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Genetic variation in a peripheral and declining population of black-tailed prairie dogs (*Cynomys ludovicianus*) from Mexico

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Across North America, anthropogenically induced habitat fragmentation has led to a severe decline in populations of black-tailed prairie dogs (*Cynomys ludovicianus*). Although the area occupied by black-tailed prairie dogs in Chihuahua, northwestern Mexico, also has recently declined, this site remains comparatively unaffected by human disturbance. Cytochrome-*b* sequences and 10 nuclear microsatellite loci were analyzed across 13 colonies to test if due to large size, absence of plague, and protected status, the black-tailed prairie dogs from Janos possess relatively high levels of genetic variation and low genetic structure; or if recent population decline and peripheral effects result in relatively low genetic variation and high genetic structure. Analysis suggests moderate mitochondrial genetic variation relative to other sciurids, and not significantly different nuclear genetic variation relative to other populations of prairie dogs. Furthermore, in accordance to black-tailed prairie dog social organization, genetic structure among local populations was significant, and within-colony variation was higher than among-colony variation for both markers. F_{ST} was higher for mitochondrial than for nuclear DNA related to female philopatry and male-biased gene flow. Finally, a negative correlation between genetic differentiation as a function of colony area and population size found for nuclear microsatellite loci suggests an increased effect of genetic drift in smaller and less-dense colonies because of recent habitat fragmentation. In conclusion, despite being a peripheral and declining population, Janos black-tailed prairie dogs retained genetic variation that has been maintained by their social structure and dispersal pattern.

Key words: black-tailed prairie dog, *Cynomys ludovicianus*, cytochrome *b*, habitat fragmentation, microsatellite, peripheral population, population genetics—empirical

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Population decline has been directly linked to the effects of habitat fragmentation on genetic viability (Frankham et al. 2002). It has been suggested that the isolation of populations reduces genetic variation via the decrease of effective population size and the disruption of gene flow, which may in turn, promote genetic drift and inbreeding (e.g., Barrat et al. 1999; Goodman et al. 2001; Grativol et al. 2001; Williams et al. 2003; Martínez-Cruz et al. 2004; Hulová and Sedlácek 2008). As a consequence, this decreases fitness and intensifies the risk of extinction (Saunders et al. 1991; Frankham et al. 2002; DiBattista 2008). Nevertheless, the effects of habitat fragmentation may depend on a number of other factors, including population bottleneck intensity, duration, time since occurrence, and population dynamics (such as metapopulation structure). Furthermore, the dispersal ability of any given species may prevent isolation, thus circumventing both genetic drift and inbreeding (Antolin et al. 2001; Schwartz et al. 2005; Purrenhage et al. 2009).

To assess the genetic viability of populations threatened by habitat fragmentation, measurements of genetic variation, differentiation, and structure are very important. These measurements provide valuable information for the design of conservation strategies, which aim to restore population connectivity, prevent further loss of genetic variation, and reduce the risk of extinction (Frankham et al. 2002). Populations of black-tailed prairie dogs (*Cynomys ludovicianus*) have long been subjected to habitat fragmentation. This is particularly evident in the United States where eradication

campaigns were implemented during the early 1900s (Hoogland 1996). In combination with subsequent outbreaks of plague (*Yersinia pestis*), the geographic range of black-tailed prairie dogs was reduced by as much as approximately 98% (Hoogland 1996; Trudeau et al. 2004; Slobodchikoff et al. 2009). Until recently, the black-tailed prairie dog population of the short-grass prairies of Janos, Chihuahua, Mexico, was considered the largest remaining black-tailed prairie dog population in North America (Ceballos et al. 2010). Following drought, cattle overgrazing, agriculture, poisoning, and scrubland expansion, this peripheral population also has suffered a serious decline, reducing its geographic range by approximately 73% (Ceballos et al. 1993, 2010; Ávila-Flores 2009). The rapid fragmentation and ongoing reduction of this population has raised serious concerns stimulating interest both in the conservation of this species and the preservation of the grasslands it inhabits. Given that this population has been subject to extensive anthropogenic disturbance, and prairie dog disappearance has been linked to concomitant losses of biodiversity from grassland ecosystems, this population is considered threatened (Mexico—SEMARNAT 2002) and the grassland it occupies has been designated a Mexican Biosphere Reserve (Dinerstein et al. 2000; Miller et al. 2000; Lomolino and Smith 2001; SEMARNAT 2009).

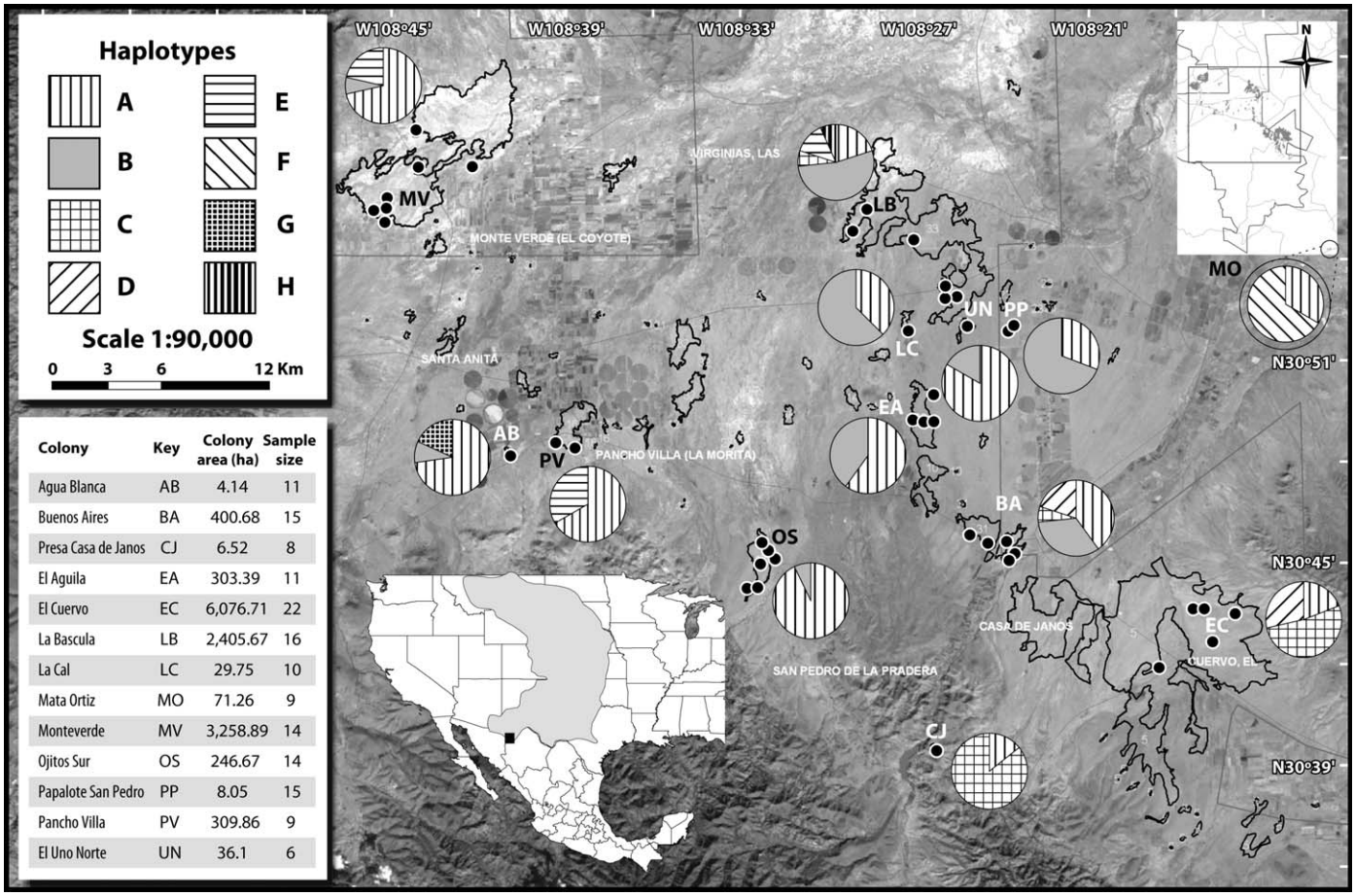
Black-tailed prairie dog colonies from Janos, Chihuahua, are located at the southernmost part of the species distribution and, therefore, constitute a peripheral population. Peripheral populations are genetically and morphologically divergent from core populations (Gaines et al. 1997) and, thus, differentiation may be crucial in response to, and adaptation to, environmental change (Lesica and Allendorf 1995). This is of potential significance to conservation strategies, and some field investigations have addressed the genetic variability among them without reaching consensus whether peripheral populations possess more or less genetic variation than core populations (Bouzat and Johnson 2004; Garner et al. 2004; Arnaud-Haond et al. 2006; Eckert et al. 2008; Kark et al. 2008; List et al. 2010; Rogell et al. 2010). Previous black-tailed prairie dog population genetics and landscape genetic studies have focused on colonies within the core area of the species distribution (Colorado, Montana, New Mexico, and South Dakota) overlooking genetic conservation value of peripheral black-tailed prairie dog populations (Chesser 1983; Foltz and Hoogland 1983; Daley 1992; Dobson et al. 1997, 1998, 2004; Roach et al. 2001; Trudeau et al. 2004; Jones and Britten 2010; Magle et al. 2010; Sackett et al. 2012). According to these studies, significant genetic structure in black-tailed prairie dog populations is related to complex social organization (presence of family groups within colonies—Chesser 1983; Dobson et al. 1997, 1998, 2004), female philopatry (Chesser 1991a, 1991b), connectivity between colonies (male-biased dispersal), time since colonization, and presence of plague (*Y. pestis*—Roach et al. 2001; Trudeau et al. 2004; Antolin et al. 2006).

The Janos black-tailed prairie dog population represents an important refuge for the conservation of this species because of its large area, absence of plague, extreme southern distribution, and protected status (Ceballos et al. 2005). However, genetic variation at this site is unknown. To further assess the value of the Janos population as a refuge for species persistence in the long term, it is important to assess its genetic variation. The purpose of this study was to measure the genetic variability and structure of a black-tailed prairie dog population located at the southernmost edge of its geographical range. There are 2 contrasting hypotheses regarding genetic variation and genetic structure of the population: due to large size, absence of plague, protected status, and the fact that genetic variation is maintained by social structure and dispersal patterns, we expect that the black-tailed prairie dogs from Janos will possess a relatively high level of genetic variation and genetic structure will be affected only by social organization and dispersal patterns; in contrast, recent population decline and peripheral effects (small effective population size and isolation) could result in relatively low genetic variation and an increase in genetic structure.

To test these predictions we used 2 molecular markers targeting maternally inherited mitochondrial DNA (mtDNA) and biparentally inherited nuclear DNA, which distinguish between female and male dynamics (Wilson et al. 1985; Gaines et al. 1997; Frankham et al. 2002; Arbogast et al. 2005; Hedrick 2005). Furthermore, black-tailed prairie dogs have complex social organization where each colony is subdivided into family groups called coteries, gene flow is male biased, and females are philopatric (Hoogland 1996; Slobodchikoff et al. 2009), so we expect to find significant genetic structure for both molecular markers, significantly more within- than among-colony variation because of the effect of social organization on genetic structure, and a higher F_{ST}-value for maternally inherited DNA than for biparentally inherited DNA because of female philopatry. Finally, considering that habitat fragmentation promotes genetic differentiation in smaller and less-dense colonies (Frankham et al. 2002), we expect to find a negative correlation between genetic differentiation, colony area, and population size.

Materials and Methods

Sample collection.—Black-tailed prairie dogs from 13 colonies located at Janos and Nuevo Casas Grandes counties in northwestern Chihuahua, Mexico, were captured, following guidelines of the American Society of Mammalogists (Sikes et al. 2011) and the Secretaria del Medio Ambiente y Recursos Naturales (SEMARNAT; permit SGPA/DGVS/04142/07), between May and July 2007 (Fig. 1). Sampling colonies were selected according to their area (Pacheco et al. 2009) taking 4 arbitrarily defined categories considering their order of magnitude in hectares: $<$ 9.9 ha, 10–99.9 ha, 100–999.9 ha, and $>$ 1,000 ha (Table 1; Fig. 1). As stated above, black-tailed prairie dogs are highly social mammals. Social aggregation has been reported to increase within-colony genetic variation and generate genetic structure within each colony, whereas female relatedness may bias inbreeding estimation (Chesser 1983, 1991a, 1991b; Sugg et al. 1996; Dobson et al. 1997, 1998,



Colony	Key	Colony area (ha)	Sample size
Agua Blanca	AB	4.14	11
Buenos Aires	BA	400.68	15
Presa Casa de Janos	CJ	6.52	8
El Aguila	EA	303.39	11
El Cuervo	EC	6,076.71	22
La Bascula	LB	2,405.67	16
La Cal	LC	29.75	10
Mata Ortiz	MO	71.26	9
Monteverde	MV	3,258.89	14
Ojitos Sur	OS	246.67	14
Papalote San Pedro	PP	8.05	15
Pancho Villa	PV	309.86	9
El Uno Norte	UN	36.1	6

Fig. 1.—Spatial location of the sampled colonies within the Janos, Chihuahua, Mexico, black-tailed prairie dog (*Cynomys ludovicianus*) population. The black square within the species distribution from southern Canada to northern Mexico (shaded gray within map of United States and Mexico) represents the location of the Janos population in the southernmost area of the species distribution. Colony borders are depicted by black lines and dots represent the capture sites on the 13 colonies sampled. Information on colony name (colony and key), colony area, and sample size are given in the table in the lower left corner. Pie graphs represent the 8 haplotypes (A–H) present and their frequency in each colony.

2004). Therefore, when performing studies that aim to assess the genetic relation between colonies within a certain area, sampling design has to consider the bias introduced by social organization. Family groups within a colony can be fixed for different alleles due to female philopatry. Hence, sampling individuals from different family groups within a colony will provide a better representation of the genetic variation present at the colony level. To sample individuals from different family groups within each colony, traps were set in plots that were placed at least 150 m apart consistent with the reported black-tailed prairie dog coterie home-range area (Hoogland 1996). Each plot consisted of a 4 × 4 grid with 25-m separation between each trap, and between 1 and 7 plots were set in each colony according to its area. Between 0.5 and 1 ml of blood was collected from the femoral artery of 1–3 black-tailed prairie dogs per plot. In total, 160 (93 females and 67 males) black-tailed prairie dog blood samples were collected. Blood was transferred into a 1.5-ml Eppendorff tube containing 20 µl of 10 mM ethylenediaminetetraacetic acid and sealed with a scrub-cap (Higuchi 1989). All samples were subsequently stored at –20°C until required for further processing. DNA was extracted using the Bio-Rad Blood Kit (Bio-Rad Laboratories Inc., Hercules, California) according to the manufacturer's instructions. DNA integrity and quality were assessed through electrophoresis in 1% agarose gels using a 100–base pair (bp) ladder and stained with ethidium bromide.

Polymerase chain reaction for mtDNA cytochrome b.—Mitochondrial DNA cytochrome-*b* (*Cytb*) sequences were obtained using the primers L14724 (5′-TGAAAAA-YCATCGTTGT-3′) and H15915 (5′-TCTTCATTTY-WGGTTTACAAGAC-3′—Harrison et al. 2003). In order to enhance amplification quality, 4 internal primers were designed using the program FastPCR (Kalendar et al. 2011—Forward-1, 5′-CATGAAACATTGGAGTTGTCC-3′; Forward-2, 5′-TTGCAGCCCTAGTCATAGTCCACC-3′; Reverse-1, 5′-GATATTTGACCTCAAGGGAGG-3′; and Reverse-2, 5′-TTTGTTGGGGATAGATCGTAGG-3′). Polymerase chain reaction amplifications were performed in a 25-µl reaction volume using 10 ng/µl of target DNA, 1× buffer, 3 mM of $MgCl_2$, 0.15 mM of deoxynucleoside triphosphates, 0.4 µM of each primer (Invitrogen, Carlsbad, California), and 2 U/µl of *Taq* DNA polymerase (Promega, Fitchburg, Wisconsin). We

Table 1.—Colony name, relative density, population size, genetic diversity, and genetic structure for mitochondrial cytochrome-*b* (*Cytb*) sequences and nuclear microsatellite loci for black-tailed prairie dogs (*Cynomys ludovicianus*) from 13 colonies from Janos, Chihuahua, Mexico. *Cytb* mitochondrial DNA (mtDNA): n = sample size; h = haplotype number; Hd = haplotypic diversity, and π = nucleotide diversity. Microsatellite nuclear markers: n = sample size; A = allelic richness with rarefaction; H_E = expected heterozygosity; H_O = observed heterozygosity, and F_{IS} = inbreeding coefficient. The colony that showed the highest mitochondrial and nuclear genetic variation was EC, and the colonies that had the smallest values of genetic variation were UN for nuclear microsatellites and OS for mtDNA.

Colony name	Relative density (individuals/ha)[a]	Population size (area[individuals/ha])[b]	*Cytb*					Microsatellites					
			n	h	Hd	π	F_{ST}[c]	n	A	H_O (*SD*)	H_E (*SD*)	F_{IS}	G''_{ST}[c]
AB	39	159.9	11	3	0.472	0.0005	0.091	11	2.4	0.309 (0.16)	0.448 (0.15)	0.321[d]	0.216
CJ	8.6	55.9	7	2	0.285	0.0003	0.401	8	2.2	0.375 (0.17)	0.499 (0.10)	0.262[d]	0.232
PP	16.4	132.8	14	2	0.439	0.0004	0.172	15	2.6	0.547 (0.15)	0.574 (0.07)	0.049	0.103
LC	8.6	256.3	8	2	0.535	0.0005	0.101	10	2.4	0.380 (0.27)	0.527 (0.05)	0.291[d]	0.258
UN	5.6	203.2	6	2	0.333	0.0003	0.041	6	1.6	0.233 (0.29)	0.276 (0.25)	0.167	0.395
MO	2.6	185.4	9	2	0.5	0.0013	0.329	9	2.4	0.433 (0.20)	0.510 (0.13)	0.158	0.098
OS	1.35	333	14	2	0.143	0.0001	0.103	14	2.3	0.436 (0.17)	0.528 (0.09)	0.181[d]	0.096
EA	8.8	2,682.1	11	2	0.545	0.0005	0.046	11	2.8	0.509 (0.13)	0.606 (0.07)	0.167[d]	0.032
PV	6.7	2,082.5	9	2	0.5	0.0009	0.031	9	2.4	0.500 (0.26)	0.556 (0.11)	0.107	0.185
BA	3	1,202.1	15	4	0.733	0.0032	0.029	15	2.6	0.447 (0.20)	0.572 (0.12)	0.225[d]	0.108
LB	9.07	21,819.7	17	5	0.639	0.001	0.147	16	2.5	0.475 (0.20)	0.543 (0.07)	0.128	0.07
MV	8.7	28,319.8	14	3	0.473	0.0007	0.023	14	2.6	0.464 (0.16)	0.565 (0.07)	0.197[d]	0.083
EC	3.5	21,146.9	22	3	0.654	0.0038	0.214	22	3.0	0.514 (0.14)	0.618 (0.09)	0.172[d]	0.005
All	8.5	111,533.6	157	8	0.704 (0.03)	0.0016 (0.001)	0.236	160	3.4 (0.8)	0.449 (0.11)	0.525 (0.11)	0.18[d]	0.272 (0.06)

[a] Relative density data provided by C. Sánchez-Giraldo (Sánchez-Giraldo 2012) and R. Ávila-Flores (Ávila-Flores 2009).

[b] Colony area according to Pacheco et al. (2009).

[c] Colony genetic differentiation estimated through pairwise F_{ST} for *Cytb* sequences, and G''_{ST} for nuclear microsatellite loci comparing each colony to the rest of the sample.

[d] Significant departures from Hardy–Weinberg equilibrium ($P < 0.05$).

used an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, California) with the following program: 3 min at 94°C, then 35 cycles of 30 s at 96°C, 60 s at 52°C, and 120 s at 72°C followed by 240 s at 72°C and a final step of 4°C.

The presence, size, and quality of polymerase chain reaction products were confirmed by 1% agarose gel electrophoresis. All polymerase chain reaction products were sequenced at the UWHTSeq FinchLab, Washington University (www.htseq.org).

Polymerase chain reaction for microsatellites.—Ten nuclear microsatellite loci specifically designed for *C. ludovicianus* (A2, A8, A101, A104, A119, C116, D1, D2, D115, and D120—Jones et al. 2005) were polymerase chain reaction amplified in a 20-µl reaction volume using 10 ng/µl of target DNA, 1× buffer, 0.4 mM of deoxinucleoside triphosphates, 0.8 µM of forward primer labeled with HEX fluorescence (Invitrogen), 0.8 µM of reverse primer (Invitrogen), 2–3.75 mM of $MgCl_2$, and 2 U/µl of *Taq* DNA polymerase (Promega). We used an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems) with the following program: 94°C for 180 s, followed by 35 cycles at 94°C for 60 s, annealing temperature as reported by Jones et al. (2005) for each primer for 30 s, and 72°C for 30 s, followed by 240 s at 72°C, and a final step of 4°C. All polymerase chain reactions included a negative control to detect possible contamination. Polymerase chain reaction products were confirmed by electrophoresis in 1.5% agarose gels. The products were dried (SpeedVac Jouan REC10.10.-RCT0; Thermo Scientific, Waltham, Massachusetts) and genotyped in an ABI Prism 3100 sequencer (Applied Biosystems).

Data analyses for mtDNA.—Sequences were assembled using the program *consed* 6.0 (Ewing et al. 1998; Gordon et al. 1998), which displays the quality of each base on the sequence and checked by eye and aligned using BioEdit version 7.1.3 (Hall 1999). We performed a BLAST in GenBank to corroborate that obtained sequences corresponded to the black-tailed prairie dog *Cytb* region. A genealogy for the mitochondrial sequences was constructed to depict relationships among haplotypes; we used the maximum-likelihood method with the approximate likelihood ratio test and bootstrap support values implemented in PhyML (Guindon and Gascuel 2003), including *Cynomys gunnisoni* as outgroup (AF157930—Harrison et al. 2003). Two *Cytb* sequences of *C. ludovicianus* (AF157892 and AF157890) and 3 *Cytb* sequences of *C. mexicanus* (AF157841, AF157842, and AF157847—Harrison et al. 2003) downloaded from GenBank also were included to build the genealogy. jModelTest 0.1.1 (Posada 2008) was used to estimate the substitution model before using PhyML. All analyses were performed excluding sites with missing data. To further explore the relationships between haplotypes, a minimum spanning network at 95% confidence level was constructed using TCS 2.1 (Clement et al. 2000).

We estimated DNA sequence genetic variation (segregating sites [*S*], haplotype diversity [*Hd*], and nucleotide diversity [π]) with the program DnaSP version 5 (Librado and Rozas 2009). To assess mitochondrial genetic structure and genetic differentiation, analysis of molecular variance (AMOVA) and pairwise F_{ST} were estimated with the program Arlequin version 3.0 (Weir and Cockerham 1984; Excoffier et al. 1992, 2005). Isolation by distance was tested through a Mantel test using 9,999 permutations with the program R (R Development Core Team 2009) ade4 library (Dray and Dufour 2007), using the multiple regressions and the linear geographic distances between colonies. Geographic distances were obtained using the mean capture site coordinate per colony with the Geographic Distance Matrix Generator version 1.2.3 (Ersts 2011).

To test for the effect of habitat fragmentation and genetic drift on population differentiation, pairwise F_{ST}-values between each individual colony and the rest of the population were estimated using DnaSP version 5 (Librado and Rozas 2009). A linear regression was performed between F_{ST}-values and ecological data ($\log_{10}$ of colony area and $\log_{10}$ of population size) obtained from the literature (Ávila-Flores 2009; Pacheco et al. 2009; Sánchez-Giraldo 2012), using R (R Development Core Team 2009). Population size was estimated through reported values of relative density (individuals/ha—Ávila-Flores 2009; Sánchez-Giraldo 2012) multiplied by colony area estimated by Pacheco et al. (2009).

Data analyses for microsatellites.—Allele frequencies were determined by direct calculation. Traditional genetic variation estimates (expected heterozygosity [H_E] and observed heterozygosity [H_O]) were obtained with programs GENEPOP version 4.0 (Raymond and Rousset 1995) and Arlequin version 3.0 (Excoffier et al. 2005). We also estimated allelic richness with correction for sample size through rarefaction using the software HP-Rare 1.0 (Kalinowski 2004, 2005). We performed an exact test for Hardy–Weinberg equilibrium per locus using Markov chain Monte Carlo with 10,000 dememorizations, 20 batches, and 5,000 iterations per batch, and estimated deviations from Hardy–Weinberg equilibrium for each colony through F_{IS} (Weir and Cockerham 1984), as well as linkage disequilibrium (LD) using the EM algorithm present in the program GENEPOP version 4.0 (Raymond and Rousset 1995). To control for artifacts associated with the presence of null alleles, the Brookfield method (Brookfield 1996) as implemented in the software MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used. Moreover, estimates of F_{ST} confidence intervals considering and without considering null alleles were estimated and compared using the program FreeNA (Chapuis and Estoup 2007).

Genetic structure was examined using AMOVA in Arlequin version 3.0 (Excoffier et al. 1992, 2005), with 1,000 repetitions and confidence intervals based on 20,000 repetitions. In addition, Meirmans and Hedrick (2011) reported a dependency on the amount of within-population variation and the maximum value of any genetic structure and genetic differentiation estimate (G_{ST}, F_{ST}, R_{ST}, θ, and ϕ_{ST}), so Hedrick's standardized G_{ST} (G''_{ST}—Meirmans and Hedrick

2011) also was estimated with the program GENODIVE 2.0b21 (Meirmans and Van Tienderen 2004), applying the default values and 999 iterations to obtain significance values. This genetic structure measure is corrected by the maximum heterozygosity (H_{MAX}), therefore providing an unbiased estimate. The software *Structure* 2.2 (Pritchard et al. 2000) was used as an alternative approach to explore genetic structure. The program employs a model-based Bayesian analysis that allows groups to be clustered without regard to their original sampling site. We performed and analyzed previous *Structure* 2.2 runs in order to determine the quantity of burn-in chains and Markov chain Monte Carlo simulations that maximize the results' posterior likelihood. These tests determined that 250,000 burn-in chains and 500,000 Markov chain Monte Carlo simulations with 30 repetitions for each K (number of clusters), where $K = 15$, were adequate to maximize posterior distributions. We considered the admixed populations and correlated allelic frequency models, because colonies share common ancestry and there is gene flow between them. Finally, to determine the optimal number of clusters (K) the ΔK test proposed by Evanno et al. (2005) as estimated with *Structure* HARVESTER (Earl and von Holdt 2011) was implemented.

Genetic differentiation between colonies was estimated through pairwise R_{ST} (Weir and Cockerham 1984; Holsinger and Weir 2009) obtained with the program Arlequin version 3.0 (Excoffier et al. 2005) using the default values provided by the program and 100 iterations to obtain significance values. Additionally, between-colony pairwise G''_{ST}-values (Meirmans and Hedrick 2011) were estimated using the program GENODIVE 2.0b21 (Meirmans and Van Tienderen 2004), applying the default values provided by the program and 999 iterations to obtain significance values.

Isolation by distance was tested through a Mantel test with 2,000 permutations in the program FSTAT version 2.9.3 (Goudet 2001), using the multiple regressions and the linear geographic distances between colonies.

To test for the effect of habitat fragmentation and genetic drift on population structure, pairwise G''_{ST}-values between each individual colony and the rest of the population were obtained using GENODIVE 2.0b21 (Meirmans and Van Tienderen 2004). A linear regression was performed between these G''_{ST}-values and ecological data ($\log_{10}$ of colony area and $\log_{10}$ of population size) obtained from the literature and estimated as stated above, using the R (R Development Core Team 2009) ade4 library (Dray and Dufour 2007).

Results

Mitochondrial DNA.—The DNA for 1,140 bp of the mitochondrial *Cytb* region of 157 samples was sequenced. BLAST analysis on GenBank corroborated that the sequences obtained matched black-tailed prairie dog (*C. ludovicianus* and *C. mexicanus*; subgenus *Cynomys*) *Cytb* sequences. In terms of sequence composition, 1,124 were invariable sites, 13 were segregating sites, and 3 sites were excluded because of missing data. Moreover, of the 13 mutations found, 9 were synonymous substitutions and 4 (sites 695, 700, 895, and 905) were replacement substitutions. However, 5 sites were potentially parsimony informative. Eight distinct haplotypes (A–H) were found and sequences were deposited in GenBank with accession numbers JQ885584, JQ885585, JQ885586, JQ885587, JQ885588, JQ885589, JQ885590, and JQ885591 (Supporting Information S1, DOI: 10.1644/12-MAMM-A-099.S1).

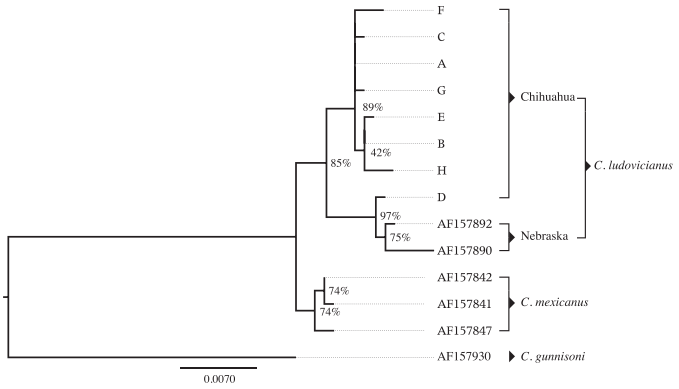


Fig. 2.—Maximum-likelihood genealogy from Janos, Chihuahua, Mexico, black-tailed prairie dog (*Cynomys ludovicianus*) cytochrome-*b* (*Cytb*) haplotypes (A–H) with bootstrap branch support values. Values near nodes are bootstrap values greater than 40%. Other sequences used were from Harrison et al. (2003) and obtained from GenBank.

Regarding the phylogenetic relationship between haplotypes, the genealogy obtained through maximum likelihood (Fig. 2) depicted that haplotype D is more related to the haplotypes of *C. ludovicianus* from Nebraska (Harrison et al. 2003) than to the rest of the haplotypes found in the population. The other 7 haplotypes constitute a monophyletic group. Branch bootstrap values gave good support to the external nodes, but there was not good resolution on the phylogenetic relationship between haplotypes A, B, C, E, F, G, and H, which were closely related. Haplotypes from *C. ludovicianus* and *C. mexicanus* formed 2 well-differentiated monophyletic groups in the genealogy. The haplotype network, in agreement with the maximum-likelihood genealogy, depicted 1 group of closely related haplotypes. Haplotype D was the most divergent (Supporting Information S2, DOI: 10.1644/12-MAMM-A-099.S2).

Haplotypes A and B were the most common, found in 46.5% and 24.8% of the individuals throughout the colonies. In addition, 3 haplotypes (C, D, and E) represented 12.1%, 5.7%, and 5.1% of the amplified samples. The rest of the haplotypes (F–H) accounted for 5.7% of the samples and were found in only 1 colony each (F: MO, $n = 4$; G: AB, $n = 2$; and H: LB, $n = 1$). Furthermore, none of the colonies was fixed for only 1 haplotype (Table 1; Fig. 1). Haplotype diversity for the 13 sampling colonies was 0.704 (Table 1). Mean nucleotide diversity was 0.00162 ($SD = 0.001$).

Genetic structure analysis points toward significant overall mitochondrial genetic structure ($F_{ST} = 0.271$, $P < 0.0001$;

Table 2.—Components of an analysis of molecular variance (AMOVA—Excoffier et al. 1992) of mitochondrial DNA cytochrome-*b* sequences (mtDNA AMOVA; F_{ST}) and nuclear microsatellite loci (microsatellite AMOVA; ϕ_{ST}) from 13 colonies of black-tailed prairie dog (*Cynomys ludovicianus*) from Janos, Chihuahua, Mexico. Significant values of genetic structure (F_{ST} and ϕ_{ST}) and significant inbreeding coefficients (ϕ_{IS}, R_{IS}, ϕ_{IT}, and R_{IT}) values were found within the population.

Source of variation	% variation	*F* coefficients	
mtDNA AMOVA			
Among colonies	27.1	F_{ST}	0.271
Within colonies	72.8		
Microsatellite AMOVA			
Among colonies	10.3	ϕ_{ST}	0.103
Within colonies	16.1	ϕ_{IS}	0.180
Between individuals within colonies	73.6	ϕ_{IT}	0.264

Table 2). The AMOVA revealed that there was more within-colony (72.8%) than among-colony (27.1%) variation.

Analysis of genetic differentiation (pairwise F_{ST} [Supporting Information S3, DOI: 10.1644/12-MAMM-A-099.S3]) showed that 3 colonies were highly differentiated (significant pairwise F_{ST} for all comparisons; CJ, MO, and EC), 4 colonies had intermediate differentiation (> 50% pairwise F_{ST} comparisons were significant), and 6 colonies presented low values of genetic differentiation (≤ 50% pairwise F_{ST} comparisons were significant). Minimum estimated distances between colonies ranged from 1.4 to 30.8 km and isolation by distance was nonsignificant ($P = 0.137$). Furthermore, *Cytb* genetic differentiation was not related to colony area or population size ($R^2_{11} = 0.087$, $P = 0.328$, and $R^2_{11} = 0.136$, $P = 0.215$, respectively).

Microsatellites.—All 10 analyzed loci were polymorphic (2–4 alleles per locus), rendering a total of 34 alleles. Furthermore, all loci were polymorphic for all black-tailed prairie dog colonies, except UN, which was monomorphic for 4 of the loci. Tests for artifacts associated with microsatellite loci did not detect the presence of null alleles or linkage disequilibrium (data not shown). Nevertheless, 3 of 10 loci deviated from Hardy–Weinberg equilibrium (A101, A104, and C116), and 8 of the 13 sampled colonies showed heterozygote deficiency (Table 1).

In terms of genetic variation, allelic richness (*A*) estimated through rarefaction resulted in 1.6–3.0 mean alleles per colony, and no private alleles were found. Mean genetic diversity (H_E) was 0.525 ($SD = 0.11$), with values ranging from 0.276 ($SD = 0.25$) to 0.618 ($SD = 0.09$).

Nuclear genetic structure ($F_{ST} = 0.103$, $P < 0.0001$; $G''_{ST} = 0.272 \pm 0.06$ *SE*, $P = 0.001$) and fixation index ($F_{IS} = 0.18$ and $F_{IT} = 0.264$; $P < 0.0001$) values were significant (Table 2), with the highest percentage of variation distributed between individuals within colonies (73.6). Once correcting for the no-admixture prior because of the presence of 2 mitochondrial lineages in the population and setting parameters as described in methods, the test of Evanno et al. (2005) indicated the presence of 2 clusters ($K = 2$ [Supporting Information S4, DOI: 10.1644/12-MAMM-A-099.S4]). From the total number of individuals 56.9% were assigned within 95% confidence to either cluster. This result suggests high admixture within the population. Within the assigned individuals, most samples from colonies CJ, LC, and UN were assigned to cluster 1 (100% from CJ and LC, and 83.3% from UN), whereas cluster 2 was composed of individuals from all remaining colonies. When examining genetic structure within each cluster, cluster 1 presented low genetic structure ($F_{ST} = 0.038$), and cluster 2 showed a value closer to that estimated for the entire sample ($F_{ST} = 0.117$).

Finally, genetic differentiation was significant for most pairwise comparisons (Supporting Information S5, DOI: 10.1644/12-MAMM-A-099.S5) and isolation by distance, estimated through a Mantel test, was nonsignificant ($r = –0.189$, $P = 0.095$). Genetic differentiation (G''_{ST}) was significant and negatively correlated to colony area ($R^2_{11} = 0.333$, $P = 0.023$; Fig. 3A) and population size ($R^2_{11} = 0.360$, $P = 0.03$; Fig. 3B).

Discussion

The black-tailed prairie dog population from Janos possesses certain characteristics (large size, absence of plague, protected status, and peripheral and declining population) that allowed the proposition of 2 distinct predictions regarding its genetic variation and genetic structure. We expected to find that the black-tailed prairie dogs from Janos possess a relatively high level of genetic variation and low genetic structure because of the large size of the population, the absence of plague, and protected status. Nevertheless, there are other factors about this population such as recent population decline and peripheral effects (small effective population size and isolation) that could result in relatively low genetic variation and an increase in genetic structure.

Our results show that despite the fact that the black-tailed prairie dog population of Janos is peripheral, its genetic diversity is comparable to that reported for other parts of the range, and this population may serve, not only because of its ecological importance (Ceballos et al. 2005) but because of its genetic variation, as a refuge for the species in the long term. As expected, there was significant genetic structure for both molecular markers, significantly more within- than among-colony variation for both markers in accordance with black-tailed prairie dog social organization, and higher F_{ST}-value for maternally inherited DNA than for biparentally inherited DNA related to female philopatry and male-biased gene flow. Finally, colony area and population size had a significant negative correlation to genetic differentiation for nuclear microsatellite loci, but not for *Cytb*. These results point toward an increased effect of genetic drift in smaller and less-dense colonies because of recent habitat fragmentation only on nuclear markers, whereas genetic differentiation for maternally inherited DNA can be related to female philopatry.

The genealogic relationship between *Cytb* haplotypes implies that the black-tailed prairie dogs of Janos represent a population where admixture or introgression, or both, or

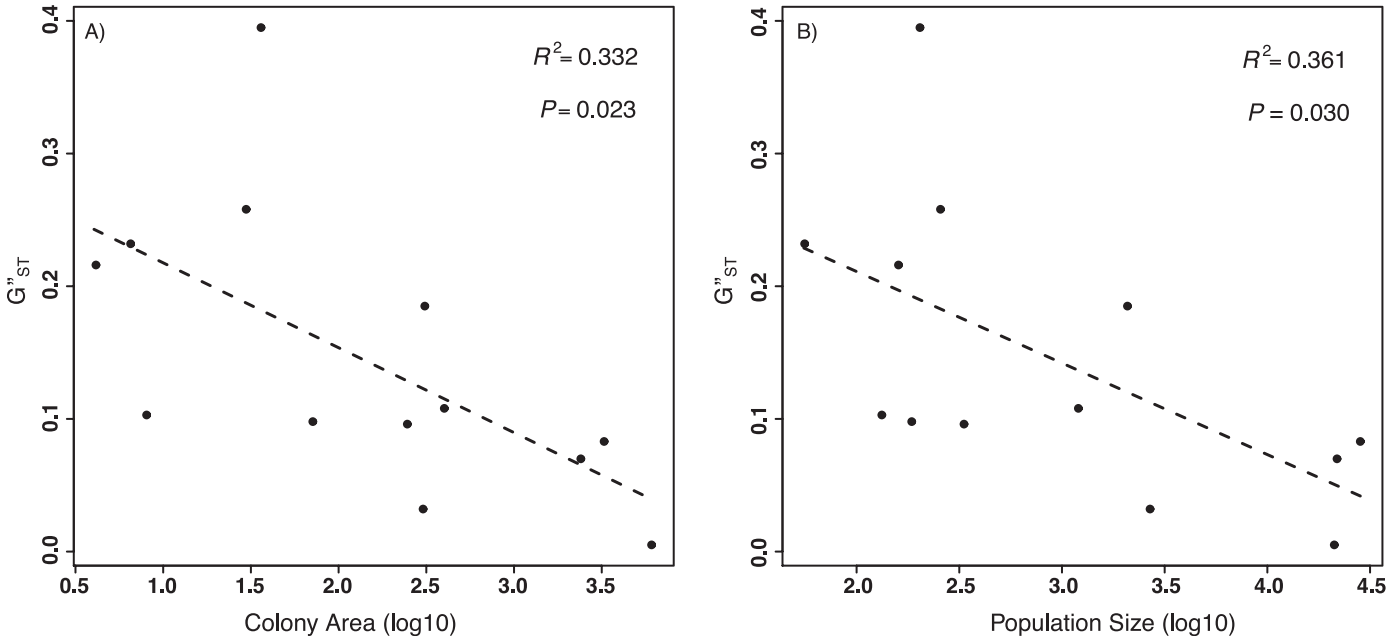


Fig. 3.—Linear regression for black-tailed prairie dog (*Cynomys ludovicianus*) microsatellite genetic differentiation (pairwise G''_{ST}—Meirmans and Hedrick 2011). A) Colony area ($\log_{10}$) and B) population size ($\log_{10}$). Significance (R^2 and *P*-value) for each regression is depicted on the upper right corner of each graph. Both regressions were negative and significant.

incomplete lineage sorting between 2 mitochondrial lineages has occurred. Studies addressing the phylogeographic history of black-tailed prairie dog populations throughout their geographic range are, nevertheless, absent. Therefore, data from Sciuridae phylogeographic studies, conducted on a larger geographical scale where a great genetic subdivision could be observed (Hewitt 2001), were used for comparison (Table 3). Nucleotide diversity was lower in Janos than reported for other species, but the presence of 8 haplotypes and 2 lineages in a small area of the species distribution suggests a moderate amount of variation. Variation at this locus relates to maternal inheritance of mtDNA and female philopatry that contribute to large effective female population sizes, which is reflected in high genetic variation (Chesser and Baker 1996).

Nuclear genetic diversity (H_E) was neither significantly lower when compared with other black-tailed prairie dog populations throughout their range or with other prairie dog species (*C. parvidens* and *C. gunnisoni* [Table 3; Haynie et al. 2003]). These results imply that the genetic diversity is within the range reported for other colonies and other members of the genera. This contrasts with the concept that peripheral populations possess less genetic variability than populations located at the center of the distribution (Eckert et al. 2008).

Relative to the nonsignificant F_{IS}-values reported for colonies in Colorado, the values from Janos were significant and similar to those found in Montana. Previous studies have reported that the presence of coteries increases genetic structure and F_{IS}-values (Chesser 1983, 1991a, 1991b; Dobson et al. 1997, 1998, 2004). In addition, significant F_{IS}-values have been associated with the Wahlund effect and population bottlenecks (Chesser 1983; Sugg et al. 1996). Despite the fact that trapping was planned to reduce this type of sampling bias, the Wahlund effect cannot be completely discarded. To distinguish between significant F_{IS}-values related to habitat fragmentation and artifacts associated to the Wahlund effect, sampling family groups within colonies should be considered in further research.

Higher global F_{ST}-values for *Cytb* than for microsatellite DNA markers (0.233 and 0.103) are consistent with female philopatry and male-biased gene flow (Hoogland 1996). The presence of male-biased gene flow is further corroborated by high admixture between the clusters obtained with *Structure* (Pritchard et al. 2000) and the lack of private alleles for nuclear loci. Nevertheless, significant pairwise genetic differentiation between colonies contrasts with this idea and, together with a major portion of the genetic variation within colonies, may relate to differential allele fixation within each family group (Dobson et al. 2004).

Consistent with previous findings (Chesser 1983; Hulová and Sedlácek 2008), the Mantel test was nonsignificant, suggesting no isolation by distance for both types of molecular markers. Similarly, lack of isolation by distance in prairie dogs and other sciurids has been linked to the effect of genetic drift, founder events, and mutation.

Lomolino and Smith (2001) stated that colony area is the main factor affecting extinction risk in plague-free black-tailed prairie dog populations and in Janos nuclear microsatellite genetic structure had a negative correlation with population size and colony area. Contrasting results for each molecular marker support the concept of genetic differentiation in microsatellites because of habitat fragmentation, and a stronger effect of genetic drift in smaller and less-dense populations

Table 3.—Population genetic studies performed on different species belonging to the family Sciuridae, including *Cynomys ludovicianus*. A) Data for cytochrome-*b* (*Cytb*), including sample size (*n*), number of localities, haplotype number (*h*), nucleotide diversity (π), and haplotype diversity (*Hd*). B) Data for microsatellites, including sample size (*n*), number of microsatellite loci, allelic richness (*A*), genetic diversity (expected heterozygosity [H_E]), Student's *t*-test *P*-value, inbreeding coefficient (F_{IS}), and genetic structure (F_{ST}).

Reference	Species	Study site	*n*	No. localities	*h*	π (*SD*)	*Hd* (*SD*)	No. loci	*A* (*SD*) (alleles per locus)	H_E (*SD*)	Student's *t*-test *P*-value	F_{IS}	F_{ST}
A) *Cytb*													
Bell et al. 2010	*Xerospermophilus tereticaudus*	Mojave and Sonora deserts	38	14	16	0.00789 (0.0011)[a]	—						
	X. mohavensis	Mojave Desert	46	11	8	0.00171 (0.00026)[a]	—						
Cook et al. 2010	*Spermophilus parryii*	Kodiak and Aleutian archipielagos and Kavalga Island	75	12	18	0.0072 (0.00026)[a]	—						
Grill et al. 2009	*Sciurus vulgaris*	Europe	83	5	21	0.00672 (0.00114)	0.736 (0.049)						
Moncrief et al. 2010	*Sciurus niger*	Eastern North America	102	18	22	0–0.007	—						
Current analysis	*C. ludovicianus*	Chihuahua, Mexico	152	1	8	0.0016 (0.001)	0.704 (0.03)						
B) Microsatellite													
Haynie et al. 2003	*C. gunnisoni*	Arizona	378					7	4.29 (1.7) (2–6)	0.49 (0.26)	0.435	—	—
Haynie et al. 2003	*C. parvidens*	Utah	145					7	3 (1) (2–4)	0.33 (0.3) (1996)	0.215	—	—
			225							0.34 (0.3) (1997)	0.195	—	—
Roach et al. 2001	*C. ludovicianus*	Colorado (Pawnee National Park)	129					7	3.9 (0.61)[b] (4–13)	0.602 (0.09)[b]	0.069	0.02	0.12
Jones and Britten 2010	*C. ludovicianus*	Montana	112					14	3.4 (1.16) (2—6)	0.54 (0.2)	0.971	0.17	0.12–0.19
Magle et al. 2010	*C. ludovicianus*	Colorado (urban)	101					10	3.1 (0.63)[b] (2.4–3.9)	0.524 (0.1)[b]	0.69	0.007	0.24
Sackett et al. 2012	*C. ludovicianus*	Colorado (urban)	510					11	9.3 (6–14)	0.663	—	—	0.109
Current analysis	*C. ludovicianus*	Chihuahua, Mexico	160					10	3.4 (0.8) (2–4)	0.53 (0.11)	—	0.18	0.10

[a] Nucleotide diversity values estimated through popset obtained from GenBank using DnaSP version 5.0 (Librado and Rozas 2009) when information was not available.

[b] Mean and standard deviation estimated from reported data when information was not available.

(Frankham et al. 2002), whereas female philopatry had a stronger effect on mtDNA.

Black-tailed prairie dogs from Janos possess a good amount of genetic variation despite being a peripheral and declining population. Although recent habitat fragmentation has affected genetic differentiation between colonies, social organization and its strong influence on genetic structure has preserved the genetic variation within colonies. To further assess the importance of this population and determine if this peripheral population is genetically divergent from core populations we need to go from local to regional scales. Fundamental information for understanding the evolutionary and ecological relationships between locations throughout the species distribution is needed to advance further black-tailed prairie dog conservation.

Resumen

En Norte América, la fragmentación del hábitat inducida por el hombre ha provocado una reducción considerable de las poblaciones de perros llaneros de cola negra (*Cynomys ludovicianus*). Aunque el área ocupada por las colonias de perros llaneros en Chihuahua, al noroeste de México, también se ha reducido recientemente, esta población ha sido menos afectada por la perturbación humana. Se analizaron secuencias del *citocromo b* (*Cytb*) y 10 microsatélites nucleares en 13 colonias para poner a prueba si la población de perros llaneros de cola negra de Janos posee niveles de variación genética relativamente altos y baja estructura genética debido a su gran tamaño, ausencia de peste y estatus de protección; o si la disminución poblacional reciente y los efectos periféricos han resultado en la presencia de baja variación y alta estructura genética. Los análisis sugieren que la variación genética mitocondrial es moderada con relación a otros sciúridos y que la variación genética nuclear no es significativamente distinta en relación a otras poblaciones de perros llaneros. Además, de acuerdo con la organización social de los perros llaneros de cola negra, la estructura genética entre poblaciones locales fue significativa y la variación hacia el interior de las colonias fue mayor que entre las colonias para ambos marcadores. Con relación a la filopatría de las hembras y la dispersión sesgada hacia los machos, la F_{ST} fue mayor para el ADN mitocondrial que para el ADN nuclear. Finalmente, para los marcadores nucleares se encontró una correlación negativa entre la diferenciación genética y el área de la colonia y el tamaño poblacional, lo que sugiere un incremento en el efecto de la deriva génica en las colonias más pequeñas y menos densas como resultado de la fragmentación del hábitat reciente. En conclusión, la estructura social y el patrón de dispersión ha mantenido la variación genética de los perros llaneros de cola negra de Janos a pesar de considerarse una población periférica y en declive.

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Supporting Information

Supporting Information S1.—Sequence name, GenBank accession number, sample size (*n*), and segregating sites (base pairs) for the 8 cytochorme-*b* (*Cytb*) haplotypes found in black-tailed prairie dogs (*Cynomys ludovicianus*) from Janos, Chihuahua, Mexico. There were 13 segregating sites, of which 9 were synonymous changes (sites 126, 198, 216, 486, 501, 648, 699, 912, and 1047) and 4 were amino acid replacement changes (sites 695, 700, 895, and 905). Haplotype D presented the highest number of differences (9) with respect to the most common nucleotide in the other haplotypes.
Found at DOI: 10.1644/12-MAMM-A-099.S1

Supporting Information S2.—Haplotype network overlaid on sampled black-tailed prairie dog (*Cynomys ludovicianus*) colonies from Janos, Chihuahua, Mexico. On top is an unweighted pair-group method using arithmetic means (UPGMA) phenogram depicting genetic differentiation estimated through Nei's genetic distance. The size of each pie graph represents the frequency of each haplotype (letters A–H); texture corresponds to each cluster of the UPGMA phenogram.
Found at DOI: 10.1644/12-MAMM-A-099.S2

Supporting Information S3.—Genetic differentiation for 13 black-tailed prairie dog (*Cynomys ludovicianus*) colonies from Janos, Chihuahua, Mexico, obtained through pairwise F_{ST}-values for cytochrome-*b* (*Cytb*) sequences.
Found at DOI: 10.1644/12-MAMM-A-099.S3

Supporting Information S4.—Bayesian clustering analysis of black-tailed prairie dogs (*Cynomys ludovicianus*) from Janos, Chihuahua, Mexico, as detected by *Structure* 2.2. Individuals from 13 colonies grouped in 2 genetic clusters ($K = 2$). White represents cluster 1, and dark gray represents cluster 2. Fifty percent of individuals show admixture, and most individuals of colonies CJ, LC, and UN were assigned to cluster 1.
Found at DOI: 10.1644/12-MAMM-A-099.S4

Supporting Information S5.—Genetic differentiation for 13 black-tailed prairie dog (*Cynomys ludovicianus*) colonies from Janos, Chihuahua, Mexico, obtained through 10 nuclear microsatellite loci. Below diagonal are pairwise R_{ST} estimates (* nonsignificant values; $P > 0.05$); above diagonal are Hedrick's standardized G_{ST} (G''_{ST}). High genetic differentiation between colonies was found, and the highest significant values were present in colonies CJ, LC, and UN.
Found at DOI: 10.1644/12-MAMM-A-099.S5

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