Journal of Mammalogy, 93(4):1061–1074, 2012

Spatiotemporal population genetics of the endangered Perote ground squirrel (*Xerospermophilus perotensis*) in a fragmented landscape

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The Perote ground squirrel (*Xerospermophilus perotensis*) is endemic to Mexico and is currently at risk of extinction. Its original range comprised 5,250 km^2 in an area known as the Oriental Basin, between the states of Puebla and Veracruz. By the end of the 20th century, however, extensive agricultural practices, overgrazing, and urbanization had already restricted suitable habitat for this species to only 16 localities. Temporal changes in genetic diversity and structure in this species were assessed from 34 museum specimens from 5 historical populations (1990–1992) and 44 individuals from 3 current populations (2007) using the mitochondrial (mtDNA) control region and 5 nuclear microsatellites. We observed a general trend (significant in some cases and nonsignificant in others) suggesting a decrease in genetic diversity within populations and an increase in genetic structure between them in recent years for both sets of markers. A Bayesian skyline reconstruction for the mtDNA sequences was congruent with a recent demographic decline scenario. If genetic drift is the predominant evolutionary force in Perote ground squirrels, then the loss of genetic diversity could intensify in a few generations even if the effective population sizes remain constant. Urgent measures to increase the effective population sizes and maintain the genetic cohesion among populations are critical to the conservation of this species. Because no protected areas are planned in the Oriental Basin, we suggest translocating individuals between populations to avoid further loss of genetic diversity in the short run. In the long run, however, it will be necessary to devise a strategy to introduce individuals into suitable patches of habitat and to spatially link these populations so that genetic exchange can take place without the need for management assistance.

Key words: conservation, ground squirrel, Mexico, microsatellites, mitochondrial DNA, population genetics, *Xerospermophilus perotensis*

DOI: 10.1644/11-MAMM-A-371.1

Habitat fragmentation is the transformation of a large expanse of habitat into a mosaic of smaller patches surrounded by a matrix of habitats unlike the original (Saunders et al. 1991; Wilcove et al. 1986). Considered to be one of the main causes of loss of biological diversity (Di Battista 2008; Meffe and Carroll 1997), habitat fragmentation may not only produce a reduction in the total number and geographic range of species, but also isolate and differentiate populations previously in genetic communication, thus challenging their evolutionary trajectories and long-term survival (Clarke and Young 2000; Lacy 1997; Young et al. 1996). Whenever there is restricted gene flow, fragmentation could lead to the loss of genetic diversity and inbreeding within fragments (Frankham et al. 2010). This in turn may affect individual fitness and increase the probability of extinction of a species (Boyce 1992; Gilpin and Soulé 1986; Reed and Frankham 2003; Roelke et al. 1993).

Museum specimens have proven to be valuable sources of DNA for addressing changes in genetic diversity and structure in populations that have declined in recent years due to anthropogenic activities (Bouzat et al. 1998; Glenn et al. 1999; Larson et al. 2002; Muñoz-Fuentes et al. 2005; Nyström et al. 2006). In this respect, current populations are expected to show reductions in their mean heterozygosity and a greater loss of alleles when compared with historical populations (Hedrick 2010). Similarly, changes in genetic diversity and structure in declining populations are expected to be more drastic in mitochondrial than in nuclear loci, because the former have smaller effective sizes and chance changes resulting in fixation

or loss will accumulate at a much faster rate (Crow and Kimura 1970). Other factors contributing to a higher mitochondrial than nuclear genetic structure are differences in the mutation rate between both genomes and sex-specific life histories (Ballard and Whitlock 2004). Results derived from genetic comparisons between museum specimens and specimens from current populations using both mitochondrial and nuclear markers are critical to understanding the natural history, ecology, and evolution of endangered species, and to developing appropriate plans for their management and conservation (Leonard 2008).

Ground squirrel species are Holarctic, and their distribution includes Eurasia, from central Europe to China, Mongolia, and Siberia, and western North America, from Alaska to central Mexico (Ellerman and Morrison-Scott 1966; Hall 1981; Hoffmann et al. 1993; McKenna and Bell 1997; Thorington and Hoffmann 2005). Ground squirrels are found in a wide range of habitats that include tropical, subtropical, and temperate forests, deserts, woodlands, prairies, steppes, and tundra (Howell 1938; Ognev 1947; Wilson and Ruff 1999; Yensen and Valdés-Alarcón 1999). Ground squirrels play a key role in the dynamics and conservation of the pastureland they inhabit and have a profound impact on the biotic and abiotic characteristics of the ecosystem (Facka et al. 2008; Laundré 1998; Whitford and Kay 1999).

The Perote ground squirrel (*Xerospermophilus perotensis*) is endemic to Mexico. Its geographic range is restricted to the arid plains of the Oriental Basin, between the states of Puebla and Veracruz, at 2,200–2,700 m elevation (Hall 1981; Valdéz and Ceballos 1997). This basin is almost completely surrounded by a belt of high-elevation mountains, encompassing an area of 5,250 km^2. Perote ground squirrels live in strong associations with alkaline grasslands, arid scrubs, and hilly and rocky areas, where they commonly dig their burrows (Best and Ceballos 1995; Valdéz and Ceballos 1997). This species might have diverged from its closest relative, the spotted ground squirrel (*X. spilosoma*), some 1.2–3.3 million years ago (mya) as a result of climate shifts during the Pleistocene (Harrison et al. 2003).

Perote ground squirrels are currently at risk of extinction. Extensive agricultural practices, overgrazing, and urbanization occurring in the Oriental Basin have jeopardized the species' existence (Valdéz and Ceballos 1997). It remains unclear when such activities began to reduce this species' historical range. Evidence suggests that Spanish conquistadors began to use this zone for agricultural purposes in the 16th century, but extensive land conversion did not really start until the 19th century, when the Mexican Interoceanic Railroad was constructed (Gerez-Fernández 1985). As a result of the Mexican Revolution, large haciendas in the Oriental Basin were fragmented in the 1930s, thus favoring the development of new land for cultivation and grazing. With the construction of Federal Highway 140 (Acatzingo–Xalapa) in the 1950s came a higher influx of traffic, further deteriorating a large part of this habitat (Lima-Muñiz 1975). By the end of the 20th century, intense habitat destruction had restricted suitable habitat for this species to only 16 localities, many of which were confined to narrow strips (20–50 m) in developed areas and separated from each other by a mean distance of 15.8 km (Valdéz and Ceballos 1997).

Despite their listing as an endangered species by Mexican federal law (Secretaría de Desarrollo Social 1994; Secretaría de Medio Ambiente y Recursos Naturales 2001) and the International Union for Conservation of Nature (2008), Perote ground squirrels have received little attention with respect to their conservation, in part because little is known about them. Although incipient data on their natural history and ecology are presently available, information on their population genetics is totally lacking. In this study we used mitochondrial and nuclear markers to evaluate the evolutionary consequences of habitat fragmentation in Perote ground squirrels through comparison of museum and current samples. We specifically addressed the changes in genetic diversity, genetic structure, and effective population sizes of this species over a 15-year period to design appropriate management strategies for its conservation.

Materials and Methods

Sampling and DNA extraction.—Skin tissue was removed from 34 museum specimens collected from 5 populations (historical Tepeyahualco [HTY]: $n = 5$; historical Totalco [HTO]: $n = 15$; historical Perote [HPE]: $n = 12$; historical Ciudad Serdán [HCS]: $n = 1$; and historical San Salvador [HSS]: $n = 1$) between 1990 and 1992 (Appendix I). These samples were stored in tubes with phosphate-buffered saline solution at 55°C until they were completely hydrated. Additionally, in 2007 we captured 44 squirrels from 3 populations (current Tepeyahualco [CTY]: $n = 16$; current Totalco [CTO]: $n = 14$; and current Pizarro [CPZ]: $n = 14$) using Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with oats. Hairs and tissue samples from the tip of the tail of each of these individuals were stored in paper envelopes and tubes with 70% ethanol, respectively. Captured individuals were marked with gentian violet and subsequently released in the burrows where they had been found. Sampling locations (Fig. 1) correspond to sites where it was possible to obtain well-preserved museum specimens for genetic analyses and find current individuals according to the study by Valdéz and Ceballos (1997). The only exception was CPZ, which had not been recorded previously and was fortuitously found during our fieldwork. We used the DNeasy Tissue Kit (Qiagen Co., Valencia, California) to extract DNA from historical and current samples.

Mitochondrial DNA sequencing.—Primers L15933 and H637 (Oshida et al. 2001) were used to amplify the complete mitochondrial (mtDNA) control region in current samples. Historical samples were more difficult to amplify, perhaps due to some mtDNA degradation and fragmentation. For this reason we designed 4 internal primers (CR1R, GGTAGG GGATAGTCATTTGG; CR1F, CCAAATGACTAT CCCCTACC; CR2R, GGTGAGTCCCTGCATCCCCC; and CR2F, GGGGGATGCAGGGACTCACC) based on the current

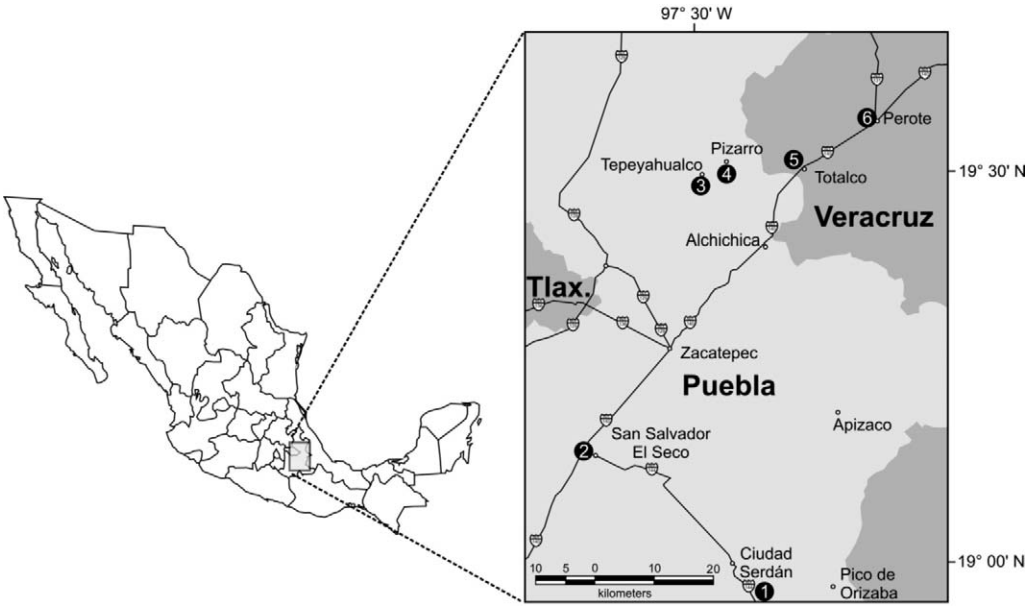

Fig. 1.—Location of the study sites in the Oriental Basin, between the states of Puebla and Veracruz, Mexico. 1) Ciudad Serdán (HCS); 2) San Salvador (HSS); 3) Tepeyahualco (HTY and CTY); 4) Pizarro (CPZ); 5) Totalco (HTO and CTO); and 6) Perote (HPE). HCS = historical Ciudad Serdán; HSS = historical San Salvador; HTY = historical Tepeyahualco; CTY = current Tepeyahualco; CPZ = current Pizarro; HTO = historical Totalco; CTO = current Totalco; HPE = historical Perote.

mtDNA sequences (see below) to amplify this region in the historical samples. In this respect, internal primers were joined to the original external primers in the following manner: L15933/CR1R, CR1F/CR2R, and CR2F/H637.

Polymerase chain reaction was performed in 25-µl reaction volumes containing 25 ng/µl of DNA, 1.25 µl of 10× buffer, 2.5 mM of $MgCl_2$, 0.2 mM of deoxynucleoside triphosphates, 0.4 µM of forward and reverse primers, and 3 units of *Taq* DNA polymerase (Applied Biosystems Inc., Foster City, California). All amplification reactions consisted of 35 cycles of 30 s at 95°C, 45 s at 50°C, and 2 min at 72°C, with an initial denaturation step of 95°C for 3 min and final extension of 72°C for 10 min.

Amplified fragments were sequenced twice in both directions (forward and reverse) by Macrogen Inc. (Seoul, Korea) to ensure convergence among parameters and then compared with homologous sequences in the GenBank to verify their authenticity. Multiple sequence alignments were performed using the ClustalW method as implemented in BioEdit version 7.0.5 (Hall 1999).

Microsatellite typing.—A total of 6 nuclear microsatellite loci from each sample were amplified by polymerase chain reaction. One of these loci (IGS-110b) was previously isolated in northern Idaho ground squirrels (*Spermophilus brunneus brunneus*—May et al. 1997) and the remaining 5 loci (A104, A2, A8, D115, and D2) in black-tailed prairie dogs (*Cynomys ludovicianus*—Jones et al. 2005). Polymerase chain reaction was carried out in 12-µl reaction volumes containing 25 ng/µl of DNA, 1.25 µl of 10× buffer, 1.5 mM of $MgCl_2$, 0.2 mM of deoxynucleoside triphosphates, 0.4 µM of forward and reverse primers, and 3 units of *Taq* DNA polymerase (Applied Biosystems). Polymerase chain reaction amplifications were performed following protocols in May et al. (1997) and Jones et al. (2005), except for loci A104 and D2, which were amplified using annealing temperatures of 54°C and 58°C, respectively.

Microsatellite fragments were analyzed on an ABI 100 automatic sequencer in 10-µl reaction mixes containing 9.25 µl of deionized formamide, 0.25 µl of GeneScan 600 LIZ Size Standard (Applied Biosystems), and 0.5 µl of polymerase chain reaction products, with an initial denaturation step of 95°C. We used Peak Scanner version 1.0 (Applied Biosystems) to precisely calculate the sizes of the fragments and discard ambiguous or low-quality amplified genotypes.

Mitochondrial DNA statistical analyses.—Haplotype diversity (h) and nucleotide diversity (π) within each population were calculated in DnaSP version 5.10.01 (Rozas and Rozas 1995). We used Arlequin version 3.5 (Excoffier and Lischer 2010) to estimate genetic differentiation levels between population pairs (F_{ST}) after 1,000 permutations. The effective number of dispersing females between populations was estimated from the expression:

$$N_{e.f}m = \frac{1 - F_{ST}}{2F_{ST}}, \quad (1)$$

where $N_{e.f}$ is the female effective population size and m is the proportion of migrants between populations (Wright 1921). Spatial structure patterns were further discussed after building a minimum spanning network with a 95% confidence limit in TCS version 1.2.1 (Clement et al. 2000).

In BEAST version 1.6.1 (Drummond and Rambaut 2007) we performed a Bayesian skyline reconstruction to estimate recent population size changes based on the mtDNA sequences. BEAUti parameters consisted of a HKY + I model

Table 1.—Distribution of historical and current Perote ground squirrel (*Xerospermophilus perotensis*) mitochondrial DNA control region haplotypes. The number of individuals with each haplotype and the total and unique number of haplotypes per population are shown. HTY = historical Tepeyahualco; HTO = historical Totalco; HPE = historical Perote; HCS = historical Ciudad Serdán; HSS = historical San Salvador; CTY = current Tepeyahualco; CTO = current Totalco; CPZ = current Pizarro.

	Historical populations					Current populations		
Haplotype	HTY	HTO	HPE	HCS	HSS	CTY	CTO	CPZ
*Xp*A	01	03	10		01		11	
*Xp*B							03	
*Xp*C								04
*Xp*D		08						05
*Xp*E	02					03		04
*Xp*F	01					13		01
*Xp*G		01						
*Xp*H		02	01					
*Xp*I		01						
*Xp*J			01					
*Xp*K	01							
*Xp*L				01				
n	05	15	12	01	01	16	14	14
Total haplotypes	04	05	03	01	01	02	02	04
Unique haplotypes	01	02	01	01	00	00	01	01

of sequence evolution, as determined in jModelTest version 0.1.1 (Posada 2008) using Akaike's information criterion (Akaike 1973), and strict clock conditions. Markov chain Monte Carlo replicates comprised 1,000,000,000 steps sampled every 10,000 steps. Tracer version 1.5 (Rambaut and Drummond 2007) was used to analyze the resulting posterior sample and assess high effective sample sizes (>1,000). Because this program yields estimates of $N_{e.f}T$, we assumed a generation time of $T = 3$ years (Sherman and Morton 1984; Zammuto 1987) to rescale this parameter and obtain $N_{e.f}$ estimates over time. Uncertainty in the estimates was indicated by 95% highest posterior density intervals (HPDIs).

Nuclear statistical analyses.—Linkage disequilibrium tests to assess independence among loci, allelic richness (A) and observed heterozygosity (H_O) and expected heterozygosity (H_E) were obtained in Fstat version 2.9.3.2 (Goudet 1995). To avoid sample-size artifacts (Leberg 1992, 2002; Petit et al. 1998), allelic richness estimates were based on the smallest number of individuals typed for any locus ($n = 5$) after 1,000 permutations.

Inbreeding coefficient (F_{IS}) levels within populations and genetic differentiation (F_{ST}) levels between them also were calculated in Fstat after 1,000 permutations. We preferred to use F_{ST} rather than R_{ST} (Slatkin 1995) because this index has a lower variance and performs better with small numbers of loci and samples (Gaggiotti et al. 1999). We used MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) to evaluate the presence of null alleles that also could cause departures from Hardy–Weinberg expectations. In cases where null alleles were likely, we estimated an unbiased F_{ST} (Weir 1996) after correcting for null alleles following the ENA method as implemented in FreeNA (Chapuis and Estoup 2007). We estimated gene flow as the number of genetic migrants between populations from the expression:

$$N_e m = \frac{1 - F_{ST}}{4F_{ST}}, \quad (2)$$

where N_e is the effective population size (Wright 1921).

Population structure for the total data set was further investigated in Structure version 2.3.3 (Pritchard et al. 2000), incorporating the sampling locations from each individual in the analysis (HTY, HTO, HPE, HCS, HSS, CTY, CTO, or CPZ) and assuming admixture and correlated allele frequencies as initial parameters. The number of possible clusters (K) ranged from 1 to 10, with 1,000,000 Markov chain Monte Carlo repetitions, 100,000 burn-in iterations, and 10 simulations per K. The most likely number of clusters was determined using the modal value of ΔK as recommended by Evanno et al. (2005). For the identified K-value, we used CLUMPP (Jakobsson and Rosenberg 2007) to determine the population assignment probability of each individual across all simulations.

For both mtDNA and nuclear data we conducted independent t-tests to find genetic differences between particular temporal data sets. Special attention was paid to the populations that came from Tepeyahualco (HTY and CTY) and Totalco (HTO and CTO), because we had historical and current samples from both locations. Significant values for multiple tests were determined after sequential Bonferroni adjustments (Rice 1989).

Results

Mitochondrial DNA statistical analyses.—Of the total data set we sequenced 1,061 base pairs (bp) from the mtDNA control region, thus obtaining 12 haplotypes that were designated *Xp*A, *Xp*B, *Xp*C, *Xp*D, *Xp*E, *Xp*F, *Xp*G, *Xp*H, *Xp*I, *Xp*J, *Xp*K, and *Xp*L (GenBank accession numbers JQ326958–JQ326969). This region showed 21 variable sites, 14 of which were C–T transitions and 6 of which were A–G transitions; the only transversion (A–C) was observed in haplotype *Xp*L, which was dramatically different from the rest and alone contributed to 5 C–T transitions and 2 A–G transitions.

We found 10 haplotypes from 34 historical samples, but only 6 haplotypes in the 44 current samples (Table 1). A total of 6 historical haplotypes (*Xp*G, *Xp*H, *Xp*I, *Xp*J, *Xp*K, and *Xp*L) and 2 current haplotypes (*Xp*B and *Xp*C) were unique to each temporal data set, whereas haplotypes *Xp*A, *Xp*D, *Xp*E, and *Xp*F were shared between both data sets. A decrease in the number of haplotypes was observed in both Tepeyahualco and Totalco. A total of 4 haplotypes were found in HTY (*Xp*A, *Xp*E, *Xp*F, and *Xp*K), whereas only 2 haplotypes were detected in CTY (*Xp*E and *Xp*F), even when the sample size in the latter was 3 times larger ($n = 5$ versus $n = 16$, respectively). Similarly, 5 haplotypes were observed in HTO (*Xp*A, *Xp*D, *Xp*G, *Xp*H, and *Xp*I), whereas only 2 haplotypes were observed in CTO (*Xp*A and *Xp*B), with both samples having a similar size ($n = 15$ versus $n = 14$, respectively).

Table 2.—Historical and current Perote ground squirrel (*Xerospermophilus perotensis*) mitochondrial DNA genetic diversity. HTY = historical Tepeyahualco; HTO = historical Totalco; HPE = historical Perote; CTY = current Tepeyahualco; CTO = current Totalco; CPZ = current Pizarro; n = sample size; h = haplotype diversity: π = nucleotide diversity. Historical Ciudad Serdán (HCS) and historical San Salvador (HSS) were excluded from the analysis because they had only 1 individual ($n = 1$).

	n	h (*SD*)	π (*SD*)
Historical populations			
HTY	05	0.900 (0.161)	0.00226 (0.00058)
HTO	15	0.695 (0.109)	0.00133 (0.00035)
HPE	12	0.318 (0.164)	0.00063 (0.00039)
$\bar{X}$		0.638 (0.295)	0.00141 (0.00082)
Current populations			
CTY	16	0.325 (0.125)	0.00123 (0.00047)
CTO	14	0.363 (0.130)	0.00034 (0.00012)
CPZ	14	0.758 (0.060)	0.00242 (0.00033)
$\bar{X}$		0.482 (0.240)	0.00133 (0.00104)

Haplotype diversity ranged from 0.318 to 0.900 in the historical populations, and from 0.325 to 0.758 in the current populations (Table 2). Historical populations had a higher, but not significantly different, mean haplotype diversity than current populations ($h = 0.638$ versus $h = 0.482$, respectively; unpaired $t_4 = 0.709$; 2-sided $P = 0.517$). HTY displayed a significantly higher haplotype diversity than CTY (95% confidence interval [95% *CI*] = 0.578–1.000 versus 95% *CI* = 0.075–0.575, respectively; $P < 0.05$). Although HTO had a higher haplotype diversity than CTO, differences were not significant (95% *CI* = 0.477–0.913 versus 95% *CI* = 0.103–0.623, respectively; $P > 0.05$).

Nucleotide diversity ranged from 0.00063 to 0.00226 in the historical populations, and from 0.00034 to 0.00242 in the current populations (Table 2). Although historical populations presented a higher mean nucleotide diversity than current populations, differences were not significant either (π = 0.00141 versus π = 0.00133, respectively; unpaired t_4 = 0.100; 2-sided $P = 0.925$). HTY showed a higher, but not significantly different, nucleotide diversity than CTY (95% *CI* = 0.00011–0.00376 versus 95% *CI* = 0.00029–0.00217, respectively; $P > 0.05$). HTO, on the other hand, displayed a significantly higher nucleotide diversity than CTO (95% *CI* = 0.00063–0.00203 versus 95% *CI* = 0.00010–0.00058, respectively; $P < 0.05$).

Genetic differentiation ranged from $F_{ST} = 0.193$ to $F_{ST} = 0.371$ in the historical populations, and from $F_{ST} = 0.399$ to $F_{ST} = 0.657$ in the current populations (Table 3). Although no statistical differences were observed, mean F_{ST}-values were lower in the historical populations when compared with those in the current populations ($F_{ST} = 0.306$ versus $F_{ST} = 0.499$, respectively; unpaired $t_4 = 1.959$; 2-sided $P = 0.122$). Using equation 1, the estimated effective number of dispersing females ranged from $N_{e.f}m = 0.848$ to $N_{e.f}m = 2.091$ in the historical populations, and from $N_{e.f}m = 0.261$ to $N_{e.f}m = 0.753$ in the current populations. Mean differences were not significant either ($N_{e.f}m = 1.282$ versus $N_{e.f}m = 0.550$, respectively; unpaired $t_4 = 1.699$; 2-sided $P = 0.165$). HTY was not genetically different from CTY ($F_{ST} = 0.301$, $P > 0.05$). We did, however, find significant differences between HTO and CTO ($F_{ST} = 0.369$, $P < 0.05$).

Table 3.—Mitochondrial DNA pairwise F_{ST}-values (below diagonals) and effective number of dispersing females ($N_{e.f}m$) values (above diagonals) between historical and current Perote ground squirrel (*Xerospermophilus perotensis*) populations. HTY = historical Tepeyahualco ($n = 5$); HTO = historical Totalco ($n = 15$); HPE = historical Perote ($n = 12$); CTY = current Tepeyahualco ($n = 16$); CTO = current Totalco ($n = 14$); CPZ = current Pizarro ($n = 14$). Historical Ciudad Serdán (HCS) and historical San Salvador (HSS) were excluded from the analysis because they had only 1 individual ($n = 1$).

	Historical populations			Current populations		
	HTY	HTO	HPE	CTY	CTO	CPZ
Historical populations						
HTY		2.091	0.908	1.161	0.984	7.565
HTO	0.193^{B}		0.848	0.514	0.855	4.354
HPE	0.355^{B}	0.371*		0.237	37.962	0.606
Current populations						
CTY	0.301^{B}	0.493*	0.678*		0.261	0.753
CTO	0.337^{B}	0.369*	0.013^{NS}	0.657*		0.636
CPZ	0.062^{NS}	0.103*	0.452*	0.399*	0.440*	

	Historical populations		Current populations	
	F_{ST}	$N_{e.f}m$	F_{ST}	$N_{e.f}m$
$\bar{X}$	0.306	1.282	0.499	0.550
SD	0.098	0.701	0.139	0.257

^{NS} Nonsignificant; ^{B} nonsignificant after Bonferroni correction; * $P < 0.05$ after Bonferroni correction.

The minimum spanning network revealed that haplotypes *Xp*B, *Xp*D, *Xp*E, *Xp*G, *Xp*H, and *Xp*J were connected to the most frequent one, *Xp*A, after 1–3 mutational steps (Fig. 2). Haplotypes *Xp*K and *Xp*F were connected to *Xp*D after 1 and 2 mutational steps, respectively, whereas haplotypes *Xp*C and *Xp*I were connected to *Xp*G after 1 mutational step. The southernmost haplotype, *Xp*L, was very different from the other haplotypes, because it was connected to *Xp*K after 10 mutational steps. At a spatial scale, almost all haplotypes were either unique to a particular population or found in neighboring populations forming a continuous distribution. The only exception was haplotype *Xp*A, which was found in Tepeyahualco (HTY only), Totalco (HTO and CTO), Perote (HPE), and San Salvador (HSS), but could not be found in the geographically intermediate population of Pizarro (CPZ).

Finally, the Bayesian skyline reconstruction suggests that Perote ground squirrels have undergone a slight population decline in recent years (Fig. 3). In this regard, the female effective population size reached $N_{e.f} = 71.4$ (95% HPDI = 25.8–225.8) individuals in 1977 and decreased to $N_{e.f} = 14.2$ (95% HPDI = 1.0–181.6) individuals in 2007.

Nuclear statistical analyses.—Of the total data set we found 13 alleles in locus A104; 7 alleles in loci A2, D115, and D2;

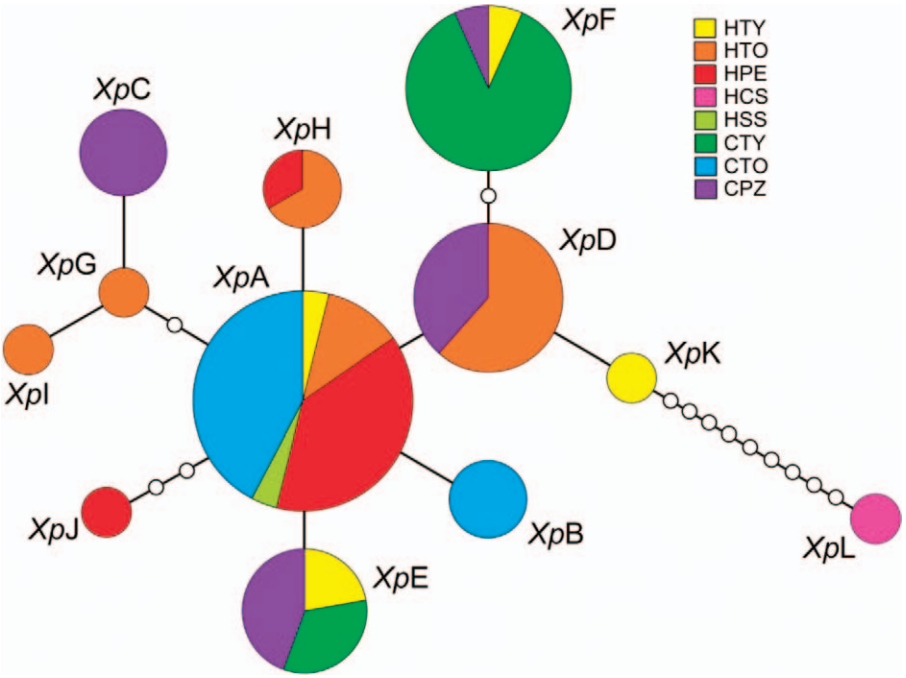

Fig. 2.—Minimum spanning network of historical and current Perote ground squirrel (*Xerospermophilus perotensis*) mitochondrial DNA control region haplotypes. Lines represent a single mutational step, with missing haplotypes represented by small circles. HTY = historical Tepeyahualco; HTO = historical Totalco; HPE = historical Perote; HCS = historical Ciudad Serdán; HSS = historical San Salvador; CTY = current Tepeyahualco; CTO = current Totalco; CPZ = current Pizarro.

and 5 alleles in locus A8 (Fig. 4). Locus IGS-110b showed no genetic variation (all amplified fragments corresponded to an allele of 117 bp) and therefore was not included in later genetic studies. We were not able to amplify locus D2 in 5 individuals from HPE.

Overall, the historical data set ($n = 34$) presented a total of 35 alleles, 3 of which were private with respect to the current data set (allele 196 in locus A104, allele 205 in locus A2, and allele 321 in locus D2). The current data set ($n = 44$), on the other hand, presented a total of 36 alleles, 4 of which were private with respect to the historical data set (alleles 168, 188, and 190 in locus A104, and allele 219 in locus A2; Fig. 4).

We assumed independence among loci because no tests of linkage disequilibrium were significant at the $P < 0.05$ level. Although loci A104 and A8 exhibited high null allele frequencies ($p_n > 0.05$) in 4 (HPE, CTY, CTO, and CPZ) of the 6 populations examined, we decided to include these loci in further analyses because they showed no evidence for significant departures of Hardy–Weinberg expectations after Bonferroni correction (Table 4).

Overall allelic richness ranged from 3.717 to 4.247 in the historical populations, and from 3.259 to 4.150 in the current populations (Table 4). No significant differences were observed between historical and current populations (A = 3.988 versus A = 3.680, respectively; unpaired $t_{28} = 0.932$; 2-sided $P = 0.359$), HTY and CTY (A = 4.000 versus A = 4.150, respectively; paired $t_4 = 0.296$; 2-sided $P = 0.782$), and HTO and CTO (A = 4.247 versus A = 3.631, respectively; paired $t_4 = 2.243$; 2-sided $P = 0.088$).

Overall expected heterozygosity ranged from 0.686 to 0.735 in the current populations (Table 4). No significant differences were observed between historical and current populations ($H_E = 0.709$ versus $H_E = 0.664$, respectively; unpaired $t_{28} = 1.203$; 2-sided $P = 0.239$), HTY and CTY ($H_E = 0.730$ versus $H_E = 0.735$, respectively; paired $t_4 = 0.091$; 2-sided $P = 0.932$), and

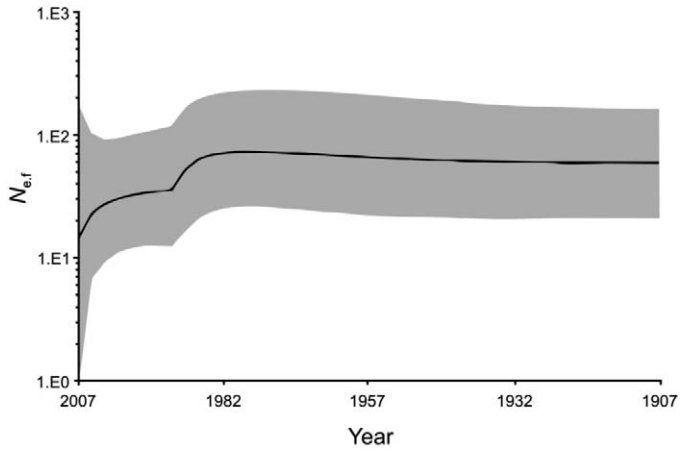

Fig. 3.—Bayesian skyline reconstruction obtained by analyzing Perote ground squirrel (*Xerospermophilus perotensis*) mitochondrial DNA control region sequences sampled at different times. We assumed a generation time of $T = 3$ years to rescale the $N_{e.f}T$ parameter on the y-axis, where $N_{e.f}$ is female effective population size, and obtain $N_{e.f}$ estimates over time. The thick solid line represents the $N_{e.f}$ median and the gray area the 95% highest posterior density intervals (HPDIs).

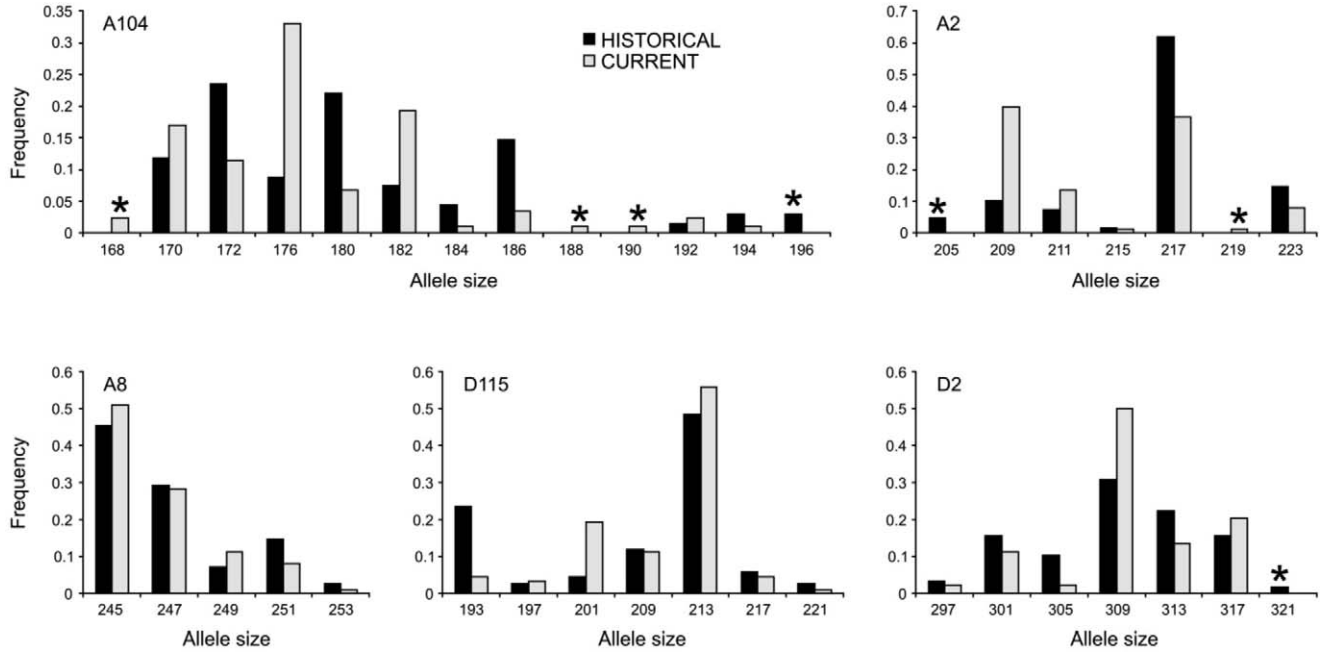

Fig. 4.—Microsatellite loci (A104, A2, A8, D115, and D2) allele frequencies for historical and current data sets. Asterisks (*) indicate private alleles per temporal data set.

HTO and CTO ($H_E = 0.711$ versus $H_E = 0.640$, respectively; paired $t_4 = 1.396$; 2-sided $P = 0.235$).

Overall inbreeding coefficients ranged from $F_{IS} = 0.068$ to $F_{IS} = 0.204$ in the historical populations, and from $F_{IS} = 0.077$ to $F_{IS} = 0.167$ in the current populations (Table 4). As in previous analyses, no significant differences were observed between historical and current populations ($F_{IS} = 0.118$ versus $F_{IS} = 0.132$, respectively; unpaired $t_{28} = 0.278$; 2-sided $P = 0.783$), HTY and CTY ($F_{IS} = 0.068$ versus $F_{IS} = 0.167$, respectively; paired $t_4 = 0.856$; 2-sided $P = 0.440$), and HTO and CTO ($F_{IS} = 0.081$ versus $F_{IS} = 0.152$, respectively; paired $t_4 = 0.832$; 2-sided $P = 0.452$).

Overall genetic differentiation ranged from $F_{ST} = 0.008$ to $F_{ST} = 0.074$ in the historical populations, and from $F_{ST} = 0.024$ to $F_{ST} = 0.091$ in the current populations (Table 5). Although no significant differences were found between historical and current populations ($F_{ST} = 0.037$ versus $F_{ST} = 0.060$, respectively; unpaired $t_4 = 0.813$; 2-sided $P = 0.462$), we highlight the fact that pairwise F_{ST}-values between historical populations were not significantly different ($P > 0.05$), as opposed to pairwise F_{ST}-values between current populations, which showed some degree of genetic differentiation ($P < 0.05$). Using equation 2, the estimated number of effective migrants ranged from $N_em = 3.628$ to $N_em = 31.500$ in the historical populations, and from $N_em = 2.997$ to $N_em = 10.667$ in the current populations ($N_em = 14.570$ versus $N_em = 5.940$, respectively; unpaired $t_4 = 0.969$; 2-sided $P = 0.388$). HTY and CTY were not genetically different ($F_{ST} = 0.032$, $P > 0.05$), but HTO was significantly different from CTO ($F_{ST} = 0.018$, $P < 0.05$).

The genotypic assignment analysis suggests that 3 populations best fit the total data set (Fig. 5). In general, HTY, HCS, HSS, and CTY were assigned to 1 cluster, HTO and HPE to a 2nd cluster, and CTO and CPZ to a 3rd cluster. Results derived from this analysis gave a higher resolution, perhaps due to prior location information for each individual, than F_{ST}-values. For example, individuals from HTO and HPE were clearly differentiated from HTY, as opposed to the F_{ST} statistic that suggested no genetic structure between these populations. Some similarities with the F_{ST}-values also were found, because HTY and CTY were assigned to the same cluster, and HTO and CTO were, to some extent, differentiated. The genotypic assignment test also shows that, even though CTO and CPZ were grouped in the same cluster, some individuals from CTO (1CTO, 7CTO, 11CTO, and 12CTO) belong to the same gene pool as HTO and HPE.

Discussion

Species inhabiting geographic ranges that are not only restricted but also strongly impacted by human activities are more prone to extinction. This has been the case for the Perote ground squirrel, where intense habitat destruction and fragmentation within the Oriental Basin have led to small and isolated populations with fewer opportunities for genetic interchange. Understanding the patterns of genetic variation and demographic collapse in fragmented populations is important for designing management strategies to mitigate the loss of genetic diversity.

Genetic diversity.—With their rapid generation times and large effective population sizes, sciurids are expected to have high numbers of haplotypes (Bromham et al. 1996; Li et al.

TABLE 4.—Historical and current Perote ground squirrel (*Xerospermophilus perotensis*) nuclear genetic diversity. HTY = historical Tepeyahualco; HTO = historical Totalco; HPE = historical Perote; CTY = current Tepeyahualco; CTO = current Totalco; CPZ = current Pizarro; *n* = sample size; A = allelic richness based on a sample size of 5 diploid individuals; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = deviation from Hardy–Weinberg proportions. Historical Ciudad Serdán (HCS) and historical San Salvador (HSS) were excluded from the analysis because they had only 1 individual ($n = 1$).

	Historical populations														
	HTY					HTO					HPE				
Locus	*n*	A	H_O	H_E	F_{IS}	*n*	A	H_O	H_E	F_{IS}	*n*	A	H_O	H_E	F_{IS}
A104	5	4.000	0.400	0.775	0.484^{NS}	15	5.916	0.667	0.881	0.243^{NS}	12	3.735	0.167	0.739	0.774^{B}†
A2	5	4.000	0.800	0.775	-0.032^{NS}	15	3.165	0.600	0.524	-0.145^{NS}	12	3.409	0.500	0.542	0.077^{NS}
A8	5	5.000	1.000	0.800	-0.250^{NS}	15	3.348	0.600	0.624	0.038^{NS}	12	3.320	0.500	0.693	0.279^{NS}
D115	5	2.000	0.600	0.450	-0.333^{NS}	15	4.083	0.667	0.719	0.073^{NS}	12	3.488	0.750	0.689	-0.088^{NS}
D2	5	5.000	0.600	0.850	0.294^{NS}	15	4.723	0.733	0.807	0.091^{NS}	7	4.635	0.857	0.821	-0.043^{NS}
Overall		4.000	0.680	0.730	0.068^{NS}		4.247	0.653	0.711	0.081^{NS}		3.717	0.527	0.686	0.204^{B}

	Historical populations			
	A	H_O	H_E	F_{IS}
$\bar{X}$	3.988	0.620	0.709	0.118
SD	0.265	0.082	0.022	0.075

^{NS} Nonsignificant; ^{B} nonsignificant after Bonferroni correction; † $p_n > 0.05$.

1996), as seen in Japanese giant flying squirrels (*Petaurista leucogenys*, $n = 24$—Oshida et al. 2001), Eurasian red squirrels (*Sciurus vulgaris*, $n = 23$—Trizio et al. 2005), American red squirrels (*Tamiasciurus hudsonicus*, $n = 35$—Wilson et al. 2005), red and white flying squirrels (*P. alborufus lena*, $n = 36$—Oshida et al. 2011), and Indian giant flying squirrels (*P. philippensis grandis*, $n = 33$—Oshida et al. 2011). Our results, however, suggest that habitat fragmentation may produce a negative effect in this respect because historical samples were already affected by habitat fragmentation and had a low number of haplotypes when examined, and because current samples had fewer haplotypes than historical samples, even though the former had a larger sample size. These findings also are consistent with *S. b. brunneus* and southern Idaho ground squirrels (*S. b. endemicus*), which have undergone severe habitat fragmentation and have only 3 and 9 haplotypes, respectively (Garner et al. 2005). In addition to this information, current Perote ground squirrels presented a reduced mitochondrial variation ($h = 0.482$, $\pi = 0.00133$) when compared with other sciurids from nonfragmented habitats,

TABLE 5.—Nuclear pairwise F_{ST}-values (below diagonals) and number of effective migrants (N_em) values (above diagonals) between historical and current Perote ground squirrel (*Xerospermophilus perotensis*) populations after correcting for null alleles. HTY = historical Tepeyahualco ($n = 5$); HTO = historical Totalco ($n = 15$); HPE = historical Perote ($n = 12$); CTY = current Tepeyahualco ($n = 16$); CTO = current Totalco ($n = 14$); CPZ = current Pizarro ($n = 14$). Historical Ciudad Serdán (HCS) and historical San Salvador (HSS) were excluded from the analysis because they had only 1 individual ($n = 1$).

	Historical populations			Current populations		
	HTY	HTO	HPE	CTY	CTO	CPZ
Historical populations						
HTY		8.583	3.628	8.063	6.660	6.064
HTO	0.030^{B}		31.500	2.482	14.139	3.540
HPE	0.074^{B}	0.008^{B}		2.568	4.636	2.750
Current populations						
CTY	0.032^{NS}	0.112*	0.107*		2.997	4.156
CTO	0.039^{B}	0.018*	0.057*	0.091*		10.667
CPZ	0.043^{B}	0.076*	0.100*	0.064*	0.024*	

	Historical populations		Current populations	
	F_{ST}	N_em	F_{ST}	N_em
$\bar{X}$	0.037	14.570	0.060	5.940
SD	0.034	14.869	0.034	4.135

^{NS} Nonsignificant; ^{B} nonsignificant after Bonferroni correction; * $P < 0.05$ after Bonferroni correction.

Table 4.—Extended.

Current populations														
CTY					CTO					CPZ				
n	A	H_O	H_E	F_{IS}	*n*	A	H_O	H_E	F_{IS}	*n*	A	H_O	H_E	F_{IS}
16	4.507	0.500	0.754	0.337†	14	5.214	0.429	0.835	0.487^{B}†	14	4.138	0.357	0.742	0.519^{B}†
16	4.181	0.625	0.742	0.157^{NS}	14	2.886	0.571	0.602	0.050^{NS}	14	2.357	0.643	0.549	-0.170^{NS}
16	4.066	0.500	0.769	0.350^{B}†	14	1.973	0.214	0.396	0.458^{NS}	14	3.141	0.429	0.555	0.228^{NS}
16	3.835	0.750	0.683	-0.098^{NS}	14	4.305	0.786	0.684	-0.149^{NS}	14	3.260	0.571	0.552	-0.035^{NS}
16	4.159	0.688	0.729	0.057^{NS}	14	3.776	0.714	0.684	-0.044^{NS}	14	3.397	0.786	0.618	-0.271^{NS}
	4.150	0.613	0.735	0.167^{B}		3.631	0.543	0.640	0.152^{B}		3.259	0.557	0.603	0.077^{NS}

Current populations			
A	H_O	H_E	F_{IS}
3.680	0.571	0.664	0.132
0.448	0.037	0.062	0.048

such as *S. vulgaris* ($h = 0.864$, $\pi = 0.018$—Trizio et al. 2005) and *T. hudsonicus* ($h = 0.648$, $\pi = 0.009$—Wilson et al. 2005).

Interestingly, Perote ground squirrels have increased nuclear variation relative to other sciurids with declining populations. For example, current Perote ground squirrel populations exhibited a mean allelic richness of 3.680 and a mean expected heterozygosity of 0.664, whereas these estimators were lower in *S. b. brunneus* ($A = 3.61$, $H_E = 0.58$—Garner et al. 2005), *S. b. endemicus* ($A = 3.02$, $H_E = 0.43$—Garner et al. 2005), Siberian flying squirrels (*Pteromys volans*, $A = 3.48$, $H_E = 0.656$—Selonen et al. 2005), and European ground squirrels (*Spermophilus citellus*, $H_E = 0.372$—Hulová and Sedláček 2008). In addition, and contrary to expectations, nuclear variation estimators in current Perote ground squirrels were very similar to those in other sciurids facing no conservation issues (Trizio et al. 2005). It is also important to consider the possible effects of ascertainment bias in microsatellites when comparing genetic diversity across different species. In this study we used microsatellite markers that were originally developed for *S. b. brunneus* (IGS-110b—May et al.1997) and *C. ludovicianus* (A104, A2, A8, D115, and D2—Jones et al. 2005) to describe the nuclear genetic variation in Perote ground squirrels. Nevertheless, no genetic variation was observed at locus IGS-110b, whereas loci A104 and A8 had a high frequency of null alleles that were possibly not amplified through standard polymerase chain reaction. For this reason, nuclear variation in Perote ground squirrels could be underestimated in this study.

Genetic structure.—Mitochondrial and nuclear genetic differentiation values were much higher in current Perote

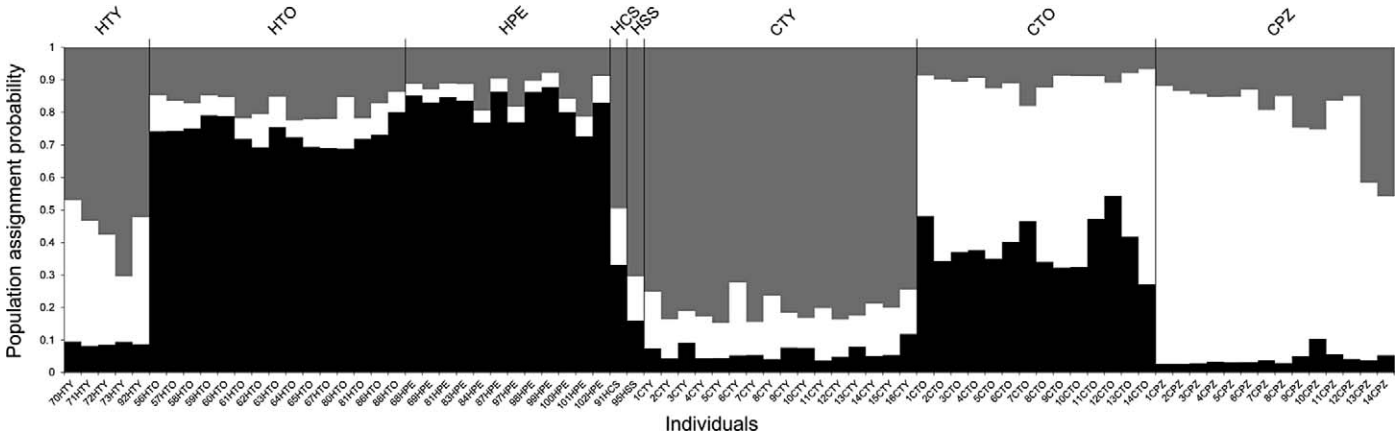

Fig. 5.—Population assignment test of historical and current Perote ground squirrels (*Xerospermophilus perotensis*). Genetic population clusters are coded with different colors. Individual assignment probabilities to particular clusters also are indicated. HTY = historical Tepeyahualco; HTO = historical Totalco; HPE = historical Perote; HCS = historical Ciudad Serdán; HSS = historical San Salvador; CTY = current Tepeyahualco; CTO = current Totalco; CPZ = current Pizarro.

ground squirrel populations (mitochondrial $F_{ST} = 0.499$ and nuclear $F_{ST} = 0.060$) when compared, for example, with those of *S. vulgaris* (mitochondrial $F_{ST} = 0.100$ and nuclear $F_{ST} = 0.041$—Trizio et al. 2005), whose populations are separated by continuous forest habitats. Current nuclear differentiation values for Perote ground squirrels were, however, lower than those found in *S. b. brunneus* ($F_{ST} = 0.125$—Garner et al. 2005), *S. b. endemicus* ($F_{ST} = 0.200$—Garner et al. 2005), *P. volans* ($F_{ST} = 0.130$—Selonen et al. 2005), and *S. citellus* ($F_{ST} = 0.158$—Hulová and Sedláček 2008), all of which experience some degree of habitat fragmentation.

Although certain precautions must be taken when comparing mitochondrial and nuclear markers because they resolve genetic structure at different time depths (Bossart and Prowell 1998; Crandall et al. 2000), the greater mitochondrial to nuclear differentiation in current Perote ground squirrels suggests that female gene flow between populations is more restricted than male gene flow, that genetic drift has been more intense in mitochondrial than in nuclear loci, or that a combination of both factors is possible. The latter seems the most reasonable scenario given that female philopatry has been previously documented in ground squirrels (e.g., nursing females have limited dispersal because they defend their territory to gather food near their burrows and to protect their young against infanticide [Tomich 1982]), and that mitochondrial loci, indeed, have much smaller effective sizes than do nuclear loci (the mitochondria is haploid and inherited only through female lineages [Crow and Kimura 1970]). Female philopatry is further supported by the minimum spanning network, where haplotypes were either unique to a particular population or found in neighboring populations at a local spatial scale. The only exception, haplotype *Xp*A, which may be considered as an ancestral haplotype because it was located at the center of the network and found at a high frequency in the total data set (Avise 2000), could have spread throughout the Oriental Basin during an early demographic expansion event in the Pleistocene some 1.2–3.3 mya with the formation of vast plains and the opening of new ecological niches in the Central Mexican Plateau (Harrison et al. 2003). The high homoplasy rate in microsatellite markers also could explain the lesser nuclear differentiation relative to the mitochondria, because 2 microsatellite gene copies with the same state may not be identical by descent (Angers et al. 2000; Chapuis and Estoup 2007; Estoup et al. 2002). However, when we performed further genetic differentiation analyses for the nuclear loci assuming a stepwise mutation model (data not shown), we obtained results similar to those reported in this study.

The genotypic assignment test suggests that nuclear (possibly male) gene flow also has been occurring in neighboring populations, but perhaps at a greater spatial scale than mitochondrial gene flow. For example, the southernmost population, HCS, formed a cluster together with HTY, even though they are separated from each other by a geographic distance of 70 km. Sex-biased dispersal leading to dissimilar patterns in the home ranges between sexes is not uncommon in squirrels. In some cases, males must temporarily vacate their territories to locate spatially dispersed receptive females (Lane et al. 2009). Aggression by adults against yearling males also could promote large-scale juvenile male migration to other populations (Neuhaus 2006).

Even though Perote ground squirrels are likely to form family groups within individual burrow systems, no inbreeding coefficient value for any population was significantly different from zero, implying that inbreeding had little effect on genetic structure. This result also is supported by the fact that rodents avoid mating among relatives, mainly through genetically based recognition mechanisms and olfactory capabilities that play an important role in kin discrimination (Clarke and Faulkes 1999; Mateo 2003; Sherborne et al. 2007). In some other cases, male ground squirrels may also disperse to peripheral populations after mating to either avoid producing offspring with their descendants or increase the quality of their territory (Nunes 2007). Current inbreeding coefficient values in Perote ground squirrels ($F_{IS} = 0.132$) were higher than those found in *S. vulgaris* ($F_{IS} = 0.056$—Trizio et al. 2005) and *P. volans* ($F_{IS} = -0.005$—Selonen et al. 2005), which come from nonfragmented and fragmented habitats, respectively. Caution, however, is advised when comparing F_{IS}-values across species because, once again, the presence of null alleles could decrease the overall observed heterozygosity and, thus, overestimate the F_{IS} parameter.

Effective population size.—We found evidence of a recent population decline through the Bayesian skyline reconstruction, suggesting that the female effective population size has decreased over the last 30 years, when extensive agricultural practices and urbanization were already taking place in the Oriental Basin. Assuming an equal ratio between females and males, the current effective population size of Perote ground squirrels should be $N_e = 28.4$ (95% HPDI = 2.0–363.2), which is lower than the effective population size estimated for *S. b. brunneus*, a ground squirrel that not only has a similar natural history but also has undergone recent and severe habitat fragmentation (Gavin et al. 1999). Several explanations are possible for the apparent population decline in Perote ground squirrels. First, populations along Federal Highway 140 (Acatzingo–Xalapa) have been heavily reduced in area because natural vegetation has been highly modified by agriculture, overgrazing, and urbanization (Valdéz and Ceballos 1997). Second, reduced immigration rates may produce low population densities in negatively impacted areas (Gavin et al. 1999). Third, the introduction of exotic predators, mainly domestic dogs, in the Oriental Basin could have a demographic impact on Perote ground squirrel populations (Best and Ceballos 1995). All these hypotheses, however, should be carefully explored in the future.

Final considerations and implications for conservation.—In this study we used mitochondrial and nuclear genetic markers to understand the natural history, ecology, and evolution of the Perote ground squirrel, an endangered species about which little is known. Furthermore, we examined changes in genetic diversity, genetic structure, and effective population sizes in

this species over a 15-year period to design effective management strategies for its conservation.

Although current nuclear variation in Perote ground squirrels is comparable to that of other sciurids facing no conservation issues, it should not escape our attention that current mitochondrial variation in this species has been severely impacted by habitat fragmentation. In addition, we observed a general trend (significant in some cases and nonsignificant in others) suggesting a decrease in genetic diversity within populations and an increase in genetic structure between them in recent years for both sets of markers. The lack of statistical power in some of these tests and the apparent increase in nuclear diversity (allelic richness and expected heterozygosity) in the particular case of Tepeyahualco could be due to the narrow generation gap between the historical and current data sets, the small sample sizes used in this study, or the effect of numerical outliers. Through the implementation of null allele corrections and Bonferroni adjustments we controlled for type I errors (i.e., false positives) that could lead to spurious inferences and, thus, we are confident significant results are true positives. Nevertheless, given the inherent nature of Perote ground squirrels, it was not possible to increase the sample size in our study to control for type II errors (i.e., false negatives).

If we take into account these considerations there is little doubt Perote ground squirrels are facing long-term conservation issues. If genetic drift is the predominant evolutionary force in this species, then the loss of genetic diversity could intensify in a few generations even if the effective population sizes remain constant. An integral conservation plan for this species should be focused on increasing the effective population sizes and maintaining the genetic cohesion among populations to minimize the evolutionary consequences of habitat fragmentation. Conservation strategies may consider the current populations of CTY, CTO, and CPZ as distinct management units, because it is clear they retain nuclear genetic diversity from 3 distinct gene pools. Protecting the core area where these populations are located also is essential to conserving as much mitochondrial variation as possible and may prove useful for future reintroductions or the establishment of biological corridors. Because no protected areas are planned in the Oriental Basin we suggest translocating individuals between populations to avoid further loss of genetic diversity in the short run, although careful examination of functional genes and morphology is recommended to avoid outbreeding depression in case populations have recently developed local adaptations to their environment. In the long run, however, it will be necessary to devise a strategy to introduce individuals into suitable patches of habitat and to spatially link these populations so that genetic exchange can take place without the need for management assistance.

Resumen

La ardilla terrestre del Perote (*Xerospermophilus perotensis*) es endémica de México y actualmente se encuentra en peligro de extinción. Su distribución original comprendía 5,250 km^2 en un área conocida como el Valle del Oriental, entre los estados de Puebla y Veracruz. No obstante, a finales del siglo XX actividades como la agricultura extensiva, el sobrepastoreo y la urbanización ya habían restringido el hábitat tipo de esta especie a sólo 16 localidades. Cambios temporales en la diversidad y estructura genética en esta especie fueron evaluados en 34 ejemplares de museo de 5 poblaciones históricas (1990–1992) y 44 individuos de 3 poblaciones actuales (2007) utilizando la región control mitocondrial (ADNmt) y 5 microsatélites nucleares. Observamos una tendencia general (significativa en algunos casos y no significativa en otros) indicando una reducción en diversidad genética dentro de las poblaciones y un aumento en estructura genética entre ellas en años recientes para ambos juegos de marcadores. Una reconstrucción Bayesiana de horizonte para las secuencias de ADNmt fue congruente con un escenario de reducción demográfica reciente. Si la deriva génica es la fuerza predominante en las ardillas terrestres del Perote, entonces la pérdida de diversidad genética podría intensificarse en algunas generaciones aún si los tamaños efectivos poblacionales se mantienen constantes. Medidas urgentes para incrementar los tamaños efectivos poblacionales y mantener la cohesión genética entre las poblaciones son críticas para la conservación de esta especie. Debido a que no existen planes para crear áreas protegidas en el Valle del Oriental sugerimos translocar individuos entre poblaciones para evitar una mayor pérdida de diversidad genética en el corto plazo. En el largo plazo, sin embargo, se requiere estructurar una estrategia para introducir individuos en parches de hábitat tipo y vincular espacialmente estas poblaciones para que el intercambio genético se lleve a cabo sin asistencia de manejo.

Acknowledgments

This project was funded by PAPIIT IN224309-3 granted to LEE. Historical samples were collected by M. Valdéz and curated by F. Cervantes at the National Mammal Collection of the Universidad Nacional Autónoma de México. We thank P. Arias and R. González for their assistance in the field, as well as J. Ortega and G. Castellanos for kindly providing the microsatellite primers used in this study. We are grateful to V. Souza, L. Espinosa, E. Aguirre, and P. Padilla for their support in the laboratory. M. Rodríguez contributed to the graphic design of the figures. We finally thank D. Hein, B. Rivera, R. Fitak, and 2 anonymous reviewers for their valuable comments and suggestions to increase the academic value of this study.

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Submitted 30 October 2011. Accepted 22 January 2012.

Associate Editor was Victor Sánchez-Cordero.

APPENDIX I

Voucher numbers of Perote ground squirrel (*Xerospermophilus perotensis*) museum specimens from the National Mammal Collection of the Universidad Nacional Autónoma de México. Localities where historical individuals were trapped correspond to Fig. 1. Year of collection is indicated in parentheses.

Tepeyahualco (HTY).—70 (1990), 71 (1990), 72 (1990), 73 (1990), 92 (1991).

Totalco (HTO).—56 (1990), 57 (1990), 58 (1990), 59 (1990), 60 (1990), 61 (1990), 62 (1990), 63 (1990), 64 (1990), 65 (1990), 67 (1990), 80 (1991), 82 (1991), 86 (1991), 88 (1991).

Perote (HPE).—68 (1990), 69 (1990), 81 (1991), 83 (1991), 84 (1991), 87 (1990), 97 (1992), 98 (1991), 99 (1991), 100 (1992), 101 (1992), 102 (1992).

Ciudad Serdán (HCS).—91 (1991).

San Salvador (HSS).—95 (1991).