# A TALE OF TWO CACTI:

# STUDIES IN *ASTROPHYTUM ASTERIAS*

# AND *LOPHOPHORA WILLIAMSII*

A Dissertation

by

MARTIN KILMAN TERRY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Botany

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| | Alan E. Pepper |
| Committee Members, | Thomas DeWitt |
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December 2005

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## ABSTRACT

A Tale of Two Cacti:

Studies in *Astrophytum asterias* and *Lophophora williamsii*.

(December 2005)

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Dr. Alan E. Pepper

*Astrophytum asterias* (star cactus) and *Lophophora williamsii* (peyote) are sympatric species in the Tamaulipecan thornscrub of South Texas and adjacent Mexico. Peyote has been excavated from two archaeological sites: Shumla Caves, Texas, and CM-79 in Coahuila. We report new radiocarbon dates: a mean of 5195 ± 20 $^{14}$C years BP for the Shumla Caves specimens, and 835 ± 35 $^{14}$C years BP for the CM-79 specimen. The Shumla Caves specimens were not intact peyote tops, but manufactured effigies thereof.

Published data on the geographic ranges of *L. williamsii* and *A. asterias* are of varying quality and accuracy. We report the results of extensive research to document extant U.S. populations by county, drawing specific conclusions about where each species currently occurs, where its occurrence is uncertain and where it is unlikely, based on herbarium specimens, verifiable reports in the primary literature and interviews with knowledgeable individuals.

Dwindling of populations of peyote is partly due to improper harvesting, namely cutting off the top of the plant so deeply below ground level that the plant is unable to regenerate new stems, and consequently dies. We describe the anatomy of the cactus shoot (stem) and root, and suggest how this new knowledge can be utilized to determine "how deep is too deep" to cut if harvesting of peyote is to be done sustainably.

We report the first population genetics study on endangered *A. asterias*, with five microsatellite markers in populations sampled at four locations in South Texas. A battery of tests and measurements indicated that in most populations heterozygosity was high, F-statistics were low, and *Nm* was >1. With one exception, these populations appear not to be undergoing excessive inbreeding, despite small population sizes.

Data from two *L. williamsii* microsatellite loci are presented. *L. williamsii*, which reproduces autogamously, exhibits a single homozygous genotype within a given population. West Texas *L. williamsii* plants differ from South Texas plants in the identity of the single allele (or single genotype) at each locus. The ability of microsatellite markers to separate West Texas from South Texas plants suggests utility of microsatellites for infraspecific taxonomic studies in *Lophophora*.

## ACKNOWLEDGEMENTS

People who contributed, directly or indirectly, knowingly or unknowingly, positively or negatively, to the present work, include the following (in semi-random order):

Marilyn Terry enhanced the quality and the enjoyment of the fieldwork, and managed to put up (cheerfully for the most part) with the many inconveniences associated with her husband's obligation to finish the research and dissertation under a rigid deadline.

Richard Evan Schultes introduced me to ethnobotany in his signature course, *Plants and Human Affairs*. Schultes also demonstrated, in the way he molded his career to follow in the geographic and professional footsteps of Richard Spruce, how a dead hero can serve as a perennial mentor who only encourages and cannot annoy.

K. Trout redesigned and tweaked the maps in this dissertation until they did what they were intended to do. Trout has also been a constant source of insightful comments and helpful information, both from the literature and from his own experience with cacti.

A. Michael Powell, in addition to being a friend and colleague, has provided invaluable information on the taxonomy and biogeography of cacti. Shirley Powell has been supportive as both friend and photographer (Figs. 4.14 and 4.15).

Robert ("RT") Turner helped with transects in the field and DNA manipulations in the lab, and his sense of humor keeps the people around him sane – or appropriately nutty, as the situation demands.

Billie Turner was always ready to indulge in the delights of fieldwork, and was a whirling dervish of applied knowledge in the herbarium.

Pierre Gambart sussed out and had photographed the essential pages of Rouhier's 1926 D. Pharm. thesis in Paris. He also supplied helpful photographs of cacti in cultivation and a European perspective on the world of cacti and cactophiles.

James Manhart, my Committee Co-Chair, saw immediately on the day I met him that this cactus project would be interesting, and has subsequently given to the project far more than could reasonably be expected of him. My hat is off to both Jim and his wife Nanette for putting me up – and putting up with me – during the long hot summer of the "deadline for data".

Alan Pepper, my other Committee Co-Chair, keeps everyone on their toes and up to date with the cutting edge of science. Alan is one of those rare individuals who is truly fluent and happy both in the field and in the lab.

James Woolley, Committee member, teaches systematics with aplomb. He also writes in a laconic style very different from my own, as seen in many of the forcibly revised passages of this document. (Listen for the groans of the sentences suffering from restrained construction and anemic word choice.)

Thomas DeWitt, Committee member, conveyed an interest in population genetics that was patently contagious.

Of great value in regard to both the *Astrophytum* work and the *Lophophora* work was the cheerful willingness of our colleagues in the Texas Parks and Wildlife Department to consult with us frequently on an *ad hoc* basis about the ever-changing state of the landscape (botanical, political and social) in South Texas. In particular, Dana Price, Jackie Poole, and their consultant Gena Janssen, were helpful on many occasions.

For help on the ground in the Tamaulipecan thornscrub we particularly thank Lisa Williams of the South Texas office of The Nature Conservancy for giving us the literal and figurative keys to the gate, Tom Patterson for showing Marilyn and me a *Lophophora* population in the dark when we ran out of daylight, Chris Best for his knowledge of the plants and the terrain and his *simpático* sense of humor, and Anna Strong for her absolutely crucial help in locating and sampling accessible populations of *Astrophytum* when they were in flower.

For patiently struggling through countless rewrites of the peyote archaeobotany manuscript, I thank Marvin Rowe, Phil Dering and Karen Steelman.

I am grateful to James Mauseth for being able to grasp the concept of "anatomy as conservation" and cheerfully investing his energy into the manuscript that became Chapter IV.

For the generous cooperation of private landowners who opened their minds and their gates to our various research projects, I simply say, "Thank you very much!" to all.

For support of the archaeobotanical radiocarbon dating work on peyote, we thank E. Phelps (Witte Museum) and W. Fitzhugh (Smithsonian Institution) for access to the Shumla Caves and Cuatro Ciénegas peyote specimens, respectively. This work was supported by a National Science Foundation Archaeometry Grant No. 0209312, University of California Office of the President/CAMS minigrant program, and a grant from the Summerlee Foundation. Radiocarbon analyses were performed under the auspices of the U. S. DOE by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

For support of the population genetics work on *Astrophytum asterias*, we thank Loretta Pressly, her colleagues at the U.S. Fish and Wildlife Service, and their counterparts at the U.S. Geological Survey, for providing the wherewithal to get the work done, in the form of the grant "Population genetics of endangered plant species in the lower Rio Grande corridor", USGS Cooperative Agreement # 02HQAG0113.

For support of the *Lophophora* fieldwork and/or labwork, we gratefully acknowledge a research grant from the Cactus and Succulent Society of America, a travel grant from the L.T. Jordan Institute for International Awareness, a research enhancement grant to Dr. James Manhart from the TAMU College of Science, a research enhancement grant from Sul Ross State University, and minigrants for lab supplies from the TAMU Department of Biology.

Last, a nameless administrator in Alpine, in an unexplained fit of adhockery, promulgated the implicit new ruling that two doctoral degrees in the life sciences are not sufficient to qualify one to teach biology at Sul Ross. Without such arbitrary interference and the concomitant constraints imposed on the structure and extent of the research by the short contractual deadline he imposed, I would undoubtedly have designed and executed some of the studies described in this dissertation in a different manner. But the unintended good from that proverbial ill wind is that without the deadline, those studies would have taken considerably longer than they actually did.

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# CHAPTER I

# INTRODUCTION

## *Astrophytum asterias* and *Lophophora williamsii*: two cacti with entangled fates

This dissertation examines aspects of two cactus species: (1) *Astrophytum asterias* (Zuccarini) Lemaire 1868 (star cactus), which is federally listed as an endangered species (U.S. Fish and Wildlife Service 1993, 2003), and (2) *Lophophora williamsii* (Lemaire ex Salm-Dyck) J.M. Coulter 1894 (peyote), which is not endangered at the species level, but which in many locations is endangered at the population level. These two cacti provide ongoing case studies in the conservation of "hunted species" – *Lophophora williamsii* being commercially hunted for its psychoactive and/or religious properties, and *Astrophytum asterias* being hunted both commercially as a cactus collector's prize and incidentally in conjunction with commercial peyote harvesting. Second, these two cacti are superficially similar in gross stem morphology to the extent that they are frequently not distinguished from each other in the field by humans whose economic activities may adversely affect one or both species. Third, these two species are not merely sympatric; they frequently share the same habitat, sometimes growing only a few centimeters apart, increasing the probability that an inexperienced person collecting one will also inadvertently collect the other. Accordingly, some of the

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This dissertation follows the style of *Molecular Ecology*.

chapters in this dissertation, such as the ones on geographical distributions (Chapter III) and DNA sequence-based population genetics (paired Chapters V and VI), present similar treatments of both species. But these two species also have their individual characteristics that they do not share. For example, *Lophophora williamsii* – but not *Astrophytum asterias* – has been excavated from archaeological sites, yielding archaeobotanical information about the use of *L. williamsii* in human prehistory (Chapter II). Similarly, the study on the anatomy of root vs. shoot (Chapter IV) is focused almost entirely on *L. williamsii*, as the method of harvesting and its consequences for the survival of the decapitated cactus have for decades been the subject of debate with regard to *L. williamsii*, but are not relevant to *A. asterias*, given its completely different root and stem morphology. In the reporting on the population genetics studies in Chapters V and VI, there is clearly more emphasis – and substantially more data – on the endangered species *A. asterias* than on *L. williamsii*. In Chapter VI, preliminary (and, we believe, representative) data on *L. williamsii* are presented for purposes of comparison to the *A. asterias* data, to illustrate the genetic consequences of the starkly different breeding systems of the two species. The *L. williamsii* data may also have infraspecific taxonomic implications.

**Fig. 1.1** Endangered *Astrophytum asterias* (star cactus), in habitat in Starr County, Texas.

### *Astrophytum asterias* and *Lophophora williamsii*: similarities and differences

*Astrophytum asterias*, commonly known as star cactus, is a small, spineless cactus with a single, low, dome-shaped stem that becomes flat or depressed during drought conditions.

In the wild, star cactus grows to 7 cm tall and 15 cm in diameter. Plants are variable in color, and may be green, grayish-green, yellow, orange (to almost red) or brown, patterned with minute whitish to yellowish epidermal scales. Each plant normally has eight triangular stem ribs separated by narrow sulci. The areoles radiate from their origin at the apical meristem, along a line down the middle of each rib, and bear tufts of short, whitish hairs. The pale yellow flowers with orange-red interior bases appear from mid-March through May (Figs. 1.1, 1.2) (Benson 1982; Damude and Poole 1990). Star cactus was listed as endangered by the US Fish and Wildlife Service (USFWS) in 1993 due to

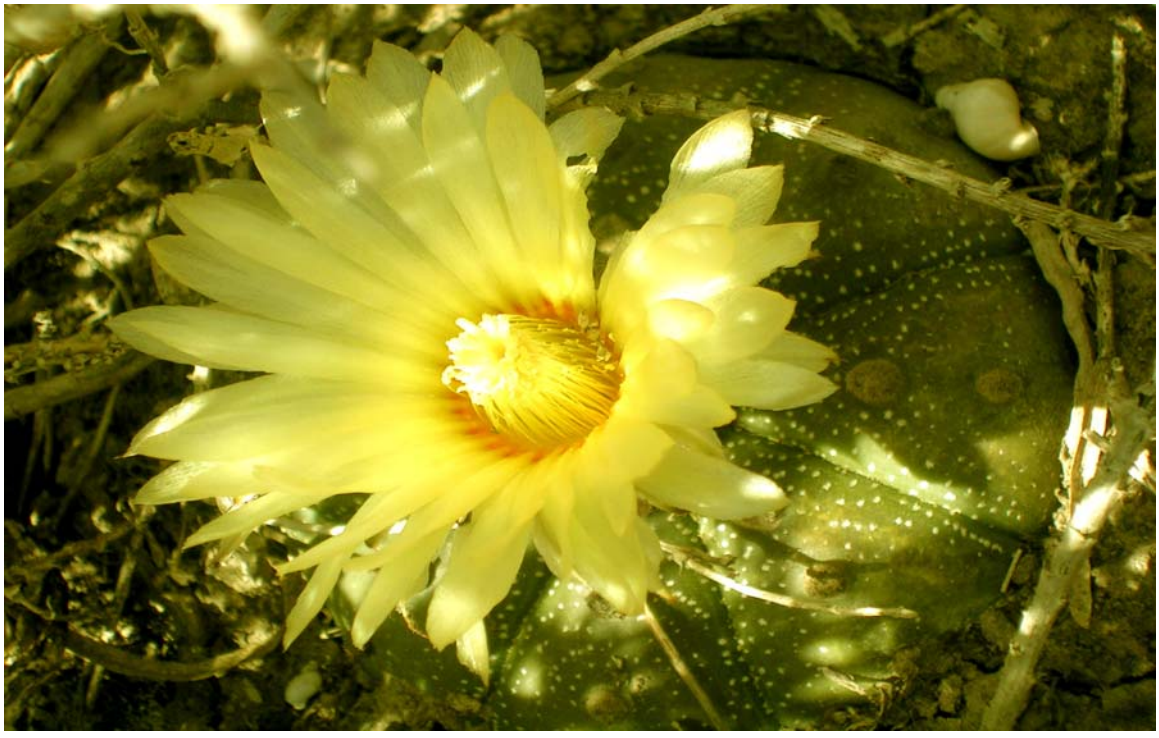

**Fig. 1.2** *Astrophytum asterias* in habitat, showing interior of flower with red throat.

its few populations and high degree of threat from collecting, and a recovery plan for the species was issued by USFWS in 2003. *A. asterias* is also listed in Appendix I of the Convention on International Trade in Endangered Species (CITES 2005). Star cactus has for decades been an extremely popular collector's item. Even though it is easily grown from seed, plants continue to be taken from the wild (Weniger 1970; Janssen et al. 2004).

*Lophophora williamsii*, commonly known as peyote, resembles star cactus in its size, shape and lack of spines. However, peyote is bluish-green and lacks the tiny whitish epidermal scales found on star cactus. Peyote has ribs numbering 5-13 (most often exactly 5, 8 or 13, following the Fibonacci series), the number increasing with size and age. Star cactus, in contrast, generally has exactly 8 ribs throughout life. Peyote's ribs may extend toward the base in a spiral conformation not seen in star cactus. Mature specimens of *L. williamsii* may have pronounced tubercles (Fig. 1.3), which give the ribs an irregular appearance not observed in *A. asterias*. The flowers of South Texas peyote appear pale pink to almost white in color (Fig. 1.4), whereas star cactus has brilliant yellow flowers with a red throat (Fig. 1.2).

**Fig. 1.3** *Lophophora williamsii* (peyote), in habitat in Starr County, Texas.
Old plant with 13 ribs surrounded by younger plants showing variation in number of ribs. The younger plants are most likely progeny of the old plant. Most or all of them probably originated from seed from the parent plant, but some may have arisen as vegetative clones of the parent plant, from lateral branching of the subterranean stem of the parent plant.

**Fig. 1.4** Peyote in flower, Starr County, Texas.

Peyote has a large, conical taproot, whereas star cactus has a very small taproot with multiple, relatively large lateral roots, most of which branch from the proximal portion of the taproot, giving the impression of a fibrous root system as seen in monocots (Fig. 1.5).

**Fig. 1.5** Taproot of peyote (left) and roots of star cactus (right). Peyote has a large subterranean stem and tapering taproot, with few lateral roots. In contrast, star cactus has a diminutive taproot, with more numerous, relatively large, lateral roots bearing many small, multiply branched, secondary branches.

## Threats to star cactus and peyote

### *The commercial peyote trade*

It is widely recognized that the commercial harvesting of peyote plants for sale to the Native American Church over the past century has resulted in a decrease in the number, size, extent and density of peyote populations in South Texas (Anderson 1995, Moreno

2005). Less obvious is the effect of the peyote trade on star cactus. The licensed peyote distributors in Starr County maintain devotional peyote gardens at their places of business, offering their Native American Church customers the opportunity to visit and pray. These gardens include specimens of both peyote and star cactus (Fig. 1.6)**.** It is unclear whether the star cactus plants in these gardens are collected deliberately or accidentally by the harvesters who sell peyote wholesale to the licensed distributors. On infrequent occasions the NAC members take a specimen of star cactus back to their homes as a (presumably nonconsumable) souvenir from the "peyote gardens" (meaning the Tamaulipecan thornscrub ecoregion) of South Texas. Though the intention is to maintain these star cacti indefinitely in cultivation, damage to the root system that occurs in the collection process assures that most of these plants dug up from their natural habitat will die in a few months. But even if some of these "exiled" plants survive, the act of removing such a plant from the population of which it was an element, renders it effectively dead in terms of its potential contribution to the genetics of the wild population.

**Fig. 1.6** Star cactus (center) growing with peyote in a peyote distributor's devotional garden.

Star cactus is currently known from only a few populations in Texas (Janssen et al. 2004) and a few more in Mexico (Martinez Avalos 2002). Recent field surveys estimate the total number of known individuals of star cactus in the U.S. (i.e., in Starr County, Texas) at no more than about 2400 individuals spread over seven properties (Janssen et al. 2004).

The licensed peyote distributors in Starr County report that peyote harvesters rarely bring in specimens of star cactus; one distributor estimated that one plant in a thousand

(0.1% of the peyote harvest) might be star cactus. In recent years, the regulated annual harvest of peyote in Texas has fluctuated around 2,000,000 buttons (Texas Department of Public Safety, unpublished data 2005)**.** Incidental harvest of star cactus at a rate of 0.1% of peyote harvests would thus result in an annual "take" of nearly 2,000 individuals – a figure of the same order of magnitude as the total number of individuals of star cactus currently known to exist in documented populations in Texas.

*Damage by herbivores*

One might expect that the presence of high concentrations of alkaloids in *L. williamsii* would render the plants sufficiently unpalatable to afford some protection from herbivory. However, this is not always the case. Moderate to severe damage (including the total obliteration of the central apical meristem in some individuals) was evident in populations of peyote in Starr County in December 2004 (M. Terry, personal observation). It should be noted, however, that all of the 25 herbivore-damaged peyote plants observed had survived by September 2005, and those that had lost their apical meristem had by that time begun to develop new stems with new apical meristems by basal branching from aerial or subterranean stems (Fig. 1.7). This outcome would suggest that the damage caused by (non-human) herbivores in wild populations of *L. williamsii* is not life-threatening to the plants.

**Fig. 1.7** *Lophophora williamsii* showing absence of apical meristem and surrounding tissue (large depression at center of plant) due to damage by herbivore ca. nine months prior to time of photograph. New stem branch is erupting through the epidermis from an areole at the base of the crown (left side of plant).

The effects of herbivory on the endangered *Astrophytum asterias* are of greater concern. The recent occurrence of damage to numerous plants by herbivores (L. Williams, personal communication 2002) may be creating a negative impact on effective population size ($N_e$) by increasing the rate of mortality by making the plants vulnerable to infections of the open wounds (especially when rainfall occurs prior to callus formation) and probably by suppression of seed production due to damage to the apical

meristem (Fig. 1.8). The occurrence of regenerated stems appears to be rare in wild populations of *A. asterias* (M. Terry, personal observation), suggesting that nonfatal but irreversible damage to an apical meristem is likely to result in a permanently sterile individual.

**Fig. 1.8** Damage to the stem, including the apical meristem region, of *Astrophytum asterias*, by an unknown but evidently common herbivore. The damage here is very recent, as evidenced by the small green pieces of unpalatable dermal tissue which the herbivore left unconsumed on the ground just above the gnawed star cactus specimen.

**Habitat and ecology of *Astrophytum asterias* and *Lophophora williamsii***

Within the Tamaulipecan thornscrub, star cactus grows in gravelly clays or loams, on gentle slopes in sparsely vegetated openings between shrub thickets within mesquite-blackbrush thorn shrublands (Fig. 1.9). Associates of both *Astrophytum* and *Lophophora* in South Texas include the shrubs *Prosopis glandulosa, Castela erecta, Acacia rigidula, Ziziphus obtusifolia, Koeberlinia spinosa, Forestiera angustifolia, Guaiacum angustifolium, Karwinskia humboldtiana* and *Varilla texana;* the grasses *Bouteloua trifida*, *Monanthochloë littoralis*, *Aristida* spp. and *Hilaria belangeri*; and numerous cacti, including *Opuntia leptocaulis, Echinocactus texensis, Mammillaria heyderi* and *Echinocereus reichenbachii* (Damude and Poole 1990; The Nature Conservancy of Texas, unpublished data; M. Terry, personal observation). *Castela erecta* and *Opuntia leptocaulis* have been documented as important nurse shrubs for star cactus in Mexico (Martinez Avalos 2002). In Texas populations, *Varilla texana* is also a very common nurse plant for star cactus (M. Terry, personal observation).

**Fig. 1.9** Area of Tamaulipecan thornscrub, habitat for peyote and star cactus.

*Astrophytum* and *Lophophora* may be found in close proximity within such thornscrub habitat, sometimes growing together under the same nurse shrub (Fig. 1.10)**.** More often, however, the two species appear to use slightly different microhabitats. For example, we have more often observed *Lophophora* near the base of shrubs while *Astrophytum* more often grows farther out under the edge of the shrub's canopy or even in the open. There also appear to be edaphic preferences that tend to separate the two

species, with *Astrophytum* preferring coarser, more gravelly soils and *Lophophora* preferring finer, more clayey soils with less gravel. Further investigation is needed to characterize more precisely how these two cacti partition the habitat.

**Fig. 1.10** Peyote (left) and star cactus (right) growing together under canopy of *Krameria ramosissima.*

**Geographic ranges of *Astrophytum asterias* and *Lophophora williamsii* in the U.S.**

The range of *Lophophora williamsii* includes both the Tamaulipecan thornscrub and the Chihuahuan desert (Rouhier 1926, 1927; Anderson 1996a), while *Astrophytum asterias* has a much more restricted range, essentially limited to the Tamaulipecan thornscrub (Damude and Poole 1990; Martinez Avalos 2002; Sanchez-Mejorada et al. 1986). The ranges of the two species overlap in the Lower Rio Grande Valley of South Texas, USA, and in northern Tamaulipas and Nuevo Leon, Mexico. A county-by-county delineation of the geographic ranges of these two species in the U.S. is presented in Chapter III. The objective is to define the range of each species within the limits of what can be currently and reliably documented, based on herbarium specimens, specific locations reported in the primary literature, and recent observations of knowledgeable individuals. The null hypothesis is that the previously published descriptions of the geographic ranges of these species are currently accurate. The alternative hypothesis is that they are not. Specific inaccuracies (which call for rejection of the null hypothesis) will be noted.

**Phylogenetic relationships**

*Astrophytum asterias* and *Lophophora williamsii* are both members of the tribe Cacteae within the subfamily Cactoideae of the family Cactaceae (Anderson 2001). The phylogenetic relationships among members of the Cacteae have been examined by Butterworth et al. (2002) using chloroplast DNA sequences based on those portions of the *rpl16* intron that could be sequenced. In their analysis, *Astrophytum capricorne* and

*Astrophytum myriostigma* fell into the "*Echinocactus* clade" (which also contained *Echinocactus horizonthalonius*, *Echinocactus ingens* and *Homalocephala texensis*). The latter clade formed a sister clade to all the rest of the members of the Cacteae except the "*Aztekium* clade" (which, in addition to *Aztekium hintoni* and *Aztekium ritteri*, also contained *Geohintonia mexicana*), the most basal clade of the tribe. The two recognized *Lophophora* species (the wide-ranging *L. williamsii* and the Mexican endemic *L. diffusa*), along with *Acharagma aguirreana*, *Acharagma roseana* and *Obregonia denegrii*, constituted the "*Lophophora* clade". The latter clade was the fourth most basal clade in the tribe Cacteae, separated from the *Astrophytum*-containing *Echinocactus* clade by a monogeneric clade consisting of three species of *Sclerocactus*. An unexpected finding was that the genus *Lophophora* was polyphyletic: *L. williamsii* was grouped with the two *Acharagma* species, while *L. diffusa* was grouped with *Obregonia denegrii*. This result, which suggests the need for a total rethinking of the genus *Lophophora*, clearly requires confirmation or modification based on further molecular studies.

Phylogenetic relationships among ill-defined geographic races of the species *L. williamsii* have been problematic for decades (Klüver 1928; Anderson 1961). Such races (in some cases distinguished by nothing more than flower color) have been variously recognized as separate species (Habermann 1974) or varieties (Weniger 1970), and the possibility of a "compromise" of resorting to the subspecies (an intermediate taxonomic level seldom used in botany) for some of these races has been discussed among cactus systematists (G. Rowley, personal communication 2005). The current consensus in the U.S. is not to recognize such geographic races at all (Anderson 2001). The preliminary

microsatellite data reported in Chapter VI suggest potential utility of microsatellites as genetic markers for clarifying the relationships among such races, particularly those which are clearly geographically disjunct.

**Anatomy of root and shoot, and its relation to peyote conservation**

It has long been argued that traditional peyote harvesting techniques are optimal for ensuring regrowth of harvestable stem tissue. Yet there is perennial controversy among stakeholders in the peyote trade about how deeply a harvester can cut the subterranean portion of the peyote plant in the process of removing the pharmacologically active top of the stem, without jeopardizing the plant's capability to regenerate new stems with harvestable crowns. It is known that in *L. williamsii* adventitious roots commonly develop from stem tissue, particularly regenerating stem tissue (Terry and Mauseth, unpublished data 2005), but the notion that adventitious stem tissue would develop from root tissue is problematic and such development has not been observed empirically. Chapter IV explores the anatomical distinction between stem and root to provide a basis for resolving the practical question of how deeply the peyoteros may cut when they harvest peyote, if this resource is to be managed in a sustainable mode.

**Population genetics studies**

Molecular population genetics studies in cacti are just beginning to be evident in the literature, and suitable molecular markers such as microsatellites have only recently been isolated (Otero-Arnaiz *et al.* 2004; Otero-Arnaiz *et al.* 2005). Most published studies have examined genetic variation indirectly, using phenotypic characters such as allozymes (Hamrick *et al.* 2002). It should be noted that almost all of the few studies published to date on the population genetics of cacti have been done in predominantly outcrossing species. The findings in those species, reviewed by Hamrick *et al*. (2002), were that, while most of the genetic diversity occurred within populations, the observed genetic diversity within populations was somewhat lower than expected. The authors attributed the excessive homozygosity within populations to local biparental inbreeding and/or a Wahlund effect associated with undetected population substructure. In the same studies, the genetic diversity among populations was generally "quite low". At the species level, however, the cactus species in the studies reviewed by Hamrick *et al.* (2002) showed high genetic diversity in comparison to other plant species. In one study where the cactus species investigated (*Pachycereus schottii*) reproduced both by outcrossing and (predominantly) by vegetative cloning through passive dispersal of stem fragments, the majority of the genetic diversity was within populations, but there was also "appreciable heterogeneity" among populations (Parker & Hamrick 1992).

In Chapter V of this dissertation, a number of conservation-related questions are addressed through studies of population genetics using microsatellite allele data. *Astrophytum asterias* will be examined in some detail as a species known to be an

obligate outcrosser (Rowley 1958; Strong & Williamson 2005). A modicum of comparative data will be presented on *Lophophora williamsii* as a reasonably closely related species (*vide supra*) whose breeding system appears to be markedly different, involving a high degree of selfing (Rowley 1980; M. Terry, personal observation).

# CHAPTER II

# ARCHAEOBOTANY OF *LOPHOPHORA WILLIAMSII*

## Synopsis

*Lophophora williamsii* (peyote), a psychoactive cactus native to the Chihuahuan Desert, is used throughout North America for medicinal and ceremonial purposes. Peyote has been adequately documented at only two archaeological sites. We determined a mean age of 5195 ± 20 $^{14}$C years BP for all three specimens from Shumla Caves in southwest Texas, and an age of 835 ± 35 $^{14}$C years BP for a specimen from shelter CM-79 near Cuatro Ciénegas, Coahuila, Mexico. The Shumla Caves specimens are composed of an aggregate of ground peyote mixed with other plant material; they appear to be manufactured peyote effigies. This study documents modification of peyote by inhabitants of the Chihuahuan Desert ca. 6,000 calendar years ago and use of the dried entire crowns of the plant in the Late Prehistoric Period.

## Introduction

Peyote (*Lophophora williamsii*) is a small (normally less than 5 cm high with a diameter seldom more than 8 cm), chalky blue-green, spineless globular cactus native to the Chihuahuan Desert and Tamaulipecan thornscrub of northeast Mexico and adjacent south and southwest Texas. A map of the area showing the geographic distribution of peyote

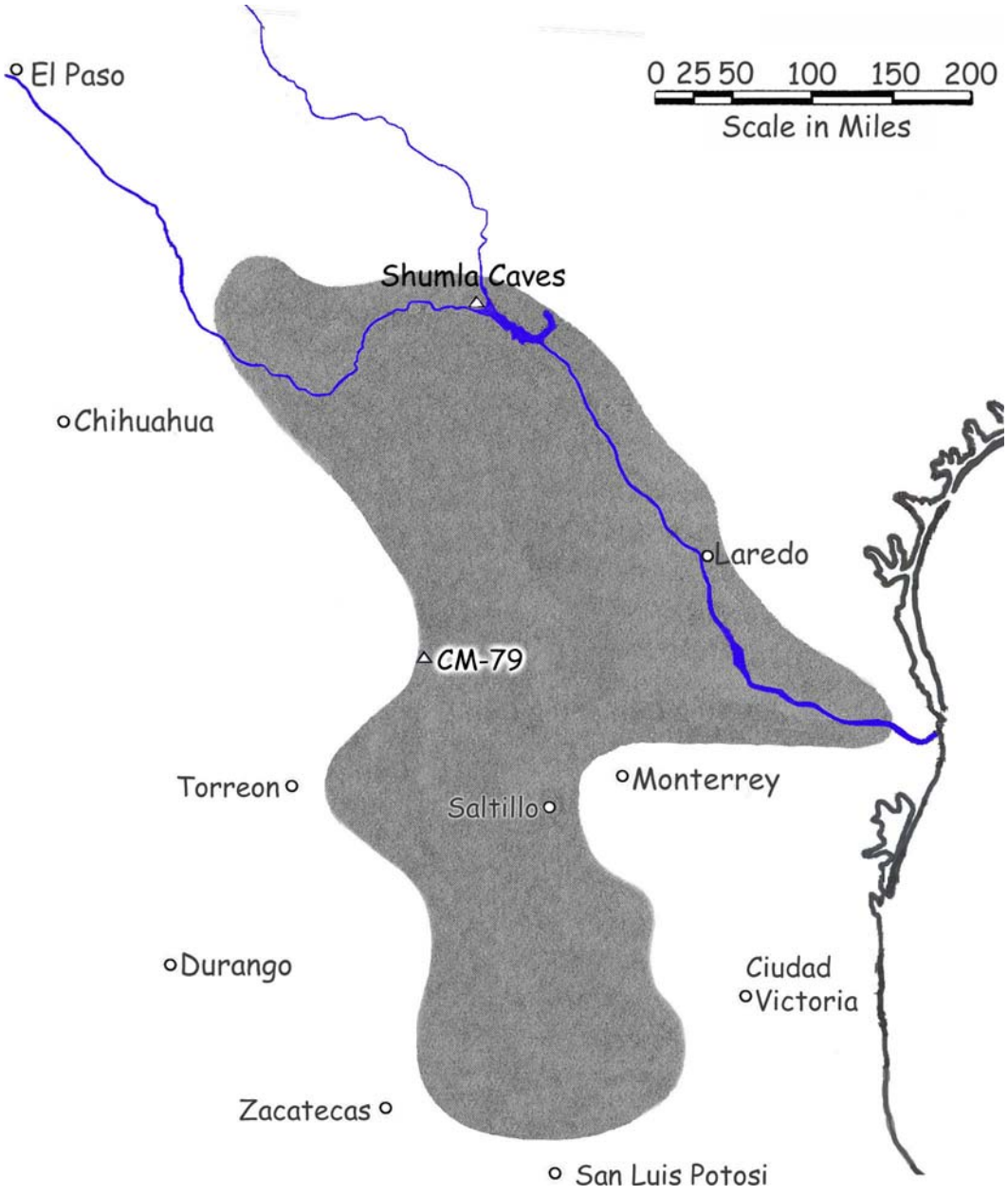


**Fig. 2.1** Locations of the two archaeological sites (denoted by triangles) where peyote has been recovered, documented and preserved. Sites are Shumla Caves, Texas (on the Rio Grande near the mouth of the Pecos River) and CM-79, Coahuila (about 50 miles west of the town of Cuatro Ciénegas). Gray shading indicates the currently recognized geographic range of the peyote cactus, modified from Anderson (1996a) by K. Trout (unpublished).

and the location of the two archaeological sites of interest is presented in Figure 2.1. The plants contain a high concentration of mescaline, an alkaloid which produces perceptual and other psychic effects characteristic of substances called “hallucinogens” by some and “entheogens” by others – the choice of terms being more a reflection of religion and politics than of science (Aberle 1966; Anderson 1996a; Huxley 1954; Litowitz 1983; Ott, 1995; Rouhier 1927). In historical accounts peyote has been applied topically as an analgesic, packed into puncture wounds as an antimicrobial agent, and taken orally for any of numerous purposes including foretelling the future, finding lost articles, stimulating the nervous system, suppressing appetite, treating gynecological and respiratory conditions, contacting supernatural beings and as anti-witch protection (Anderson 1996b; Arlegui 1851; Bye 1979; Cardenas 1945; Estrada y Flores 1946; La Barre 1957; Leon 1611; Nentvig 1971; Parsons 1974; Sahagun 1829; Schultes 1938a; Stewart 1987). The soil in the natural habitat of peyote is generally shallow and calcareous, often with limestone outcroppings. Plants grow singly or in clusters that may result from post-harvest regeneration of new stems or from seed production by a parent plant. Peyote plants prefer partially shaded conditions, and thus tend to grow under the canopy of nurse shrubs such as acacias and mesquites. Figure 2.2 shows a cluster of peyote plants growing *in situ* in the Chihuahuan Desert. Under drought conditions, which occur frequently in the area, peyote crowns may contract to a level below the surface of the ground as the tissues lose moisture (Morgan 1983a).

**Fig. 2.2** *Lophophora williamsii* (peyote) in its Chiuhuahuan Desert habitat. This cluster of five contiguous individuals growing among limestone boulders is ca. 150 mm long.

Currently, peyote is widely used by indigenous peoples for its medicinal and psychoactive properties (Anderson 1996a; Schultes 1998). Although the geographical area where peyote grows naturally in the U.S. is limited (see Chapter III), its use has extended to numerous tribes throughout North America, as far north as the Northwest Territory of Canada (B. Sangrey, personal communication 2005). Peyote is harvested by cutting off

the aerial crown at approximately ground level. After collection, the crowns dry to hard brownish disks called buttons (Fig. 2.3 a & b).

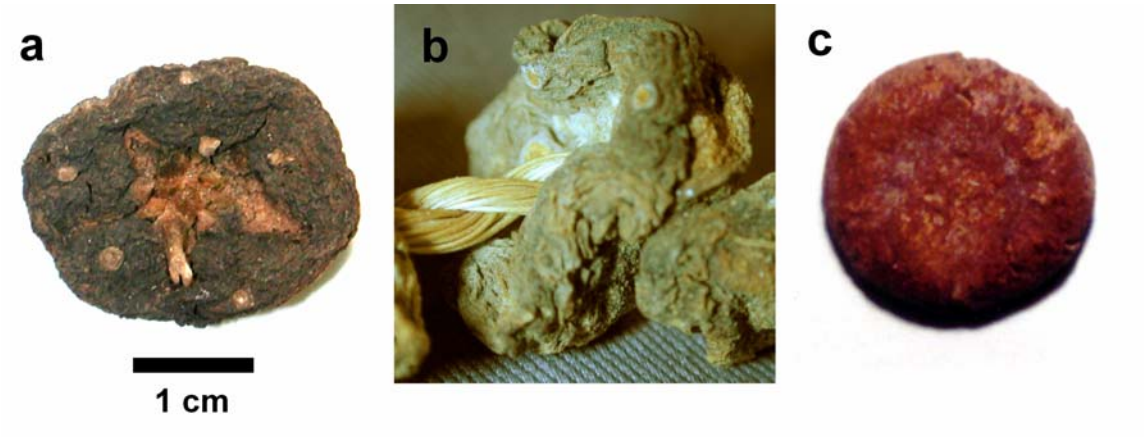


**Fig. 2.3** Peyote morphology. Shown here are (a) a modern peyote button, consisting of the dried crown of a peyote cactus harvested in Starr County, Texas; (b) a late Prehistoric peyote button from site CM-79, near Cuatro Ciénegas, Coahuila (with portions of adjacent buttons that were strung on the same cord); and (c) a middle Archaic manufactured peyote specimen from Shumla Caves, near Comstock, Texas, showing differences in structure from (a) and (b). Preservation of organic materials in the Chihuahuan Desert of northeast Mexico and southwest Texas is excellent, and the lack of normal peyote morphology in (c) is not due to deterioration or the ancient age of the specimen.

Many American Indian groups either were using peyote or were familiar with its uses, from pre-Conquest to the 19$^{th}$ century: Acaxee, Aztec, Caddo, Carrizo, Cazcan, Coahuilteco, Cora, Guachichil, Huichol, Jumano, Julimeno, Karankawa, Lagunero, Lipan and Mescalero Apache, Opata, Otomi, Pima Bajo, Tamaulipeco, Tarahumara, Tarascan, Tlascalan, Tepehuan, Tonkawa, and Zacateco (Shonle 1925; Stewart 1987).

The only two archaeological sites where peyote has been found, preserved in museum collections, and discussed in the literature are Shumla Caves in southwest Texas and shelter CM-79 near Cuatro Ciénegas, Coahuila, Mexico. Peyote has also been reported by Woolsey at Fields Shelter (Campbell 1958), by Hicks at a shelter in Crockett County, Texas (McGregor 1991), and by Sayles at several Texas sites (Sayles 1935), but it appears that no voucher specimens are currently available. Here we describe ancient modification of the Shumla Caves peyote and report radiocarbon dates on four specimens of excavated peyote, documenting its use for ca. 6,000 calendar years.

Previous reports of radiocarbon dates on archaeological peyote have suffered from lack of documentation and clarity. Indeed, the impetus to take another look at the few peyote specimens that had been the subject of previous studies by others, stemmed from a disappointment in the quality of the published reports (Furst 1989; Adovasio & Fry 1976; Bruhn et al. 2002), which presented radiocarbon dates on archaeological peyote with no supporting data. The various omissions of essential information by previous investigators left large lacunae of uncertainty that cast doubt on the accuracy of some of the published dates. The relationship between our current results and the previously published data are presented with a focus on the peyote specimens from Shumla Caves and Cuatro Ciénegas.

**Archaeological setting**

Prehistoric people occupied the Lower Pecos River region beginning at least 10,000 radiocarbon years ago (Turpin 1991). The surface geology is dominated by outcrops of heavily eroded cretaceous limestone with many canyons formed by the three major rivers – Devils, Pecos and Rio Grande – and their tributaries. In these canyons, differential erosion formed many cavities, overhangs, and rock shelters. These natural shelters have provided protection, not only for the extensive rock art located in the region, but for people who occupied the shelters over many millennia.

Material remains are common because the dry rock shelters provide for unusually good preservation. Many types of remains are represented, e.g., "the atlatl, large oval and stemmed (but not fluted) projectile points, the grooved club, …net carrying frame and/or conical burden basket, fiber sandals, both twined and coiled basketry,… plaited matting, fur cloth, twisted fiber, and cordage" (Taylor 1956).

The plant material from Shumla Caves, a series of nine caves including 41VV113, was excavated in 1933 by G. C. Martin (no date), and its stratigraphic and associated archaeological context is unknown. The site report for Shumla Cave No. 5 (41VV113) contains descriptions of nine burials, but does not mention peyote associated with them. The published report, however, mentions a single "mummified" specimen of peyote among the perishable items excavated from Cave No. 5 (Martin no date). Typical of early excavations, no information concerning the age of the Shumla Caves peyote can be ascertained from stratigraphy.

At shelter CM-79 near Cuatro Ciénegas, Coahuila, Mexico, nine buttons (dried tops of peyote plants) had been collected but not radiocarbon dated; their age was inferred from dates on associated matting (Adovasio and Fry 1976; Bruhn et al. 1978; Taylor 1988). The peyote recovered from CM-79 was excavated in 1941 by W. W. Taylor (1988). He describes CM-79 as a burial cave containing three secondary interments. Included in the numerous burial goods were nine peyote buttons strung on a single piece of cordage. Perhaps because two of the burials had been disturbed by looting activities, there is very little detail given regarding the artifacts recovered from the shelter.

**Materials and methods**

We removed approximately 10 mg of material from the interior of each of the three Shumla Caves peyote specimens. We also collected approximately 50 mg from the interior of one of the Cuatro Ciénegas peyote specimens. An acid-base-acid pretreatment was performed on the plant material: two soaks in hot (90˚C) 1 M HCl, followed by two soaks in hot 1 M NaOH, and two additional hot soaks in 1 M HCl. Afterwards, the sample was repeatedly rinsed with ultrapure distilled, de-ionized water. Remaining plant material was combusted to $CO_2$ and converted to graphite for an accelerator mass spectrometer target. A split of the $CO_2$ was taken for stable isotope analysis ($\delta^{13}C$). Radiocarbon measurement was conducted at Lawrence Livermore National Laboratory's Center for Accelerator Mass Spectrometry (CAMS).

## Results

### *New radiocarbon dates*

New radiocarbon dates on three altered peyote specimens excavated at Shumla Caves, Val Verde County, Texas, plus the first direct radiocarbon date on one of the specimens of natural peyote excavated from shelter CM-79, near Cuatro Ciénegas, Coahuila, are shown (corrected for $\delta^{13}C$) in Table 2.1. The three Shumla Caves specimens have statistically indistinguishable radiocarbon ages of 5160 ± 45, 5200 ± 35, and 5210 ± 35 $^{14}C$ years BP, with a weighted mean of 5195 ± 20 $^{14}C$ years BP, calibrated to 4045 – 3960 BC (2σ), calculated using the "R_Combine" function of the OxCal Calibration Program (Bronk Ramsey 2000; Stuiver et al. 1998). The radiocarbon date for the Cuatro Ciénegas peyote is 835 ± 35 $^{14}C$ years BP, calibrated to 1070-1280 AD (2σ).

**Table 2.1** Radiocarbon dates of archaeological peyote specimens from Shumla Caves, Texas, and CM-79 (Cuatro Ciénegas), Coahuila.

| CAMS # | Location | Carbon (mg) | $\delta^{13}C$ (‰) | Radiocarbon Age* (years BP) | 2σ Calibrated Ages |
|---|---|---|---|---|---|
| 86846 | Shumla Caves | 0.90 | -14.68 | 5160 ± 45 | 4220 – 3800 BC |
| 86045 | Shumla Caves | 0.65 | -21.7 | 5200 ± 35 | 4200 – 3950 BC |
| 86046 | Shumla Caves | 0.91 | -21.8 | 5210 ± 35 | 4220 – 3950 BC |
| Mean | Shumla Caves | - | - | 5195 ±20 | 4045 - 3960 BC |
| 96157 | Cuatro Ciénegas | 0.13 | -10.8 | 835 ± 35 | 1070 - 1280 AD |

*$\delta^{13}C$ corrected

## Discussion

### *Dating the antiquity of peyote use by inference*

The antiquity of peyote use had previously been estimated from four principal types of information. (1) From a sixteenth century history of Mexico (Sahagun 1829), peyote use by the Chichimecos people was inferred to date back to 300 BC (Rouhier 1927; Schultes 1938b). (2) Archeological ceramic artifacts with peyote motifs, from Colima, Mexico, date from 100 BC to 300 AD (Furst 1974). (3) The radiocarbon dating of other plant materials found in the same archeological site as peyote specimens yielded a date-by-association of 810-1070 AD at Cuatro Ciénegas (Adovasio and Fry 1976). (4) More recently, a particular genre (Pecos River style) of rock art found in the Lower Pecos River region of southwest Texas that sometimes incorporates peyote motifs (Boyd 1998) have been dated to between 2750 and 4200 $^{14}$C years BP (e.g., Rowe 2001; Rowe and Steelman 2002). All these earlier estimates for the antiquity of peyote use in the Chihuahuan Desert substantially underestimated the ages determined by our direct radiocarbon dating of the Shumla Caves specimens.

### *The Shumla Caves peyote specimens*

In a book review, Furst (1989) intercalated the comment that a direct radiocarbon date on one of the peyote specimens from Shumla Caves “unexpectedly added six millennia” to the oldest age then thought to apply to archaeological peyote. This is an oblique reference to a range of calendar dates from 810 AD to 1070 AD, which had been

reported to date peyote from the Cuatros Ciénegas CM-79 shelter (Adovasio and Fry 1976; P.T. Furst, personal communication 2003). (The various problems with these dates of Adovasio & Fry (1976) are discussed below in the section on the Cuatro Ciénegas peyote specimens.) Calculating back six millennia (in calendar years) from 810-1070 AD, one obtains a date of approximately 5000 BC – which is about 1,000 calendar years older than our mean date on the Shumla Caves specimens. Furst (1996) more recently published the explicit date of 5000 BC as the oldest date for peyote use, which is consistent with the implicit date that he had previously published (Furst 1989). We attempted in 2002 to locate the data at UCLA, where Rainer Berger had performed the radiocarbon dating (Furst 1989). Berger, by this time reported to be retired in France and chronically ill, did not respond to communications (M. Terry, personal observation 2002). Further enquiries revealed that, because the UCLA laboratory identification number was not available, the radiocarbon data that could serve either to confirm or to correct the 5000 BC date reported by Furst, were now irretrievable from archives of the former UCLA radiocarbon laboratory (R.E. Taylor, personal communication 2003). This situation demonstrates the importance of reporting radiocarbon laboratory numbers, measured and corrected radiocarbon dates (including their uncertainty intervals), fractionation ($\delta^{13}C$ values), calibrated dates (where dates expressed in calendar years are calculated from radiocarbon dates expressed in $^{14}C$ years BP), and the program used in calibrating the dates. None of these critical points was addressed in the date of 5000 BC reported by Furst (1989, 1996).

Bruhn et al. (2002) radiocarbon dated two of the three Shumla Caves peyote specimens, and reported a mean age of 5700 years, but with no laboratory identification number (nor any indication of the laboratory where the work was done) and no $\delta^{13}C$ value. They did not report the two individual radiocarbon dates and their corresponding uncertainties. Nor did they indicate any type of units for years, to denote whether the dates were calibrated or not. This omission is significant, as the calibrated date differs from the uncalibrated date by over 700 $^{14}C$ years. In the absence of such documentation, one cannot tell whether their unreported dates were internally consistent, or whether one date may have agreed with our three internally consistent dates documented here. In a personal communication, Bruhn added the following information to the published date: the radiocarbon ages of the two samples are 5030 ± 65 and 4885 ± 60 $^{14}C$ years BP, with a weighted mean of 4952 ± 44 $^{14}C$ years BP for the two samples (J.G. Bruhn, personal communication 2004). This is ca. 250 radiocarbon years more recent than the average of our three dates of 5195 ± 20 $^{14}C$ years BP. However, we also dated each of the same three Shumla Caves peyote samples <u>without pretreatment</u> (i.e., without removing any humic acid contamination). The dates thus obtained were 4995 ± 40, 4515 ± 40 and 4670 ± 40 $^{14}C$ years BP. Perhaps Bruhn et al.'s (2002) pretreatment was insufficient to remove all the humic acid contamination.

*The Cuatro Ciénegas peyote specimens*

The first reported date for archaeological peyote, which was reported for peyote specimens from the CM-79 site near Cuatro Ciénegas, Coahuila, consisted of a range of

uncalibrated calendar dates from 810 AD to 1070 AD (Adovasio & Fry 1976). The three radiocarbon dates on which Adovasio & Fry's range of dates was based, were later corrected for $\delta^{13}C$ and published as 1200 ± 70, 1000 ± 60 and 920 ± 75 $^{14}C$ years BP by Walter Taylor, who excavated these peyote specimens in 1941 (Taylor 1988). Adovasio & Fry (1976) neglected to note that these dates were not obtained on the peyote itself, but rather on a series of three pieces of matting recovered from the same shelter as the peyote (Bruhn et al. 1978; Taylor 1988). Unlike these dates obtained from associated materials, our radiocarbon date of 835 ± 35 $^{14}C$ years BP was obtained directly from a CM-79 peyote button. A comparison of our data to the calibrated, $\delta^{13}C$-corrected radiocarbon dates on the matting (Smithsonian Carbon-Dating Laboratory, 1972) show that the three dated CM-79 matting specimens were respectively ca. one, two and four centuries older than the peyote found in the same burial cave. In view of the fact that the cave was inferred to contain three secondary burials (Taylor 1988), the differences between the ages of the matting and that of the peyote may reflect differences in dates of primary and/or secondary interment. These differences also illustrate the pitfalls of dating by association.

*Modification of the Shumla Caves specimens*

After careful inspection, we concluded that the Shumla Caves specimens, which have been described as 'peyote buttons' (Bruhn et al. 2002) and 'well-preserved plants' (Furst 1989) by previous investigators, are *not* simply peyote buttons (the dried crowns of peyote plants). Instead, they appear to be manufactured effigies of peyote, mixed with non-cactus plant material and contrived to resemble peyote in a stylized way. One can infer from the

results of Bruhn et al. (2002) that the Shumla Caves specimens do contain peyote tissue or an extract thereof, as they have a high (2%) mescaline concentration uniquely characteristic of *Lophophora*, which exceeds concentrations in other plants of this region by orders of magnitude. But, the Shumla Caves specimens lack the essential morphological characteristics of *Lophophora*. These include: (1) areoles (small round structures that produce the characteristic tufts of silk-like trichomes or hairs; (2) sulci (furrows) demarcating the ribs of the plant; (3) roughly parallel corrugations of the epidermal tissue; (4) irregular three-dimensional deformations of the crown; and (5) organized vascular and parenchymal tissue structure normally visible in the cross section where the top of the plant was cut in the process of harvesting. (Features 3 and 4 are associated with desiccation.) All these morphological features are seen in modern peyote buttons (Fig. 2.3 a) and also in the Cuatro Ciénegas archaeological specimens (Fig. 2.3 b), but not in the Shumla Caves specimens (Fig. 2.3 c and Fig. 2.4).

Perhaps the most telling comparison is obtained from magnified views of external surfaces of the specimens. Instead of the normal tissue structure observed in modern peyote buttons and the Cuatro Ciénegas buttons, what one sees in the Shumla Caves specimens is an agglutination of plant particles and fibers—including pieces of material that are clearly from vascular plants other than peyote—randomly arranged in a pattern that is devoid of any obvious structure (Fig. 2.4).

The upper surface of these specimens is covered with a relatively thick (up to 1 mm), hard, smooth outer layer that appears to consist of a waxy or proteinaceous substance. This layer may have resulted from baking and/or the addition of a coating agent, which imparts a finished effect to the surface. No such layer is observed on the upper surface of actual peyote buttons. For the Shumla Caves specimens, the only *morphological* aspects suggestive of peyote are their size and very approximate shape – the shape of the Shumla Caves specimens being much more similar to that of a crown of a living peyote plant than a dried peyote button.

Another unexpected finding was that one of the Shumla Caves specimens had small flecks of yellow pigment on the upper/lateral surface. Transmission electron microscopy of yellow specks revealed a lack of cellular structure, suggesting that the yellow color may be an anthropogenic paint. Whether these crafted peyote effigies were intended for consumption (in which case perhaps the yellow pigment was accidentally rather than intentionally applied) or for a symbolic ceremonial purpose, as with “Chief” peyote buttons placed on the center of the altar in modern ceremonies of the Native American Church, is unknown.

**Fig. 2.4** Photomicrograph (20X) of the flat, rough lower surface of archaeological manufactured peyote button. This is the same Shumla Caves peyote specimen whose rounded, smooth upper surface is pictured in Fig. 3 c. Randomly arranged fragments of fibrous tissue from plants other than peyote appear to have been incorporated into the matrix of peyote tissue.

*Stable isotope values*

In addition to morphology, stable carbon isotope ratios for material from the Shumla Caves specimens confirm that the latter are not composed of pure peyote material. A modern dried peyote crown had a $\delta^{13}C$ value of –14.61 ‰ (Steelman and Rowe 2002). In addition, one of the peyote buttons from Cuatro Ciénegas was determined to have a $\delta^{13}C$ value of –10.8 ‰. Both values are characteristic of cacti with Crassulacean acid metabolism (CAM), commonly having $\delta^{13}C$ values from –20 to –10 ‰. In contrast, $C_3$

plants typically have a mean $\delta^{13}C$ value of –27 ‰ (Boutton 1991). The $\delta^{13}C$ values for two of the Shumla Caves specimens were intermediate at –21.7 and –21.8 ‰, suggesting that they contain a mixture of CAM peyote tissue and other $C_3$ plant material. This shift in the $\delta^{13}C$ values is consistent with our visual observation of non-cactus plant material in the Shumla Caves specimens. The $\delta^{13}C$ value of –14.68 ‰ for the third Shumla Caves specimen falls within the expected range for cacti, suggesting that a lesser amount of $C_3$ material was included in the sample taken from that specimen for stable carbon isotope measurement.

**Conclusions**

Peyote has been recovered from two archaeological sites near localities where the plant occurs naturally and where perishable materials are unusually well preserved, namely in the Chihuahuan Desert of Texas and Coahuila. Our radiocarbon-dating results on the three Shumla Caves specimens place this psychoactive and medicinally valuable plant in human habitations more than 5000 $^{14}C$ years BP, corresponding to a calibrated date of ca. 6000 calendar years ago. Of the Cuatro Ciénegas specimens, which are natural peyote buttons strung together on a cord and presumably all of the same age, the specimen we dated (the first of these specimens ever directly radiocarbon dated) is much more recent, at 835 ± 35 $^{14}C$ years BP, in the Late Prehistoric Period. The mean age of the three Shumla Caves specimens is 5195 ± 20 $^{14}C$ years BP, in the Middle Archaic Period. Unlike the Cuatro Ciénegas peyote buttons, the three Shumla Caves peyote

specimens are modified and contain fragments of other plants in addition to peyote material. This mixture had been molded into a discoid form superficially resembling the crown of a living peyote plant. The cultural significance of these manufactured mescaline-containing peyote effigies is intriguing but presently unclear.

# CHAPTER III

# GEOGRAPHIC DISTRIBUTION OF *LOPHOPHORA WILLIAMSII* AND *ASTROPHYTUM ASTERIAS* IN THE U.S.

## *Lophophora williamsii*

The clarity and detail of our understanding of the current geographic distribution of *L. williamsii* north of the Rio Grande varies enormously from one region to another. A general impression of the range of this species can be obtained by inspecting the findings of Rouhier (1927) and Anderson (1996a), as shown superimposed in Figure 3.1.

Further information on the distribution of *Lophophora williamsii*, by county, can be found in Turner et al. (2003). However, that atlas shows several geographic datapoints for which no voucher specimens can currently be located, and for which we have been able to find no specific locations in the primary literature. Examples are the two locations shown as dots in Hidalgo County in South Texas (Turner et al. 2003). Given the history of changes in land use that replaced most of the peyote habitat in Hidalgo County with farmland and cities during the 20$^{th}$ Century, these apparently unsupported data points may well mark the sites of historical rather than extant populations. One plausible explanation for the lack of voucher specimens for these and other *L. williamsii* location data points in Turner et al. (2003) is that the voucher specimens in question were among the many specimens of *L. williamsii* that were stolen from the TEX herbarium in the 1960's. These thefts occurred despite the fact that B. Tharp, then

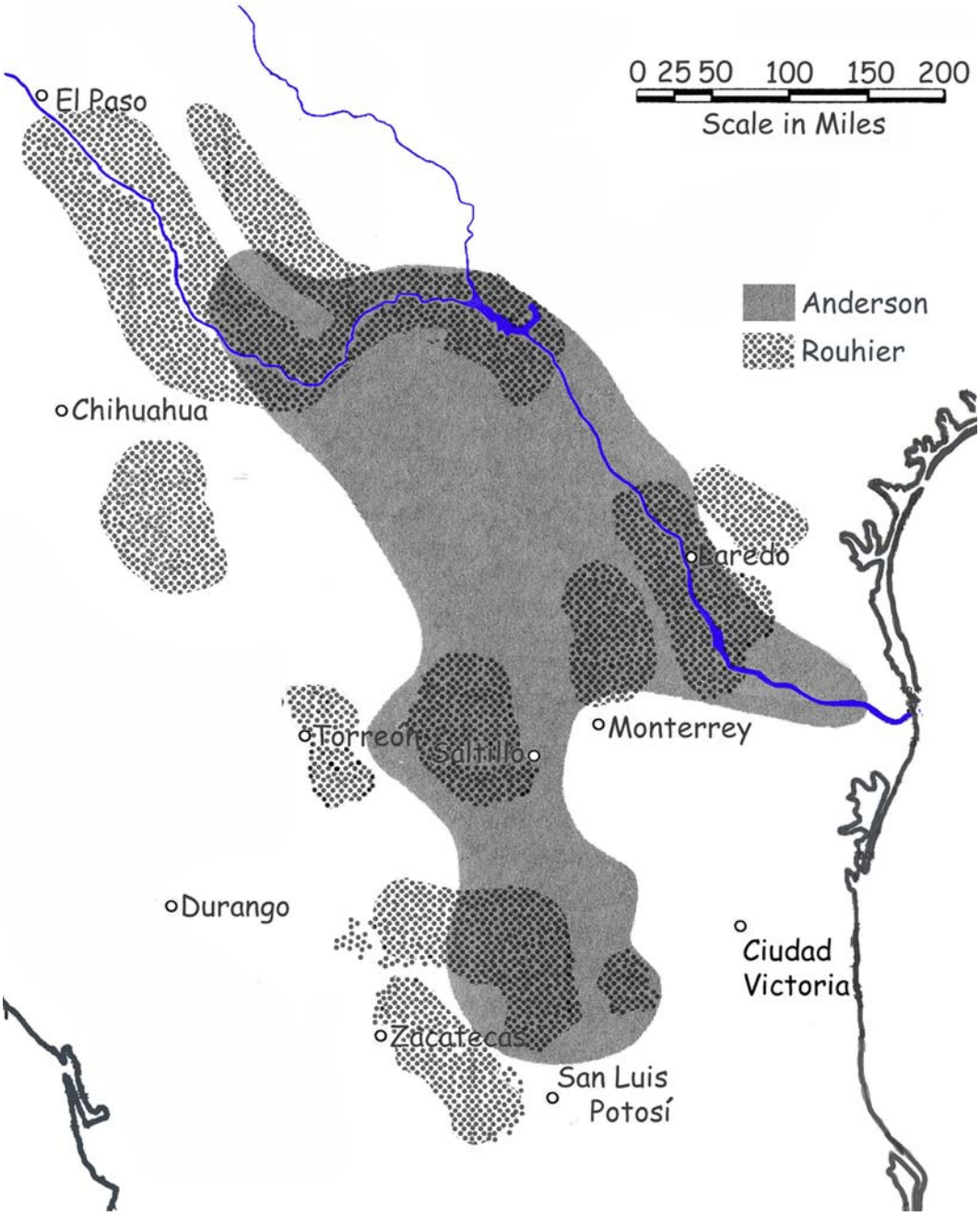


**Fig. 3.1** Geographic distribution of *Lophophora williamsii*. This map (K. Trout, unpublished data 2005) merges adapted versions of maps of Rouhier (1927), whose representation of the range of the species is denoted by disjunct stippled areas, and Anderson (1996a), whose conception of the range is shown as the single, continuous, solid gray area.

curator of the TEX herbarium, was reported to have routinely soaked specimens of *L. williamsii* in a toxic solution before mounting them on herbarium sheets, and then to have written “POISON” on the herbarium sheets (B.L. Turner, personal communication 2005).

Other sources of published data (of varying quality and reliability) on the distribution of *L. williamsii* in the U.S. include Anderson (1961, 1969, 1996a), Benson (1982), Havard (1885), Morgan (1976, 1984), Powell & Weedin (2004), Rouhier (1926, 1927), Schaefer (2000), Schultes (1937), Schultz & Runyon (1930), Stewart (1987) and Weniger (1970, 1984). Of these published works, the most accurate and comprehensive in our view – and also the most parsimonious in the size of the geographic range of *L. williamsii* that they portray – are those of Anderson (e.g., 1969) and Stewart (1987).

We have personally collected or examined herbarium specimens from the following Texas counties: Starr, Zapata, Jim Hogg, Webb, “Kinney and Maverick”, Val Verde, Brewster and Presidio. These county records will be considered in geographic order, proceeding upstream along the Rio Grande, generally from south and/or east to north and/or west (Fig. 3.2).

*Starr, Zapata, Jim Hogg and Webb Counties*

In these counties the Bordas Escarpment, marked by caliche outcrops and calcareous soils, and the adjoining Aguilares Plain and the Breaks of the Rio Grande, which are characterized by rolling terrain broken by occasional low caliche hills called *lomas*, still

support numerous, geographically extensive populations of *Lophophora williamsii*. Accordingly, it is in these counties where commercial peyote harvesting continues as it has since the latter half of the 19th Century (Morgan 1983a, 1983b), though with indications of decreasing abundance of the plant in recent years (Morgan 1983b; Anderson 1995; Moreno 2005).

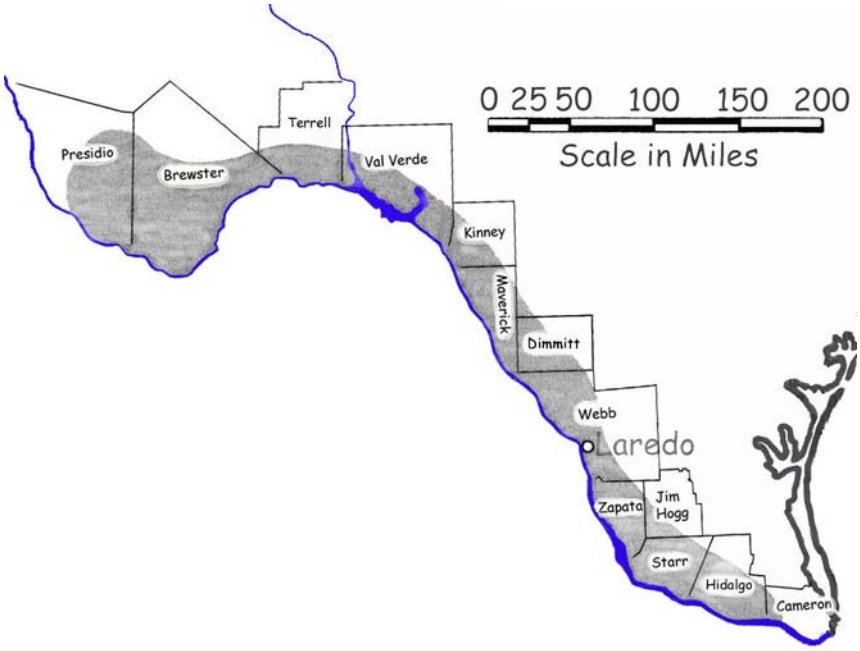


**Fig. 3.2** U.S. portion of Anderson's (1996a) range of *L. williamsii*, adapted with superimposition of Texas border counties (K. Trout, unpublished data 2005).

Herbarium specimens of *L. williamsii* from one or more of these four counties may be found in major herbaria that have U.S.-collected *L. williamsii* specimens in their collections. The TEX collection includes a specimen from Starr County (TEX

00286286), as does the UNM collection (UNM 35396). (UNM is where D. Weniger's personal collection of cactus herbarium specimens is deposited). The BRIT collection includes a Zapata County *L. williamsii* specimen (Laredo Junior College No. 24, collected by Vergara et al., 7 NOV 1961). At the University of Texas at Austin, a Jim Hogg County specimen is found in the LL collection (LL 00286284), and a Webb County specimen is found in the TEX collection (TEX 00286285). Additional specimens from Starr County and other locations are found in the RSA-POM herbarium (where E.F. Anderson's specimens of *L. williamsii* are deposited).

*"Kinney and Maverick Counties"*

Documentation for the (historical?) existence of *Lophophora* in Maverick and/or Kinney County is based on a single herbarium specimen (UNM 48838). The herbarium sheet for this specimen notes the collection location as "Kinney and Maverick Counties, Del Rio-Eagle Pass areas". The collector is identified as Horst Kuenzler, and what would appear to be his collection number is given as 3844. The date of collection is noted as "Spring 1971". When we enquired about the details of the location where this specimen was collected, in a letter to Mr. Kuenzler in 2003, Mr. Kuenzler made a special trip to the UNM herbarium to examine the specimen. The unexpected result of his examination was that Mr. Kuenzler determined that he was not in fact the collector of the specimen (H. Kuenzler, personal communication 2004). He could only speculate that the specimen may have been collected by a Mr. Prince Pierce or a Mr. Luke Vortman, and we have been unable to locate either of them for comment. The handwriting on the

herbarium sheet is that of E. Castetter, curator of the UNM herbarium until his retirement in the early 1970's, and it was not unusual for Castetter to assign his own collection number to an accession if the collector did not supply his own personal collection number (J. Mygatt, personal communication 2005). Based on what is known of Castetter's collection numbers in the year 1970, it seems likely that Castetter supplied his own collection number in the absence of any such number from the actual collector – whoever that might have been. So the UNM specimen remains clouded with uncertainty; if the notation as to the identity of the collector is in error, the collection location (as vague as it is) could also be in error. At the moment, there appears to be no obvious way to resolve this problem, and until such time as more field work is done in that region, it seems prudent to suspend judgment about the occurrence of *Lophophora* in Kinney and Maverick Counties.

*Val Verde County*

The situation in Val Verde County is somewhat clearer. In the US herbarium in Washington, D.C., there is a specimen of *L. williamsii* (US 00206877) collected at "the mouth of the Pecos River" by W. Lloyd in 1890. That collection site is probably on land that currently lies within the Amistad National Recreation Area (administered by the National Park Service), but its exact location – even if the population has survived – is unknown (J. Labadie, personal communication 2002). We collected an *L. williamsii* specimen (SRSC, M&M Terry No. 270) from a small population in the Lower Pecos region of Val Verde County in 2001. Our impression is that *L. williamsii* is not common

in Val Verde County, and that its current distribution is extremely patchy, with small numbers of small populations occurring behind locked gates on private land.

*Brewster County*

The historical occurrence of *L. williamsii* in Brewster County has been documented with two herbarium specimens in SRSC: one collected by B. Warnock (No. 18498) at Chilicotal Mountain in Big Bend National Park in1961, and another collected by D. Smith (No. 2300) at the same location in 1972. Unfortunately, the location of this extremely small population (only about a dozen individuals), which was situated within a few steps of the Glenn Springs Road in Big Bend National Park, was published by Warnock (1970), and when Smith revisited the site in 1980, he found that the population had been extirpated (D. Smith, personal communication 2001). Though other populations of peyote were known to investigators who worked in Big Bend National Park in the 1970's (D. Easterla, personal communication 1975; R. Wauer, personal communication 2001), our experience of interviewing the most senior and most knowledgeable members of the Park staff revealed that there is at present no one on the Park staff who knows where a surviving population is.

There are reports (of varying credibility) of a few scattered populations elsewhere in Brewster County, but as the undocumented populations are anecdotally reported to be located on private land to which authorized access is limited, confirmation of their existence is problematic.

*Presidio County*

*Lophophora* has been known to occur in Presidio County since the 19th Century (Havard 1885), and the Shafter population has been known for over a century (Mooney 1897; McAllister 1954). The population at that location was one of ten that Anderson chose to represent the genus in his Ph.D. dissertation (1961), and we likewise chose specimens from that population as sources of DNA samples representing the northwestern extremity of the currently documented geographic range of the genus. Herbarium specimens from this location are available at SRSC (B. Warnock No. 000; M&M Terry No. 471), UNM (D. Weniger No. 400), and RSA-POM (E.F. Anderson No. 925).

As in the case of Brewster County, at least one other population has been unattributably reported to exist in Presidio County, but botanical confirmation and documentation are lacking due to the difficulty of obtaining legal access to private land.

*Questionable historical locations*

If one examines the published maps showing the U.S. distribution of *Lophophora* (Fig. 3.1), they give the impression of a more extensive historical geographic range than can currently be documented. Statements from the literature in some cases appear to lend support to the accuracy of these maps. One trivial but likely explanation of that fact is the converse possibility that the maps were drawn secondarily as visual representations of the verbal assertions found in the literature – regardless of whether the assertions themselves were valid. Another possibility is that the maps and verbal assertions did accurately reflect the historical distribution of *Lophophora*, but that the cactus was

subsequently extirpated from many of those areas and therefore cannot now be documented to occur there. Still another possibility is that the cactus was not extirpated from those areas, but that subsequent changes in land use practices – namely fencing and locked gates – increasingly excluded would-be collectors (both American Indians and botanists) from the locations where peyote had been harvested historically, until ultimately the exact locations were forgotten. This unresolved question of whether or to what extent the maps based on historical information are currently accurate, should become clear as land tenure in Texas continues to change and more landowners open their properties to botanical research projects.

Hidalgo County, immediately east of Starr County, appears to have had populations of *Lophophora williamsii* historically (Schultz & Runyon 1930; Weniger 1970; Schaefer 2000; Turner et al. 2003; J.H. Everitt, personal communication 2005; T. Patterson, personal communication 2005). We are not aware of any herbarium specimens to document such historical populations, however. It is a fact that much of the brush country in western Hidalgo County (where suitable habitat for *Lophophora* once existed, just as fragments of viable habitat still exist in adjacent eastern Starr County) was converted into citrus plantations and urban sprawl in the latter half of the 20$^{th}$ Century. This transformation must have destroyed most of the historical habitat of *Lophophora* in Hidalgo County. In our view, the only plausible hope of documenting any present occurrence of the cactus in Hidalgo County would be to discover a remnant population on intact ranchland on the western edge of the county.

Terrell County, between Val Verde and Brewster Counties, is shown as part of the continuum of the historical range of *L. williamsii* along the Rio Grande in maps such as those of Rouhier and Anderson (Fig. 3.1). However, we find no specimen in any herbarium collection to document the occurrence of this species in Terrell County. The current County Judge, Leo Smith, who was a commercial cactus collector in the environs of Sanderson ("the Cactus Capital of Texas" according to the Chamber of Commerce) before he was elected Judge, has never encountered a specimen of *L. williamsii* in all his cactus collecting activities in Terrell County (L. Smith, personal communication 2004).

Ward County comes to our attention here in regard to the purported historical occurrence of peyote near the town of Pyote. According to local legend and anecdotal accounts by local residents, peyote grew in the vicinity of this small town until about 1900, when an unspecified group of Indians came from the west and dug up all the plants (Stewart, 1987). Looking at the terrain today, it is difficult to believe that peyote ever existed there, as there is no obvious habitat that would be appropriate (M. Terry, personal observation), and the town of Pyote is more than 150 km from the nearest documented population of *Lophophora*.

Perhaps the most intriguing part of Rouhier's distribution map – where it differs most conspicuously from the map of Anderson – consists of the northwestern extremities of the distribution of *Lophophora* (Fig. 3.1). One of Rouhier's extensions of *Lophophora*'s range continues north through the Davis Mountains, the Apache Mountains and the Delaware Mountains, up as far as the New Mexico border. In this regard there is a historical reference to the travels of Quanah Parker, last Chief of the Comanche, who

was reportedly accustomed to visiting the area of Mitre Peak, a few miles northwest of Alpine in northern Brewster County, to replenish his peyote supplies (Schaefer 2000). Perhaps Parker had a temporary garden of transplanted peyote there, or perhaps he rendezvoused there with an associate who delivered peyote to him there from a distant collection site. Be that as it may, there appears to be no suitable habitat for *Lophophora* around Mitre Peak (an igneous formation), and it seems highly improbable that peyote would grow there naturally.

Rouhier's other extension of the range of *Lophophora* goes up the valley of the Rio Grande to El Paso, and beyond into southern New Mexico in the vicinity of Deming. Both of these apparent extensions of the currently recognized range of the genus are interesting in relation to Rouhier's (1927) claim that peyote occurs naturally in southern New Mexico, where peyote has never been reported by any botanist. The extension that encompasses the Delaware Mountains reflects a quoted passage from an unspecified work of the American anthropologist James Mooney, to the effect that the "Salt Plains Mountains", now called the Delaware Mountains, were "rich in peyotes" when Mooney visited West Texas in 1897 (Rouhier 1927). It is notable that the substance of this quote, which we have found to be untraceable to its primary source in an exhaustive examination of the Mooney papers in the National Anthropology Archives in the Smithsonian Institution, was reported by Rouhier to be based not on Mooney's personal observations, but rather on a statement by one of Mooney's American Indian informants. The only modern datum on this question is the observation of R. Worthington, who worked on the flora of the central Delaware Mountains in connection with the

establishment of the windfarm that is operating there now. He observed neither *Lophophora* nor geological formations compatible with *Lophophora* in the Delaware Mountains (R. Worthington, personal communication 2001).

In summary, Rouhier's map is evidently inaccurate in regard to its extensions to the north and west beyond Anderson's range. Anderson's map provides more reasonable delineations of the documentable U.S. range of *Lophophora*, though several of the counties included in his range lack current documentation (*vide supra*), and certain areas included in his range (notably Cameron County, Dimmitt County, and all but the extreme northern portion of Maverick County) are geologically and edaphically unsuitable to *Lophophora*, in that they lack limestone outcroppings and associated calcareous soils. Our interpretation is that these latter areas were included by Anderson only to make the northern/eastern boundary of the range a smooth line running more or less parallel to the Rio Grande and in particular remaining consistently on the U.S. side of the river – even where it would have been more accurate to show the boundary of the range of the cactus deviating into Coahuila on the Mexican side of the river. This is an instance of the map having been drawn so as not to violate the enunciated general principle that "[i]n the United States, *L. williamsii* is found in the Rio Grande region of Texas…" (Anderson 1996a), which implied sacrificing accuracy in favor of the ideal of a Rio Grande valley uniformly filled with peyote habitat on both sides of the river as far upstream as the Big Bend region. In regard to the prospects of finding peyote where the historical maps or other historical sources suggest that it once existed, there is no

shortage of intriguing suggestions, but there is little or no credible evidence to corroborate them in the present circumstances.

### *Astrophytum asterias*

The geographic range of *Astrophytum asterias* (as broadly and historically understood) and its relation to the range of *Lophophora williamsii* are portrayed in Figure 3.3. With *A. asterias*, as with *L. williamsii*, we are clearly dealing with a species that is primarily Mexican in its distribution. With *A. asterias* the situation is even more extreme than in the case of *L. williamsii*, as current knowledge of the range of star cactus in the U.S. is that the plant is restricted to Starr County, Texas (Clover 1932, 1937; Zimmerman & Parfitt 2003; Janssen et al. 2004). Assertions that *A. asterias* occurred historically to the east, in Hidalgo County (Weniger 1970) – and even farther east, in western Cameron County (Benson 1969) – are not supported by voucher specimens and have been found to be impossible to confirm at present (Janssen et al. 2004).

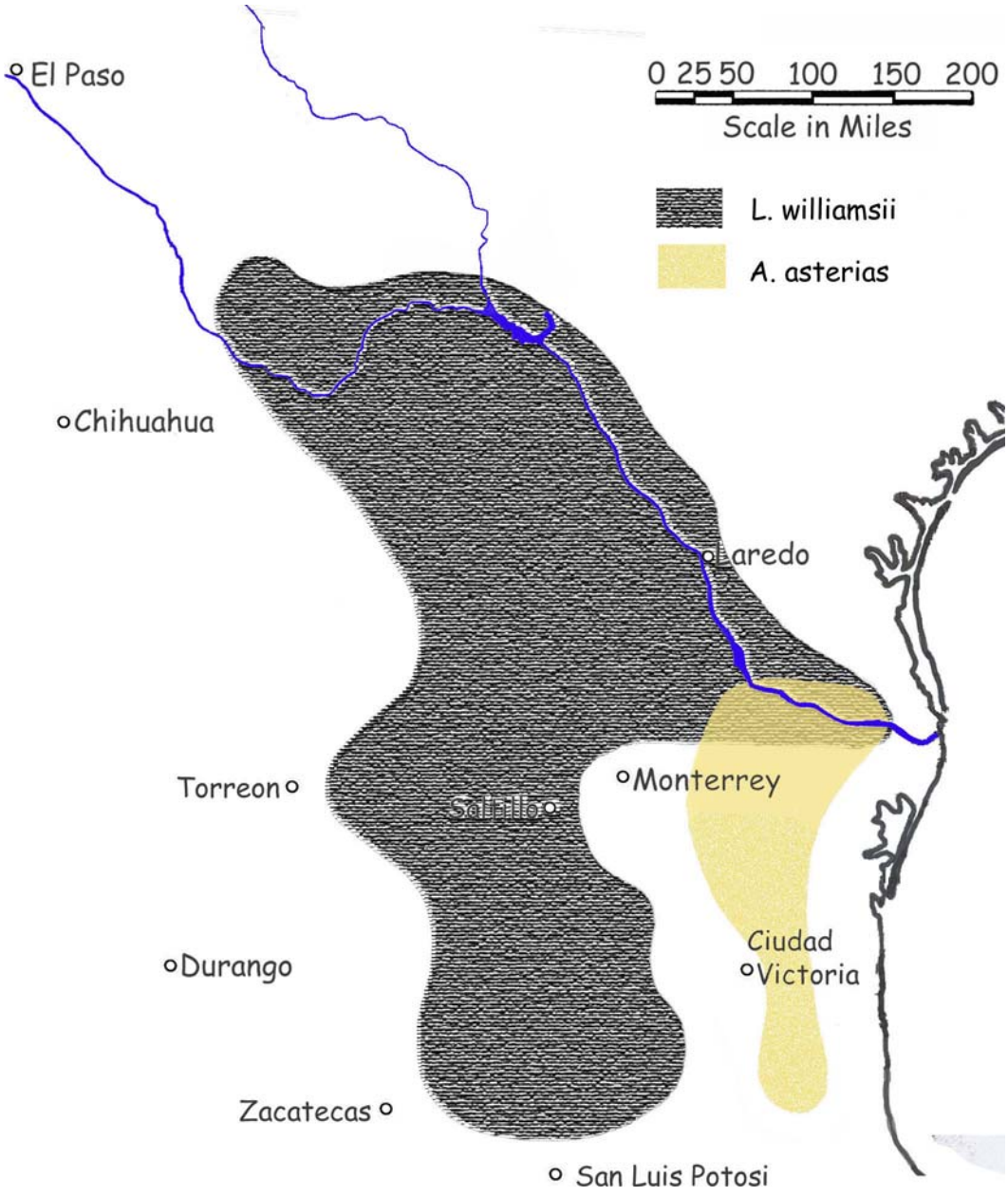


**Fig. 3.3** Geographic distribution of *Astrophytum asterias* (yellowish area) and *Lophophora williamsii* (rough charcoal gray area). In the Mexican portions of their respective ranges, the two species are mostly allopatric. They are sympatric only in the northern extremity of the Tamaulipecan thornscrub ecoregion, specifically in Starr County, Texas. The U.S. range of *A. asterias* is shown as broader than Starr County to allow for the (questionable) historical occurrence of this species in nearby counties along the Rio Grande.

# CHAPTER IV

# ROOT-SHOOT ANATOMY AND POST-HARVEST VEGETATIVE CLONAL DEVELOPMENT IN *LOPHOPHORA WILLIAMSII*: IMPLICATIONS FOR CONSERVATION

## Introduction

Federal law provides protection for the use of peyote for bona fide religious ceremonial purposes by members of the Native American Church (NAC). The supply of peyote for such purposes is regulated by the Drug Enforcement Administration (DEA) and the Texas Department of Public Safety. The regulated commerce in peyote begins with the harvest of peyote from wild populations by licensed peyote distributors or their agents. Commercial quantities of peyote occur in the U.S. only in Starr, Zapata, Webb and Jim Hogg Counties in South Texas, and all four currently licensed peyote distributors are based where peyote grows in those counties, within 70 km of the Rio Grande. Historically the peyote distributors have gained access to harvestable populations of peyote through peyote-specific lease agreements with private landowners. Most of the actual harvesting of peyote is done by contract laborers who are paid in accordance with the number and size of freshly cut "buttons" (tops of stems) of peyote that they deliver to the licensed distributors.

The proper technique for harvesting peyote is that the crown (i.e., the aerial, photosynthetic portion of the stem) of the peyote cactus is cut off at or immediately below its base, and the subterranean portion of the plant, including all or most of the

subterranean portion of the stem, is left in the ground to regenerate one or more new crowns. Such harvesting of the commercially valuable crown of the cactus may be accomplished by cutting through the plant transversely at the level of the surface of the ground, at or near the interface of the green crown and the brown subterranean portion of the stem, using a machete, a cutting tool with a broad flat blade and a handle about 60 cm long (such as a hand edger), or virtually any kind of knife (M. Terry, personal observation: Fig. 4.1a-c).

**Fig. 4.1a** Proper peyote harvesting technique, showing cutting tool and angle of cut. Plant is cut transversely at base of crown, i.e., at ground level.

**Fig. 4.1b** Proper peyote harvesting technique, immediately after cut. Cut has been made parallel to ground surface, and harvested crown (at bottom of photo, about 5 cm in diameter, with 8 ribs) has been removed from subterranean portion of stem (above, remaining in the ground). Cut surface shows cross section of vascular cylinder (ring of yellow tissue near center of stem), pith within the vascular cylinder, and yellowish green parenchymal tissue of the thick cortex of the stem (between the vascular ring and the very thin outer layers of juxtaposed hypodermis and epidermis).

**Fig. 4.1c** Proper peyote harvesting technique, showing manual collection of cut crown. Underside of cut surface of harvested crown (left) shows very narrow (2-3 mm wide) ring of bark at perimeter of cut surface, indicating that the cut was made just below base of crown, in uppermost portion of subterranean stem. Cortical parenchyma of crown (left) is slightly greener (due to higher chlorophyll content) than cortical parenchyma of subterranean stem (right), which is slightly more yellowish.


**Fig. 4.2** Peyote plant immediately after most of crown has been harvested. Shown are cut just above base of harvested crown (green tissue at top), subterranean stem (brown bark-covered tissue immediately below cut base of crown) capable of regenerating new crowns, and tapering taproot (bottom part of plant with a few visible lateral roots).

**Peyote harvest – regeneration of crown(s) by decapitated plant**

When proper harvesting technique is adhered to, the decapitated subterranean portion of the stem and the more distal taproot of the cactus (Fig. 2) remain intact and *in situ*, where the viable subterranean stem tissue will normally begin to regenerate one or more new crowns by lateral branching from axillary (areolar) buds within a few months after loss of the apical meristem (Fig. 4.3)**.** Such regenerated crowns may in turn be sustainably harvested after they reach maturity, some years later (Fig. 4.4).

**Fig 4.3** Two young peyote crowns ("pups") regenerating by lateral branching from base of cut crown. Cut surface of crown is becoming creased by impinging growth of pups.

**Fig. 4.4** Repeated harvesting of successive crowns from the same peyote plant. Individual on left bears notches indicating stem regeneration after having its crown harvested three times in the past. Individual on right has never had its crown harvested (the distal end of the root broke off when the plant was dug up for examination).

**Peyote harvest – degeneration and death of the decapitated plant**

Not every peyote plant responds in the same manner to removal of its apical meristem along with the crown at harvest. The simplest response is that described above: regeneration of one or more new crowns by lateral branching from (usually subterranean) stem tissue (Figs. 4.3 & 4.4). But frequently we observe a more complex, gradual response, which begins with simple lateral branching from the remaining stem of the decapitated plant. The difference is that the new stem branches, instead of remaining dependent on the taproot of the original plant, put down their own adventitious taproots and eventually become independent plants that detach themselves from the original plant, which degenerates and dies in this process.

The unusual aspect of this second type of response of peyote to removal of its apical meristem in the harvesting process, is that the regeneration of new stems and the generation of new adventitious taproots from the new stem branches proceed in a seamless developmental process until at some point it must be recognized that the new, increasingly autonomous shoot-root units have become independent vegetative clones of the original plant. That is to say, where we started with a single decapitated plant undergoing development of new stem branches, we end up with what must be recognized as a parent plant with clonal progeny.

Another remarkable feature of this process is that as the clonal progeny become larger and more nutritionally independent, the stem tissue connection between the parent plant and each of the vegetative clones degenerates to a slender tube consisting mostly of vascular tissue, while the parent plant (which has no photosynthetic capabilities of its own) steadily decreases in size and density as the nutrients stored in its parenchymal tissues are depleted and utilized by the growing clonal offspring. In the final phase of the process, the connection between the parent plant and the now nutritionally independent progeny disintegrates, and what is left of the decapitated parent plant dies.

This series of events is depicted in Figures 4.5-4.13. All the photos shown are of specimens that we collected from a relict population, most of which was destroyed in the process of land clearance for a housing development, near Rio Grande City, Starr County, Texas.

**Fig. 4.5** Peyote plant with a fairly normal-looking stem and taproot, with no lateral stem branches. The asymmetrical annular constriction just above the point where the long lateral root emerges, may be a scar reflecting the harvesting of the original crown several years ago. This mature plant has a crown ca. 5 cm in diameter with 8 ribs.

**Fig. 4.6** Another normal plant with a single long lateral root and no obvious evidence of previous harvesting. The symmetry of the annular constrictions on the long subterranean stem suggests reduced growth rates in periods of winter and/or drought. The crown of this mature plant measures ca. 6 cm in diameter and has eight ribs. The shallow, elongated indentation in the side of the crown facing the reader is a scar from tissue sampling for DNA analysis 18 months previously.

**Fig. 4.7** A good example of a plant that was decapitated exactly once, several years ago. The size of the new crown is ca. 5 cm in diameter. Note that the crown-bearing lateral branch is markedly offset from the center of the original subterranean stem seen at the base of the lateral branch.

**Fig. 4.8** *L. williamsii* plant showing evidence of having been previously harvested at least twice. The small sizes of the two crowns on lateral branches from the original stem suggest that the most recent harvesting of this plant was perhaps 2-3 years ago. (The larger of the two crowns is about 2.5 cm in diameter. Both are 5-ribbed.)

**Fig. 4.9** *L. williamsii* plant that has been harvested several times. Only one of the two new crowns is visible from this view, but the two adventitious taproots, each originating in a stem branch bearing a crown, are both visible (protruding downward to bottom edge of image). Each of the two crown-bearing stems has recently developed by lateral budding from a previous harvest-associated lateral branch that was itself decapitated near ground level. The two new crowns, each with its own adventitious taproot, are well on their way to becoming independent of the parent rootstock. The original/parental plant, represented by its bark-covered taproot protruding to bottom left from the original subterranean stem (also bark-covered), is degenerating, but its subterranean stem is still alive and still attached to the subterranean stems of the two clonal progeny.

**Fig. 4.10** Two vegetative clones that originated as lateral branches of the stem of a decapitated parent plant. The parent plant is degenerating but still clearly recognizable as the bark-covered subterranean stem with taproot extending down between and beyond the taproots of the two new plants. There is still a connection (not shown directly, but inferable from the inter-adherence of the plants in the photo) between the new plants and the parental plant, but each of the young plants has its own functional taproot and is virtually independent of the parental rootstock by now.

**Fig. 4.11** Pair of “sister” vegetative clones similar to those described in Fig. 4.10. The difference in that here the original rootstock (remaining portion of subterranean stem plus root) is shorter and stockier and still has functional lateral roots emerging from the taproot. Thus the decapitated parent plant appears still to be contributing significantly to the nourishment of its two clonal progeny.

**Fig. 4.12** Three clonal sister peyote plants – each anatomically and functionally developed with its individual crown and adventitious taproot. All three vegetative clones are still attached to the parental subterranean stem (the dark brown structure visible between the taproots of the middle and right clones).

**Fig. 4.13** The amorphous, decaying mass (right) that is tenuously attached to the subterranean stem of the live cactus (left), is the dead remnant of the subterranean stem of the parent plant. The parent plant was decapitated by peyoteros some years ago. The young specimen that developed from the then-living parental subterranean stem, is now fully formed, with its own taproot, and is fully independent. The very low density of the attached mass of dead parental tissue suggests that it is in an advanced stage of decomposition.

This natural process of vegetative clone production in response to the excision of the apical meristem that occurs when peyote crowns are harvested, is remarkably congruent with the folk belief that harvesting peyote results in an increase in the number of plants in a population, and that where one plant grew before harvesting, several plants may be found after allowing an adequate period of time for the peyote to "grow back". A necessary condition for this process to occur, however, is that the harvesting be done in a manner that does not preclude the production of new stems from the subterranean stem of a plant from which the crown has been harvested. What does this mean in terms that could serve as a practical guideline for sustainable harvesting?

The answer must be based on an understanding of the anatomy of the root and stem of *Lophophora williamsii*. We begin with the observation that of all the people working with this plant – and here we include peyote distributors, members of the Native American Church, cactus hobbyists in countries where peyote cultivation is legal, and yes, even botanists – very nearly zero appreciate the distinction between true root and subterranean stem. Yet this distinction is crucial to an understanding of how to harvest peyote so that it will "grow back", because, as far as we have been able to determine from observations to date, new stem branches will develop only from stem tissue, not from root tissue. Therefore, if in harvesting the crown one cuts so deeply below the crown that all or most of the subterranean stem tissue is removed along with the crown, then there will be no possibility of new stem development, and the stemless root left in the ground will simply perish. With that as the operating premise, let us now consider the anatomy of the root and the shoot of *L. williamsii*, and how to distinguish between

them. (The shoot by definition encompasses the stem and all the structures to which it gives rise, such as leaves, flowers and fruit, but in this discussion we shall focus on the shoot as stem.)

**Anatomy of the root and shoot of *L. williamsii***

Ideally we should like to be able to identify the shoot-root transition zone in order that at least some of the shoot remains with the root of the plant left in the ground after harvest. Unfortunately, the plants normally protrude only 1-3 cm above the surface of the ground and have a large subterranean shoot that tapers gradually until it ends in a taproot. The shoot-root transition zone does not occur near the soil level where the seed germinated but instead occurs at various depths (higher in smaller plants, deeper in larger plants). It is not possible to identify the shoot/root transition zone merely by examining an intact plant with the naked eye.

In most seed plants, young shoots and roots can be distinguished from each other because the shoot has a pith and cortex whereas the root lacks both these structures (Mauseth 1988). However, several other cacti grow like peyote – having a large below-ground shoot that tapers into a large taproot – and the shoot-root nature of those structures has not been clarified (Stone-Palmquist and Mauseth 2002). Early works on the anatomy of *Lophophora* (Rouhier 1927; Bravo 1931; Janot & Bernier 1933) gave fair to very good anatomical descriptions of root and shoot, but even Bravo, whose description of the subterranean stem and root of *Lophophora* was the best available in its day, admitted that “[it] is most difficult to know in which region the stem ends and in

which the root begins…" (Bravo 1931). Because better harvest techniques may aid the survival of this species – or, more specifically, may aid the survival of this species in areas subject to intensive harvesting for human consumption, as in South Texas – we undertook a histologic study of the anatomy of roots and shoots in *L. williamsii* to determine if there are reliable criteria for distinguishing the root from the shoot (and particularly the subterranean portion of the shoot). We especially looked for criteria that could be used in the field.

*Materials and methods*

Plants were collected by M. Terry (DEA Researchers Registration No. RT0269591) from a wild population of *L. williamsii* near Rio Grande City in Starr County, Texas, or, in the case of one specimen (the very large plant), donated for research purposes by law enforcement personnel. Specimens examined included two very small plants, two plants of intermediate size and one very large plant (Table 4.1).

Plants were dissected with care being taken to obtain samples of material that was definitely root (provisionally defined as the region below the uppermost point at which a lateral root had emerged), definitely shoot (namely the aerial portion of the shoot – specifically known as the crown, *sensu* Schultes (1938a) – which has a blue-gray to blue-green epidermis, photosynthetic tissue and axillary buds), and definitely hypocotyl (the transition zone between shoot and root – hypocotyl samples being obtained by taking numerous samples between obvious root and obvious shoot). In all but the smallest, youngest plants, the plant material that was easily recognizable as root was

**Table 4.1** Dimensions of the three adult plants studied, listed from shortest to longest. All values are in millimeters. Shoots of plants #1212 and #1213 were sampled at ground level and at two below-ground levels; plant #1214 was sampled only at one below-ground level. The root of each plant was sampled only at the top of the root, where it most resembled a portion of shoot.

| Sample | Plant length | Aerial height | Plant diam at soil level | Shoot cortex thickness | Shoot pith diam | Root cortex-like zone thickness | Root pith-like region diam |
|---|---|---|---|---|---|---|---|
| Plant 1212, at soil level | 135 | 24 | 55 | 20 | 4 | | |
| 1212, at -20 mm | | | | 13 | 6 | | |
| 1212, at -25 mm | | | | 9 | 6 | | |
| 1212, root, -35 mm | | | | | | 1.5 | 10 |
| Plant 1214, at soil level | 140 | 44 | 78 | 32 | 10 | | |
| 1214, at -35 mm | | | | 6 | 27 | | |
| 1214, root, -41mm | | | | | | 3.0 | 27 |
| Plant 1213, at soil level | 160 | 40 | 59 | 14 | 6 | | |
| 1213, at -20 mm | | | | 14 | 6 | | |
| 1213, at -35 mm | | | | 9 | 8 | | |
| 1213, root, -45 mm | | | | | | 1.0 | 3.4 |

located at least 35 mm below soil level (45 mm below in plant #1213; Table 4.1). Since the nature of the higher subterranean portions of *L. williamsii* was unknown, samples were taken from all plants examined, with the position of each sample being carefully measured with the soil level (taken as the level of the base of the crown) as reference.

As tissue samples were obtained during dissection, they were immediately immersed in Navashin's solution, then aspirated in a vacuum chamber to remove air and permit rapid penetration of fixative. Tissues were fixed for 24 hours, dehydrated through mixtures of ethanol and tertiary butanol, then embedded in Paraplast Plus. After microtoming, sections were stained with Safranin and Fast Green by a procedure designed especially for cacti (Mauseth et al., 1985).

*Results*

All plants tapered gradually from an unbranched aerial shoot to a region of subterranean shoot, then to hypocotyl, and finally to taproot. The taper was uniform in most plants, without any abrupt change in diameter that might indicate the boundary between shoot and hypocotyl or between hypocotyl and root. The two smallest plants that we examined were only 31mm long (9 mm above ground, 22 mm below ground) and 50 mm long (10 mm above ground), so seeds must have germinated at or slightly below ground level. However, in the three adults we examined, the root/shoot junction was located at least 35 mm below the soil level (45 mm below in plant #1213), so plants of *Lophophora williamsii* must have contractile roots pulling the root/shoot junction deeper as the plant ages. All above-ground portions of shoot were covered with a blue epidermis; all

subterranean portions were covered with thin, flaking brown bark. Ribs and axillary buds (often called areoles in cacti) were obvious on all above-ground portions, and withered areoles were occasionally detected (as much as 17 mm below soil level on plant #1213). Lateral roots emerged only from areas that were later shown to be taproot or hypocotyl, not from shoot tissue. But one plant examined in the field but not dissected and studied here had a root emerging from its side at about the same level as a lateral stem branch; that root might have emerged from the hypocotyl rather than the shoot but that is not known. This isolated field observation should be interpreted in light of the possibility that some of what appear to be uppermost lateral roots of *Lophophora* may turn out to be adventitious roots emerging from subterranean stem tissue. Whether ordinary subterranean stem tissue can produce adventitious roots, or whether adventitious roots can be produced only by regenerative lateral branches from a plant whose apical meristem has been removed, is an anatomical issue still to be resolved.

Structure of the root

Very young regions of root (less than 0.5 mm in diameter) had an organization typical of most dicots. There was an epidermis, a thin cortex only a few cells thick, endodermis and vascular tissue consisting of small bundles of primary phloem alternating with arms of protoxylem, and metaxylem occupied the very center. An important point is that metaxylem contained significant amounts of living xylem parenchyma cells, it did not consist entirely of dead tracheary elements. This organization was found in only the two smallest, youngest plants, in samples taken from closest to root tips. Older portions of roots (2.5 to 30 mm in diameter) had altered their organization. They had lost their

epidermis and cortex, and parenchyma cells in the metaxylem had begun to proliferate. Secondary xylem (wood) and phloem were present, but epidermis, cortex and endodermis had been replaced by a bark consisting of thin flakes of cork. Just interior to the bark was a band of parenchyma that appeared to be cortex but which was really secondary phloem parenchyma. This cortex-like region was about 0.3 to 3.0 mm thick and extended inward from the bark almost to the vascular cambium. Thickness was correlated with root size: roots less than 4.0 mm in diameter had a cortex-like region only about 0.3 to 0.4 mm thick; roots about 10-12 mm in diameter had a cortex-like region 1.0 mm thick; and very large roots 30 mm in diameter had a cortex-like region 3.0 mm thick. The cortex-like region was recognizable as secondary phloem only because it had traces of collapsed sieve tube members in it. There were no vascular bundles in the cortex-like region other than very rare connections with lateral roots, and these were oriented vertically rather than radially or tangentially.

Metaxylem parenchyma proliferated in roots, producing such abundant amounts of parenchyma that the center of the root appeared to have a pith. Metaxylem vessel elements were pushed apart (such proliferation in an otherwise mature tissue is called dilatation), and parenchyma cells in the innermost, first-formed wood also underwent dilatation growth. This pith-like region could be identified as dilatated xylem (rather than true pith) by the presence of isolated vessel elements within it; these were easily visible with a handlens and dissecting microscope. In roots about 2.5 to 5.0 mm in diameter, the pith-like region was about 0.5 to 1.2 mm in diameter, but it was 3.4 mm in diameter in roots 12 mm wide, 10.0 mm in diameter in roots 22 mm wide, and 27 mm

wide in roots 35 mm wide. Dilatation occurred in both the innermost, first-formed secondary xylem as well as the middle regions, but the outer regions of secondary xylem (the outermost 1.0 – 2.0 mm) had ordinary wood organization.

Root wood consisted of a ray system and an axial system (containing axially oriented cells such as vessel elements). Rays were extremely narrow, only 7.3 sd 2.3 µm wide and consisted of large, rounded parenchyma cells. The axial system consisted of vessel elements, paratracheal parenchyma in immediate contact with the vessels, and wide-band tracheids (WBTs). Wide-band tracheids are an unusual type of cell found in almost all cacti; they are short (range in *Lophophora*: 315 to 525 µm), broad spindle-shaped tracheids with secondary walls that are annular or helical (Mauseth et al., 1995; Mauseth, 2004). There were no fibers in the wood. Just as rays were narrow, so were axial masses (98.4 sd 54 µm wide), and cross sections of root wood appeared to be rather solid when viewed with the naked eye or by dissecting microscope.

Slender lateral roots (1-3 mm diameter) emerged from taproots, but were extremely sparse, with only two or three present on any plant. This could be in part because most lateral roots in this species are ephemeral (emerging in response to moisture, then being shed in conditions of drought), small, and fragile (M. Terry, personal observation). The result is that all but the few largest lateral roots are broken off and left in the ground when one uproots the plant from its natural growth site in habitat – no matter how carefully one goes about extracting the plant. When one grows the plant in loose, friable soil under artificial conditions, more lateral roots and their finer branches remain intact when the plant is depotted. The plants used in this study, however, were recently

uprooted from the gravelly soil of their habitat and this would account for some reduction in the number of intact (and therefore observed) lateral roots.

Structure of the shoot

Young regions of shoot differed from older regions by still having epidermis but lacking secondary xylem, phloem and bark. The above-ground, photosynthetic portions of the two smallest plants were only 15 mm in diameter, two plants were 55 and 59 mm in diameter and an exceptionally large, old plant was 78 mm in diameter at the point between the aerial and subterranean portions of the shoot (Table 4.1).

Epidermis was present on all aerial portions of shoots and had a blue-gray color. Hypodermis consisted of one layer of parenchyma cells. Both epidermis and hypodermis cells definitely did not have thickened walls so the shoot surface was very soft. Shoot cortex was always much thicker than the root's cortex-like region of secondary phloem. The thinnest cortex in an adult plant was 6 mm (in an old, below-ground portion of plant #1214) and the thickest was 32 mm at soil level in the same plant. Cortex was only 1.5 mm thick in the seedlings. The outermost cortex cells were columnar and aligned in palisades, the palisade cortex was about 3.5 mm thick. Cells of the inner cortex (located between the base of the palisade cortex and the phloem) consisted of large, rounded parenchyma cells. Cortical bundles were abundant throughout the inner cortex, extending to the base of the palisade cortex, and each bundle contained both xylem and phloem. Cortical bundles were easily visible by handlens and dissecting microscope.

A slender pith was present in the center of all stems. It was only 1.5 mm in diameter in seedlings, from 4 to 6 mm in plants of medium size, and 10 to 27 mm in diameter in

the largest plant (i.e., plant #1214, which had the greatest girth). It consisted of just parenchyma cells with very rare spherical crystals and no mucilage. There were no medullary bundles at all and no dilatated metaxylem. The lack of xylem in the pith was easily visible by handlens and dissecting microscope: with both of these, shoot pith looked very clean and homogeneous whereas the root's pith-like region was coarse and granular due to xylem in the dilatated region.

Young shoots had a ring of collateral vascular bundles located between pith and cortex, older shoots had secondary xylem and phloem as well. Secondary xylem in shoots was similar to root wood. Rays were narrow (149 sd 134 µm, just one or two cells wide) and consisted of just parenchyma cells with no sclerification at all. The axial system consisted of small numbers of vessels and paratracheal parenchyma but large amounts of WBTs. As in roots, axial masses were narrow, only about 318 sd 179 µm wide. No xylem fibers were present in any sample. Due to the narrow rays and axial masses and the lack of fibers, shoot wood resembled root and the two could not be distinguished if a microscope view contained only wood and no other tissues. Secondary phloem in shoots did not produce a cortex-like region as it did in roots; instead, as the sieve tube members stopped conducting, phloem collapsed into a thin, tangential band.

All subterranean portions of *Lophophora* shoots were covered by bark similar to that on older portions of roots. An unusual feature was that shoot bark occasionally contained crystals and vascular bundles, indicating that the cork cambium had arisen deeply enough in the shoot cortex to cut across cortical bundles; however, both crystals and vascular bundles were too small to be visible with a handlens examination of bark.

Several plants had shrunken, withered tubercles on their subterranean portions. They were wrinkled and covered with bark just like all other subterranean portions, but their centers contained living parenchyma cells and an apical meristem, apparently the shoot apical meristem of the tubercle. The lowest one found on each adult plant was 15 mm below soil level on plant #1212, 30 mm on plant #1213, and 22 mm on plant #1214.

Withered tubercles were identifiable on subterranean, bark-covered portions of two of the adult plants. Tubercles were reliably identifiable only in the uppermost 4.0 mm of subterranean shoot; below that, they had withered so much that the only visible remnants were peg-like structures 2.0 – 3.0 mm in diameter and 1.0 – 2.0 mm tall, which appeared to be deteriorated areolar tufts of trichomes. Bark was sufficiently wrinkled and rough that some of its irregularities resembled withered tubercles, making identification of tubercles difficult. Subterranean withered tubercles should have been aligned with the rows of tubercles on the aerial portions (Fig. 4.14), but there were only two cases in which a row of withered tubercles could be identified by their areolar tufts (Fig. 4.15). We followed rows of tubercles from the aerial portions of shoots down into subterranean portions but usually could not find any identifiable withered tubercles, apparently the areolar tufts abscise from the plant.

**Fig. 4.14** *L. williamsii* young tubercle with areole bearing a tuft of woolly trichomes. The area of the central apical meristem (not shown) from which the tubercles with their areoles emerge radially, was located immediately below the field of the photograph.

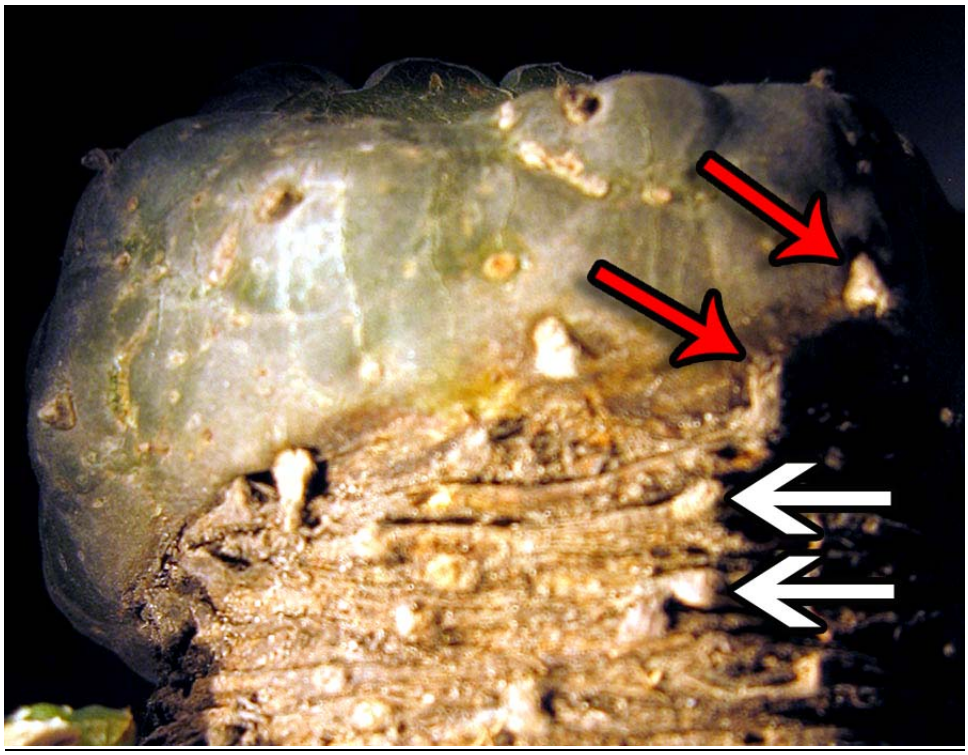

**Fig. 4.15** *L. williamsii* tubercles with trichome-tufted areoles on lower portion of crown (red arrows). On the corrugated brown surface of the brown subterranean stem are two light-colored protuberances (white arrows) situated on a diagonal curve containing the two tufted areoles in the crown above. These protuberances are the visible remnants of areolar tufts of stem tubercles, which are the source of lateral branches of stem that develop in response to removal of the plant's shoot apical meristem.

Structure of the hypocotyl

The hypocotyl is the short (less than 10 mm long) region located between the seedling shoot and the seedling root. The structure of the hypocotyl in *L. williamsii* had characters of both the root and shoot. The center of all hypocotyls was root-like because it consisted of dilatated metaxylem and innermost secondary xylem, so it too was pith-like. It could be identified as not being a true pith by the presence of vessel elements and WBTs interspersed with the parenchyma cells. The outermost regions were true cortex,

and even though a hypocotyl is not a part of the shoot, the hypocotyls of *Lophophora* had cortical bundles. Hypocotyl cortex width was wider than that of the cortex-like region in roots, narrower than the true cortex of shoots in each plant. All hypocotyl samples had abundant secondary xylem and phloem, which was similar to that in both roots and shoots. Hypocotyl bark was similar to that of shoots, having occasional bits of cortical bundle that had been cut off by a cork cambium that was located deep within the cortex.

**Discussion**

This study shows that roots and shoots of *Lophophora williamsii* differ significantly in several features. At least in fresh plants, the two organs can be distinguished easily and reliably using just a handlens or dissecting microscope (Table 4.2). Both root and shoot have an outer region that resembles cortex, but the true cortex of shoots has a granular appearance because it contains numerous cortical bundles (Fig. 4.16), as is true of many cacti (Sajeva and Mauseth 1991; Mauseth and Sajeva 1992). In contrast, the outer region of roots resembles cortex but is in fact an accumulation of secondary phloem, which has a very smooth appearance as seen with a handlens. The vascular bundles of lateral roots pass through this cortex-like region of secondary phloem, but because lateral roots are so sparse and because their vascular bundles are oriented vertically, there is little chance of confusing the shoot and root outer tissues. Roots of other cacti also have this outermost

**Table 4.2** Distinguishing characters of shoots and roots of *L. williamsii*.

| | Shoots | Roots |
|---|---|---|
| Outer tissues | True cortex; appears granular due to presence of cortical bundles. At least 5 mm or more thick. | Cortex-like region; appears smooth due to lack of cortical bundles. At most only 3 mm thick. |
| Center | True pith; appears smooth due to lack of medullary bundles and lack of dilatated metaxylem. Width is not a reliable criterion. | Pith-like region; appears granular due to dilatated metaxylem and innermost secondary xylem. |
| Withered tubercles | Sometimes present, not always easy to identify if bark is rough. | Never present. |
| Lateral roots | Never present on shoots? May be confused with post-harvest adventitious roots. | Common on taproots, but could be absent. |

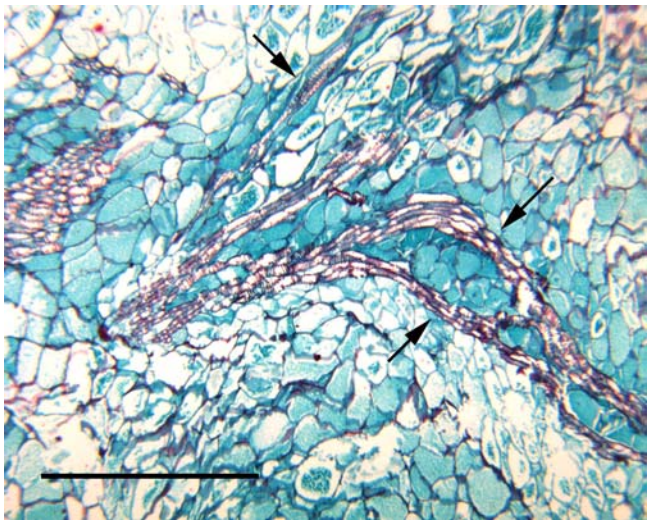

**Fig. 4.16** Cortex of shoot of *L. williamsii*. Arrows indicate cortical bundles running through the parenchyma. All visible parenchyma cells are cortex parenchyma. Scale bar (lower left) = 1 mm.

cortex-like region (Stone-Palmquist and Mauseth 2002). The true cortex of the root of *L. williamsii* is pure parenchyma (Fig. 4.17), not secondary phloem.

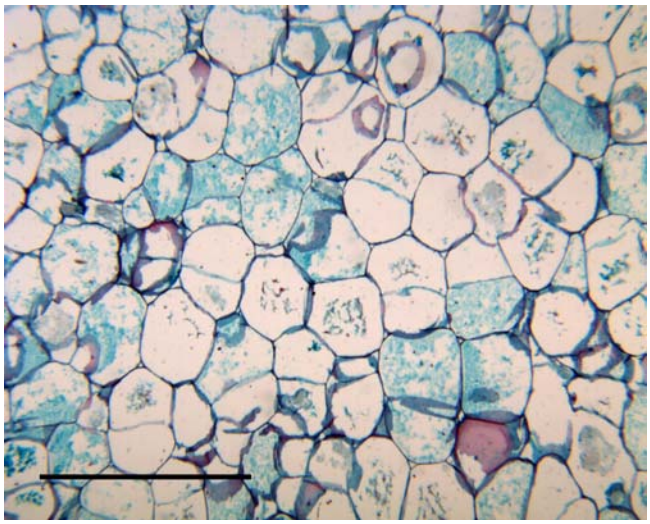

**Fig. 4.17** Cortex of root of *L. williamsii*. All cells are cortical parenchyma cells. Absence of cortical bundles is conspicuous. Scale bar = 1 mm.

The center of shoots is occupied by true pith, which is homogeneous in appearance due to the lack of medullary bundles in *Lophophora williamsii* (Fig. 4.18). Medullary

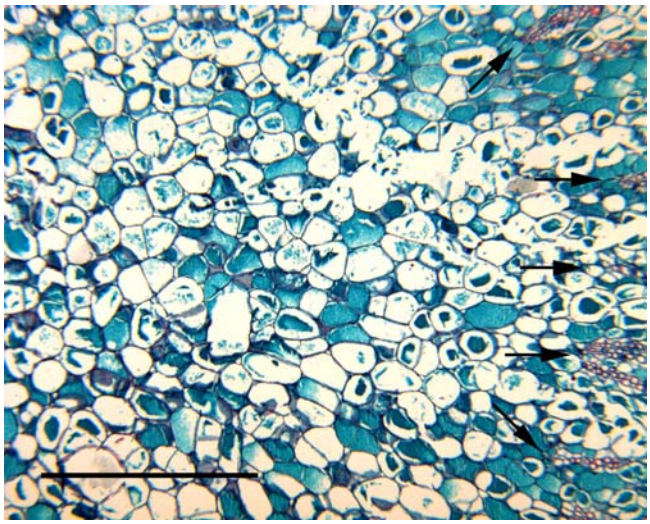

**Fig. 4.18** Pith of center of shoot of *L. williamsii*. The pith is seen to consist of pure parenchyma (center and left) without xylem. Arrows (right) indicate the innermost primary xylem of the vascular bundles in the ring of bundles at the perimeter of the pith. Only part of the ring of vascular bundles is shown (right), and all the cells on the left are true pith. Scale bar = 1 mm.

bundles are common in many species of cacti but lacking in others (Mauseth 1993). In the roots of most species, metaxylem either has no parenchyma or if it does, the parenchyma does not undergo proliferation, so roots of most plants have no pith-like region at all and can easily be distinguished from shoots (Mauseth 1988). The pith-like region of roots in *L. williamsii* makes the roots look like shoots at first glance or with just the naked eye, but because it originates by cell division in root metaxylem (Fig. 4.19), it has a granular appearance when examined with a handlens.

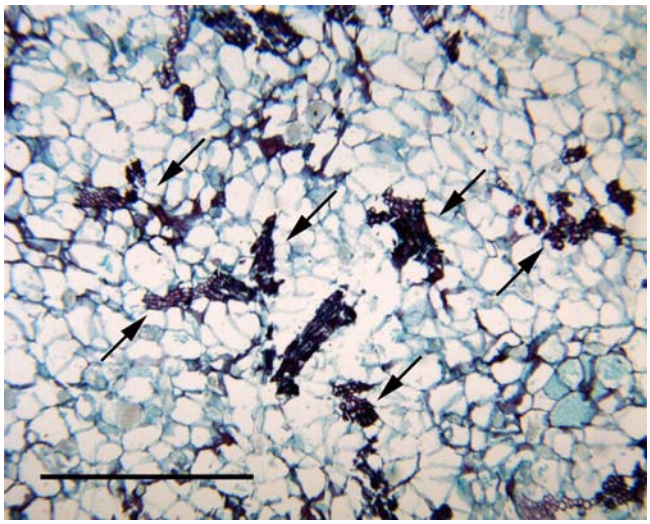

**Fig. 4.19** Dilatated metaxylem of center of root of *L. williamsii*. All arrows indicate masses of dark-staining metaxylem tracheary elements. All parenchyma cells in the image are metaxylem parenchyma cells, not pith. Scale bar = 1 mm.

Two characters might be useful for distinguishing shoots from roots without cutting plants open to examine the cortex-like regions and pith-like regions. Lateral roots emerged from the sides of other roots and from the sides of hypocotyls, but none was seen on any part of the three adult shoots we examined. It is possible that shoots had produced adventitious roots which had either broken off when the plants were collected or which had abscised before collection. No remnants of such roots were seen when we examined the sides of subterranean portions of shoots with a dissecting microscope, but these plant parts were so wrinkled and bark-covered that we could have missed any that were present. However, as we examined the microscope slides of subterranean portions of shoots, we did not encounter any vascular bundles that would have indicated

adventitious roots had been present. One plant, examined in the field, had a root emerging from its side at about the same level as a lateral branch; if that root was emerging from the shoot rather than the hypocotyl then shoots as well as roots might bear roots. Now that anatomical characters can be used to distinguish roots from shoots, it will be possible to examine more plants in the field to see how frequently shoots bear roots from their sides, and the extent to which such adventitious root development is associated with branching of subterranean stem tissue in response to peyote harvesting or removal of the apical meristem by natural processes.

If the below-ground portion of the plant has withered tubercles, it must be part of the shoot rather than root or hypocotyl. However, we did not find any withered tubercles on one of our adult specimens despite a search with a dissecting microscope. Apparently they either wither so much that they become unrecognizable or they abscise, so their absence cannot be used as proof that the structure is either hypocotyl or root. When trying to find withered tubercles in the field, search in a line continuous with the line formed by the rows of tubercles in the aerial shoot, because all tubercles are formed in rows (just as on the ribs of columnar cacti; Mauseth 2000).

If a plant remnant is to sprout and continue growing after its top has been harvested, the presence and health of these withered tubercles is important. If a plant is harvested by being cut too low, only root or hypocotyl will remain in the ground and neither of these have axillary buds, so neither can produce a bud to replace the harvested shoot. If the plant is harvested by being cut through the subterranean shoot, and if the remaining portion of shoot has healthy tubercles – withered but with an axillary bud – then the

remaining portion should be able to sprout and grow and be ready for harvesting again in a few years. But if the remaining shoot has abscised all its tubercles, or if they have withered so much that they are no longer healthy, then the remaining piece of plant will not be able to sprout and will instead eventually die for lack of photosynthetic tissues. Tubercles located higher on the subterranean portion of the shoot are younger and presumably healthier than those lower down, deeper in the soil and closer to the root. If plants are harvested by cutting the subterranean shoot rather high – closer to soil level – the greater the chances are that the residual piece of plant will have healthy tubercles and will be able to re-sprout.

With the information discovered in the current study, we now have the tools to examine in detail the sprouting potential after different types of harvesting. A set of plants could be harvested at various depths below soil level with the certainty that all had been cut high enough that some shoot tissue had been left on the remaining plant portion. The harvested top of each could be examined for withered tubercles. Presumably if plants are cut so high that their harvested tops have several recognizable withered tubercles, then the remaining portion also has at least a few tubercles and will sprout. But if plants are cut so low that the harvested tops have few or no identifiable withered tubercles near the cut – and if the cut does pass through shoot tissue not root tissue – then probably the remaining shoot also has few or no tubercles capable of sprouting. It is even possible to try cutting the plants through the hypocotyl to see if it is capable of forming adventitious shoot buds; that capacity is rare in hypocotyls but is known to occur in a few species.

Preliminary data on plants under greenhouse conditions were collected on 11 plants over a period of three years (not synchronically). Six plants were cut low (approximately 1.5 crown diameters below the base of the crown, and in every case at least 50 mm below the base of the crown). The distal subterranean portions of those plants were then observed for at least eight months. No new stem branches were observed on any of the six low-cut plants. The other five plants were cut high (at about the base of the crown, and in no case more than 5 mm below the base of the crown) and similarly observed. One or more crown-bearing lateral branches from the decapitated subterranean stem were observed within five months on three of the six plants, and within eight months on all five high-cut plants.

Future studies include a similar greenhouse experiment on regrowth, with substantially larger numbers of plants and with varying measured depths of cut expressed as a fraction (or multiple) of crown diameter, so that crown diameter could be used as a practical guide for harvesting peyote in the field. It will be noted whether the cut goes through root, stem, or hypocotyl. That titration of the effect of depth of cut on regrowth in the greenhouse will be followed by a similar experiment conducted in the field, with individually identified and permanently marked plants. It is anticipated that results in the field may differ from greenhouse results, due to harsher environmental conditions in the field and the possibility that some of the smaller plants in the field may have been harvested previously, perhaps leaving less than the critical mass of subterranean stem tissue needed to regenerate viable photosynthetic stem tissue. These factors may affect regeneration and survivorship in all experimental groups.

# CHAPTER V

# POPULATION GENETICS OF *ASTROPHYTUM ASTERIAS*

## Introduction

Because some of the U.S. populations of *Astrophytum asterias* (like some of the Mexican populations) have been subject to human predation (in the form of cactus "collection") since early in the 20th Century (Clover 1932), it would be reasonable to expect that the more accessible of these populations would now be reduced in size. In addition to collecting, other pressures on *Astrophytum* populations are associated with changes in land use, including agricultural practices such as root-plowing, residential development (B. Treviño, personal communication 1998), commercial development, and a general trend toward conversion of large ranches into smaller tracts with diverse uses – the latter phenomenon being an effect of the rapidly increasing human population of South Texas generally and Rio Grande City in particular. These changes in land use may result in the outright annihilation of star cactus populations and loss of habitat (as when a population is bulldozed in the process of real estate development or root-plowed in the conversion of brush to pasture). Or they may cause more subtle effects such as habitat fragmentation (which results in the breaking up of large populations into subpopulations separated from each other by terrain devoid of *Astrophytum*, with the result that gene flow among the subpopulations is impeded) or habitat degradation (as when overgrazing brings about selective changes in the composition of the plant community, and denudation of ground cover and trail formation promote increased soil

erosion), which will in turn render the land increasingly unsuitable for survival and reproduction of *Astrophytum*. Accordingly, one might expect that some of the U.S. populations of this cactus would exhibit the genetic effects of small population size associated with a bottleneck. Such effects would include decreased heterozygosity (increased homozygosity), fixation of alleles, and loss of alleles – all indications of decreased genetic diversity, which is often accompanied by loss of fitness and decreased "evolutionary potential". Roughly speaking, depletion of the population's genetic assets decreases the probability that the population will survive in the face of long-term adverse changes in environmental conditions. Detection of genetic results that may be indicative of a bottleneck is one of the objectives of this study.

We undertook a study of *A. asterias* individuals from four U.S. locations on three properties, using initially six microsatellite markers captured from the genomic DNA of an individual in one of the four locations. The results are interpreted to determine the degree of genetic structure within and among the four demes defined by location, and to indicate which, if any, of the demes constitute distinct populations (as opposed to genetically indistinguishable subpopulations of a single population that lacks significant structure). The importance of structure is that its presence by definition indicates nonrandomness of breeding among the sampled individuals, resulting in deviation from Hardy-Weinberg equilibrium (Haldane 1954). Having determined that a degree of genetic structure is present, we shall seek to provide a historical biological explanation for it.

We also evaluate and address other issues such as reproductive biology (outcrossing vs. selfing) and gene flow in the form of gametic migration (transport of pollen by pollinators) and zygotic migration (transport of seed by animals). The suitability of these demes/populations as sources of seed for reintroduction of *Astrophytum asterias* into suitable habitat within the historical range of the species or restoration/augmentation of extant but decimated populations is discussed.

## Materials and methods

### *Demes, individuals and plant tissue sampled*

Ninety-four individuals were sampled from the four US locations that were both known and accessible on April 8, 2005. These are denoted Min, Tne, Tnw and Esc, and are located in Starr County, Texas. Due to the potential for poaching, the exact locations of the study sites are not disclosed as per the request of the property owners and conservation agencies involved (USFWS, TPWD). The demes of *A. asterias* at all four locations are small in area (3 hectares or less), and no two demes are contiguous, though three of them (Min, Tne and Tnw) were all within 1.3 km of each other, while the fourth (Esc) was about 9-10 km distant from the other three. The exact distances among these demes are presented in the half-matrix in Table 5.1. The distribution of the 94 sampled individuals among the locations was as follows. Min: 42. Tne: 26. Tnw: 12. Esc: 14. The number of individuals sampled was determined by and equal to the number of individuals found in flower on the day sampling took place. This constraint of limiting

**Table 5.1** Geographic distances among the four sampled locations in Starr County, Texas. Distances between the members of each pair of locations are in kilometers.

| | Min | Tne | Tnw | Esc |
|---|---|---|---|---|
| Min | -- | | | |
| Tne | 0.82 | -- | | |
| Tnw | 1.27 | 0.45 | -- | |
| Esc | 10.33 | 9.50 | 9.05 | -- |

sampling to plants in bloom was imposed as a consequence of the decision to use the least invasive method of tissue sampling possible, which was to collect one tepal from each individual in flower. The normal sampling method for cacti not in flower, viz., taking biopsy samples of stem tissue, was rejected as too invasive, due to the risk of mortality from infection of the open wound. The individual tepals collected were placed into vials containing desiccant pellets (t.h.e. Desiccant, EMD Chemicals), where they were stored dry at room temperature until DNA extraction.

*DNA extraction procedure*

*A. asterias* tissue (one desiccated tepal) was ground for 3-4 sec. in a 1.5-ml Eppendorf tube (WVR catalog No. 20170-620) with a blue Teflon pestle that fits the apex of the Eppendorf tube (WVR catalog No. KT95050-99). Extraction buffer (*vide infra*), 0.5 ml,

was added to the Eppendorf tube and the tissue in extraction buffer was ground forcefully until most lumps were broken up (ca. 10 sec.). The cap of the Eppendorf tube was closed and the tube was agitated vigorously by “raking” it rapidly and forcefully five to 10 times across an 80- or 96-well polyproplylene freezer rack for Eppendorf tubes, so that the tissue in extraction buffer appears very frothy. (At this point the solution can be left at ambient temperature for several hours or days. The DNA is stable in the extraction buffer.)

The extraction buffer containing the DNA was spun for 4 min. at full speed in a microcentrifuge to remove solids. Supernatant, 450 µl, was carefully removed (at which point the first tube, with its pellet of non-DNA-containing solids, was discarded), and the supernatant was deposited into a fresh Eppendorf tube with 450 µl isopropyl alcohol (IPA) at room temperature. The new tube was inverted to mix the extraction buffer supernatant and IPA, and the solution was left to incubate for 10 min. at room temperature. Then the solution was spun for 5 min. at maximum speed at room temperature, and the supernatant was discarded. The tube with its DNA pellet was inverted onto a paper towel and left for the pellet to air dry at room temperature for a few minutes. Then 0.5 ml of Super TE (*vide infra*) was added to the tube, and the pellet was resuspended (often requiring the manual use of another small teflon pestle), followed by vortexing for 20 sec. (If necessary, the procedure may be interrupted at this point and the solution stored frozen at -20˚C. Barring any such interruption, the solution was allowed to sit for 5 min. at room temperature.)

The tube was spun for 20 sec. to remove any undissolved solids (probably mostly polysaccharides), and the supernatant (ca. 450 µl) was transferred by pipette to a new tube containing 50 µl of 3 M NaOAc, pH 5.2. The tube was mixed by inversion, and 500 µl of room-temperature IPA was added to the tube, which was again inverted gently to mix, then allowed to incubate at room temperature for 10 min. The tube was then spun for 10 min. at room temperature, and the supernatant discarded, leaving a small white pellet in the tube. The pellet was washed twice with 1 ml cold (-20˚C) 80% ethanol, spinning at full speed for 20 sec. after each ethanol wash. The pellet was then dried in a speed vac under low heat. The pellet was stored in 100 µl 0.1x TE at 4˚C overnight to help soften the pellet and dissolve the DNA. On the following day the tube was gently flicked to dissolve and mix any remnant of a visible pellet, at which point a 5-µl sample of the DNA solution was electrophoresed on a 1.5% agarose gel to confirm the presence of extracted DNA. The DNA solution was then stored at -20˚C until needed.

The extraction buffer for the DNA extraction procedure consists of 200 mM Tris pH 7.5, 250 mM NaCl, 25mM EDTA pH 8, and 0.5% SDS. Super TE consists of 50 mM Tris pH 7.5 and 10 mM EDTA pH 8. The 0.1x TE consists of 1mM Tris pH 7.5 and 0.1 mM EDTA.

*Microsatellite development*

A library of six distinct microsatellite loci was developed from *A. asterias* genomic DNA from an individual in the Tne population. Information on these loci, including

number of alleles detected, primer sequences and melting temperatures, size range of the amplicon, and fluorescent labels employed on the primers is presented in Table 5.2.

**Table 5.2** *Astrophytum asterias* microsatellite loci. Data shown include name of locus, sequences of forward and reverse primers, size range of amplicon, number of alleles detected (*n*), identity of fluorescent label on 5' end of forward primer, and predicted melting temperature (salt-adjusted).

| Locus name | Primer sequences (5'→3') | *n* | Size range | Fluorescent label | Melting temp. |
|---|---|---|---|---|---|
| *AaB6* | CATGCGAACAGATTGAAAAGAGGG<br>ACTCAGGAAAGACTTACACCATGG | 7 | 83-99 | HEX | 64˚C |
| *AaH11* | GAAGAAACACTTCTGCAAGTAGATG<br>GATTTCCATCACCATCTTGTCAGC | 13 | 83-109 | FAM | 63˚c |
| *AaA3* | GCAAGCAAGAGTATGGTGAATTGG<br>AGTTATTTTCACGGTAACACACATGG | 10 | 138-168 | FAM | 64˚C |
| *AaG3* | CTAACAGAGAATCCAAGGCTTTTCC<br>AATCGCCAGCCGAGGGAGAC | 4 | 127-133 | HEX | 64˚C |
| *AaC3* | ACGGTCCAGTCACATAACATTCC<br>AACTAATATCATGCTGCGTCGTTAG | 11 | 88-108 | FAM | 63˚C |
| *AaD9* | CTGTTTAGTTCTCTCGTCTTCACC<br>CTCCGCTTTTACTGCTAGCACC | 6 | 135-143 | HEX | 64˚C |

The method for microsatellite capture and cloning is derived from the biotinylated-oligonucleotide capture concept of Kijas *et al.* (1994) and Prochazka (1996), with some of the modifications described by Reddy *et al.* (2001). The essence of the modified method used in our laboratory for capturing microsatellite loci from plant DNA, similar to that described in Pepper and Norwood (2001), is as follows: Genomic DNA (ca. 2 µg)

extracted from tissue of a representative individual of *Astrophytum asterias* from population Tne was digested with restriction endonucleases, which yielded a population of blunt-ended restriction fragments, most of which were between 500 and 1000 bp in length. The restriction fragments were ligated to adaptor primers AP11 and AP12, and the adaptor-ligated products were amplified by PCR using AP11 as the only primer. Short (40-60 bp) biotinylated oligonucleotide probes with various dinucleotide and trinucleotide repeat sequences were then hybridized in 6x SSC with denatured (95˚C for 5 min.) preamplified genomic DNA from the previous step. Annealing of the biotinylated probes and the genomic DNA was carried out at 60˚C for 1 h. The hybridized genomic DNA fragments with biotinylated probes were then incubated with streptavidin-coated magnetic beads (Promega), so that the biotin (bound to the oligonucleotides containing microsatellite-like repeat sequences, which were hybridized with genomic DNA fragments containing complementary repeat sequences) reacted with and became bound to the streptavidin on the magnetic beads, thus capturing the microsatellite-containing genomic DNA fragments annealed to the biotinylated oligonucleotides. A series of washings with 6x SSC and 0.1% SDS removed the non-microsatellite-containing fragments of genomic DNA that were not hybridized with the biotinylated probes bound to the streptavidin on the magnetic beads. Captured genomic DNA fragments were then eluted from the beads by incubation with 0.1 M NaOH at 60˚C. The solution was then neutralized with 1 M Tris buffer and desalted with a size-exclusion column, which also removed the short oligonucleotide probes. The desalted DNA was then amplified by a second round of PCR (30 cycles), and the PCR products

were cloned by ligation into a cloning vector (Invitrogen pCR Blunt II Topo vector) and transformed into *E. coli*, which was grown out on agar plates. In this system recombinant colonies likely to contain microsatellite inserts are positively selected by inactivation of the *ccdB* (control of cell death) gene, so that only the insert-bearing *E. coli* produced viable colonies. Colonies were transferred to 96-well plates with freezing medium and stored at -80˚C until needed for PCR and sequencing.

Seventy-one clones were sequenced, and from those sequences 29 primer pairs were designed and screened (first without fluorescent labeling, then, for the primer pairs that amplified some locus in genomic DNA of *A. asterias*, with fluorescent labeling of the 5' end of the forward primer), to obtain the six polymorphic loci shown in Table 5.2. The genotypic data from one (*AaA3*) of the six good loci were excluded from the analyses due to the fact that *AaA3* exhibited linkage disequilibrium with *AaC3*, which fact may have biased the results in some of the population genetic tests and measurements. So the net yield of the screening process was actually five usable loci.

The microsatellite loci were amplified from *A. asterias* genomic DNA samples by polymerase chain reactions (PCRs) performed on a Stratagene Robo-Cycler, with the following components in the PCR reaction: 1x PCR buffer, combined dNTPs (0.2 mM each), 1 mM $MgSO_4$, 0.5 units KOD Hot Start DNA polymerase (Novagen), combined forward and reverse fluorescent primer solution (0.3 µM each primer), template (5-50 ng genomic DNA), and double-distilled water q.s. for a total reaction volume of 25 µl. The PCR regimen began with denaturation at 94˚C for 2 min, followed by 30 cycles as follows: denaturation at 94˚C for 30 sec, annealing at 55˚C for 15 sec, and extension at

68˚C for 20 sec. The last cycle was followed by a prolonged extension phase at 68˚C for 10 min. The fluorescent PCR reaction product was diluted as appropriate (usually 1:30 but sometimes with less dilution) to give a strong enough (but not excessively strong) signal for readable peaks on the electropherograms of the DNA fragments. Then 1 µl of the diluted PCR product was added to 9 µl of High Di formamide (Applied Biosystems) with 0.1 µl of ROX 400HD dye (Applied Biosystems) as an internal size standard. The resulting samples were denatured for 2 min at 95˚C and subjected to fragment analysis by capillary electrophoresis in a 4.5% denaturing polyacrylamide gel on an ABI 3100 DNA sequencer in Genescan mode, which detected the fluorescent-labeled primers and size standard, permitting determination of DNA fragment length to the nearest single nucleotide. A combination of Genescan and Genotyper software (Applied Biosystems) was used to determine allele size and homozygosity/heterozygosity.

*Genetic variation within and among populations*

Population genetic parameters were estimated from the allele data, and subsequent analyses performed, using various programs in Genepop on the Web (based on Raymond & Rousset 1995; see Appendix 1 for input data). In the Genepop analyses, we provisionally treated the four sampled demes (Min, Tne, Tnw, and Esc, identified by their geographic locations) as independent populations. However, when referring to the *F*-statistics of Wright (1965), we employ Wright's terminology, whereby our four geographically defined "populations" (or demes) become Wright's "subpopulations",

and our total composite sample (94 individuals from our four “populations”) becomes Wright’s single “population”.

We first used Genepop to test for linkage disequilibrium between pairs of loci. Finding a significant P value for linkage disequilibrium involving the *AaA3* and *AaC3* loci, we excluded the genotypic and allelic data for the *AaA3* locus from subsequent tests and measurements, unless we knew that the program for a given test was robust to linkage disequilibrium. This meant that in effect we used only five loci to calculate or estimate allele frequencies, Wright’s (1965) *F* coefficients $F_{IS}$, $F_{ST}$ and $F_{IT}$, (where $F_{IS}$ measures genetic variation among individuals within subpopulations, $F_{ST}$ measures variation among subpopulations, and $F_{IT}$ measures variation among all sampled individuals across the totality of subpopulations), *Rho*-statistics such as $Rho_{ST}$ (which estimates $R_{ST}$, the modified version of Wright’s $F_{ST}$ (Slatkin 1995; Rousset 1996) designed for use with such stepwise mutation processes as are postulated to apply to microsatellites), and *Nm* (where *N* is the effective population size and *m* is the effective proportion of the population replaced by migrants in each generation), which affords a measure of gene flow under the island model (Wright 1943), using the private allele method of Slatkin (1985) modified by Barton and Slatkin (1986). We used various Genepop programs to test for Hardy-Weinberg disequilibrium, heterozygote deficiency, heterozygote excess, allelic differentiation (across all populations and between all pairs of populations), and genotypic differentiation (across all populations and between all pairs of populations).

*Decreased heterozygosity and population bottlenecking*

Using Genepop, we tested for decreased heterozygosity with particular interest, as it is often associated with small effective population size associated with bottlenecks – which would not be an unexpected finding in these *Astrophytum* populations, given the history of overcollection of plants and adverse changes in land use over the past several decades. In primarily outcrossing species such as *A. asterias*, decreased heterozygosity is often associated with an increase in expression of detrimental recessive genes, which may likewise bring about a decrease in fitness, which in turn may result in a decreased survival rate – all of which, if allowed to continue unabated, would predictably push the population into a self-reinforcing causal chain of events known as the "extinction vortex" (Soule & Orians 2001).

*Genetic differentiation and geographic distance (isolation by distance)*

We used the Isolde program in Genepop to test for isolation by distance (Wright 1943). This program measures the correlation of genetic differentiation between the members of each pair of populations (as measured by $F_{ST}$) and the geographic distance between the members of each pair of populations.

*Estimation of the number of populations and population structure*

Commonly one of the most difficult questions to resolve in population genetics is, given a set of genetic data collected from a set of spatially/geographically clustered individuals, how many genetically distinct populations do the sampled individuals actually comprise? We adopted a Bayesian approach to this problem, using the software package called "*structure*" (Pritchard et al. 2000), which utilizes a model-based clustering method that assumes that the allele data exhibit Hardy-Weinberg equilibrium and no linkage disequilibrium. An iterative Markov chain Monte Carlo (MCMC) algorithm, for which we set the parameters to include a burn-in of 100,000 iterations, followed by a run of 1,000,000 iterations, identified one or more modes of maximum posterior probability for each assumed value of K, where K is the number of populations that best reflects the genetic structure, if any, in the genotypic data for all sampled individuals. Each of these output values from the MCMC algorithm (in the form of Ln P(D), which is the natural log of the posterior probability that the data fit the assumed value of K), which were generated with the sequentially assumed values of K, was then used to calculate a posterior probability for each value of K tested. Generally with this method the value of K with the highest posterior probability is selected as the number of populations most likely to fit the genotypic data. We carried out this procedure for assumed values of K ranging from 1 through 4. In one set of 16 runs (four runs for each of the four demes) we excluded the geographic origin data of the sampled individuals in order to obtain a purely genetic analysis of all 94 sampled individuals, which is the most appropriate mode in which to test assumed values of K. In a subsequent, comparable set

of 16 runs, we incorporated the geographic origin data of the sampled individuals into the data set, with the specific objective of obtaining numbered Fst values for each population, to evaluate how different each population was from mean allele frequencies.

**Results**

The six *Astrophytum asterias* loci examined exhibited a total of 51 detectable alleles. The number of alleles per locus ranged from four to 13, with a mean of 8.5 alleles per locus. A maximum of two alleles per individual (as homozygotes and heterozygotes) were detected, from which fact we infer diploidy in the sampled set of individuals.

Summary information on the four geographic populations sampled, including numbers of individuals sampled, $A_O$ (number of alleles per locus in a population), $H_E$ (expected heterozygosity) and $H_O$ (observed heterozygosity), is presented in Table 5.3.

**Table 5.3** Summary population data. Included are name of population defined by geography, number of individuals sampled per population (sample size), mean number of alleles observed per locus for a given population ($A_O$), mean expected heterozygosity across all loci ($H_E$), and mean observed heterozygosity across all loci ($H_O$).

| Population | Sample size | $A_O$ | $H_E$ | $H_O$ |
|---|---|---|---|---|
| Min | 42 | 7.2 | 0.675 | 0.619 |
| Tne | 26 | 6.7 | 0.616 | 0.647 |
| Tnw | 12 | 5.2 | 0.612 | 0.653 |
| Esc | 14 | 4.7 | 0.548 | 0.393 |

*Genetic variation within and among populations*

For purposes of the following discussion we shall define degrees of statistical significance conventionally as follows: A result is not significant (NS) if the probability $P \geq 0.05$. It is significant if $P < 0.05$, and is highly significant if $P < 0.01$.

We tested all pairs of the original six loci for genotypic disequilibrium (linkage disequilibrium) in each population in Genepop. The *AaA3-AaC3* locus pair showed significant ($P = 0.04$) genotypic disequilibrium in the Min population, and approached significance in both the Tnw population ($P = 0.09$) and the Esc population ($P = 0.06$). Fisher's method for determining a P-value across all populations yielded a significant ($P = 0.03$) result for genotypic (linkage) disequilibrium for the *AaA3-AaC3* locus pair only. In order to avoid potential confounding effects associated with linkage disequilibrium in subsequent tests and measurements (as some of the programs are explicitly based on the assumption of no linkage disequilibrium), we excluded the data for locus *AaA3* from subsequent analyses. I.e., the dataset was reduced from six loci to five loci for all other analyses. When the test for linkage equilibrium was run using the five-locus dataset, there were no significant results, from which we infer that the linkage disequilibrium involved the *AaA3-AaC3* locus pair only, and that it was resolved by the removal of one member of that pair.

The exact Hardy-Weinberg test of Haldane (1954) for nonrandom breeding was applied to each locus across all populations. A significant deviation from Hardy-Weinberg equilibrium was found for locus *AaC3* ($P = 0.02$), and locus *AaH11* showed a highly significant ($P < 0.01$) deviation from Hardy-Weinberg equilibrium. When the

same test was applied to each population across all loci, populations Min and Esc respectively showed significant ($P = 0.02$) and highly significant ($P < 0.01$) deviations from Hardy-Weinberg equilibrium. When the test was applied globally across all loci and all populations, the overall result was a highly significant ($P < 0.01$) Hardy-Weinberg disequilibrium.

The results for the Hardy-Weinberg test for heterozygote deficiency, when applied to each locus, by population, are presented in Table 5.4.

**Table 5.4** Results of Hardy-Weinberg test for heterozygote deficiency, testing each locus in each population. Significant and highly significant P values are shown numerically in the table. "NS" indicates a nonsignificant P value.

| Locus | Min pop | Tne pop | Tnw pop | Esc pop |
|---|---|---|---|---|
| *AaB6* | NS | NS | NS | NS |
| *AaH11* | 0.0000 | NS | NS | 0.0000 |
| *AaG3* | NS | NS | NS | NS |
| *AaC3* | 0.007 | NS | NS | NS |
| *AaD9* | NS | NS | NS | NS |

The same test for heterozygote deficiency for each population, tested at each locus, gave results as shown in Table 5.5.

**Table 5.5** Results of Hardy-Weinberg test for heterozygote deficiency, testing each population at each locus. Significant and highly significant P values are shown numerically in the table. "NS" indicates a nonsignificant P value.

| Population | Locus *AaB6* | Locus *AaH11* | Locus *AaG3* | Locus *AaC3* | Locus *AaD9* |
|---|---|---|---|---|---|
| Min | NS | 0.0000 | NS | 0.007 | NS |
| Tne | NS | NS | NS | NS | NS |
| Tnw | NS | NS | NS | NS | NS |
| Esc | NS | 0.0000 | NS | NS | NS |

When the Hardy-Weinberg global test for heterozygote deficiency was applied in the multi-locus mode to each population, it gave highly significant (P = 0.0000) results for the Min and Esc populations, and non-significant results for the Tne and Tnw populations. When the same test was applied in the multi-population mode to each locus, the results were highly significant (P = 0.0000) at locus *AaH11* and significant (P = 0.02) at locus *AaC3*. The overall test for all populations and all loci gave a highly significant (P = 0.0000) result.

The Hardy-Weinberg test for heterozygote excess, when applied by locus in each population, yielded only one significant (P = 0.04) result, at locus *AaG3* in population Min. The result was identical when the test was applied by population at each locus. When the Hardy-Weinberg global test for heterozygote excess was applied in the multi-locus mode to each population, it gave no significant result for any population. When the

same test was applied in the multi-population mode to each locus, only locus *AaG3* gave a significant (P = 0.02) result. The overall result for all loci and all populations was not significant.

In the Genepop test for genic (i.e., allelic) differentiation for all populations at each locus, significant P values were obtained for locus *AaB6* (P = 0.047) and locus *AaD9* (P = 0.04); and highly significant P values were obtained for loci *AaH11* (P = 0.0000), *AaG3* (P = 0.001) and *AaC3* (P = 0.0000). Fisher's combination test for genic (allelic) differentiation across all loci in all populations gave a Chi-square value of infinity and a non-numerical "highly significant" value of P.

Results of the test for genic (allelic) differentiation for all **pairs** of populations at each locus and across all loci are presented in Table 5.6. Fisher's method for calculating a P value for genic differentiation for each population pair across all loci yielded significant results for the Tne-Tnw population pair (the two geographically closest populations) (P = 0.03) and for the Tnw-Esc pair (P = 0.012), and highly significant results (P < 0.01) for the other four population pairs.

**Table 5.6** Genic (allelic) differentiation for all pairs of populations, by locus and across all loci.

| Locus | Population pair | Significant (*) & highly significant (**) results |
|---|---|---|
| B6 | Min-Tnw | * P = 0.04 |
| B6 | Tnw-Esc | ** P = 0.006 |
| H11 | Min-Tne | ** P = 0.001 |
| H11 | Min-Esc | ** P = 0.0002 |
| H11 | Tne-Esc | ** P = 0.00009 |
| G3 | Min-Tnw | ** P = 0.0005 |
| G3 | Min-Esc | * P = 0.03 |
| C3 | Min-Tne | ** P = 0.0008 |
| C3 | Min-Esc | ** P = 0.002 |
| C3 | Tne-Esc | ** P = 0.002 |
| D9 | Min-Esc | * P = 0.04 |
| D9 | Tne-Tnw | * P = 0.045 |
| All loci | Min-Tne | ** P = 0.00001 |
| All loci | Min-Tnw | ** P = 0.002 |
| All loci | Min-Esc | ** P = 0.00000 |
| All loci | Tne-Tnw | * P = 0.03 |
| All loci | Tne-Esc | ** P = 0.00002 |
| All loci | Tnw-Esc | * P = 0.012 |

The log-likelihood-based Genepop test for genotypic differentiation for all populations at each locus (Goudet et al. 1996) gave significant results for loci *AaB6* (P = 0.045) and *AaD9* (P = 0.04), and highly significant results for the remaining loci *AaH11* (P = 0.001), *AaG3* (P = 0.0003) and *AaC3* (P = 0.0001). Fisher's method for testing for genotypic differentiation for all populations across all loci gave a highly significant result (Chi-square = 60; P = 0.0000).

Results of the Genepop test for genotypic differentiation for all **pairs** of populations are shown in Table 5.7. Fisher's method for testing each population pair across all loci

**Table 5.7** Genotypic differentiation for all pairs of populations.

| Locus | Population pair | Significant (*) & highly significant (**) results |
|---|---|---|
| B6 | Min-Tnw | * P = 0.02 |
| B6 | Tnw-Esc | * P = 0.02 |
| H11 | Min-Tne | ** P = 0.007 |
| H11 | Min-Esc | ** P = 0.004 |
| H11 | Tne-Esc | ** P = 0.0007 |
| G3 | Min-Tnw | ** P = 0.0005 |
| G3 | Tne-Tnw | * P = 0.03 |
| G3 | Min-Esc | * P = 0.015 |
| C3 | Min-Tne | ** P = 0.001 |
| C3 | Min-Esc | ** P = 0.004 |
| C3 | Tne-Esc | ** P = 0.005 |
| D9 | Min-Esc | * P = 0.02 |
| All loci | Min-Tne | ** P = 0.00008 |
| All loci | Min-Tnw | ** P = 0.002 |
| All loci | Tne-Tnw | * P = 0.04 |
| All loci | Min-Esc | ** P = 0.00003 |
| All loci | Tne-Esc | ** P = 0.0002 |

yielded a significant result (P = 0.04) for the Tnw-Tne pair of populations that are very close to each other geographically. The results of this test for the other five population pairs were all highly significant: P = 0.0002 for Tne-Min, P = 0.001 for Tnw-Min, P = 0.00000 for Esc-Min, P = 0.00003 for Esc-Tne, and P = 0.004 for Esc- Tnw.

The Barton & Slatkin (1986) program for estimating *Nm* based on the frequencies of private alleles used the mean sample size (mean size of the four population samples = 94 ÷ 4 = 23.5) to calculate the mean frequency of private alleles in the four populations, which is 0.041. The program yielded the following results for the three regression curves of Barton & Slatkin (1986): number of migrants for $N_{moy}$ = 10 was 7.72; number of migrants for $N_{moy}$ = 25 was 3.00; number of migrants for $N_{moy}$ = 50 was 1.93. The number of migrants per generation after correcting for size on the appropriate regression curve was 3.19.

In regard to quantifying genetic correlation and diversity based on allele frequency among individuals within populations, the computed values of $F_{IS}$ for each locus in each population are presented in Table 5.8.

**Table 5.8** Allele frequency-based correlation and variation among the individuals in each population. The matrix consists of $F_{IS}$ values for each locus (*x*-axis) in each population (*y*-axis). The figures in the bottom row are the $F_{IS}$ values for all sampled individuals (from all populations combined) for each locus. The figures in the last column are the $F_{IS}$ values for all loci combined for each population.

| Loci: / Pops | B6 | H11 | G3 | C3 | D9 | All loci |
|---|---|---|---|---|---|---|
| Min | 0.0838 | 0.3148 | -0.1979 | 0.1841 | 0.0534 | 0.1124 |
| Tne | -0.1450 | -0.0676 | -0.0502 | 0.0177 | -0.0549 | -0.0537 |
| Tnw | -0.0645 | -0.0386 | -0.1856 | -0.1111 | -0.0312 | -0.0888 |
| Esc | 0.1000 | 0.7500 | -0.0879 | 0.3627 | 0.4694 | 0.3226 |
| All pops | 0.0239 | 0.2276 | -0.1371 | 0.1192 | 0.0714 | |

In regard to quantifying genetic correlation and diversity based on allele size among individuals within populations, the computed values of $Rho_{IS}$ (a parameter comparable to Wright's (1965) $F_{IS}$ but based on allele sizes rather than allele frequencies) for each locus in each population are presented in Table 5.9.

**Table 5.9** Allele size-based correlation and variation among the individuals in each population. The matrix consists of $Rho_{IS}$ values for each locus (*x*-axis) in each population (*y*-axis). The figures in the bottom row are the $Rho_{IS}$ values for all sampled individuals (from all populations combined) for each locus. The figures in the last column are the $Rho_{IS}$ values for all sampled individuals (all loci combined) for each population.

| Loci: / Populations | B6 | H11 | G3 | C3 | D9 | All loci |
|---|---|---|---|---|---|---|
| Min | -0.0134 | 0.3816 | -0.2398 | 0.1583 | -0.0206 | 0.1376 |
| Tne | -0.2523 | 0.0750 | -0.0311 | -0.1459 | -0.0095 | -0.0243 |
| Tnw | -0.0820 | -0.0216 | -0.2774 | 0.0256 | -0.3113 | 0.0089 |
| Esc | 0.1122 | 0.1242 | -0.5986 | -0.3825 | 0.8102 | 0.0232 |
| All pops | -0.0885 | 0.2350 | -0.2425 | 0.0319 | 0.0408 | |

In order to obtain a quantitative notion of the degree of genetic differentiation (or population substructure) at the individual and subpopulation levels relative to the entire population sampled, single-locus F-statistics based on allele **identity** were estimated for all geographically defined populations (or subpopulations *sensu* Wright (1965)) according to the method of Weir & Cockerham (1984). The results are shown in Table 5.10.

**Table 5.10** *F*-statistics based on allele identity, for all populations. $Fwc_{IS}$, $Fwc_{ST}$ and $Fwc_{IT}$ correspond to Wright's (1965) $F_{IS}$, $F_{ST}$ and $F_{IT}$, respectively.

| Locus | $Fwc_{IS}$ | $Fwc_{ST}$ | $Fwc_{IT}$ |
|---|---|---|---|
| B6 | 0.0257 | 0.0487 | 0.0732 |
| H11 | 0.2274 | 0.0268 | 0.2481 |
| G3 | -0.1249 | 0.0485 | -0.0703 |
| C3 | 0.1354 | 0.0538 | 0.1820 |
| D9 | 0.0761 | 0.0185 | 0.0932 |
| All loci: | 0.0819 | 0.0410 | 0.1196 |

Single-locus $F_{ST}$ based on allele **identity** was also estimated for all **pairs** of populations using the method of Weir and Cockerham (1984). Estimates for the five loci are shown in a series of five mini-tables collectively designated Table 5.11.

**Table 5.11** Estimates of $F_{ST}$ for all pairs of populations at each locus. Each mini-table displays the six pairwise $F_{ST}$ values for a given locus. The numerical notation used here for populations (viz., Pop 1, Pop 2, Pop 3 and Pop 4) corresponds exactly to the alphabetical notation used elsewhere: Min, Tne, Tnw and Esc, in that order.

Estimates for each locus:

*AaB6*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0295 | | |
| 3 | 0.1140 | 0.0224 | |
| 4 | -0.0164 | 0.0694 | 0.1921 |

*AaC3*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0342 | | |
| 3 | -0.0111 | 0.0326 | |
| 4 | 0.1105 | 0.1263 | 0.0851 |

*AaH11*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0242 | | |
| 3 | 0.0038 | 0.0202 | |
| 4 | 0.0471 | 0.0559 | -0.0137 |

*AaD9*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0359 | | |
| 3 | -0.0156 | 0.0675 | |
| 4 | 0.0173 | -0.0130 | 0.0111 |

*AaG3*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0208 | | |
| 3 | 0.1439 | 0.0691 | |
| 4 | 0.0517 | -0.0087 | 0.0061 |

Estimates for all loci:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0282 | | |
| 3 | 0.0482 | 0.0392 | |
| 4 | 0.0479 | 0.0586 | 0.0544 |

*Rho*-statistics (the equivalent of *F*-statistics, but based on allele size rather than allele frequency) were estimated for all populations according to the method of Michalakis & Excoffier (1996). *Rho*-statistics are closely related to the *R*-statistics which Slatkin (1995) proposed to incorporate the stepwise mutation theory for microsatellites into measures of differentiation within a population. The results are shown in Table 5.12.

**Table 5.12** Rho-statistics based on allele size, for all populations. $Rho_{IS}$, $Rho_{ST}$ and $Rho_{IT}$ estimate Slatkin's (1995) $R_{IS}$, $R_{ST}$ and $R_{IT}$, respectively.

| Locus | $Rho_{IS}$ | $Rho_{ST}$ | $Rho_{IT}$ |
|---|---|---|---|
| *AaB6* | -0.0864 | 0.0610 | -0.0201 |
| *AaH11* | 0.2379 | -0.0171 | 0.2249 |
| *AaG3* | -0.2410 | 0.1083 | -0.1066 |
| *AaC3* | 0.0348 | 0.0372 | 0.0708 |
| *AaD9* | 0.0459 | 0.0078 | 0.0534 |
| All loci: | 0.0721 | 0.0147 | 0.0858 |

$Rho_{ST}$ (as defined by Rousset 1996) based on allele **size** was estimated for all **pairs** of populations according to the method of Michalakis & Excoffier (1996). The results are shown in Table 5.13.

**Table 5.13** Estimates of $Rho_{ST}$ for all pairs of populations at each locus. Each mini-table displays the six pairwise $Rho_{ST}$ values for a given locus. $Rho_{ST}$ estimates Slatkin's (1995) $R_{ST}$. The numerical notation used here for populations (viz., Pop 1, Pop 2, Pop 3 and Pop 4) corresponds exactly to the alphabetical notation used elsewhere: Min, Tne, Tnw and Esc, in that order.

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Estimates for each locus:

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*AaB6*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0080 | | |
| 3 | 0.2124 | 0.0853 | |
| 4 | -0.0218 | 0.0134 | 0.3096 |

*AaC3*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | -0.0149 | | |
| 3 | -0.0091 | 0.0117 | |
| 4 | 0.1114 | 0.1820 | 0.0378 |

*AaH11*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | -0.0062 | | |
| 3 | -0.0326 | -0.0290 | |
| 4 | -0.0294 | 0.0089 | -0.0209 |

*AaD9*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0155 | | |
| 3 | -0.0144 | 0.0421 | |
| 4 | 0.0118 | -0.0362 | 0.0271 |

*AaG3*:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.0529 | | |
| 3 | 0.2518 | 0.0875 | |
| 4 | 0.1401 | 0.0077 | 0.0105 |

Estimates for all loci:

| Pop | 1 | 2 | 3 |
|---|---|---|---|
| 2 | -0.0042 | | |
| 3 | 0.0220 | 0.0150 | |
| 4 | 0.0175 | 0.0417 | 0.0671 |

---

*Genetic distance and geographic distance (isolation by distance)*

The Isolde program in Genepop tests for a correlation between genetic differentiation between populations (measured as $F_{ST}$) and the geographic distance between populations – Wright's (1943) concept of isolation by distance. The input data consist of the geographic distances between populations (Table 5.1) and the between-population $F_{ST}$ data (across all loci) from Table 5.11, which are reformatted in a half-matrix below in Table 5.14.

**Table 5.14** Pairwise $F_{ST}$ values (calculated across all five loci) among the four sampled populations in Starr County, Texas.

| | Min | Tne | Tnw | Esc |
|---|---|---|---|---|
| Min | -- | | | |
| Tne | 0.0282 | -- | | |
| Tnw | 0.0482 | 0.0392 | -- | |
| Esc | 0.0479 | 0.0586 | 0.0544 | -- |

The results of the isolation-by-distance analysis were negative. I.e., the two single-tailed tests yielded statistically insignificant P values, indicating the lack of a significant correlation between interpopulational genetic differentiation and interpopulational geographic distance.

*Number of populations; genetic structure within and among populations*

The results of 16 runs of the *structure* program (Pritchard et al. 2000) where only the genotypic data were used in the algorithm (i.e., where non-genetic population data such as collection location data were ignored) are shown, along with the results of 16 runs where collection location data were incorporated into the algorithm, in Table 5.15.

The key items of note in the first 16 rows of Table 5.15 are the values of K (assumed number of populations) in the second column and the computed values of Ln P(D) (the natural logarithm of the posterior probability that the genotypic data fit the assumed value of K) in the third column. When only the genetic data were considered, the values of Ln P(D) were highest (with values ca. -1370) when K = 1 was assumed (in the top four data rows of Table 5.15, for runs designated 1-4 no geo). That is to say, the posterior probability of a good fit of the data was maximized when we assumed that all sampled individuals belong to a single population (i.e., that there is little genetic structure among the 94 individuals sampled from the four geographic locations). Even though the maximum values of Ln P(D) were slightly higher (with values ca. -1360) when geographic population data were included and a value of K = 4 was assumed (in the bottom four rows of Table 5.15, for runs designated 13-16 w/ geo) – which would appear to imply four distinct populations corresponding to the four sampled geographic locations is a better "solution" than a single all-inclusive population – such inclusion of the geographic data in the *structure* parameter set is not appropriate for an unbiased determination of the number of populations comprised by the individuals genotyped in a dataset (J.K. Pritchard, personal communication 2005). Therefore we shall rely on the

**Table 5.15** Results of 32 runs of *structure* program. The main parameters of each run included a burn-in of 100,000 iterations, followed by 1,000,000 iterations. The first 16 runs (designated "no geo") were done without taking the geographic origin of the individuals into account. The last 16 runs (designated "w/ geo") incorporated the geographic origin data into the algorithm. K is the assumed number of populations for each run. Ln P(D) is an estimate of ln(P(X|K)), the natural logarithm of the posterior probability P that the genotypic data X fit the assumed value of K. Var(Ln P(D)) is the variance of Ln P(D).

| Run name | K | Ln P(D) | Var[LnP(D)] | $\alpha$ | Fst_1 | Fst_2 | Fst_3 | Fst_4 |
|---|---|---|---|---|---|---|---|---|
| 1 no geo | 1 | -1371.8 | 9.6 | - | 0.0012 | - | - | - |
| 2 no geo | 1 | -1371.3 | 10.5 | - | 0.0011 | - | - | - |
| 3 no geo | 1 | -1376.3 | 17.9 | - | 0.0019 | - | - | - |
| 4 no geo | 1 | -1375.0 | 7.1 | - | 0.0022 | - | - | - |
| 5 no geo | 2 | -1390.6 | 47.1 | 5.44 | 0.0112 | 0.0153 | - | - |
| 6 no geo | 2 | -1486.9 | 273.7 | 2.56 | 0.0307 | 0.0451 | - | - |
| 7 no geo | 2 | -1439.5 | 177.6 | 2.23 | 0.0190 | 0.0520 | - | - |
| 8 no geo | 2 | -1474.8 | 243.3 | 3.82 | 0.0306 | 0.0429 | - | - |
| 9 no geo | 3 | -1724.2 | 762.4 | 3.45 | 0.0527 | 0.0261 | 0.1032 | - |
| 10 no geo | 3 | -1695.0 | 719.6 | 1.48 | 0.0403 | 0.0531 | 0.0364 | - |
| 11 no geo | 3 | -1521.6 | 322.2 | 4.57 | 0.0209 | 0.0190 | 0.0172 | - |
| 12 no geo | 3 | -1527.6 | 339.9 | 4.49 | 0.0277 | 0.0300 | 0.0181 | - |
| 13 no geo | 4 | -1735.7 | 763.4 | 5.29 | 0.0361 | 0.0291 | 0.0276 | 0.0250 |
| 14 no geo | 4 | -1959.1 | 1264.2 | 2.15 | 0.0660 | 0.0566 | 0.0545 | 0.0406 |
| 15 no geo | 4 | -1925.9 | 1227.2 | 0.92 | 0.0513 | 0.0719 | 0.0519 | 0.0550 |
| 16 no geo | 4 | -1780.2 | 925.4 | 0.86 | 0.0758 | 0.0558 | 0.0308 | 0.0537 |
| 1 w/ geo | 1 | -1374.9 | 16.1 | - | 0.0022 | - | - | - |
| 2 w/ geo | 1 | -1378.9 | 22.1 | - | 0.0020 | - | - | - |
| 3 w/ geo | 1 | -1374.0 | 14.1 | - | 0.0022 | - | - | - |
| 4 w/ geo | 1 | -1370.4 | 11.1 | - | 0.0005 | - | - | - |
| 5 w/ geo | 2 | -1367.1 | 53.9 | 5.77 | 0.0216 | 0.0233 | - | - |
| 6 w/ geo | 2 | -1367.2 | 56.6 | 3.77 | 0.0216 | 0.0255 | - | - |
| 7 w/ geo | 2 | -1368.8 | 58.0 | 5.43 | 0.0210 | 0.0254 | - | - |
| 8 w/ geo | 2 | -1366.1 | 52.4 | 5.13 | 0.0182 | 0.0277 | - | - |
| 9 w/ geo | 3 | -1393.8 | 123.7 | 4.42 | 0.0230 | 0.0092 | 0.0319 | - |
| 10 w/ geo | 3 | -1373.8 | 93.6 | 2.96 | 0.0261 | 0.0175 | 0.0312 | - |
| 11 w/ geo | 3 | -1374.4 | 98.3 | 3.22 | 0.0249 | 0.0197 | 0.0383 | - |
| 12 w/ geo | 3 | -1372.4 | 93.4 | 2.67 | 0.0238 | 0.0171 | 0.0372 | - |
| 13 w/ geo | 4 | -1351.6 | 95.9 | - | 0.0289 | 0.0223 | 0.0139 | 0.0806 |
| 14 w/ geo | 4 | -1360.7 | 109.1 | - | 0.0245 | 0.0179 | 0.0101 | 0.0847 |
| 15 w/ geo | 4 | -1360.7 | 112.7 | - | 0.0262 | 0.0219 | 0.0155 | 0.0791 |
| 16 w/ geo | 4 | -1354.3 | 101.1 | - | 0.0263 | 0.0217 | 0.0185 | 0.0789 |

value of K = 1 determined from the first 16 runs of the program, without using the geographic data, as the proper output of this program in regard to the number of populations.

The important result from the last 16 runs in Fig. 5.15 is that the highest values of the population-specific F-statistics generated by the *structure* program are those of Fst_4 when geographic data were used in conjunction with the genotypic data (in the last four rows of the table, in the last column). These elevated values (where the "4" refers to the Esc population) indicate a moderately greater degree of structure in the Esc geographic population than in any of the others.

**Discussion**

*Sampling limitations, generation time, and detection of a possible bottleneck*

A negative consequence of restricting sampling to all those individuals found in flower on the day of sample collection was that it limited sampling to a small minority (5-10%) of the individuals in each population. It also specifically excluded from sampling all sexually immature individuals. That means it excluded from sampling a large percentage of the individuals of the generation consisting of the progeny of the generation of individuals that were sampled. This is an important consideration because any genetic changes that could be attributed to a very recent or ongoing bottleneck, might be manifested only in individuals young enough to be the progeny of the adults comprising

the newly bottlenecked population, and at least the juvenile individuals of this younger generation were systematically excluded by the sampling regimen.

Although we have no hard data either on the average number of years it takes a newly germinated *A. asterias* plant to reach sexual maturity in its natural habitat, or on the average lifespan of individuals in habitat, we suspect that these plants live for several decades in habitat as they do in cultivation (P. Gambart, personal communication 2005), which would imply that many of the mature plants that we sampled would have been engendered prior to any recently initiated bottleneck. This would in turn imply that our sample might not include enough individuals with genotypes that would reflect a current bottleneck, with the result that such a bottleneck might escape detection in our analysis (as in Friar et al. 2000).

*Hardy-Weinberg equilibrium, heterozygote deficiency, and heterozygote excess*

The deviations from Hardy-Weinburg equilibrium identified with Haldane's (1954) exact test at loci *AaH11* (highly significant) and *AaC3* (significant) are of interest in part because *AaH11* is the most allele-rich locus (with 13 alleles) and *AaC3* is the second-most allele-rich locus (with 11 alleles). When this test was applied to the four sampled populations, the most conspicuous results were the significant result in the Min population and the highly significant result in the Esc population for Hardy-Weinberg disequilibrium. In this regard we should note that the Esc population has the distinction of being both small and apparently isolated by considerable distance from other populations. The highly significant result of this test applied globally across all loci and

all populations suggests the presence of structure within and/or among the presumptive populations.

The Hardy-Weinberg test for heterozygote deficiency showed highly significant results at the same two loci (*AaH11* and *AaC3*) and in the same populations (Min and Esc) that were highlighted by the Haldane test. The results for populations-by-loci and loci-by-populations are mathematically congruent; thus, the two applications of the test are redundant. However, the two different presentations of the results provide ease of inspection as to where heterozygote deficiencies occur among the populations on the one hand, and among the loci on the other hand. The heterozygote deficiency detected in the Esc population is a typical finding in a small (and therefore predictably inbred) population, where homozygosity tends to increase with inbreeding. If it is true that this population is also isolated geographically from other such populations, then there would be minimal gene flow into the population to mitigate the effects of inbreeding. This result is consistent with the low value of $H_O$ compared to $H_E$ for the Esc population in Table 5.3. The heterozygote deficiency detected in the Min population, on the other hand, may be an artifact of the sampling regimen. The fact that we sampled every individual in flower on the day of collection resulted in the sampling of spatially clustered individuals in the Min population. It is reasonable to assume that many such closely spaced plants would be closely related – and indeed inbred – with each other. Furthermore, it is not unreasonable to expect that synchronicity of flowering would occur among inbred individuals, which would result in such inbred individuals being sampled together. Thus, in a highly clustered population such as Min, our sampling

regimen may have predisposed our sample to contain a disproportionately high percentage of inbred individuals, which would have been disposed to heterozygote deficiency, which would then constitute a sort of “pseudo-Wahlund” effect.

The significant results in the Hardy-Weinberg test for heterozygote excess for locus *AaG3* only (in the Min population *per se* and across all populations) are compatible with the $F_{IS}$ values (Table 5.8) and the $Rho_{IS}$ values (Table 5.9), which are uniformly negative for each individual population and for all populations combined, at locus *AaG3* – which is true at no other locus. As to the cause of the excess of heterozygotes in the *A. asterias* plants we sampled, Pudovkin et al. (1996) observed that when the effective population size is small, allelic frequencies in male and female parents may differ due to binomial sampling error, resulting in an excess of heterozygotes (relative to Hardy-Weinberg equilibrium) in the progeny. Another relevant observation is that breeding systems involving self-incompatibility may result in an excess of heterozygotes (Hedrick 2000). *Astrophytum asterias* has been shown experimentally to be an obligate outcrosser, and a likely basis of this is a mechanism of self-incompatibility (Strong & Williamson 2005). Why a significant excess of heterozygotes would occur only at locus *AaG3*, is not obvious. It may be relevant that a feature of *AaG3* that sets it apart from the other loci we developed, is that it is the least polymorphic, having only four alleles, each differing from the next by a single dinucleotide repeat.

*Genic (allelic) and genotypic differentiation*

An interesting aspect of the results of the test for genic (allelic) differentiation for all populations at each locus is that all five loci showed P values that were very nearly significant (*AaB6*), significant (*AaD9*), or highly significant (*AaH11*, *AaG3* and *AaC3*). This suggests that all five loci contribute in varying nontrivial degrees to genetic structure in the sampled populations. This conclusion is further supported by the results of the test for genic (allelic) differentiation for all pairs of populations, where all five pairs of loci showed significant (the Tne-Tnw pair) or highly significant (all four other pairs) P values. Further independent confirmation is provided by the "G-like" test (Goudet et al. 1996) for genotypic differentiation for all populations, which similarly yielded significant (*AaB6* and *AaD9*) or highly significant (*AaH11*, *AaG3* and *AaC3*) results for all five loci. Likewise, the test for genotypic differentiation for all pairs of populations gave results that were either significant (the Tne-Tnw pair, which populations are geographically very close together) or highly significant (the other four pairs of populations) for all five pairs of loci.

*Gene flow:* Nm *based on private alleles*

The most important feature of the results of the Barton & Slatkin (1986) program for determining the number of migrants per generation (*Nm*) based on the number of private alleles in each geographic population, is that *Nm* is slightly greater than 1. This suggests that flying insect pollinators of *A. asterias* (A. Strong, personal communication 2005)

and/or birds that ingest the seeds and transport them enterically are effecting measurable gene flow among the four geographically separated populations.

$F_{IS}$ and $Rho_{IS}$

The $F_{IS}$ values quantifying genetic correlation and diversity based on allele frequency (Table 5.8) reveal that most of the genetic diversity among individuals within a given population is associated with the loci *AaH11*, *AaG3* and *AaC3*, in that order. In terms of populations, elevation of $F_{IS}$ values is most marked in the Esc population, with very high values of $F_{IS}$ in the Esc population row at the *AaH11*, *AaC3* and *AaD9* loci. The elevated values of $F_{IS}$ appear to be associated with high levels of homozygosity in the Esc population at these loci. The Min population has the second-highest $F_{IS}$ values overall, which may be attributable to the sampling of closely spaced – and closely related – individuals in flower in that location at the time of sampling. The neighboring demes Tne and Tnw show the lowest values of $F_{IS}$, and thus the lowest degree of genetic diversity (based on allele identity) among the individuals comprising those demes.

The values of $Rho_{IS}$, which measure the correlation and variation among individuals within each population, based on allele size (Table 5.9), are somewhat more diffuse than is the case with $F_{IS}$. By population, the trends remain similar to those exhibited by $F_{IS}$ in Table 5.8. Wright (1978) considered $F_{ST}$ values from 0 to 0.05 to denote *little* genetic differentiation, values from 0.05 to 0.15 to denote *moderate* genetic differentiation, values from 0.15 to 0.25 to denote *great* genetic differentiation, and values above 0.25 to denote *very great* genetic differentiation. Assuming that such guidelines for

interpretation can be extended from $F_{ST}$ to $F_{IS}$ – and hence to $Rho_{IS}$ – then inspection of Table 5.9 for "elevated" values of $Rho_{IS}$, defined as those greater than 0.15, reveals that such values indicating "*great* genetic differentiation" occur at the following alleles, by population. Min: *AaH11* and *AaC3*. Tne: no loci. Tnw: no loci. Esc: *AaD9*. The highest values of $Rho_{IS}$ occur as follows, by population. Esc: highest (0.8102 at locus *AaD9*). Min: 2nd highest (0.3816 at locus *AaH11*) and 3rd highest (0.1583 at locus *AaC3*). These observations collectively suggest that Esc has the greatest, and Min the 2nd greatest, degree of internal (inter-individual) variation. In the program that calculates values of $Rho_{IS}$ across all populations, the results show a greatly elevated value (0.2350) only at locus *AaH11*.

*Wright's F-statistics and related Rho-statistics*

Considering now the Weir & Cockerham (1984) derived versions of Wright's *F*-statistics (Table 5.10), we observe that the values most elevated are those of $Fwc_{IT}$ (highest mean value in the table), with values almost as high for $Fwc_{IS}$, and somewhat lower values for $Fwc_{ST}$. These results indicate that (1) the individuals sampled in this study show a moderate amount of genetic differentiation among themselves *in toto* (i.e., when all 94 sampled individuals are considered as a single population group without regard to division into subpopulations); (2) the individuals sampled show almost as much genetic differentiation among themselves within the confines of their subpopulation groups; but (3) there is notably less genetic differentiation among the

subpopulations (our geographic populations or demes) themselves, compared to the amount of genetic differentiation among the individuals that comprise these groups.

When all pairs of populations were compared at each locus in terms of $F_{ST}$ values (Table 5.11), the majority of the results were low ($F_{ST} < 0.05$), indicating "*little* genetic differentiation" among populations (Wright 1978). The exceptions (where $F_{ST} > 0.05$, indicating "*moderate* genetic differentiation" according to Wright's guidelines) among the 30 pairs of populations tested (6 population pairs at each of 5 loci), were population pairs Min-Tnw, Tne-Esc and Tnw-Esc at locus *AaB6*; Tne-Esc at locus *AaH11*; Min-Tnw, Min-Esc and Tne-Tnw at locus *AaG3*; Min-Esc, Tne-Esc and Tnw-Esc at locus *AaC3*; and Tne-Tnw at locus *AaD9*. The interesting trends here are that the Esc population, the most isolated by distance from the other three populations, is moderately differentiated from one of the other three populations in seven population pairs covering four of the five loci (all except *AaD9*), whereas Tne and Tnw, which are the closest to each other geographically, show moderate differentiation from each other at only one locus (*AaD9*). Populations Min and Tne, which are also quite close to each other geographically, showed no $F_{ST}$ value indicating moderate differentiation from each other (i.e., no pairwise $F_{ST} \geq 0.05$) at any of the five loci. These results are consistent with the principle of isolation by distance, such that differentiation between populations due to genetic drift is proportional to the geographic distance between the populations. The highest value of $F_{ST}$ (0.1921, indicating *great* genetic differentiation) was that of the Tnw-Esc population pair at locus *AaB6*. The conspicuous elevation of this value probably reflects a combination of the already noted high degree of differentiation of the

Esc population and the fact that Tnw and Esc had the smallest sample sizes (12 and 14 individuals respectively).

The *Rho*-statistics based on allele size (Table 5.12) showed average magnitudes that ranked in the same order as the *F*-statistics of Weir and Cockerham (1984) in Table 5.10, viz., $Rho_{IT} > Rho_{IS} > Rho_{ST}$. At all five loci one or more *Rho*-statistics showed moderate differentiation (i.e., *Rho*-statistic ≥ 0.05): $Rho_{ST}$ at *AaB6* and *AaG3*, $Rho_{IS}$ at *AaH11*, and $Rho_{IT}$ at *AaH11*, *AaC3* and *AaD9*. As with the F-statistics, the Rho-statistics generally indicated greater genetic variation among individuals (both within populations and across all populations) than among populations.

Among the estimates of $Rho_{ST}$ for all pairs of populations at each locus (Table 5.13), values indicating moderate differentiation (where $Rho_{ST} \geq 0.05$) occur as follows. Min-Tnw, Tne-Tnw and Tnw-Esc at locus *AaB6*; Min-Tne, Min-Tnw, Min-Esc and Tne-Tnw at locus *AaG3*; and Min-Esc and Tne-Esc at locus *AaC3*. These results are similar to those seen with $F_{ST}$ above (Table 5.11), as the elevated values indicative of moderate differentiation between members of a pair of populations are observed at the same loci (*AaB6*, *AaG3* and *AaC3*) and for the same population pairs, viz., Esc paired with each of the other three populations (one or more such pairings occur at each of the three indicated loci), and pairs involving the other three populations at two of the same loci (*AaB6* and *AaG3*). These results suggest that, for this dataset, $F_{ST}$ (based on allele identity and the infinite alleles model of microsatellite mutation) and $Rho_{ST}$ (based on allele size and the stepwise mutation model of microsatellite mutation) behave rather similarly.

*Structure and the number of populations*

The results of the Bayesian approach (*structure* program: Pritchard et al 2000) to the problem of determining the number of genetically distinct populations formed by 94 individuals sampled from demes at four geographic locations, with an array of known microsatellite genotypes at five loci, are somewhat equivocal. The *structure* algorithm is explicitly predicated on the assumption that the input data are in Hardy-Weinberg equilibrium. We know from Haldane's exact test for nonrandom mating that this dataset exhibits a highly significant Hardy-Weinberg disequilibrium. We do not know exactly how such a violation of this underlying assumption of *structure* might affect the performance of the program. However, we do know that Hardy-Weinberg equilibrium is rarely observed in nature, so the program must be somewhat robust to Hardy-Weinberg disequilibrium if it actually produces useful results with real datasets, as Pritchard et al. (2000) claim.

The *structure* output data are adequate to infer that there is a small but measurable degree of structure among the demes at the four geographic locations sampled. Furthermore, based on the geographic population-specific Fst values computed by the program, the Esc deme shows more genetic differentiation (from drift and inbreeding) in relation to the mean allele frequencies of the individuals from all sampled demes combined, than any of the other demes. At the same time, when the most appropriate parameter set (excluding the geographic data) is used in the *structure* program, the one-population model is best supported by the genotypic data, signifying that, on the basis of their genotypic data, the four geographically separated demes (Min, Tne, Tnw and Esc)

are more appropriately perceived as components of a single population than as four (or three or two) independent populations. This result suggests that the plants in these four demes are likely descendents of a single large population that historically covered much more of southern Starr County than it does today. This conclusion is independently supported by the generally low $F_{ST}$ (and $Rho_{ST}$) values for most population pairs, as well as by the finite positive value of *Nm*, which indicates non-negligible levels of gene flow among the populations. Even the negative results of the Genepop isolation-by-distance program suggest that these four subpopulations are genetically quite similar – which likewise suggests recent descent from a more or less continuous ancestral population.

*Conservation implications of the population genetic data*

In regard to the planning of conservation measures to implement the USFWS Recovery Plan for *Astrophytum asterias*, there are some clear conclusions. On the one hand, most of the subpopulations (demes) that we sampled in this study are surprisingly healthy in terms of levels of heterozygosity and genetic diversity. On the other hand, current small effective population size ($N_e$) is a concern in even the largest of these subpopulations. The Esc subpopulation is in particular need of restoration/augmentation. This is evident from its high degree of homozygosity at several loci, its notably elevated values of Fst_4 in the *structure* program (indicating a moderate degree of drift away from the mean allele frequencies of all four subpopulations combined), its small population size, and the small area of suitable habitat currently available to this population (not to mention the vulnerability to domestic herbivores on that particular property). The obverse of this

conclusion is that the Esc population would not be genetically suitable as a source of seed for other reintroduction/augmentation programs. The most salutary direction for the managed flow of genes would be from the larger, more heterozygous populations with low $F_{ST}$ values (such as Min and Tne) to the smaller, more homozygous populations with high $F_{ST}$ values (such as Esc).

*Future studies*

*Astrophytum asterias* has an extremely limited geographic distribution in the U.S. (*vide supra,* Chapter III), consisting of a fraction of Starr County, Texas, which constitutes the northeastern extremity of the range of this primarily Mexican species. Because the U.S. populations are geographic outliers at the edge of the range and therefore probably subject to special environmental selection pressures associated with the extreme conditions encountered at the spatial edge of viability for the species, it would be reasonable to expect that these populations may also be genetic outliers, vis-à-vis the populations in Mexico that are more geographically central to the overall distribution of the species. Evaluation of this hypothesis will be carried out in a future study when *A. asterias* tissues or DNA samples become available from Mexican populations. Such a study will also reveal whether the genetic composition of Mexican populations would be sufficiently compatible with what we know about the genetic composition of U.S. populations to make advisable the use of Mexican genotypes in reintroduction or augmentation of U.S. populations, and *vice versa*.

# CHAPTER VI

# POPULATION GENETICS OF *LOPHOPHORA WILLIAMSII*

## Introduction

The combination of time constraints and an unusually low yield of good primer pairs precluded a study of the population genetics of *Lophophora williamsii* of the same scope and depth as the study on *Astrophytum asterias* reported in the previous chapter. In the present chapter we shall provide comparative microsatellite genotypic data on 24 individuals of *L. williamsii* from three widely separated geographic locations, at one "normal" locus (*Lw14*) and one apparently compound locus (*Lw42*). These preliminary results on *L. williamsii* are compared to the results on *A. asterias* in terms of the contrasting breeding systems of these two species.

## Materials and methods

Tissue samples were obtained by stem biopsy from a total of 24 individuals of *L. williamsii*: eight from a population in Starr County (RES), eight from a population in Val Verde County (LTR), and eight from a population in Presidio County (STR). All laboratory procedures, including DNA extraction, microsatellite capture, cloning, sequencing, primer design, primer screening (including screening of fluorescent dye-labeled primers), determination of allele size and genotyping, were identical to those employed with *Astrophytum* (see previous chapter).

**Results**

Information on the two *Lophophora* loci examined, including primer sequences and melting temperatures, size range of the amplicon, and fluorescent labels employed on the primers, is given in Table 6.1.

**Table 6.1** *Lophophora williamsii* microsatellite loci. Data shown include name of locus, sequences of forward and reverse primers, size range of amplicon, number of alleles (*n*), identity of fluorescent label on 5' end of forward primer, and annealing temperature (salt-adjusted).

| Locus name | Primer sequences (5'→3') | *n* | Size range | Fluorescent label | Annealing temp. |
|---|---|---|---|---|---|
| *Lw14* | TCTGCGAATTCAGCGTAAAGTAGG<br>GTGTAGCACTCCCTCACGC | 2 | 159-168 | HEX | 64˚C |
| *Lw42* | GAATGAGCAGAAAAGCCTCGAAG<br>CAGATTTCTCGCCTCTCTCAGC | 2 | 152-164 | FAM | 63˚C |

The problematic nature of locus *Lw42* can be seen in the electropherograms generated by the Genescan-Genotyper software (Applied Biosystems). First, to establish what one normally expects to see for a homozygote, we look at a typical electropherogram for locus *Lw14* (Fig. 6.1).

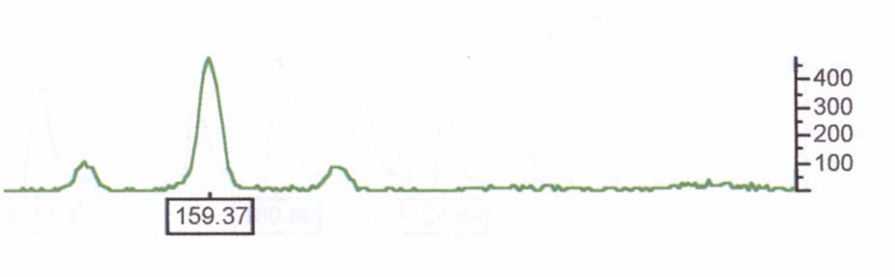


**Fig. 6.1** Electropherogram of a typical homozygote: *L. williamsii* individual #6 at locus *Lw14*. The single major peak, labeled 159.37, indicates a homozygote of an allele that measures 159 basepairs (bp) in length. The minor peaks, arranged symmetrically on either side of the major peak, are typical "stutter peaks", indicating the production of small quantities of PCR product that differ from the actual allele by exactly plus one or minus one repeat sequence. In this case, as the *Lw14* locus is a microsatellite composed of trinucleotide repeats, the stutter peak to the left of the 159-bp allele peak signifies a DNA fragment 156 bp long, while the stutter peak to the right indicates a fragment 162 bp long.

Since there is not a single heterozygote among the 24 individuals genotyped at the *Lw14* locus, we shall refer to the *Astrophytum* data to obtain electropherograms typical of heterozygotes (Figs. 6.2 and 6.3).

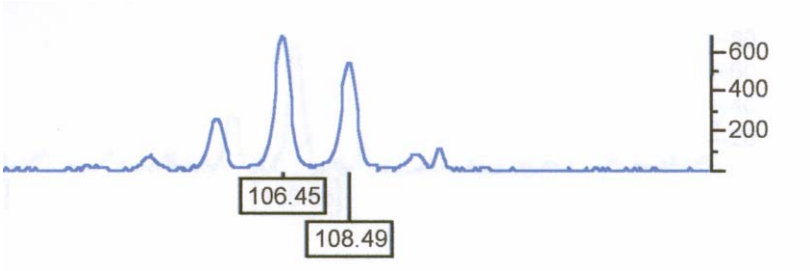


**Fig. 6.2** Electropherogram of a typical heterozygote: *A. asterias* individual #16 at locus *AaH11*. The allele peaks of the heterozygote are the two labeled 106.45 and 108.49, which are interpreted to indicate alleles whose actual lengths are 107 and 109 bp. When the two alleles of a heterozygote differ in length by a single repeat, as is the case here (the *AaH11* locus being a microsatellite composed of dinucleotide repeats), the first of the two allele peaks (i.e., that of the smaller allele, on the left) is normally slightly greater in magnitude than the second peak (representing the larger allele, on the right). Stutter peaks are visible on both sides of the two allele peaks.

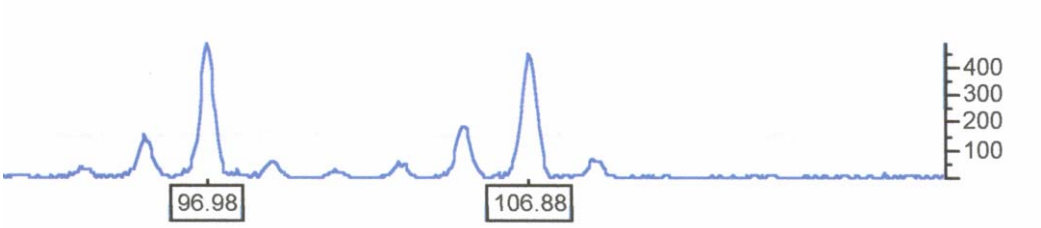


**Fig. 6.3** Electropherogram of a typical heterozygote: *A. asterias* individual #18 at locus *AaH11*. The allele peaks of the heterozygote are the two labeled 96.98 and 106.88, which are interpreted to indicate alleles whose actual lengths are 97 and 107 bp. When the two alleles of a heterozygote differ in length by more than a single repeat, as is the case here, each of the two allele peaks is bracketed by its own symmetrical series of stutter peaks on either side, so that each allele has the gestalt of a homozygote peak (Fig. 6.1). Here a continuous series of stutter peaks is visible between the labeled peak of the smaller allele (left) and that of the larger allele (right).

And now for something completely different, we compare the preceding normal tracings with some representative electropherograms from *L. williamsii*, locus *Lw42* (Figs. 6.4-6.6).

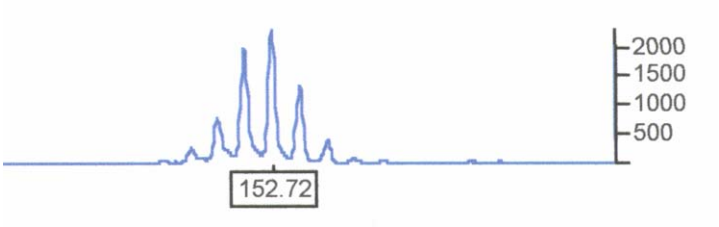


**Fig. 6.4** Ambiguous genotype of *L. williamsii* individual #22 at locus *Lw42*. In the absence of other relevant data, one could provisionally make a case for interpreting this electropherogram tracing as either (a) a homozygote (allele size 153 bp) with unusually high stutter peaks before it and after it or (b) a heterozygote (allele sizes 153 and 155 bp) with a high stutter peak preceding the peak of the smaller allele and a low peak for the larger allele. But see other relevant data that follow (Figs. 6.5 and 6.6).

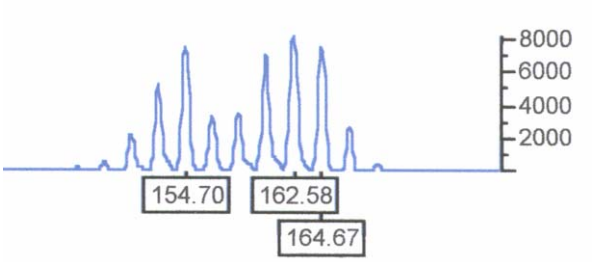


**Fig. 6.5** Ambiguous genotype of *L. williamsii* individual #1 at locus *Lw42*. This electropherogram tracing could possibly be interpreted as a heterozygote with the smaller allele at the peak labeled 154.70, and with unusually exaggerated stutter peaks immediately before and immediately after the peak of the larger allele (labeled 162.58). But one could also interpret it as an amalgam of three alleles: a homozygote with an allele size of 155 (peak labeled 154.70) plus a heterozygote with allele sizes 163 and 165 (peaks labeled 162.58 and 164.67, respectively). If the latter is an accurate interpretation, then what we have here is a compound locus, i.e., a situation where the primers designed for locus *Lw42* are in fact amplifying *Lw42* plus some other microsatellite locus in the genome, which results in electropherograms with more than two alleles.

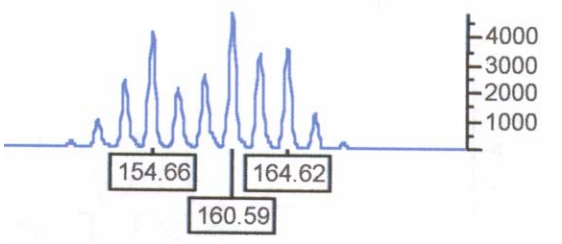


**Fig. 6.6** Uninterpretable genotype of *L. williamsii* individual #2 at locus *Lw42*. The only difference between this tracing and the one in Fig. 6.5 is that here there appear to be three allele peaks (the three tallest peaks, labeled 154.66, 160.59 and 164.62). If we discard the possibility of triploidy, then this electropherogram is uninterpretable as a single-locus genotype. The most likely interpretation would appear to be that *Lw42* is a compound locus, and what we are seeing are the superimposed genotypes of (1) the locus from which the *Lw42* primer pair was designed and (2) some other locus – possibly a duplicate of the first locus, but one that has mutated independently to produce some different alleles – such that the second locus is being amplified by the same *Lw42* primers that amplify the first *Lw42* locus.

Thus, the genotype results for *L. williamsii* include data for a "normal" microsatellite locus, *Lw14*, the genotypes of which are unusual only in that all the individuals tested are homozygotes, and an anomalous locus, *Lw42*, the genotypes of which range from the unusual to the uninterpretable (at least in terms of single-locus diploid genotypes).

While the genotypic data for *Lw42* cannot be analyzed as ordinary diploid allele data – and *L. williamsii* is known to be diploid (Powell and Weedin 2004) – these data are not totally devoid of analytical value. The *Lw42* genotypic electropherogram tracings from Genotyper can be categorized into recognizable gestalts with reasonably uniform characteristics, as follows:

(A) We can group the genotypes of the type seen in Fig. 6.4, where a major peak of ca. 153 bp is bracketed between two high-magnitude peaks and some obvious stutter peaks that occur at intervals of 2 bp. This group of similar genotypes we shall designate as Type A.

(B) We can recognize a second distinct group of genotypes as seen in Figs. 6.5 and 6.6. These genotypes have a high-amplitude peak at ca.155 bp, followed by three high-amplitude peaks at ca. 161, 163 and 165 bp, with apparent stutter peaks at intervals of 2 bp between, before, and after the mentioned major peaks. This group of similar genotypes we shall call Type B.

The genotypic data at loci *Lw14* and *Lw42* for all 24 sampled individuals of *L. williamsii* are presented in Table 6.2.

**Table 6.2** Genotypic data for 24 individuals of *L. williamsii* from three distantly separated populations at two loci. Figures in the first column are individual plant identification numbers. Population identifiers are given in the second column as one of the following: RES indicates Starr County, LTR indicates Val Verde County, and STR indicates Presidio County. In the third column are the six-digit, two-allele genotypes (each allele designated by its 3-digit length in bp) for locus *Lw14*. In the fourth column are genotypes defined qualitatively above as Type A or Type B, for locus *Lw42*. Missing data for an allele are indicated by 000.

| Indiv. | Pop. | *Lw14* | *Lw42* |
|---|---|---|---|
| 1 | RES | 159159 | Type B |
| 2 | RES | 159159 | Type B |
| 3 | RES | 000000 | Type B |
| 4 | RES | 159159 | Type B |
| 5 | RES | 159159 | Type B |
| 6 | RES | 159159 | Type B |
| 7 | RES | 159159 | Type B |
| 8 | RES | 159159 | Type B |
| 9 | LTR | 165165 | Type A |
| 10 | LTR | 165165 | Type A |
| 11 | LTR | 165165 | Type A |
| 12 | LTR | 165165 | Type A |
| 13 | LTR | 165165 | Type A |
| 14 | LTR | 165165 | Type A |
| 15 | LTR | 165165 | Type A |
| 16 | LTR | 165165 | Type A |
| 17 | STR | 000000 | Type A |
| 18 | STR | 165165 | Type A |
| 19 | STR | 165165 | Type A |
| 20 | STR | 165165 | Type A |
| 21 | STR | 165165 | Type A |
| 22 | STR | 165165 | Type A |
| 23 | STR | 165165 | Type A |
| 24 | STR | 165165 | Type A |

This dataset cannot be analyzed with the usual programs for population genetic tests and measurements. However, inspection of the data suggests that each population is genotypically uniform; every sampled individual within each population has exactly the same genotype for each locus. Furthermore, for locus *Lw14*, which has "normal" electropherogram tracings, we observe that every individual in each population is homozygotic. Finally, the results at both loci show an absolute genetic distinction between the Starr County, South Texas population (RES, where all individuals are homozygotes for the 159-bp allele at locus *Lw14* and have the Type B genotype at locus *Lw42*) and the two West Texas populations (LTR and STR, where all individuals are homozygotes for the 165-bp allele at locus *Lw14* and have the Type A genotype at locus *Lw42*).

An incidental finding that merits mention is that the primers designed for the *Astrophytum asterias* locus *AaH11* amplified a locus in genomic *Lophophora williamsii* DNA of individuals from the two West Texas populations (STR and LTR) and from two South Texas populations (RES and LLN). Sequencing of the PCR product revealed that the *Lophophora* locus amplified by the *AaH11* primers did contain a microsatellite, but a completely different one from the microsatellite at the *AaH11* locus in *Astrophytum*. Nor was there any similarity between the flanking sequences or the microsatellites of the two primer pairs designed for *Lophophora* (loci *Lw14* and *Lw42*) and those of the *Lophophora* locus amplified by the *Astrophytum* primers for locus *AaH11*.

**Discussion**

The results for *Lophophora* can be understood most clearly in comparison with the *Astrophytum* data. We shall examine some key differences.

*Lophophora* and *Astrophytum* have radically different breeding systems, *Astrophytum asterias* being an obligate outcrossing species (Rowley 1958; Strong & Williamson 2005), whereas to a large extent – possibly close to 100 percent – *Lophophora williamsii* reproduces by self-fertilization (Rowley 1980; Terry, personal observation). The possibility of apomixis in *Lophophora* has not yet been ruled out by experimentation, but what is clear is that *Lophophora* plants in complete isolation produce fertile seed. Accordingly, for *Astrophytum* we expect to see genetic data that are the product of outcrossing per flying insect pollinators; the pattern would include mixing of alleles within populations (and some mixing between populations, decreasing with interpopulational distance), normal levels of heterozygosity (except in small, isolated populations like Esc), and maintenance of relatively large numbers of alleles at most loci. And we do in fact observe such phenomena in the *Astrophytum* data for the four South Texas populations studied. In contrast, with a primarily autogamous cactus like *Lophophora*, we would expect to see minimal mixing of alleles within a population, virtually no mixing of alleles between populations, very low levels of heterozygosity, and relatively low numbers of alleles per locus, as many ancestral alleles would have gone to fixation in the form of homozygotes or gone to extinction, due to the extreme degree of inbreeding which selfing constitutes. And that is indeed what we observe in the *Lophophora* data: genetic monotony confined to a single homozygotic genotype

within each population, with some variation between distant populations, but variation only in the sense of a shift from 100% homozygotes of one allele in one population to 100% homozygotes of a different allele in another population (the difference in identity of the fixed alleles being attributable to genetic drift). It is remarkable that this pattern holds true even for locus *Lw42*, where the genotypes are characterized non-allelically in terms of their recognizable gestalts with numerically defined peaks in the electropherograms. However, we cannot say with certainty what constitutes a homozygote in the case of locus *Lw42*. The electropherograms appear to be composites of more than one locus, and with so many peaks we could have a mixture of homozyotes and/or fixed heterozygotes.

Another factor underlying the stark differences between the *Astrophytum* data and the *Lophophora* data may be seen in the distances among populations in the two studies. In the case of *Astrophytum*, all four populations were clustered in a geographic area that constitutes a fraction of Starr County, such that the greatest distance between any two populations (Min and Esc) was only 10 kilometers, and the shortest distance between any two populations (Tne and Tnw) was less than half a kilometer. In the case of *Lophophora*, on the other hand, the three populations studied were scattered along the valley of the Rio Grande over hundreds of kilometers. The distance between the two most distant populations (RES and STR) was on the order of 570 km, while the distance between the two closest populations (LTR and STR) was about 240 km. The point here is that, apart from the different breeding systems and the potential reach of pollinators in the case of *Astrophytum* or vertebrate seed carriers for either species, the distances

among the *Lophophora* populations are so great that the amount of gene flow from one to another must be vanishingly close to zero. The degree of isolation by distance among the *Lophophora* populations dwarfs any notion of isolation by distance as applied to the *Astrophytum* populations in Starr County.

The seemingly absolute distinction between South Texas *L. williamsii* and West Texas *L. williamsii*, as revealed in this preliminary study using microsatellite loci, suggests that microsatellites may have utility for addressing the taxonomic problems – both interspecific and infraspecific – that have plagued students of this genus for many decades. The fact that the Val Verde County plants show the same uniform genotypes as the Presidio County plants, and that both West Texas populations are genotypically and allelically distinct from the Starr County plants, suggests a West Texas vs. South Texas genetic demarcation. This is particularly intriguing in light of the successive treatments of a taxon that was first described as a specious species (*L. echinata*) by Croizat (1944), then described, more appropriately, as a variety (*L. williamsii* var. *echinata*) by Bravo (1967), and, most recently, was declared to be a variety (*L. williamsii* var. *echinata*) that explicitly included both Chihuahuan and Coahuiltecan plants and some West Texas plants that exhibit the morphology of the Presidio County and Val Verde County plants (Weniger 1970, 1984). The fact that there are few morphological characters (including stem dimensions, stem color and flower color) and physiological characters (including frost resistance, resistance to all-day exposure to full sun, and possibly alkaloid content) that separate the Trans-Pecos *L. williamsii* from the South Texas *L. williamsii*, has made most students of the genus *Lophophora* reluctant to follow Weniger in granting varietal

recognition to the West Texas plants (which occur at the northern and western extremity of the range of the species). Now, however, the preliminary data reported here, which are totally concordant with the notion that there are clear genetic differences between South Texas and West Texas populations of *L. williamsii*, suggest that data on several more microsatellite loci, using genomic DNA samples from adequate numbers of individuals from Mexican populations in Chihuahua and Coahuila as well as South Texas and West Texas, could lend support to – or negate – the proposition that separate varietal status for the West Texas plants and certain groups of northern Mexican plants is justified. In the event that these northern and western populations of *L. williamsii* were found to constitute a valid natural group meriting varietal recognition, the nomenclatural problem would already have been solved, as such a variety would clearly fit within the existing description of *L. williamsii* var. *echinata*, *sensu* Weniger (1970), as adapted from Bravo (1967), as modified from Croizat (1944), based on a photograph in Schultes (1940).

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## SUMMARY AND CONCLUSIONS

*Lophophora williamsii* (peyote) has been excavated from two archaeological sites: Shumla Caves in southwest Texas and CM-79 in Coahuila. We report new radiocarbon dates: a mean age of 5195 ± 20 $^{14}$C years BP for the three Shumla Caves specimens (one of which had not been previously dated, and none of which had been previously dated with adequate published documentation), and an age of 835 ± 35 $^{14}$C years BP for the CM-79 specimen (which had not been previously dated). Contrary to previous reports, the Shumla Caves specimens were discovered not to be intact desiccated peyote tops, but rather manufactured effigies of peyote tops, consisting of a triturated mixture of peyote and other plant material.

Published data on the geographic ranges of *L. williamsii* and *A. asterias* are of varying quality and accuracy, probably due to obsolescence in several cases. We report the results of extensive research to document extant U.S. populations by county, drawing specific conclusions about where each species currently occurs, where its occurrence is uncertain, and where it is unlikely. These conclusions are based on credible herbarium specimens, verifiable specific locations in the primary literature, and recent interviews with knowledgeable individuals.

Dwindling of populations of peyote is partly due to improper harvesting, namely cutting off the top of the plant so deeply below ground level that the plant is unable to regenerate new stems, and consequently dies. We describe in detail the anatomy of the shoot (both aerial and subterranean stem) and root of *L. williamsii*, and suggest how this

new knowledge can be utilized in future empirical studies to determine “how deep is too deep” to cut if the harvesting of peyote is to be done sustainably.

We report the first population genetics study on endangered *A. asterias*, with five microsatellite markers in populations sampled at four locations in South Texas. The results of a battery of standard population genetics tests and measurements indicated that in most of the sampled populations heterozygosity was high (indicating a high level of random outcrossing), F-statistics were low (indicating low levels of genetic structure due to drift and/or inbreeding), and *Nm* was slightly greater than 1 (indicating low but finite levels of gene flow among populations). With the exception of the Esc population, the sampled populations appear not to be undergoing excessive inbreeding, despite small population sizes. Seed from these populations (except for Esc) may be used for reintroduction of *A. asterias* into suitable historical habitat and augmentation of extant populations.

Data at two *L.williamsii* microsatellite loci were generated from genomic DNA of plants from one South Texas population and two geographically disjunct West Texas populations. *L. williamsii*, which reproduces autogamously, exhibited a single homozygous genotype within a given population. West Texas *L. williamsii* plants differed from South Texas plants in the identity of the single allele at each locus. The ability of microsatellite markers to separate West Texas from South Texas plants with absolute consistency suggests that microsatellites may have utility for infraspecific taxonomic studies in *Lophophora*.

## REFERENCES


Aberle DF (1966) *The Peyote Religion Among the Navajo*. Aldine, Chicago.

Adovasio JM, Fry GF (1976) Prehistoric psychotropic drug use in northeastern Mexico and Trans-Pecos Texas. *Economic Botany*, **30**, 94-96.

Anderson EF (1961) *A Taxonomic Revision of* Ariocarpus, Lophophora, Pelecyphora, *and* Obregonia (*Family Cactaceae*). Ph.D. Dissertation, Claremont Graduate School, Claremont, California.

Anderson EF (1969) The biogeography, ecology, and taxonomy of *Lophophora* (Cactaceae). *Brittonia*, **21**, 299-310.

Anderson EF (1995) The "peyote gardens" of South Texas: a conservation crisis? *Cactus and Succulent Journal*, **67**, 67-73.

Anderson EF (1996a) *Peyote: The Divine Cactus*. University of Arizona Press, Tucson.

Anderson EF (1996b) Peyote and its derivatives as medicine. In: *Yearbook of Cross-Cultural Medicine and Psychotherapy 1995* (eds Winkelman M, Andritzky W), pp. 369-379. Verlag für Wissenschaft und Bildung, Berlin.

Anderson EF (2001) *The Cactus Family*. Timber Press, Portland, Oregon.

Arlegui J (1851 [1737]) *Crónica de la Provincia de N. S. P. S. Francisco de Zacatecas.* Jose Bernardo de Hogal, Mexico. Reprinted Calle de los Rebeldes Num. 2, Mexico.

Barton NH, Slatkin M (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409-415.

Benson L (1969) Cactaceae. In: Lundell CL et al., *Flora of Texas*, **Vol. 2**, Part II, pp. 288-289. Texas Research Foundation, Renner, Texas.

Benson L (1982) *The Cacti of the United States and Canada*. Stanford University Press, Stanford, California.

Boutton TW (1991) Stable carbon isotope ratios of natural materials. In: *Carbon Isotope Techniques* (eds Coleman DC, Fry B), p. 177. Academic Press, San Diego.

Boyd CE (1998) Pictographic evidence of peyotism in the Lower Pecos, Texas Archaic. In: *The Archaeology of Rock-Art* (eds Chippendale C, Taçon PSC), pp. 229-246. University Press, Cambridge, U.K.

Bravo H (1931) Nota acerca de la histología del peyote, *Lophophora willliamsii* Lemaire. *Anales del Instituto de Biología de la Universidad de Mexico*, **2**, 3-14.

Bravo H (1967) Una revision del genero *Lophophora*. *Cactaceas y Suculentas Mexicanas*, **12**, 8-17.

Bronk Ramsey C (2000) 2000 OxCal Program v3.5. University of Oxford Radiocarbon Accelerator Unit. Electronic document, http://www.rlaha.ox.ac.uk/oxcal/oxcal.htm.

Bruhn JG, Lindgren JE, Holmstedt B, Adovasio JM (1978) Peyote alkaloids: identification in a prehistoric specimen of *Lophophora* from Coahuila, Mexico. *Science*, **199**, 1437-1438.

Bruhn JG, De Smet PAGM, El-Seedi HR, Beck O (2002) Mescaline use for 5700 years. *The Lancet*, **359**, 1866.

Butterworth CA, Cota-Sanchez JH, Wallace RS (2002) Molecular systematics of tribe Cacteae (Cactaceae: Cactoideae): a phylogeny based on rpl16 intron sequence variation. *Systematic Botany*, **27**, 257-270.

Bye R (1979) Hallucinogenic plants of the Tarahumara. *Journal of Ethnopharmacology*, **1,** 23-48.

Campbell TN (1958) Origin of the mescal bean cult. *American Anthropologist*, **60**, 156-160.

Cardenas J de (1945 [1591]) *Problemas y Secretos Maravillosos de las Indias*. Ediciones Cultura Hispánica, Madrid. [Originally published in Mexico.]

CITES (2005) Convention on International Trade in Endangered Species of Wild Fauna and Flora, Appendix I. CITES Secretariat, Geneva.

Clover EU (1932) *Astrophytum asterias* in the United States. *Rhodora*, **34**, 227-228.

Clover EU (1937) Vegetational survey of the Lower Rio Grande Valley, Texas. *Madroño*, **4**, 41-66, 77-100.

Croizat L (1944) A study of the genus *Lophophora*. *Desert Plant Life*, **16**, 43-44.

Damude N, Poole JM (1990) Status report on *Echinocactus asterias* (*Astrophytum asterias*). Report prepared for U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Estrada y Flores A de (1946 [1652]) *San Pedro Teocaltiche* (ed. Rea V). Biblioteca Aportación Histórica, Mexico City, Mexico.

Everitt JH (2005) Personal communication. Kika de la Garza Agricultural Research Center, Weslaco, Texas.

Friar EA, Ladoux T, Roalson EH, Robichaux RH (2000) Microsatellite analysis of a population crash and bottleneck in the Mauna Kea silversword, *Argyroxiphium sandwicense* ssp. *sandwicense* (Asteraceae), and its implications for reintroduction. *Molecular Ecology*, **9**, 2027-2034.

Furst PT (1974) Hallucinogens in precolumbian art. In: *Art and Environment in Native America* (eds King ME, Traylor IR), pp 55-107. Special Publications of the Museum, No. 7. Texas Technical University, Lubbock, Texas.

Furst PT (1989) [Review of] *Peyote Religion: A History* (ed. Stewart OC). *American Ethnologist*, **16**, 386-387.

Furst PT (1996) Myth as history, history as myth. In: *People of the Peyote* (eds Schaefer SB, Furst PT), pp. 26-60. University of New Mexico Press, Albuquerque.

Goudet J, Raymond M, De Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 933-1940.

Habermann V (1974) *Lophophora fricii* Habermann species nova. *Kactusy*, **10**, 123-127.

Haldane JBS (1954) An exact test for randomness of mating. *Journal of Genetics*, **52**, 631-635.

Hamrick JL, Nason JD, Fleming TH, Nassar JM (2002) Genetic diversity in columnar cacti. In: *Columnar Cacti and Their Mutualists: Evolution, Ecology, and Conservation* (eds Fleming TH, Valiente-Banuet A), pp. 122-133. University of Arizona Press, Tucson.

Havard V (1885) Report on the flora of western and southern Texas. *Proceedings of the U.S. National Museum*, **8**, 449-533.

Hedrick PW (2000) *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, Massachusetts.

Huxley A (1954) *The Doors of Perception*. Chatto & Windus, London.

Janot M, Bernier M (1933) Essai de localisation des alkaloides dans le peyotl. *Bulletin des Sciences Pharmacologiques*, **40**, 145-153.

Janssen GK, Poole JM, Williamson PS (2004) The Research and Recovery of Star Cactus (*Astrophytum asterias*). Section 6 interim report. Texas Parks and Wildlife Department, Austin.

Kijas JM, Fowler JC, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16,** 656-662.

Klüver H (1928) *Mescal: The Divine Plant and Its Psychological Effects*. Kegan Paul, Trench, Trubner & Co., London.

Kuenzler H (2004) Personal communication. Belen, New Mexico.

Labadie J (2002) Personal communication. National Park Service, Del Rio, Texas.

La Barre W (1957) Mescalism and peyotism. *American Anthropologist*, **59**, 708-711.

Leon M de (1611) *Camino del Cielo en Lengua Mexicana*. Diego Lopez davalos, Mexico City, Mexico.

Litowitz T (1983) Hallucinogens. In: *Clinical Management of Poisoning and Drug Overdose* (eds Haddad L, Winchester J). W. B. Saunders, Philadelphia.

Martin GC (no date) *Archaeological Exploration of the Shumla Caves*. Big Bend Basket Maker Papers No. 3, pp. 19-22, 78. Witte Memorial Museum, San Antonio.

Martinez Avalos JG (2002) Distribución y estimación del tamaño de las poblaciones de *Astrophytum asterias* (Zucc.) Lem. (Cactaceae) a partir de la densidad, en la región noreste de Mexico. Proceedings of the Texas Plant Conservation Conference, Austin.

Mauseth JD, Montenegro G, Walckowiak AM (1985) Host infection and flower formation by the parasite *Tristerix aphyllus* (Loranthaceae). *Canadian Journal of Botany*, **63**, 567 – 581.

Mauseth JD (1988) *Plant Anatomy*. Benjamin/Cummings. Menlo Park, California.

Mauseth JD, Sajeva M (1992) Cortical bundles in the persistent, photosynthetic stems of cacti. *Annals of Botany*, **70**, 317-324.

Mauseth JD (1993) Medullary bundles and the evolution of cacti. *American Journal of Botany*, **80**, 928-932.

Mauseth JD, Uozumi Y, Plemons BJ, Landrum JV (1995) Structural and systematic study of an unusual tracheid type in cacti. *Journal of Plant Research*, **108**, 517 – 526.

Mauseth JD (2000) Theoretical aspects of surface-to-volume ratios and water-storage capacities of succulent shoots. *American Journal of Botany*, **88**, 1107-1115.

Mauseth JD (2004) Wide-band tracheids are present in almost all species of Cactaceae. *The Journal of Plant Research*, **117**, 69-76.

McAllister G (1954) Peyote at Shafter, Texas, in the Big Bend. *Cactus and Succulent Journal (US)*, **26**, 146-147.

McGregor R (1991) Threaded and twined matting: a late introduction into the Lower Pecos. In *Papers on Lower Pecos Prehistory* (ed. Turpin SA), pp. 141-148. Texas Archeological Research Laboratory Studies in Archeology 8, University of Texas, Austin.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, **142**, 1061-1064.

Mooney J (1897) Unpublished field notes on work in West Texas. In: "James Mooney papers", National Anthropological Archives, Smithsonian Institution, Washington, D.C.

Moreno S (2005) For Native Americans, peyote is dwindling. Austin American-Statesman, Monday, September 19, 2005, p. B3.

Morgan GR (1976) *Man, Plant and Religion: Peyote Trade on the Mustang Plains of Texas.* Ph.D. Dissertation, University of Colorado, Boulder.

Morgan GR (1983a) The biogeography of peyote in South Texas. *Botanical Museum Leaflets*, **29,** 73-86. Harvard University, Cambridge.

Morgan GR (1983b) Hispano-Indian trade of an Indian ceremonial plant, peyote (*Lophophora williamsii*), on the Mustang Plains of Texas. *Journal of Ethnopharmacology*, **9**, 319-321.

Morgan GR (1984) Peyote Trade in South Texas. *Southwestern Historical Quarterly*, **87**, 269-296.

The Nature Conservancy of Texas (2004) Unpublished data. South Texas Office of The Nature Conservancy, Mission.

Nentvig J (1971 [1767]) *Descriptión Geográfica, Natural y Curiosa de la Provincia de Sonora* (ed. Viveros G). Archivo General del la Nación, Mexico City.

Otero-Arnaiz A, Cruz-Sanders J, Casas A, Hamrick JL (2004) Isolation and characterization of microsatellites in the columnar cactus: *Polaskia chichipe* and cross-species amplification within the tribe Pachycereeae (Cactaceae). *Molecular Ecology Notes*, **4**, 265-267.

Otero-Arnaiz A, Casas A, Hamrick JL, Cruse-Sanders J (2005) Genetic variation of *Polaskia chichipe* (Cactaceae) under domestication in the Tehuacán Valley, central Mexico. *Molecular Ecology*, **14**, 1603-1611.

Ott J (1995) *The Age of Entheogens & The Angels' Dictionary*. Natural Products, Kennewick, WA.

Parker KC, Hamrick JL (1992) Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schottii*. *American Journal of Botany*, **79**, 86-96.

Parsons EC (1974 [1939]) *Pueblo Indian Religion*. University of Chicago Press, Chicago.

Pepper AE, Norwood LE (2001) Evolution of *Caulanthus amplexicaulis* var *barbarae* (Brassicaceae) a rare serpentine endemic plant: a molecular phylogenetic perspective. *American Journal of Botany*, **88**, 1479-1489.

Powell AM, Weedin JF (2004) *Cacti of the Trans-Pecos & Adjacent Areas*. Texas Tech University Press, Lubbock.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 170-181.

Prochazka M (1996) Microsatellite hybrid capture technique for simultaneous isolation of various STR markers. *Genome Research*, **6**, 646-649.

Pudovkin AI, Zatkin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote excess in progeny. *Genetics*, **144**, 383-387.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.

Reddy OUK, Pepper AE, Abdurakhmonov I, Saha S, Jenkins JN, Brooks T, Bolek Y, El-Zyk KM (2001) New dinucleotide and trinucleotide microsatellite marker resources for cotton genome research. *Journal of Cotton Science*, **5**, 103-113.

Rouhier A (1926) *Monographie du Peyotl* Echinocactus Williamsii *Lem.* Thèse pour l'obtention du diplôme de Docteur de l'Université de Paris (Pharmacie). Paris.

Rouhier A (1927) *Le Peyotl – La Plante Qui Fait les Yeux Emerveilles*. Doin, Paris.

Rousset F (1996) Equilibrium values of measure of population subdivision for stepwise mutation processes. *Genetics*, **142**, 1357-1362.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219-1228.

Rowe MW (2001) Dating by AMS Radiocarbon Analysis. In: *Handbook of Rock Art Research* (ed. Whitley DS), pp. 139-166. Altamira, New York.

Rowe MW, Steelman KL (2002) Radiocarbon dating of rock paintings using plasma-chemical extraction. *American Laboratory*, **34**, 15-19.

Rowley G (1958) *Astrophytum asterias* Lem. *National Cactus & Succulent Journal*, **13**, 7-8.

Rowley G (1980) Pollination syndromes and cactus taxonomy. *The Cactus and Succulent Journal of Great Britain*, **42**, 95-98.

Sahagun B de (1829 [1582]) *Historia General de las Cosas de Nueva España*, Tomo **II**, Libro VIII, Cap. V; Tomo **III**, Libro X, Cap. XXIX. Carlos Maria de Bustamante, Mexico City.

Sajeva M, Mauseth JD (1991) Leaflike structure in the photosynthetic, succulent stems of cacti. *Annals of Botany*, **68**, 405-411.

Sanchez-Mejorada H, Anderson EF, Taylor NP, Taylor R. (1986) Succulent plant conservation studies and training in Mexico. World Wildlife Fund, Washington, D.C.

Sayles EB (1935) An archaeological survey of Texas, *Medallion Papers* **17**. Gila Pueblo, Globe, AZ.

Schaefer SB (2000) The peyote religion and Mescalero Apache: an ethnohistorical view from West Texas. *The Journal of Big Bend Studies*, **12**, 51-70.

Schultes RE (1937) Peyote and plants used in the peyote ceremony. *Botanical Museum Leaflets, Harvard University*, **4**, 129-152.

Schultes RE (1938a) The appeal of peyote (*Lophophora williamsii)* as a medicine. *American Anthropologist*, New Series, **40**, 698-715.

Schultes RE (1938b) Peyote – an American Indian heritage from Mexico. *El Mexico Antiguo*, **4,** 199-208.

Schultes RE (1940) The aboriginal therapeutic uses of Lophophora Williamsii. *Cactus and Succulent Journal*, **12**, 177-181.

Schultes RE (1998) Antiquity of the use of New World hallucinogens. *The Heffter Review of Psychedelic Research*, **1,** 1-7.

Schultz ED, Runyon R (1930) *Texas Cacti: A popular and scientific account of the Cacti native of Texas*. Texas Academy of Science, San Antonio.

Shonle R (1925) Peyote, the giver of visions. *American Anthropologist*, **27**, 53-75.

Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457-462.

Smithsonian Carbon-Dating Laboratory (1972) Smithsonian Institution Archives, Record Unit 387, Box 1, laboratory notebook entries for June 16, June 30 and July 3, 1972. Washington, D.C.

Soule ME, Orians GH (2001) Conservation biology research: Its challenges and contexts. In: Soule ME, Orians GH (eds.), *Conservation Biology: Research Priorities for the Next Decade.* Island Press, Washington, D.C..

Steelman KL, Rowe MW (2002) Potential for virtually nondestructive radiocarbon and stable carbon isotopic analyses on perishable archaeological artifacts. In: *Archaeological Chemistry VI: Materials, Methods, and Meaning,* (ed. Jakes KA), pp. 8-21. American Chemical Society, Washington, D.C.

Stewart OC (1987) *Peyote Religion*: *A History*. University of Oklahoma Press, Norman.

Stone-Palmquist M and Mauseth JD (2002) The structure of enlarged storage roots in cacti. *The International Journal of Plant Research*, **163,** 89-98.

Strong A, Williamson P (2005) Reproductive Biology of Star Cactus (*Astrophytum asterias*). Proceedings, Texas Plant Conservation Conference, Ladybird Johnson Wildflower Center, Austin.

Stuiver M, Reimer PJ, Bard E, Beck JW, Burr GW, Hughen KA, Kromer B, McCormac G, van der Plicht J, Spurk M (1998) INTCAL98 radiocarbon age calibration, 24000-0 cal BP. *Radiocarbon*, **40**, 1041-1083.

Taylor WW (1956) Some implications of the $^{14}C$ dates from a cave in Coahuila, Mexico. *Bulletin of the Texas Archeological Society*, **27**, 215-234.

Taylor WW (1988) *Contributions to Coahuila Archaeology with an Introduction to the Coahuila Project*. Research Paper No. 52, pp.36, 159. Center for Archaeological Investigations, Southern Illinois University, Carbondale.

Terry M, Mauseth J (2005) unpublished data. Department of Biology, Sul Ross State University, Alpine, and Department of Biology, University of Texas at Austin.

Texas Department of Public Safety (2005) Peyote sales totals and distributors of Texas. Unpublished data. Austin, Texas.

Trout K (2005) Unpublished data. Trout's Notes, Boonville, California.

Turner BL, Nichols H, Denny G, Doron O (2003) *Atlas of the Vascular Plants of Texas*. BRIT Press, Fort Worth.

Turpin SA (1991) Time out of mind: the radiocarbon chronology of the Lower Pecos River Region. In *Papers on Lower Pecos Prehistory* (ed. Turpin SA), pp. 1-51. Texas Archeological Research Laboratory Studies in Archeology 8, The University of Texas, Austin.

U.S. Fish and Wildlife Service (1993) Endangered and threatened wildlife and plants; determination of endangered status for the plant *Astrophytum asterias* (star cactus). *Federal Register*, **58**, 53804-53807.

U.S. Fish and Wildlife Service (2003) Recovery Plan for Star Cactus (*Astrophytum asterias*). U.S. Fish and Wildlife Service, Albuquerque, New Mexico. i-vii + 38pp., A1-19, B1-8.

Warnock BH (1970) *Wildflowers of the Big Bend Country, Texas.* Sul Ross State University, Alpine, Texas.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.

Weniger D (1970) *Cacti of the Southwest: Texas, New Mexico, Oklahoma, Arkansas, and Lousiana*. University of Texas Press, Austin.

Weniger D (1984) *Cacti of Texas and Neighboring States: A Field Guide*. University of Texas Press, Austin.

Wright S. (1943) Isolation by distance. *Genetics*, **28**, 114-138.

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, **19**, 395-420.

Wright S (1978) *Evolution and the Genetics of Populations. Volume **4**. Variability within and among Natural Populations.* University of Chicago Press, Chicago.

Zimmerman AD, Parfitt BD (2003) *Astrophytum*. In: Editorial Committee (eds.) *Flora of North America*, Vol. **4**, pp. 191-192. Oxford University Press, New York.

# APPENDIX

**Appendix 1** Raw genotypic data for *Astrophytum asterias* (four populations, six loci). The data are in the format of input data for Genepop. Each six-digit data cell represents a genotype of the type $A_xA_y$, where $A_x$ (the first allele) is uniquely identified by its size *x* expressed as its three-digit length in basepairs, and $A_y$ (the second allele) is similarly identified by its size *y* expressed as its three-digit length in basepairs. Where $x = y$, the genotype is homozygotic. Where $x \neq y$, the genotype is heterozygotic. Identification numbers of individuals are in the far left column. Names of populations to which the individuals belong are in the second column. The final six columns, from left to right, are the genotypes of the six loci: *AaB6, AaH11, AaA3, AaG3, AaC3 and AaD9,* in that order. The intermediate rows without data (designated "pop") are separators between data of different populations.

| | | ***AaB6*** | ***AaH11*** | ***AaA3*** | ***AaG3*** | ***AaC3*** | ***AaD9*** |
|---|---|---|---|---|---|---|---|
| **1** | **Min** | **, 087091** | **083083** | **150156** | **127133** | **092092** | **137139** |
| **2** | **Min** | **, 087091** | **107107** | **156156** | **127129** | **090090** | **135139** |
| **3** | **Min** | **, 087091** | **097105** | **156162** | **129129** | **098100** | **137139** |
| **4** | **Min** | **, 083091** | **103103** | **156156** | **129133** | **090092** | **137137** |
| **5** | **Min** | **, 083087** | **097101** | **156162** | **129129** | **092092** | **139139** |
| **6** | **Min** | **, 083083** | **107107** | **150156** | **129133** | **090098** | **135139** |
| **7** | **Min** | **, 083091** | **101101** | **156162** | **129133** | **092092** | **139143** |
| **8** | **Min** | **, 091091** | **087109** | **153162** | **129133** | **092096** | **139139** |
| **9** | **Min** | **, 083087** | **101101** | **156162** | **133133** | **092096** | **137139** |
| **10** | **Min** | **, 083087** | **105105** | **156156** | **129129** | **098098** | **137139** |
| **11** | **Min** | **, 083083** | **095105** | **156156** | **129129** | **092096** | **137137** |
| **12** | **Min** | **, 083091** | **087101** | **156162** | **133133** | **092096** | **139139** |
| **13** | **Min** | **, 091091** | **097097** | **153156** | **127133** | **090100** | **137139** |
| **14** | **Min** | **, 083083** | **105105** | **153156** | **129133** | **092098** | **139139** |
| **15** | **Min** | **, 083093** | **105107** | **156162** | **129131** | **088092** | **139139** |
| **16** | **Min** | **, 087087** | **107109** | **153156** | **127133** | **092106** | **137139** |
| **17** | **Min** | **, 083083** | **097105** | **153156** | **129133** | **090098** | **139139** |
| **18** | **Min** | **, 083089** | **097107** | **162162** | **129133** | **094096** | **137139** |
| **19** | **Min** | **, 083083** | **097097** | **153165** | **129133** | **090096** | **139139** |
| **20** | **Min** | **, 083083** | **099105** | **150165** | **129133** | **094094** | **138139** |
| **21** | **Min** | **, 083089** | **097097** | **156165** | **129133** | **096096** | **138139** |
| **22** | **Min** | **, 083083** | **097107** | **144156** | **127129** | **098106** | **135139** |
| **23** | **Min** | **, 083091** | **083105** | **138156** | **133133** | **098104** | **135139** |
| **24** | **Min** | **, 083083** | **083089** | **156162** | **129133** | **100104** | **139139** |
| **25** | **Min** | **, 087087** | **083101** | **156156** | **131133** | **090090** | **139139** |

**26 Min , 083083 083097 156156 127129 104104 139139**
**28 Min , 083093 105105 156168 133133 104104 139139**
**29 Min , 083089 089089 156162 129133 090104 139139**
**30 Min , 083083 083083 153162 129133 090104 139139**
**31 Min , 083087 099105 153156 129133 090104 139139**
**32 Min , 087091 105105 153156 129133 090092 139139**
**33 Min , 083087 097105 156165 129133 090104 138139**
**34 Min , 087091 097099 156168 133133 090092 139139**
**35 Min , 083087 083099 153153 129133 090090 139139**
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**37 Min , 083087 087105 150156 127133 090092 139139**
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**39 Min , 083093 095095 156156 127133 092096 139139**
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**41 Min , 083091 095097 156156 129133 090090 135139**
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**47 Tne , 083083 095105 153153 129129 092094 135139**
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**49 Tne , 083091 105107 156159 127133 092098 139139**
**50 Tne , 083083 095105 156159 129133 094098 139139**
**51 Tne , 083099 103105 156162 129129 092092 139139**
**52 Tne , 083083 099101 153162 129133 090092 139139**
**53 Tne , 083083 087103 153153 129133 098098 139139**
**54 Tne , 083091 097103 156162 127129 098100 139139**
**55 Tne , 083083 097107 153156 127129 092094 139139**
**56 Tne , 083091 095103 150156 127131 090092 139139**
**57 Tne , 083083 083097 162162 127129 092094 139143**
**58 Tne , 083083 087103 150156 129133 098098 139139**
**59 Tne , 083083 107107 156162 129133 092102 139139**
**60 Tne , 083087 103105 156156 129133 098098 139139**
**61 Tne , 083093 095107 156156 127133 090090 139139**
**62 Tne , 083087 097103 153156 133133 098104 139139**
**63 Tne , 083093 087101 156165 131133 090104 135139**
**64 Tne , 083091 103105 156165 129133 090092 139139**
**65 Tne , 083087 105105 156156 127129 098098 137139**
**66 Tne , 083083 099103 153156 129129 090092 139139**
**67 Tne , 083083 103107 153156 129129 090098 139139**
**68 Tne , 083097 087091 156165 129131 092098 139139**
**69 Tne , 087091 097103 150156 127127 090098 139139**

**pop**
**70 Tnw , 083083 095097 156162 127127 092104 135139**
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**82 Esc , 083091 097109 156159 127129 092092 139139**
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**85 Esc , 083087 099099 156156 127133 092092 139139**
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**95 Esc , 083091 083107 153156 127133 090100 139139**
**96 Esc , 091091 097097 153156 129129 090090 139139**

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# VITA

Martin Kilman Terry emerged from the weeds (aka invasive species) of Texas with an interest in biology whose ontogeny involved metamorphosis and persistence through phases of dormancy over several decades. Attempts were made to educate him at Harvard (A.B., 1971) and Texas A&M (D.V.M., 1976; Ph.D. in Veterinary Toxicology, 1981). He has worked as an educator and in pharmaceutical regulatory affairs in North and South America, Africa and Europe. Forsaking the political machinations of Washington for the more complex politics of small-town rural Texas in the mid-1990's, he and his wife Marilyn moved into a cabin on the family farm north of College Station, where he finally cut the umbilical cord to the pharmaceutical industry and returned to Texas A&M as a recycled graduate student in Botany. In 2003 he accepted an academic appointment in the Department of Biology, Sul Ross State University, Alpine, TX 79830, where he may be reached currently (mterry@sulross.edu). He enjoys the interactions with the students, collaboration with colleagues in botany, chemistry and archaeology, and the opportunities for studying plants in their natural habitats without having to resort to the interstate highway system. Last year he and some colleagues founded the Cactus Conservation Institute (www.cactusconservation.org), which focuses primarily on protection of vulnerable species of cacti of the Tamaulipecan thornscrub of South Texas and adjacent Mexico. He continues to work with the Texas A&M group that is generating the population genetics data on several endangered species in the Tamaulipecan thornscrub, where star cactus is the star of the Starr County cactus flora.