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RESEARCH ARTICLE

Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot

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Abstract The ecology of a species strongly influences genetic variation and population structure. This interaction has important conservation implications because taxa with low dispersal capability and inability to use different habitats are more susceptible to anthropogenic stressors. Ocelots (*Leopardus pardalis albescens*) and bobcats (*Lynx rufus texensis*) are sympatric in Texas and northeastern Mexico; however, their ecology and conservation status are markedly different. We used 10 microsatellite loci and a 397-bp segment of the mitochondrial control region to examine how historical and ecological differences in these two species have influenced current patterns of genetic diversity in a landscape heavily altered by anthropogenic activities. Substantially higher genetic diversity (heterozygosity and haplotype diversity) and population connectivity was observed for bobcats in comparison to ocelots. The level of divergence among proximate ocelot populations (<30 km) was greater than between bobcat populations separated by >100 km. Ocelot populations in the US have never recovered from reductions experienced during the twentieth century, and their low genetic variation and substantial isolation are exacerbated by strong preference for dense native thornshrub and avoidance of open habitat. In contrast, despite continued legal harvesting and frequent road-related mortality, bobcats have maintained wide distribution, high abundance, and population connectivity. Our study illustrates that sympatric species with a similar niche can still have sufficient ecological differences to alter their response to anthropogenic change. Sensitive species, such as the ocelot, require additional conservation actions to sustain populations. Ecological differences among species occupying a similar guild are important to consider when developing conservation plans.

Keywords Felidae · Microsatellites · Population structure · Dispersal · Ecology

Introduction

Concordant patterns of genetic diversity across unrelated taxa reflect similar responses to biogeographic processes associated with major environmental and ecological perturbations (Avise 1994, 2000). However, evolutionary mechanisms, including natural selection, sexual selection, and genetic drift often work at local scales (Sugg et al. 1996; Hedrick 2011). Variance in ecological, behavioral, and physiological traits strongly impact the dynamics of populations, thus influencing their divergence. For species

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with narrow habitat preference, limited dispersal and low fecundity, reductions in population size and distribution combined with habitat loss and degradation can culminate in a landscape mosaic of small, fragmented populations in which genetic drift and inbreeding contributes to loss of diversity, increased divergence, and demographic instability (Hedrick 2011). In contrast, the use of a broad variety of habitat types within patchy environments, dispersal between patches, and high fecundity facilitate the recovery of populations from external pressures (e.g., drought, disease, habitat loss, and harvesting), particularly in fragmented landscapes (Gårdmark et al. 2003).

The dichotomy between habitat specialists and habitat generalists is broadly defined by species-specific differences in niche breadth (Rosenzweig 1981; Whittaker 1998; Büchi and Vuilleumier 2014). Specialists exhibit a relatively narrow use of resources or physiological tolerances that can restrict the dispersal ability of an organism to cross unsuitable habitat (Whittaker 1998). Thus, disturbance and habitat fragmentation can generate major landscape barriers for specialists, while sympatric populations of generalists with a wider niche breadth may be unaffected. Habitat specialization influences distribution and abundance of a species as well as its ability to respond to disturbance (MacArthur 1972), making specialists more susceptible to extinction (Henle et al. 2004). It is predicted that the impact of population crashes and landscape alterations are different for habitat specialists compared to habitat generalists (Branch et al. 2003; Gårdmark et al. 2003). Therefore, habitat use and life history traits can significantly impact both patterns of genetic variation and how species recover from population reductions.

The ocelot (*Leopardus pardalis albescens*) and bobcat (*Lynx rufus texensis*) are sympatric in southern United States (U.S.) and northeastern Mexico, with markedly different habitat use, fecundity, and dispersal (Tewes 1986; Tewes and Everett 1986; Laack et al. 2005; Horne et al. 2009; Sunquist and Sunquist 2002). In this region, both species are at the periphery of their respective distributions (Fig. 1). The ocelot is a Neotropical felid, distributed as far north as southern United States (Murray and Gardner 1997). In contrast, the bobcat is a Nearctic felid, and its southern distribution only extends into central Mexico (Larivière and Walton 1997).

During nineteenth and early twentieth centuries, removal of over 95 % of native Tamaulipan brushland, development, and uncontrolled harvest extirpated ocelots from most of Texas (Jahrsdoerfer and Leslie 1988; Schmidly 2002, 2004). Currently, only two small isolated ocelot populations persist in southern Texas. Although in Central and South America ocelots are common and often the most abundant felid with broader habitat use, in their northernmost range they are restricted to dense thornshrub habitat (Tewes and Everett 1986; Sunquist and Sunquist 2002; Schmidly 2002, 2004; Haines et al. 2006c; Horne et al. 2009). In comparison, despite being historically exposed to the same anthropogenic pressures, the ecologically flexible bobcat remains abundant and widely distributed throughout Texas utilizing diverse habitats in all ecoregions within the state (Sunquist and Sunquist 2002; Schmidly 2004), as well as most of the United States. Even areas dominated by either agriculture or substantial suburban development often have high bobcat densities (Schmidly 2004; Heilbrun et al. 2006; Ruell et al. 2009).

Population size reductions and habitat fragmentation have been major drivers of the loss of both genetic variation and connectivity in populations of numerous felids, including Asiatic lions (*Panthera leo persica*), Amur leopards (*P. pardus orientalis*), Eurasian lynx (*L. lynx*), mountain lions (*Puma concolor*), Iberian lynx (*L. pardinus*) and Florida panthers (*P. concolor coryi*) (Roelke et al. 1993; Freeman et al. 2001; Uphyrkina et al. 2002; Palomares et al. 2002; Schmidt et al. 2009; Casas-Marce et al. 2013). Several pieces of genetic evidence suggest that the two remaining ocelot populations in Texas have responded negatively to habitat fragmentation, with inability to disperse between habitat patches (Janecka et al. 2011). Estimates of effective population size (N_E) are low for both of these populations (Janecka et al. 2008). In comparison to populations in northern Mexico, the Texas populations show lower heterozygosity for microsatellite loci and less mitochondrial haplotype diversity (Janecka et al. 2007c, 2011). Moreover, genetic variation in historical samples from Texas is higher than seen in the current populations (Janecka et al. 2014).

In contrast, the bobcat, a felid species sympatric with ocelots in southern Texas and parts of Mexico, appears to have responded differently to landscape changes. Although limited in scope, a localized genetic study (Janecka et al. 2006a, 2007a) on bobcat at the Welder Wildlife Refuge in southern Texas revealed estimates of expected heterozygosity and numbers of alleles at 12 microsatellite loci to be twice to three times that previously reported for ocelots in Texas. This suggests that bobcats may be less impacted by habitat fragmentation in areas where they are sympatric with ocelot, partly because these two species differ in their habitat requirements (Horne et al. 2009). A comparison of genetic diversity of sympatric species with differing habitat ecology and population dynamics can yield valuable insights for conservation and management (Branch et al. 2003).

We hypothesized the bobcat, a habitat generalist, will have higher genetic diversity and greater population connectivity than the ocelot, a habitat specialist, within the same landscape. We used 10 autosomal microsatellite loci and the mitochondrial (mtDNA) control region to test this hypothesis by directly comparing the genetic diversity of sympatric ocelot and bobcat populations occupying the same areas of southern Texas and northern Mexico. We discuss historical,

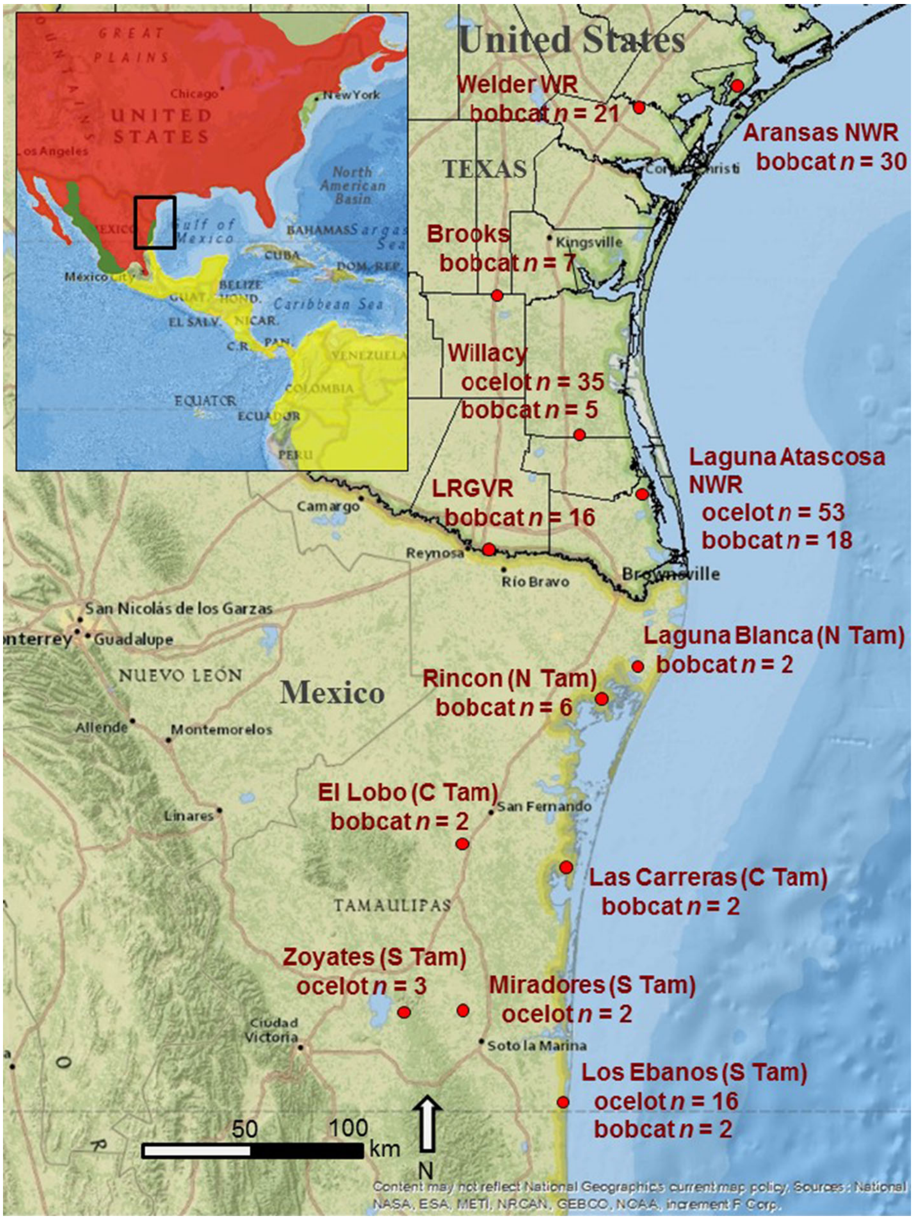

Fig. 1 Map of study sites. Localities sampled (1994–2005) and sample sizes for ocelot and bobcat populations examined in this study. For inset distribution map based on Sunquist and Sunquist (2002), *red* represents bobcat range, *yellow ocelot* range, and *dark green* areas of overlap. *WR* Wildlife Refuge, *NWR* National Wildlife Refuge, *LRGV* Lower Rio Grande Valley Refuge system, *N Tam* Northern Tamaulipas, *C Tam* Central Tamaulipas, *S Tam* Southern Tamaulipas

anthropogenic, and ecological factors that may have been important in forming the patterns observed. Studies that increase our understanding of how sympatric species respond to and recover from anthropogenic changes are critical for evaluating human-induced threats to populations and for designing effective management strategies that conserve a broad array of taxa in an ecological community.

Methods

Samples

Ocelots ($n = 109$) and bobcats ($n = 112$) from southern Texas and northeastern Mexico were used to compare patterns of genetic diversity (Fig. 1). Of these samples, microsatellite data was generated for 70 ocelots and 95 bobcats and mtDNA sequences for 78 ocelots and 69 bobcats. The samples were collected during various ecological studies on ocelot and bobcat from 1994 to 2005 and maintained at Texas A&M University-Kingsville (Caso 1994; Laack 1991; Blankenship 2000; Shindle and Tewes 2000; Haines et al. 2005a; Laack et al. 2005; Haines et al. 2006a). This study used only archived samples; therefore, no individuals were handled in this research. A portion of the samples obtained were collected from road-kills found in the study areas. Specific sites (Fig. 1) included: (1) Laguna Atascosa National Wildlife Refuge, Cameron County, Texas (LANWR), (2) private ranches in northern Willacy County, Texas (Willacy), (3) Brooks County area in Texas (Brooks), (4) Lower Rio Grande Valley National Wildlife Refuge, Texas (LRGV), (5) Rob and Bessie Welder Wildlife Refuge, San Patricio County, Texas (Welder), (6) Aransas National Wildlife Refuge, Texas (ANWR), (7) northern Tamaulipas, Mexico (N Tamaulipas) including Laguna Blanca and Rincon, (8) El Lobo and Las Carreras in central Tamaulipas, Mexico (C Tamaulipas), and (9) Zoyates, Miradores, and Los Ebanos in southern Tamaulipas, Mexico (S Tamaulipas).

For the bobcat samples from Mexico, we extracted DNA and performed two iterations of whole genome amplification using Phi29 DNA polymerase as described in Janecka et al. (2006b, 2007b) in Mexico City, Mexico. The synthetically derived CITES-exempt DNA was used for downstream analysis. Janecka et al. (2006b, 2007b) tested this method and showed genotypes and sequences derived from whole genome amplified synthetic DNA are identical to the original template DNA. The bobcat samples from Mexico were not initially stored in a buffer and had higher levels of DNA degradation so only mtDNA sequences were successfully generated for those individuals.

Microsatellite genotyping and analysis

DNA extractions were performed with a PureGene® DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA). Following methods of Janecka et al. (2008), 10 microsatellite loci (FCA008, FCA023, FCA043, FCA045, FCA077, FCA082, FCA090, FCA096, FCA126, and FCA132) were used to genotype 95 bobcats (e.g., for the remaining 17 bobcats only mtDNA data was generated as described below). These loci were originally isolated in the domestic cat (*Felis catus*) by Menotti-Raymond et al. (1999). Positive and negative controls were included in genotyping plates and no contamination or genotyping errors were observed. At least two individuals previously genotyped were included to ensure alleles were consistently sized across runs. For ocelots, we used a microsatellite data set from a previous study (Janecka et al. 2011), but the analysis was limited to the three primary populations (LANWR, Willacy, S Tamaulipas) in the Tamaulipas Biotic Province and the 10 loci above.

Measures of genetic variability, including observed heterozygosity (H_O), expected heterozygosity (H_E), mean number of alleles (A_N), number of private alleles (A_P), unbiased expected heterozygosity (uH_E), and the fixation index (F_I) were estimated using GENALEX 6.4 (Peakall and Smouse 2012) and allelic richness (A_R) using FSTAT 2.9.3 (Goudet 1995). The Student's t test was used to test for significant differences in A_N and H_O between populations with >10 samples. Tests for linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 3.1 (Guo and Thompson 1992). Populations were tested for deviations from equilibrium at each locus and across all loci. The Bonferroni method was used to correct for multiple comparisons (Rice 1989).

The global $F_{ST\text{-}nuc}$ (nuclear markers) from AMOVA and pairwise $F_{ST\text{-}nuc}$ estimates derived from microsatellites were tested for significance with 10,000 permutations in GENALEX. The $F_{ST\text{-}nuc}$ among populations was analyzed using principle coordinate analysis (PCoA) to visualize the relative levels of similarity. The Mantel permutation test in GENALEX was employed to estimate isolation by distance through comparisons of linearized $F_{ST\text{-}nuc}$ versus geographic distance for bobcats (Mantel 1967; Slatkin and Barton 1989). Assignment tests in GENALEX were conducted by estimating the probability of individuals originating from each of the populations. Previous studies have shown that the portion of individuals assigned to a population from which they were not sampled (i.e., misassigned) is positively correlated with dispersal (Rannala and Mountain 1997; Paetkau et al. 2004). Proportion of misassigned individuals was compared between populations and the likelihoods of the two highest assignments were plotted.

Bayesian model-based clustering in STRUCTURE 2.3.4 was used to explore population structure without regard to geographic origin (Pritchard et al. 2000). This approach applies a Bayesian algorithm to estimate the likelihood of K genetic clusters (synonymous with "populations") and the portion of individual genetic variation (Q) attributed to each of the clusters, based on LD and HWE. The likelihood was estimated for $K = 1$–8 using the admixture model and correlated allele frequencies for five independent runs with 1,000,000 Markov chain Mater Carlo generations after a burn-in of 400,000 iterations. The F_{st}, alpha, and likelihood were examined across runs for convergence. The most likely number of clusters was determined by estimating the posterior probability (PP) for each K as recommended by Pritchard et al. (2000) and the *ad hoc* statistic *Delta K* of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012). The composition of the genetic clusters were compared to the geographic origin of samples.

Mitochondrial sequencing and analysis

A 436-base pair (bp) fragment of the mitochondrial control region was PCR amplified using primers from Jae-Heup et al. (2001) that were modified to match the ocelot and bobcat mtDNA sequence (F primer, 5′CTC AAC TAT CCG AAA GAG CTT; R primer, 5′CCT GTG GAA CAT TAG GAA TT). After trimming primer sequences and eliminating low quality base reads, this segment aligned with positions 16,832 to 17,009 and 1 to 218 positions in the domestic cat mitochondrial genome (GenBank

Accession U20753). This section is located in the central conserved region between repetitive sequences I and II (Jae-Heup et al. 2001). The PCR amplification and sequencing followed methods of Janecka et al. (2011). Consensus sequences, derived from reads in both directions, were assembled using SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.).

Sequences were aligned using the default settings in CLUSTAL-X (Thompson et al. 1997). Numbers of variable sites (V_S), number of haplotypes (N_{HAP}), haplotype diversity (D_{HAP}), nucleotide diversity (π), and mean number of nucleotide differences were calculated in DNASP 4.10.8 (Nei and Li 1979; Rozas and Rozas 1999). A minimum spanning network of haplotypes was constructed and plotted to represent relationships among haplotypes using TCS 1.21 (Clement et al. 2000). Departures of haplotype frequencies from neutral evolution were tested using the Tajima's *D* and Fu and Li's *F* in DNASP (Tajima 1989; Fu and Li 1993).

Population differentiation was examined using two methods. First, an exact test for population differentiation based on haplotype frequencies was implemented in ARLEQUIN 3.5 (Excoffier et al. 2005). Second, population structure was tested using pairwise $F_{ST\text{-}mtd}$ (mitochondrial) estimates derived from the control region sequences in ARLEQUIN. Estimates of $F_{ST\text{-}mtd}$ were tested for significance against the null distribution obtained from 1000 permutations (Excoffier et al. 1992).

Results

Microsatellite variation and structure

Composite microsatellite genotypes from 95 bobcats and 82 ocelots were used to estimate patterns of genetic diversity within and between populations (Table 1). Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5b2k6. Only one locus was out of HWE in one ocelot population (FCA 132 in LANWR; Table S1) and two loci in the bobcat population in ANWR (FCA077 and FCA096 (Table S2). Ocelots in Texas had 38 % lower A_N, 52 % lower A_R, and 34 % lower H_O relative to bobcats ($P = 1.04 \times 10^{-10}$, 6.68×10^{-12}, $P = 0.005$, respectively). The difference was greatest in the LANWR population that had the highest sample size of ocelots ($P = 0.0000115$ for A_N, $P = 9.93 \times 10^{-7}$ for A_R, $P = 0.00462$ for H_O). In this area, genetic diversity for bobcats was twice that of ocelots, despite a 2.5-fold greater number of ocelots sampled ($n = 42$ and $n = 17$, respectively). In bobcats, all 10 loci were variable in the six populations sampled. In contrast, there were two loci (FCA043 and FCA096) that had no variation in one of the ocelot populations (LANWR). The S Tamaulipas ocelot population at Los Ebanos had a somewhat higher A_N and A_R than observed in the southern Texas populations, but both A_N and A_R were still below that found for bobcats ($P = 0.0292$ and $P = 0.00132$, respectively) (Table 1). A

Table 1 Microsatellite diversity of ocelot and bobcat populations at 10 loci in southern Texas and northeastern Mexico

	Autosomal Microsatellites							
	N	A_N	A_R	A_P	H_O	H_E	F_I	MA (%)
Ocelot								
Texas	70	5.1	3.9	n.a.	0.490	0.470	−0.029	n.a.
Laguna Atascosa NWR	42	2.7	2.4	4	0.381	0.362	−0.036	0
Willacy	28	3.4	3.1	2	0.600	0.577	−0.021	0
Mexico, Los Ebanos	12	4.0	3.7	12	0.610	0.586	−0.022	8
Bobcat								
Texas	95	8.2	8.1	n.a.	0.742	0.776	0.042	n.a.
Laguna Atascosa NWR	17	6.3	5.8	1	0.706	0.762	0.068	65
Willacy	4	3.4	n.a.	1	0.708	0.616	−0.129	50
Brooks	7	5.4	5.2	1	0.776	0.749	−0.041	100
Lower Rio Grande VRS	16	5.6	5.1	1	0.753	0.687	−0.107	19
Welder WR	21	6.3	5.7	1	0.744	0.752	0.010	38
Aransas NWR	30	6.7	5.6	4	0.756	0.729	−0.034	20

Bobcat samples from Mexico had higher levels of DNA degradation and therefore microsatellite analysis was not successful for these individuals. Allelic richness was estimated for population with more than 5 sampled individuals

NWR National Wildlife Refuge, *VRS* Valley Refuge system, *N* sample number, A_N mean number of alleles, A_R allelic richness, A_P private alleles, H_O observed heterozygosity, H_E expected heterozygosity, uH_E unbiased expected heterozygosity, F_I fixation index, *MA* population missassignments greater number of private alleles was seen in ocelot populations relative to bobcats, suggesting lower gene flow.

In the AMOVA, most genetic variation for ocelots was partitioned among populations, and the overall $F_{ST\text{-}nuc} = 0.214$ ($P = 0.001$) was 5-fold higher than for bobcats ($F_{ST\text{-}nuc} = 0.041$, $P = 0.001$). For the two areas where both species co-occurred (LANWR and Willacy), the ocelot pairwise $F_{ST\text{-}nuc}$ was greater (0.194 for ocelot vs 0.017 for bobcat) (Table 2). The $F_{ST\text{-}nuc}$ between these nearby ocelot populations, separated by only 20 km, was nearly 3-fold higher than between the most distant bobcat populations located ~350 km apart (LRGV and ANWR, $F_{ST\text{-}nuc} = 0.068$). The highest $F_{ST\text{-}nuc}$ observed for ocelot was between LANWR and the S Tamaulipas site at Los Ebanos ($F_{ST\text{-}nuc} = 0.345$, $P = 0.001$). Bobcat populations that did not have significant pairwise $F_{ST\text{-}nuc}$ values were LANWR, Willacy, and Brooks. The most divergent bobcat population, based on $F_{ST\text{-}nuc}$, analysis was the LRGV; it had the highest estimate in comparison with the nearby Brooks population (pairwise $F_{ST\text{-}nuc} = 0.087$, $P = 0.001$) (Fig. 2; Table 2). The second most divergent bobcat population was ANWR (pairwise $F_{ST\text{-}nuc}$ 0.059 with Willacy and 0.068 with LRGV). Although the significance of several other pairwise $F_{ST\text{-}nuc}$ values among the bobcat populations suggested some structure, estimates were low, ranging from 0.015 to 0.040. The principle coordinate analysis illustrates the substantial divergence of the ocelot populations relative to bobcats (Fig. 2).

Table 2 Estimates of differentiation and gene flow among ocelot (A) and bobcat (B) populations based on 10 autosomal microsatellite loci

Ocelot	LANWR	Willacy	S Tam (MX)
A			
LANWR	–	0.001	0.001
Willacy	0.194^{a}	–	0.001
S Tam (MX)	0.345^{a}	0.102^{a}	–

Bobcat	LANWR	Willacy	Brooks	LRGV	Welder	ANWR
B						
LANWR	–	0.191	0.424	0.001	0.028	0.001
Willacy	0.017	–	0.078	0.005	0.033	0.005
Brooks	0.001	0.034	–	0.001	0.213	0.017
LRGV	0.046^{a}	0.085^{a}	0.087^{a}	–	0.001	0.001
Welder	0.015^{a}	0.034^{a}	0.008	0.069^{a}	–	0.001
ANWR	0.040^{a}	0.059^{a}	0.029^{a}	0.068^{a}	0.026^{a}	–

Pair-wise $F_{ST\text{-}nuc}$ values are in the bottom left portion of each matrix. The top right portions show the respective P values

LANWR Laguna Atascosa National Wildlife Refuge, *S. Tam* Southern Tamaulipas, *LRGV* Lower Rio Grande Valley refuge system, *ANWR* Aransas National Wildlife Refuge

[a] Significant difference

The isolation by distance model was rejected ($P = 0.679$, $P = 0.186$, respectively, Figure S1) for both ocelot and bobcat suggesting that landscape, anthropomorphic, and habitat features better explain the patterns in genetic diversity than geographic distance.

Assignment tests that estimated the likelihood of individuals originating in each population based on genotypes also revealed much greater connectivity in bobcats. Among all ocelots sampled, only one out of 82 individuals was misassigned (~1 % rate) (Table S3). In contrast, 39 % of bobcats were misassigned (37 of 95) (Table S4). For bobcats, the lowest misassignment ratio was in the LRGV bobcats (19 %), and the highest in LANWR bobcats (65 %), consistent with the $F_{ST\text{-}nuc}$ pairwise estimates (Table 1). The higher level of assignment to the correct populations in ocelots was clearly observed when the log of probability of the two most likely populations was plotted (Fig. 3).

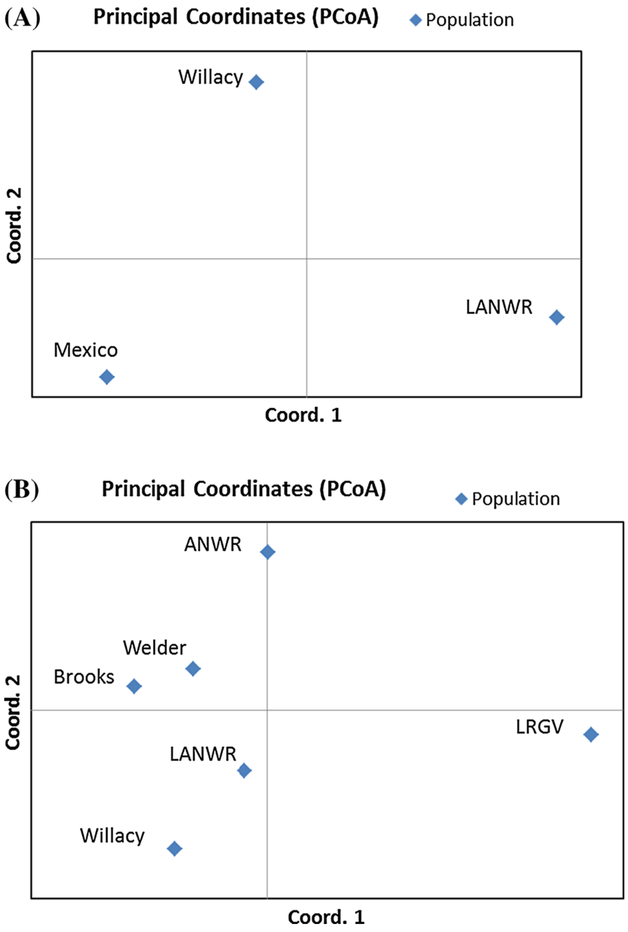

Fig. 2 Principle coordinate analysis. Plots derived from principle coordinate analysis of pairwise $F_{ST\text{-}nuc}$ estimates from microsatellite data for **a** ocelot and **b** bobcat populations to visualize levels of divergence

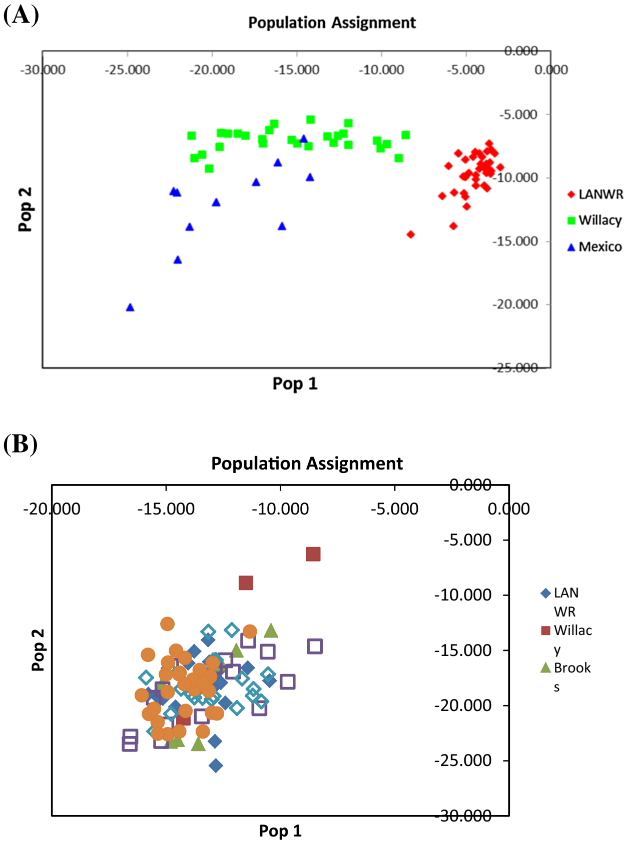

Fig. 3 Population assignments. Likelihood of population assignment for each individual to all respective populations based on microsatellite allele frequencies for **a** ocelots and **b** bobcats

Model-based clustering for bobcats without regard for geographic origin consistently partitioned individuals into $K = 3$ genetic clusters (likelihood *Ln* probability of data, $Ln[K] = -3106.6$, $PP = 0.99$, $\Delta K = 6.62$) (Fig. 4a, Table S5). Under this model, the first cluster consisted of LRGV, second cluster of ANWR, while the last cluster included all remaining localities in Texas (i.e., LANWR, Willacy, Brooks, and Welder). For the ocelot, there was disagreement in the number of *K* clusters between the ΔK statistic implemented by STRUCTURE HARVESTER and the *PP* estimate from Pritchard et al. (2000) (Fig. 4b; Table S5). The ΔK statistic was highest for $K = 2$ ($PP = 0$, $Ln[K] = -1455.2$, $\Delta K = 109.5$) with one cluster consisting of only LANWR and the second cluster composed of Willacy and S Tamaulipas. The placement of LANWR and Willacy individuals into separate clusters, is consistent with the high divergence between these two nearby populations. In contrast to ΔK interpretation, the *PP* estimate was highest for $K = 5$ clusters ($PP = 0.99$, $Ln[K] = -1390.6$, $\Delta K = 4.2$), with cluster 1 composed primarily of LANWR, whereas, clusters 2 and 3 of Willacy, and clusters 4 and 5 of S Tamaulipas. For $K = 3$ the genetic partitioning by STRUCTURE corresponded to the three sampling localities. In all STRUCTURE scenarios, ocelots from the two adjacent sites in Texas (Willacy and LANWR) were always partitioned into different genetic clusters, whereas bobcats from these two areas were grouped together in the same cluster, along with bobcats from Brooks and Welder (Fig. 4a, b).

Mitochondrial variation and structure

We sequenced and aligned 397-bp fragments of the control region for 69 bobcats and compared it with the orthologous region previously sequenced for 78 ocelots examined by Janecka et al. (2014). New sequences were deposited in GenBank under accession numbers KU981028-KU981039. There were two insertions in the bobcat sequence. In ocelots, only four haplotypes differing at three variable sites were observed, and each haplotype differed from another by a single mutation yielding a very simple network (Fig. 5). In Texas, all but five ocelots from Willacy had the same haplotype, whereas all four haplotypes were observed in S Tamaulipas despite the smaller sample size. Haplotype and nucleotide diversity in ocelots were highest in the S Tamaulipas population ($D_{HAP} = 0.6790$, $\pi = 0.0029$) and lowest in LANWR, which was fixed for the most common haplotype (Tables 3, 4). Willacy samples were collected over three periods (1984–1990, $N = 8$; 1994–1998, $N = 16$; 2005, $N = 10$). By 2005, the low frequency haplotype two was no longer detected at this site. Tajima's *D* (-0.854, $P > 0.10$) and Fu and Li's *F* (0.373, $P > 0.10$) tests of neutrality were not significant for the ocelot populations.

In contrast to ocelots, bobcats exhibited high levels of diversity, with 11 variable sites (Table 3) distributed among 12 haplotypes (Tables 4). The haplotype network was more complex reflecting the higher level of diversity (Fig. 6). Overall haplotype and nucleotide diversity was $D_{HAP} = 0.813$ and $\pi = 0.0069$. Because bobcats are distributed in more areas of Texas, we were able to sample a greater number of populations, thus partially contributing to the higher number of haplotypes observed. However, for most bobcat populations, we sequenced substantially fewer bobcats than the ocelots sampled in the two Texas populations with low diversity. Despite the smaller bobcat sample size per site, the observed mtDNA diversity within each locality was greater than in ocelots.

Higher bobcat genetic diversity was particularly striking for the three areas where we had both ocelot and bobcat samples. In LANWR, Willacy, and central Tamaulipas, the haplotype diversity in bobcats was $D_{HAP} = 0.833$, 0.700, and 0.736 compared to ocelot values of 0, 0.258, 0.679, for the same populations, respectively. The lowest bobcat diversity observed was in the LRGV with $D_{HAP} = 0.3330$ and $\pi = 0.0042$. We were able to sequence a small number

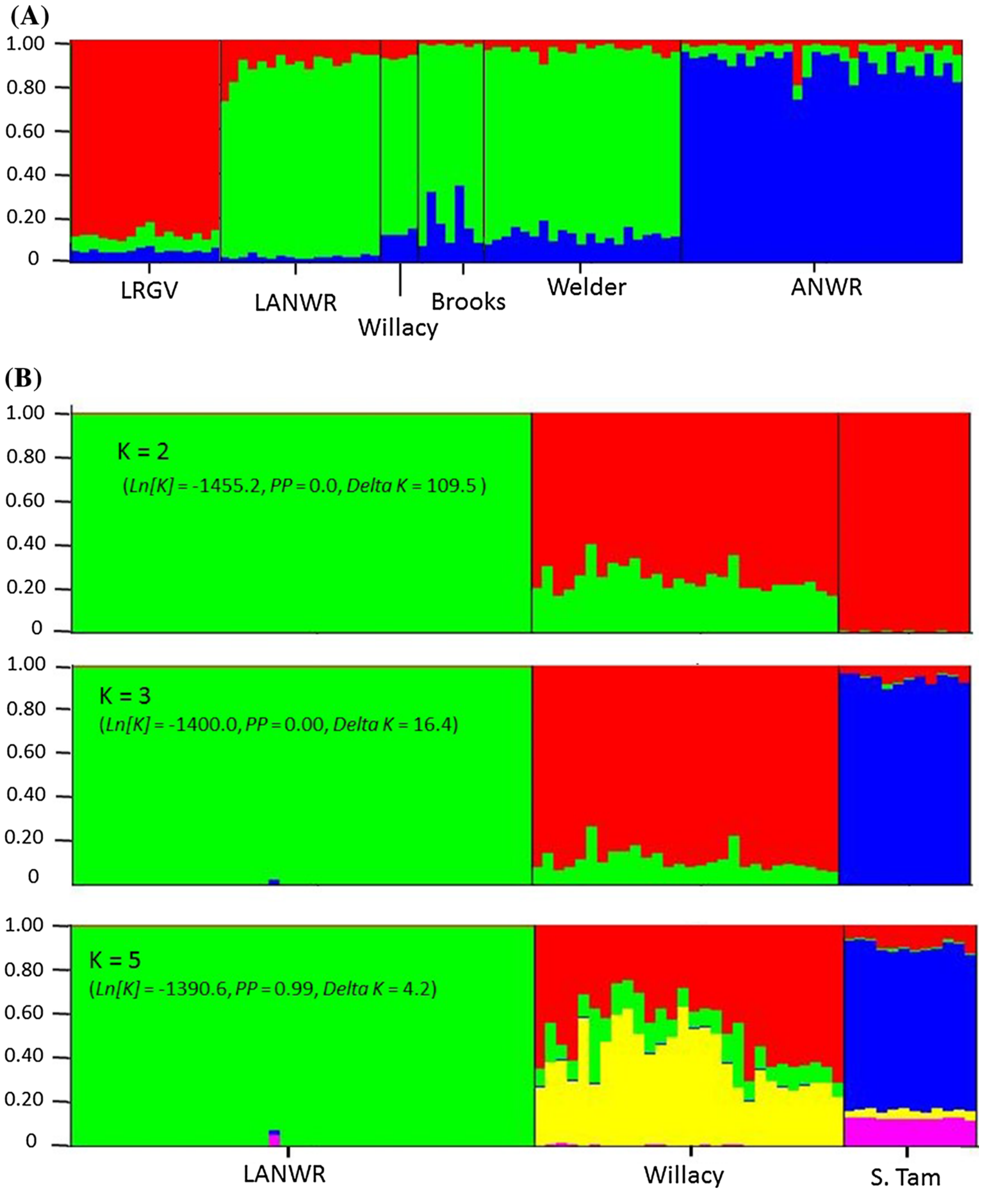

Fig. 4 STRUCTURE plots. Bayesian model-based clustering of individuals without regard to sampling location estimated in STRUCTURE from microsatellite data for the bobcat **a** and ocelot **b**. For bobcats the posterior probability (*PP*) and *Delta* K (Evanno et al. 2005) supported $K = 3$ genetic clusters and therefore we show only one graph. Microsatellite data was not available for bobcats from Mexico. For ocelots, because the methods for estimating *K* did not agree, we show graphs for $K = 2$, 3, and 5 and provide the *Ln[K], PP*, and *Delta K*

of bobcats south of the Rio Grande River; N Tamaulipas and C Tamaulipas had diversity similar to Texas. In S Tamaulipas only two individuals were sequenced, and both had the same haplotype that was present in all bobcat populations. There was an average of 2.5 nucleotide differences among bobcat sequences. Tajima's *D* (0.289, $P > 0.10$) and Fu and Li's *F* (-0.545, $P > 0.10$) tests were not significant in bobcat populations.

No significant differences in haplotype and nucleotide diversity were observed among bobcat populations. When all samples were pooled, haplotype and nucleotide diversities in bobcats were significantly ($P < 0.05$) higher than seen for ocelots. The LANWR and Willacy ocelot populations had significantly ($P < 0.05$) lower haplotype and nucleotide diversities compared to the LANWR and Willacy bobcat populations. There was no significant difference in haplotype diversity between ocelot and bobcat populations in Mexico.

The $F_{ST\text{-}mtd}$ estimates among the ocelot populations were significant between LANWR and S Tamaulipas and between Willacy and S Tamaulipas ($F_{ST\text{-}mtd} = 0.291$, $P > 0.001$ and $F_{ST} = 0.134$, $P = 0.015$, respectively) (Table 5). The $F_{ST\text{-}mtd}$ between LANWR and Willacy was high ($F_{ST\text{-}mtd} = 0.102$) and nearly significant $P = 0.063$. There was substantially less divergence between bobcat populations (Table 5). The only significant bobcat pairwise $F_{ST\text{-}mtd}$ was between Welder and LRGV ($F_{ST\text{-}mtd} = 0.230$, $P = 0.024$). Similar to the microsatellite data, the highest bobcat $F_{ST\text{-}mtd}$ values were observed when LRGV was compared to other populations.

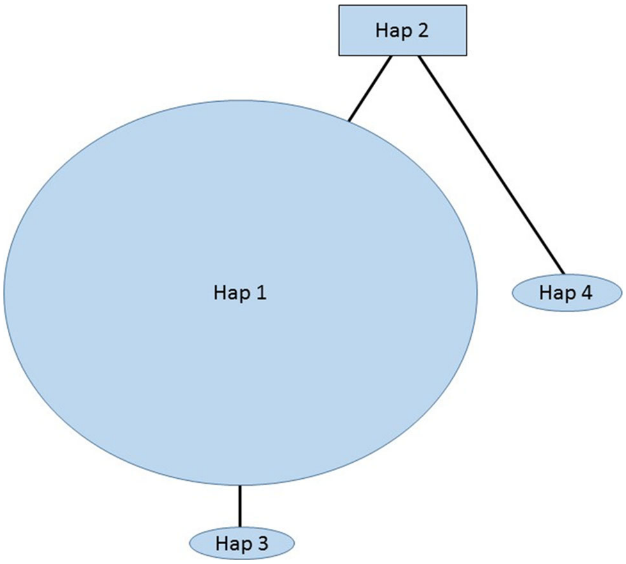

Fig. 5 Ocelot haplotypes. Minimum spanning network showing the most parsimonious mutation pathway between the 4 observed ocelot mtDNA control region haplotypes. Size of haplotypes is proportional to their frequency among all samples. The haplotype numbers correspond to those assigned in Table 4. The *square* represents the potential ancestral haplotype identified by the TCS program

Discussion

Differences in ocelot and bobcat genetic variation

Low ocelot genetic diversity was previously reported for the relict populations in the U.S. (Janecka et al. 2008, 2011, 2014). To these findings we add a direct comparison of genetic variation with a sympatric felid that occupies a similar ecological niche. We observed higher diversity and population connectivity at both nuclear and mitochondrial loci in bobcat populations. In contrast to the bobcat, the ocelot had substantially lower levels of genetic diversity and very limited dispersal across the fragmented habitat. In LANWR, the site with the lowest ocelot diversity, bobcats had among the highest diversity observed in this study. Bobcat samples from Mexico had high DNA degradation, thus precluding an examination of microsatellite variation. Nevertheless, mtDNA control region sequences from bobcats south of the Rio Grande River in Mexico had similar diversity to their Texas counterparts. In contrast, ocelot populations in Texas had substantially lower diversity relative to those occurring in Mexico.

Table 3 Mitochondrial diversity observed in a 397-bp pair portion of the control region for ocelot and bobcat populations sampled 1994–2005

Locality	*N*	V_S	N_{HAP}	D_{HAP}	*SD*	π	*SD*
Ocelot							
All samples	78	3	4	0.257	0.063	0.0008	0.0002
Texas	60	1	2	0.155	0.060	0.0039	0.0002
Laguna Atascosa NWR	26	0	1	0	0	0	0
Willacy	34	1	2[a]	0.258	0.086	0.0007	0.0022
Southern Tamaulipas, MX	13	3	4	0.679	0.112	0.0029	0.0006
Central Tamaulipas, MX	5	0	1	0	0	0	0
Bobcat							
All samples	69	11	12	0.813	0.025	0.0069	0.0004
Texas	55	11	11	0.834	0.026	0.0069	0.0005
Laguna Atascosa NWR	12	6	5	0.833	0.069	0.0065	0.0008
Willacy	5	5	3	0.700	0.218	0.0056	0.0026
Brooks	7	7	4	0.810	0.130	0.0008	0.0019
Lower Rio Grande VRS	6	5	2	0.333	0.215	0.0042	0.0027
Welder Wildlife Refuge	13	7	5	0.705	0.122	0.0052	0.0014
Aransas NWR	12	8	5	0.803	0.078	0.0086	0.0009
Mexico	14	6	4	0.736	0.075	0.0069	0.0007
Northern Tamaulipas MX	8	6	4	0.750	0.139	0.0075	0.0016
Central Tamaulipas, MX	4	4	2	0.667	0.204	0.0067	0.0021
Southern Tamaulipas, MX	2	0	1	0	0	0	0

[a] Only one of the haplotypes was observed in the Willacy population after 1999

NWR National Wildlife Refuge, *VRS* Valley Refuge system, *N* number of individuals, V_S variable sites, N_{HAP} number of haplotypes, D_{HAP} haplotype diversity, *SD* standard deviation, π nucleotide diversity

Table 4 Mitochondrial control region haplotype frequencies in ocelot (A) and bobcat (B) populations sampled 1994–2005 in Texas and northeastern Mexico

Haplotype Ocelot	LANWR	Willacy	S Tam (MX)
A			
Hap 1	1.000	0.853	0.538
Hap 2	0	0.147	0.154
Hap 3	0	0	0.231
Hap 4	0	0	0.077

Haplotype Bobcat	LANWR	Willacy	Brooks	LRGV	Welder	ANWR	N Tam (MX)	C Tam (MX)	S Tam (MX)
B									
Hap 1	0.167	0.600	0	0.833	0	0	0.500	0	0
Hap 2	0.250	0.200	0.143	0	0.538	0.083	0.125	0.500	0
Hap 3	0.167	0	0.286	0	0.077	0	0	0	0
Hap 4	0.083	0	0	0	0	0	0	0	0
Hap 5	0.333	0.200	0.429	0.167	0.154	0.333	0.250	0.500	1.000
Hap 6	0	0	0.143	0	0	0	0	0	0
Hap 7	0	0	0	0	0.154	0	0	0	0
Hap 8	0	0	0	0	0.077	0	0	0	0
Hap 9	0	0	0	0	0	0.333	0	0	0
Hap 10	0	0	0	0	0	0.083	0	0	0
Hap 11	0	0	0	0	0	0.167	0	0	0
Hap 12	0	0	0	0	0	0	0.125	0	0

LANWR Laguna Atascosa National Wildlife Refuge, *S Tam* Southern Tamaulipas, LRGV Lower Rio Grande Valley Refuge system, *ANWR* Aransas National Wildlife Refuge

All three ocelot populations were divergent, with the most significant differences between the two closest populations, LANWR and Willacy. In an effort to obtain directly comparable data for ocelot that was generated for bobcats, only 10 microsatellite loci were used from the Janecka et al. (2011) microsatellite data set. However, with all 26 variable loci the patterns of divergence (Janecka et al. 2011) are similar to what we observed. Estimates of genetic diversity within and between the Texas populations suggest a lack of dispersal for ocelots in Texas, which is in sharp contrast to the pattern observed for bobcats. In LANWR, which contains the most isolated, genetically depauperate ocelots in the U.S., bobcats exhibit among the highest rates of gene flow with connectivity to populations that are >100 km away. Indeed, our data suggests that the LANWR bobcats are part of a large panmictic population that includes Willacy, Welder, and Brooks.

The bobcat populations that exhibited higher levels of divergence were LRGV and ANWR. The LRGV Refuge system consists primarily of small, disconnected habitat patches adjacent to the Rio Grande River (Fisher 1998). These habitat patches are very isolated and located near Brownsville, Texas, Cameron County, an area reported to have the largest human footprint in the World based on population density, land transformation, and power infrastructure (Sanderson et al. 2002). The rate of human development and agriculture in both Cameron and Willacy counties has been dramatic (Fig. 7). It is so severe that it reduces bobcat connectivity (Fisher 1998), a species that normally shows tolerance to substantial levels of anthropogenic activities and habitat alterations. The other more divergent bobcat population was ANWR, which occurs entirely on a Blackjack Peninsula. This refuge has high-quality habitat, but is surrounded on three sides by Copano Bay, Saint Charles Bay, and San Antonio Bay. The nearest population sampled on Welder Wildlife Refuge is ~50 km southwest of ANWR, on the opposite side of Saint Charles Bay, and the other bobcat populations are located farther south. The area directly northwest of Blackjack Peninsula and bordering ANWR is cropland with limited dispersal cover. The combination of geography and cropland likely contributes to reduced migration into and out of ANWR.

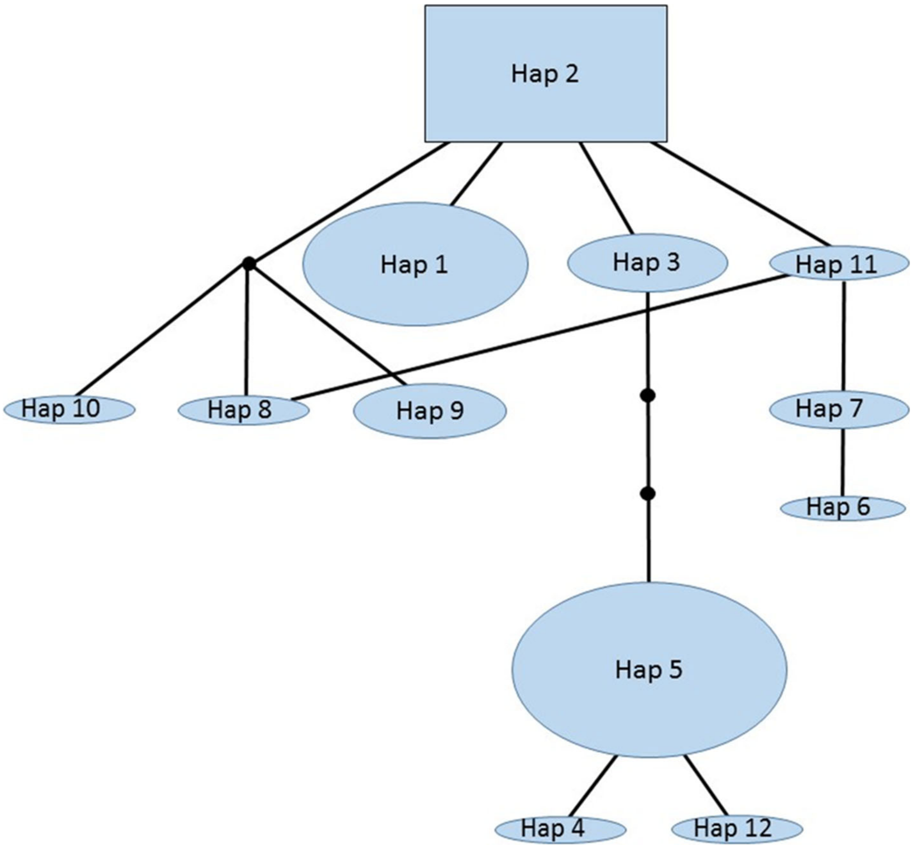

Fig. 6 Bobcat haplotypes. Minimum spanning networks showing the most parsimonious mutation pathway between observed bobcat mtDNA control region haplotypes. Size of haplotypes is proportional to their frequency among all samples. *Black dots* in pathway represent intermediate haplotypes that were not observed during this study. The haplotype numbers correspond to those assigned in Table 4. The *square* represents the potential ancestral haplotype identified by the TCS program

Ocelot and bobcat ecological differences

Differences in genetic structure between the bobcat and ocelot can be partly explained by differences in their response to human activities and habitat fragmentation. The effects of landscape level changes on populations are largely the result of species ecology (i.e., habitat use and population dynamics), and as suggested by Didham (2010), species demonstrating habitat specialization are more sensitive to fragmentation. Bobcats are habitat generalists and occur in all ecological zones of Texas (Sunquist and Sunquist 2002; Schmidly 2004). They use many different habitat types and are often found in close proximity to human dominated areas including towns, rural subdivisions, roads, and agricultural fields (Larivière and Walton 1997). Bobcats can occur even in highly isolated patches along the Rio Grande River (Fisher 1998).

In contrast, ocelots have more specific habitat requirements than bobcats (Shindle and Tewes 1998; Horne et al. 2009). In Texas, they prefer dense thornshrub with >85 % canopy cover (Horne et al. 2009), and are severely restricted by highly fragmented landscapes surrounding LANWR (Harveson et al. 2004; Jackson and Zimmerman 2005; Tremblay et al. 2005; Haines et al. 2006c) (Fig. 8). During >30 years of live-trapping and camera-trapping, only two ocelots have been documented in habitat patches isolated by croplands in the Lower Rio Grande Valley, and there has not been a single successful dispersal event observed (i.e., one in which the dispersing individual produced offspring in the new population) (Tewes 1986; Laack 1991; Caso 1994; Shindle and Tewes 2000; Haines et al. 2005a, b; Laack et al. 2005; Haines et al. 2006a, b, c). Interspecific interactions may further isolate ocelot populations (Horne et al. 2009), especially in areas where bobcat densities are high and the habitat is suboptimal, yet potentially useful for ocelots. Therefore, high bobcat and coyote (*Canis latrans*) densities around the two relict populations of ocelot may further reduce the already low likelihood that unoccupied habitat patches in Texas will be recolonized by ocelots. Habitat specialists like the ocelot are predicted to decline at a faster rate than generalists when their primary habitat is removed (Büchi and Vuilleumier 2014).

Ocelot and bobcat population historical differences

In the early 1900s, ocelots were found in parts of central and eastern Texas, whereas bobcats had an even wider distribution (Schmidly 2002; Janecka et al. 2014). Unregulated harvesting of both felids occurred during this period, along

Table 5 Estimates of differentiation and gene flow derived from the mitochondrial control region among ocelot (A) and bobcat (B) populations

Ocelot	LANWR	Willacy	S Tam (MX)
A			
LANWR	–	0.063	0.000
Willacy	0.102	–	0.015
S Tam (MX)	0.291[a]	0.134[a]	–

Bobcat	LANWR	Willacy	Brooks	LRGV	Welder	ANWR	N Tam (Mx)
B							
LANWR	–	0.229	0.771	0.13	0.078	0.269	0.477
Willacy	0.050	–	0.204	0.699	0.203	0.354	0.823
Brooks	0	0.123	–	0.062	0.149	0.272	0.332
LRGV	0.186	0	0.268	–	0.024	0.230	0.430
Welder	0.103	0.072	0.089	0.230[a]	–	0.172	0.113
ANWR	0.025	0	0.039	0.068	0.053	–	0.479
N Tam (Mx)	0	0	0.010	0	0.097	0	–

Pair-wise $F_{ST\text{-}mtDNA}$ is in the bottom left portion of each matrix and the respective *P*-values are in the top right. Bobcats from Rincon and Laguna Blanca were pooled into the N Tam group (Northern Tamaulipas, Mexico) due to their proximity

LANWR Laguna Atascosa National Wildlife Refuge, *S Tam* Southern Tamaulipas, *LRGV* Lower Rio Grande Valley Refuge system, *ANWR* Aransas National Wildlife Refuge

[a] Significant difference

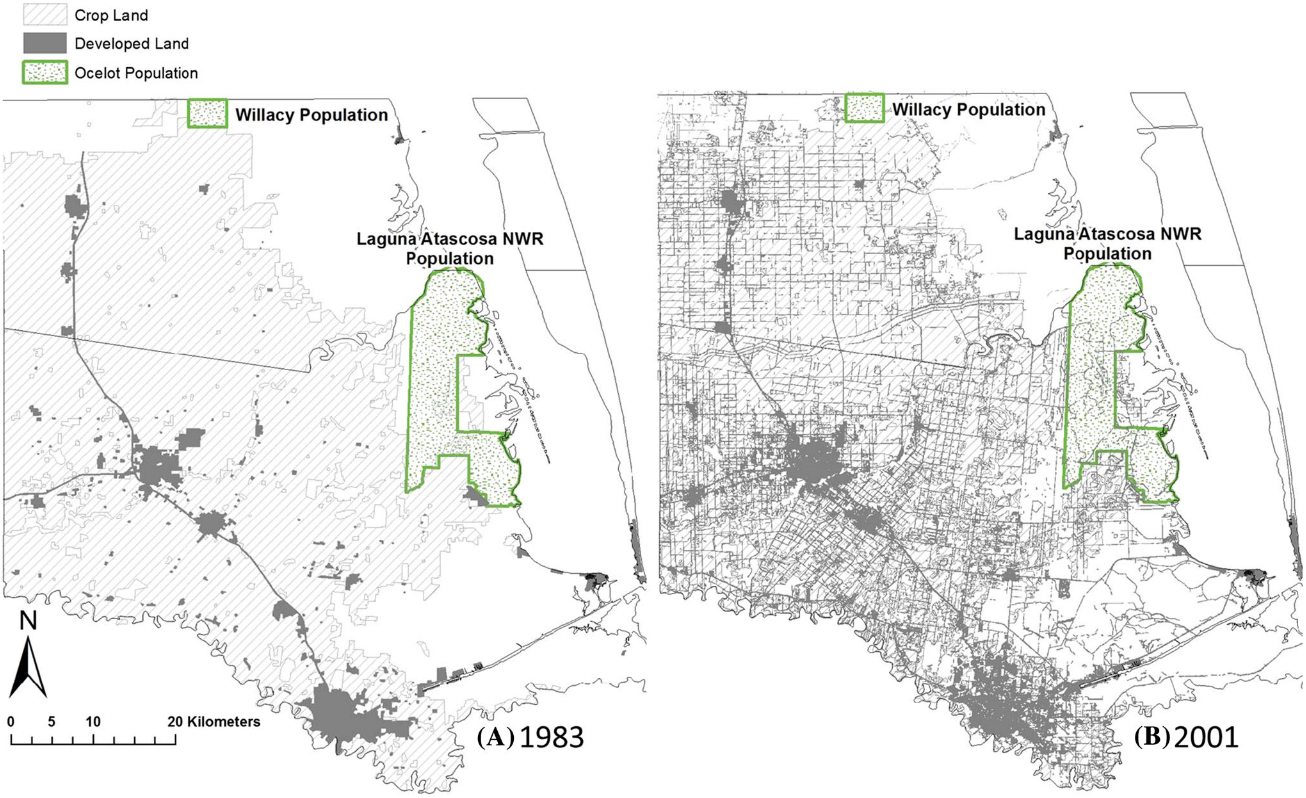

Fig. 7 Development in southern Texas. Change in the human footprint (i.e., developed land and crop land) from **a** the early 1980s to **b** the early 2000s in Cameron County, Texas (contains Laguna Atascosa NWR ocelot population) and Willacy County, Texas, (contains Willacy ocelot population) United States. Data were sourced from Haines et al. (2008), Homer et al. (2007) and Price et al. (2006)

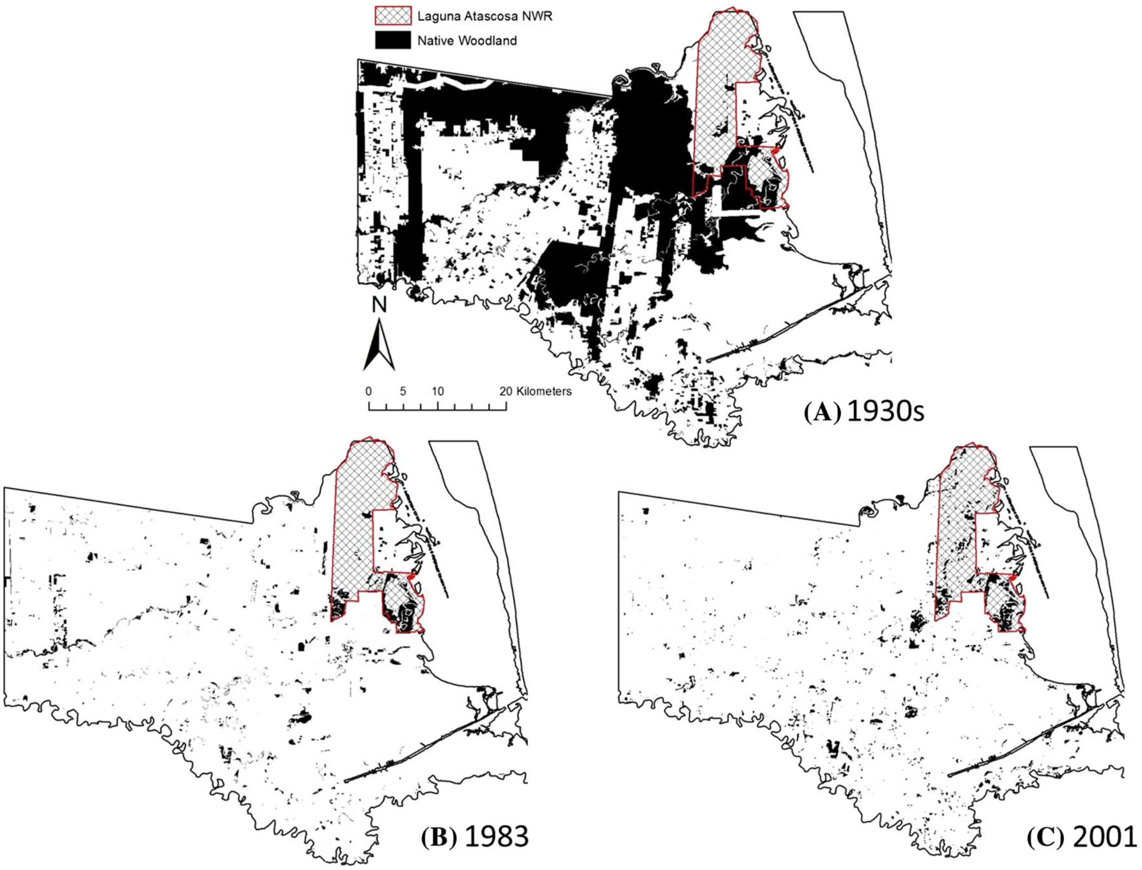

Fig. 8 Habitat in Cameron County. Extent of native woodland habitat from **a** the mid-1930s, **b** 1983 and **c** 2001 in Cameron County, Texas, United States. Data were sourced from Tremblay et al. (2005) and Haines et al. (2008)

with major habitat modifications (Tewes and Everett 1986; Schmidly 2002) (Fig. 8). Lack of ocelot habitat combined with low fecundity resulted in extremely small, fragmented ocelot populations in Texas that have not recovered to their former size and distribution. In contrast, despite continued legal hunting and trapping of bobcats, this felid remains widely distributed and abundant in Texas because of its broad habitat use, ability to occupy areas impacted by humans, and high reproductive output (Larivière and Walton 1997; Laack et al. 2005; Horne et al. 2009). Because of the inability of ocelots to disperse within Texas, they have lost variation and are isolated. In contrast, bobcats have maintained higher abundance and wide distribution, which is reflected in their higher genetic diversity and gene flow.

The small population size and isolation of the two remnant ocelot populations has led to loss of diversity through genetic drift and inbreeding (Janecka et al. 2008, 2011, 2014). Unless conservation interventions are implemented, this trend in Texas will continue because the Rio Grande Valley is one of the fastest growing regions in the U.S. (United States Census Bureau 2010). Since the 1930s, ocelot habitat in southern Texas has declined dramatically and the remnant islands that are left are becoming more fragmented and isolated in a landscape widely dominated with anthropogenic activity (Figs. 7, 8).

Genetic factors play a role in the viability of small populations (Frankham and Ralls 1998; Frankham 2005). Traits that decrease fitness (i.e., sperm abnormalities, heart defects, disease susceptibility, and suppressed reproductive rates) are known to increase in frequency in small, isolated populations, causing inbreeding depression (Reed et al. 2003; Reed and Frankham 2003; Frankham 2005). This has been empirically shown in the Florida panther, cheetah (*Acynonix jubatus*), African lion (*P. leo nubica*), Asiatic lion, and many other inbred populations of naturally outbreeding organisms (O'Brien et al. 1985; O'Brien and Evermann 1988; Wildt et al. 1987; O'Brien et al. 1987; Roelke et al. 1993). Conservation actions designed to restore genetic diversity and avoid inbreeding depression, such as trapping and translocating ocelots between the two populations in Texas, and supplementing both with ocelots from northeastern Mexico, need to be implemented immediately to ensure persistence of ocelots in the U.S. This recommendation was also suggested by Haines et al. (2006c) from habitat-based population viability analysis that evaluated different recovery strategies.

Bobcat interchange among populations in Texas seems to be occurring based on our analysis of genetic variation. High levels of genetic diversity and gene flow, similar to other regions of the US (Croteau et al. 2012; Reding et al. 2012), illustrates the resilience and adaptability of bobcats under increasing anthropogenic changes to ecosystems in southern Texas. However, despite their resilience, the highest sources of mortality in southern Texas are anthropogenic (e.g., road-kills; Haines et al. 2005a; Blankenship et al. 2006), and some studies have indicated that bobcats tend to avoid urban areas with low prey abundance and habitat, thereby reducing gene flow (Crooks 2002; Riley et al. 2003, 2006, 2010; Lee et al. 2012). Evidence from our data suggest that bobcat dispersal is indeed reduced where anthropogenic impacts to the landscape are excessive, as seen in parts of the Lower Rio Grande Valley. Even in this extreme case, however, bobcats appear to be considerably less impacted than ocelots. However, wildlife agencies should be cautious in interpreting high variation and connectivity in a species because there can be substantial lag time before changes in demography are manifested in genetic diversity.

Landscapes are changing as a result of anthropogenic processes, some of which are creating a mosaic of habitat patches. Such fragmentation can have both ecological (Didham 2010; Gubbi et al. 2012) and genetic (Delaney et al. 2010) consequences. As indicated by Henle et al. (2004), species differ in their sensitivity to habitat fragmentation and human activity (Rogala et al. 2011). Some of the predictors (e.g., dispersal power, ecological specialization, population size) outlined by these authors may help explain the difference in genetic response shown by ocelots and bobcats. Unlike bobcats that occupy a broad range of habitat types, including urban settings, ocelots show a strong preference for dense thornshrub, which was once more abundant in southern Texas. This habitat specialization in combination with small population sizes and an inability to disperse across barriers, such as highways and open areas, probably explains why ocelots have been unable to recover from previous population reductions and habitat fragmentation. In contrast, despite habitat alterations and continued harvesting of bobcats, this species has maintained a wide distribution, high abundance, and population connectivity. The patterns of genetic variation and gene flow observed for these two sympatric species of felids suggests that using a surrogate species, such as the bobcat, to predict the response of another species to potential barriers to dispersal across a fragmented landscape should be approached with caution. For endangered species like the ocelot, sustainability of fragmented populations requires careful attention to factors that might confound their management and conservation.

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