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Population Genetics of *Agave cocui*: Evidence for Low Genetic Diversity at the Southern Geographic Limit of Genus *Agave*

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Abstract

The *Agave* genus embraces many species with outstanding ecological and economic importance in the arid regions of the Americas. Even though this genus covers a broad geographic distribution, our knowledge on the population genetics of species is concentrated in taxa located in North America. Recently, it has been demonstrated that plant domestication decreases levels of genetic diversity in managed populations and increases population structure with respect to wild populations. We examined levels of allozyme diversity ($N = 17$ loci) and population structure of *Agave cocui*, the species at the southern limit of distribution of the genus. We sampled 7 wild populations ($N = 30$–35 individuals per population) representative of the geographic distribution of the species in Venezuela. Among the agaves studied, *A. cocui* has some of the lowest estimates of genetic diversity (H_e[species] = 0.059, H_e[population] = 0.054) reported until present. We propose that this condition is probably linked to the recent origin of this species in arid and semiarid regions of Colombia and Venezuela, probably through one or a few founder events. The lowest estimates of genetic diversity were associated with small populations in very restricted arid patches; but also with overexploitation of rosettes for production of fermented drinks and fibers. Santa Cruz de Pecaya, one of the 2 centers of economic use of agaves in northwestern Venezuela presented one of the lowest values of genetic variability, a sign suggesting that human impact represents a significant threat to the available genetic pool that this species possesses in the region.

Key words: *Agavaceae, allozymes, genetic structure, recent origin, Venezuela*

Agaves, together with cacti, have outstanding ecological roles and economic importance in arid and semiarid regions in the Americas. They provide water, food, and roosting sites for a wide variety of organisms associated with these environments (Fleming and Valiente-Banuet 2002; Rocha et al. 2006). Also, they have been used by humans for hundreds of years as sources of food, medicine, fodder for domestic animals, fibers, and production of nonalcoholic and alcoholic beverages (Callen 1965; Gentry 1982; Colunga-García Marín et al. 1986, 1996; Casas et al. 1999; Casas and Barbera 2002; Cházaro and Hernández 2006). As a consequence of these uses, anthropogenic pressures during prehistoric and modern times on numerous agave species have generated impacts of variable magnitude on their demographic traits and population genetics.

In the last decade, an increasing number of studies have centered their interest on the morphological, physiological, and genetic consequences of in situ management of populations and cultivation practices in wild species of plants associated with arid environments in Mesoamerica (for a review, see Casas et al. 2007). In relation to the effects of plant domestication on genetic diversity parameters in cacti and agaves, the available data indicate that domestication practices can generate contrasting results, depending on the species under study and the patterns of manipulation imposed by the users. For cacti, Casas et al. (2006, 2007) found that in situ population management and cultivation practices can decrease levels of allozyme genetic diversity (*Escontria chiotilla*: H_e[wild] = 0.134, H_e[managed in situ] = 0.110; *Polaskia chichipe*: H_e[wild] = 0.505, H_e[managed in situ] = 0.504, H_e[cultivated] = 0.476). On the other hand, in columnar cacti-like *Stenocereus stellatus*, cultivation practices can increase phenotypic and allozyme genetic variation with respect to wild populations (H_e[wild] = 0.253; H_e[managed in situ] = 0.270; H_e[cultivated] = 0.289).

In the case of agaves, most studies indicate that human-manipulated populations have lower genetic diversity than

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wild populations, and genetic structure is higher among anthropogenic populations than among natural ones. Several examples in support of this pattern exist for species studied in Arizona, USA. For instance, anthropogenic populations of 2 species, *A. murpheyi* (H_e[species level] = 0.098 and H_e[population level] = 0.089) and *A. delamateri* (H_e[species level] = 0.067; H_e[population level] = 0.056), presented very low levels of allozyme genetic variability at both species and population level (Parker et al. 2007). For *A. parryi*, Parker et al. (2010) demonstrated that anthropogenic populations consistently had lower levels of genetic diversity than wild populations for both allozymes (H_e[population level—anthropogenic] = 0.079, H_e[population level—wild] = 0.114) and microsatellites (H_e[population level—anthropogenic] = 0.433, H_e[population level—wild] = 0.621). Wild populations of this species were less differentiated (G_{ST} = 0.130) than anthropogenic populations (G_{ST} = 0.292), suggesting that human manipulation can create complex spatial patterns of genetic relatedness among populations that do not reflex the geographic relationship among localities. The only exception to this pattern was found by Vargas-Ponce et al. (2009) in *A. angustifolia*, for which traditional landraces had a genetic diversity estimate (H = 0.442) similar to wild populations (H = 0.428).

Another factor that seems to play a role at explaining levels of genetic diversity in *Agave* is the geographic distribution of the species. For instance, species analyzed in Mexico, that is, the region with the highest richness of both species (~200) and endemics (~150), and hence considered to be the group's center of origin (García-Mendoza 2004a; Scheinvar 2008), have higher allozyme variability (*A. victoria-reginae*: H_e[species level] = 0.334, Martínez-Palacio et al. 1999; *A. lechuguilla*: H_e[species level] = 0.394, Silva-Montellano and Eguiarte 2003) than agaves examined in the northwestern limits of the distributional range of the genus (see species mentioned above). Genetic diversity estimates of Mexican agaves (*A. angustifolia*, *A. celsii*, *A. cerulata*, *A. cupreata*, *A. cupreata*, *A. deserti*, *A. difformis*, *A. garcia-mendozae*, *A. hidalgeuensis*, *A. karwinskii*, *A. potatorum*, *A. striata*, *A. subsimplex*, *A. xylonacantha*, *A. cupreata*, *A. tequilana*) using other genetic markers also show between moderate and high values of genetic variation (ISSR: N = 9, mean H_e[species level] = 0.249 (range: 0.199–0.331), random amplified polymorphic DNA: N = 4, mean H_e[species level] = 0.186 (range: 0.143–0.210); Scheinvar 2008).

A decline of genetic variation in peripheral species of genus *Agave* could be interpreted as indirect evidence in support of the central–marginal hypothesis. This hypothesis explains low levels of genetic diversity in peripheral populations of plants and animals as a consequence of smaller effective population size and greater geographical isolation in comparison with geographically central populations (Lesica and Allendorf 1995; Eckert et al. 2008). Species of *Agave* in the periphery of the distribution of the genus probably originated through founder events, which imply the foundation of new populations with a small number of individuals that became isolated from the source populations, evolving separately from them. Therefore, we should expect to find low levels of genetic variation in species of *Agave* distributed in Central America, the Caribbean, and northern South America when compared with Mexican taxa; however, there is no information available on estimates of genetic diversity and the effects of population management practices on these estimates for agaves in those regions.

Agave cocui (Trelease) occupies the southeastern limits of distribution of the genus in northern South America and nearby islands (García-Mendoza 2002). This species mainly inhabits arid and semiarid areas and rocky outcrops in northern Venezuela and western Colombia. Pre-Hispanic people of at least 3 different ethnic groups, Jiraharas, Ayamanes, and Xaguas, have used *A. cocui* for the last 5000–1000 years (CETIC-Fundacite-Coro 2007). The most outstanding uses given to this plant have been, and continue being, production of fibers and fermentation of an alcoholic beverage known as "cocuy de penca," a spirit similar to those produced in Mexico from agaves. Most of the economic activity around this species has occurred in northwestern Venezuela, where users have extracted specimens for the last 150 years without developing cultivation practices (Savedra et al. 2006). In year 2000, the Venezuelan government legalized the commercialization of cocuy de penca and a few localities in Falcón and Lara states received the Denomination of Origin for production of this spirit. Since then, the cultivation of *A. cocui* has been implemented in those localities at a minor scale, but projects for major development of the cocuy de penca industry are being formulated for both states (CETIC-Fundacite-Coro 2007). Before these development projects are initiated, we need to determine what levels of genetic diversity characterize wild and traditionally exploited populations of *A. cocui* in Venezuela, how much genetic structure is present in these populations, and how levels of genetic diversity compare with agaves in Mexico and United States. The geographic location of this species in the southern limits of the genus suggests that it should be of recent origin, through one or a few founder events, probably derived from progenitor species in Southern Mexico, Central America, or the Caribbean, and therefore, it has had less evolutionary time to build-up genetic diversity compared with many of the older Mexican taxa. Also, in concordance with the patterns observed for anthropogenic populations of other agaves examined until present, we predict that the lowest levels of genetic diversity should be found in localities where *A. cocui* has been under human pressure for extraction of plants.

Materials and Methods

Study Species

Agave cocui is a monocarpic succulent rosette. Leaves are green to grayish, curved, 80–135 cm length and 20–40 cm width, and their edges and tips possess reddish spines. Flowering initiates in September and ends in February (Figueredo 2010). Flowers are yellow hermaphrodite and grouped in umbels (N = 10–44) over lateral peduncles that

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grow along a 4–5 m long central axis (Hoyos 1985; Lemus 2003). Within an umbel, male and female functions are separated in time. Stamens mature one day before the pistil. Like other members of the genus, *A. cocui* is self-incompatible and reproduces both sexually and asexually (Lemus 2003; Infante et al. 2006). Flowers are visited by bees, wasps, butterflies, several birds including hummingbirds, and nectar-feeding bats; however, the latter seem to be the most effective pollinators (Lemus 2003). Fruits are capsules with numerous (147 ± 44.1 standard deviation, Lemus 2003) flattened black seeds dispersed by wind. Vegetative buds called bulbils emerge from the base of unfertilized flowers (Figueredo 2010). In addition, small rosettes can be formed asexually at the base of adult plants.

Study Area and Tissue Sampling

The main arid and semiarid regions and areas with rocky outcrops inhabited by *A. cocui* in Venezuela are represented in this study (Figure 1). A total of 7 populations were sampled, collecting viable seeds from $N = 30–35$ individuals per population (Table 1).

Santa Cruz de Pecaya (SCP) is a locality in Falcón state, where a significant process of extraction of agave rosettes has occurred for many decades and continues occurring at high intensity at the present time. Until year 2006, 17 producers of cocuy de penca around that locality extracted nearly 37 000 plants per year without replacement to cover the local demand of this liquor (Padilla et al. 2007). The remaining populations are located in sites where no exploitation of this species has been reported. Dominant vegetation of most of the sampling sites consisted mainly of thorny scrubs and spiny shrubs (Huber and Alarcón 1988). In the case of rocky outcrops, like La Mona (LM) and La Victoria (LV), the landscape is dominated by grasses and agaves.

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 Instituto Venezolano de Investigaciones Científicas (IVIC), Altos de Pipe, Caracas, Venezuela. Seedlings were ready for enzyme extraction when they were about 1.5 cm tall (±3-month-old seedlings). For each population, we analyzed one seedling per sampled individual plant.

Electrophoresis Procedures

Seedlings were ground using sand, cold mortar, and pestle. A PVP-phosphate extraction buffer was added to the tissue to solubilize and stabilize the enzymes (Wendel and Parks 1982). Chromatography paper wicks (Whatman 3 mm, Maidstone) 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 (Starch Art, Smithville, TX). Combinations of 4 buffer systems and 12 enzyme systems were used to resolve 17 putative loci. Buffers and enzyme systems included the following: buffer 4, isocitrate dehydrogenase (*Idh*-1), malate dehydrogenase (*Mdh*-1 and *Mdh*-2), 6-phosphogluconate dehydrogenase (*6-Pgdh*-1 and *6-Pgdh*-2), phosphoglucoisomerase (*Pgi*-1), phosphoglucomutase (*Pgm*-1 and *Pgm*-2); buffer 8-, aspartate aminotransferase (*Aat*-1 and *Aat*-2), diaphorase (*Dia*-1), menadione reductase (*Mnr*-1 and *Mnr*-2); buffer 11, adenylate kinase (*Ak*-1), uridine diphosphoglucose

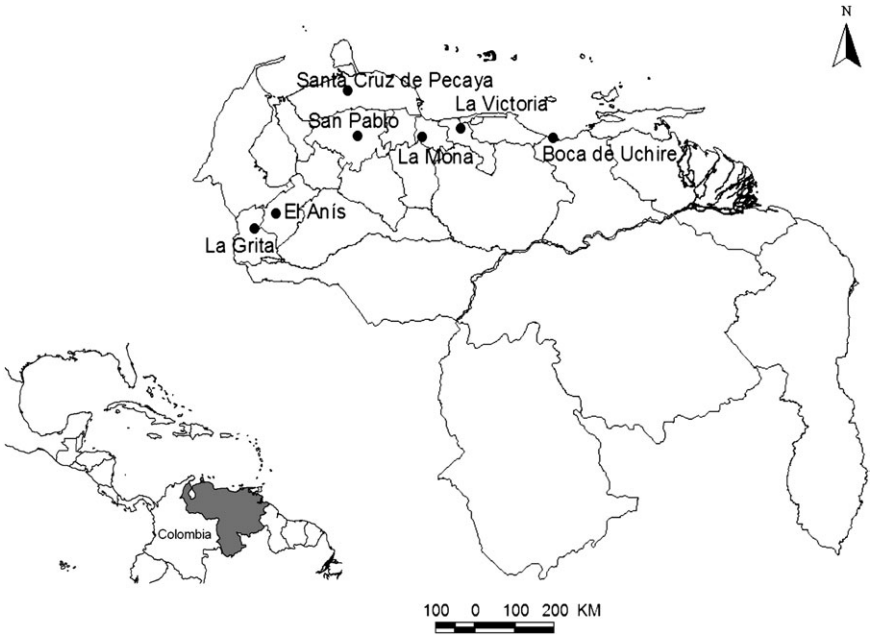

Figure 1. Distribution map of populations of *Agave cocui* sampled for this study in Venezuela.

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Table 1 Sampling sites for *Agave cocui* in Venezuela

Locality	Mean annual precipitation (mm)	Geographic coordinates	Altitude (m)
El Anís (EA)	528.6	08°27′47.6″N, 71°29′32.3″W	516
La Grita (LG)	—	08°08′15.4″N, 71°58′36.70″W	1290
Santa Cruz de Pecaya (SCP)[a]	463.5	11°05′25.91″N, 69°53′00.85″W	237
San Pablo (SP)	510.4	10°07′52.4″N, 69°39′43.0″W	937
La Mona (LM)	1143.2	10°06′35.9″N, 68°11′09.6″W	765
La Victoria (LV)	987.6	10°17′30.8″N, 67°19′50.8″W	697
Boca de Uchire (BU)	618.2	10°05′17.5″N, 65°14′32.5″W	10

Acronyms of localities in parenthesis.

[a] Population under human pressure for extraction of rosettes.

pyrophosphorylase (*Ugpp*-1); and buffer 34, alcohol dehydrogenase (*Adh*-1), glutamate dehydrogenase (*Gdh*-1). 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.

Data Analysis

Allele frequencies and standard genetic diversity parameters following Hedrick (1985) and Berg and Hamrick (1997) were estimated at the species and population level. Allele frequencies were estimated using the program GENALEX v. 6.2 (Peakall and Smouse 2006). Departures from Hardy–Weinberg (H-W) expectations were examined for each locus and population with the H-W exact test proposed by Rousset and Raymond (1995) using the program GENEPOP on the Web (http://genepop.curtin.edu.au/). The following estimates of genetic diversity 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 ($A_e = 1/\sum p_i^2$, where p_i is the frequency of the ith allele), observed heterozygosity (H_o) and expected heterozygosity or genetic diversity ($H_e = 1 - \Sigma\, p_i^2$). These 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 (SEs). All these parameters were estimated using the program POPGENE v. 1.32 (Yeh et al. 1997). Nei's parameters of genetic diversity (Nei 1973, 1977) were estimated for each polymorphic locus, including total genetic diversity (H_T), mean genetic diversity within populations (H_S), and the proportion of genetic diversity due to differences among populations ($G_{ST} = (H_T - H_S)/H_T$). These estimates were obtained using the program FSTAT v. 2.9.3.2 (Goudet 1995). Wright's (1978) F_{IS} was estimated for each locus using the program TFPGA (Miller 1997).

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. This test was conducted with the program POPGENE v. 1.32. A positive correlation between the 2 variables is indicative of isolation by distance. A Mantel test of association (9000 permutations) between pairwise $F_{ST}/(1 - F_{ST})$ and ln of geographic distance matrices was conducted with the program TFPGA v. 1.3 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 using POPGENE v. 1.32 to generate average clusterings using the UPGMA method. The dendrogram obtained using this procedure was created with the program MEGA v. 4.0 (Tamura et al. 2007) and helped to understand the genetic relationship among populations.

Results

Genetic Diversity

Allozyme banding segregation patterns indicate that *A. cocui* is a diploid species

Forty-one alleles at 17 loci were resolved across all populations. Only 5 private alleles were detected, 4 of them in population EA, in the Andes, and one in population LV, in the north central portion of the country. At the species level, 70.6% of the loci were polymorphic (Table 2). The average number of alleles per locus (A_s) was 2.41 and per polymorphic locus (AP_s) was 3.00. The average effective number of alleles per locus (A_{es}) was 1.07. 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 low ($H_{es} = 0.059$). Across polymorphic loci ($N = 12$), Nei's (1973) H_e values ranged between 0.031 and 0.170. Mean within-population genetic diversity estimates obtained across all populations surveyed were considerably low: $P_p = 38.7 \pm 7.1$ (range: 11.8–58.8), $A_p = 1.529 \pm 0.118$ (range: 1.118–2.000), $AP_p = 2.313 \pm 0.104$ (range: 2.000–2.889), $A_{ep} = 1.069 \pm 0.018$ (range: 1.016–1.149), $H_{ep} = 0.054 \pm 0.013$ (range: 0.014–0.105). The lowest levels of genetic variation across parameters corresponded to population LG, in a small Andean xeric patch, and population SCP, the population under human pressure for agave extraction. The highest value of expected heterozygosity was observed in population BU, in the central coast.

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Table 2 Summary of genetic diversity estimates at the species and population level for *Agave cocui* based on 17 allozyme loci

Level	*N*	%*P*	*A*	*AP*	A_e	H_o	H_e
Species	229	70.59	2.412	3.000	1.066	0.050	0.059
Population							
Santa Cruz de Pecaya (SCP)[a]	35	17.65	1.235	2.333	1.017	0.015	0.018
San Pablo (SP)	34	47.06	1.588	2.250	1.069	0.036	0.055
La Grita (LG)	30	11.76	1.118	2.000	1.016	0.015	0.014
El Anís (EA)	30	52.94	2.000	2.889	1.112	0.078	0.087
La Victoria (LV)	35	58.82	1.765	2.300	1.077	0.058	0.063
La Mona (LM)	30	29.41	1.353	2.200	1.046	0.042	0.039
Boca de Uchire (BU)	35	52.94	1.647	2.222	1.149	0.106	0.105
Mean (±1 SE)		38.65 (7.13)	1.529 (0.118)	2.313 (0.104)	1.069 (0.018)	0.050 (0.013)	0.054 (0.013)

[a] Population under past and present human pressure for extraction of rosettes.

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, H_o = mean observed heterozygosity, H_e = mean expected heterozygosity, SE = standard error.

Genetic Structure

Mean observed heterozygosities (H_{op}) across loci were equal or slightly lower than mean expected values (H_{ep}) for all populations, with exception of population LM, where H_{op} was slightly above H_{ep} (Table 2). The H-W exact test per locus and population did not detect heterozygous deficit in 80.5% of the examined cases. The only significant departures from equilibrium were detected in population LV for locus *Aat-2*, population SPM for locus *Aat-1*, population SCP for locus *Ak-1*, and population SP for loci *Mnr-2* and *Aat-1*. The mean F_{IS} across loci was not significantly different from zero (F_{IS} = 0.085 ± 0.006; 95% confidence interval: −0.024 to 0.204), indicating absence of inbreeding or genetic subdivision within populations.

Nei's total genetic diversity for polymorphic loci was H_T = 0.087 (0.013 SE) and most of that diversity was found within populations, H_S = 0.081 (0.011). Mean G_{ST} across loci was 0.057 ± 0.014. We detected a significant positive relationship between H_T and G_{ST} estimates across polymorphic loci (Pearson r = 0.65, $P < 0.05$). For 2 loci with relative high H_T values, we obtained 2 of the highest G_{ST} estimates (AAT-1: H_T = 0.116, G_{ST} = 0.097; MNR-2: H_T = 0.160, G_{ST} = 0.163). The regression coefficient of the linear regression analysis of $F_{ST}/(1 - F_{ST})$ on ln of geographic distance (Figure 2) was positive (β = 0.03) and explained 19% of the variation in $F_{ST}/(1 - F_{ST})$. The association between pairwise ln geographic distances and pairwise $F_{ST}/(1 - F_{ST})$ values was close to be significant (Mantel test, Z = 8.89, r = 0.43, P = 0.08), suggesting that isolation by distance should not be completely discarded as the model that better explains the genetic relationship between the populations analyzed. Remarkably, population pairs with the largest separation distances had considerable variation in the $F_{ST}/(1 - F_{ST})$ values (Figure 2).

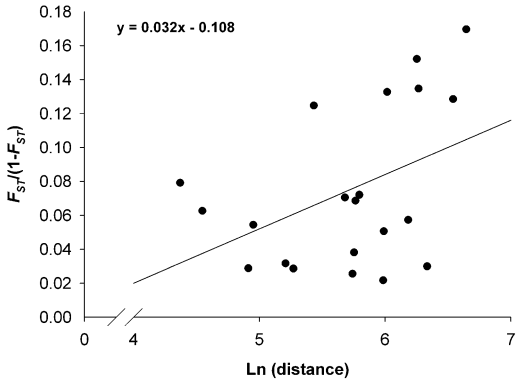

Figure 2. Differentiation among *Agave cocui* populations. Multilocus estimates of pairwise differentiation ($F_{ST}/[1 - F_{ST}]$) are plotted against the natural logarithm of pairwise geographic distances (in kilometers) according to Rousset (1997). R^2 = 0.187.

Nei's identity (*I*) values varied between 0.980 and 0.993 (data available upon request), averaging 0.990 ± 0.001. The lowest genetic identities were associated with population pairs including population BU, located on the central coast of Venezuela and well separated from the remaining populations, all of them in western portion of the country. Nei's genetic distance (*D*) averaged 0.005 ± 0.001, with pairwise values varying between 0.001 and 0.016. The UPGMA dendrogram based on genetic distances between populations (Figure 3) separated population BU, in the eastern coast, from the remaining populations in the west. One of the Andean populations (EA) was separated from the remaining populations in the cluster referred to. The rest of the populations within that cluster were not grouped according to their geographic distances.

Discussion

Among the *Agave* species examined for genetic diversity until present, *A. cocui* stands as one of the species with the lowest genetic variability at both species and population level. We hypothesize that this condition could be related to its recent origin in northern South America, probably through one or few founder events, and differences observed in genetic variability among populations should be determined by a combination of ecological and anthropogenic factors.

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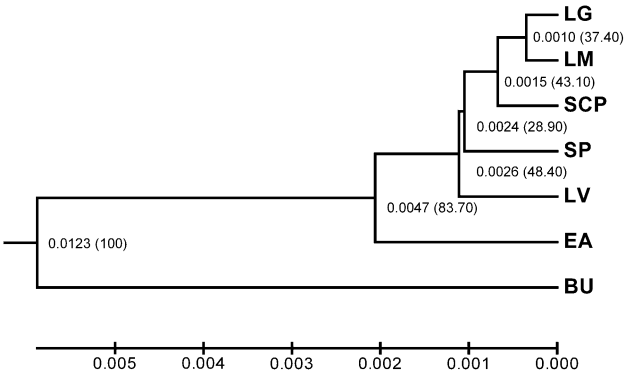

Figure 3. UPGMA cluster based on Nei's (1972) genetic distances (*D*) estimated among 7 populations of *Agave cocui*. Populations were sampled across the Venezuelan range of the species. Numbers are Nei's genetic distances and in parenthesis results of 1000 bootstrapping random replicates. For names of populations see Table 1.

The Agavaceae originated and radiated in Mexico 20–26 million years ago (Ma) (García-Mendoza and Galván 1995; Good-Avila et al. 2006). The genus *Agave* also appeared and diversified in Mexico 8 to 10 Ma (Good-Avila et al. 2006). Species of this genus distributed in northern South America are most likely the result of the most recent colonization events, either through the Caribbean or through Central America, which began after species radiated in Mexico. One biogeographic evidence that suggests this hypothetical "north–south" colonization pattern is the gradual reduction in number of native species from Mexico ($N \sim 150$) to Venezuela ($N = 2$) (García-Mendoza 2004b). A recent origin through one or few colonization events with a limited number of individuals (founder events) should be evidenced by a relatively low overall level of genetic diversity across loci (Lande 1999). Species level expected heterozygosity for *A. cocui* was 5-fold lower than species estimates found for Mexican agaves (H_e[species level] = 0.334–0.394; Martínez-Palacio et al. 1999; Silva-Montellano and Eguiarte 2003) and similar or even lower than estimates found for anthropogenic populations of agaves in Arizona (H_e[population level] = 0.056–0.089; Parker et al. 2007, 2010). In agreement with our results, a study conducted by Dávila et al. (2007) to evaluate the utility of ISSR markers to examine genetic relationships between *A. cocui* and other members of the genus showed that *A. cocui* had a substantially lower percentage of polymorphic loci (36.0%) than the Mexican *A. angustifolia* (85.3%).

Peripheral species of *Agave* examined for genetic diversity, first in Arizona (USA) and now in Venezuela, have the lowest levels of variation reported until now for the genus. These results are concordant with the central–marginal hypothesis, frequently used to explain why genetic diversity declines from populations located in the center of the geographical range of a species toward the periphery of its distribution (Lesica and Allendorf 1995; Eckert et al. 2008). According to this hypothesis, 2 key genetic parameters, effective population size (*Ne*) and the rate of gene flow (*m*), should be highest at the range center and lowest at range margins, resulting in lower genetic diversity in geographically peripheral populations. And this pattern is expected to be intensified if peripheral populations experience more frequent population bottlenecks than those in central environments. *Agave cocui*, located at the southern and most recently occupied geographic limits of the genus, quite probably originated by sporadic colonization events from a species distributed at northern latitudes. We should expect that the marginal population from which this new species started to evolve began as a relatively small and isolated group of individuals, with only a fraction of the genetic variation present in the source population.

Other plant genera associated with dry ecosystems in the Neotropics show a similar trend. A reduction of genetic diversity from the center of origin to the southern edges of its geographic distribution is exhibited by the genus *Stenocereus*. This lineage of 22 columnar cacti evolved in the Tehuacán-Cuicatlán Valley, Mexico, about 8.52 Ma (Dávila-Aranda et al. 2002; Barba-Montoya 2009), a time of origin similar to the one proposed for genus *Agave*. Central American countries have one or 2 species of *Stenocereus*, but only 1 species reached northern South America (Hunt 1999). Species level allozyme variation in Mexican taxa varied between $H_e = 0.201$ in *S. thurberii* (Hamrick et al. 2002) to $H_T = 0.592$ in *S. pruinosus* (H_T: Nei's total genetic diversity, Parra et al. 2008). In the case of *S. griseus*, distributed in Colombia, Venezuela and the Netherlands Antilles, the species level estimate of genetic diversity was below values ($H_e = 0.182$; Nassar et al. 2003) found in all the Mexican taxa. *Guaiacum officinale*, the species of Lignum vitae with the most extreme southern distribution in this genus composed of 5 taxa, has substantially lower allozyme variation ($H_e = 0.200$ in Venezuela, Nassar 2010) than *G. sanctum* ($H_e = 0.329$ in Costa Rica, Fuchs and Hamrick 2010), a species present in Florida, Central America and the Caribbean.

Estimates of genetic diversity in *A. cocui* varied substantially among the examined populations ($P = 11.8–58.8$, $A = 1.118–2.000$, $A_e = 1.016–1.149$, $H_e = 0.014–0.105$). Even though this species is mainly pollinated by nectar-feeding bats, one of them capable of flying over long distances (Soriano et al. 2000; Newton et al. 2003), these differences in genetic diversity suggest that pollen-mediated gene flow among populations is restricted. The hypothetical migratory route proposed for *Leptonycteris curasoae* (Glossophaginae) along a north–south axis of arid regions in western Venezuela includes *A. cocui* populations (SCP, SP, EA, LG) that show variable estimates of genetic diversity. Seed dispersal is mediated by wind, but populations of *A. cocui* are discretely distributed in arid patches and rocky outcrops with hundreds of kilometers of separation, and seeds would not be able to travel over such distances. Our average estimate of genetic structure for the species was quite low ($G_{ST} = 0.057$), suggesting substantial historical gene flow among populations; but many of the loci used for this calculation had very low H_T values, which were associated with low G_{ST} estimates.

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If we consider that loci with higher H_T values can give us more representative estimates of genetic structure for this species, then we should focus on the G_{ST} estimates obtained from *Aat-1* (0.097) and *Mnr-2* (0.163), which would be considered to represent the result of high and moderate levels of gene flow, respectively. We think that humans are probably the main dispersal agents responsible for moving genes of *A. cocui* among populations and promoting colonization events during the time people have used this plant in northern South America. In Arizona, patterns of genetic similarity among populations of *A. parryi* are attributed to a complex anthropogenic history (Parker et al. 2010). Anthropogenic populations of this species come from distant (39–132 km) and diverse sources indicating that translocation of individuals over long distances was, and still is, a common practice among agave users. We need to understand well the history of the man–agave interaction in Venezuela in order to understand the genetic relationships among populations of this species.

The lowest levels of genetic diversity recorded in some of the populations examined can be explained on the basis of ecological life-history traits and anthropogenic impact. Small population size has been associated with low genetic variation in plants and animals in general (Ellstrand and Elam 1993; Frankham et al. 2005; Letelier 2007). Population LG, in the Andean region, and LM, in the Central region, are 2 of the smallest and more spatially isolated populations examined in Venezuela (Figueredo 2010). These populations have some of the lowest estimates of genetic variability recorded in this study across all parameters considered. LG is the smallest (<29 km^2) locality inhabited by *A. cocui* in an archipelago of xeric patches in the Andean region isolated from each other by humid ecosystems (Soriano and Ruiz 2003). LM is located in a small rocky outcrop (<10 km^2) surrounded by evergreen forests and agricultural areas. SCP was the other population with very low genetic variability across most parameters estimated. Historically, this locality and others in southern latitudes were inhabited by the Jiraharas, Ayamanes, and Xaguas 5000–1000 years ago, 3 ethnic groups that used the agave in their daily lives. Unfortunately, no available records exist that can give us an accurate notion of the level of use and population management exerted by those human groups in the past; but we know that for the last decades SCP has been a locality with high level of human pressure on *A. cocui* to produce cocuy de penca (Padilla et al. 2007). Until year 2006, 17 local producers of this spirit used nearly 37 000 rosettes per year to cover the liquor's regional demand (Padilla et al. 2007). Unregulated extraction of rosettes without replacement for many years has probably contributed to reduce the population size and number of reproductive individuals that produce seeds, generating as a consequence a decrease in the available genetic pool in that locality. Identification and evaluation of additional localities where populations of *A. cocui* are being used in the region are needed to corroborate the effect of human manipulation on levels of genetic diversity of this species. Finally, the 2 populations with the highest values of genetic diversity were BU in the central coast and EA in the largest (>300 km^2) arid patch in the Andes. There are no historical records of agave extraction by humans for commercial purposes in these populations. Even though we do not have estimates of the total area covered by these populations in comparison with the other wild populations surveyed, they are located in a matrix of xeric vegetation comparatively larger. It would be important to complement the information generated in this study with genetic diversity estimates of populations of *A. cocui* in Colombia, where this species is restricted to the arid coastal zone, between Santa Marta and the Guajira peninsula and also in the inter-Andean arid patches of Chicamocha and Tatacoa, in the south (Albesiano and Fernández-Alonso 2006).

The recently formulated plans for a major development of the cocuy de penca industry in northwestern Venezuela call for the need to take into account information on the genetic status of the populations of *A. cocui* and the impact that unregulated extraction of plants can have on levels of genetic diversity in this species. This is a species with naturally low levels of genetic variation, a condition that puts a narrow limit to the results expected from population management and artificial selection initiatives. The populations of BU, EA, LV, and SP are the main reservoirs of genetic diversity for this species and the main candidates as sources of material to create a germplasm bank. To guarantee their survival, these populations should be given special official protection. The genetic erosion evidenced in SCP as a consequence of decades of overexploitation without replacement is a good example of what can happen to other natural populations in Venezuela if no measures are taken to conduct appropriate population management measures. In this regard, Venezuelan initiatives to generate a sustainable and adequate use of *A. cocui* should take into consideration the wide experience and knowledge accumulated on the use of agaves in Mexico and the conservation and legal protection of their germplasm diversity (Colunga-García Marín and May-Pat 1993, 1997; Colunga-García Marín et al. 1996; Colunga-García Marín and Zizumbo-Villarreal 2007; Vargas-Ponce et al. 2007, 2009).

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