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Original Article

Population Genetics of Jaguarundis in Mexico: Implications for Future Research and Conservation

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ABSTRACT The jaguarundi (*Puma yagouaroundi*) is a Neotropical felid that ranges from northern Mexico to South America. The population trend for jaguarundis is declining, yet much remains unknown about their ecology. We live-trapped 11 jaguarundis during 1991–2004 in Tamaulipas, Mexico, and we collected blood for genetic analyses. Our objectives were to 1) estimate neutral diversity using microsatellite and mitochondrial (mtDNA) markers, 2) estimate potentially adaptive diversity in coat coloration via the melanocortin-1 receptor gene (MC1R), and 3) provide recommendations on the use of genetic markers for noninvasive studies. We observed moderate levels of heterozygosity ($H_E = 0.49$, SD = 0.22) and number of alleles ($A = 4.00$, SD = 1.65) at 12 feline microsatellite loci. The probability of identifying 2 different jaguarundis as the same individual was low using only 4–7 loci ($P_{(ID)} < 0.001$ and $P_{(ID)sib} < 0.01$, respectively). We observed one mtDNA haplotype, indicating no mtDNA diversity. However, we documented diversity with the MC1R assay. The frequency of the melanistic mutation (gray phenotype) was 0.33, whereas, the frequency was 0.67 for the ancestral allele (red phenotype). Microsatellite diversity of our sampled jaguarundis was less than that of sympatric populations of ocelots (*Leopardus pardalis*) in Tamaulipas; however, mtDNA diversity was much lower. The frequency of the melanistic mutation was lower in our sample than was previously reported in captive jaguarundis. Our microsatellite loci provided adequate diversity to implement noninvasive genetic tools to better understand the ecology of the elusive jaguarundi. To our knowledge, this work is the first genetic evaluation of wild jaguarundis and provides baseline information for future research and conservation. © 2013 The Wildlife Society.

KEY WORDS felidae, jaguarundi, melanism, melanocortin-1 receptor, microsatellite loci, mitochondrial DNA, *Puma yagouaroundi*.

The jaguarundi (*Puma yagouaroundi*) is a Neotropical felid that is distributed from northern Mexico to Argentina (de Oliveira 1998, Caso et al. 2008). The population trend for jaguarundis is declining, and they are of conservation concern in Argentina and Mexico (Caso et al. 2008). These felids are medium-sized, and exhibit elusive, territorial, and diurnal behavior (Konecny 1989, de Oliveira 1998). Additionally, jaguarundis display characteristics consistent with generalist species in that they feed on multiple prey items (Tófoli et al. 2009, Bianchi et al. 2011, Silva-Pereira et al. 2011), and occupy a variety of habitats (Konecny 1989, Caso 1994, de Oliveira 1998, Caso et al. 2008).

Much remains unknown about the ecology of jaguarundis (Tófoli et al. 2009), largely because they are extremely difficult to capture (Caso 1994). Noninvasive genetic techniques are a useful alternative for researching and monitoring difficult-to-capture species (Taberlet et al. 2001, Waits and Paetkau 2005, Beja-Pereira et al. 2009, Sugimoto et al. 2012) such as jaguarundis (e.g., Roques et al. 2011). However, noninvasive methods rely on the availability of DNA markers, which need to exhibit diversity to be useful. We initiated a field study in Tamaulipas, Mexico, to evaluate jaguarundi genetic characteristics. Our objectives were as follows: 1) estimate neutral diversity using microsatellite and mitochondrial (mtDNA) markers, 2) estimate potentially adaptive diversity at a gene associated with melanism

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(melanocortin-1 receptor [MC1R]; Eizirik et al. 2003), and 3) provide recommendations on the use of genetic markers for noninvasive studies. Previous genetic work on jaguarundis was limited to captive individuals (Eizirik et al. 2003, Moreno et al. 2006). To our knowledge, this study is the first genetic evaluation of free-ranging jaguarundis, thus providing baseline information.

STUDY AREA

We trapped jaguarundis on Los Ebanos Ranch in Tamaulipas, Mexico, during 1991–2004. Los Ebanos Ranch was located adjacent to the Gulf of Mexico, and the primary land use was cattle ranching (Caso 1994). Woody vegetation had been removed in a strip pattern for cattle forage production; however, native plant communities remained (Fig. 1). Native vegetation was characterized as low tropical forest (Caso 1994). Annual temperatures on Los Ebanos Ranch ranged from approximately −5° C to 34° C, and mean annual precipitation was approximately 92 cm (Caso 1994).

MATERIALS AND METHODS

To capture jaguarundis, we used wire Tomahawk box traps (Tomahawk Live Trap Co., Hazelhurst, WI) with an attachment for live bait (e.g., domestic chickens; *Gallus* spp.). We immobilized captured individuals using 20 mg/kg of ketamine combined with 5 mg/kg of xylazine, or 5 mg/kg of tiletamine–zolazepam (Zoletil 50; Caso 1994). We determined age (i.e., subad, ad) of jaguarundis based on tooth eruption and wear, sexual development, and body size (Caso 1994). We attached very-high-frequency radiocollars, recorded morphological characteristics, and collected blood. We placed whole-blood in lysis buffer (Longmire et al. 1997) and stored it at room temperature until DNA extraction. Our trapping techniques were approved by the Mexican Ministry of Environmental and Natural Resources (SEMARNAT; permit DGVS-10022-2004).

We extracted DNA from blood samples using a commercial kit (DNeasy kit; Qiagen, Valencia, CA), and amplified multiple regions of DNA using 3 separate approaches. First, we amplified a panel of 12 nuclear microsatellite loci (FCA008, FCA035, FCA043, FCA045, FCA077, FCA082, FCA090, FCA096, FCA132, FCA133, FCA176, FCA205) described by Menotti-Raymond et al. (1999). We amplified loci in 10-μl reaction volumes containing 5 μl AmpliTaq Gold PCR Master Mix (Applied Biosystems, Foster City, CA), 0.24 μM of each primer, and 1–2 μl of DNA. Our polymerase chain reaction (PCR) conditions consisted of an initial denaturation at 94° C for 10 minutes, 20 cycles of 94° C for 15 seconds, 55° C for 15 seconds, 72° C for 30 seconds, followed by 35 cycles of 89° C for 15 seconds, 55° C for 15 seconds, and 72° C for 30 seconds, with a final extension of 72° C for 30 minutes. We added PCR product from each individual to a denaturing formamide and size standard mixture (Hi-Di Formamide, GeneScan ROX 500; Applied Biosystems). We then loaded samples onto an ABI 3130xl DNA analyzer (Applied Biosystems) for fragment separation. All sample sets loaded onto the ABI 3130xl contained a positive and negative control to evaluate run consistency and document contamination, if present. We sized fragments using GeneMapper v4.0 (Applied Biosystems).

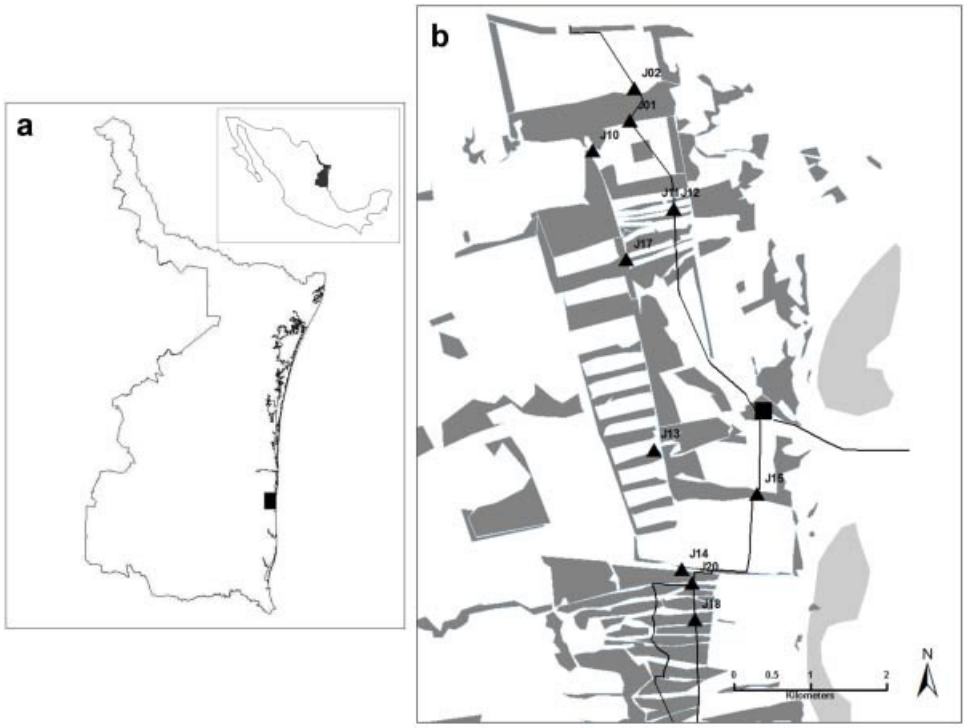

Figure 1. Part (a): Inset is a map of Mexico with Tamaulipas highlighted in black. Larger map is of Tamaulipas with Los Ebanos Ranch indicated by black square. Part (b): Spatial distribution of trapped jaguarundis ($n = 11$) during 1991–2004 on Los Ebanos Ranch. Triangles are trapping locations, square represents Los Ebanos headquarters, and black line is the main road. Dark and light gray patches indicate low tropical forest and salt lagoons, respectively.

Second, we amplified a 437 base-pair segment of the mtDNA control region using primers PAN-OCELOT-F (5′-CTCAACTATCCGAAAGAGCTT-3′), and PAN-OCELOT-R (5′-CCTGTGGAACATTAGGAATT-3′; Jae-Heup et al. 2001, Janečka et al. 2011). We amplified sequences in 25 μl reaction volumes containing 12.5 μl AmpliTaq Gold PCR Master Mix (Applied Biosystems), 0.10 μM of each primer, 1.0 μl of $MgCl_2$, 1.0 μl of bovine serum albumin, and 1.5 μl of DNA. Our PCR conditions consisted of 94° C for 10 minutes followed by 40 cycles of 94° C for 20 seconds, 58° C for 30 seconds, 72° C for 60 seconds, and a final extension at 72° C for 10 minutes. The PCR products were electrophoresed on 1% agarose gels containing ethidium bromide, and viewed using ultraviolet light. We purified PCR products from successful reactions using an enzymatic method (ExoSAP-IT; USB Corporation, Wilmington, MD), which we then used as template for sequencing reactions (BigDye Terminator Cycle Sequencing kit 1.1; Applied Biosystems). We sequenced each sample in both directions on an ABI 3130xl DNA analyzer (Applied Biosystems), and assembled and edited sequences using SEQUENCHER v4.5 (Gene Codes, Ann Arbor, MI). We deposited mtDNA sequences into GenBank (accession no. JX901159–JX901168).

Lastly, we assayed individuals for diversity at the MC1R gene. Jaguarundis display 2 phenotypic variants in coat color, red and gray (de Oliveira 1998, Eizirik et al. 2003). Gray coat color, or melanism, is the nonancestral form and is expressed because of a genetic mutation along the MC1R gene (Eizirik et al. 2003). Specifically, the MC1R-Δ24 mutation is the in-frame deletion that results in abnormal formation of pigment amino acids in jaguarundis (Eizirik et al. 2003). The MC1R assay developed by Eizirik et al. (2003) provides an easy means to detect the MC1R-Δ24 mutation without DNA sequencing. We amplified DNA fragments at the MC1R gene using primers ExtDel-F1 (5′-CTGCACTCGCCCATGTATTA-3′) and ExtDel-R1 (5′-CCACAGACCAGCACGTCAAT-3′; Eizirik et al. 2003), and followed PCR conditions described in Eizirik et al. (2003; table S2); the forward primer was fluorescent-labeled to facilitate detection of the fragment on our automated sequencer. We applied PCR products to a denaturing formamide and size standard mixture (Hi-Di Formamide, GeneScan ROX 500; Applied Biosystems). We then loaded samples onto an ABI 3130xl DNA analyzer (Applied Biosystems) for fragment separation. We sized fragments using GeneMapper v4.0 (Applied Biosystems).

DATA ANALYSIS

We examined our microsatellite data for genotyping errors and estimated multiple measures of genetic diversity. First, we used MICRO-CHECKER v2.2.3 to test for genotyping errors associated with stuttering, large allele dropout, and null alleles (Van Oosterhout et al. 2004). Second, we estimated observed (H_O) and expected heterozygosity (H_E; Nei 1987), and the number of alleles per locus (A) using ARLEQUIN v3.5 (Excoffier and Lischer 2010). Third, we tested Hardy–Weinberg expectations using F_{IS} (Weir and Cockerham 1984), and assessed statistical significance (2-sided) by performing 1,023 permutations of alleles among individuals. Finally, we computed the probability of identity using the unbiased ($P_{(ID)}$) and sibs ($P_{(ID)sib}$) estimators in GIMLET (Valiere 2002). The probability of identity refers to the probability of randomly sampling 2 individuals from a population with the same genotype at multiple loci (Waits et al. 2001). We calculated both $P_{(ID)}$ and $P_{(ID)sib}$ to establish a range of loci that could reliably discern individuals in a sample of unrelated and related individuals (Waits et al. 2001).

We were unable to perform analyses of mtDNA diversity because we observed only one mtDNA haplotype; however, we documented variation with the MC1R assay. We compared the phenotypic coat color of each jaguarundi with our genotypes, and calculated allele frequencies for the MC1R-Δ24 mutation and ancestral allele (Eizirik et al. 2003).

RESULTS

We sampled blood from 11 different jaguarundis (Table 1; Fig. 1), which consisted of 8 gray and 3 red phenotypes (Fig. 2). We were able to discriminate among individuals because radiocollars remained on individuals for the duration of the study. We gathered microsatellite genotypes for all

Table 1. Sex, approximate age, date of capture, coat color phenotype, and melanocortin-1 receptor (MC1R) genotype for 11 jaguarundis sampled during 1991–2004 on Los Ebanos Ranch, Tamaulipas, Mexico. MC1R genotypes follow the notation of Eizirik et al. (2003), whereby Δ24 and + indicate the deletion (i.e., melanistic mutation) and ancestral allele, respectively.

Individual	Sex	Age	Date of capture	Coat color phenotype	MC1R genotype
J1	F	Ad	20 Jul 1991	Gray	Δ24/+
J2	M	Subad	5 Apr 1992	Red	+/+
J10	M	Ad	13 Oct 1995	Gray	
J11	F	Ad	2 Nov 1995	Gray	
J12	M	Ad	4 Nov 1995	Gray	Δ24/+
J13	F	Ad	5 Nov 1995	Gray	Δ24/+
J14	M	Ad	19 Oct 1996	Red	+/+
J15	M	Ad	23 Oct 1996	Gray	Δ24/+
J17[a]	M	Subad	27 Oct 1999	Red	
J18	F	Ad	11 Oct 2000	Gray	
J20	M	Subad	6 Nov 2004	Gray	

[a] Individual in which we did not acquire a sequence of the mitochondrial DNA control region.

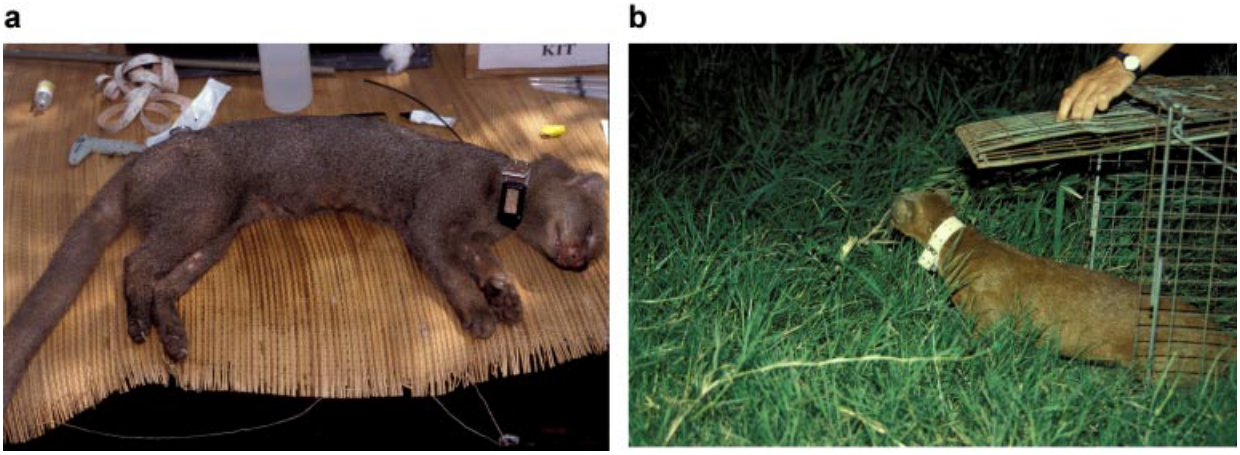

Figure 2. Photos of a gray (Part a) and red (Part b) coat colored jaguarundis from Los Ebanos Ranch, Tamaulipas, Mexico, sampled during 1991–2004. Photos were taken by Arturo Caso.

individuals (2% missing data), and negative controls indicated no contamination. We successfully sequenced the mtDNA control region for 10 individuals. Unfortunately, we were only able to amplify alleles for 6 individuals using the MC1R assay because we exhausted our supply of extracted DNA, and did not have an opportunity to re-extract samples.

The microsatellite panel we screened amplified well using our PCR conditions, and displayed moderate levels of genetic diversity. MICRO-CHECKER (Van Oosterhout et al. 2004) indicated no evidence of genotyping errors due to stuttering or large allele dropout, and suggested that null alleles might be present at only locus FCA133 (Table 2). Observed heterozygosity and H_E ranged from 0% to 73% and 9% to 71%, with a mean of 42% and 49%, respectively (Table 2). Estimates of A ranged from 2 to 8 with a mean of 4.00. We observed a relatively high F_{IS}, which suggests a departure from Hardy–Weinberg expectations, but the estimate was not statistically significant ($F_{IS} = 0.11$, $P = 0.14$). The microsatellite panel appeared to provide adequate diversity to discern individuals with a low probability of error ($P_{(ID)} < 0.001$) using only 4 of the 12 loci (Table 2). However, to discriminate among siblings, our data suggested that 3 additional loci were needed (i.e., 7 of 12) to maintain a low probability of error ($P_{(ID)sib} < 0.01$).

Table 2. Microsatellite loci, allele size range in base pairs (bp), number of alleles (A), and observed (H_O) and expected (H_E) heterozygosity for jaguarundis ($n = 11$) sampled during 1991–2004 on Los Ebanos Ranch, Tamaulipas, Mexico.

Locus	Size range (bp)	A	H_O	H_E
FCA008[a,b]	127–141	5	0.73	0.71
FCA082	237–245	4	0.36	0.40
FCA090	101–105	2	0.00	0.21
FCA132	164–178	2	0.09	0.09
FCA133[c]	138–142	3	0.10	0.53
FCA176[a,b]	213–239	8	0.64	0.65
FCA045	157–161	3	0.18	0.18
FCA035[b]	126–140	4	0.64	0.57
FCA043[a,b]	110–124	5	0.64	0.70
FCA077[a,b]	132–138	4	0.73	0.71
FCA096[b]	198–218	5	0.55	0.53
FCA205[b]	102–108	3	0.45	0.57
Mean		4.00	0.42	0.49
SD[d]		1.65	0.27	0.22

[a] Combination of loci producing a low probability of identification error for a sample of unrelated individuals ($P_{(ID)} < 0.001$).
[b] Combination of loci producing a low probability of identification error for a sample including siblings ($P_{(ID)sib} < 0.01$).
[c] Locus where null alleles may be present based on analyses from MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004).
[d] SD over loci.

Similar to our microsatellite panel, our mtDNA sequences amplified well with our PCR conditions. However, we documented no diversity in our mtDNA sequences in that we observed one mtDNA haplotype. Although we gathered data for only 6 individuals with the MC1R assay, we documented genetic diversity (Table 1). Four of the 6 individuals exhibited a gray phenotype, while the remaining 2 exhibited a red phenotype. All gray individuals were heterozygous with the ancestral allele and the MC1R-Δ24 mutation. The red individuals were homozygous with the ancestral allele. Therefore, the frequency of the MC1R-Δ24 mutation in our sample was 0.33, whereas, the frequency was 0.67 for the ancestral allele.

DISCUSSION

The microsatellite results for our sampled jaguarundis indicated moderate levels of diversity and a high F_{IS} value. Our observed F_{IS} may suggest population genetic structure, or could be an artifact of our geographically restricted sample. Sampling at relatively fine spatial scales can result in spatial autocorrelation among individuals, particularly under common genetic gradients such as isolation-by-distance (Hardy and Vekemans 1999). A subset of our loci (FCA008, 045, 077, 096) were the same used by Moreno et al. (2006) for captive jaguarundis, but our estimates of A were much lower ($\bar{x}A = 4.25$) than estimates from the captive sample ($\bar{x}A = 10.75$; Moreno et al. 2006). However, Moreno et al. (2006) sampled 36 zoo animals from 5 different states in Brazil, which may have inflated estimates. Similarly, our

estimates of H_E and A were lower than estimates from a sympatric population of ocelots (*Leopardus pardalis*; $H_E = 0.64$, $A = 4.64$; Janečka et al. 2011). Many more microsatellites were used in Janečka et al. (2011), making strict comparisons tenuous. A comparative study using similar microsatellite loci for ocelots and jaguarundis could provide insight regarding the similarities or differences between these 2 sympatric species.

In contrast to microsatellite results, we documented no mtDNA diversity for our sampled jaguarundis. We observed only one mtDNA haplotype, while sympatric ocelots exhibited 4 haplotypes with 3 variable sites (Janečka et al. 2011). Geographic extent and sample sizes from both populations were comparable (jaguarundi $n = 10$, ocelot $n = 15$; Janečka et al. 2011), and each species was sequenced at the same location along the mtDNA control region. The comparison of jaguarundi and ocelot mtDNA diversity was somewhat contrary to our expectations. Ocelots are considered habitat specialists, and nearly exclusively use mature thornshrub or forest habitat (Caso 1994, Horne et al. 2009). In contrast, jaguarundis use multiple vegetation types, including thornshrub, forest, grassland, and pasture (Konecny 1989, Caso 1994, Caso et al. 2008). We expected the more generalist jaguarundis to exhibit levels of genetic diversity comparable to or greater than ocelots, but this expectation was not supported by our data.

The MC1R assay indicated diversity among red and gray colored jaguarundis, and that the ancestral allele (red phenotype) occurred at a higher frequency than did the melanistic mutation (gray phenotype). The frequency of the melanistic mutation in our sample was much lower than reported for captive jaguarundis (Eizirik et al. 2003). A broader application of the MC1R assay in free-ranging populations would provide a robust evaluation of assortative mating, and potentially reveal information regarding the adaptive significance of coat color.

The reason for the discrepancy between our microsatellite (moderate diversity) and mtDNA (no diversity) results is unknown, but may be a result of our sample. For example, factors such as our restricted spatial extent, sampling relatives (i.e., parent–offspring, full-siblings), or aggregating samples over time could have biased estimates. However, 8 of the 11 individuals were adults sampled within a period of 9 years, and all subadults sampled were males (Table 1). Jaguarundis classified as adults were likely post-dispersal in that their home ranges were smaller (e.g., 8.5–8.8 km^2) than a subadult male presumably dispersing (e.g., 14.32 km^2; Caso 1994). Additionally, jaguarundis have lived 10 years in captivity (Sunquist and Sunquist 2002), mean litter size is approximately 1.9 kittens, and litters are produced about every 1.5 years (de Oliveira 1998). Therefore, it appears our sample was not disproportionately influenced by family groups or temporal aggregation over multiple generations.

Alternatively, the disparity between our microsatellite and mtDNA diversity estimates could be indicative of the demographic history of jaguarundis. One hypothesis is that jaguarundis may have experienced a pattern similar to the puma (*Puma concolor*), which was extirpated from North America approximately 10–17,000 years ago and subsequently re-colonized from South American populations (Culver et al. 2000). An extirpation and re-expansion history can produce a pattern of low mtDNA diversity, and moderate–high nuclear microsatellite diversity because of differences in effective population size. Effective population size for mtDNA is much lower than for nuclear microsatellite DNA because mtDNA is inherited through the mother's lineage only, whereas nuclear DNA is bi-parentally inherited (Allendorf and Luikart 2007). Consequently, population extirpations or bottlenecks have a greater negative effect on mtDNA diversity compared with nuclear microsatellite diversity (Allendorf and Luikart 2007). An alternative explanation regarding our mtDNA and microsatellite results is that anthropogenic factors, such as recent development, habitat loss, and fragmentation, have negatively influenced population size (e.g., bottleneck) or genetic connectivity. Finally, the moderate–no genetic diversity we observed in jaguarundis may be consistent with the central-marginal hypothesis (Eckert et al. 2008), where populations located near the center of a species' geographic range tend to exhibit higher levels of diversity than do populations located at the periphery of the range (Schwartz et al. 2003). Our study area is near the northern edge of the jaguarundi range (de Oliveira 1998, Caso et al. 2008), but additional data are clearly needed to evaluate these hypotheses.

MANAGEMENT IMPLICATIONS

To our knowledge, this work represents the first genetic evaluation of free-ranging jaguarundis. We have provided estimates of genetic diversity for a peripheral population in northern Mexico, where jaguarundis are of conservation concern (Caso et al. 2008). We have also offered potentially fruitful directions for future research. In terms of conservation and management, the most useful part of this study is likely our microsatellite results. Our microsatellite panel displayed reasonable levels of diversity and, thus, strong power to discriminate among individuals with a low probability of error using only 4–7 loci (Table 2). A range of 4–7 loci is similar to what Paetkau (2004) suggested to achieve high accuracy of individual identification in noninvasive genetic studies. Because only 4–7 loci are likely required, noninvasive genetic tools are a useful and feasible option to assist jaguarundi population monitoring and estimation while avoiding the difficulties associated with live-capture. This is important because jaguarundis display extremely elusive behavior. For example, mean capture rate is as low as 1 individual/1,320 trap-nights (A. Caso, unpublished data). By implementing noninvasive techniques, and avoiding the elusive nature of jaguarundis, we can advance our ecological understanding of this felid, which is essential for future conservation and management efforts.

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