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ORIGIN OF THE TEXAS GULF COAST ISLAND POPULATIONS OF *AMBROSIA PSILOSTACHYA*: A NUMERICAL STUDY USING TERPENOID DATA

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Abstract—The volatile terpene and sesquiterpene lactone patterns for 20 populations of *Ambrosia psilostachya* from the Texas Mainland and Gulf Coast Islands and 7 populations of *A. cumanensis* from near Vera Cruz, Mexico, were determined and the resulting volatile terpene data were analysed by numerical classification methods. The terpene data indicated that the Texas Gulf Coast islands populations of *A. psilostachya* are genetically closer to the Vera Cruz, Mexico populations of *A. cumanensis* than they are to the Texas mainland populations of *A. psilostachya.*

INTRODUCTION

Ambrosia psilostachya DC. (Compositae), a ragweed which is distributed from Canada to central Mexico typically displays an extremely variable morphology. However, the populations of this species which occur on a series of barrier islands paralleling the Texas Gulf coast are all morphologically similar, suggesting that they were derived from a single, localized source. The islands populations characteristically possess a thick, rather scabrous leaf and display similar habits; whereas the mainland populations have generally less-thick non-scabrous leaves with considerable intra- and inter-populational variation. We previously observed[1,2] that the Texas islands populations of *A. psilostachya* also differed in their sesquiterpene lactone chemistry from the mainland populations. The island populations produced a series of three dilactone-pseudoguaianolides: psilostachyin A, B, and C; whereas the latter were characterized by two or more of seven known monolactone pseudoguaianolides (see Fig. 1 and Table 1). The island chain is known to have been formed *de novo* within the last 5000 years as a result of post-Pleistocene sea-level changes.[3,4] The islands were initiated as submerged bars at points where the rivers emptied into the Gulf of Mexico and increased in size by long-shore drift and beach accretion. Under the influence of the prevailing winds, sediment-carrying water currents flow in a southwesterly direction along the upper Texas coast;[5] thus the northern islands probably attained large sizes as much as 2000 years earlier than the others.[3,4]

We previously suggested[2] that the islands were colonized by *A. psilostachya* from the south because of the unlikely chance of establishment of seeds on the northern islands from the adjacent mainland; this mainland is a black-waxy coastal prairie covered at climax

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[1] H. E. MILLER, Ph.D. Thesis, University of Texas at Austin, Austin (1967).

[2] H. E. MILLER, T. J. MABRY, B. L. TURNER and W. W. PAYNE, *Am. J. Bot.* **55**, 316 (1968).

[3] E. A. LOHSE, In *Finding Ancient Shorelines*, special publication No. 3, *Soc. Econ. Paloen. and Miner.*, Tulsa, Okla. (1955).

[4] R. J. LEBLANC and W. D. HODGSEN, *Gulf. Coast Assoc. Geol. Soc. Trans.* **9**, 197 (1959).

[5] F. M. BULLARD, *Texas Geol. Soc. Amer. Bull.* **53**, 1021 (1942).

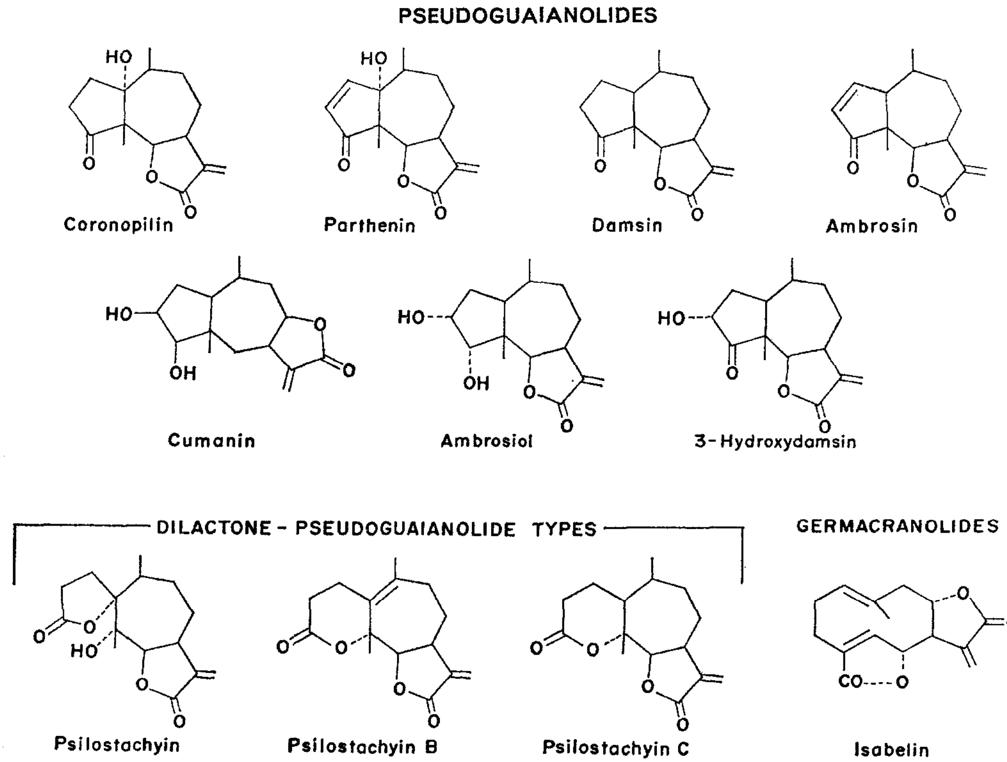

FIG. 1. SESQUITERPENE LACTONES WHICH HAVE BEEN IDENTIFIED FROM *A. psilostachya.*

by an *Andropogon* grassland which probably precluded the existence of the mostly weedy *A. psilostachya* 5000 years ago. In contrast, the south Texas coastal mainland contains an area of intruding sand dunes of Pleistocene origin;[6] these dunes could have supported ragweed populations 3000 to 5000 years ago when the islands were first open for colonization. On the basis of earlier sesquiterpene lactone studies, we proposed[2] (in 1968) that *A. psilostachya* plants in this mainland dunes area probably served as the gene pool from which the islands were colonized. Indeed, in these earlier studies one population of *A. psilostachya* which occurs in the mainland dunes area was found to contain the same psilostachyin series of sesquiterpene dilactones that characterize the island populations. However, we had also noted that the populations of *A. cumanensis* Kunth. from near Vera Cruz, Mexico, produced the same sesquiterpene dilactones as the island populations of *A. psilostachya.* This latter finding offered a second proposal for the origin of the island populations of *A. psilostachya*: namely, that they were derived from *A. cumanensis. A. cumanensis* and *A. psilostachya* occur sympatrically in Mexico and are considered to be members of the same species complex; they appear on morphological, cytological and geographical grounds to be closely related species.[2,7] In fact, it was previously noted that the Texas islands populations of *A. psilotachya* are morphologically more similar to *A. cumanensis* than are the mainland collections of *A. psilostachya* from elsewhere in the U.S.A.[2]

[6] W. A. PRICE, *Bull. Amer. Assoc. Petr. Geol.* **17,** 907 (1933).
[7] W. W. PAYNE, *J. Arnold Arb.* **45,** 401 (1964).

In order to bring additional evidence to bear upon the question of the origin of the island populations of *A. psilostachya* we determined the volatile oil patterns of 20 populations of *A. psilostachya* and 7 of *A. cumanensis*. The volatile oil data were subjected to numerical analyses in order to determine the levels of chemical similarities among all the populations. Finally the volatile oil and sesquiterpene lactone data for all the populations were compared. The results of these recent investigations are reported here.

RESULTS AND DISCUSSION

The Volatile Oils

The volatile oils of 20 populations of *A. psilostachya* and 7 of *A. cumanensis* were obtained by steam distillation of fresh plant material; the steam distillates were analysed by GLC and, in many instances, individual constituents were isolated by preparative GLC and characterized by IR and/or NMR spectroscopy; these included: myrcene, *trans*-ocimene, limonene, β-phellandrene, α-terpineol, cineole, α-pinene, camphene, β-pinene, Δ^3-carene, sabinol, borneol, bornyl acetate, camphor, humulene, δ-cadinene, caryophyllene, β-cubebene. In the present study quantitative volatile oil data were not employed in the numerical analyses because, when 15 plants from each of three populations were individually analysed for their volatiles, all three populations showed considerable intrapopulational quantitative variation but none varied qualitatively. In addition plants from population No. 6 were analysed at different stages of development; again only quantitative variation was observed. Finally, plants from two of the island populations of *A. psilostachya* (Nos. 27 and 30) were transplated to Austin, Texas; the volatile constituents of the second year of growth were qualitatively but not quantitatively identical to those found in the populations before transplantation. Therefore, using only qualitative variations in the volatile oil chemistry, similarity ratios were calculated for the 20 populations of *A. psilostachya* and 7 of *A. cumanensis*; these ratios were then used to cluster the populations by the single-linkage method.

Sesquiterpene Lactones

The sesquiterpene lactones were extracted from plants belonging to several populations including all of those for which volatile oil data were obtained; the crude syrups were analysed by NMR by standard procedures.[1,2,8]

Correlation of the Volatile Oil and Sesquiterpene Lactone Data

The numerical analysis of the volatile oil data clustered the 27 populations into three groups; with few exceptions each group was found to be characterized by one of the three structurally distinct types of sesquiterpene lactones known to occur in these species (Fig. 1 and Table 1). The three groups of populations were designated the Port Isabel Group, the Texas Mainland Group and the Texas Islands—Vera Cruz, Mexico Group (Fig. 2).

1. *The Port Isabel group*. Three populations of *A. psilostachya* (Nos. 25, 26 and 40) which were collected near Port Isabel, Texas, showed a high level of similarity and were distinguished by the presence of camphor and another volatile component designated No. 27. Populations Nos. 23 and 35, which occur more than 70 miles north of Port Isabel (see Fig. 3), were also linked to this group by the presence of these same two substances. In

[8] T. J. MABRY, in *Phytochemical Phylogeny* (edited by J. B. HARBORNE), Chap. 13, pp. 269–300, Academic Press, New York (1970).

TABLE 1. SESQUITERPENE LACTONES OF

Population No.	Location	Per cent of known compound Psilostachyin	Psilostachyin B	Psilostachyin C	Ambrosiol	Parthenin	Coronopolin	3-Hydroxydamsin	Ambrosin	Isabelin
6	Austin, Texas	—	—	—	40	—	60	—	—	—
7	Austin, Texas	—	—	—	40	—	60	—	—	—
9	Mustang I., Texas	40	30	30	—	—	—	—	—	—
10	Tampico, Mexico	—	—	—	—	30	70	—	—	—
12	Tempeche, Mexico†	30	60	10	—	—	—	—	—	—
13	Tuxpan, Mexico†	20	70	10	—	—	—	—	—	—
14	Poza Rica, Mexico†	20	70	10	—	—	—	—	—	—
15	Papantla, Mexico†	10	80	10	—	—	—	—	—	—
16	Papantla, Mexico†	50	40	10	—	—	—	—	—	—
17	Tecolutla, Mexico	—	—	—	—	30	70	—	—	—
18	Nautla, Mexico†	20	70	10	—	—	—	—	—	—
19	Vera Cruz, Mexico†	20	70	10	—	—	—	—	—	—
20	Vera Cruz, Mexico†	10	80	10	—	—	—	—	—	—
21	Vera Cruz, Mexico†	10	80	10	—	—	—	—	—	—
23	Kingsville, Texas	10	—	—	—	—	—	—	—	90
24	Raymondville, Texas	—	—	—	80	—	—	—	20	—
25	Port Isabel, Texas	20	—	20	—	—	—	—	—	60
26	Port Isabel, Texas	10	—	10	—	—	—	—	—	80
27	Padre Isl., Texas	30	50	20	—	—	—	—	—	—
28	Padre Isl., Texas	30	50	20	—	—	—	—	—	—

* See refs. 2 and 9 for the characteristic NMR signals used to identify the sesquiterpene lactones.
† *A. cumanensis*; unmarked populations are *A. psilostachya*.

addition, population No. 35 contained a major volatile oil component designated No. 96, a substance otherwise detected only in population No. 25.

All of the above mentioned populations (Nos. 23, 25, 26, 35 and 40) were found to contain the novel sesquiterpene dilactone isabelin; this substance belongs to the germacranolide type of sesquiterpene lactones which are typically found in *A. artemisiifolia.* Since the latter species is known[9] to produce isabelin and related germacranolides and is sympatric in several areas of south Texas with *A. psilostachya,* the presence of isabelin in these few isolated populations of *A. psilostachya* which constitute the Port Isabel Group may be the result of hybridization between the two species.

2. *The Texas Mainland group.* Eight mainland populations of *A. psilostachya* from central and south Texas formed a single cluster which was initiated at the 0·979 similarity level by populations Nos. 6 and 60. Populations Nos. 33, 34 and 41 were also linked to the cluster at relatively high levels of similarity, and lastly populations Nos. 36, 37 and 24 were joined to the cluster. With the exception of the monoterpene β-phellandrene none of the volatile oil constituents were unique to the mainland group. However, populations Nos. 24, 35 and 37 produced two volatile oil components (designated Nos. 87 and 89) which distinguished these south Texas populations from the more northern members of the group.

[9] T. H. PORTER, T. J. MABRY, H. YOSHIOKA and N. H. FISCHER, *Phytochem.* 9, 199 (1970).

A. psilostachya AND *A. cumanensis**

Population No.	Location	Per cent of known compound: Psilostachyin	Psilostachyin B	Psilostachyin C	Ambrosiol	Parthenin	Coronopolin	3-Hydroxydamsin	Ambrosin	Isabelin
29	Mustang Isl., Texas	40	40	20	—	—	—	—	—	—
30	Galveston Isl., Texas	30	50	20	—	—	—	—	—	—
31	Matagorda I., Texas	20	50	30	—	—	—	—	—	—
32	Padre Isl., Texas	—	—	—	70	—	—	30	—	—
33	Blanco Co., Texas	—	—	—	—	50	50	—	—	—
34	Kendall Co., Texas	—	—	—	—	40	60	—	—	—
35	Kenedy Co., Texas	20	—	10	—	—	—	—	—	70
36	Kenedy Co., Texas	—	—	—	100	—	—	—	—	—
37	Willacy Co., Texas	10	50	40	—	—	—	—	—	—
38	Port Mansfield, Texas	40	40	20	—	—	—	—	—	70
40	Port Isabel, Texas	10	—	20	—	—	—	—	—	—
41	Kleberg Co., Texas	20	70	10	—	—	—	—	—	—
45	Orizaba, Mexico†	10	80	10	—	—	—	—	—	—
47	Vera Cruz, Mexico†	30	60	10	—	—	—	—	—	—
48	Vera Cruz, Mexico†	20	60	20	—	—	—	—	—	—
53	Anton Lizardo, Mexico†	30	60	10	—	—	—	—	—	—
54	Cordoba, Mexico†	—	70	30	—	—	—	—	—	—
55	Cordoba, Mexico†	10	80	10	—	—	—	—	—	—
57	Saltillo, Mexico	—	—	—	—	30	70	—	—	—
60	Austin, Texas	—	—	—	70	—	30	—	—	—

On the basis of their sesquiterpene lactones this mainland group was divided into two types: six inland populations contained the monolactone ambrosiol as a major component, while populations Nos. 37 and 41, both of which occur within 30 miles of the coast, produced the sesquiterpene dilactones which are typical for the island populations. In our earlier study,[2] we had also reported the presence of dilactones in a population from south Texas; this same population was re-collected in the present investigation and is now designated as No. 37. The volatile oil data for both populations Nos. 37 and 41 clearly included them with the other mainland populations. In our previous proposal for the origin of populations of *A. psilostachya* which occur on the Texas Gulf coast islands we suggested that population No. 37 was representative of the gene pool which gave rise to the island populations some 3000 years ago. Significantly, however, none of the present islands populations produces a volatile oil chemistry that corresponds to that of population No. 37 or, for that matter, of any population analysed to date on the Texas mainland. Thus, the presence of sesquiterpene dilactones in a few small mainland populations which occur near the coast (i.e. Nos. 37 and 41) probably represents some gene flow from the islands to the mainland. In support of this suggestion, we might point out that the prevailing winds blow landward

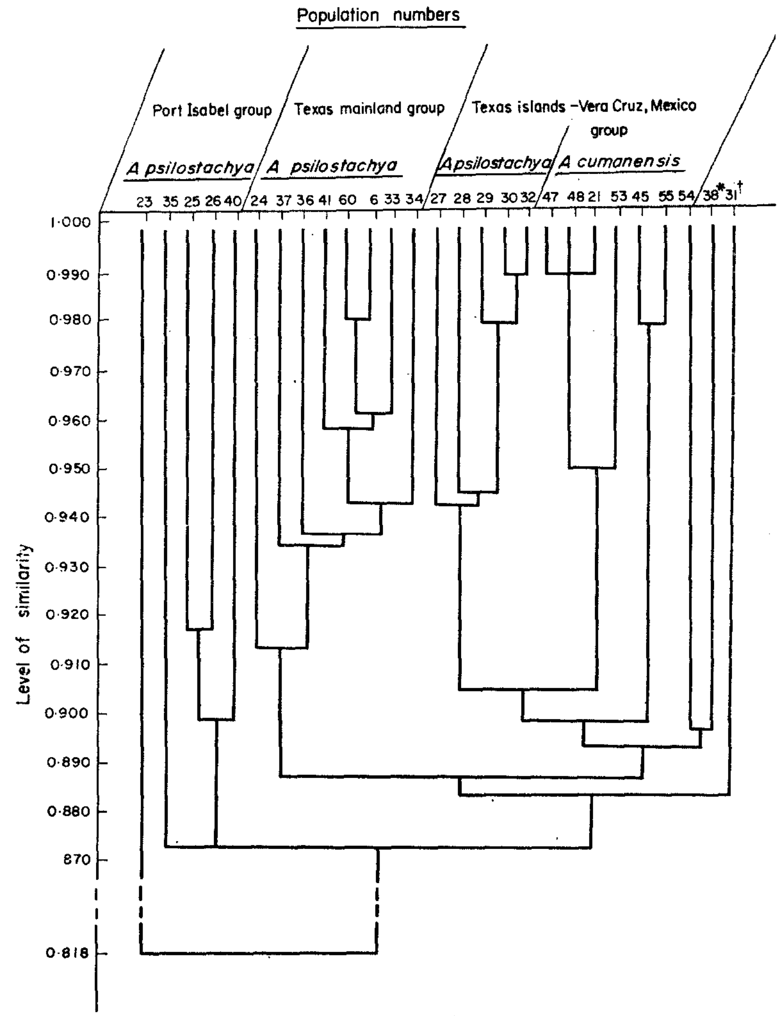

FIG. 2. DENDROGRAM OF 20 POPULATIONS OF *A. psilostachya* AND SEVEN OF *A. cumanensis*. THE SIMILARITY MEASURES WERE BASED ON THE QUALITATIVE VARIATIONS IN THE VOLATILE OILS.

* Texas Mainland population of *A. psilostachya*.

† Texas Island population of *A. psilostachya*.

ten months of the year including the period of July through September during which time anthesis takes place in *A. psilostachya*, a species highly specialized for wind pollination.

3. *The Texas Islands–Vera Cruz, Mexico group.* The Texas Gulf coast islands populations of *A. psilostachya* and the Vera Cruz, Mexico, populations of *A. cumanensis* constitute a group based on both volatile oil and sesquiterpene lactone chemistry. This group also includes population No. 38 which was collected in the Texas mainland dunes region. The clusters derived by the numerical analysis of the volatile oil data indicate that all of these populations (except No. 31) have high levels of similarity. The four Mexican coastal populations of *A. cumanensis* formed a cluster at a similarity ratio of 0·952; these then clustered with the five Texas islands populations even before they clustered with the inland Mexican populations Nos. 45 and 55 at the 0·899 level. Population No. 54 was linked to

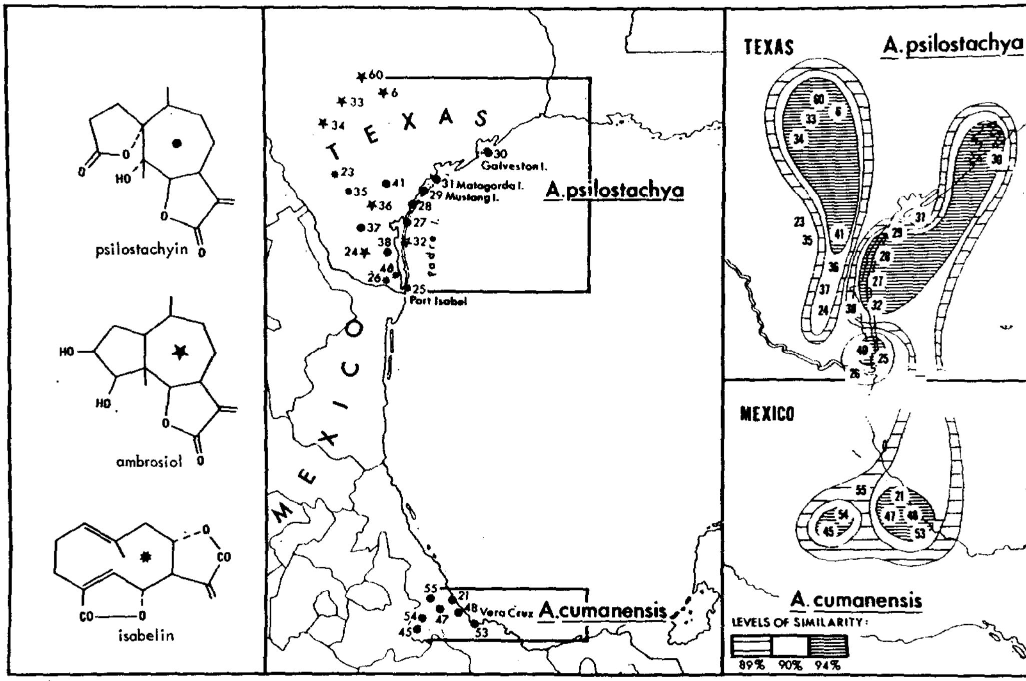

FIG. 3. THE LEFT-HAND PORTION OF THE FIGURE SHOWS THE CHARACTERISTIC SESQUITERPENE LACTONE CHEMISTRY OF 20 POPULATIONS OF *A. psilostachya* AND SEVEN POPULATIONS OF *A. cumanensis*, WHILE THE RIGHT-HAND PORTION PRESENTS THREE LEVELS OF SIMILARITY FOR THE VOLATILE OIL CLUSTERING PATTERNS FOR THE SAME POPULATIONS.

the Texas mainland population No. 38 at the 0·989 similarity level and these two were linked to the island-Mexico cluster at the 0·894 level; both of these populations are peripheral members of the island-Mexico group based on the volatile oil data.

Population No. 31 from Matagorda Island was most similar to population No. 28 from Padre Island, but clustered only at the 0·882 level, in part because of the presence of camphor and component 27, compounds which are characteristic of the Port Isabel populations.

The Texas islands–Mexico group populations generally produced more sesquiterpene alcohols than did those belonging to the Texas mainland and Port Isabel groups. Two sesquiterpene alcohols, components Nos. 63 and 65, were produced by all members of the Texas islands–Mexico group and δ-cadinene, a sesquiterpene hydrocarbon, was produced by all the island populations plus population No. 38, and three Mexican populations (Nos. 53–55). Only the seven Mexican populations produced component No. 97, another sesquiterpene alcohol.

With one exception (population No. 32), all the island populations of *A. psilostachya* (more than 20 including those from our earlier study[2]) and the Mexican populations of *A. cumanensis* contained the psilostachyin series of dilactones.

CONCLUSION

Both the volatile oil and sesquiterpene lactone data obtained in the present study indicate a closer genetic relationship between the Texas islands populations of *A. psilostachya* and the Vera Cruz, Mexico, populations of *A. cumanensis* (Fig. 3) than between the island and mainland populations of *A. psilostachya.*

EXPERIMENTAL

Sampling. Populations of *Ambrosia psilostachya* were sampled in Texas and Mexico from July to early November 1968, and populations of *A. cumanensis* were collected in the state of Vera Cruz, Mexico in April and November 1968. Because of the tendency of *A. psilostachya* to form clonal populations a population was defined as one which contained plants from ten different rootstocks. The ten plants were pooled to give a population sample. Plant material for volatile oil analysis was kept fresh in plastic bags on ice. A 50 g sample of whole plant material was collected and air-dried for sesquiterpene lactone analysis. Voucher specimens for all populations are deposited in the University of Texas at Austin Herbarium. Populations Nos. 6, 28, and 29 were re-collected in June 1969, for analysis of the intrapopulational quantitative variations in the volatile oils; fifteen individual plants from each population were analysed separately by GLC and the resultant data were used to determine the range, mean, and standard deviation of each component.

Volatile oil pattern analysis. The volatile oils were obtained from fresh plant material by steam distillation for two hours in a modified refluxing condenser in which the terpenoids were trapped in a layer of Et_2O. The samples were stored in N_2 at $-18°$. Bulk quantities of fresh plant material were steam distilled in a non-refluxing unit for 90 min; the resultant 20 gallons of water were extracted with CH_2Cl_2, yielding approximately 20 ml of oil which were used in the isolation and identification of individual components. All volatile oil samples were analysed on a Varian 152OC gas chromatograph equipped with a hydrogen flame ionization detector, a Varian model 476 digital integrator, and a $381 \times 0{\cdot}32$ cm. O.D. stainless steel column, vibrator-packed with 4% PEG:1% DEGA on 100 mesh Gas Chrom Q (Applied Science Laboratories). The injector temperature was 140°; the helium carrier gas flow rate was 25 ml/min; and the temperature was programmed for 45° to 200° at 3°/min.

Isolation and identification of the volatile oil components. The total oil sample was initially separated into polar and non-polar fractions by elution through a silica gel column first with pentane, then CH_2Cl_2, and finally methanol (a modification of the procedure of Kugler and Kovats).[10] The volatile oil components of each fraction were then isolated by preparative-scale GLC employing an Aerograph Autoprep 700 equipped with a thermal conductivity detector and various 0·64 cm o.d. packed copper columns. The individual components were condensed on the walls of 0·32 cm o.d. teflon tubing as they were eluted from the gas-chromatograph. Each isolated component was checked for purity on at least two GLC 0·32 cm o.d. packed stainless steel columns of different polarity and on silica gel G or 5% $AgNO_3$ impregnated silica gel G TLC plates using as solvents pentane or hexane for the former plates and hexane:ether:acetic acid (95:5:1) for the latter. The IR spectra of pure components were recorded as thin films on salt plates or in Beckman silver chloride Extrocells. A component was considered positively identified if it had: (1) the same IR spectrum as a known compound, and (2) the same GLC retention times (on two columns of different polarity) and TLC R_f values as the known compound.

Extraction and detection of sesquiterpene lactones. Approximately 50 g of whole dried plant material were extracted with $CHCl_3$; the extract was worked up by standard procedures[2,8,11] and analyzed by NMR spectroscopy using previously described procedures.[2,8]

Quantitation of volatile oils for numerical analyses. A Varian model 476 digital integrator was employed to quantitate relative peak areas. Those peaks not analysed by IR were compared on the basis of their GLC retention times. Among the 27 populations, 98 different components were assigned numbers which represented the same compounds on all chromatograms. The numbered components always accounted for more than 90 per cent of the total oil in each sample.

Similarity measure.[12] A similarity measure based only on the qualitative variation in the volatile oils were employed. The program SRPRAB[13] (provided by Dr. Robert P. Adams)* was written in Fortran IV and computations were carried out on the Control Data 6600 computer of the University of Texas at Austin. The technique employed is designated as follows:

$$SR = 1{\cdot}0 - \sum_{i=1}^{n} RD_{x(y)i} \Big/ \sum_{i=1}^{n} CW_i$$

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[10] E. KUGLER and E. ZU KOVATS, *Helv. Chim. Acta* **46**, 1480 (1963).

[11] H. YOSHIOKA, T. J. MABRY and H. E. MILLER, *Chem. Comm.* 1679 (1968).

[12] For more details, see J. L. POTTER, Ph.D. Thesis, University of Texas, Austin (1970).

[13] R. P. ADAMS, Ph.D. Thesis, University of Texas, Austin (1969).

where SR is the similarity ratio; $RD_{x_iy_i}$ is the relative dissimilarity between taxon x and taxon y for the ith volatile component; and CW is the character weight. $RD_{x_iy_i} = 1$ if i is present and greater than a trace in one taxon but absent in the other, *or* $= 0$ if x_i and y_i are both trace components. $CW_i = 1$ if either x_i or y_i are non-trace components, and $CW = 0{\cdot}5$ if x_i and y_i are both trace components (i.e. the matching of trace components contributes only half as much to the similarity ratio as the matching of non-trace components). No comparison was made if x_i was a trace and y_i was absent. A component was considered a trace if it constituted less than 0·3 per cent of the total oil. The similarity ratio of two taxa then ranges from 0 (no volatile constituents in common) to 1·0 (a sample compared with itself).

The similarity ratios were clustered by the single linkage technique, a procedure which arranges the pairs of populations into clusters based on their similarity to each other, starting with the most similar pair. A new member is linked to an established cluster by means of its similarity to any one member of that group.

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Key Word Index—Ambrosia psilostachya; Compositae; systematics; geographical distribution; evolution terpenes; sesquiterpenoid lactones; monoterpenes.