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PAPER CHROMATOGRAPHY FOR DETERMINING PALATABILITY DIFFERENCES IN VARIOUS STRAINS OF BIG SAGEBRUSH

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ABSTRACT

Two-dimensional phenolic extraction on 9-inch squares of chromatographic paper was discovered to be a simple laboratory technique for quickly classifying more than 100 foliage collections of big sagebrush from over the Intermountain area into two major palatability classes. These agreed readily with field observations of preferences by deer and livestock on winter ranges. The technique was also in close agreement with the observed preferences by deer of 10 geographic sources transplanted to a common area having a uniform soil within their winter range. It was also useful for quickly recognizing four subgroups in subspecies of *Artemisia tridentata vaseyana* and two subgroups in *Artemisia tridentata tridentata*.

The solvent system for the first dimension was n-butanol, acetone, water (4:1:3) and for the second dimension acetic acid and water (15:85). Chromatograms were viewed under longwave ultraviolet light before and after exposure to ammonia fumes and in daylight following the application of ammonia. The strong association that was found between chromatographic variation and palatability suggests that the procedure may be used to predetermine the grazing potential of any collection of big sagebrush.

The most obvious chromatographic characteristic was the size and intensity of the blue in spot 9. It ranged from large to small and iridescence varied from brilliant to dull. This spot was successfully used to classify all big sagebrush samples into the two basic groups with the other spots being used to make subgroups.

Introduction

Common big sagebrush (*Artemisia tridentata*) is one of the most widely distributed and abundant shrubs of the Western United States (Hall and Clements 1923; Beetle 1960; Dayton 1931; and Plummer et al. (1968). It is particularly abundant over much of the Great Basin and in many areas may comprise up to 90 percent of the shrubby vegetation. It is an important browse for big game on their winter ranges. (The nomenclature in this publication has followed Holmgren and Reveal 1966.)

Past observations have revealed that there is considerable variation in the palatability of big sagebrush for big game and livestock. This variation in palatability is often associated with the geographical source of the sagebrush, but in some cases palatable and unpalatable plants may be found growing on a common site. These observations indicate that there may be a real opportunity for development of improved strains through selection and breeding.

Apparently, considerable genetic diversification has occurred in the big sagebrush complex as a result of hybridization and back crossing. Genetic makeup is probably associated with dissimilarities in the chemistry of the plants involved; thus, if chemical differences are detected in plants collected from various geographical sources, this indicates that some degree of genetic divergence exists. Furthermore, the amount of chemical difference may be an index of the extent to which strains have become separated. Such differences in the chemistry of plants have been utilized extensively in recent years to supplement morphological differences in the grouping of closely related plants in some of the major taxa. For example, Hollis (1966, 1967a, 1967b, and 1967c) made comparative analyses of the polyphenols from leaf extracts of *Eucalyptus* using paper chromatography. As a result of these analyses, he resolved many of the problems relating to the taxonomy of this genus. The solution of these problems was not possible on the basis of morphological characters alone.

Similar studies on the genus *Baptisia* were conducted by Alston and Turner (1962, 1963) and Brehm and Alston (1964). In addition, Holbo (1965) completed a comparable study of *Artemisia* section *Tridentatae* in which he readily separated the individual species by chromatography. Using thin-layer chromatography, Brunner¹ showed that strain variation in big sagebrush could be readily detected. Consequently, it appeared likely that similar techniques could be used to separate strains of big sagebrush into palatability classes.

¹J. Brunner. Some observations on *Artemisia* in Nevada. Bureau of Land Management, Las Vegas, Nevada. (Manuscript in preparation.)

Experimental Procedure

From February through September 1969, more than 100 foliage collections of big sagebrush were taken from widely scattered areas in Utah, Nevada, Idaho, Wyoming, and Colorado. We obtained these from a wide variety of sites so that the collections would be fairly representative of the species distribution. In addition to using them in the chemical analyses described below, the degree of grazing by big game was determined to discover if palatability, or preference, was associated with geographical source or ecotype. Observations were made at locations where grazing pressure had not been severe enough to force utilization of unpalatable plants. Generally, the degree of grazing was determined in January, but some observations were made in February and March, and a few were made in April. Palatability was given a low rating where less than 15 percent of the current growth was removed, medium where utilization was 15 to 40 percent, and high where utilization was more than 40 percent. The degree of herbage removal was determined by the technique described by Pechanec and Pickford (1937).

Since we were aware that palatability may be affected by soil or other environmental factors, about 100 plants under 2 years old were collected from each of ten geographical sources of big sagebrush. These plants (totaling about 1,000) were transplanted from their natural sites to comparable randomized rows spaced 3 feet apart on State Fish and Game land located on deer winter range near Price, Utah. Transplanting was done in April and early May of 1968. Plants from three of these sources had been observed to be especially palatable to deer; plants from seven sources had been categorized as unpalatable while growing on their natural sites. The degree of grazing on these transplanted rows was determined in January 1969 and 1970 by the same technique described above.

Foliage collections from plants of the more than 100 sources were placed in brown paper bags and dried in the absence of light. Then, by use of a mortar and pestle, 0.5 grams of the dried leaves were pulverized and placed in brown 30 ml. bottles, and 7.0 ml. of absolute methanol was added to extract phenolic constituents. After 24 hours at room temperature, the extract was decanted and concentrated by evaporation to 2.0 ml. Twenty-five μ l of this extract was applied to duplicate 9-inch squares of Whatman No. 3 MM chromatographic grade filter paper in two dimensions. The solvent system for the first dimension was n-butanol, acetone, water (4:1:3) and for the second dimension, acetic acid, water (15:85). Chromatograms were viewed under longwave ultraviolet light before and after exposure to ammonia fumes and in daylight following the application of ammonia in order to note the appearance and color changes of the resulting spots. Each spot was given an arbitrary number for identification purposes and the R_f value of each was computed for both directions of the finished chromatogram.

$$R_f = \frac{\text{Distance of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

The R_f value of a given spot is then expressed as:

$$R_f = R_f \text{ (first dimension)} / R_f \text{ (second dimension)}$$

Results

CHROMATOGRAMS

As might be expected when dealing with a large and complex species such as big sagebrush, considerable chromatographic variation was found. Presently, the plant collections have been divided into two major groups, I and II. Group I has 4 subgroups (Ia, Ib, Ic, and Id). Group II has 2 subgroups (IIa and IIb). These groups are based on differences in the chromatographic spots. Chromatograms of each collection always display a basic compliment of ten spots (1, 2, 3, 4, 7, 8, 9, 11, 13, 27) plus varying combinations from an additional eleven spots (5, 6, 10, 12, 14, 16, 22, 25, 26, 33, 36) (table 1 and figs. 1-6). Some of these spots exhibit marked differences in size and intensity of color; therefore, the chromatograms were organized into groups taking into consideration both qualitative and quantitative variations.

Table 1.-- R_f values and color of the chromatographic spots in *Artemisia tridentata*

Spot no.	R_f	Color		
		Ultraviolet	NH ₃ + Ultraviolet	NH ₃ + Daylight
1	.53/.46	Blue	Yellow-green	Gray
2	.90/.78	Violet	Violet	--
3	.88/.71	Blue-green	Blue-green	--
4	.87/.71	Violet	Violet-brown	Yellow
5	.47/.11	Yellow or Yellowish-brown	Yellow or Yellowish-brown	--
6	.28/.13	Yellow or Yellowish-brown	Yellow or Yellowish-brown	--
7	.32/.08	Violet	Violet	Yellow-brown
8	.51/.87	Dark blue	Dark blue	--
9	.54/.78	Light iridescent blue-green	Light iridescent blue-green	--
10	.18/.84	Blue	Blue	--
11	.34/.75	Blue	Yellow-green	Yellow
12	.26/.71	--	Blue	--
13	.29/.64	Pink	Yellow-pink	--
14	.35/.59	Blue or violet	Gray-blue or violet	--
16	.32/.90	Dark blue	Dark blue	--
22	.32/.84	Blue	Blue-green	--
25	.83/.67	Blue-green	Blue-green	--
26	.38/.73	Blue	Blue	--
27	.51/.57	Blue	Yellow-green	Gray
33	.75/.60	Pink	Pink	--
36	.26/.84	Bright blue-green	Bright blue-green	--

Figures 1-6.--Representative two-dimensional chromatograms of methanol-soluble extracts from the leaves of the six subgroups (under groups I and II) of big sagebrush. For spot coloration and R_f values, see table 1.

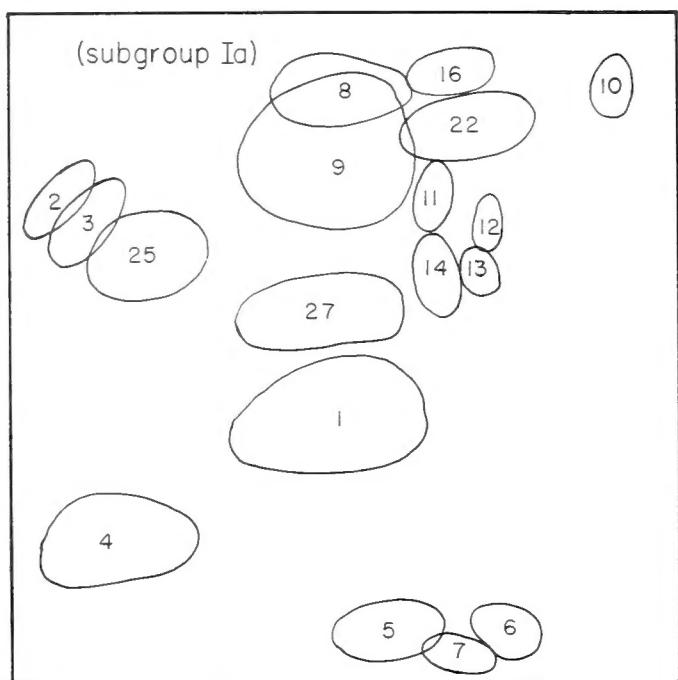


Figure 1

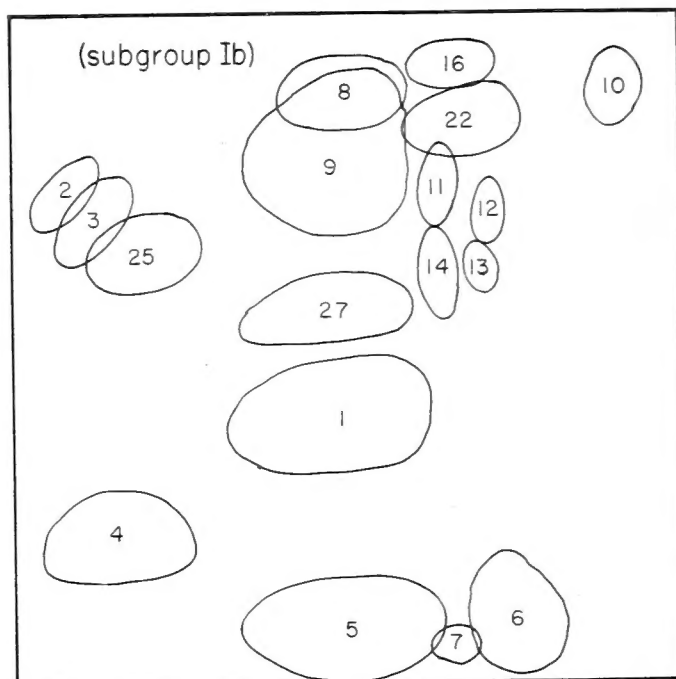


Figure 2

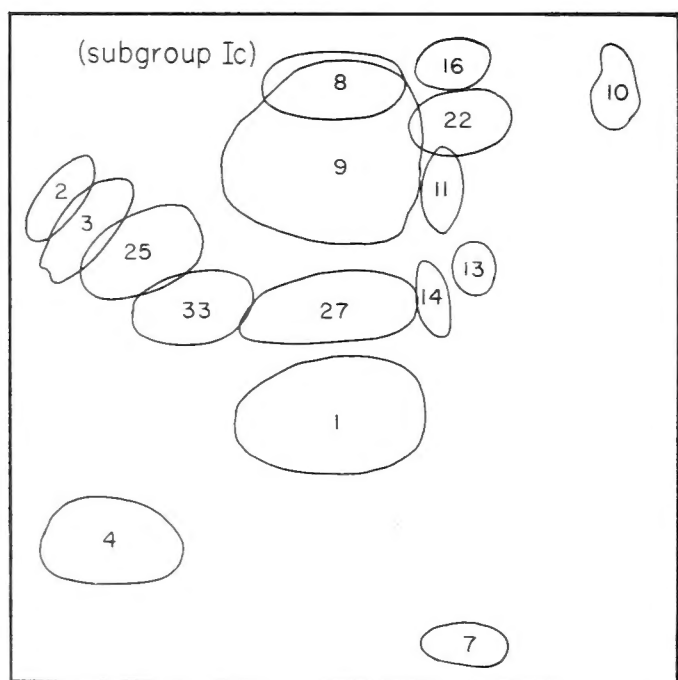


Figure 3

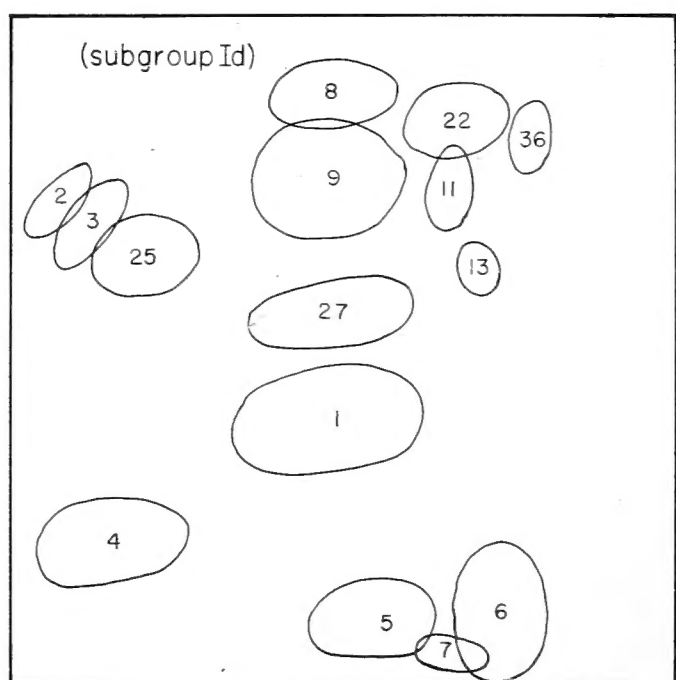


Figure 4

HANKS, DAVID L., BRUNNER, JAMES R., CHRISTENSEN, DONALD R.,
and PLUMMER, A. PERRY

1971. Paper chromatography for determining palatability differences in
various strains of big sagebrush, USDA Forest Serv. Res. Pap.
INT-101, 9 p., illus.

Two-dimensional phenolic extraction was found to be a simple labora-
tory technique for quickly classifying big sagebrush (*Artemisia triden-*
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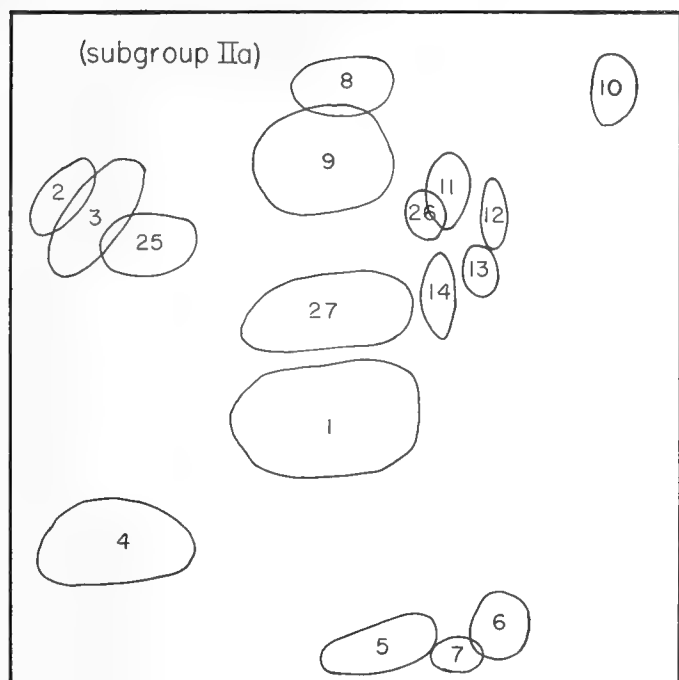


Figure 5

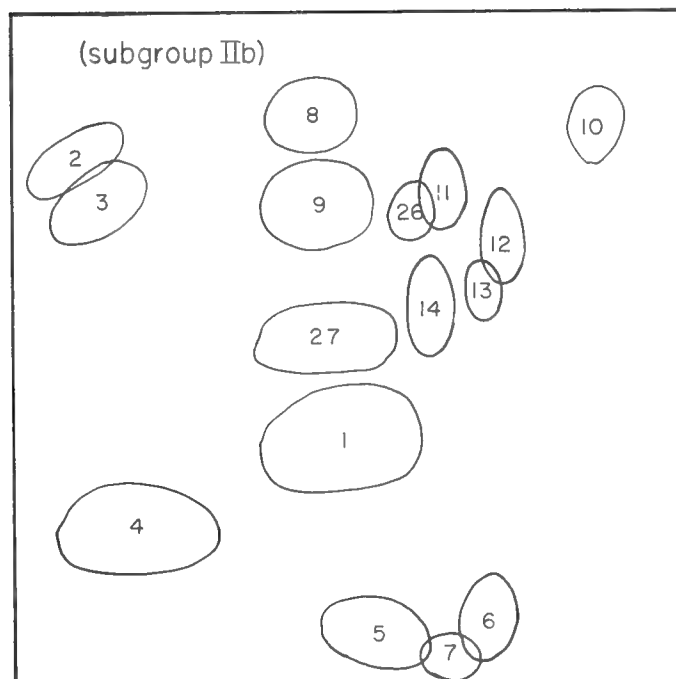


Figure 6

Chromatograms of all collections in Group I contain a large, iridescent blue spot 9 ($R_f = .54/.78$) which is the most prominent in the entire chromatogram and most characteristic of this major group. In addition, displayed in all specimens of Group I were spots 25 and either 16 or 22 ($.83/.67$, $.32/.90$, and $.32/.84$, respectively). On the other hand, spot 9 of Group II is usually smaller and always less brilliant than in Group I. Furthermore, spot 16 was always absent from the chromatograms of Group II and 22 was found only occasionally. However, spots 5, 6, and 26 ($.47/.11$, $.28/.13$, and $.38/.73$, respectively) were always present in Group II as shown in figures 5 and 6.

Group I.--This major group was subdivided into its four distinct subgroups of Ia, Ib, Ic, and Id (figs. 1-4), on the basis of the following characteristics. In addition to the brilliancy of spot 9, and the presence of spots 16, 22, and 25 mentioned previously, chromatograms of Ia contain spots 5, 6, 10, 12, and 14 (fig. 1). Spots 5 and 6 of this group are relatively small and dull and yellowish-brown in color.

Subgroup Ib differs from Ia only in the size and color of spots 5 and 6, which in Ib are large and bright yellow (fig. 2).

Chromatograms of Ic lack spots 5, 6, and 12 but always contain the prominent pink spot, 33. Spot 9, however, is larger and more brilliant in Ic than in any of the other 3 subgroups of group I (fig. 3).

Subgroup Ic appears to be made up of collections of subspecies *A. tridentata vaseyana* or closely related intermediates within group I. Since this subspecies is such a polymorphic complex, it is possible that Ia and Ib, as well as Ic, may be considered within it.

Group Id lacks spots 10, 12, 14, and 16. Here, spot 9 is intermediate in size but remains highly iridescent. In Id, spots 5 and 6 are large and bright yellow as in Ib. In addition, a bright blue-green spot, 36, is prominent in subgroup Id (fig. 4).

Group II.--This major group was divided into the two subgroups IIa and IIb (figs. 5 and 6), both of which lack spots 16 and 36 but contain spots 5, 6, and 26.

In subgroup IIa, spot 9 is smaller than in any of the previously described subgroups except Id where it is of similar size. However, the brilliancy of this spot in IIa is decidedly less intense than that found in any of the collections placed in group I; nevertheless, spot 9 in IIa remains somewhat iridescent. Spots 12, 14, and 25 occur in this subgroup (fig. 5). In both IIa and IIb, spots 5 and 6 are small and dull yellowish-brown. They are similar in size and color to those found in Ia.

In subgroup IIb, spot 9 is very small, often not exceeding one-half inch in diameter, and it exhibits little or no iridescence. In most chromatograms of subgroup IIb, spots 12 and 25 are missing; and when present they are small and only faintly colored. Spot 14 is always present in IIb, although occasionally the color of this spot is dark violet rather than the usual blue-gray found in all other groups (fig. 6). On the basis of morphological examinations, it seems apparent that collections representing sources in subgroups IIa and IIb would be in the subgenus *A. tridentata tridentata*. Further observations may show IIa to be considerably integrated with Ia.

GRAZING

The groupings already described are related to the degree of grazing by deer and livestock. However, observations indicate that plants in group I are much more palatable than those in group II (fig. 7). This was true on the row plantings on State Fish and Game lands northwest of Price, Utah, in January 1969 and 1970, and also where natural representatives of these two major groups were found on the local winter range.



Figure 7.--Comparison of subgroup Ic from Hobble Creek (left) with subgroup IIb from Indianola, in January 1970, when grazing averaged 60 and 5 percent, respectively. Plants of these sources had attained a similar height of about 24 inches when deer began to graze them in mid-November.

We are confident that differences in palatability exist within groups I and II, but our observations have not yet been intensive or refined enough to detect this and correlate it definitely with chromatographic analyses.

Three of the ten collections planted near Price chromatographed as group I. By mid-January in both 1969 and 1970, all of these were grazed by deer to an average of about 60 percent. None of the big sagebrush plants were grazed less than 40 percent; and on several of the plants, grazing was in excess of 80 percent. In contrast, of the seven collections chromatographed as group II, none were grazed more than 40 percent, and most were grazed less than 5 percent.

All collections from individual *A. tridentata* plants or populations on winter ranges which had been grazed in excess of 50 percent invariably chromatographed as group I except for the single exception represented by a collection from Utah County (Hobble Creek trial site) and involving some big sagebrush transplants. Moreover, all collections from plants or populations observed to be relatively unpalatable on the open range, chromatographed as group II. This was especially noticeable in the area from which collections of subgroups Id and IIa were taken in northwestern Nevada where these two subgroups grow in an intermixed population on a foothill winter range (fig. 8). Subgroup Id on this same range was palatable to the cattle that graze on the area, but IIa was unpalatable and grazed very little. By March 15, 1968, subgroup Id on this range had been grazed slightly in excess of 60 percent, and grazing on IIa was less than 15 percent. The partiality for Id was also exhibited by sheep and deer in that area. The difference between subgroups Id and IIa was also evident on the transplanted areas near Price, Utah. A similar selectivity was also noted where subgroups Ia and Ib intermix with IIb in Ephraim Canyon. Under these intermix conditions, Ia and Ib were grazed in excess of 70 percent by late January of 1969 and 1970, but none of the plants in subgroup IIb were grazed more than 15 percent.



Id

IIa

Figure 8.--Marked differences were noted in the size of *Artemisia tridentata* subgroups Id (left) and IIa (right) as a result of cattle grazing in the Jackson mountains in northwestern Nevada.

The most obvious chromatographic characteristic of collections that were selected by deer and livestock was the presence of a large and highly iridescent spot 9. This marker is particularly obvious in subgroups Ia, Ib, and Ic where its brilliance is considerably greater than in the unpalatable subgroups; however, this distinction is not so clear in regard to Id and IIa. Since subgroups Id and IIa are found in close physical association, the use of additional characters is frequently necessary. Other indicators of palatability for deer and livestock are the presence of spots 16 and 22 in subgroups Ia, Ib, and Ic; the presence of 22 in Id; and the absence of 26 in all groups.

Discussion

At this point in our study of the big sagebrush complex, we believe it is significant that chromatograms of the various collections allow rather well-defined groupings. The existence of these groups not only supports our initial hypothesis regarding the occurrence of genetic divergence within subspecies of big sagebrush, but provides evidence that this genetic change is following quite discrete lines. However, we must point out that some of the collections do not fall into any of the designated groupings but appear to occupy positions intermediate between them. Chromatograms of these intermediates most commonly contain both spot 22, found primarily in group I, and 26 occurring in group II; but such chromatograms lack spot 16 found in group I, and spot 9 is usually intermediate in brilliancy. This combination suggests hybridization between groups I and II and the retention by the progeny of some characteristics from each parent. Nevertheless, the majority of collections can be readily separated into the previously described groupings by the methods given.

In regard to the ecological distribution of the major groups and subgroups, the following observations have been made. Collections of group I were obtained primarily in mountain habitats. Those of subgroup Ic were obtained in the lower mountains and higher foothill areas. Individuals from subgroups Ia and Ib of the lower foothills area have come from areas extending from the lower limits of Ic to the base of the foothills where they overlap with subgroup IIb, the prevalent big sagebrush (*A. tridentata tridentata*) of the more extensive lowlands.

The two subgroups Ia and Ib occupy the lower foothill areas, and it appears that Ia predominates in the upper portion while most collections of Ib have come from the lower part. However, specimens of each have been collected throughout this entire range.

Subgroups Id and IIa have only been collected from areas on the Jackson and Pine Forest mountains in northwest Nevada where they occupy similar habitats.

Chromatograms of the tall bushes commonly observed growing along such places as fencerows and arroyos differ slightly from those of IIb, among which they are frequently found. However, too few collections have been analyzed to warrant the formation of a separate subgroup at this time, although it appears likely that this may be advisable following a more thorough study. In the present paper, this tall fencerow type is included in IIb. Thus, on the basis of source material, it seems significant that the distribution pattern fits fairly close to the chromatographic divisions, especially between highland and lowland sources.

There is little likelihood that phenols observed in the big sagebrush of this study are responsible for the relative palatability of these plants. However, the strong association that has been found between chromatographic variation and palatability suggests that this laboratory procedure may be utilized to quickly evaluate the grazing potential of any collection of big sagebrush. Consequently, chromatography can be a useful tool in selecting strains of big sagebrush for specific purposes.

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Logan, Utah (in cooperation with Utah State University)

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