American Journal of Botany 90(11): 1628–1637. 2003.

POPULATION GENETIC STRUCTURE OF VENEZUELAN CHIROPTEROPHILOUS COLUMNAR CACTI (CACTACEAE)[1]

JAFET M. NASSAR,[2,3,5] J. L. HAMRICK,[4] AND THEODORE H. FLEMING[3]

[2]Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Apdo. Postal 21827, Caracas 1020-A, Venezuela;
[3]Biology Department, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124 USA; and
[4]Departments of Botany and Genetics, University of Georgia,
2502 Plant Sciences, Athens, Georgia 30602 USA

We conducted allozyme surveys of three Venezuelan self-incompatible chiropterophilous columnar cacti: two diploid species, *Stenocereus griseus* and *Cereus repandus*, and one tetraploid, *Pilosocereus lanuginosus*. The three cacti are pollinated by bats, and both bats and birds disperse seeds. Population sampling comprised two spatial scales: all Venezuelan arid zones (macrogeographic) and two arid regions in northwestern Venezuela (regional). Ten to 15 populations and 17–23 loci were analyzed per species. Estimates of genetic diversity were compared with those of other allozyme surveys in the Cactaceae to examine how bat-mediated gene dispersal affects the population genetic attributes of the three cacti. Genetic diversity was high for both diploid (P_s = 94.1–100, P_p = 56.7–72.3, H_s = 0.182–0.242, H_p = 0.161–0.205) and tetraploid (P_s = 93.1, P_p = 76.1, H_s = 0.274, H_p = 0.253) species. Within-population heterozygote deficit was detected in the three cacti at macrogeographic (F_{IS} = 0.145–0.182) and regional (F_{IS} = 0.057–0.174) levels. Low genetic differentiation was detected at both macrogeographic (G_{ST} = 0.043–0.126) and regional (G_{ST} = 0.009–0.061) levels for the three species, suggesting substantial gene flow among populations. Gene exchange among populations seems to be regulated by distance among populations. Our results support the hypothesis that bat-mediated gene dispersal confers high levels of genetic exchange among populations of the three columnar cacti, a process that enhances levels of genetic diversity within their populations.

Key words: allozymes; bats; Cactaceae; *Cereus*; columnar cacti; *Pilosocereus*; population structure; *Stenocereus*; Venezuela.

In recent years, a growing interest in the evolution, ecology, and conservation of columnar cacti has generated a substantial volume of information on the reproductive biology of these plants (e.g., Fleming et al., 1996, 2001; Sahley, 1996; Valiente-Banuet et al., 1996; Nassar et al., 1997; Casas et al., 1999). Today we know that many columnar cacti are partially or completely dependent on bats for pollination. But what are the population genetic consequences of being a chiropterophilous cactus? Empirical evidence suggests that pollen-mediated gene flow is often the predominant form of gene flow in outcrossing plants (Ellstrand, 1992; Ennos, 1994; but see Oddou-Muratorio et al., 2001). Gene flow models predict that the level of population subdivision in a species should be constrained by the extent of genetic exchange among populations, an effect that could be accentuated by the existence of long-distance migrants (Wright, 1943; Slatkin and Maruyama, 1975; Slatkin, 1985). Assuming that plant-feeding bats can transport pollen, and probably seeds, far away from the donor plants, we would expect this process to promote low levels of plant population subdivision and substantial within-population genetic variation. But despite the almost intuitive connection between a plant's dispersal capabilities and levels of population differentiation, the importance of gene flow in shaping the genetic structure of plant populations remains controversial (Hamrick, 1982; Loveless and Hamrick, 1984). This is in part due to the lack of control for a broad spectrum of historical, taxonomic, and ecological backgrounds that differentiate sets of plant species that have been the subject of population structure comparisons. In the case of chiropterophilous cacti, we need to examine patterns of genetic variation in species pollinated by bats and to compare observed patterns with those described for other cactus species that share similar life history and ecological traits with bat cacti, but that rely on different gene-dispersal vectors (e.g., bees, butterflies, hawk moths, and birds).

Attributes of genetic diversity of long-lived woody plants adapted to arid conditions have been poorly examined (Keys and Smith, 1994; Cortés and Hunziker, 1997; Lia et al., 1999; Martínez-Palacios et al., 1999; Nassar et al., 2002). Levels and patterns of allozyme variation have been investigated for only five species of columnar cacti, four North American taxa, *Pachycereus schottii* (Engelmann) Hunt, *Carnegiea gigantea* (Engelmann) Britton & Rose, *Stenocereus thurberi* (Engelmann) Buxbaum, and *Pachycereus pringlei* (Watson) Britton & Rose (Hamrick et al., 2002; Nason et al., 2002), and one Peruvian cactus, *Weberbauerocereus weberbaueri* (Schumann ex Vaupel) Backeberg (Sahley, 1996). *Pachycereus pringlei* and *W. weberbaueri* are autotetraploid; the rest are diploid. With the exception of *P. schottii*, a moth-pollinated cactus (Fleming and Holland, 1998), all other species include nectar-feeding bats among their pollination and seed-dispersal agents (Fleming et al., 1996, 2001; Sahley, 1996; Fleming and Nassar, 2002; Sosa and Fleming, 2002). Overall, North American bat cacti maintain high percentages of polymorphic loci (P_S = 83.8–93.3), intermediate to high number of alleles per polymorphic locus (AP_s = 2.79–3.42), and average to high levels of heterozygosity (H_{ES} = 0.129–0.212; Hamrick et al., 2002). Besides this, most of the genetic diversity in these species resides within populations (G_{ST} = 0.075–0.128). These obser-

[1] Manuscript received 11 February 2003; revision accepted 9 May 2003.

This research was funded by NSF Supplementary Grant DEB-9421673 (to T. H. F.), NSF Grant DEB-9420254 (to J. L. H.), a Tropical Biology Fellowship from the University of Miami, and a Cactus and Succulent Society of America Research Grant (to J. M. N.). Andy Tull provided greenhouse assistance at the University of Georgia. PROVITA provided logistic support in the field. Instituto Jardín Botánico de Venezuela provided permits for collection in Venezuela. We are thankful to Janet Castro, Mindy Burke, and Rebecca Pappert for field and laboratory assistance. Kathryn Rodríguez-Clark, Omar Cornejo, and two anonymous reviewers gave valuable input on the final version of the manuscript.

[5] E-mail: jnassar@oikos.ivic.ve.

vations contrast with the relatively high levels of genetic structure reported for *P. schotti* (G_{ST} = 0.431; Nason et al., 2002), the moth-pollinated cactus. Altogether, the genetic structure attributes observed for North American bat cacti are consistent with the foraging behavior characteristic of cactophilous nectar-feeding bats and their potential for long-distance movements (Horner et al., 1998; Fleming and Nassar, 2002). However, because other floral visitors (e.g., doves, hummingbirds, honey bees, solitary bees, and hawk moths) also pollinate the species examined, we cannot attribute the patterns of genetic diversity observed to bats exclusively. Ideally, to make more accurate inferences on the influence of flower-visiting bats on the population genetics of columnar cacti, we would need to focus on species exclusively dependent on these vectors for their sexual reproduction.

The highest levels of bat specialization among cacti have been observed in species restricted to Neotropical regions, including Mexico (Valiente-Banuet et al., 1996, 1997a, b), Central America (Tschapka et al., 1999), the Caribbean (Petit, 1995), and Venezuela (Nassar et al., 1997). In Venezuela, strict bat pollination has been identified in at least five cactus species with similar reproductive characteristics and genetic self-incompatibility. This number represents about 63% of all the Venezuelan columnar cactus species. Three of them, *Stenocereus griseus* (Haworth) Buxbaum, *Cereus repandus* (Linnaeus) Miller, and *Pilosocereus lanuginosus* (Linnaeus) Byles & Rowley, also have similar geographic distributions and habitat affinities across Venezuela and are relatively common species (Ponce, 1989). The southern long-nosed bat, *Leptonycteris curasoae* (Glossophaginae, Phyllostomidae), is an active pollinator and seed disperser of the three species (Nassar et al., 1997). This bat can forage among locations separated by up to 14 km and fly as much as 100 km in a single night (Horner et al., 1998). On the other hand, seed dispersal of the three cacti is shared between bats and birds (Sosa and Soriano, 1993; Soriano et al., 1999); however, bird-mediated gene dispersal of these species is assumed to be spatially restricted based on the home range (<12 ha) assigned to them in Venezuelan arid zones (Bosque, 1984).

The main goal of this study was to examine and compare levels and patterns of allozyme variation in *S. griseus*, *C. repandus*, and *P. lanuginosus* across their geographic ranges in Venezuela. We hypothesized that patterns of genetic variation in Venezuelan chiropterophilous cacti should reflect the long-distance gene-dispersal properties of their obligate floral visitors, namely high levels of genetic variation within populations and relatively low levels of population differentiation. We compared our estimates of genetic diversity and structure, based on seedling samples, with values reported for other cacti surveyed on the same spatial scales but with contrasting gene-dispersal systems.

MATERIALS AND METHODS

Study systems—*Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* are columnar cacti distributed in northern South America and part of the Caribbean (Ponce, 1989; Zappi, 1994). *Stenocereus griseus* and *C. repandus* behave genetically as diploid species, while *P. lanuginosus* showed the allozyme banding segregation pattern of an autotetraploid (this study). Although their geographic distributions do not overlap completely, the three species cover similar spatial ranges, mostly matching the distribution of arid and semiarid lands in Venezuela (Ponce, 1989) (Fig. 1a). *Stenocereus griseus* and *C. repandus* are found below 1000 m, while *P. lanuginosus* can be found as high as 1800 m in the Andes. The three species are multi-branched, 3–6 m tall, and differ in spatial arrangement of branches and some morphological attributes (Nassar et al., 1997). Flowers are hermaphrodite, tubular-campanulate, with white internal tepals, night-blooming, with few flowers in anthesis per individual per night. *Stenocereus griseus* produces flowers during most of the year (9–12 mo). In contrast, *C. repandus* and *P. lanuginosus* produce flowers and fruits seasonally (J. M. Nassar, unpublished data). The three species are self-incompatible based on hand-pollination experiments and nectar-feeding bats are nearly the only effective pollinators of these cacti (Petit, 1995; Nassar et al., 1997). Fruits are multi-seeded (>1000) berries, and dispersal is mainly performed by bats and also several birds (Bosque, 1984; Sosa and Soriano, 1993; Soriano et al., 1999). Although sexual reproduction is the main reproductive mechanism, these cacti can occasionally spread vegetatively when branches fall from adult individuals and develop root systems. In their natural habitats, population densities vary markedly, from 18 to 1315 individuals/ha. The highest densities occur mostly for *S. griseus* and *C. repandus*, which form vegetation units locally known as ''cardonales.''

Study sites—The study was conducted in the arid and semi-arid lands of Venezuela (Fig. 1a). These lands represent most of the Peri-Caribbean Dry Region described by Sarmiento (1976). This region includes most of the coastline, insular areas, and an extensive zone along a north-south axis of continuous xeric formations and small arid patches in western Venezuela, from Paraguaná Peninsula to Ureña, on the frontier with Colombia. The vegetation associated with these regions consists mainly of thorny scrubs, spiny shrubs, and dry forest (Huber and Alarcón, 1988). Sampled localities and geographic information related to study sites are indicated in Fig. 1 and Table 1.

Two spatial scales were considered for genetic diversity analyses: (1) a macrogeographic scale, including populations from throughout Venezuela (Fig. 1a) and (2) a regional scale, which considers populations within two geographically restricted areas, the mainland region (Fig. 1b) and the peninsula region (Fig. 1c). The mainland region (9°49′–10°19′ N and 69°28′–70°06′ W) is located in a low mountainous zone (500–1000 m). The peninsula region (11°56′–11°58′ N and 69°56′–70°01′ W) encompasses the Paraguaná Peninsula, a large extension of flatlands in northwestern Venezuela. Both regions occur within a 56 × 70 km area, approximately.

Sampling procedures—Tissues used for allozyme analyses were obtained from seedlings of the three species. At the macrogeographic scale, 15 populations of *S. griseus*, 14 of *C. repandus*, and 10 of *P. lanuginosus* were selected. For the mainland region, four, five, and two populations were chosen, respectively. For the peninsula region, five, four, and two populations were sampled, respectively. At each site and when population densities allowed, 48 adult individuals of each species were selected. Conspecific plants were at least 10 m apart to be eligible for sampling. Viable seeds from one fruit per individual were collected and stored under dry conditions. Seeds were germinated directly in trays with potting soil and placed in the greenhouse facilities at the University of Georgia, Athens, Georgia, USA. Seedlings were ready for enzyme extraction when they were about 1 cm tall. One seedling was used from each individual sampled from each population, for a total of 717 seedlings of *S. griseus*, 648 of *C. repandus*, and 430 of *P. lanuginosus*.

Electrophoretic procedures—Seedlings were ground using sand, cold mortar, and pestle. Two polyvinylpyrrolidone-phosphate extraction buffers, one for *S. griseus* (Wendel and Parks, 1982) and the other for *C. repandus* and *P. lanuginosus* (Mitton et al., 1979), were added to the tissue to solubilize and stabilize the enzymes. Chromatography paper wicks (Whatman 3 MM, Maidstone, UK) were then soaked with the protein extract, placed into microtest plates, and stored at −70°C until analysis. Horizontal electrophoresis was conducted on 10% potato starch gels (Sigma, St. Louis, Missouri, USA). Combinations of four buffer systems and 15 enzyme systems were used to resolve 18, 17, and 23 putative loci for *S. griseus*, *C. repandus*, and *P. lanuginosus*, respectively. Buffers and enzyme systems included the following: buffer 4, isocitrate dehydrogenase (*Idh*-1), 6-phosphogluconate dehydrogenase (*6-Pgdh*-1, *6-Pgdh*-2, and *6-Pgdh*-3); buffer 8, aspartate aminotransferase (*Aat*-1 and *Aat*-2), alcohol dehydrogenase (*Adh*-1), fluorescent esterase (*Fe*-1

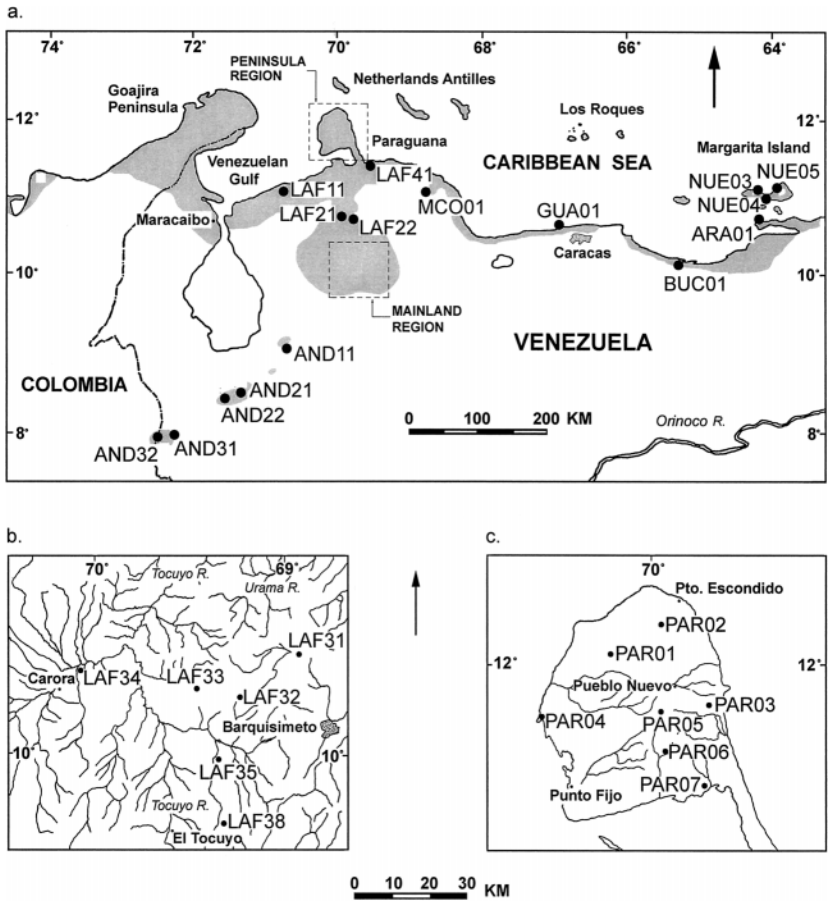

Fig. 1. Maps of Venezuela (a), mainland region (b), and peninsula region (c) showing locations where populations of *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* were sampled. Shaded areas correspond to arid and semiarid lands in Venezuela (Sarmiento, 1976).

and *Fe*-2), glutamate dehydrogenase (*Gdh*-1 and *Gdh*-2), menadione reductase (*Mnr*-1, and *Mnr*-2, and *Mnr*-3), phosphoglucoisomerase (*Pgi*-1 and *Pgi*-2), triosephosphate isomerase (*Tpi*-1, *Tpi*-2, and *Tpi*-3); buffer 11, adenylate kinase (*Ak*-1), malate dehydrogenase (*Mdh*-1, *Mdh*-2, *Mdh*-3, and *Mdh*-4), uridine diphosphoglucose pyrophosphorylase (*Ugpp*-1 and *Ugpp*-2); and buffer 34, diaphorase (*Dia*-1 and *Dia*-2), leucine aminopeptidase (*Lap*-1), and phosphoglucomutase (*Pgm*-1 and *Pgm*-2). Buffer recipes and stains are modified from Soltis et al. (1983) and Mitton et al. (1979), with the exception of recipes for *Aat* and *Dia* (Cheliak and Pitel, 1984). Loci and alleles were designated by relative protein mobility, with lower numbers assigned to those farther from the origin. For the tetraploid species, banding patterns were examined for relative band intensities interpreted as corresponding to genotypes of different allelic dosage.

Data analysis—Allele frequencies and standard genetic diversity parameters following Hedrick (1985) and Berg and Hamrick (1997) were estimated at the species (subscript ''s''), regional (Mainland and Peninsula, subscript ''r''), and population (subscript ''p'') levels for the three species. For this purpose, we used the program LYNSPROG, written by M. D. Loveless (College of Wooster, Wooster, Ohio, USA) and A. F. Schnabel (University of Indiana, South Bend, Indiana, USA). 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 ($A_e = 1/\Sigma f_i^2$, where f_i is the frequency of the *i*th allele), observed heterozygosity (H_o), and Nei's (1973) gene diversity ($H = 1 - \Sigma f_i^2$). Estimates were calculated for each locus and averaged over all loci. Population level estimates 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). In addition, we determined the percentage of alleles found per population relative to the total number of alleles scored for the species (% Al).

Measures of the spatial partitioning of genetic variation were performed at both macrogeographic and regional scale. At macrogeographic scale, we included populations from all over Venezuela. Because populations are close to each other (<50 km) within the peninsula and mainland regions, we randomly chose one population from each of these areas to maintain the macrogeographic range of analysis. We repeated this subsampling method for all possible combinations of populations from the two regions. At the regional level, all the populations within each the mainland and peninsula regions were analyzed. Nei's parameter of population differentiation G_{ST}, which is the proportion of total genetic diversity (H_T) from differences among populations, was estimated for each polymorphic locus (Nei, 1973, 1977). This method to estimate population differentiation is applicable to organisms of any ploidy (Nei, 1973). Differences in allele frequencies among populations were examined for each polymorphic locus using a heterogeneity chi-square analysis, $\chi^2 = 2NG_{ST}(a - 1)$, df = $(a - 1)(n - 1)$; for *a* alleles, *n* populations, *N* = total individuals, and df = degrees of freedom (Workman and Niswander, 1970). Chi-square values and degrees of freedom were summed over all loci to conduct overall tests for the multilocus estimates of G_{ST} (Berg and Hamrick, 1997). Wright's (1978) within-population inbreeding coefficient (F_{IS}) was also estimated for each polymorphic locus and its significance tested by random permutations of genes among individuals within populations using the program SPAGeDi 1.0 (Hardy and Vekemans, 2002), which accepts genotype data of any ploidy level. Overall means and standard errors for G_{ST} and F_{IS}

TABLE 1. Sampling locations in Venezuela of *Stenocereus griseus* (S), *Cereus repandus* (C), and *Pilosocereus lanuginosus* (P).[a]

Code	Locality	Latitude N	Longitude W	Altitude (m)	Species
AND11	Mesa Esnujaque	09°03′10″	70°42′14″	~1745	P
AND21	Caparú	08°29′31″	71°19′49″	800–850	S, C
AND22	Puente Real	08°28′06″	71°24′22″	~400	C
AND31	Michelena	07°57′30″	72°14′22″	~1250	P
AND32	Ureña	07°51′40″	72°26′14″	350–400	S
ARA01	Salina Araya	10°36′36″	64°12′20″	20–40	S
BUC01	Boca de Uchire	10°06′24″	65°22′31″	~50	C
GUA01	La Guaira	10°53′10″	67°02′30″	200–300	S
LAF11	Sta. Rosa	11°07′36″	69°32′02″	~60	S, C
LAF21	Juan Gil	10°53′00″	69°45′26″	200–250	S
LAF22	Maporal	10°50′26″	69°40′00″	700–750	C
LAF31	Mesa de Urigua	10°13′46″	69°27′30″	700–750	S, C
LAF32	Banco de Baragua	10°07′49″	69°35′03″	750–800	S, C
LAF33	Turturia	10°08′06″	69°43′02″	600–650	C
LAF34	Aregue	10°13′18″	70°03′20″	350–400	S, C, P
LAF35	Guadalupe	10°02′00″	69°40′30″	550–600	S, C
LAF38	San José	09°51′10″	69°39′06″	700-750	P
LAF41	Mataruca	11°27′36″	69°32′02″	40–60	P
MCO01	Maicillar	11°12′28″	68°50′04″	80–100	P
NUE03	Comején	11°13′12″	64°13′30″	~20	P
NUE04	La Restinga	10°58′40″	64°09′28″	0–20	C
NUE05	Altagracia	11°07′00″	63°55′00″	60–80	P
PAR01	San José de Acaboa	12°00′56″	70°06′36″	~60	S, C, P
PAR02	El Vínculo	12°05′12″	69°55′30″	~20	S
PAR03	Charaima	11°54′40″	69°51′06″	20–80	S
PAR04	El Pico	11°51′26″	70°16′37″	0–20	S
PAR05	Adaure	11°52′56″	69°58′20″	120–160	S, P
PAR06	Santa Ana	11°47′00″	69°58′33″	~40	C
PAR07	Tacuato	11°42′40″	69°50′16″	0–20	C

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

estimates were obtained by jackknifing over loci (Weir, 1996). We performed jackknifing procedures across populations of each species to calculate variance and standard errors of the G_{ST} estimates and compared mean G_{ST} estimates among the columnar cacti species using a one-way ANOVA.

Isolation by distance was tested using Rousset's (1997) method, 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 (Mantel and Valand, 1970; Heywood, 1991). Nei's (1972) genetic identities (*I*) and distances (*D*) were estimated between all pairs of populations of each species to generate average clusterings using the neighbor-joining method (Romesburg, 1984). Dendrograms plotted using this procedure helped in understanding the genetic relationship among populations. Mantel tests and neighbor-joining clustering were performed using the programs NTSYS-pc version 1.8 (Rohlf, 1993) and Mega version 2.1 (Kumar et al., 1993), respectively.

RESULTS

Genetic diversity—Allozyme banding segregation patterns indicated that *S. griseus* and *C. repandus* are diploids, while *P. lanuginosus* behaves genetically as an autotetraploid. Only locus *Mdh*-2 in *C. repandus* exhibited duplication of bands in all populations. In *P. lanuginosus*, 18 out of 21 polymorphic loci had balanced and unbalanced heterozygote banding patterns, suggesting tetrasomic inheritance. Loci *Ako-1*, *Mnr-2*, and *Tpi-3*, which were monomorphic for most populations, only had a few unbalanced heterozygotes. There was no evidence of fixed heterozygotes normally seen in allotetraploid species. Ten, 11, and 13 enzyme systems were assayed for *C. repandus*, *S. griseus*, and *P. lanuginosus*, respectively, and these systems generated 17, 18, and 23 putative loci, respectively. Estimated allele frequencies for the three species are provided as Supplementary Data accompanying the online version of this paper.

Genetic diversity results at the species, region, and population levels are summarized in Table 2. A total of 60, 63, and 72 alleles were resolved for *C. repandus*, *S. griseus*, and *P. lanuginosus*, respectively. At the species level, 21 (91.3%), 16 (94.1%), and 18 (100.0%) loci were polymorphic for *P. lanuginosus*, *C. repandus*, and *S. griseus*, respectively. The three species had comparable average number of alleles per locus (A_s = 3.3–3.5) and per polymorphic locus (AP_s = 3.5–3.7). The average effective number of alleles per locus (A_{es}) varied slightly among species, from 1.35 in *S. griseus* to 1.47 in *C. repandus*. The marked decay in the number of alleles per locus from *A* to A_{es} is to the substantial proportion of loci (46–67%) with one common allele ($f_i > 0.95$) and several rare alleles for each of the three species. Overall gene diversity was high for the two diploid (H_s = 0.182–0.242) and the tetraploid (H_s = 0.274) species.

At the regional level, estimates of genetic diversity in the peninsula region were higher than in the mainland region for parameters P_r (Mann-Whitney *U* test; $U = 18.3$, $P = 0.04$), A_{er} ($U = 20$, $P = 0.01$) and H_{er} ($U = 20$, $P = 0.01$) in *S. griseus* and parameters P_r ($U = 14$, $P = 0.05$), A_{er} ($U = 14.2$, $P = 0.03$), and H_{er} ($U = 14.2$, $P = 0.03$) in *C. repandus*. For the same parameters estimated in *P. lanuginosus*, the highest values were obtained in the mainland region; however, no significant differences among regions were detected.

Average percentage of alleles (Al%) captured in a given population ranged from 51% for *S. griseus* to 72% for *P. lan-*

TABLE 2. Summary of genetic diversity estimates at the species, region, and population levels for *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* in Venezuela, based on 18, 17, and 23 allozyme loci, respectively.

Level	*N*	*p*	*A*	A_p	A_e	%A1	H_o	*H*
Stenocereus griseus								
Species	717	100.0	3.50	3.50	1.35	63[a]	—	0.182
Region								
Mainland								
Pooled (4 sites)	192	61.1	2.00	2.64	1.29	57.1	0.148	0.162
Population mean		52.8	1.69	2.31	1.28	—	0.144	0.153
SE		2.8	0.06	0.07	0.01	—	0.003	0.003
Peninsula								
Pooled (5 sites)	238	88.9	2.50	2.69	1.36	71.4	0.165	0.185
Population mean		63.3	1.91	2.45	1.35	—	0.163	0.179
SE		2.8	0.04	0.07	0.01	—	0.004	0.004
Population								
15 sites								
Mean	47–48	56.7	1.78	2.36	1.30	51.32	0.145	0.161
SE		2.3	0.05	0.04	0.02	1.43	0.007	0.006
Range		44.4–72.2	1.56–2.11	2.11–2.60	1.19–1.38	42.9–60.3	0.089–0.187	0.110–0.195
Cereus repandus								
Species	648	94.1	3.53	3.69	1.47	60[a]	—	0.242
Region								
Mainland								
Pooled (5 sites)	240	82.4	2.47	2.79	1.34	6.83	0.165	0.176
Population mean		70.6	1.96	2.37	1.32	—	0.161	0.170
SE		1.86	0.04	0.06	0.01	—	0.003	0.003
Peninsula								
Pooled (3 sites)	141	88.2	2.65	2.87	1.50	80.0	0.223	0.270
Population mean		78.4	2.18	2.50	1.46	—	0.217	0.249
SE		2.0	0.09	0.13	0.00	—	0.007	0.008
Population								
14 sites								
Mean	35–48	72.3	2.05	2.44	1.38	58.0	0.179	0.205
SE		2.3	0.06	0.05	0.02	1.75	0.007	0.009
Range		58.8–88.2	1.71–2.53	2.20–2.77	1.30–1.47	48.3–71.7	0.154–0.243	0.164–0.265
Pilosocereus lanuginosus[b]								
Species	431	91.3	3.30	3.52	1.43	72[a]	—	0.274
Region								
Mainland								
Pooled (2 sites)	96	87.0	2.61	2.85	1.44	83.3	0.350	0.278
Population mean		80.4	2.39	2.73	1.44	—	0.349	0.276
SE		2.2	0.09	0.06	0.01	—	0.002	0.003
Peninsula								
Pooled (2 sites)	91	87.0	2.65	2.90	1.42	90.3	0.328	0.264
Population mean		78.3	2.33	2.68	1.41	—	0.328	0.260
SE		8.7	0.24	0.12	0.01	—	0.001	0.009
Population								
10 sites								
Mean	25–48	76.1	2.29	2.69	1.41	72.0	0.320	0.253
SE		2.5	0.06	0.03	0.02	1.98	0.014	0.01
Range		65.2–87.0	2.09–2.61	2.56–2.85	1.34–1.48	65.3–81.9	0.240–0.380	0.191–0.295

Notes: *N* = number of individuals, *p* = proportion of polymorphic loci, *A* = mean number of alleles per locus, A_p = 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* = mean gene diversity, SE = standard error.

[a] Total number of alleles for the species.

[b] Tetraploid species.

uginosus (Table 2). For *P. lanuginosus*, the tetraploid cactus, the proportion of individuals possessing three different alleles at least at one locus was relatively high across populations, with population MOC01 having the highest proportion (85.4%) and Margarita Island and the Andean populations having the lowest proportions (42.6–58.3%). Individuals with four different alleles at a locus were less common (<10.4%) across populations. The lowest estimates of genetic diversity for all the parameters examined at the population level were obtained for *S. griseus*, P_p = 56.7%, A_p = 1.78, AP_p = 2.36,

TABLE 3. Wright's (1978) within-population inbreeding coefficient (F_{IS}) and Nei's (1973) statistic of population differentiation (G_{ST}) estimated for 19, 17, and 21 polymorphic loci in *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* in Venezuela, respectively. Estimates were conducted at macrogeographic and regional scales (mainland and peninsula). For the macrogeographic scale, only one population was considered from the mainland and peninsula regions (see Results: Genetic structure for explanation).

Species	Macrogeographic range			Mainland region			Peninsula region		
	N	F_{IS}	G_{ST}	*N*	F_{IS}	G_{ST}	*N*	F_{IS}	G_{ST}
Stenocereus griseus	8			4			5		
Mean		0.145**	0.092**		0.057	0.044**		0.154**	0.028**
SE		0.048	0.030		0.029	0.018		0.067	0.006
Cereus repandus	7			5			3		
Mean		0.182**	0.126**		0.093*	0.022**		0.128**	0.061**
SE		0.052	0.023		0.056	0.007		0.057	0.022
Pilosocereus lanuginosus[a]	7			2			2		
Mean		0.176**	0.043**		0.171**	0.009*		0.174**	0.011**
SE		0.033	0.007		0.045	0.001		0.044	0.001

Notes: *N* = number of populations, F_{IS} = within population inbreeding coefficient, G_{ST} = proportion of total genetic diversity found among populations. Means and standard errors were calculated by jackknifing across loci.

* $P < 0.05$; ** $P < 0.001$.

[a] Autotetraploid species.

$A_{ep} = 1.30$, $H_o = 0.145$ and $H_p = 0.161$. On the other hand, *P. lanuginosus* had the highest values for all parameters, $P_p = 76.1\%$, $A_p = 2.29$, $AP_p = 2.69$, $A_{ep} = 1.41$, $H_{op} = 0.320$, and $H_p = 0.253$. *Cereus repandus* had intermediate average estimates. Population-level estimates differed significantly among species for P_p (one-way ANOVA; $F = 19.0$, df = 2, $P < 0.001$), A_p ($F = 15.6$, df = 2, $P < 0.001$), AP_p ($F = 13.7$, df = 2, $P < 0.001$), A_{ep} ($F = 12.7$, df = 2, $P < 0.001$), and H_p ($F = 28.5$, df = 2, $P < 0.001$). Overall, genetic diversity estimates varied slightly among populations within species, with the widest ranges of variation occurring in P_p and H_p for all cacti.

From a geographic perspective, and with few exceptions, the three species converged in having the highest levels of genetic diversity for all parameters in populations located in northwestern Venezuela (Fig. 1). For diploid cacti, populations PAR04, PAR05, PAR01, PAR07, LAF11, LAF22, LAF34, and LAF35 had the highest P_p (67.0–88.24%) and H_p (0.182–0.265) values, while most Andean and northeastern populations including AND21, AND22, AND32, GUA01, BUC01, and ARA01 had comparatively lower estimates of P_p (44.0–64.7%) and H_p (0.110–0.180). For the tetraploid cactus, populations PAR05, MOC01, LAF41, LAF38, and LAF34 had the highest estimates of P_p (78.3–87.0%) and H_p (0.271–0.295), while Andean and Margarita Island populations including AND11, AND31, NUE03, and NUE05 had the lowest values of P_p (65.2–69.6%) and H_p (0.191–0.253).

Genetic structure—A substantial proportion of the observed heterozygosities calculated per locus and population were significantly smaller than expected heterozygosities under Hardy-Weinberg equilibrium in the three species, suggesting a moderate deficiency of heterozygotes across species (data available upon request from J. M. N.). For *S. griseus*, 19 of 130 (14.6%) fixation indices were positive and significantly different from zero ($P < 0.05$). For *C. repandus*, 30 of 148 (20.2%) fixation indices were positive and significantly different from zero ($P < 0.05$). For *P. lanuginosus*, 89 of 173 (51.4%) fixation indices were positive and significantly different from zero ($P < 0.05$). Mean estimates of Wright's (1978) within-population inbreeding coefficient (F_{IS}) were positive and low to moderate for the three cacti at both spatial scales (Table 3).

At the macrogeographic level, allele frequency heterogeneity among populations was detected for 89% of the polymorphic loci in *S. griseus*, 88% in *C. repandus*, and 76% in *P. lanuginosus* (data available upon request from J. M. N.). In the mainland region, allele frequency heterogeneity among populations was detected for 63.6% of polymorphic loci in *S. griseus*, 35.7% in *C. repandus*, and 10.5% in *P. lanuginosus*. In the peninsula region, allele frequency heterogeneity among populations was detected for 62.5% of polymorphic loci in *S. griseus*, 80.0% in *C. repandus*, and 41.2% in *P. lanuginosus*. *Stenocereus griseus* had 17 rare alleles restricted to single populations, followed by *C. repandus* with 12, and *P. lanuginosus* with three rare alleles (data available upon request from J. M. N.). At the macrogeographic level, G_{ST} estimates were relatively low for the three cacti (Table 3). *Pilosocereus lanuginosus* had the lowest average G_{ST} (0.043) across loci, followed by *S. griseus* (0.092), and *C. repandus* (0.126). Because only one population was chosen randomly from each regional subset (mainland and peninsula) for the macrogeographic analysis, the reported results are only one outcome of the various possible combinations of populations. To determine the range of variation of macrogeographic G_{ST} estimates, multilocus estimates of G_{ST} were calculated for all possible combinations of populations between the mainland and peninsula regions. The average (± 1 SE) multilocus G_{ST} values obtained from this operation were 0.046 $\pm$0.001 for *P. lanuginosus*, 0.096 $\pm$ 0.001 for *S. griseus*, and 0.115 $\pm$ 0.002 for *C. repandus*. At the regional level, no significant differences in multilocus G_{ST} estimates were found between mainland and peninsula subsets for the three cacti (95% confidence intervals based on jackknifing across loci). Mean G_{ST} estimates obtained by jackknifing across populations for the three columnar cacti differed among species (one-way ANOVA, $F = 128.2$, $P < 0.0001$), with *C. repandus* having the highest mean G_{ST} ($G_{ST} = 0.124 \pm 0.005$), followed by *S. griseus* ($G_{ST} = 0.090 \pm 0.004$) and *P. lanuginosus* ($G_{ST} = 0.042 \pm 0.002$). A hummingbird-pollinated Venezuelan cactus (*Melocactus curvispinus*, $G_{ST} = 0.188 \pm 0.004$; Nassar et al., 2001) showed comparatively more population subdivision than *S. griseus* ($t = 17.3$, $P < 0.0001$, df = 15), *C. repandus* ($t = 10.3$, $P < 0.0001$, df = 14), and *P. lanuginosus* ($t = 28.7$, $P < 0.0001$, df = 14). An insect-pollinated Venezuelan cactus (*Pereskia guamacho*, $G_{ST} = 0.111 \pm 0.003$; Nassar et al., 2002) also showed more pop-

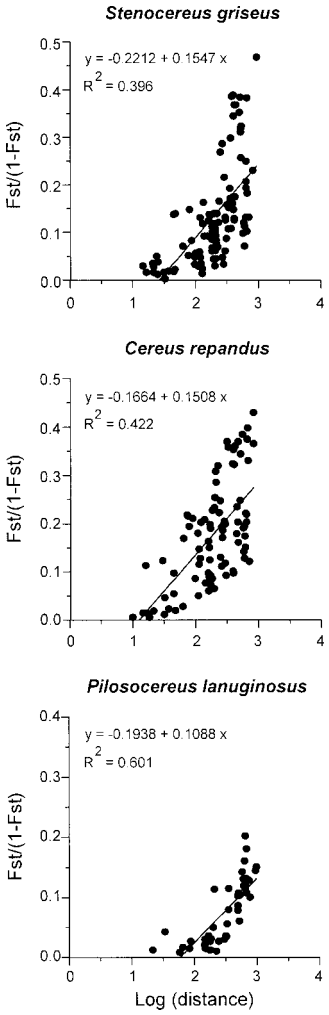

Fig. 2. Differentiation among populations of *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus*. Multilocus estimates of pairwise differentiation ($F_{ST}/(1 - F_{ST})$) are plotted against the natural logarithm of pairwise geographic distances (in kilometers) according to Rousset (1997).

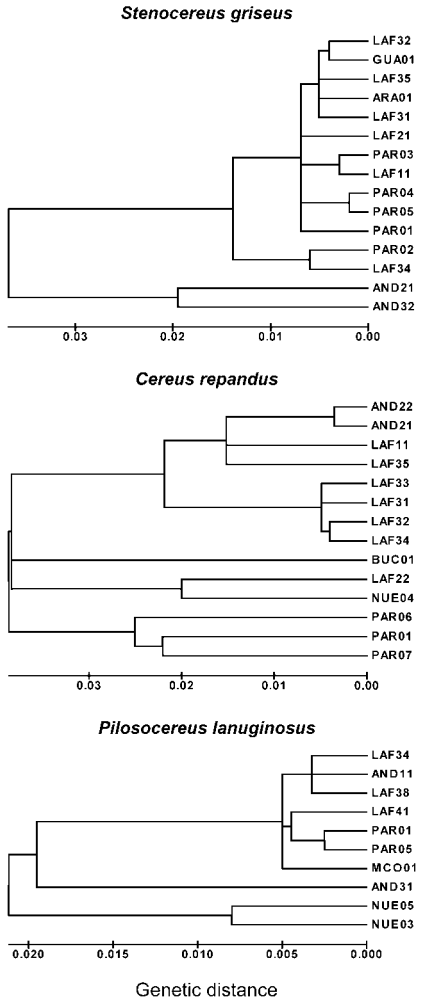

Fig. 3. Neighbor-joining clusters based on Nei's (1972) genetic distances (*D*) estimated among 15, 14, and 10 populations of *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus*, respectively. Populations were sampled across the Venezuelan range of the three species. Population codes follow Table 1.

ulation subdivision than *S. griseus* ($t = 4.3$, $P < 0.001$, df = 14) and *P. lanuginosus* ($t = 19.2$, $P < 0.0001$, df = 13), but lower population structure than *C. repandus* ($t = 2.6$, $P = 0.02$, df = 13).

Isolation by distance was detected at the macrogeographic level for the three columnar cacti (Fig. 2). The association between pairwise $\log_{10}$ geographic distances and pairwise $F_{ST}/(1 - F_{ST})$ values was positive and highly significant for the three species of cactus at the macrogeographic scale (Mantel test; $r = 0.63$, one-tailed $P < 0.001$ for *S. griseus*; $r = 0.65$, one-tailed $P < 0.001$ for *C. repandus*; $r = 0.78$, one-tailed $P < 0.001$ for *P. lanuginosus*). Regression coefficients were positive for the three species ($\beta = 0.155$ for *S. griseus*, $\beta = 0.151$ for *C. repandus*, and $\beta = 0.109$ for *P. lanuginosus*), and regression lines explained 39.6%, 42.2%, and 60.1% of the variation in $F_{ST}/(1 - F_{ST})$, respectively. Population pairs with the largest geographic separations had considerable variation in $F_{ST}/(1 - F_{ST})$ values for *S. griseus* and *C. repandus*. When meaningful to calculate, no significant associations between pairwise $\log_{10}$ geographic distances and pairwise $F_{ST}/(1 - F_{ST})$ values were detected at the regional level ($r = 0.67$, one-tailed $P < 0.21$ for *S. griseus* in the mainland subset; $r = -0.43$, one-tailed $P < 0.1$ for *S. griseus* in the peninsula subset; $r = 0.53$, one-tailed $P < 0.13$ for *C. repandus* in the mainland subset). This was mainly due to the low number of possible pairwise comparisons that could be conducted within regional subsets, thus reducing the statistical power to reject the null hypotheses of no isolation by distance.

Nei's (1972) genetic identities (*I*) were relatively high for all species (mean ± 1 SE; $I = 0.966 \pm 0.002$ for *S. griseus*, $I = 0.940 \pm 0.004$ for *C. repandus*, and $I = 0.978 \pm 0.003$ for *P. lanuginosus*), as expected for conspecific populations (Gottlieb, 1977; Crawford, 1989). The lowest genetic identities were mostly associated with population pairs including Andean populations. Neighbor-joining dendrograms based on Nei's genetic distances between populations partially matched geographic relationships among populations in the three species (Fig. 3). The trees for *P. lanuginosus* and *C. repandus* had the best correspondence between genetic and geographic distances. In *S. griseus*, some groupings were incongruent with geographic relationships, especially between eastern (ARA01, GUA01) and midwestern (LAF31, LAF32, LAF35) popula-

tions. Well-defined geographic units of closely related populations included Andean populations (AND-), midwestern (LAF-) and eastern (NUE-, ARA1, BUC1) regions, the Paraguaná Peninsula cluster (PAR-), and populations in western Venezuela, including the Andes, and the mainland and peninsula subsets. Andean (AND-) and Margarita Island (NUE-) populations had the highest genetic differences with respect to other populations.

DISCUSSION

Genetic diversity of columnar cacti—Our results indicate that *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* have quite high levels of genetic diversity. Compared to flowering plants in general ($N = 655$, $P_s = 51.3\%$, $AP_s = 1.97$, $H_s = 0.15$) and long-lived woody species in particular ($N = 191$, $P_s = 65.0\%$, $AP_s = 2.22$, $H_s = 0.177$), Venezuelan chiropterophilous cacti ranked at the highest levels of genetic diversity (Hamrick et al., 1992). Among long-lived woody plants adapted to xeric environments, the three species had intermediate to high values of gene diversity ($H_p = 0.17$–0.29 in the Zygophyllaceae, Cortés and Hunziker, 1997; $H_p = 0.28$–0.39 in the Agavaceae, Eguiarte et al., 2000). Within the Cactaceae, species- and population-level estimates of genetic variation for Venezuelan columnar cacti were similar to average values obtained from four North American species, *Carnegiea gigantea*, *Stenocereus thurberi*, *Pachycereus pringlei*, and *Lophocereus schotii*, and one Peruvian species, *Weberbauerocereus weberbaueri* ($P_s = 89.8\%$, $P_p = 58.9\%$, $AP_s = 3.09$, $AP_p = 2.45$, $A_{es} = 1.33$, $A_{ep} = 1.27$, $H_s = 0.189$, $H_p = 0.177$; Hamrick et al., 2002). These species share with Venezuelan cacti similar life-history and ecological traits, including long-lived woody condition, columnar form, a predominantly animal-outcrossing breeding system, and self-incompatibility, with the exception of *P. pringlei* and *W. weberbaueri* (Murawski et al., 1994; Fleming et al., 1996; Sahley, 1996; Nassar et al., 1997, 2002). All columnar cacti surveyed up to now form a compact group with respect to their genetic diversity profiles: very high percentage of polymorphic loci, high number of alleles per polymorphic locus, and intermediate to high heterozygosity. Except for the moth-pollinated *P. schotii*, all other species have bats as facultative or obligate gene-dispersal agents. With the exception of *C. gigantea*, all other bat cacti had comparatively higher within-population genetic variation than the moth cactus ($P_p = 49.5\%$, $AP_p = 2.33$, $H_p = 0.144$; Hamrick et al., 2002). Among North American bat cacti, *C. gigantea* is the species with the northernmost distribution and the one that least relies on bats as pollinator agents (Fleming et al., 2001). Altogether, the available data on columnar cacti suggest that bat-mediated gene dispersal enhances genetic variation in this group of plants, probably by facilitating genetic exchange among populations.

The highest values of genetic diversity in the three Venezuelan columnar cacti occurred in populations located in northwestern Venezuela. The same observation was reported by Nassar et al. (2002) for the cactus *Pereskia guamacho*, surveyed over the same geographic range. In general, large and continuously distributed habitats facilitate the maintenance of high levels of genetic diversity in plants (Barrett and Kohn, 1991; Hamrick and Godt, 1989, 1996; Ellstrand and Elam, 1993). The most extensive arid zones in Venezuela are located in northwestern Venezuela (Sarmiento, 1976). This region has remained dry and suitable for xerophilous vegetation at least since the late Tertiary (Ochsenius, 1983), despite a history of cyclic expansions and contractions (Gentry, 1982; Schubert, 1988; Rull, 1996). It is possible that this paleoclimatic condition might have contributed to the existence of stable and genetically diverse populations of cacti in the region. Other less extensive arid zones in Venezuela, like the isolated arid patches in the Andes and the eastern coastline, contain columnar cacti populations with some of the lowest within-population genetic diversity values observed in this study.

Regional comparisons showed that *S. griseus* and *C. repandus* had more genetic diversity in the peninsula subset than in the mainland subset. This result is contrary to expectations, considering that populations on the Paraguaná Peninsula are currently more geographically isolated than mainland populations. However, this was not the situation during the Late Pleistocene (13 000–18 000 yr BP), when the Caribbean Sea was substantially lower and an extensive land bridge connected the Paraguaná Peninsula with continental Venezuela (Ochsenius, 1983). Contemporary gene flow between the Paraguaná Peninsula and adjacent mainland is possible, because nectar-feeding bats that pollinate and disperse columnar cacti in Venezuela and roost in the peninsula have been shown to fly across the isthmus towards the mainland (Martino et al., 1998). It is feasible that during these long-distance flights, bats could transport viable cactus pollen and seeds between the two regions. This does not seem to be the case for the hummingbird-pollinated cactus, *Melocactus curvispinus* (Nassar et al., 2001), and an insect-pollinated cactus, *Pereskia guamacho* (Nassar et al., 2002), surveyed for allozyme diversity in the same Venezuelan locations. Those species had significantly more genetic variation in the mainland region than in the peninsula region, a pattern suggesting comparatively more limited gene-dispersal capabilities than chiropterophilous cacti.

Genetic structure of columnar cacti—Fixation indices and F_{IS} statistics for the three species indicated that there is a slight to moderate deficiency of heterozygous individuals within populations at both macrogeographic and regional scales. It is not clear why. Because these cacti are self-incompatible and are nearly obligate outcrossers, the possibility that inbreeding caused the deviations from H-W equilibrium is low. Biparental inbreeding could contribute to reduced heterozygosity in relatively small and partially isolated populations (Handel, 1983; Heywood, 1991), but estimates of biparental inbreeding conducted on populations of *S. griseus* and *C. repandus* using allozyme markers and following methods by Ritland and Jain (1981) indicated a very low percentage of consanguineous matings ($t_m - t_s = 0.02$, where t_m is the family level multilocus outcrossing rate and t_s is the single-locus outcrossing rate averaged across loci) for the two species (Nassar, 1999).

Significant but low genetic differentiation among populations was detected at both macrogeographic and regional scales. Between 4% and 13% of the total genetic diversity (H_T) of the species was found among populations. Mean G_{ST} estimates for the two regional subsets were somewhat lower (0.009–0.044) than those found at the macrogeographic level, indicating that there is little interpopulation differentiation within arid regions for the three species. *Pilosocereus lanuginosus*, the autopolyploid cactus, had the lowest level of population structure. This pattern is in agreement with the fact that gene flow events in a tetraploid species involve the movement of twice the number of genes transported in a diploid species. Overall, such levels of population structure are substantially lower than mean G_{ST}

values reported for 655 plants across taxa (0.228), but similar to estimates obtained from 195 long-lived woody plants (0.084) (Hamrick et al., 1992). Low population structure in bat cacti is consistent with the flight capabilities of bats in general and the southern long-nosed bat, *Leptonycteris curasoae*, in particular. This nectar-feeding bat pollinates and disperses columnar cacti in North America (Fleming et al., 1996), the Netherlands Antilles (Petit, 1995), and northern South America (Nassar et al., 1997) and can forage among locations separated by up to 14 km and fly as much as 100 km in a single night (Horner et al., 1998). Compared to bats, birds that disperse cactus seeds in Venezuela seem to have a relatively restricted radius of activity based on their reported home ranges (<12 ha) and their resident status (Bosque, 1984). Cactus species with spatially restricted gene-dispersal systems, such as the insect-pollinated *P. guamacho* (G_{ST} = 0.112; Nassar et al., 2002), the hummingbird-pollinated *M. curvispinus* (G_{ST} = 0.189; Nassar et al., 2001), and the moth-pollinated *P. schottii* (F_{ST} = 0.431; Nason et al., 2002), have comparatively more genetic structure than North American bat-pollinated cacti (G_{ST} = 0.075–0.128; Hamrick et al., 2002) and the Venezuelan columnar cacti considered in this study. Overall, bat-mediated gene dispersal within the Cactaceae appears to confer higher levels of gene exchange among populations than other animal-mediated gene-dispersal systems.

For the three Venezuelan columnar cacti, genetic differentiation among populations increased with geographic distance at the macrogeographic scale. This result indicates that isolation by distance determines the mode that genes move across the landscape for the three species. Levels of genetic identity observed for the three cacti ($I > 0.94$) are within the range of values reported for conspecific populations of plants (Gottlieb, 1977; Crawford, 1989). The topology of neighbor-joining trees based on Nei's genetic distances (D) corresponded with important geographic relationships between populations of the three cacti. Populations located in Margarita Island (*C. repandus* and *P. lanuginosus*), the Andean arid patches (*S. griseus*, *C. repandus*), and the Paraguaná Peninsula (*C. repandus*) were grouped together and well separated from the others, reflecting how water and topographic isolation can influence genetic relatedness among populations. Also, populations distributed in the mainland region (*C. repandus*), the central coast (*S. griseus*), and western Venezuela (*C. repandus* and *P. lanuginosus*), tended to be grouped together, reflecting their geographic relationships. Overall, at the level of resolution allowed by allozyme analyses, geographic barriers that separate populations of the three species do not seem important enough to promote speciation in these cacti.

In summary, the results of this research indicate that Venezuelan chiropterophilous cacti, and in general all bat columnar cacti studied to date, form a relatively uniform group in terms of their genetic diversity attributes. Bat-mediated gene dispersal is associated with high levels of genetic diversity and low levels of interpopulation differentiation across cactus species. From a conservation perspective, there are two important elements. First, because most of the total genetic variation detected in Venezuelan columnar cacti resides within populations, the risk of negatively affecting the species' gene pool by localized population extinctions should be relatively low. However, the concentration of high levels of genetic diversity for the three cacti in northwestern Venezuela should be taken into consideration for the delimitation of conservation areas containing plant genetic diversity reservoirs within arid and semiarid zones in northern South America.

LITERATURE CITED

BARRETT, S. C., AND R. KOHN. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. *In* D. A. Falk and K. E. Holsinger [eds.], Genetics and conservation of rare plants, 3–30. Oxford University Press, New York, New York, USA.

BERG, E. E., AND J. L. HAMRICK. 1997. Quantification of genetic diversity at allozyme loci. *Canadian Journal of Forestry Research* 27: 415–424.

BOSQUE, C. A. 1984. Structure and diversity of arid zone bird communities in Venezuela. Ph.D. dissertation, University of Washington, Seattle, Washington, USA.

CASAS, A., A. VALIENTE-BANUET, A. ROJAS-MARTÍNEZ, AND P. DÁVILA. 1999. Reproductive biology and the process of domestication of the columnar cactus *Stenocereus stellatus* in Central Mexico. *American Journal of Botany* 86: 534–542.

CHELIAK, W. M., AND J. A. PITEL. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National Forestry Institute, Information Report P1-X-42. Canadian Forestry Service, Agriculture Canada, Ottawa, Ontario, Canada.

CORTÉS, M. C., AND J. H. HUNZIKER. 1997. Isozymes in *Larrea divaricata* and *Larrea tridentata* (Zygophyllaceae): a study of two amphitropical vicariants and autopolyploidy. *Genetica* 101: 115–124.

CRAWFORD, D. J. 1989. Enzyme electrophoresis and plant systematics. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 146–164. Dioscorides, Portland, Oregon, USA.

EGUIARTE, L. E., V. SOUZA, AND A. SILVA-MONTELLANO. 2000. Evolución de la familia Agavaceae: filogenia, biología reproductiva y genética de poblaciones. *Boletín de la Sociedad Botánica de México* 66: 131–150.

ELLSTRAND, N. C. 1992. Gene flow among seed plant populations. *New Forests* 6: 241–256.

ELLSTRAND, N. C., AND D. R. ELAM. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.

ENNOS, R. A. 1994. Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* 72: 250–259.

FLEMING, T. H., AND J. N. HOLLAND. 1998. The evolution of obligate mutualisms: the senita and senita moth. *Oecologia* 114: 368–378.

FLEMING, T. H., AND J. M. NASSAR. 2002. Population biology of the lesser long-nosed bat *Leptonycteris curasoae*, in Mexico and northern South America. *In* T. H. Fleming and A. Valiente-Banuet [eds.], Columnar cacti and their mutualists: evolution, ecology and conservation, 283–305. University of Arizona Press, Tucson, Arizona, USA.

FLEMING, T. H., C. T. SAHLEY, J. N. HOLLAND, J. D. NASON, AND J. L. HAMRICK. 2001. Sonoran desert columnar cacti and the evolution of generalized pollination systems. *Ecological Monographs* 71: 511–530.

FLEMING, T. H., M. D. TUTTLE, AND M. A. HORNER. 1996. Pollination biology and the relative importance of nocturnal and diurnal pollinators in 3 species of Sonoran Desert columnar cacti. *Southwestern Naturalist* 41: 257–269.

GENTRY, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* 69: 557–593.

GOTTLIEB, L. D. 1977. Electrophoretic evidence and plant systematics. *Annals of the Missouri Botanical Garden* 64: 161–180.

HAMRICK, J. L. 1982. Plant population genetics and evolution. *American Journal of Botany* 69: 1685–1693.

HAMRICK, J. L., AND M. J. W. GODT. 1989. Allozyme diversity in plant species. *In* A. H. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Population genetics, breeding and genetic resources, 43–63. Sinauer, Sunderland, Massachusetts, USA.

HAMRICK, J. L., AND M. J. W. GODT. 1996. Conservation genetics of endemic plant species. *In* J. L. Avise and J. L. Hamrick [eds.], Conservation genetics: case histories from nature, 281–304. Chapman and Hall, New York, New York, USA.

HAMRICK, J. L., M. J. W. GODT, AND S. L. SHERMAN-BROYLES. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.

HAMRICK, J. L., J. D. NASON, T. H. FLEMING, AND J. M. NASSAR. 2002. Genetic diversity in columnar cacti. *In* T. H. Fleming and A. Valiente-Banuet [eds.], Columnar cacti and their mutualists: evolution, ecology and conservation, 122–133. University of Arizona Press, Tucson, Arizona, USA.

HANDEL, S. N. 1983. Pollination ecology, plant population structure, and gene flow. *In* L. Real [ed.], Pollination biology, 163–211. Academic Press, Orlando, Florida, USA.

HARDY, O. J., AND X. VEKEMANS. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618–620.

HEDRICK, P. W. 1985. Genetics of populations. Jones and Bartlett, Boston, Massachusetts, USA.

HEYWOOD, J. S. 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* 22: 335–355.

HORNER, M. A., T. H. FLEMING, AND C. T. SAHLEY. 1998. Foraging behavior and energetics of a nectar-feeding bat *Leptonycteris curasoae* (Chiroptera: Phyllostomidae). *Journal of Zoology* 244: 575–586.

HUBER, O., AND C. ALARCÓN. 1988. Mapa de vegetación. M.A.R.N.R., Caracas, Venezuela.

KEYS, R. N., AND S. E. SMITH. 1994. Mating system parameters and population genetic structure in pioneer populations of *Prosopis velutina* (Leguminosae). *American Journal of Botany* 81: 1013–1020.

KUMAR, S., K. TAMURA, AND M. NEI. 1993. MEGA: molecular evolutionary genetics analysis. Pennsylvania State University, University Park, Pennsylvania, USA.

LI, C. C., AND D. G. HORVITZ. 1953. Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics* 5: 107–117.

LIA, V., C. I. COMAS, M. C. CORTÉS, AND J. H. HUNZIKER. 1999. Isozyme variation in *Larrea ameghinoi* and *Larrea nitida* (Zygophyllaceae): genetic diversity and its bearing on their relationship. *Genetica* 106: 197–207.

LOVELESS, M. D., AND J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

MANTEL, N., AND R. S. VALAND. 1970. A technique of nonparametric multivariate analysis. *Biometrics* 26: 547–558.

MARTÍNEZ-PALACIOS, A. L., L. E. EGUIARTE, AND G. R. FURNIER. 1999. Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan Desert. *American Journal of Botany* 86: 1093–1098.

MARTINO, A., A. ARENDS, AND J. ARANGUREN. 1998. Reproductive pattern of *Leptonycteris curasoae* Miller (Chiroptera: Phyllostomidae) in northern Venezuela. *Mammalia* 62: 69–76.

MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphism detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.

MURAWSKI, D. A., T. H. FLEMING, K. RITLAND, AND J. L. HAMRICK. 1994. Mating system of *Pachycereus pringlei*: an autotetraploid cactus. *Heredity* 72: 86–94.

NASON, J. D., J. L. HAMRICK, AND T. H. FLEMING. 2002. Historical vicariance and post-glacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert cactus. *Evolution* 56: 2214–2226.

NASSAR, J. M. 1999. Comparative population genetic structure of Venezuelan cacti and estimates of their mating systems. Ph.D. dissertation, University of Miami, Coral Gables, Florida, USA.

NASSAR, J. M., J. L. HAMRICK, AND T. H. FLEMING. 2001. Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). *Heredity* 87: 69–79.

NASSAR, J. M., J. L. HAMRICK, AND T. H. FLEMING. 2002. Allozyme diversity and genetic structure of the leafy cactus (*Pereskia guamacho* [Cactaceae]). *Journal of Heredity* 93: 193–200.

NASSAR, J. M., N. RAMÍREZ, AND O. LINARES. 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. *American Journal of Botany* 84: 918–927.

NEI, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.

NEI, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.

OCHSENIUS, C. 1983. Aridity and biogeography in northernmost South America during the Late Pleistocene (Peri-Caribbean Arid Belt, 62°–74°W). *Zentralblatt fär Geologie und Paläontologie, Teil I* (3/4): 264–278.

ODDOU-MURATORIO, S., R. J. PETIT, B. LE GUERROUE, D. GUESNET, AND B. DEMESURE. 2001. Pollen- versus seed-mediated gene flow in a scattered forest tree species. *Evolution* 55: 1123–1135.

PETIT, S. 1995. The pollinators of two species of columnar cacti in Curaçao, Netherlands Antilles. *Biotropica* 27: 538–541.

PONCE, M. 1989. Distribución de las cactáceas en Venezuela y su ámbito mundial (Trabajo Especial de Ascenso a Profesor Agregado). Universidad Central de Venezuela, Facultad de Agronomía, Maracay, Venezuela.

RITLAND, K., AND S. K. JAIN. 1981. A model for estimation of outcrossing rate and gene frequencies based on *n* independent loci. *Heredity* 47: 37–54.

ROHLF, F. J. 1993. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 1.80. Exeter Software, Setauket, New York, USA.

ROMESBURG, H. C. 1984. Cluster analysis for researchers. Lifetime Learning Publications, Belmont, California, USA.

ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.

RULL, V. 1996. Late Pleistocene and Holocene climates of Venezuela. *Quaternary International* 31: 85–94.

SAHLEY, C. T. 1996. Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). *American Journal of Botany* 83: 1329–1336.

SARMIENTO, G. 1976. Evolution of arid vegetation in tropical America. *In* D. W. Goodall [ed.], Evolution of desert biota, 65–100. University of Texas Press, Austin, Texas, USA.

SCHUBERT, C. 1988. Climatic changes during the last glacial maximum in northern South America and the Caribbean: a review. *Interciencia* 13: 128–137.

SLATKIN, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393–430.

SLATKIN, M., AND T. MARUYAMA. 1975. The influence of gene flow on genetic distance. *American Naturalist* 109: 597–601.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27.

SORIANO, P. J., M. E. NARANJO, C. RENGIFO, M. FIGUERA, M. RONDÓN, AND R. L. RUIZ. 1999. Aves consumidoras de frutos de cactáceas columnares del enclave semiárido de Lagunillas, *Mérida, Venezuela*. *Ecotropicos* 12: 91–100.

SOSA, V. J., AND T. H. FLEMING. 2002. Why are columnar cacti associated with nurse plants? *In* T. H. Fleming and A. Valiente-Banuet [eds.], Columnar cacti and their mutualists: evolution, ecology and conservation, 306–323. University of Arizona Press, Tucson, Arizona, USA.

SOSA, M., AND P. J. SORIANO. 1993. Solapamiento de dieta entre *Leptonycteris curasoae* y *Glossophaga longirostris* (Mammalia: Chiroptera). *Revista de Biología Tropical* 41: 529–532.

TSCHAPKA, M., O. VON HELVERSEN, AND W. BARTHLOTT. 1999. Bat pollination of *Weberocereus tunilla*, an epiphytic rain forest cactus with functional flagelliflory. *Plant Biology* 1: 554–559.

VALIENTE-BANUET, A., M. C. ARIZMENDI, A. ROJAS-MARTÍNEZ, AND L. DOMÍNGUEZ-CANSECO. 1996. Ecological relationships between columnar cacti and nectar-feeding bats in México. *Journal of Tropical Ecology* 12: 103–119.

VALIENTE-BANUET, A., A. ROJAS-MARTÍNEZ, M. C. ARIZMENDI, AND P. DÁVILA. 1997a. Pollination biology of two columnar cacti (*Neobuxbaumia mezcalensis* and *Neobuxbaumia macrocephala*) in the Tehuacán Valley, Central Mexico. *American Journal of Botany* 84: 452–455.

VALIENTE-BANUET, A., A. ROJAS-MARTÍNEZ, A. CASAS, M. C. ARIZMENDI, AND P. DÁVILA. 1997b. Pollination biology of two winter-blooming giant columnar cacti in the Tehuacán Valley, Mexico. *Journal of Arid Environments* 37: 331–342.

WEIR, B. S. 1996. Genetic data analysis, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

WENDEL, J. F., AND C. R. PARKS. 1982. Genetic control of isozyme variation in *Camelia japonica* L. *Journal of Heredity* 73: 197–204.

WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* 22: 24–49.

WRIGHT, S. 1931. Evolution in Mendelian populations. *Genetics* 16: 97–159.

WRIGHT, S. 1943. Isolation by distance. *Genetics* 28: 114–138.

WRIGHT, S. 1978. Evolution and genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, Illinois, USA.

ZAPPI, D. C. 1994. *Pilosocereus* (Cactaceae), the genus in Brazil. Succulent plant research, vol. 3. Royal Botanic Gardens, Kew, UK.