

Gene Flow Versus Selection Pressure and Ancestral Differentiation in the Composition of Species: Analysis of Populational Variation of *Juniperus ashei* Buch. Using Terpenoid Data

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Summary. Twenty-four populations of *Juniperus ashei* were sampled throughout the range of this taxon and the terpenoids of the foliage were analyzed by gas/liquid chromatography. Population differentiation was investigated by analysis of variance and numerical taxonomy. Three south Texas and one Mexican population clustered together with the rest of the taxon appearing fairly uniform. No evidence was found of hybridization or introgression with other taxa. Disjunct populations in Oklahoma and the Ozarks, which have been genetically isolated from the central population for thousands of years, showed no signs of differentiation nor genetic drift. The present pattern of distribution probably dates from the Pleistocene. The south Texas and Mexican populations appear to be the primitive elements of the species. Populational differences have apparently been maintained in adjacent populations in spite of seemingly large gene flow and conversely, chemical uniformity is being maintained in many disjunct populations where there is little or no gene flow.

Key words: *Juniperus ashei* - Cupressaceae - Gymnospermae - Terpenes - Population Differentiation - Pleistocene - Relict - Numerical Taxonomy

INTRODUCTION

The effects of species migrations on populational differentiation has always been of interest in the study of evolution, yet few investigations have been made in enough detail to elucidate the principles involved (Ehrlich & Raven, 1969). Questions concerning the amount of populational differentiation versus the amount of gene flow and the rate and directions of population differentiation in disjunct, relict populations are still unanswered. The purpose of this paper is to analyze the terpenoids of continuous and disjunct, relict

and recent, populations of *Juniperus ashei* Buch. so as to gain insight into these questions. This taxon has been analyzed extensively by Adams & Turner (1970) in central Texas, Hall (1952, 1953), and Hall & Carr (1962). The results reported by Adams & Turner differed considerably from the work of Hall. Neither introgression nor hybridization with other taxa was found by Adams & Turner (1970). The principal pattern of population differentiation in central Texas is shown in Fig.1, based on 39 terpenoid compounds, F weighted. The divergent populations are all on the edges of the distribution of *J. ashei* and show populational differentiation in spite of supposedly high gene flow in this dioecious, wind-pollinated taxon. Analysis based on 11 morphological characters showed a similar pattern of populational differentiation. Hypotheses advanced to explain the variation were: 1. Gene flow has been directed by winds predominately from the south during the pollination period combined with north-south migration of birds (seed dispersers); 2. Selection factors may be common to the peripheral populations while different selection is operating on the central populations; 3. The small "non-random" or selected samples (5 trees per population) may have led to these unusual results; 4. The divergent populations may be relicts.

Since the northern, disjunct populations in Oklahoma and Arkansas (see Fig.2) were not sampled in the initial study and only 5 trees per population were sampled, additional field work was undertaken to overcome these shortcomings. This paper reports on the results of sampling 15 trees per population location as shown in Fig.2. The morphological characters are still being scored and will be reported on later. The literature has been adequately reviewed by Adams & Turner (1970).

MATERIALS AND METHODS

Fig.2. shows the distribution of *J.ashei* and the populations where 15 trees were sampled in December, 1970 with the exception of the population in Mexico where only one tree was found and sampled. The sampling methods are given in Adams & Turner (1970). Voucher specimens are filed at Colorado State University. All samples were placed in a random sequence for distillation and analyses by assigning random numbers (3 digits) from a random number table and then ranking the numbers from low to high as suggested by Adams (1972). These procedures convert the temperal changes in foliage, oils, columns, etc., to random variables. Therefore population differentia-

tion patterns can be easily separated from experimental errors in the statistical analyses phase. The volatile terpenoids were steam distilled for two hours as outlined by Adams (1970) and the extracts were kept at -20 C until analyzed by gas/liquid chromatography.

The individual compounds were separated on a Varian 1840 gas/liquid chromatograph with FID, using a 200 foot x .02 inch capillary wall coated open tubular column (WCOT), 8% PEG 20M. Carrier gas flow, N₂ = 8.6 ml/min (25 cm/sec), make-up gas, N₂ = 15 ml/min, H₂ = 25 ml/min, air = 250 ml/min. One microliter samples were injected and split 1/20. The chromatograph was temperature programmed as follows: isothermal

80 C for 8.2 min. 3 C/min linear increase for 41 min, 2 C/min linear increase for 4.1 min then isothermal at 219.4 C for 20.5 min.

The identities of the terpenoids of *J.ashei* are given in von Rudloff (1968) and Adams & Turner (1970). The individual peaks were quantified with an Infotronics digital integrator with automatic punched output to an IBM O26 keypunch.

One hundred and fifty-two compounds were subjected to analysis of variance (ANOVA) to determine which characters showed significant differences among the populations. Forty-one compounds had highly significant F ratios (F = variance among populations/variance within populations). Fifty-four compounds had F ratios greater than 1.0 and thus were used to compute F - 1 weighted mean character difference (MCD) similarity measures as formulated in Adams (1975) which is very similar to the F weighting used in the original study (1970). Clustering was by single linkage (nearest neighbor) as previously used.

RESULTS

A contoured similarity map of the populations (Fig.3) reveals that the disjunct populations of *J.ashei* in Oklahoma and Arkansas are not different from the large central Texas population. The basic pattern of differentiation in the central Texas region is the same as revealed in the previous study (cf.Fig.1), but notice that the tree sampled from northern Mexico (OTU 116) clusters well with the 3 divergent Texas populations. The uniformity of *J.ashei* is also shown in the phenogram (Fig.4). Tree 116 from Mexico clusters with the divergent populations (12, 13, 17), while the other populations are all very similar. There is probably no significance to the differences in the order of the entry of those OTUs in the phenogram between .92 and .88 similarity.

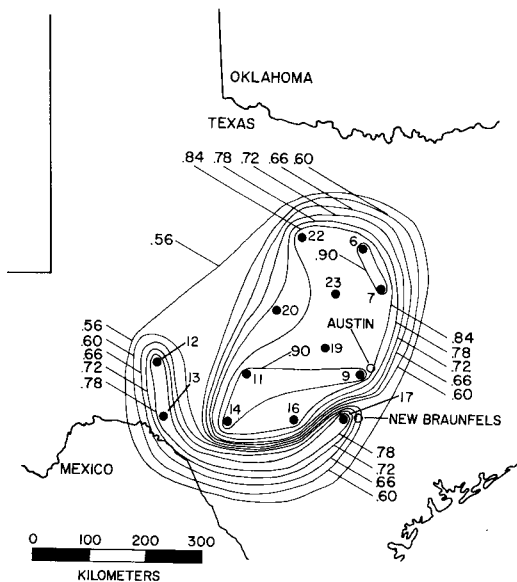


Fig.1. Contoured Similarity of *Juniperus Ashei* Based on 39 Terpenoid Characters, F-Weighted from Adams and Turner (1970). Notice the areas of populational differentiation at the lower right and left (southeast and southwest). These results were based on samples of 5 trees per population

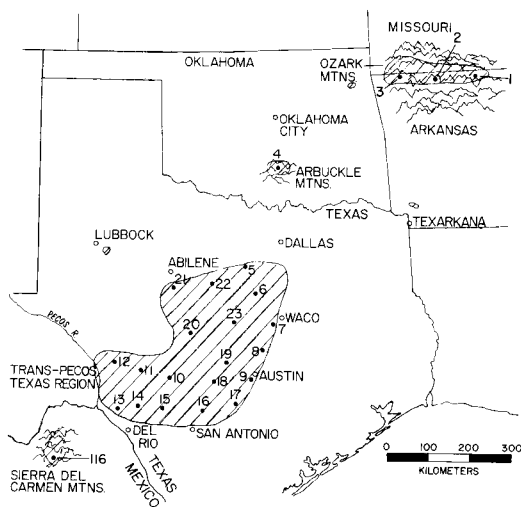


Fig.2. Distribution of *J. Ashei* and Populations Sampled for the Current Study. Most of the populations in central Texas are the same as sampled previously. The dashed line in Mexico indicates that the distribution is not precisely known

There is no evidence of populational differentiation, hybridization, or introgression of the disjunct populations in Oklahoma and Arkansas. Preliminary analyses of a few trees from the northeastern Oklahoma and southwestern Arkansas populations showed these populations to be very similar to the other Oklahoma and Arkansas populations.

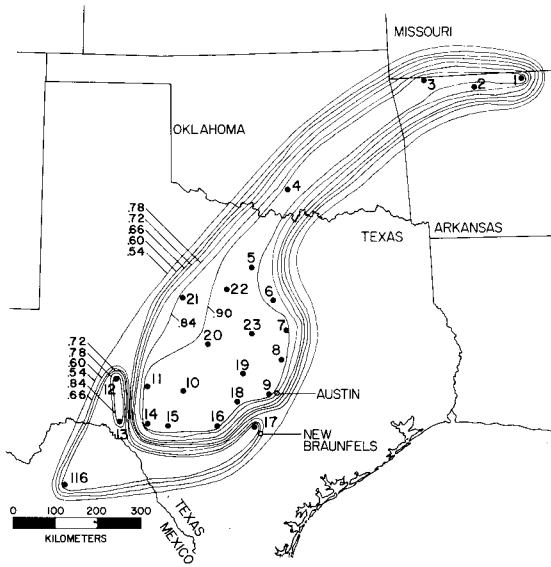


Fig.3. Contoured Similarity Based on 54 Terpenoid Characters, F-1 Weighted. The contour intervals show a pattern of uniformity in the central Texas, Oklahoma, and Arkansas populations. Notice that the same three populations previously shown as being different now cluster with the tree from Mexico

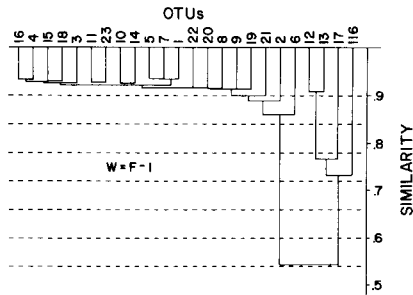


Fig.4. A Phenogram Based on the 54 Terpenoid Characters, F-1 Weighted and Clustered by Single Linkage. The dashed lines show the contour levels used in Fig.3. The relict populations are quite apparently different in this diagram

DISCUSSION

The pattern of population differentiation revealed in this study raises several interesting points about the evolutionary history of this taxon. Of the four hypotheses advanced in the previous study for the parallel divergence of the peripheral populations, two are fairly improbable. That some common selection factor may be operating in these populations (and the Mexican population) appears to be unlikely. In comparing the habitat around population 17 with the western populations 12 and 13 and the Mexican population (116), one is immediately impressed with the differences in vegetation

types. The eastern site has many elements of the eastern flora as well as the central Texas flora while the western sites are dominated by the Chihuahuan desert flora elements. The principal difference in climate seems to be in moisture with the eastern sites receiving about 81 cm of rain annually while the western sites receive 35 to 46 cm (Arbingast et al. 1967). This leads to a higher evaporation rate in the western sites and less relative humidity (37% vs. 50%, annual, 6 pm). January and July temperature are very similar, although it is a little warmer in the western sites. Changes in the vegetation going west from population 17 (New Braunfels) are apparent in a matter of a few kilometers as the climate gets drier as one goes west. Although most visible indicators point to considerable differences between the habitats of the eastern population (17) and the western and Mexico sites, there could be some cryptic common selection pressure.

Small "non-random" sampling errors were certainly possible when only 5 trees were used (Adams & Turner, 1970) but this study using 15 samples per population reconfirms the same pattern in those populations which were resampled. Flake et al. (1969, 1973) found similar results with *J. virginiana* in successive sampling. In addition, the samples in the current study were analyzed in random order to eliminate procedural errors. Therefore it seems very unlikely that the results are due to any "non-random" errors.

Although the predominately southerly winds and bird migrations may be important in maintaining these unique gene combinations, the most likely explanation for the pattern of differentiation is that the populations are ancestral relicts. The presence of more elongate glands in the divergent populations is likely more primitive than the round glands which are characteristic of *J. ashei* throughout the rest of its range. So far as known, *J. ashei* is the only species in the genus with round glands. The plants from the divergent populations have a stronger central axis and more slender foliage than is typical of *J. ashei*.

The occurrence of the Mexican population is not unexpected when one considers the migrations of species before, during and after the Pleistocene. The influence of Pleistocene climatic fluctuations on the expansions and contractions of species ranges is well documented. These effects have been amplified recently by the work on rat middens and palynology in the southwestern United States (King, 1973; Mehringer et al. 1970; Van Devender & King, 1971; Wells, 1965, 1966, 1970; Wells & Berger, 1967; Wright, 1970; Whitehead, 1972). The floristic affinities between the Edwards plateau of central Texas and the Sierra Madre of Nuevo Leon and Coahuila has

existed since the Tertiary (Axelrod, 1958). King (1973) has suggested that "The western Missouri Ozarks (*and presumably northern Arkansas*) are covered by open pine-parkland from at least 40,000 BP until the start of the last full glacial 20,000-25,000 BP, then with boreal spruce forest until at least 13,500 BP". These dates correlate well with paleotemperatures computed from ratios of O^{18}/O^{16} of deep-sea cores (Emiliani, 1971). Bryant (1969) thought the full-glacial vegetation of central Texas to be pine-dominated woodland and Hafsten (1961) views the Llano Estacado (northwestern Texas) as a boreal woodland of spruce and pine. Wells (1966) indicated that the life zones in the Chihuahuan desert (Chisos Mountains area) were probably compressed during the full Wisconsin but the more montane, mesophilic elements probably descended only about 400 meters with the woodland zone descending about 800 meters. *Juniperus pinchotii* was apparently widespread with pinyon in what is now desert (Wells, 1966). *Juniperus pinchotii* is currently found in a more mesic region (western Edwards plateau) and at about 1500 meters in the trans-Pecos Texas region. If central Texas was a pine woodland which intergraded into boreal pine-spruce to the northwest (Llano Estacado) and intergraded into the boreal spruce forest in the Ozarks, where was *J. ashei*? Recent evidence (Adams, 1975) shows that *J. pinchotii* from just south of Sierra del Carmen (from the same site as *J. ashei* tree 116) is very similar to populations several hundred kilometers north in northcentral to west Texas.

It seems likely that either 1. *J. ashei* retreated from the Ozarks, Oklahoma, and central Texas into Mexico during the Pleistocene, 2. small pockets of trees survived the Pleistocene on rocky, southfacing slopes, or 3. *J. ashei* never occurred in central Texas, Oklahoma or the Ozarks until after the Wisconsin. If the divergent populations, 12, 13, and 17, are considered as relicts along with the Mexican refugia population, then the central Texas, Oklahoma, and Ozark populations might have developed rapidly from the south during a drier, warmer period (Hypsithermal?) following the last full glaciation which Emiliani (1971) has reduced to 10,000 BP! This would explain the lack of populational differentiation in the populations in Oklahoma and the Ozarks.

If small pickets of trees did survive the full glacial in Oklahoma and the Ozarks, we should find some evidence of genetic drift or insular effects in the disjunct populations. It seems improbable that a fully representative sample of the preglacial (or Tertiary?) population would have survived in each population. In order to obtain answers to these kinds of questions which involve estimates of genetic variation, we

are currently analyzing several iso-enzyme systems to investigate genetic drift, long distance dispersal and populational differentiation in *J. ashei*.

In any case, it is clear from the data presented that populations can be quite uniform in spite of being genetically isolated over hundreds of kilometers and on the other hand, populations which are adjacent (a few kilometers) still maintain themselves distinct according to ancestral make-up or accumulated gene differences. These observations support the hypothesis of Ehrlich & Raven (1969) that selection pressure and past evolutionary history are more important than gene-flow in maintaining species (or populations). This conclusion is really borne out in the New Braunfels population. A tree examined only 15 km west of population 17, was chemically and morphologically of the central Texas type. One would expect that in this obligatory outbreeding species that this small distance would present no barrier to gene flow. Yet it appears that, although the ancestral population (at New Braunfels) is practically surrounded by the divergent populations, the ancestral genetic make-up accumulated at least 10,000 BP., still persists!

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