Allozyme Diversity and Genetic Structure of the Leafy Cactus (*Pereskia guamacho* [Cactaceae])

J. M. Nassar, J. L. Hamrick, and T. H. Fleming

We examined levels of genetic variation and genetic structure in the leafy cactus (*Pereskia guamacho*) in arid and semiarid zones in Venezuela. We surveyed genetic diversity within 17 populations using 19 allozyme loci. Genetic diversity was relatively high at both the species (P_s = 89%, A_s = 3.26, AP_s = 3.53, H_{es} = 0.24) and population (P_p = 63%, A_p = 1.90, AP_p = 2.42, H_{ep} = 0.20) levels. A significant deficit of heterozygote individuals was detected within populations in the Paraguana Peninsula region (F_{IS} = 0.301). Relatively low levels of population differentiation were detected at macrogeographic (G_{ST} = 0.112) and regional levels (G_{ST} = 0.044 for peninsula region and G_{ST} = 0.074 for mainland region), suggesting substantial genetic exchange among populations; however, gene flow in this species seems to be regulated by the distance among populations. Overall, estimates of genetic diversity found in *P. guamacho* are concordant with the pattern observed for other cacti surveyed, namely high levels of polymorphism and genetic diversity with one common allele and several rare alleles per locus. Differences in gene dispersal systems between this species and other cacti studied were not reflected in the patterns of genetic diversity observed. The concentration of the highest estimates of genetic variation in northwestern Venezuela suggests a potential reservoir of plant genetic diversity within xerophilous ecosystems in northern South America.

Our knowledge of the levels and spatial distribution of genetic diversity in long-lived woody plants is primarily based on studies of northern temperate taxa, many of them conifers, and Neotropical tree species. According to these studies, long-lived woody plants with broad geographic ranges and predominantly outcrossing breeding systems possess higher levels of allozyme diversity than taxa with other combinations of traits (Hamrick and Godt 1989, 1996b; Hamrick et al. 1992). Long-lived woody species also have less population differentiation, on average, than herbaceous and short-lived woody taxa (Hamrick et al. 1992). Despite their recognized ecological importance in arid environments, xerophilous woody species are not well represented in the plant genetic diversity literature (Cortés and Hunziker 1997; Hamrick et al. 2002; Keys and Smith 1994; Lia et al. 1999; Martínez-Palacios et al. 1999).

The Cactaceae are among the flowering plants that dominate the vegetation of arid and semiarid zones of the New World. Although about 1600 species of cacti are recognized (Gibson and Nobel 1990), fewer than 15 taxa have been surveyed for allozyme diversity (Fleming et al. 1998; Hamrick et al. 2002; Nassar 1999; Neel et al. 1996; Parker and Hamrick 1992; Sahley 1996). Furthermore, the available data are biased toward columnar cacti (subfamily Cactoideae), while members of subfamilies Pereskioideae and Opuntioideae have been rarely examined (Hamrick and Godt 1997; Sternberg et al. 1977). Overall, the few cacti studied have relatively high levels of allozyme diversity. Cacti pollinated and dispersed by bats have relatively low levels of population differentiation, in agreement with the vagile nature of their pollen and seed vectors. On the other hand, we know almost nothing about the genetic diversity of cacti with other life forms and gene dispersal systems.

Among cacti, the genus *Pereskia* represents the group that most closely resembles the characteristics of long-lived woody species in other plant families, including multiple branched woody stems that bear true leaves. These and other anatomical, morphological, and physiological traits found in several species of *Pereskia* are considered to be primitive for cacti (Gibson and Nobel 1990; Leuenberger 1986). This genus is widely distributed

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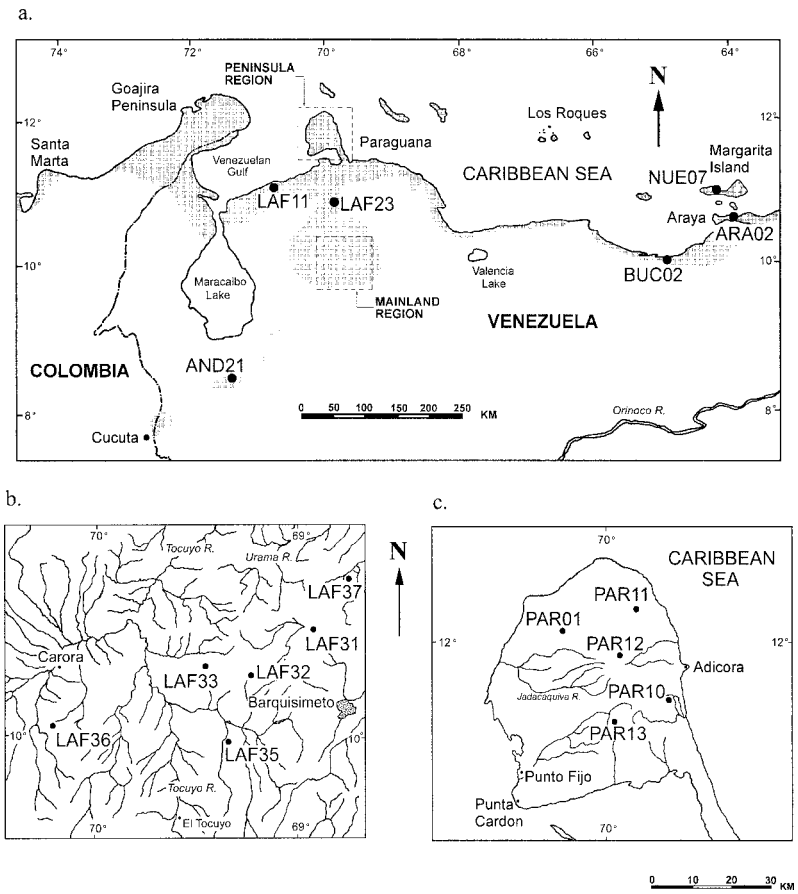

Figure 1. Maps of **(a)** northwestern and central Venezuela, **(b)** mainland region, and **(c)** peninsula region showing locations where populations of *P. guamacho* were sampled. Shaded areas correspond to arid and semiarid lands in Venezuela (Sarmiento 1976).

in the western hemisphere, from Mexico to northern Argentina (Leuenberger 1986). Although considerable variation exists in plant and floral morphology across species, many reproductive and ecological traits are held in common, including a deciduous condition, self-incompatibility, insect pollination, seasonal flowering and fruiting, and animal-mediated seed dispersal. In Venezuela, *Pereskia* is represented by two shrub- and treelike taxa, *P. aculeata* and *P. guamacho.* The later is frequently observed in arid and semiarid habitats, although it is also present in less xeric environments, including dry forests (Leuenberger 1986; Ponce 1989).

The evolutionary history of *P. guamacho* has been characterized by a cyclic process of habitat expansion and contraction. During the late Tertiary and Quaternary periods, the climate of northern South America was characterized by expansion of arid landscapes corresponding to glacial periods and their contraction during interglacial intervals (Gentry 1982; Ochsenius 1983; Raven and Axelrod 1975; Schubert 1988). In northern Venezuela, the narrow arid and semiarid territory extending from the Guajira Peninsula (northeast Colombia) to the Paria Peninsula (northwest Venezuela), also known as the "peri-Caribbean arid belt," has maintained its xeric condition at least since the last glacial maximum (13,000–18,000 years BP) (Ochsenius 1983; Schubert 1988). This region represents the remnant of a potentially broader expanse of arid lands inhabited by *P. guamacho* and other xerophytic vegetation during past glacial episodes. The distribution of genetic diversity within the arid belt should be influenced by the spatial availability of suitable habitats and the connectedness among them, since these factors determine the size and temporal stability of the populations (Barrett and Kohn 1991; Ellstrand and Elam 1993; Hamrick and Godt 1989, 1996b). According to this, the highest levels of genetic diversity for *P. guamacho* should occur in the northwestern portion of the arid belt (Falcón and Lara states), where the largest extensions of continuous arid lands are located. At least three other cactus species in Venezuela have been found to concentrate the highest levels of genetic variation in that region (Nassar 1999).

This study examined levels and patterns of allozyme diversity in 17 populations of *P. guamacho* over a spatial scale comprising most of its natural distribution within arid environments in Venezuela. Based on its woody nature and obligate outcrossing condition, this species should have relatively high levels of genetic diversity within its populations and low genetic structure among populations. On the other hand, insect pollination might restrict pollen-mediated gene flow (Levin 1981; Levin and Kerster 1974), producing more genetic differentiation among populations than is found among chiropterophilous cacti with similar mating systems (Hamrick et al. 2002; Nassar 1999). If gene flow between populations is spatially restricted by the limited capability of gene vectors to move pollen and seeds, we should find an association between genetic differentiation and geographic distances between populations (isolation by distance). Estimates of genetic diversity were compared with values obtained from other cactus species previously studied in the same areas, but differing from *P. guamacho* in several life-history traits, including life form and potential for gene dispersal. Particular attention was given to the spatial distribution of genetic variation, which could support the existence of a reservoir of genetic diversity for xerophytic vegetation in northwestern Venezuela. Specific objectives of this study were (1) to estimate levels of genetic diversity at the species, regional, and population levels; (2) to examine patterns of genetic diversity within and among populations; and (3) to test if the observed pattern of population differentiation is indicative of isolation by distance.

Materials and Methods

Study System

Pereskia guamacho F. A. C. Weber is distributed in northeastern Colombia, Bonaire Island, and Venezuela (Leuenberger 1986). It is widely distributed in northern and central Venezuela, reaching its southern limits on the frontier with Colombia to the west and in the mid-Orinoco river (7°50′ N) to the east (Ponce 1989) (Figure 1a). Although it is fairly common in arid and semiarid zones, *P. guamacho* is also found in xerophilous Andean patches, deciduous and semideciduous forests, gallery forests, and disturbed areas, mostly below 1000 m of elevation.

Table 1. Sampling locations of *P. guamacho*[a]

Code	Locality	State	Latitude N	Longitude W	Altitude (m)	Individuals/ ha
AND21	Caparú	Mérida	08°29′31″	71°19′49″	800–850	40.0
ARA02	Via Chacopata	Sucre	10°39′22″	63°46′12″	0–20	50.0
BUC02	Basílica San José	Anzoátegui	10°03′26″	65°01′30″	50	—
LAF11	Sta. Rosa	Falcón	11°07′36″	69°32′02″	60	75.0
LAF23	María Díaz	Falcón	10°53′06″	69°41′26″	250	25.0
LAF31	Mesa de Urigua	Lara	10°13′46″	69°27′30″	700–750	162.5
LAF32	Banco de Baragua	Lara	10°07′49″	69°35′03″	750–800	22.5
LAF33	Turturia	Lara	10°08′06″	69°43′02″	600–650	42.5
LAF35	Guadalupe	Lara	10°02′00″	69°40′30″	550–600	—
LAF36	Los Arangues	Lara	10°01′36″	70°03′06″	550–600	85.0
LAF37	Potrero de Ramiro	Lara	10°21′14″	69°27′06″	700–750	190.0
NUE07	Comején	Nueva Esparta	11°13′12″	64°13′30″	20	57.5
PAR01	San José de Acaboa	Falcón	12°00′56″	70°06′36″	60	30.0
PAR10	Salina del Diablo	Falcón	11°52′30″	69°49′49″	0–20	85.0
PAR11	El Vínculo	Falcón	12°05′12″	69°55′30″	20	70.0
PAR12	Balsamar	Falcón	11°59′15″	69°54′27″	80	62.5
PAR13	Santa Ana	Falcón	11°47′00″	69°58′33″	100	22.5

[a] Populations encoded LAF3 correspond to the mainland region and populations encoded PAR correspond to the peninsula region.

P. guamacho ($2n = 22$; Leuenberger 1986), locally known as "guamacho" or "supi," is a treelike cactus, 3–8 m tall, with small semisucculent leaves and well-developed spines. Flowers are solitary or fascicled, hermaphroditic, bright yellow, diurnal, and last less than 1 day. This species is the only one in the genus that flowers in a leafless condition at the end of the dry season (March–May) (Leuenberger 1986; Nassar JM, unpublished data). Hundreds to thousands of flowers per individual undergo anthesis in a relatively short period (less than 1 month) between the late dry season and the beginning of the rains (April–May). This species is self-incompatible and pollination is performed by medium-size and large bees and butterflies (Leuenberger 1986; Ramírez N, personal communication). Fruit maturation is slow and occurs during the rainy season (July–October). Presumably seed dispersal is mainly by terrestrial mammals. Besides sexual reproduction, this species has been successfully reproduced from stalks in plant nurseries, but its clonal capabilities under natural conditions are unknown. Individuals can be solitary or may form loose patches of many trees on flatlands or on irregular ground.

Study Sites

This study only considered populations located in the arid and semiarid lands of Venezuela (Figure 1a). Included were most of the coastline, one island, an extensive zone in northwestern Venezuela, and one xeric patch in the Andes. Study site names, geographic coordinates, altitude, and population density information are summarized in Table 1. Two spatial scales were considered for the genetic diversity survey: a macrogeographic level, including populations throughout Venezuela (Figure 1a), and a regional level, considering only populations within two geographically restricted areas, the mainland region (Figure 1b) and the peninsula region (Figure 1c). The mainland region (9°49′–10°19′ N and 69°28′–70°06′ W) is located in a low-elevation mountainous area (500–1000 m) in the largest extension of continuous arid lands in the country. The peninsula region (11°56′–11°58′ N and 69°56′–70°01′ W) encompasses the Paraguana Peninsula, on the northwestern coast. The two regional subsets are each contained in a 56 km × 70 km area, approximately.

Sampling Procedures

Seventeen populations were selected at the macrogeographic level. Six and five populations were chosen from the mainland and peninsula regions, respectively. Forty-eight individuals separated by a minimum of 10 m were selected from each locality. A few well-developed leaves from young twigs were clipped from each plant, wrapped in aluminum foil, transferred into liquid nitrogen, and kept frozen in an ultracold freezer at −70°C until analysis. A total of 816 samples were used in the study.

Electrophoresis Procedures

Leaf samples were cut into small pieces and ground with a pinch of sand, liquid nitrogen, and a cold mortar and pestle. A PVP-phosphate extraction buffer (Wendel and Parks 1982) was added to solubilize and stabilize enzymes. The crude protein extract was soaked into chromatography paper wicks (Whatman 3 MM, Maidstone, UK), placed into microtest plates, and stored at −70°C until analysis. Horizontal electrophoresis was conducted on 10% potato starch gels (Sigma, St. Louis, MO). Combinations of 5 buffer systems and 11 enzyme systems were used to resolve 19 putative loci: buffer 4, isocitrate dehydrogenase (*Idh-1*), shikimate dehydrogenase (*Skdh-1*), and 6-phosphate dehydrogenase (*6-Pgdh-1* and *6-Pgdh-2*); buffer 7, diaphorase (*Dia-1*); buffer 8, triose-phosphate isomerase (*Tpi-1, Tpi-2,* and *Tpi-3*); buffer 11, malate dehydrogenase (*Mdh-1, Mdh-2,* and *Mdh-3*), phosphoglucose isomerase (*Pgi-1* and *Pgi-2*), and uridine diphosphoglucose pyrophosphorylase (*Ugpp-1* and *Ugpp-2*); and buffer 34, superoxide dismutase (*Sod-1*), colorimetric esterase (*Ce-1*), and phosphoglucomutase (*Pgm-1* and *Pgm-2*). Buffer recipes and stains are modified from Soltis et al. (1983) with the exception of buffer 34 (Mitton et al. 1979) and enzyme system *Dia* (Cheliak and Pitel 1984). Loci and alleles were designated by relative protein mobility, with lower numbers assigned to those farther from the origin.

Data Analyses

Allele frequencies and standard genetic diversity parameters following Hedrick (1985) and Berg and Hamrick (1997) were estimated at the species (subscript "s"), regional (subscript "r"), and population (subscript "p") levels. For this purpose we used LYNSPROG, written by M. D. Loveless (College of Wooster, Wooster, Ohio) and A. F. Schnabel (University of Indiana, South Bend, Indiana). The following estimates were obtained: proportion of polymorphic loci (P), mean number of alleles per locus (A), and per polymorphic locus (AP), effective number of alleles per locus ($A_e = 1/\Sigma p_i^2$, where p_i is the frequency of the ith allele), observed heterozygosity (H_o), and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$). These estimates were calculated for each locus and averaged over all loci. Population level estimates of these parameters were averaged over all populations to obtain means and standard errors. Departures from Hardy–Weinberg expectations were evaluated for each polymorphic locus in each population by calculating Wright's (1931) fixation index ($F = 1 - [H_o/H_e]$). Significant deviations of F from zero were tested using a chi-squared test formulated in terms of the fixation index F (Li and Horvitz 1953). The percentage of alleles found per population relative to the total number of alleles scored for the species (%Al) was also calculated.

Measures of spatial partitioning of ge-

netic variation were performed at two spatial scales, macrogeographic and regional. In the former, we included populations over the complete Venezuelan range, with only one population randomly chosen from the mainland and peninsula regions. This procedure better balances the spatial dimensions for analyses of genetic structure. At the regional level, populations within the mainland and peninsula areas were considered separately. Nei's parameters of genetic diversity were estimated for each polymorphic locus (Nei 1973, 1977), including total genetic diversity (H_T), mean genetic diversity within populations (H_S), and the proportion of genetic diversity due to gene frequency differences among populations [$G_{ST} = (H_T - H_S)/H_T$]. Differences in allele frequencies among populations were examined for polymorphic loci using a heterogeneity chi-squared analysis, $\chi^2 = 2NG_{ST}(a - 1)$, with $(a - 1)(n - 1)$ degrees of freedom, where a = number of alleles, n = number of populations, and N = total individuals (Workman and Niswander 1970). Wright's (1978) within-population inbreeding coefficient (F_{IS}) was also estimated for each locus. Significant differences from zero were tested using a chi-squared formulated in terms of the fixation index F (Li and Horvitz 1953). Overall means and standard errors for all estimates were obtained by averaging over all polymorphic loci. In addition, standard errors were obtained by jackknifing over loci (Weir 1996). Chi-squared values and degrees of freedom were summed over all polymorphic loci to conduct overall tests for the mean multilocus estimates of G_{ST} and F_{IS} (Berg and Hamrick 1997). We performed jackknifing procedures across populations to calculate the variance and standard error of G_{ST} estimates for *P. guamacho* (insect pollinated) and compared the mean G_{ST} against equivalent estimates for three Venezuelan bat-pollinated columnar cacti (*Stenocereus griseus, Cereus repandus,* and *Pilosocereus lanuginosus*; Nassar 1999) using a two-tailed t test.

Isolation by distance was tested using Rousset's method (1997), based on the computation of a linear regression of pairwise $F_{ST}/(1 - F_{ST})$ estimates to the natural logarithm of geographic distances between pairs of populations. A positive correlation between the two variables is indicative of isolation by distance. A Mantel test of association (9000 permutations) between pairwise $F_{ST}/(1 - F_{ST})$ and $\log_{10}$ of geographic distance matrices was used to test for significance of the isolation by distance pattern (Heywood 1991). Nei's (1972) genetic identities (I) and distances (D) were estimated between all pairs of populations to generate average clusterings using the neighbor-joining method (Romesburg 1984). Dendrograms plotted using this procedure help to visualize the genetic relationship among populations. Mantel tests and the neighbor-joining clustering were performed using the program NTSYS-pc version 1.8 (Rohlf 1993).

Table 2. Summary of genetic diversity estimates at the species, region, and population level for *P. guamacho* based on 19 allozyme loci

Level	N	P	A	AP	A_e	%Al	H_o	H_e
Species	816	89.5	3.26	3.53	1.45	62[a]	—	0.239
Region								
Mainland								
Pooled	288	79.0	2.68	3.13	1.53	83.0	—	0.257
Population mean		63.7	1.99	2.55	1.46	—	0.205	0.236
SE		2.4	0.05	0.05	0.02	—	0.010	0.007
Peninsula								
Pooled	240	79.0	2.47	2.87	1.32	74.7		0.200
Population mean		67.4	1.85	2.26	1.31	—	0.146	0.190
SE		3.9	0.08	0.08	0.02	—	0.016	0.011
Population								
AND21	48	47.4	1.63	2.33	1.35	50.0	0.162	0.185
ARA02	48	47.4	1.68	2.44	1.22	51.6	0.114	0.127
BUC02	48	52.6	1.63	2.20	1.29	50.0	0.146	0.162
LAF11	48	79.0	2.16	2.47	1.40	66.1	0.187	0.225
LAF23	48	73.7	2.26	2.71	1.45	67.7	0.188	0.227
LAF31	48	68.4	2.00	2.46	1.42	61.3	0.196	0.234
LAF32	48	68.4	2.16	2.69	1.49	66.1	0.224	0.237
LAF33	48	66.7	1.94	2.42	1.48	56.5	0.211	0.246
LAF35	48	63.2	2.00	2.58	1.55	61.3	0.228	0.263
LAF36	48	63.6	2.05	2.67	1.40	62.9	0.194	0.221
LAF37	48	52.6	1.79	2.50	1.42	54.8	0.175	0.215
NUE07	48	57.9	1.79	2.36	1.22	54.8	0.128	0.140
PAR01	48	73.7	1.79	2.07	1.38	54.8	0.147	0.223
PAR10	48	57.9	1.79	2.36	1.32	54.8	0.144	0.191
PAR11	48	57.9	1.63	2.09	1.26	50.0	0.118	0.161
PAR12	48	73.7	2.05	2.43	1.27	62.9	0.159	0.174
PAR13	48	73.7	2.00	2.36	1.34	61.3	0.161	0.202
Mean		63.4	1.90	2.42	1.37	58.1	0.170	0.202
SE		2.4	0.05	0.05	0.02	1.46	0.008	0.009

N = sample size, P = proportion of polymorphic loci, A = mean number of alleles per locus, AP = mean number of alleles per polymorphic locus, A_e = mean effective number of alleles per locus, %Al = percentage of total number of alleles in the species, H_o = mean observed heterozygosity, H_e = mean expected heterozygosity, SE = standard error.

[a] Total number of alleles in the species.

Results

Genetic Diversity

Sixty-two alleles at 19 loci were resolved across all populations. At the species level, 90% of the loci were polymorphic (Table 2). The average number of alleles per locus (A_s) was 3.26 and per polymorphic locus (AP_s) was 3.53. The average effective number of alleles per locus (A_{es}) was 1.45. The decrease from A_s to A_{es} reflects the presence of uneven allele frequencies at many of the polymorphic loci. Overall genetic diversity at the species level was relatively high, H_{es} = 0.239. At the regional level, genetic diversity parameters were estimated separately for the mainland and peninsula regions (Table 2). The most marked difference between the two regions was in terms of expected heterozygosity [H_{er}(peninsula) = 0.200 and H_{er}(mainland) = 0.257]. Mean population estimates of genetic diversity in the mainland region were significantly higher than in the peninsula region (Mann–Whitney U test) for A_r ($U = 29$, $P < .01$), AP_r ($U = 29$, $P < .01$), A_{er} ($U = 30$, $P < .01$), and H_{er} ($U = 28$, $P < .01$). At the population level, the average percentage of alleles present in a given population (Al%) was 58.1%, and ranged from 50.0% (AND21, BUC2, and PAR1) to 67.7% (LAF23). Mean within-population genetic diversity estimates obtained across all populations surveyed were $P_p = 63.4 \pm 2.4$, $A_p = 1.90 \pm 0.05$, $AP_p = 2.42 \pm 0.05$, $A_{ep} = 1.37 \pm 0.02$, and $H_{ep} = 0.202 \pm 0.009$. Significant associations were not detected between population densities (Table 1) and P_p (Spearman's rank correlation, $r = -0.28$, ns), A_p ($r = -0.16$, ns), AP_p ($r = 0.13$, ns), A_{ep} ($r = -0.11$, ns), and H_{ep} ($r = -0.14$, ns).

The highest levels of genetic diversity (H_e) for the species were concentrated in

populations located in the northwestern portions of the country (LAF11, LAF23, LAF31, LAF32, LAF33, LAF35, LAF36, and LAF37), with population estimates of 0.215 or more. The mainland region had the highest pooled heterozygosity (0.257). Other populations surveyed had H_e values generally below 0.185, with the exception of the peninsula region, for which there was considerable heterogeneity among populations (H_e = 0.161–0.223). Most of the populations (86%) for which low H_e was detected were substantially separated (more than 15 km) from surrounding conspecific populations.

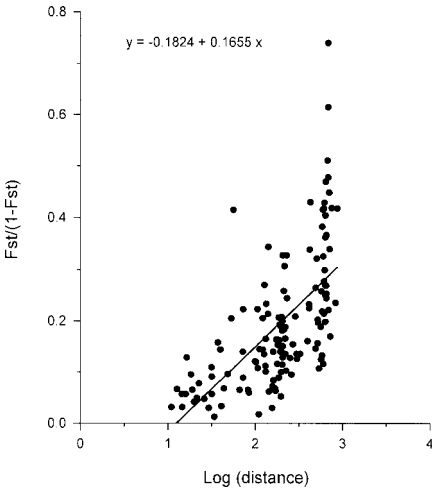

Figure 2. Differentiation among *P. guamacho* populations. Multilocus estimates of pairwise differentiation ($F_{ST}/[1 - F_{ST}]$) are plotted against the natural logarithm of pairwise geographic distances (in kilometers) according to Rousset (1997). R^2 = 0.40.

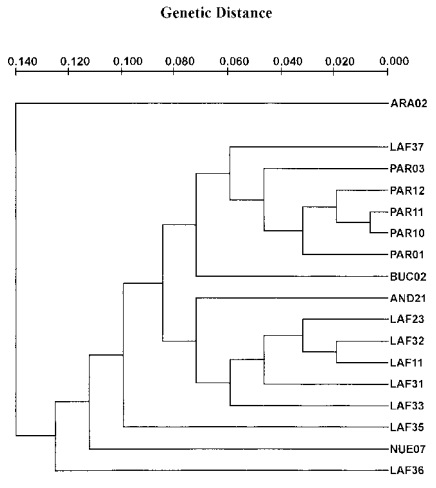

Figure 3. Neighbor-joining cluster based on Nei's (1972) genetic distances (*D*) estimated among 17 populations of *P. guamacho.* Populations were sampled across the Venezuelan range of the species. Population codes follow Table 1.

Genetic Structure

Mean observed heterozygosities (H_{op}) were lower than mean expected values (H_{ep}) for all populations, indicating a moderate heterozygote deficiency within populations (Table 2). The highest heterozygote deficit was detected in the Paraguana Peninsula. Forty-seven of 151 (31%) fixation indices were positive and, with one exception, significantly different from zero ($P < .05$). The mean F_{IS} across loci was significantly different from zero and positive at the macrogeographic level (F_{IS} = 0.180 ± 0.061; χ^2 = 176, $P < .001$) and within the mainland (F_{IS} = 0.116 ± 0.056; χ^2 = 84, $P < .01$) and peninsula regions (F_{IS} = 0.301 ± 0.085; χ^2 = 237, $P < .001$), suggesting that *P. guamacho* populations have some degree of inbreeding, population subdivision, or both.

Overall, most alleles had a wide geographic distribution in Venezuela, but frequencies varied markedly among populations (allele frequency data available upon request from J.M.N.). Significant allele frequency heterogeneity among populations was detected for 16 of 17 (94%) polymorphic loci at the macrogeographic level, 13 of 15 loci (87%) within the mainland region, and 12 of 15 loci (80%) within the peninsula region. At the macrogeographic level, the mean G_{ST} across loci was 0.112 ± 0.054 and varied between 0.013 (*Tpi-4*) and 0.441 (*Ugpp-1*). Since only one population was chosen randomly from each regional subset (mainland and peninsula) for the macrogeographic analysis, these results represent only one outcome of the various possible combinations of populations. To determine the range of variation in G_{ST} estimates, multilocus estimates of G_{ST} were calculated for all possible combinations of populations. The average multilocus G_{ST} obtained across all combinations was 0.118 ± 0.010. The two regional subsets of populations did not differ significantly in multilocus G_{ST} (95% confidence intervals jackknifing across loci); however, the mainland region (G_{ST} = 0.074 ± 0.025) showed comparatively more population subdivision than the peninsula region (G_{ST} = 0.044 ± 0.011). The mean G_{ST} estimate obtained by jackknifing across populations for *P. guamacho* (0.111 ± 0.003) was significantly higher than G_{ST} estimates obtained for *Stenocereus griseus* (0.090 ± 0.004; t = 4.3, $P < .001$, df = 14) and *Pilosocereus lanuginosus* (0.042 ± 0.002; t = 19.2, $P < .0001$, df = 13), but significantly lower than the estimate for *Cereus repandus* (0.124 ± 0.005; t = 2.6, $P < .05$, df = 13).

Isolation by distance was detected at the macrogeographic level when all the populations were included in the linear regression analysis of $F_{ST}/(1 - F_{ST})$ on $\log_{10}$ of geographic distance (Figure 2). The regression coefficient was positive (β = 0.166) and the regression line explained 39.5% of the variation in $F_{ST}/(1 - F_{ST})$. Population pairs with the largest separation distances had considerable variation in the $F_{ST}/(1 - F_{ST})$ values. The association between pairwise $\log_{10}$ geographic distances and pairwise $F_{ST}/(1 - F_{ST})$ values was positive and highly significant (Mantel test, r = 0.63, one-tailed $P < .001$). Tests conducted at the regional level relied on fewer pairwise comparisons than the one conducted at the macrogeographic level. This reduces the statistical power to reject the null hypotheses of no isolation by distance. Despite this limitation, an apparent pattern of isolation by distance was detected in the mainland region (β = 0.142; Mantel test, r = 0.64, one-tailed $P < .018$). The regression line explained 35.6% of the variation in $F_{ST}/(1 - F_{ST})$. In contrast, no isolation by distance was detected in the peninsula region (β = −0.01; Mantel test, r = −0.094, one-tailed P = .4).

Nei's identity (I) values varied between 0.860 and 0.993 (data available upon request), with a mean of 0.942 ± 0.002. The lowest genetic identities were associated with population pairs including population ARA2, located on the extreme east coast of Venezuela. The neighbor-joining dendrogram based on genetic distances between populations (Figure 3) tended to cluster populations from the same geographic regions together. The Andes population (AND21) grouped with inland populations in western Venezuela. All the populations in the Paraguana Peninsula (PAR-) were grouped in a small cluster. The central coast population (BUC02) grouped with the peninsula populations. Population ARA2 (Araya Peninsula), on the eastern coast, had the highest levels of genetic differentiation observed with respect to the populations studied. When ARA2 was removed from the macrogeographic analysis of genetic structure, G_{ST} dropped from 0.112 to 0.095 and the mean I value increased slightly to 0.948.

Discussion

Compared to average values for other plant taxa with a broad variety of life-history and ecological traits (N = 655; P_s = 51.3, AP_s = 1.97, H_{es} = 0.15), *P. guamacho* had more genetic diversity for all the parameters estimated (Hamrick and Godt 1989; Hamrick et al. 1992). The same pat-

tern holds when this species is compared with other long-lived woody plants with similar life-history traits (N = 191; P_s = 65.0, AP_s = 2.22, H_{es} = 0.177; Hamrick et al. 1992). Similar or even higher levels of genetic diversity have been reported for other long-lived desert perennials in the Zygophyllaceae (H_{ep} = 0.17–0.29; Cortés and Hunziker 1997) and the Agavaceae (H_{ep} = 0.28–0.39; reviewed by Eguiarte et al. 2000). If only polymorphic loci are considered, however, levels of total [H_T (average across loci) = 0.261 ± 0.054] and within-population [H_S (average across loci) = 0.215 ± 0.043] genetic diversity for *P. guamacho* were relatively low for the high values of *P* and *AP* found in the species. This is due to many loci having a single common allele and several low-frequency ones. Within the Cactaceae, levels of genetic variation estimated for *P. guamacho* were concordant with average values (N = 8; P_s = 91.8, AP_s = 3.36, H_{es} = 0.207) reported for other species surveyed under similar protocols in North America and Venezuela (Hamrick et al. 2002; Nassar 1999; Nassar et al. 2001; Parker and Hamrick 1992). Among cacti, levels of genetic variation in *P. guamacho* resembled values found in columnar cacti from Venezuela, Mexico, and the southwest United States (P_s = 83.8–100, AP_s = 2.79–3.69, H_{es} = 0.129–0.274; Hamrick et al. 2002; Nassar 1999). These cacti share with *Pereskia* a predominantly animal-outcrossing breeding system, even though one of them is capable of selfing (Fleming et al. 1996; Murawski et al. 1994; Nassar 1999; Nassar et al. 1997).

The highest estimates of genetic diversity observed in *P. guamacho* were concentrated in northwestern Venezuela (Falcón State and the northern part of Lara State). Three other species of Venezuelan cacti, *S. griseus, C. repandus,* and *P. lanuginosus,* also had the highest levels of genetic variation in that region (Nassar 1999). This land forms the largest area of continuous arid and semiarid territory of the "peri-Caribbean arid belt" in northern South America (Ochsenius 1983). The dry paleoclimate of the region has been associated with cyclic expansions and contractions of arid conditions that started in the late Tertiary and continued during the Quaternary periods (Gentry 1982; Ochsenius 1983; Raven and Axelrod 1975; Rull 1996; Schubert 1988). The last of these events occurred during the Wisconsin glaciation, which reached its maximum between 13,000 and 18,000 years BP (Ochsenius 1983; Rull 1996; Schubert 1988). A substantial area of that historically arid belt remains arid or semiarid, especially the first 200 km in the north-south axis, starting on the Paraguana Peninsula. All that area was potentially continuously suitable for *P. guamacho* and other xerophytic plant species. Large, continuously distributed habitats should facilitate the establishment of large, temporally stable populations and the maintenance of relatively high levels of genetic variation (Barrett and Kohn 1991; Ellstrand and Elam 1993; Hamrick and Godt 1989, 1996b). Populations located in narrower arid and semiarid areas (ARA2, NUE7, AND21, and BUC2) had consistently lower levels of genetic variation than those found in the northwestern region. This pattern could be explained in part by the effect of genetic drift, provided that the effective size of the populations from the central and west coast are small enough for this factor to operate. The growing evidence indicating the concentration of genetic variation in northwestern Venezuela supports the hypothesis that this territory could be a reservoir of genetic diversity for xerophytic vegetation.

Populations in the mainland region had significantly more genetic variation than populations in the peninsula region. A similar result was obtained in an equivalent regional comparison conducted between populations of the cactus *Melocactus curvispinus* in Venezuela (Nassar et al. 2001). In *Melocactus,* differences between the mainland (H_{er} = 0.157, H_{ep} = 0.111 ± 0.019) and peninsula (H_{er} = 0.093, H_{ep} = 0.080 ± 0.008) areas were not significant; however, taken together, these results suggest that the geographic isolation of the Paraguana Peninsula might have played a role in reducing genetic variation of cactus populations within this region. Historical fluctuations in sea level, associated with glacial and interglacial periods, could have significantly reduced the overall population sizes and led to a loss of genetic variation (particularly low-frequency alleles). The relative isolation of the Paraguana Peninsula from the mainland after the end of the last glaciation (from 13,000 years BP to present; Ochsenius 1983) may have prevented the reintroduction of alleles from the mainland, particularly if the dispersal capabilities of gene vectors (insects and terrestrial mammals) associated with these plants are limited across the peninsula's isthmus and over water.

A moderate deficiency of heterozygotes was detected at both the macrogeographic and regional levels. Biparental inbreeding can be important enough to reduce heterozygosity in relatively small and partially isolated populations (Handel 1983; Heywood 1991; McCauley et al. 1996). Many of the populations sampled in this study consisted of groups of individuals organized in discrete patches surrounded by other types of vegetation, evidencing spatial isolation from other conspecific populations. On the other hand, the mass flowering characteristic of this species and the proximity of individuals within a patch could reduce pollinator movement between plants. Under restricted seed dispersal, mating among near neighbors can be consanguineous, a pattern that can generate population substructuring and a deficit of heterozygotes. No information is available on how extensive seed dispersal in *Pereskia* species is. Presumably, terrestrial mammals are important seed vectors of these plants (Leuenberger BE, personal communication); however, how far they move seeds is an open question. More data on genetic substructuring and pollinator movement are needed to fully identify the causes of the heterozygote deficiency observed in many of these populations.

Significant but low genetic differentiation among populations was observed at both the macrogeographic and regional levels. Approximately 12% of the total genetic diversity (H_T) of the species was found among populations. G_{ST} values among populations within regions decreased substantially relative to that observed at the macrogeographic level, indicating that more historical gene flow occurs within geographically contiguous areas. Even though the mainland (G_{ST} = 0.074) and peninsula (G_{ST} = 0.044) regions occupy similar spatial dimensions, the irregular topography that characterizes the mainland area might reduce gene exchange among populations relative to the peninsula area. In fact, significant isolation by distance was detected within this region, but not within the peninsula. The Paraguana Peninsula is mostly dominated by flatlands that offer no topographic obstacle to gene movement among populations. A similar pattern of population differentiation was observed in *M. curvispinus* (Nassar et al. 2001) in the same localities in Venezuela [F_{ST}(mainland) = 0.324 ± 0.022, F_{ST}(peninsula) = 0.150 ± 0.021].

Compared to other plant taxa, *P. guamacho* had somewhat more genetic structure than 195 long-lived woody species (G_{ST} = 0.084), 37 animal-pollinated woody species (G_{ST} = 0.099), and 14 woody taxa

with animal-ingested seed dispersal (G_{ST} = 0.051) (Hamrick et al. 1992). Among cacti, *P. guamacho* had levels of population genetic differentiation intermediate to those reported for other species (N = 8, mean G_{ST} = 0.121, range 0.043–0.242) (Hamrick et al. 2002; Nassar 1999). Two out of three bat-pollinated columnar cacti from Venezuela had significantly less genetic differentiation among populations than *P. guamacho* (Nassar 1999); but this evidence is still insufficient to conclude that insect-pollinated cacti have more population structure than species pollinated and dispersed by vertebrates. Our overall test for isolation by distance (Rousset 1997) indicated that genetic differentiation between populations increases with geographic distance. Based on the widespread distribution of this species in northern and central Venezuela, we hypothesize that the movement of genes across a two-dimensional landscape best explains the observed population structure.

Several factors might explain the relatively low levels of population structure observed in *P. guamacho.* First, like the columnar cacti, *P. guamacho* is a predominantly outcrossing, animal-pollinated species. This mating system promotes the transfer of genes between plants and subpopulations and it has been associated with relatively low levels of population structure (Hamrick and Godt 1989; Hamrick et al. 1992). Second, seed dispersal in this species could be more extensive than is assumed. Fruits are palatable, and once ripe, many fall and accumulate on the ground, becoming available to numerous small mammals (rodents, skunks, foxes, etc.), goats, and cattle. To determine how extensive seed dispersal is, we need to identify the dispersal agents and describe their foraging patterns. Finally, among Venezuelan cacti, *P. guamacho* has one of the broadest geographic ranges. Besides being common in arid and semiarid zones, it is also present in dry forests and other habitat types. It has been shown that woody species with relatively broad, continuous distributions have less genetic variation among their populations than congeners with more restricted distributions (Hamrick and Godt 1996ab; Hamrick et al. 1992). Thus even though pollen- and seed-mediated gene flow in *P. guamacho* could be more restricted than in columnar cacti, its more continuous geographic distribution could lead to greater overall gene flow and a reduction in population differentiation.

Levels of genetic identity (average I = 0.942) estimated between population pairs of *P. guamacho* in Venezuela are within the range reported for conspecific populations of plants (Crawford 1989; Gottlieb 1977). The neighbor-joining tree based on Nei's genetic distances (D) between populations partially reflects the geographic relationships between them. With the exception of populations LAF37, LAF35, and LAF36, the dendrogram separated inland from coastal populations. One interpretation of the clustering of the coastal populations is a historically broad connection among them and substantial gene flow between central and western populations during the last glaciation period, when the Venezuelan paleoclimate was drier and arid lands were more extensive than at present. Contemporary coastal populations of *P. guamacho,* in contrast, may be experiencing a loss of genetic diversity due to decreases in population size and fragmentation occasioned in part by the history of human activity in this region. Historically, human concentrations and development in Venezuela have been concentrated in the northern portion of the country, with the coast being one of the most disturbed landscapes (PDVSA 1992). Population ARA2, on the east coast, was clearly segregated from the remaining coastal populations. This site is relatively isolated on the Araya Peninsula and had the lowest genetic diversity (H_{ep} = 0.127) of all populations examined. Under limited gene flow and small population size, the reduction of genetic variation by genetic drift could be intensified (Barrett and Kohn 1991; Ellstrand and Elam 1993; Hamrick and Godt 1989; Lande 1988).

Overall our results support the view that cacti as a group maintain high levels of genetic diversity. *P. guamacho* had levels and patterns of partitioning of genetic diversity similar to those found previously in columnar cactus species. This species had the highest levels of gene diversity in the northwestern part of its geographic distribution, as was the case for three other Venezuelan cactus species. This is a relevant finding from a conservation and land management perspective, since it identifies a potential reservoir of plant genetic diversity within xerophilous ecosystems in northern South America.

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