

CLINAL GEOGRAPHIC VARIATION IN MESCALINE CONCENTRATION AMONG TEXAS POPULATIONS OF *LOPHOPHORA WILLIAMSII* (CACTACEAE)

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ABSTRACT

A phytochemical analytical study was conducted to address the question of whether *Lophophora williamsii* (peyote) plants from Chihuahuan Desert populations in the Trans-Pecos region of West Texas exhibited higher tissue concentrations of mescaline than plants from Tamaulipan Thornscrub populations of South Texas. This question is of cultural significance to the Native American peyote religion, which involves the ingestion of peyote as a psychopharmacologically active sacrament. Tissue samples were field-collected from 10 individuals in each of four *L. williamsii* populations, two of which were located in the Chihuahuan Desert, and two of which were located in the Tamaulipan Thornscrub ecoregion. For each of the four populations, the tissue samples from 10 individual plants were pooled, the alkaloids were extracted, and the average mescaline concentration of the population was determined by HPLC. There was limited geographic variation in mescaline concentration; the highest concentration (3.52% of dry tissue) was only 27% greater than the lowest (2.77%), and the difference between the Chihuahuan Desert populations and the Tamaulipan Thornscrub populations was not significant. However, mescaline concentrations increased significantly along a gradient from southeast to northwest, i.e., from the southeasternmost Tamaulipan Thornscrub population to the northwesternmost Chihuahuan Desert population.

RESUMEN

Se hizo un estudio fitoquímico analítico para contestar esta pregunta: ¿Tienen las plantas de *Lophophora williamsii* (peyote) en las poblaciones del desierto chihuahuense en la región Trans-Pecos de Tejas concentraciones de mescalina en los tejidos más altas que las concentraciones en las plantas de poblaciones en la ecorregión matorral espinoso tamaulipeco del sur de Tejas? Esta pregunta tiene importancia cultural para la religión del peyote de los nativos americanos, la cual implica la ingestión del peyote como un sacramento psicofarmacológicamente activo. Muestras de tejido de 10 individuos por población fueron recogidas en el campo de cuatro poblaciones de *L. williamsii*, dos de las cuales estaban localizadas en el desierto chihuahuense, y dos en el matorral espinoso tamaulipeco. Para cada una de las cuatro poblaciones, las muestras de tejido de 10 plantas individuales fueron juntadas y mezcladas, los alcaloides fueron extraídos, y la concentración promedio de mescalina fue determinada por HPLC. Hubo variación limitada en la concentración de mescalina. La concentración más alta (3.52% del peso de tejido seco) fue solamente un 27% más alta que la más baja (2.77%), y la diferencia entre las poblaciones del desierto chihuahuense y las del matorral espinoso tamaulipeco no fue significativa. Sin embargo, las concentraciones de mescalina se incrementaron significativamente a lo largo de un gradiente desde el sureste hacia el noroeste, es decir, desde la población más al sureste en el matorral espinoso tamaulipeco hacia la población más al noroeste en el desierto chihuahuense.

INTRODUCTION

Lophophora williamsii (Lem. ex Salm-Dyck) J.M. Coulter. (Cactaceae) is a small (up to ca. 8 cm in diameter), spineless, globular cactus of northeastern Mexico and adjacent Texas (Figs. 1a, 1b). Commonly known as peyote, it is of cultural and economic importance for its use as a religious sacrament, based largely on the psy-



FIG. 1a. An adult peyote cactus (diameter ca. 6 cm) in habitat in Chihuahuan Desert.



FIG. 1b. Six small peyote cacti (diameter of largest individual is ca. 4 cm) in habitat in Tamaulipan Thornscrub. The circular grouping and small size indicate that these plants are regrowth resulting from the harvesting of the crown of the parent plant (not visible) by peyote cutters in recent years.

choactive properties of its principal alkaloid, mescaline, in the ceremonies of the Native American Church (NAC). Peyote is also used therapeutically by many Native American tribes as a medicinal plant for the treatment of a wide variety of conditions (Schultes 1938), though it is unclear whether therapeutic efficacy is related to the activity of mescaline in such uses of peyote. Regulated sales of wild-harvested peyote by licensed distributors in the U.S.A. total ca. 1.5 million “buttons” (harvested tops of stems) per year (Texas Department of Public Safety, *in litt.* 2011).

One reason for analyzing the tissue mescaline concentrations in four geographically disjunct populations of peyote is based in ethnobotany. Thomas Blackstar, the most senior of the elders of the NAC in the Comanche tribe (which is centered in southern Oklahoma near Lawton), stated anecdotally that there is a preference among some Native Americans (notably the Kiowa) for the “purple peyote” of West Texas (Blackstar, pers. comm.). The name “purple peyote” refers to seasonally drought-stressed plants which appear to have resorbed and metabolized much of their chlorophyll, leaving the reddish betalain pigments as the dominant chromogenic compounds (Fig. 2).

A reasonable ethnobotanical interpretation of Blackstar’s statement—and one that is considered valid by other NAC members in Oklahoma (Terry, pers. obs.)—is that the preference for peyote in this “purple” condition for ceremonial use is based on the perception that it is “stronger” (a word that suggests higher mescaline content) than ordinary, non-stressed, gray-green to blue-green peyote (as shown in Fig. 1). An illuminating fact is that this cultural preference for purple peyote is also a *de facto* geographical preference, since the purple peyote normally occurs only in the Chihuahuan Desert region of Trans-Pecos Texas and adjacent Mexico. The present study was accordingly designed to determine whether there were greater concentrations of mescaline in the Chihuahuan Desert populations of West Texas than in the Tamaulipan Thornscrub populations of South Texas (Fig. 3).

Another reason for doing this phytochemical analytical study of geographically disjunct populations of two ecologically different regions was the potential chemotaxonomic value of such a study. There has been discussion in the literature since the 1940’s about possible taxonomic differences between the Chihuahuan Desert populations and the Tamaulipan Thornscrub populations of *Lophophora williamsii* (Croizat 1944; Weniger 1970; Bravo 1971; Powell and Weedin 2004). One quantitative character that has not previously been evaluated in this regard is tissue concentration of mescaline, which might reasonably be expected to vary from one ecoregion to another by virtue of variation of the status of regulatory genes affecting the rate of biosynthesis, which would be an example of the phenotypic plasticity described by Schlichting and Smith (2002). It could be of taxonomic value to determine whether the Chihuahuan Desert populations show inherently different (in either direction, higher or lower) tissue concentrations of mescaline than the Tamaulipan Thornscrub populations. Such a quantitative phytochemical difference—or lack thereof—would add to the evidence available for evaluating the validity of recognizing of a new variety of *L. williamsii*. The original chemotaxonomic hypothesis was that there is a significant difference in the tissue mescaline concentrations of plants sampled from the two geographic regions.

Alternatively, the mescaline concentrations may increase in a gradual fashion across the entire Texas portion of the range of this species. The four populations studied fell along a rough gradient from southeast to northwest: from Starr County (RES) north to Jim Hogg County (LMR) in the Tamaulipan Thornscrub ecozone, then northwest to Val Verde County (LTR) and west to Presidio County (STR) in the Chihuahuan Desert ecozone (Fig. 3). In terms of the gradient, the appropriate ethnobotanical hypothesis is that mescaline concentrations increase along this gradient, and the appropriate chemotaxonomic hypothesis is that they differ, in either direction, along the gradient.

MATERIALS AND METHODS

Samples of aerial stem tissue (ca. 4 g fresh weight from each individual) were field-collected by biopsy from 10 individuals in each of four Texas populations (designated RES, LMR, LTR and STR) of *Lophophora williamsii*: two in the Tamaulipan Thornscrub (RES in Starr County and LMR in Jim Hogg County) and two in the Chihuahuan Desert (LTR in Val Verde County and STR in Presidio County). (See map, Fig. 3.)



FIG. 2. "Purple peyote" in habitat in Chihuahuan Desert. Plant shows reddish betalain pigment and depleted chlorophyll resulting from the severe drought of 2010–2011.

In order to eliminate the possible confounding factors of drought stress and dehydration from this geographic phytochemical analysis, we collected all the peyote tissue samples during the rainy season in July 2010 and desiccated the tissue samples prior to analysis. There is an untested but prevalent belief among NAC members that older/larger peyote plants constitute "stronger medicine" than younger/smaller plants (Teodoso Herrera, pers. comm. based on his personal experience as spiritual leader of the Native American Church of the Rio Grande, and on the opinions of his numerous NAC colleagues). Accordingly, in order to eliminate the possible confounding factor of different stages of maturity of the plants sampled for this study, we collected tissue samples exclusively from individuals with eight ribs, which are young adult plants that are typically ca. 3–5 cm in diameter (Terry et al., unpublished data).

The samples of cactus tissue were cut into small slices, and the individual samples were pooled by population and set to dry for a week on a drying rack. Once desiccated, the tissue was ground to a fine powder with a mortar and pestle.

A sample of 2.0 g of the ground dry cactus tissue from each population was then Soxhlet-extracted with 200 ml HPLC-grade methanol for 8 h. The methanol extract was evaporated to dryness, and the residue of the extract was dissolved in 200 ml HPLC-grade water, which underwent acid-base extraction with dichloromethane, as described in Ogunbodede et al. (2010). The dichloromethane was evaporated to dryness, and the residue, containing the mescaline and related alkaloids, was redissolved in 10.0 ml of methanol and stored at -20°C in a labeled vial. The four extracts representing the four populations were run on an Agilent 1260 Infinity HPLC, using 9:1 water to acetonitrile acidified with 0.1% trifluoroacetic acid as the mobile phase, and a Phenomenex Gemini $5\mu\text{C}18$ column. Samples of 1.0 μL were injected with a flow rate of 1.2 ml/min and run for 30 min. Each of the four samples was run three times and the values of area under the curve (AUC) for the three peaks per sample were averaged. A standard curve of mescaline standard was generated and used to in-

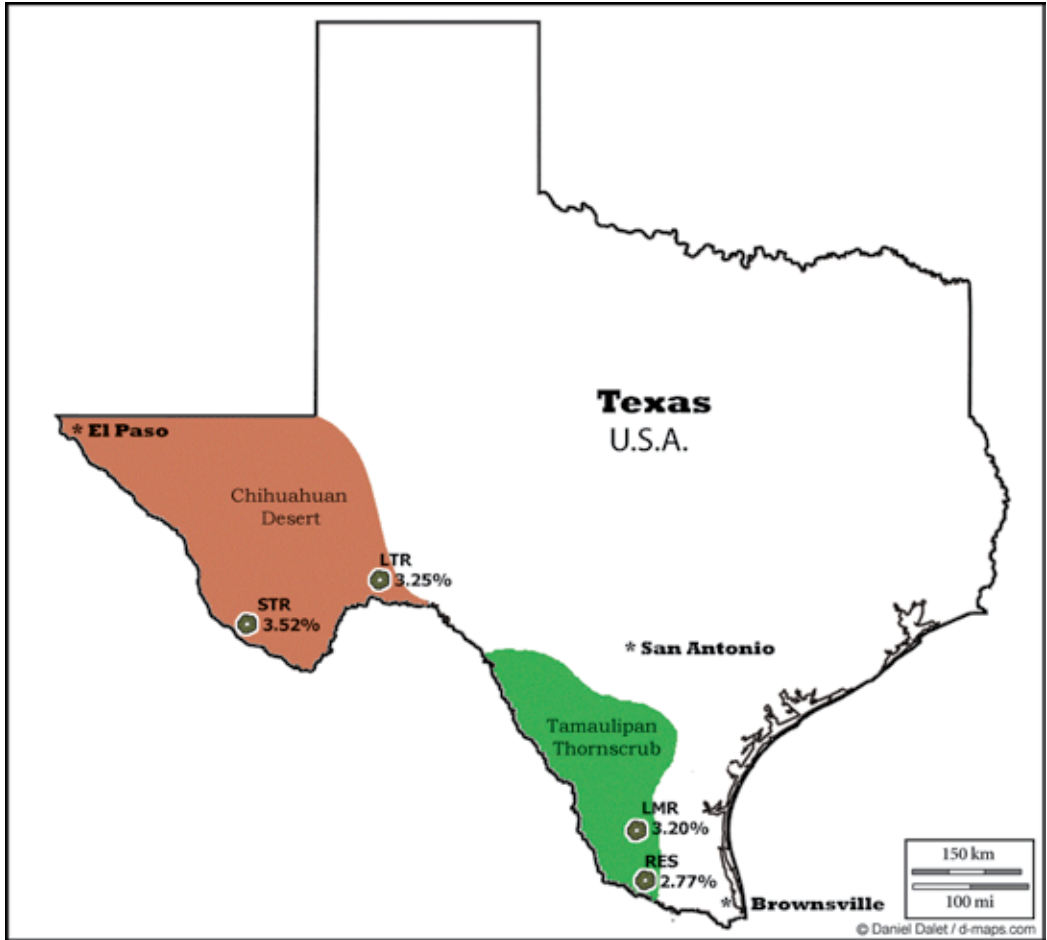


Fig. 3. Map of sampled populations of peyote, showing geographic location and average mescaline concentration of each population. Note slight and irregular—but continuous—increase in mescaline concentration from one population to the next in geographic order from southeast to northwest.

terpolate the concentration of mescaline corresponding to the mean AUC of each sample. Then back calculations were done to determine the original concentration of mescaline (as percent, w/w) in the desiccated tissue samples from the four populations of peyote.

Confirmation of the identity of mescaline in these samples was achieved with GC-MS. A sample methanol extract of *L. williamsii* from this study was evaporated to dryness under an N_2 stream at room temperature, and the residue dissolved in dichloromethane for analysis. The instrumentation was an Agilent 6890 GC equipped with a DB-5ms (0.25 mm I.D. x 30 m) column, splitless injection (250°), and an Agilent 5972 MSD (transfer line at 275°), operated in full scan mode. Helium was used as the carrier, and the oven program was 70° (with a 1 min hold), then 20°/min to 250°, with a final hold.

RESULTS

On GC-MS, the mescaline peak had an identical retention time to an authentic reference sample, and the mass spectrum matched a spectrum in the NIST database (Stein et al. 2005) with 93.5% probability of best match.

The average mescaline concentration for each population sampled is presented along with the geographic

location of the population in Fig. 3. There was limited variation among the four samples, as the highest concentration of mescaline (3.52%, in STR) was only ca. 27% greater than the lowest concentration (2.77%, in RES). A *t*-test comparing Chihuahuan Desert and Tamaulipan Thornscrub populations was not significant ($P = 0.26$)—not surprising given the small number of populations sampled and the resulting low statistical power.

However, the order of increasing mescaline concentrations in the four populations sampled matched our suggested geographic gradient exactly. The lowest concentration was in the Starr County population (RES), at the southeastern end of the gradient, and the highest concentration was in the Presidio County population (STR), at the northwestern end of the gradient. Of the 24 possible permutations in which the four different mescaline concentrations of the four populations could be ordered, only two would match the gradient: one of them with the mescaline concentrations increasing from southeast to northwest, and the other one with the mescaline concentrations decreasing from southeast to northwest.

Under the gradient version of the chemotaxonomic hypothesis, we are asking whether there are consistent differences in mescaline concentrations along the gradient in either direction, and so a two-tailed statistical test is appropriate. The probability of obtaining one of the two permutations with the geographic order of populations the same as the order of the mescaline concentrations in either direction is 2 out of 24, or $P = 0.083$, not significant but suggestive of a possible geographical pattern (cline) in mescaline tissue concentration in this species.

Under the gradient version of the ethnobotanical hypothesis, we are asking specifically whether mescaline concentrations increase from southeast to northwest in Texas, and the appropriate statistical test is therefore one-tailed. Only one of the 24 permutations of the four populations produces a match between geographic order from southeast to northwest and order of increasing mescaline concentrations, and that is the permutation obtained in this study. That outcome has a probability of 1 out of 24, or $P = 0.042$. That is to say, the mescaline concentrations significantly increase in Texas along this southeast-to-northwest gradient.

DISCUSSION AND CONCLUSIONS

It is important to understand the pharmacological relationship between mescaline concentration in the plant and the dose of mescaline ingested by a person in a peyote religious ceremony. If peyote is available from two populations, A and B, and the peyote from A contains a mescaline concentration 25% higher than that of the peyote from B—all other things being equal—a given person can attain exactly the same dose by eating 100 g of A or 125 g of B. And in a peyote meeting where each participant titrates his/her own dose to attain the desired subjective spiritual effect, the difference between peyote from A and peyote from B would not be viewed as a matter of concern (Teodoso Herrera, pers. comm.). However, if the difference in mescaline concentration were considerably greater, such that a person would have to ingest a much larger quantity of the peyote from B to attain the same dose of mescaline as a person ingesting peyote from A—as might be the case where the peyote from B consisted of small, immature plants that would taste “sweet” due to their low alkaloid content—then the peyote from B would be deemed undesirable by many members of the peyote religious community, both because of the low levels of mescaline and because eating immature plants would deny the plants the opportunity to reproduce themselves (Herrera, pers. comm.). The range of mescaline concentrations seen in this study is more reflective of the former situation, where the difference between the high and low extremes is perceptible but not objectionable.

One factor that may have affected the average mescaline concentration in the RES population of Starr County is that we found considerable evidence of previous harvesting of the peyote in that population (e.g., groups of small regrowth plants as shown in Fig. 1b). Extensive photographic documentation of such post-harvest regrowth in this population can be seen in Terry and Mauseth (2006). If it were the case that peyote crowns that grow as lateral branches from the subterranean stems of harvested plants are, at least for several years, deficient both in size and in mescaline content—as is widely believed but only partially documented (Terry et al. 2011)—then it would be reasonable to expect that a population such as RES, in an area known for intensive commercial peyote harvesting, would show evidence of such harvesting in the form of depression of the average mescaline content of the population.

While we were not able, in this study, to differentiate statistically between the mescaline concentrations of Tamaulipan Thornscrub and Chihuahuan Desert peyote populations, our results suggest a likelihood that such a difference may exist. We were also able to demonstrate a significant geographical pattern consistent with somewhat higher concentrations in populations located in the more northern and western regions of the range of the species in Texas. These first results on how tissue levels of the principal peyote alkaloid vary with geography are compatible with the anecdotal Native American perception that peyote from Texas populations of the Chihuahuan Desert is more efficacious as a pharmacologically active religious sacrament than peyote from populations farther to the south and east in the Tamaulipan Thornscrub. Geographically more extensive fieldwork is in progress to obtain *L. williamsii* tissue samples from a larger number of populations, which will afford the statistical power required for more definitive resolution of the chemotaxonomic question.

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