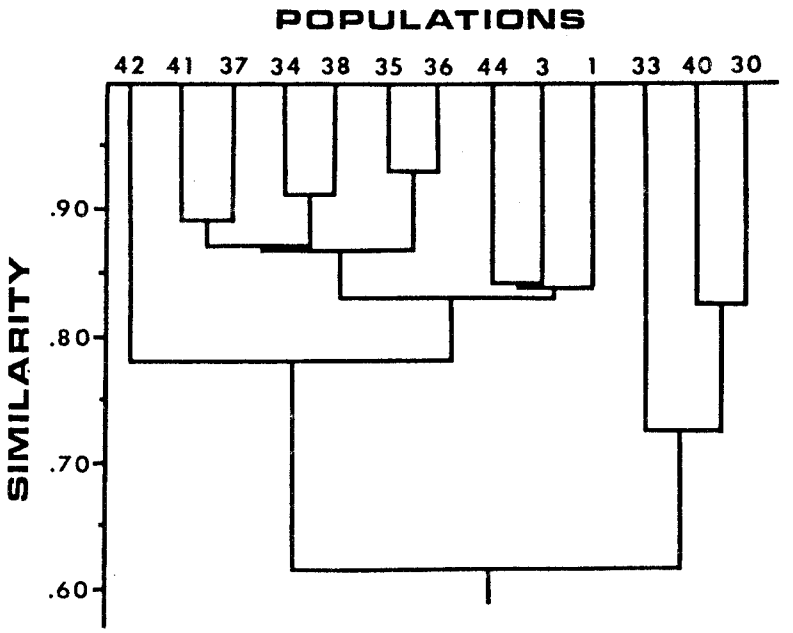

Figure 12. Dendrogram of 13 populations of *J. ashei* using F weighted, single linkage clustering involving 11 morphological characters.

much more variation among populations of *J. ashei.* Population 42 is now slightly removed from most of the populations and population 35 appears to form the center of a loosely connected cluster involving most of the populations. Nevertheless, the divergence of populations 33, 40, and 30 is still quite conspicuous, just as with the terpenoid datum. Undoubtedly the inclusion of growth habit and the subjective nature of some of the scoring of the other morphological data have contributed to this increased variation.

Composite Analysis

In order to evaluate the total trend of divergence in populations of *J. ashei,* similarity measures were computed using the aforementioned 39 terpenoid and 11 morphological characters. Weighting was by use of the F tests. Dendrographic representation is shown in figure 13. As expected, this dendrogram is somewhat intermediate between the one using only chemical data and the one using only morphological data. Nevertheless, some reinforcement is evident in the delimitation of the cluster consisting of populations 1 and 3 at the northeastern extremity of the distribution in Texas. The populations may now be divided into roughly four sets: The northeast extremity of the Texas range (1 and 3); the central region of the Edwards plateau (37, 44, 42, 41, 34, 36, and 38); population 35; and the divergent peripheral populations (33, 30, and 40).

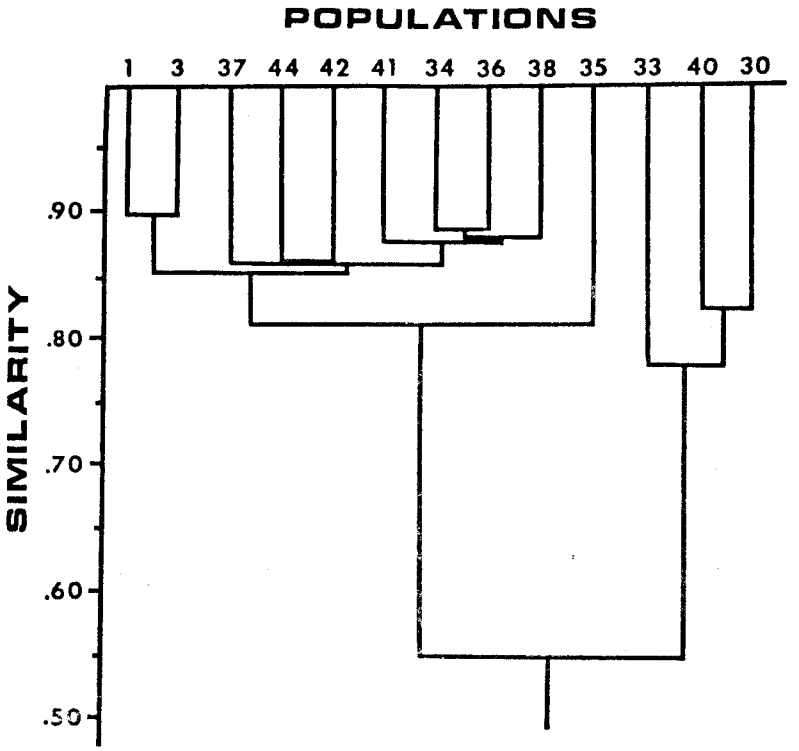

Figure 13. Dendrogram of 13 populations of *J. ashei* using F weighted, single linkage clustering involving 39 terpenoid and 11 morphological characters.

Discussion

Hall (1952) reported hybridization between *J. virginiana* and *J. ashei* in Travis Co., Texas (near populations 38 and 39). He also reported introgression of *J. virginiana* genes into *J. ashei* as far southwest as Bexar Co. In addition, Hall, McCormick, and Fogg (1962) reported widespread hybridization and introgression between *J. ashei* and *J. pinchotii* along the Devils River (near populations 30, 33) and, indeed, throughout most of West Texas. Hybridization on this scale ought to be relatively easy to detect using the terpenoids of these 3 taxa, since their terpenoids are so distinct (Fig. 2). Yet, as previously mentioned, von Rudloff, Irving, and Turner (1968; unpubl.) found no evidence of hybridization whatsoever between *J. virginiana* and *J. ashei*, even when sampling some of the same populations examined by Hall (1952). The evidence compiled in this study suggests that the variation observed in *J. ashei* can not be explained on the basis of hybridization with or introgression from either *J. virginiana* or *J. pinchotii*. In this connection it should be reemphasized that the sample selections were intentionally biased in order to find possible hybrids; yet none was found! Could such widespread hybridization have been missed even when the study was directed towards its detection?

The acceptance of hybridization and/or introgression as a significant factor in the observed variation of *J. ashei* are mitigated against for the following reasons:

1. Populations 40 (Comal Co.) and 30 (Val Verde Co.) have diverged from the

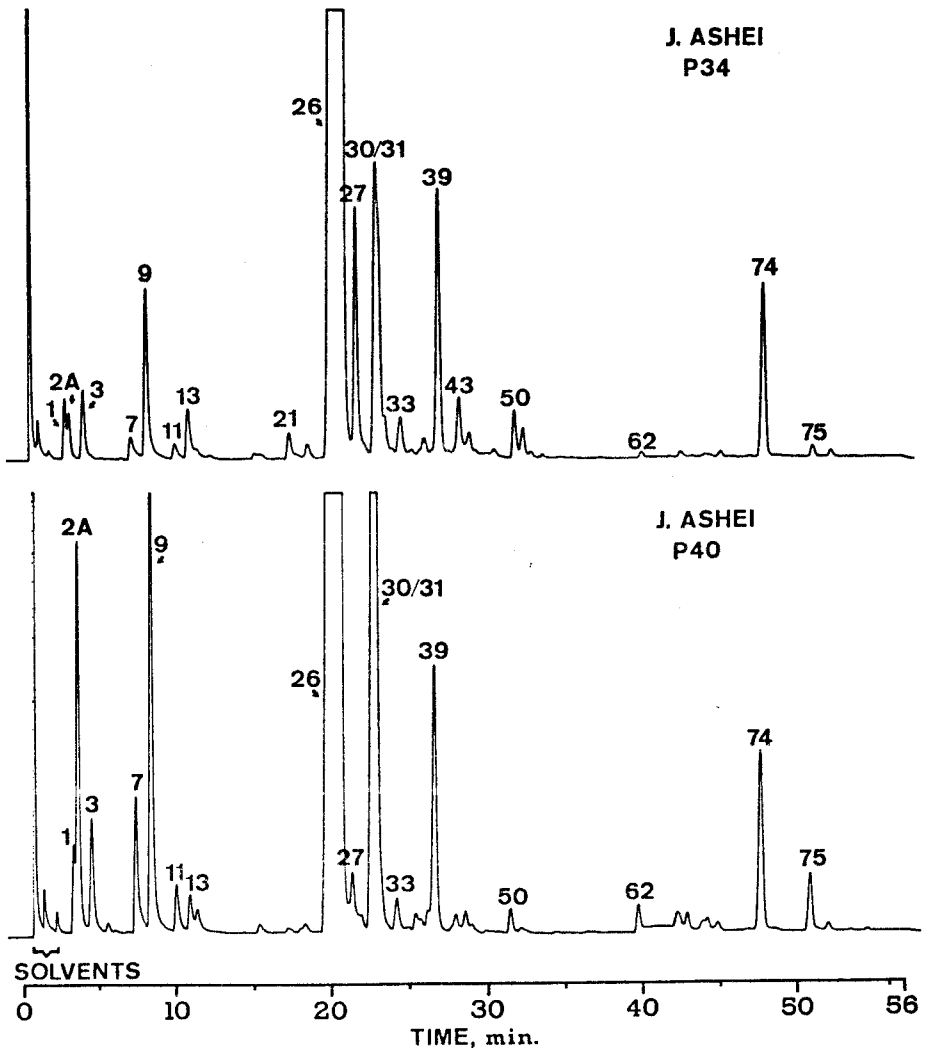

Figure 14. Gas/liquid chromatograms of the terpenoids of population 34 (typical of central Texas) and population 40 (a peripheral divergent population of *J. ashei*).

other populations of *J. ashei* more than they have diverged from each other; yet, *J. virginiana* occurs in the same general region at population 40, while *J. pinchotii* occurs together with *J. ashei* in population 30. If hybridization between very different species is the reason for the variation of populations 40 and 30, one would expect yet further divergence one from the other rather than convergence, as suggested by numerical treatment of the data.

2. If genes of another taxon are being introduced into *J. ashei* at these two sites, why are no compounds found in *J. ashei* which are characteristic of the terpenoids found in *J. virginiana* or *J. pinchotii*? Hanover (1966) has shown in *Pinus* spp. that the production of terpenes is under genetic control, being, in general, additively

inherited in the F_1. Furthermore, hybridization and/or introgression can be detected by the use of terpenoid data as is suggested by several studies (Irving, 1968) including those of the senior author working with *J. pinchotii* and *J. monosperma* (Adams, 1969a).

In short, the more probable alternative is that hybridization of *J. ashei* by *J. virginiana* or *J. pinchotii* is either nonexistent or very infrequent.

The divergence of populations 40, 30, and 33 seems more readily explained by one of the following:

1. Predominately southerly winds during the pollination period combined with northward migration of birds (which disseminate seeds of this species in early spring-personal observation) may have given rise to differentiation in this region.

2. Some common selective factor may be operating in these populations.

3. The small "non-random" or selected samples, may have led to these unusual results.

4. The populations may be relics. The presence of the more elongate glands, as in the 3 divergent populations, is very common in *Juniperus* in North America. This might indicate that populations 40, 33, and 30 represent some of the more primitive or relic populations of *J. ashei* and that the other populations have diverged from similar populations originally confined to that region.

Generally speaking, *J. ashei* is a fairly uniform species. While populations 40, 33 and 30 are somewhat different in a few characters, overall, the amount of divergence is still quite small. Figure 14 shows the gas chromatograms of *J. ashei* (population 34) and *J. ashei* (population 40). Although these few populations represent the extremes found in *J. ashei*, they are still very similar.

Considerable additional study will be necessary before many of the populational problems raised here are resolved. It should appear clear, however, that the approaches employed are unusually suited for the resolution of such problems, both at the infra- and supraspecific levels.

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