

## GENETIC DIVERSITY IN CHIHUAHUAN DESERT POPULATIONS OF CREOSOTEBUSH (ZYGOPHYLLACEAE: *LARREA TRIDENTATA*)<sup>1</sup>

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We examined isozyme variation in the dominant Chihuahuan Desert shrub, *Larrea tridentata* (creosotebush), to determine the genetic variation within and among populations, the biogeographic relationships of populations, and the potential inbreeding in the species. We surveyed 17 populations consisting of 20 to 50 individuals per population along a 1600-km north–south transect across the Chihuahuan Desert. The southernmost population was near Villa Hidalgo, Mexico, and the northernmost near Isleta Pueblo, New Mexico. All 12 isozyme loci examined were polymorphic ( $H_i = 0.416$ ), with up to nine alleles per locus. Despite high levels of variation, we detected moderate inbreeding in *L. tridentata* populations. Most variation was found within rather than among populations ( $G_{ST} = 0.118$ ). Furthermore, recently established populations in the northern limits of the Chihuahuan Desert did not show decreased levels of genetic variation ( $H_o = 0.336$ ). A significant correlation was found between pairwise genetic and geographic distances ( $r = 0.305$ ). *Larrea tridentata* showed and continues to show a massive range expansion into the arid and semi-arid regions of the American Southwest, but as shown by the high genetic variation, this expansion took place as a wave, rather than a series of founder events.

**Key words:** biogeography; Chihuahuan Desert; creosotebush; genetic variation; isozymes; *Larrea tridentata*; population genetics; Zygophyllaceae.

Anthropogenic disturbances of arid lands in Mexico and the American Southwest, in concert with climate dynamics of drought cycles, have facilitated the desertification process. Rangeland overgrazing resulting from high stocking rates of cattle, sheep, and horses in the late 19th century coupled with extended drought periods have created conditions conducive to desert shrub invasions into formerly grassland habitats (Milne et al., 2003). Over the past 200 years, this desertification has altered a large portion of the Chihuahuan Desert landscape (Humphrey, 1958; Hastings and Turner, 1965; Mainquet, 1994). Woody shrub invasion into grasslands considerably alters ecosystem dynamics (Schlesinger et al., 1990) and has been examined extensively (Branscomb, 1958; Buffinton and Herbel, 1965; York and Dick-Peddie, 1969; Whitford, 1997; Kerley and Whitford, 2000). One study in the Chihuahuan Desert (Flores and Yeaton, 2000) suggested that *Larrea tridentata* or *Prosopis* spp. (Fabaceae) are the first species to invade open areas in the grassland, with *Acacia schaffneri* (Fabaceae) next, followed by species of *Opuntia* (Cactaceae) and *Yucca* (Agavaceae). Although recent work has examined the genetic diversity in *Prosopis* spp. (Saidman and Vilaridi, 1987; Keys and Smith, 1994), *L. tridentata* offers an additional opportunity to study the invasion of these disturbed habitats by a pioneer species.

*Larrea tridentata* (DC) Coville (creosotebush) is the dominant shrub of the warm deserts of North America. It ranges from Querétaro, Central Mexico, northward to New Mexico, USA, and from Texas westward to Nevada and California. Its elevational distribution ranges from sea level to 1600 m above sea level. Although the arrival time of *L. tridentata* in North America is unknown (estimates range from 1.5 to 8.4 million years ago (my BP; Hunter et al., 2001; Lia et al., 2001), *L. tridentata* first appeared in fossil packrat middens during the late Quaternary, approximately 18 700 yr BP (Van Devender, 1990; Hunter et al., 2001). *Larrea tridentata* likely arrived in North America as a result of a long-distance dispersal event from South America, and was probably derived from an ancestral population of *L. divaricata* Cav., a relatively widespread species in Argentina, Chile, and Peru (Lia et al., 2001). During the last 11 000 yr, as the warm deserts formed in Mexico and the American Southwest, *L. tridentata* expanded its range to include the present-day distributions of the Chihuahuan, Sonoran, and Mojave Deserts (Betancourt et al., 1990). Much of this latter expansion has taken place in the last 5000 yr (Van Devender, 1990), with recent northern range extensions into central New Mexico during the last 200 yr (Hennessy et al., 1983; Humphrey, 1987; McPherson et al., 1988; Grover and Musick, 1990).

The recent and rapid expansion of *L. tridentata* throughout the warm deserts of North America has been accompanied by an unusual biogeographic pattern of change in chromosome number. Yang (1967, 1970) and Barbour (1969) established the existence of polyploid variation in the taxon in which tetraploid chromosomal races replaced diploid as *L. tridentata* moved from the Chihuahuan to the Sonoran, and hexaploid replaced tetraploid as it invaded the Mohave Desert. Poggio et al. (1989) correlated the increase in ploidy level, due to autopolyploidy, with increasing aridity in the respective de-

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TABLE 1. List of study sites and locations of *Larrea tridentata* (creosotebush) collections. Site number refers to the site depicted in Fig. 1. Site name is generally the town or physical feature nearest the collection site, Sevilleta National Wildlife Refuge (SNWR). Latitude/longitude are estimated to the nearest 15".

Site No.	Site Name	State, Country	Latitude	Longitude
1	Isleta Pueblo	New Mexico, USA	34°55'30"	106°43'45"
2	Black Butte, SNWR	New Mexico, USA	34°24'30"	106°41'00"
3	Five Points, SNWR	New Mexico, USA	34°15'00"	106°41'00"
4	Socorro	New Mexico, USA	34°07'30"	106°54'45"
5	San Marcial	New Mexico, USA	33°45'00"	107°00'45"
6	Flying X Ranch	New Mexico, USA	33°30'00"	107°13'30"
7	Caballo	New Mexico, USA	32°57'15"	107°20'00"
8	Hatch	New Mexico, USA	32°40'15"	107°02'45"
9	Las Cruces	New Mexico, USA	32°23'00"	106°47'15"
10	El Sueco	Chihuahua, Mexico	29°59'30"	106°22'15"
11	Delicias	Chihuahua, Mexico	28°25'15"	105°40'00"
12	Ciudad Jimenez	Chihuahua, Mexico	27°20'00"	104°56'00"
13	Mapimi	Durango, Mexico	26°41'00"	103°44'45"
14	Saltillo	Nuevo Leon, Mexico	25°11'30"	100°45'30"
15	Matehuala	San Luis Potosi, Mexico	23°40'30"	100°34'30"
16	La Viga	Tamaulipas, Mexico	22°45'30"	99°57'00"
17	Villa Hidalgo	San Luis Potosi, Mexico	22°37'30"	100°32'00"

serts, with the highest ploidy level (hexaploidy) occurring in the Mojave Desert.

The life history characteristics of *L. tridentata* are suited to its range expansion and persistence in the habitats that it colonizes (Reynolds, 1986). The shrub combines long-lived, clonal reproduction (Vasek, 1980) with mixed-mating sexual reproduction (Simpson et al., 1977). Vasek (1980) presented evidence that some clones in the Mojave Desert may be over 11 000 yr old. *Larrea tridentata* is largely outcrossing and insect pollinated by a number of oligolectic (21 species) as well as generalist bees (99 species; Simpson et al., 1977; Minckley et al., 2000) although the species is self-compatible and autogamy and geitongamy can occur (Rossi et al., 1999). The fruits are preadapted for epizoid dispersal and are presumably transported by mammals and birds (Hunziker et al., 1977).

Despite its major ecological importance in desert ecosystems, its unusual biogeographic history, its recent polyploid evolution, and its even more recent range expansion in the Chihuahuan Desert, population genetic variation and diversity have not been extensively surveyed for *L. tridentata*. This highlights the conspicuous paucity of information on genetic variation in desert species in general (Schuster et al., 1994). Studies of genetic diversity in *Larrea* are essential to elucidate our understanding of evolution in the taxon as well as to provide insight into the relationship of its interesting evolutionary history and ecological attributes to the amount and structuring of genetic variation at the population level. Lia et al. (1999) examined genetic variation in *L. ameghinoi* and *L. nitida* in South America. However, to date, there are only two published small-scale studies of genetic variation in *L. tridentata*: Schuster et al. (1994) examined isozyme diversity in one diploid population in Arizona; and Cortés and Hunziker (1997) examined greenhouse-germinated seedlings from three populations of diploid cytotypes and one population of a tetraploid cytotype. Nonetheless, the population genetic structure of *L. tridentata* has not been assessed throughout its range in the Chihuahuan Desert.

We set out to examine the population genetic structure of *L. tridentata* in the Chihuahuan Desert. We addressed the following questions: (1) How much genetic variation exists in diploid populations of *L. tridentata* in the Chihuahuan Desert,

and how is the variation distributed within and among populations? (2) Is variation reduced in the recently established populations at the northern limits of the Chihuahuan Desert? (3) What is the proportion of inbreeding vs. outcrossing in the mixed-mating system of *L. tridentata* in the Chihuahuan Desert? (4) What is the genetic relatedness of populations?

## MATERIALS AND METHODS

Seventeen populations of *L. tridentata* (Table 1) comprising 528 individuals (20–50 individuals per population, a small percentage of the total population) were sampled along a 1600-km north–south axis beginning at the northernmost distributional point in New Mexico at Isleta Pueblo near Albuquerque, and extending to the southern distributional boundary in central Mexico near San Luis Potosi (Fig. 1). The Isleta Pueblo population is disjunct, separated by approximately 65 km from populations in the edge of the Chihuahuan Desert proper near Socorro, New Mexico. All other populations sampled were in the continuous distribution of the Chihuahuan Desert *L. tridentata*. Young leaf materials were collected from each individual sampled and stored on ice for transport to the laboratory. Sampled individuals were greater than 25 m apart to avoid collecting from the same clone. All populations sampled were assumed to be diploid based on the studies by Yang et al. (1977) and Hunter et al. (2001).

The extraction buffer consisted of 0.1 mol/L Tris (pH 7.0), 1.0 mmol/L ethylene-diamine tetra-acetic acid (EDTA, disodium), 0.2 mol/L sucrose, 0.6% polyvinylpyrrolidone (PVP w/v), 2.0% polyethylene glycol (mw w/v), 0.1% bovine serum albumin (BSA), 2.0 mmol/L ascorbic acid (free acid), and 14 mmol/L 2-mercaptoethanol. Approximately six fresh leaves per individual were ground with 1.4 mL of extracting buffer. The supernatant was transferred into 1.5-mL microcentrifuge tubes and centrifuged at 10344 × g for 1 min at 4°C. Filter paper wicks were soaked in the centrifuged supernatant and inserted into 12.5% starch gels for electrophoresis.

Ten enzyme systems were resolved using two buffer systems. Buffer system A was morpholine-citrate pH 6.1 (= System 2 electrode buffer in Wendel and Weeden, 1989). The gel buffer was prepared by dilution of one part electrode buffer with nine parts distilled water. Buffer system B was 0.5 M Tris-verseneborate pH 8.0 (Shaw and Prasad, 1970). Buffer system A was used to resolve isocitrate dehydrogenase (IDH, EC 1.1.1.41), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.43), shikimate dehydrogenase (SDH, EC 1.1.1.25), and menadiene reductase (MNR, EC 1.6.99.2). Buffer system B was used to resolve aspartate aminotransferase (AAT, EC 2.6.1.1), triose-phosphate isomerase (TPI, EC 5.3.1.1), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), alcohol dehydrogenase (ADH, EC

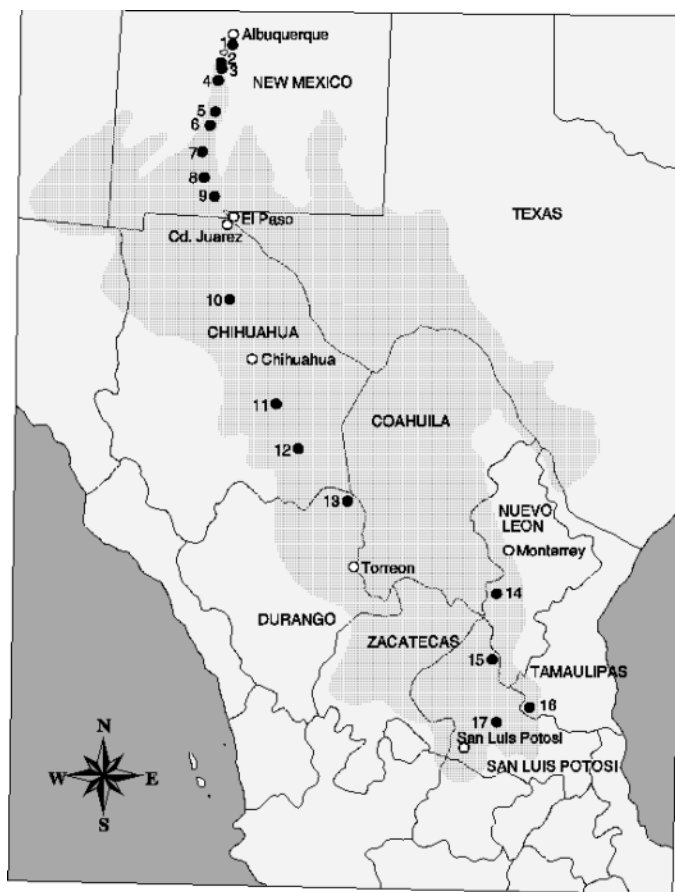


Fig. 1. Distribution of *Larrea tridentata* in the Chihuahuan Desert of the United States and Mexico. Solid black dots represent collection sites. Numbers refer to sites listed in Table 1. Shading indicates general range of the Chihuahuan Desert.

1.1.1.1), and aldolase (ALD, EC 4.1.2.13). Gels were electrophoresed overnight for approximately 17 h at 50 mV. The gels were sliced and incubated in enzyme-specific stain solutions at 37°C. Names of enzymes and staining procedures followed Weeden and Wendel (1989) and Wendel and Weeden (1989), respectively. Interpretation of the genetic basis of enzyme banding patterns relied on general patterns found in diploid angiosperms (Gottlieb, 1982; Weeden and Wendel, 1989). Individuals from different populations were run on the same gel to confirm the allelic designations for each locus.

**Analysis of genetic variation**—Several descriptive statistics were computed using the computer program Genetic Data Analysis, version 1d11 (GDA; Lewis and Zaykin, 1998), including the proportion of polymorphic loci ( $P_p$ ), the mean number of alleles per polymorphic locus ( $A_p$ ), the expected heterozygosity ( $H_e$ ), the observed heterozygosity ( $H_o$ ), and the fixation index ( $f$ ). In order to compare our results with those of Hamrick and Godt (1989), we also computed the species-level statistics  $P_s$ ,  $A_s$ ,  $A_{ep}$ , and  $H_{es}$ , and the population-level statistics  $P_p$ ,  $A_p$ ,  $A_{ep}$ , and  $H_{ep}$ .

Wright's  $F$  statistics ( $F_{IS}$ ,  $F_{IT}$ , and  $F_{ST}$ ) were estimated in GDA using the moment estimators described by Weir and Cockerham (1984). The Weir and Cockerham estimator of Wright's  $F_{ST}$  ( $\theta$ ) is more appropriate than  $G_{ST}$  for comparisons across studies because it does not depend on the sampling scheme (Cockerham and Weir, 1993). The value of  $G_{ST}$  depends both on the number of populations sampled as well as on the number of individuals sampled per population, making  $\theta$  the better estimator to use for comparisons across studies in which differences in both sampling dimensions are common. In the case of this study, however, the difference between  $G_{ST}$  and  $\theta$  is expected to be slight because of the large number of populations sampled (17)

and the large number of individuals sampled per population (average 30). We also computed Nei and Chesser's (1983) gene diversity statistics ( $H_s$ ,  $H_T$ , and  $G_{ST}$ ) using the program GeneStat-PC (version 3.31; Lewis and Whitkus, 1989).

To estimate the genetic relationship of the *L. tridentata* populations sampled, we obtained pairwise evolutionary distances in GDA using both the unbiased measure proposed by Nei (1978) and the coancestry coefficient-based distances described by Reynolds et al. (1983) and Weir (1996, pp. 194–195). We then exported the resulting distance matrices to NEXUS format for input into PAUP\* (version 4.0d64; Swofford, 1998). TBR (tree bisection/reconnection) heuristic searches were then conducted in PAUP\* using the least squares optimality criterion to obtain estimates of the phylogeny.

## RESULTS

**Genetic variation**—The 10 enzyme systems revealed 12 putative loci that were resolved and reliably scored: *6Pgd*, *Idh*, *Gpi-2*, *Pgm*, *Tpi-1*, *Tpi-2*, *Ald*, *Adh*, *Aat-1*, *Aat-2*, *Mnr*, and *Sdh*. Banding patterns for all loci were concordant with those generally reported for diploid vascular plants (Weeden and Wendel, 1989). All loci were polymorphic in at least one population. No locus had fewer than five alleles. Five loci had five alleles (*6Pgd*, *Idh*, *Tpi-2*, *Ald*, *Adh*), two loci had six alleles (*Tpi-1*, *Aat-1*), two loci had seven alleles (*Gpi*, *Pgm*), one locus had eight alleles (*Mnr*), and two loci had nine alleles (*Aat-2*, *Sdh*). Some loci were not scored for all individuals due to poor or inconsistent resolution. The table of allele frequencies and a complete list of genotypes for every locus in each population is available on <http://sevilleta.unm.edu>.

The descriptive statistics (Table 2) demonstrate a high degree of variability in the Chihuahuan Desert populations of *L. tridentata*. The proportion of polymorphic loci ( $P_p$ ) was greater than 0.75 for all populations sampled, and 100% of the loci were polymorphic for 11 of the 17 populations studied. The mean number of alleles per polymorphic locus averaged 3.89 and was 3.0 or greater for each of the 17 populations. The expected and observed heterozygosity averaged 0.362 and 0.322, respectively, resulting in a positive average fixation coefficient (0.112), reflecting the greater than expected number of homozygotes within most populations. Seven populations showed fixation coefficients near zero, thus approximating Hardy-Weinberg genotypic proportions, whereas the other ten populations all had fixation coefficients greater than 0.1. These statistics reveal an unusual coupling of high polymorphism with moderate inbreeding in most populations.

Recently established populations in the northern limits of the Chihuahuan Desert did not show decreased levels of genetic variation (Isleta Pueblo  $H_o = 0.336$ , Black Butte  $H_o = 0.280$ , Five Points  $H_o = 0.355$ , and Socorro  $H_o = 0.315$ ). Matehuala and La Viga in Central Mexico had the lowest variation with  $H_o = 0.266$  and  $H_o = 0.214$ , respectively. The Las Cruces population had the highest level of genetic variation,  $H_o = 0.431$ .

**Inbreeding**—Wright's  $F$  statistics (Table 3) also reflect the inbreeding observed within populations (Table 2). Estimates  $f$  and  $F$  (of Wright's  $F_{IS}$  and  $F_{IT}$ , respectively) were both significantly positive (95% bootstrapping confidence intervals). Despite this overall significant level of inbreeding, several individual loci (*6Pgd*, *Idh*, *Pgi*, *Tpi-1*, and *Sdh*) showed very low levels of within-population inbreeding as measured by  $f$ . The coancestry coefficient  $\theta$  was estimated to be 0.116, which was significantly different from zero (Table 3). The coefficient of population differentiation  $G_{ST}$  measured in *L. tridentata* was

TABLE 2. Descriptive statistics for Chihuahuan Desert populations of *Larrea tridentata* (creosotebush) (means are over all loci): mean sample size ( $N$ ), proportion of polymorphic loci ( $P_p$ ), mean number of alleles per polymorphic locus ( $A_p$ ), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), and fixation index ( $f$ ). The fixation index was estimated using the method of moments estimator given in Weir (1996, p. 80). The upper and lower 95% confidence limits resulted from 1000 bootstrap replicates.

Population	$N$	$P_p$	$A_p$	$H_e$	$H_o$	$f$
1. Isleta Pueblo	37.0	1.00	3.33	0.320	0.336	-0.050
2. Black Butte	48.5	0.92	4.18	0.359	0.280	0.223
3. Five Points	34.9	0.92	3.64	0.362	0.355	0.021
4. Socorro	39.4	1.00	3.83	0.358	0.315	0.123
5. San Marcial	40.0	1.00	4.33	0.441	0.302	0.319
6. Flying × Ranch	27.0	0.92	3.82	0.333	0.336	-0.009
7. Caballo	29.6	1.00	3.50	0.376	0.316	0.162
8. Hatch	29.8	1.00	3.83	0.401	0.382	0.047
9. Las Cruces	29.6	1.00	4.17	0.424	0.431	-0.016
10. El Sueco	32.0	1.00	4.82	0.436	0.304	0.304
11. Delicias	27.8	1.00	4.64	0.355	0.339	0.045
12. Ciudad Jimenez	31.0	1.00	4.58	0.387	0.312	0.196
13. Mapimi	30.2	1.00	4.58	0.386	0.329	0.150
14. Saltillo	19.4	0.89	3.13	0.286	0.342	-0.202
15. Matehuala	21.0	0.75	3.33	0.313	0.266	0.153
16. La Viga	18.8	0.83	3.00	0.265	0.214	0.196
17. Villa Hidalgo	20.0	1.00	3.42	0.343	0.308	0.102
Mean	30.4	0.95	3.89	0.362	0.322	0.112
Lower 95%		0.92	3.64	0.338	0.300	0.045
Upper 95%		0.99	4.17	0.383	0.342	0.161

very close to the value of  $\theta$  ( $G_{ST} = 0.118$ ,  $\theta = 0.116$ ). This value also indicates that most of the variation in *L. tridentata* is distributed within rather than among populations.

**Genetic differentiation**—The tree obtained (Fig. 2) using a matrix of pairwise distances estimated by Nei’s (1978) formula groups the four central Mexico populations (Saltillo, Matehuala, La Viga, and Villa Hidalgo) separately from all other populations; however, the northern Mexico and New Mexico populations were markedly interspersed. We performed a Mantel test and found a moderate correlation between genetic and geographic distances ( $r = 0.305$ ,  $P < 0.001$ ). The San Marcial and Flying × Ranch populations were notable in their exceptionally long branches, which suggest high mutation rates in these lineages under the Nei model. A similar phylogeny (Fig.

3) results from using a matrix of coancestry-based distances. The tree based on this distance measure also shows the four central Mexico populations separated from the other populations. As in the tree inferred from Nei’s genetic distance measure, populations from New Mexico and northern Mexico were interspersed. Again, there is a significant correlation between genetic and geographic distances ( $r = 0.294$ ,  $P < 0.001$ ). In this tree, only the Flying × Ranch population showed an exceptionally long branch, which indicated that this lineage had a very small population size (under the pure drift model used to estimate the genetic distances). We also performed a Mantel test for only the New Mexico populations (first 9 populations in Table 1). Neither the correlation between geographic distances and genetic distances based on coancestry genetic distances ( $r = 0.059$ ,  $P = 0.383$ ) nor Nei’s genetic distance ( $r$

TABLE 3.  $F$  statistics and gene diversity statistics of *Larrea tridentata* (creosotebush). Method of moments estimators for Wright’s  $F_{IS}$  ( $f$  – inbreeding within populations),  $F_{IT}$  ( $F$  – inbreeding seen by examining the entire collection of populations), and  $F_{ST}$  ( $\theta$  – among population inbreeding) obtained using the methodology of Weir and Cockerham (1984). The upper and lower 95% confidence limits resulted from bootstrapping across loci (1000 replicates).  $H_s$ ,  $H_t$ , and  $G_{ST}$  are the within-population gene diversity, the among-population gene diversity, and the coefficient of population differentiation, respectively (Nei and Chesser, 1983).

Locus	$f$	$F$	$\theta$	$H_s$	$H_t$	$G_{ST}$
<i>6Pgd</i>	-0.115	-0.056	0.053	0.365	0.384	0.049
<i>Idh</i>	-0.001	0.188	0.189	0.376	0.488	0.230
<i>Gpi</i>	0.034	0.055	0.023	0.269	0.275	0.020
<i>Pgm</i>	0.207	0.292	0.107	0.541	0.603	0.102
<i>Tpi-1</i>	0.056	0.103	0.050	0.212	0.224	0.053
<i>Tpi-2</i>	0.138	0.273	0.156	0.185	0.216	0.144
<i>Ald</i>	0.470	0.522	0.097	0.186	0.207	0.101
<i>Adh</i>	0.353	0.529	0.272	0.248	0.344	0.278
<i>Aat-1</i>	0.244	0.336	0.123	0.358	0.404	0.116
<i>Aat-2</i>	0.165	0.231	0.079	0.722	0.783	0.078
<i>Mnr</i>	0.254	0.282	0.037	0.236	0.245	0.038
<i>Sdh</i>	-0.032	0.117	0.144	0.702	0.813	0.137
Overall	0.124	0.226	0.116	0.367	0.416	0.118
Std. Error	—	—	—	0.055	0.062	—
Upper 95%	0.211	0.309	0.155	—	—	—
Lower 95%	0.043	0.149	0.083	—	—	—

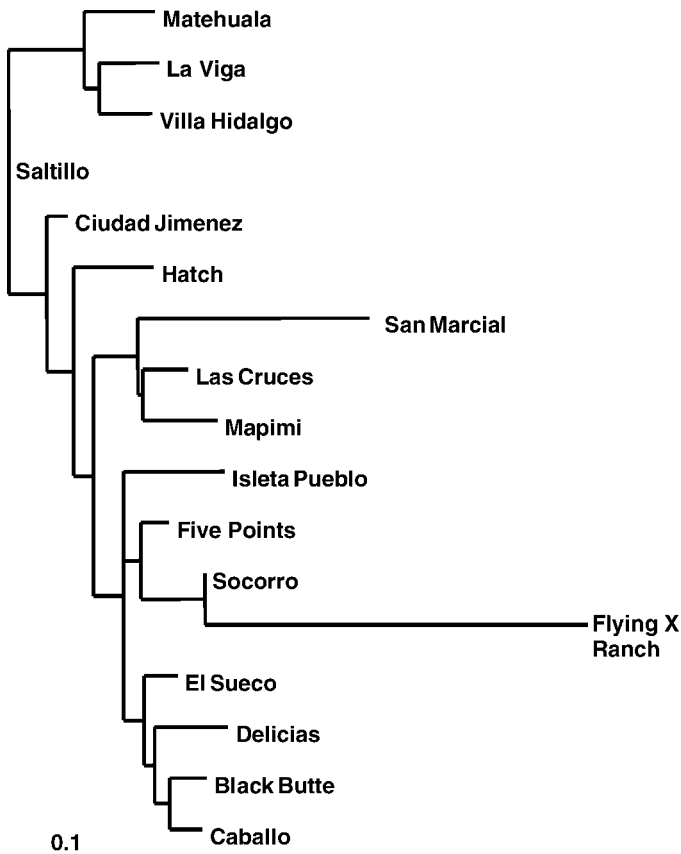


Fig. 2. The tree of *Larrea tridentata* populations using a matrix of pairwise distances estimated using Nei's (1978) formula. There is a significant correlation between genetic relationship and geographic location ( $r = 0.305$ ,  $P < 0.001$ ).

$= -0.114$ ,  $P = 0.741$ ) were significant. Thus, there was little correspondence between genetic and geographic distances except that the central Mexico populations were close both genetically and geographically. Also, the lineage leading to the Flying  $\times$  Ranch population has diverged conspicuously more than other lineages, and this can be explained in terms of either increased mutation rate or decreased population size, depending on the model used to infer genetic distances.

## DISCUSSION

**Genetic variation**—At the species level, *L. tridentata* is highly unusual compared to other long-lived perennials, widespread species, species with mixed animal-dependent breeding systems, and species with both sexual and asexual reproduction. At the species level, 100% of the loci are polymorphic, there is an average of six alleles per polymorphic locus, the effective number of alleles is 2.145, and the expected heterozygosity is 0.415 in *L. tridentata*. These figures are well above the averages reported for groups having similar life history characteristics. At the population level, *L. tridentata* is also extreme, with 95% of its loci polymorphic, an average of 3.89 alleles per locus, an effective number of alleles of 1.84, and an expected heterozygosity of 0.362. *Populus tremuloides* (Salicaceae), noted for being extreme in these measures (Cheliak and Dancik, 1982), exceeds *L. tridentata* only in expected heterozygosity (0.420 compared to 0.362). Even when com-

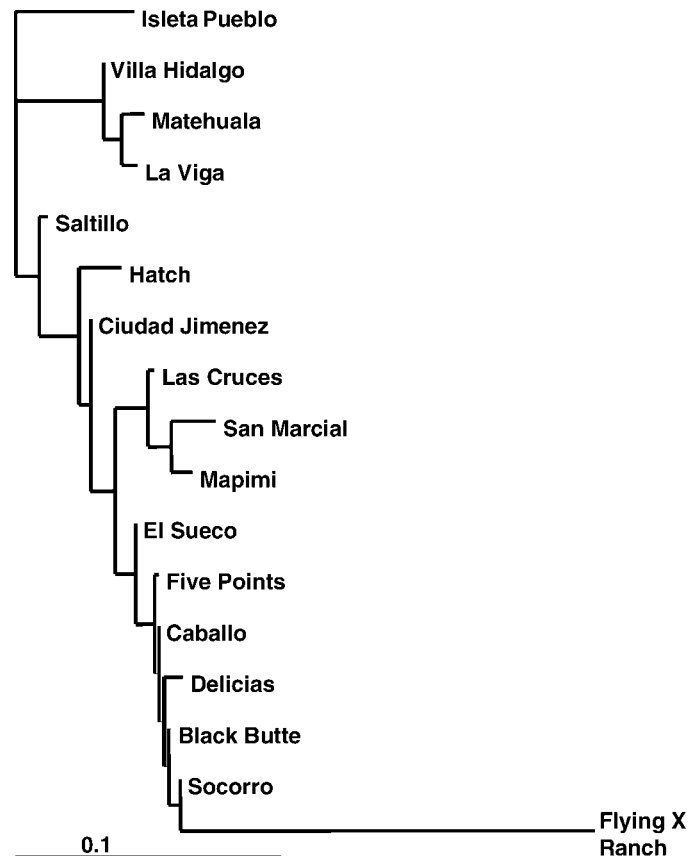


Fig. 3. The tree of *Larrea tridentata* populations obtained using a matrix of coancestry-based distances. This tree is slightly different than that of Fig. 2; however, this tree also shows a significant correlation between genetic relationship and geographic location ( $r = 0.294$ ,  $P < 0.001$ ).

pared to other studies on *L. tridentata*, our data showed strikingly higher values for every measure of genetic variation. The percentage of polymorphic loci and the genetic diversity index ( $H_e$ ) is nearly double the average for seed plants in general as well as for the values from the Sonoran Desert population of *L. tridentata*. Not one sampled locus was monomorphic or had fewer than five alleles. These values are among the highest reported for vascular plants.

Genetic variation in populations is clearly correlated to a great extent with the distributional range and life history characteristics of the particular species (Hamrick et al., 1979; Loveless and Hamrick, 1984; Hamrick and Godt, 1989). *Larrea tridentata* is a long-lived woody, desert, perennial that is widespread and reproduces both sexually and asexually. In general, long-lived woody perennials maintain higher levels of genetic variation ( $H_{es} = 0.177$  compared to  $H_{es} = 0.15$  for seed plants in general; Hamrick et al., 1992). For long-lived woody perennials, widespread species tend to have higher numbers of expected heterozygotes ( $H_{es} = 0.257$ ) than endemic ( $H_{es} = 0.078$ ), narrow-range species ( $H_{es} = 0.165$ ) or regionally spread species ( $H_{es} = 0.169$ ; Hamrick et al., 1992). Values for the widespread species are twofold higher than values for herbaceous perennials in general ( $H_{es} = 0.082$ – $0.098$ ) (Hamrick et al., 1992).

Other widespread species that are long-lived and inhabit deserts have high levels of heterozygosity. *Juniperus rigida* (Cupressaceae) ( $H_e = 0.224$ ; Huh and Huh, 2001), *Echinocereus*

*engelmannii* (Cactaceae) (tetraploid—high genetic diversity determined from banding patterns; Neel et al., 1996), and eight species of cacti ( $H_e = 0.208$ ; Nassar et al., 2001) are examples. Aside from those studies, few studies of genetic variation in desert plants exist. Eight species of *Melocactus* (Cactaceae), *Encelia farinosa* (Asteraceae), *Lophocereus schottii* (Cactaceae), and some *Prosopis* (Fabaceae) species have genetic variation levels ranging from  $H_e = 0.126$  to  $H_e = 0.230$  (Saidman and Vilardi, 1987; Parker and Hamrick, 1992; Schuster et al., 1994; Nassar et al., 2001). *Agave victoriae* (Agavaceae) has the highest mean expected heterozygote value ( $H_e = 0.335$ ; Martinez-Palacios et al., 1999).

The eight *Melocactus* (Cactaceae) species share several life history characteristics with *L. tridentata*, in that they are long-lived, mixed-mating, widespread, desert species. The mean expected heterozygosity for these eight species is 0.203 and  $G_{ST} = 0.112$ . However, *Agave* is also a desert taxon that is long lived and reproduces both sexually and asexually, yet has a high mean expected heterozygosity value ( $H_e = 0.335$ ; Martinez-Palacios et al., 1999) most similar to *L. tridentata* ( $H_e = 0.362$ ). In general, long-lived woody perennials that reproduce both sexually and asexually have high levels of genetic diversity ( $H_e = 0.215$ ; Hamrick et al., 1992). Species having at least one of the life history characteristics in common with *L. tridentata* tend to have higher than average genetic variation. Therefore, *L. tridentata* may contain very high levels of genetic variation because of its life history characteristics.

All populations of *L. tridentata* have very high levels of genetic variation, however little differentiation exists at the population level. There is no north-south geographic pattern in partitioning of genetic variation. This lack of latitudinal differentiation is consistent with results of seed albumin electrophoretic patterns of in *L. tridentata*, which showed little difference between northern and southern diploid populations (Hunziker et al., 1972). The recent, disjunct Isleta Pueblo population also did not show significantly different genetic diversity from the other populations sampled in the continuous distributional range. Similar results were found in recently established populations of *Pinus edulis* (Pinaceae) at the northern edge of its range in Colorado (Betancourt et al., 1991). Minimal reduction of genetic variation and little genetic differentiation were attributed to rapid population increases and enhancement of long-distance gene flow. Hamrick et al. (1989) also found that isolated populations of *Pinus ponderosa* (Pinaceae) maintained the same alleles and levels of genetic variation as populations within the continuous distributional range. Apparently, the recent and rapid range expansion of *L. tridentata* within the Chihuahuan Desert has maintained a large reservoir of genetic variability, suggesting that *L. tridentata* pushed north in a great wave rather than in a succession of founder events. Furthermore, this pattern rejects the hypothesis of less genetic variation in the recent northernmost populations.

Another predictor of genetic variability is population size. Species with immense populations tend to have high levels of genetic variation compared to species with smaller population sizes (Mitton, 1997). The levels of heterozygosity for neutral alleles can be given by the equation,  $H = 4N_e u / (4N_e u + 1)$ , where  $H$ ,  $N_e$ , and  $u$  are the heterozygosity, effective population size, and the neutral mutation rate, respectively (Kimura and Ohta, 1971). According to this equation, when effective population size is large, heterozygosity should be high. *Larrea tridentata* is often the dominant shrub wherever it is found

and its populations are quite large. Our results suggest that *L. tridentata* has a high outcrossing rate, resulting in a high effective population size in which high heterozygosity is expected. However, population size alone cannot account for the exceedingly high level of genetic variation shown in this study.

**Inbreeding**—Most *L. tridentata* populations studied were deficient of heterozygotes. The significantly positive values of  $f$  and  $F$  as well as  $\theta$  indicate that moderate levels of inbreeding may contribute to the population structuring of *L. tridentata* ( $\theta = 0.116$ ,  $G_{ST} = 0.118$ ). This is not unusual for a mixed-mating species. Hamrick and Godt (1989) report somewhat higher  $G_{ST}$  values for animal-pollinated, mixed-mating species than for outcrossing species ( $G_{ST} = 0.216$  and  $G_{ST} = 0.197$ , respectively). This indicates that more of the genetic variation is partitioned among, rather than within, populations of mixed-mating species, compared to outcrossing animal-pollinated species due to possible inbreeding within populations. A survey of long-lived woody perennials produced similar results (animal-pollinated, mixed-mating species,  $G_{ST} = 0.122$ ; outcrossing species,  $G_{ST} = 0.099$ ; Hamrick et al., 1992) that are more comparable to values of  $G_{ST}$  for *L. tridentata* ( $G_{ST} = 0.118$ ).

There is a significant amount of variation in values of  $f$  across populations of Chihuahuan Desert *Larrea*. Possible causes of this variation are differences in breeding system among populations. Differences in breeding system may be responsible for the excess and/or paucity of heterozygotes noted in particular populations. These differences could have resulted from variation between outcrossing and selfing rates. Simpson et al. (1977), Boyd and Brum (1983) and Rossi et al. (1999) have shown that tetraploid and hexaploid *L. tridentata* and the closely related diploid *L. divaricata* in South America are capable of facultative inbreeding although both taxa appear to be weakly self-incompatible. All the studies indicate that outcrossing is strongly favored in the populations studied but selfing can and does occur albeit at comparatively low levels. Boyd and Brum (1983) found that selfing can result in up to 28% seed set in hexaploid Mojave Desert plants. Selfing rates are influenced by the availability of pollinators and annual precipitation (Rossi et al., 1999). In periods of drought, pollinators may be reduced resulting in higher levels of selfing. Studies of diploid Chihuahuan Desert *Larrea* populations are needed to assess the interrelationship of breeding system, precipitation, and pollinator availability and their combined effect on variation in the fixation index.

**Genetic structure**—*Larrea tridentata* first appeared in the packrat midden fossil data 18 700 yr ago, however the arrival of *L. tridentata* to North America is much earlier. Molecular clock data for allozymes estimate the divergence between *L. divaricata* and *L. tridentata* to be 1.2 my BP (Cortés and Hunziker, 1997), whereas *rbcL* data place this divergence time between 4.2 and 8.4 my BP (Lia et al., 2001). Van Devender (1990) speculated that the earliest specimens of *L. tridentata* in the fossil packrat midden record were probably hexaploid. Thus the arrival of *L. tridentata* to North America must predate 18 700 yr ago to allow for the evolution of hexaploids from diploid ancestors. Regardless of its arrival time, evidence from packrat midden data suggests that *L. tridentata*'s range expanded and contracted in southwestern New Mexico several times in the late Holocene (Hunter et al., 2001) and only established itself in New Mexico within the last few centuries

(Van Devender, 1990), perhaps aided by overgrazing and fire suppression. This expansion and contraction along with the relatively recent establishment may explain the moderate correlation between geographic and genetic distances among populations. A few notable patterns arise in the phylogenies presented here that may explain the range expansion of *L. tridentata*. First, the four southernmost populations—Saltillo (14), Matehuala (15), La Viga (16), and Villa Hidalgo (17)—form a clade distinct from other populations. Second, Five points (3), Socorro (4), Flying × Ranch (6), Caballo (7), El Sueco (10), Delicias (11), and Black Butte (2) consistently group together, suggesting that El Sueco and Delicias may be the source populations for a majority of the New Mexico populations. Third, Mapimi (13), San Marcial (5), and Las Cruces (9) also consistently group together. These patterns, although lacking resolution, may reflect the range expansion and contraction in New Mexico proposed by Hunter et al. (2001). However, the lack of resolution may be due to the low differentiation between populations. Another explanation may be that *L. tridentata*'s range expansion followed a metapopulation model, in which the northern populations were founded by individuals from more than one southern population. This would not only explain the disparate relationship between the northern and southern populations, but also explain the high genetic variability in the founding populations.

In conclusion, this is a remarkably variable species with a well-developed aptitude for long-range dispersal. It also differs from other species whose natural migrations have been studied. These studies show that populations tend to have reduced levels of genetic variation at marginal populations. *Larrea tridentata* appears to be unusual in that it does not show a loss of variation as it migrated northward. This high level of genetic variation in diploid populations, magnified by autopolyploidy, undoubtedly contributed to its remarkable success in its 20th century range expansion in all the deserts of the American Southwest under different climate regimes, soil types, and land use histories. Future research will, we hope, reveal the mechanisms of how the genetic diversity of *L. tridentata* contributes to its success in range expansion and survival in newly colonized habitats.

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