Section 6 (Texas Traditional) Report Review

Attachment to letter dated APR 03 2009

TPWD signature date on report 3/5/09

Project Title: Conservation Genetics of Endangered Ocelot in Texas and Northeastern Mexico

Final or Interim Report? Interim

Grant #: 77-R

Reviewer Station: Corpus Christi ESFO and Laguna Atascosa NWR

Lead station was contacted and concurs with the following comments:
☐ Yes ☐ No ☒ Not applicable (reviewer is from lead station)

.....

Interim Report (check one):

☐ is acceptable as is

☐ is acceptable as is, but comments below need to be addressed in the next report

☐ needs revision (see comments below)

Final Report (check one):

☒ is acceptable as is

☐ is acceptable, but needs minor revision (see comments below)

☐ needs major revision (see comments below)

Comments:

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-77-R

Endangered and Threatened Species Conservation

Conservation Genetics of Endangered Ocelot in Texas and Northern Mexico

Prepared by:

Jan Janecka

Carter Smith
Executive Director

Clay Brewer, Acting
Division Director, Wildlife

5 March 2009

FINAL REPORT

STATE: ____Texas_____ **GRANT NUMBER:** ___TX E-77-R_____

GRANT TITLE: Endangered and Threatened Species Conservation

REPORTING PERIOD: ____8/1/06 to 7/31/09

PROJECT TITLE: Conservation Genetics Of Endangered Ocelot In Texas And Northern Mexico

OBJECTIVE(S):

To characterize patterns of genetic variation in the remaining ocelot populations of Texas and Northern Mexico.

Segment Objectives:

Task 1. TAMU shall analyze ocelot samples (approximately 120 individuals) obtained during previous radio-telemetry research and opportunistically collected from road-kills.

Task 2. Genomic and mitochondrial DNA shall be extracted from tissue and genotyped at 20 microsatellite loci, and a portion of the mitochondrial control region will be sequenced using previously described protocols.

Task 3. Population genetic statistics for both mtDNA and microsatellite loci shall be used to evaluate overall levels of genetic variation within populations and degree of genetic divergence between populations of ocelot in Texas and northern Mexico.

Task 4. Evidence of subdivision shall be evaluated with exact tests, pair-wise F_{st}, and AMOVA. Assignment test data shall be used to assign road-killed animals to existing populations. At the same time, assignment tests shall be used to evaluate the degree of population subdivision as a consequence of age and sex and to estimate dispersal between populations.

Task 5. Relationships among individuals within each population shall be examined and levels of inbreeding (estimated from the overall statistics related to allelic and genotypic frequencies) and estimates of effective population size will be compared across populations in both Texas and Mexico.

Summary Of Progress:

See .pdf file, entitled “TX E-77-R Final Report.”

Significant Deviations:

None

Location: Laboratory facilities at Texas A&M University, College Station, Texas.

Cost: __available upon completion of grant.

Prepared by: _Craig Farquhar_____ **Date:** _5 March 2009___

Approved by: _____ **Date:**____5 March 2009_____

C. Craig Farquhar

FINAL REPORT
Texas Parks and Wildlife Department
2/28/09 (Extended Deadline)

CONSERVATION GENETICS OF ENDANGERED OCELOT IN TEXAS AND NORTHERN MEXICO

Grant #: E-77-R
Contract #187221

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Conclusions

Ocelot (*Leopardus pardalis*) populations have been drastically reduced and fragmented in the United States, making them a high conservation priority. Only two small, isolated populations remain in the US, both of which occur in southern Texas. We examined the genetic diversity of ocelots in Texas and northeastern Mexico to estimate population parameters important for developing effective recovery strategies.

Analysis of 31 autosomal microsatellites, 12 Y-linked microsatellites, and 419-bp of the mitochondrial control region revealed lower genetic diversity in Texas. The lowest levels were observed in the Laguna Atascosa National Wildlife Refuge (Cameron population), the highest levels in Mexico (Mexico population), with intermediate variation in Willacy County, Texas (Willacy population). All tests of population structure showed very high differentiation of the two remaining Texas populations (and also between Texas and Mexico) consistent with complete isolation and no exchange of individuals for >30 years.

The lower genetic diversity in the Texas populations was consistent with the small effective population size (N_e) estimates from temporal changes in allele frequencies. The maximum N_e estimated was 14.4 in Cameron and 3.6 in Willacy, well below the critical value recommended for even short-term population viability. There was a reduction in genetic diversity during the 1990s. Genetic erosion will continue without direct intervention as a result of small population size and complete isolation.

In Texas, genetic diversity observed from historical specimens collected between 1890 and 1956 was comparable to Mexico. This strongly suggests that the low diversity in Texas is a direct result of human-caused ocelot population reductions and fragmentation during the 20th century. Recovery strategies that alleviate genetic drift, inbreeding, and loss of adaptive variation need to be implemented in Texas to avert potential negative effects on fitness. Translocations are required to minimize further loss in genetic variation. Due to the extreme divergence of the Texas populations, northeastern Mexico would be an appropriate source area, with preference given to the most genetically divergent ocelots at variable autosomal, Y-linked, and mtDNA markers.

1. Introduction

In the 19th and early 20th centuries, habitat degradation and human encroachment extirpated ocelot populations from large parts of their range in the United States, and currently only 2 fragmented populations occur in Texas (Tewes and Everett 1986; Navarro *et al*. 1993; Haines *et al*. 2005; Haines *et al*. 2006). These events lead to the listing of the ocelot as Endangered under the US Endangered Species Act (USFWS 1999). In the Tamaulipan Biotic Province (TBP), ocelots exhibit strong habitat selection for dense thorn-shrub with greater than 95% canopy cover (Shindle and Tewes 1998). In the TBP, the remaining habitat used by ocelots occurs in isolated patches in the Lower Rio Grande Valley (Tewes and Everett 1986; Navarro *et al*. 1993). One of the 2 major areas where ocelots occur is the Laguna Atascosa National Wildlife Refuge (LANWF), the other is on private ranches approximately 30 km northeast of the refuge in Willacy County. The ocelot has not recovered to its former distribution and abundance in the US, likely due to lack of habitat and dispersal between the remaining ocelot populations in Texas, as well as between Texas and northern Mexico (Navarro *et al*. 1993; Haines *et al*. 2005; Haines *et al*. in press).

Increasingly, state and federal wildlife agencies are adopting genetic methodologies in the management of their wildlife populations because of the important information that can be obtained with molecular techniques (Avise 1994; DeYoung and Honeycutt 2005). Parameters that must be taken into consideration during the drafting of successful recovery plans are population connectivity, effective population size (N_e), and estimates of rates of genetic erosion, and these can be estimated from genetic data. There is also strong evidence that genetic diversity is closely associated with population viability and the adaptability of populations to biological and environmental changes (Neigel 1996).

The original recovery plan for the ocelot emphasized determination of both existing population sizes and the amount of habitat required to sustain viable populations. Recommendations were made to increase available habitat through either restoration and/or the establishment of corridors, and by either augmenting existing populations or establishing new populations by means of translocation (USFW 1990). The plan states “the Texas population of the ocelot can be de-listed when it has reached a level that is considered demographically stable and genetically viable.” The original recovery plan clearly articulates concern over limited gene flow between populations and levels of genetic diversity within populations, and more recent comments by the recovery team have listed genetics as high priority. Recently an effort has gone underway to supplement Texas ocelot populations with individuals from Mexico. Therefore, a detailed assessment of genetic variation in the remaining ocelot populations in Texas and northern Mexico is essential to the overall goals of the ocelot recovery plan.

Previous work by Walker (1997) suggest the following: (1) The population at Laguna Atascosa NWR has significantly lower levels of heterozygosity than the other population in Texas, and both Texas populations appear to have lower levels of genetic variation than those in northern Mexico. (2) Loss of variation in the Texas populations may be the result of recent habitat fragmentation and extremely low effective population sizes, especially at Laguna Atascosa. These results allude to a potential decline in genetic viability within the remaining ocelot populations in Texas, and the population at Laguna Atascosa NWR is particularly problematic.

However, prior to devising any translocation plans for offsetting the continued loss of genetic variation in ocelot populations in Texas, several additional pieces of genetic information need to be obtained. A detailed assessment of existing levels of

genetic variation within and between remaining populations of ocelot in Texas, a determination of the number of individuals that breed each generation, and the identification of appropriate source populations for possible translocations is required. An accurate estimate of the number of successfully breeding individuals (effective population size [N_e]) is essential to population viability, because the effective population size is directly correlated to the rate at which genetic variation will erode in genetically isolated populations (Honeycutt 2000; DeYoung and Honeycutt 2005). In addition, small numbers of breeding individuals combined with lack of dispersal increase the chance of inbreeding, possibly resulting in genetic disorders such as sperm abnormalities.

The goal of this project was to further characterize patterns of genetic variation in the remaining ocelot populations in Texas, especially as it relates to the overall population size, the number of breeding individuals, movement between the remaining populations, levels of inbreeding, and evidence for a genetic bottleneck in response to more recent habitat fragmentation. We used a panel of autosomal and Y-chromosome microsatellite loci, and a segment of mitochondrial DNA (mtDNA) to address the following objectives: (1) Estimate population parameters including the population structure, gene flow, effective population size (number of breeding individuals), and changes in effective population size. (2) Estimate rates of genetic erosion in autosomal chromosomes, the Y-chromosome, and mitochondrial neutral loci. (3) Determine the extent to which more recent loss of habitat is responsible for current levels of genetic variation in Texas. This information will aid on the development of an effective recovery plan for the establishment of ocelot populations with long-term viability.

2. Methods

2.1. Study area and samples

During previous radio-telemetry studies between 1984 and 2005, blood and tissue samples were taken from 127 wild-caught ocelots (Laack 1991; Beltran and Tewes 1995; Caso 1994; Horne 1998; Shindle and Tewes 2000; Laack *et al*. 2005; Haines *et al*. 2005; Haines *et al*. 2006). All sampling sites were located in the Tamaulipas Biotic Province (TBP) in southern Texas and northeastern Mexico (Figure 1). This province extends from Tamaulipas (Mexico) to southern Texas and is characterized by thorn-shrub, scrub forests, and mixed grassland-forest habitats. This region is a transition zone between the Nearctic and Neotropical fauna (Blair 1950).

Sampling localities included areas the encompassed the two remaining Texas populations (Table 1) and Mexico. There were 63 samples from LANWR, Cameron County, Texas (n = 63, referred to as the Cameron population), (2) 39 samples from private ranches in northern Willacy County, Texas (n = 39, referred to as Willacy population). Twenty-five samples were collected from Tamaulipas, Mexico (referred to as the Mexico population). In Texas, 16 ocelots were also sampled outside of the primary areas occupied by the two populations. These included 14 sampled collected as road-kills (all in the Lower Rio Grande Valley), and 2 cats live-trapped in the Lower Rio Grande Valley, one at Santa Anna NWR n 1992, and the other in Port of Brownsville in 1998 (Table 2). Samples from road-killed ocelots (n = 14) were collected in Texas adjacent to the above localities.

Majority of the blood samples collected during radio-telemetry studies were stored in lysis buffer (Longmire *et al*. 1997). Some samples were not stored in any preservation buffer (particularly those collected prior to 1993 and some from Mexico), many of these had DNA too degraded for successful genetic analysis. The tissue

samples collected from road-kills were stored frozen or in lysis buffer. All DNA extractions were performed with a PureGene® DNA extraction kit (Gentra Systems, USA) following the supplier's protocol.

2.2. Autosomal microsatellite screening and genotyping

We selected 41 autosomal microsatellite loci (Table 3) characterized by Menotti-Raymond et al. (1999) in the domestic cat for screening to identify informative microsatellites. Microsatellite loci were selected to cover a broad part of the genome based on their location in the domestic cat whole-genome radiation hybrid map (Build 0.1 (NCBI Map Viewer). Felids have high-level of chromosomal conservation, therefore >95% of the gene order is conserved across Felidae, allowing for the application of the domestic cat map to other wild cats. Of the 41 loci screened, 31 produced good PCR amplification and clean genotypes (Table 3 and 4), and were used in the subsequent analysis of neutral autosomal variation.

The PCR amplifications were conducted in 10 µl volume. There were two reaction conditions used (see Table 2). The first contained 0.2 mM of each dNTP, 1X PCR HotMaster™ *Taq* buffer with 2.5 mM $MgCl_2$ (Eppendorf, Germany), 0.25 units of HotMaster™ *Taq* (Eppendorf), 0.24 mM forward primer labeled with a fluorescent dye, 0.24 mM reverse primer, and 20 ng DNA template. The thermocycler conditions included an initial denaturing step of 94ºC for 60 s, 30 cycles of 94ºC for 30 s, 53ºC for 30 s, 72ºC for 60 s, and a final extension step of 72ºC for 2 min. The second reaction conditions included 0.8 mM of each dNTP, 1x PCR Platinum *taq* buffer (Invitrogen, USA), 2.5 mM $MgCl_2$, 0.2 units of Platinum *taq* (Invitrogen), 0.027 mM forward primer labeled with a m13 sequence tag on the 5' end, 0.4 mM m13 primer 5' labeled with a fluorescent dye, 0.4 mM reverse primer, and 20 ng DNA template, and thermocycler conditions of 94ºC for 2 m, 40 cycles of 94ºC for 15 s, 55ºC for 30 s, 72ºC for 60 s, and a final extension step of 72ºC for 5 min.

The PCR products were genotyped on either an ABI 3100 automated sequencer (Applied Biosystems, USA) in the Laboratory of Plant Genomics and Technology, Texas A&M University and sized using GENOTYPER 2.0 (Applied Biosystems) or an ABI 3730 sequencer in the Veterinary Integrative Biosciences, Texas A&M University. A minimum of 2 previously genotyped ocelot samples were included with every sample set genotyped to ensure alleles were consistently sized.

2.3. Y-chromosome microsatellite screening and genotyping

There is a lack of available microsatellite loci on the Y chromosome for population analysis of felids. We therefore tested microsatellites recently discovered in introns of genes located on the Y chromosome of the domestic cat for applications in the ocelot. Introns were sequenced as part of Dr. William Murphy's research on felid Y chromosomes. One of Dr. Murphy's MS students (Brian Davis, Texas A&M University) designed primers flanking microsatellites present in these introns as part of his thesis research. We screened these 28 Y-linked microsatellite loci distributed in 7 genes for variation on the ocelot. The PCR conditions used included 0.8 mM of each dNTP, 1X PCR Platinum *taq* buffer (Invitrogen), 1.5 mM $MgCl_2$, 0.2 units of Platinum *taq* (Invitrogen), 0.027 mM forward primer labeled with a m13 sequence tag on the 5' end, 0.4 mM m13 sequence primer 5' labeled with a fluorescent dye, 0.4 mM reverse primer, and 20 ng DNA template, and cycles of 94ºC for 2 m, 40 cycles of 94ºC for 15 s, 58ºC for 30 s, 72ºC for 60 s, and a final extension step of 72ºC for 5 min. Alleles were genotyped as above. Of the 28 Y-linked loci screened, only 18 yielded a single PCR

product. These 18 loci were screened in 75 male ocelots, and 12 were successfully genotyped (Table 5).

2.4. Mitochondrial control region segment sequencing

A 419-bp segment of the control region was sequenced and aligned for 86 ocelots using primers from Jae-Heup et al. (2001) that were modified to complement the ocelot mitochondrial DNA sequence.

PAN-OCELOT-F primer, 5'CTCAACTATCCGAAAGAGCTT
PAN-OCELOT-R primer, 5'CCTGTG GAACATTAGGAATT

This fragment is homologous with 16,821 to 229 positions of the domestic cat mitochondrial genome and is located in the central conserved region between repetitive sequences I and II (Lopez et al. 1996, Jae-Haup et al. 2001). The PCR amplification was performed in 25 µL volumes containing 0.2 mM of each dNTP, 1X PCR buffer (10 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$, 50 mM KCl; Sigma-Aldrich™), 1.25 units of JumpStart *Taq* (Sigma-Aldrich™), 0.25 mM of forward primer, 0.25 mM of reverse primer, and 1 µL of 10–20 ng/µL DNA template. Reaction conditions included an initial denaturing step of 94˚C for 1 min, 30 cycles of 94˚C for 15 sec, 58˚C for 30 sec, 72˚C for 2 sec, and a final extension step of 72˚C for 2 min. The PCR products were sequenced using an ABI BigDye v. 1.1 Terminator Kit (Applied Biosystems) and ABI 3100 automated sequencer following the recommendations of the manufacturer (Applied Biosystems). Sequences were obtained in both directions and contiguous sequences were constructed using Sequencer v. 3.0 (Gene Codes Corporation, USA).

2.5. Genetic diversity – autosomal microsatellite analysis

Measures of genetic variability were estimated using GENALEX 6.0 (Peakall and Smouse 2001), and included observed heterozygosity (H_o), expected heterozygosity (H_e), mean number of alleles (A_m), number of effective alleles, and number of private alleles (A_p). Tests for Hardy-Weinberg equilibrium (HWE) were performed using GENEALEX. The Bonferroni method was used to correct *P*-values for multiple comparisons in the HWE (Rice 1989).

2.6. Genetic divergence between ocelot populations

To minimize temporal effects in the population structure analysis we excluded individuals captured prior to 1991. Samples were divided into three populations with a total data matrix of 94 individuals genotyped for 31 microsatellite loci: (1) Cameron (n = 42), (2) Willacy (n = 28), and (3) Tamaulipas (n = 14). Pair-wise F_{st} estimates were derived in GENEALEX. Effective numbers of migrants per generation (N_em) were estimated from the mean frequencies of private alleles by GENEPOP (Barton and Slatkin 1986; Slatkin and Barton 1986; Raymond and Rousset 1995). Assignment tests were conducted by estimating log-likelihood probabilities of individuals originating in each population using both GENECLASS 2.0 and GENEALEX (Piry *et al.* 2004). Both Bayesian and frequency methods were used to estimate assignment probabilities. Miss-assignments were compared between populations. The number of miss-assignments is directly related to dispersal between populations (Paetkau *et al.* 1995). GENECLASS was also used to detect first generation migrants in the three populations using the methods of Paetkau *et*

al. (2004). Individuals sampled outside of the study sites (e.g., road-killed ocelots and the two ocelot live-trapped outside the main population areas) were assigned to source populations using a Bayesian algorithm (Rannala and Mountain 1997)

2.7. Model-based genetic structure of individual samples

The Bayesian model-based clustering method of Pritchard *et al*. (2000) as implemented in STRUCTURE 2.1 (Pritchard *et al.* 2000) was used to assess population structure without regard to geographic origin. This approach uses a Bayesian algorithm to calculate the probabilities of a specific number of clusters (*K*, clusters are synonymous with populations) and the probabilities of individuals being assigned to each cluster. These probabilities are based solely on the allele frequencies and multi-locus genotypes. The probabilities for *K* = 1–10 clusters were estimated using the admixture model from two independent runs with a burn-in of 20,000 MCMC generations and sampling of 100,000 generations. The number of clusters was then determined from the likelihoods. The individuals composing each of the clusters were compared with their geographic origins.

2.8. Estimate of effective population size

Effective population size was estimated in NEESTIMATOR (Version 1.3; Peel *et al*. 2004) using two different temporal methods. These methods estimate N_e from the change in allele frequencies observed through time. The first approach used a Bayesian algorithm based on coalescence and was implemented by TM3 within NEESTIMATOR (Beaumont 2003; Berthier *et al*. 2002) and the second approach was a pseudo-likelihood method developed by Wang and Whitlock (2003) and implemented by MLNE within NEESTIMATOR. The N_e estimates were obtained for both Cameron and Willacy populations. There were not enough samples from Mexico for this analysis. The Cameron population was divided into two temporal groups (1986–1989, *n* = 14) and 1996–1999 (*n* = 17). The Willacy population was divided into a 1996-1998 group (n = 13) and a 2005 group (*n* = 10). Both of these division represented one ocelot generation (7 years).

2.9. MtDNA data analysis

Sequence alignments were performed in CLUSTAL-X (Thompson *et al*. 1997) and population statistics, including number of variable sites, haplotype diversity H_d, nucleotide diversity (π), and mean number of nucleotide differences, were calculated in DNASP 4.10.8 (Rozas *et al*. 2006). A minimum spanning network of haplotypes was constructed in ARLEQUIN 3.0 (Excoffier *et al*. 2006) and plotted to represent relationships among haplotypes. Tests for departure of haplotype frequencies from neutrality were performed using Tajima's D test and Fu and Li's D test in DNASP (Tajima 1989; Fu & Li 1993).

Two methods were used to compare mtDNA variation in the ocelot populations. First, an exact test for population differentiation based on haplotype frequencies was implemented in ARLEQUIN. Second, population structure was tested using pair-wise F_{st} estimates in ARLEQUIN. Estimates of F_{st} were tested for significance against the null distribution of F_{st} values obtained from 1,000 permutations.

2.10. Genetic diversity comparison between extant and historic populations

Samples were collected from ocelot specimens in the Smithsonian Institution from Texas and Mexico (1890 to 1956). The control region segment was sequenced and 11 microsatellites were genotyped for 11 specimens collected in southern Texas and 4 in northeastern Mexico. Due to the degradation of DNA in museum samples data was not available for all individuals at all loci. This is typical of studies incorporating historical samples. For the samples that we successfully analyzed, we compared genetic diversity levels between extant and historic ocelot populations, and levels of genetic divergence was tested using the mtDNA data.

3. Results and Discussion

3.1. Genetic diversity of autosomal microsatellite loci

The highest levels of genetic diversity across the 31 autosomal loci were observed in Mexico (A_m = 5.0, H_e = 0.612) with the lowest levels in the Cameron population (A_m = 2.74, H_e = 0.353; Table 4). The variation in the Willacy area was slightly below that of Mexico (A_m = 3.52, H_e = = 0.510). One of the loci (FCA208) was found to be out of HWE in all of the populations. In addition FCA023 and FCA132 were out of HWE in Cameron, along with FCA035 in Mexico. Only 77% of the loci were polymorphic in Cameron populations compared to 94% in Willacy and 97% in Mexico. Of the 62 private alleles detected at all loci, three were in Cameron, six in Willacy, and 54 in Mexico.

3.2. Genetic divergence between populations

Significant genetic structure was observed in all tests of population differentiation. In the AMOVA test, the R_{st} value was significant for population structure (P = 0.010) among the three populations. All three populations also showed significant levels of differentiation in the genic and genotypic tests implemented by GENEPOP (chi square = infinity, P = highly significant). This high level of structure was also reflected in the pair-wise F_{st} values. The highest F_{st} was between Cameron and Mexico (0.148), and the lowest was between Willacy and Mexico (0.067). The F_{st} between the two Texas populations was 0.105. The estimate of N_em obtained using the private allele method was 0.521 per generation between all pairs of populations. A N_em >1 is required to prevent divergence of populations. There were no miss-assigned individuals among the three populations, further supporting significant genetic structure (Figure 2). In all three populations, there were no first generation migrants detected.

3.3. Assignment of road-kills

All but two road-killed ocelots were assigned to the Willacy population. The two that were not (P-97-14 and P-95-15), were unable to be assigned to any population. The individual captured in Santa Anna also remained unassigned. The only ocelot assigned to Cameron originating from outside LANWR was the individual captured in Port of Brownsville.

3.4. Model-based clustering analysis of population structure

Model-based clustering without regard for geographic information consistently found K = 6 clusters among the three ocelot populations with a mean posterior probability of 1.00 for two independent runs. All individuals from Cameron County were in one cluster (Figure 3). The Willacy individuals were divided into two distinct clusters. Individuals from Tamaulipas were divided into three clusters. There were no individuals from more than one population assigned to the same cluster, consistent with the significant population differentiation observed in the other tests.

The separation of the Willacy population into two clusters was correlated with temporal distribution of samples. The first cluster included individuals (n = 14) that were captured in Willacy County between 1994 and 1998. The second cluster was composed of all individuals (n = 10) captured in 2005.

3.5. Loss of genetic diversity and genetic drift in autosomal microsatellites

We observed a pattern of loss of genetic diversity through time in both the Cameron and Willacy populations in the autosomal microsatellites. Our samples dated from 1986 to 2005 in Cameron, and from 1985 to 2005 in Willacy. To examine changes in diversity through time, we divided samples for each population into 3 groups. In Cameron, samples were separated into "Cameron 1986-91" (n = 15), "Cameron 1993–96" (n = 15) and "Cameron 1999–2005" (n = 15). The Willacy population was similarly divided into "Willacy 1984–91" (n = 14), "Willacy 1995–98" (n = 15) and "Willacy 2005" (n = 10). In both populations, we observed a reduction of the effective number of alleles (13% in Cameron and 19% in Willacy) and the heterozygosity (16% in Cameron and 21% in Willacy, Figure 4). The decline and isolation of the Cameron population appears to predate that of Willacy. Willacy retained more diversity through the 1980s, however, in the 1990s lost diversity more rapidly than Cameron.

From 1986 to 2005, the two Texas populations also exhibited greater levels population divergence as a result of genetic drift, due to the small population size and isolation. The pair-wise F_{st} values increase through time (Table 6) between all population groups. The initial F_{st} between Cameron 1986–91 and Willacy 1984–91 was 0.091, and went up to 0.128 for the later temporal groups. Similarly, the F_{st} values between both Texas populations and the Mexico population increased through the decade, from 0.130 to 0.158 in the case of Cameron, and from 0.064 to 0.110 in the case of Willacy.

3.6. Effective and census population size estimates in Texas

The N_e estimates for the Cameron population were 8.0 (*95% CI*: 3.2–23.1) and 13.9 (*95% CI*: 7.7–25.1), in Willacy they were substantially lower at 2.9 (*95% CI*: 1.7–5.6) and 3.1 (*95% CI*: 1.9–13.5). Previous studies have reported N_e/census size (N_c) ratios of 0.37 in ocelot (Ludlow and Sunquist 1987), 0.4 in tiger (Smith and McDougal 1991), and 0.25–0.5 in Florida panthers (Seal et al. 1989). Based on these studies and our N_e estimates, the census population sizes of ocelots in the two Texas populations are estimated to be 20–35 in Cameron, and 7–8 in Willacy using a N_e/N_c = 0.4. This is consistent with census size estimates from ecological studies in that area (Haines *et al*. 2006).

However, it is important to note that the N_e derived from temporal methods is the harmonic mean between the two time periods sampled. Therefore, the N_e estimate for the Cameron and Willacy populations is for 1992–1993 and 2001–2002, respectively.

The population size estimates from genetic data cannot be interpreted as representing current population sizes. We emphasize this because these estimates are frequently miss-interpreted by biologists. Nonetheless, our data suggests that the Willacy population appears more unstable and is at smaller size than Cameron.

3.7. Y-microsatellite diversity

Of the 28 Y-linked microsatellites tested in 8 male ocelots, 18 were successfully amplified. These 18 loci were screened in 75 male ocelots (Cameron n = 34, Willacy n = 31, Mexico n = 10) and clean genotypes were obtained for 12 (Table 5). However, only two loci were variable (Table 5). Three alleles were observed at SMC2, and three at SMC7 (Table 4). The Y-loci used in this study are located in the single copy non-recombinant region of the Y chromosome. Therefore, the loci represent one haplotype.

Based on the SMC2 and SMC7 microsatellites, there was a total of five Y-haplotypes observed. Only one of these was detected in Cameron, and there were three haplotypes observed in Willacy and three in Mexico. The 172/173 haplotype was the most common in both Texas populations. The haplotype diversity was lowest in Cameron (H_d = 0), intermediate in Willacy (H_d = 0.462), and highest in Mexico (H_d = 0.667). Unfortunately, because of the low number of males and the poor DNA quality of many samples from Mexico, only 3 males from Mexico were successfully haplotyped.

3.8. Mitochondrial diversity, differentiation, and drift

A 419 bp fragment of the control region was sequenced and aligned for 86 ocelots. There were 3 variable sites distributed among 4 haplotypes, and each haplotype differed by only one mutation (n = 86; Table 7 and Figure 5). The central haplotype was found in all populations and was at the highest frequencies (Table 7 and 8; Figure 4). Only 1 haplotype was observed in the Cameron population (n = 26). Two haplotypes were identified in 34 ocelots from Willacy, one of which was the haplotype observed in Cameron. Two additional haplotypes were observed in Mexico. The highest levels of haplotype and nucleotide diversity (H_d = 0.733, π = 0.00282) were observed in Mexico (Table 7). Intermediate levels were observed in Willacy during the mid-90's.

The greatest level of mtDNA differentiation was between the Cameron and Mexico populations with an F_{st} value of 0.159, and significantly different haplotype frequencies. The lowest level of differentiation was between Willacy and Cameron (F_{st} = 0.055).

There was no mtDNA diversity observed in Cameron. However, a rapid loss of diversity was observed in Willacy over a span of only 20 years. In this population, haplotype diversity decreased from 0.536 in the 1984–1990 period, to 0.233 in the 1994–1998. By 2005, there was no diversity detected in the mitochondrial control region in the Willacy population. This was consistent with the genetic erosion observed in the autosomal microsatellites.

3.9. Historical genetic diversity

The microsatellite diversity among the museum specimens sampled in Texas was higher than observed in both contemporary Texas populations. The mean number H_e in the historical Texas samples (1890–1935) was comparable to that of Mexico (0.615 vs. 0.623, respectively). Nine private alleles were present in the historical samples from Texas. In contrast, only one private allele was present in Cameron and three in Willacy (compared to 26 in Mexico). Pair-wise F_{st} was estimated to examine the change in allele

frequencies that occurred in Texas. The most genetically divergent pair of populations was Cameron (sampled 1996–1998) and Mexico (sampled 1994–1998) with F_{st} = 0.243. The lowest divergence observed was between Willacy and the historical samples from Texas (F_{st} = 0.082). The Cameron population was more divergent from the historical samples (F_{st} = 0.187), than it was from contemporary Willacy population (F_{st} = 0.129).

There were a total of four mtDNA haplotypes observed in the Texas historical samples (1890–1956), compared to only two in the contemporary populations (one of which was no longer observed in 2005, Table 6). Historical levels of genetic diversity in Texas (H_d = 0.673, π = 0.00191) were comparable to that of the extant population in Mexico. The haplotype frequencies in the historical Texas population were most similar to Willacy (1994–1998, F_{st} = -0.025), and most divergent from Cameron. A loss of genetic diversity occurred during the 20th century among ocelot populations in Texas in response to anthropogenic factors.

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Table 1. Ocelot samples analyzed during this study.

#	ID	Sex	Date Collected	Collector	Location
Laguna Atascosa					
1	M65	M	1/18/86	Laack	LANWR
2	M82	M	6/29/86	Laack	LANWR
3	F88	F	8/4/86	Laack	LANWR
4	M95	M	4/10/87	Laack	LANWR
5	M110	M	3/4/88	Laack	LANWR
6	F118	F	6/15/88	Laack	LANWR
7	M125	M	10/23/88	Laack	LANWR
8	M128	M	1/13/89	Laack	LANWR
9	M132	M	2/18/89	Laack	LANWR
10	M147	M	7/10/89	Laack	LANWR
11	F150	F	8/4/89	Laack	LANWR
12	F151	F	8/12/89	Laack	LANWR
13	F158	F	11/9/89	Laack	LANWR
14	M165	M	5/3/91	Laack	LANWR
15	M170	M	2/22/91	Laack	LANWR
16	F172	F	2/28/91	Laack	LANWR
17	M174	M	3/26/91	Laack	LANWR
18	M175	M	4/30/91	Laack	LANWR
19	F176	F	5/11/91	Laack	LANWR
20	M179	M	4/47/92	Laack	LANWR
21	F182	F	11/19/92	Laack	LANWR
22	M183	M	11/24/92	Laack	LANWR
23	F184	F	1/25/93	Laack	LANWR
24	F186	F	5/11/93	Laack	LANWR
25	F189	F	2/19/94	Laack	LANWR
26	M191	M	2/23/95	Laack	LANWR
27	M192	M	3/18/95	Laack	LANWR
28	M193	M	5/11/95	Laack	LANWR
29	F194	F	11/7/95	Laack	LANWR
30	M195	M	11/28/95	Laack	LANWR
31	F197	F	3/13/96	Laack	LANWR
32	F198	F	3/14/96	Laack	LANWR
33	M199	M	XX/XX/95	Laack	LANWR
34	F201	F	4/22/96	Laack	LANWR
35	M202	M	4/22/96	Laack	LANWR
36	M205	M	5/1/96	Laack	LANWR
37	M209	M	5/28/96	Laack	LANWR
38	F214	F	12/15/96	Laack	LANWR
39	M217	M	4/23/97	Laack	LANWR
40	M218	M	5/1/97	Laack	LANWR
41	F219	F	5/7/97	Laack	LANWR
42	M222	M	12/7/97	Laack	LANWR
43	F223	F	4/9/98	Laack	LANWR
44	M224	M	4/10/98	Laack	LANWR
45	M225	M	4/30/98	Laack	LANWR
46	M226	M	5/6/98	Laack	LANWR
47	F228	F	11/22/98	Laack	LANWR
48	F229	F	3/29/99	Laack	LANWR
49	F230	F	12/15/99	Laack	LANWR
50	F235	F	4/5/00	Laack	LANWR
51	F236	F	4/6/00	Laack	LANWR
52	M238	M	12/3/00	Laack	LANWR
53	M239	M	12/19/00	Laack	LANWR
54	M240	M	3/23/01	Laack	LANWR
55	M241	M	4/28/01	Laack	LANWR
56	F242	F	11/27/01	Laack	LANWR
57	M244	M	4/1/03	Laack	LANWR
58	M245	M	12/11/03	Laack	LANWR
59	M246	M	1/10/04	Laack	LANWR
60	M247	M	1/10/04	Laack	LANWR
61	M248	M	XX/XX/05	Laack	LANWR
62	F249	F	XX/XX/05	Laack	LANWR
63	F250	F	XX/XX/05	Laack	LANWR

#	ID	Sex	Date Collected	Collector	Location
WILLACY					
1	ER1	M	5/17/91	Beltran	Robert East
2	ER2	F	5/20/91	Beltran	Robert East
3	STC-33	M	9/29/85	Twedt	Rockefeller
4	STC-34	M	9/29/85	Twedt	Rockefeller
5	RR4	M	6/25/91	Shindle	Rockefeller
6	RR6	F	6/27/91	Shindle	Rockefeller
7	RR7	M	6/28/91	Shindle	Rockefeller
8	YT1	M	4/19/91	Beltran	Yturria
9	YT2	M	4/19/91	Beltran	Yturria
10	YT3	F	4/19/91	Beltran	Yturria
11	YT4=Y941	M	4/19/91	Beltran	Yturria
12	YT8	F	5/13/91	Beltran	Yturria
13	STC-18	M	8/23/84	Twedt	Yturria
14	STC-32	?	9/29/85	Twedt	Yturria
15	Y946	M	1/23/94	Shindle	Yturria
16	Y947	M	1/28/94	Shindle	Yturria
17	Y961	M	4/29/96	Shindle	Yturria
18	Y964	M	5/1/96	Shindle	Yturria
19	Y971	F	1/23/97	Shindle	Yturria
20	Y972	M	1/25/97	Shindle	Yturria
21	Y973	M	1/26/97	Shindle	Yturria
22	Y974	F	1/26/97	Shindle	Yturria
23	Y975	M	1/26/97	Shindle	Yturria
24	Y976	M	5/7/97	Shindle	Yturria
25	Y977	F	1/29/98	Shindle	Yturria
26	Y981	M	1/28/98	Shindle	Yturria
27	Y982	M	1/29/98	Shindle	Yturria
28	Y983	M	1/31/98	Shindle	Yturria
29	Y984	F	2/4/98	Shindle	Yturria
30	Y1-05	F	2/7/05	Grassman	Yturria
31	Y2-05	M	2/7/05	Grassman	Yturria
32	Y3-05	F	2/7/05	Grassman	Yturria
33	Y4-05	F	2/8/05	Grassman	Yturria
34	Y5-05	M	2/9/05	Grassman	Yturria
35	Y6-05	M	2/9/05	Grassman	Yturria
36	Y7-05	M	2/10/05	Grassman	Yturria
37	Y8-05	F	2/10/05	Grassman	Yturria
38	Y9-05	F	2/10/05	Grassman	Yturria
39	Y10-05	M	3/2/05	Grassman	Yturria
MEXICO					
1	Mex 1	M	6/15/91	Caso	Los Ebanos
2	Mex 2	F	6/15/91	Caso	Los Ebanos
3	Mex 3	F	6/16/91	Caso	Los Ebanos
4	Mex 4	M	6/29/91	Caso	Los Ebanos
5	Mex 5	M	1/24/92	Caso	Los Ebanos
6	Mex 6	M	3/5/92	Caso	Los Ebanos
7	Mex 7	F	3/31/92	Caso	Los Ebanos
8	Mex 9		?	Caso	Los Ebanos
9	Mex 10	F	10/9/95	Caso	Los Ebanos
10	Mex 14	M	?	Caso	Los Ebanos
11	Mex 18	F	11/22/01	Caso	Los Peritos
12	Mex 19	M	3/9/00	Caso	Los Ebanos
13	Mex 22	F	11/22/01	Caso	Los Ebanos
14	Mex 23	F	11/23/01	Caso	Los Ebanos
15	Mex 26	F	10/23/02	Caso	Los Ebanos
16	Mex 27	M	10/21/05	Caso	Los Ebanos
17	Mex 28	M	5/10/04	Caso	Los Ebanos
18	Mex 29	F	11/1/04	Caso	Los Ebanos
19	MI2	M	6/23/94	Caso	Miradores
20	MI3	F	6/23/94	Caso	Miradores
21	MI4	M	6/24/94	Caso	Miradores
22	LM2	F	xx/xx/94	Caso	La Mesa
23	Z2	M	11/xx/94	Caso	Los Zoyates
24	Z3	M	6/xx/94	Caso	Los Zoyates
25	Z4	F	3/xx/1994	Caso	Los Zoyates

Table 2. Individuals sampled outside of the primary areas occupied by the two remaining ocelot populations in Texas.

#	ID	Sex	Date Collected	Collection	Collector	Location	Comments
1	PM1	M	7/29/91	road kill	Beltran	P. Mansfield	
2	M168	M	11/16/89	road kill	Game Warde	P. Mansfield	Highway 186 1.6 km W of bridge
3	F161	F	?	road kill	?	P. Mansfield	
4	M162	M	?	road kill	?	P. Mansfield	Fetus in F161
5	PM93	?	10/xx/1993	road kill	?	P. Mansfield	
6	RK1/12/04	M	1/12/04	road kill	?	P. Mansfield	9.6 km W of Port Mansfield
7	SARITA	M	10/15/97	road kill	Shindle	Sarita	4.5 km North of Sarita
8	310-AGO-90	M	8/31/90	road kill	Tewes	Sarita	6.6 km South of Sarita
9	Y962	M	10/27/97	road kill	Shindle	Lyford	1 mi. N. of Lyford, Hwy 77
10	RK1999	?	6/17/99	road kill	D. Martinez, \	Willacy	Hw 186
11	P-97-14	M	4/7/97	road kill	Shindle	Cameron	[1]Cameron County, Hwy 106
12	F180	F	5/8/92	trapped	Laack	Santa Anna	
13	Port1	M	4/27/98	trapped	Shindle	P. Brownsville	Port Brownsville
14	P-95-150	?	5/8/95	road kill	Laack	?	
15	P-95-15	M	5/19/95	road kill	?	?	
16	17039-001	?	?	road kill	USFWS	?	

1. Near RGV Shooting Center Driveway, this is a Laguna collared cat captured initially in May 1995

Table 3. Autosomal microsatellites used in this study. Location refers to that in the domestic cat linkage map. Microsatellites in bold were selected for genetic analysis of ocelot populations. Dye labeling refers how the PCR amplicon was fluorescently labeled. Direct = 5'-labeled forward primer, m13 tag = labeled using a 5'-labeled m13 primer. Note: m13 tag adds 18 bp to the alleles. The PCR conditions refer to the 1st and 2nd conditions described in the methods.

Number	Chromosome	Position (cM)	Locus	PCR Results	Dye Labeling	PCR Conditions
1	**A1**	**62.2**	**FCA229**	**Positive**	**m13 tag**	**1**
2	**A1**	**124.5**	**FCA090**	**Positive**	**Direct**	**2**
3	A1	255.8	FCA100	Positive		
4	**A1**	**264.8**	**FCA008**	**Positive**	**Direct**	**2**
5	**A2**	**118.2**	**FCA105**	**Positive**	**Direct**	**2**
6	**A2**	**162.2**	**FCA124**	**Positive**	**m13 tag**	**1**
7	**A3**	**82.2**	**FCA208**	**Positive**	**m13 tag**	**1**
8	**A3**	**111.2**	**FCA171**	**Postive**	**m13 tag**	**1**
9	B1	178.9	FCA212	Positive		
10	**B1**	**46.9**	**FCA023**	**Positive**	**Direct**	**2**
11	**B1**	**184.9**	**FCA126**	**Positive**	**Direct**	**2**
12	B2	0	FCA275	PCR failed		
13	B2	171.3	FCA133	PCR failed		
14	**B2**	**2.2**	**FCA833**	**Positive**	**m13 tag**	**1**
15	**B3**	**44.2**	**FCA201**	**Positive**	**m13 tag**	**1**
16	**B3**	**173.2**	**FCA088**	**Positive**	**m13 tag**	**1**
17	**B4**	**0**	**FCA857**	**Positive**	**m13 tag**	**1**
18	**B4**	**151.1**	**FCA044**	**Positive**	**m13 tag**	**1**
19	**C1**	**0**	**FCA873**	**Positive**	**m13 tag**	**1**
20	C1	430.2	FCA890	PCR multi-banded		
21	C2	0	FCA568	Positive		
22	**C2**	**72.2**	**FCA077**	**Positive**	**Direct**	**2**
23	**C2**	**160.2**	**FCA117**	**Positive**	**Direct**	**2**
24	**C2**	**176.2**	**FCA043**	**Positive**	**Direct**	**2**
25	**D2**	**3.9**	**FCA165**	**Positive**	**m13 tag**	**1**
26	**D2**	**34.9**	**FCA262**	**Positive**	**m13 tag**	**1**
27	**D2**	**94.2**	**FCA035**	**Positive**	**Direct**	**2**
28	**D2**	**115.2**	**FCA078**	**Positive**	**Direct**	**2**
29	**D3**	**0**	**FCA523**	**Positive**	**m13 tag**	**1**
30	**D3**	**83.2**	**FCA249**	**Positive**	**Direct**	
31	D3	87.2	FCA026	PCR failed		
32	**D3**	**110.2**	**FCA132**	**Positive**	**Direct**	**2**
33	**D4**	**103.9**	**FCA045**	**Positive**	**Direct**	**2**
34	**E1**	**35.9**	**FCA082**	**Positive**	**Direct**	**2**
35	**E2**	**122.5**	**FCA096**	**Positive**	**Direct**	**2**
36	**E2**	**131.5**	**FCA075**	**Positive**	**m13 tag**	**1**
37	**E3**	**92.3**	**FCA1015**	**Positive**	**m13 tag**	**1**
38	F1	17.3	FCA1297	PCR multi-banded		
39	**F1**	**113.9**	**FCA1034**	**Positive**	**m13 tag**	**1**
40	F2	25.5	FCA1311	PCR multi-banded		
41	F2	171.4	FCA1048	PCR failed		

Table 4. Genetic diversity among 31 autosomal microsatellites in three ocelot populations. Abbreviations: Ca = Cameron County, Texas; Wi = Willacy County, Texas; Mx = Mexico; Ho = observed Heterozygosity, He = expected Heterozygosity; Efct = Effective Alleles.

	Sample Size			No. of Alleles			No. of Efct. Alleles			Inform. Index			H_o			H_e			Fixation Index		
Locus	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**
FCA008	60	34	24	5	4	4	4.0	3.5	2.8	1.44	1.31	1.12	0.80	0.82	0.75	0.75	0.71	0.64	-0.07	-0.16	-0.17
FCA023	60	34	24	3	3	3	1.5	2.1	1.1	0.55	0.83	0.20	0.25	0.53	0.08	0.32	0.53	0.08	0.21	0.01	-0.03
FCA035	60	34	24	3	4	7	2.3	2.8	5.8	0.92	1.17	1.83	0.67	0.74	0.54	0.57	0.65	0.83	-0.16	-0.13	0.35
FCA043	60	34	23	1	2	5	1.0	1.7	2.1	0.00	0.61	1.04	0.00	0.35	0.43	0.00	0.42	0.53	#N/A	0.15	0.18
FCA045	59	34	23	2	3	5	1.8	2.8	4.9	0.63	1.06	1.59	0.44	0.74	0.61	0.44	0.64	0.79	-0.01	-0.15	0.23
FCA077	60	34	24	2	3	6	1.4	2.2	3.5	0.48	0.84	1.42	0.27	0.44	0.58	0.30	0.54	0.71	0.11	0.18	0.18
FCA078	60	34	12	2	2	4	1.2	1.3	2.3	0.29	0.39	0.96	0.17	0.26	0.50	0.15	0.23	0.56	-0.09	-0.15	0.11
FCA082	60	34	12	3	4	5	2.4	3.4	3.9	0.95	1.29	1.48	0.52	0.88	0.83	0.58	0.70	0.75	0.11	-0.26	-0.12
FCA090	55	30	9	4	5	4	2.5	3.4	3.6	1.08	1.36	1.32	0.67	0.67	0.89	0.60	0.71	0.72	-0.12	0.06	-0.23
FCA096	59	34	19	1	2	4	1.0	1.4	3.1	0.00	0.49	1.18	0.00	0.26	0.58	0.00	0.31	0.67	#N/A	0.14	0.14
FCA105	60	33	21	3	3	9	1.5	2.1	4.4	0.52	0.83	1.78	0.38	0.67	0.67	0.31	0.53	0.77	-0.23	-0.26	0.14
FCA117	56	34	16	2	4	5	2.0	2.5	3.2	0.69	1.07	1.36	0.57	0.65	0.44	0.50	0.60	0.69	-0.14	-0.08	0.37
FCA126	60	34	24	5	6	6	3.5	4.6	3.9	1.37	1.63	1.56	0.80	0.85	0.75	0.72	0.78	0.74	-0.11	-0.09	-0.01
FCA132	60	34	23	5	4	4	1.3	2.1	2.1	0.50	0.81	0.94	0.23	0.41	0.43	0.25	0.51	0.53	0.06	0.20	0.17
FCA249	59	34	15	1	1	1	1.0	1.0	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	#N/A	#N/A	#N/A
FCA044	48	34	9	3	5	5	1.2	2.5	3.8	0.37	1.17	1.44	0.17	0.62	0.89	0.17	0.61	0.73	0.04	-0.02	-0.21
FCA075	50	37	15	2	2	6	1.5	1.9	3.1	0.51	0.66	1.34	0.30	0.54	0.67	0.33	0.47	0.68	0.10	-0.15	0.02
FCA088	50	36	15	2	2	6	1.6	1.6	3.5	0.57	0.56	1.50	0.44	0.33	0.53	0.38	0.38	0.71	-0.14	0.11	0.25
FCA124	50	37	14	4	7	7	2.9	5.2	5.9	1.20	1.74	1.84	0.70	0.89	0.93	0.66	0.81	0.83	-0.06	-0.11	-0.12
FCA165	49	35	14	4	6	9	3.3	3.6	5.1	1.27	1.50	1.86	0.78	0.66	0.93	0.69	0.72	0.80	-0.12	0.09	-0.16
FCA171	50	36	14	3	2	5	1.1	1.8	2.4	0.27	0.63	1.13	0.12	0.53	0.64	0.11	0.43	0.58	-0.05	-0.21	-0.12
FCA201	43	35	14	2	3	4	1.1	2.2	2.4	0.15	0.90	1.02	0.07	0.43	0.29	0.07	0.54	0.59	-0.04	0.20	0.52
FCA208	47	31	11	5	6	5	3.0	2.1	2.8	1.29	1.13	1.21	0.17	0.16	0.18	0.67	0.53	0.64	0.74	0.70	0.71
FCA229	50	37	15	4	6	8	3.5	3.7	5.8	1.32	1.44	1.87	0.70	0.86	0.73	0.71	0.73	0.83	0.02	-0.19	0.11
FCA262	49	37	15	1	1	3	1.0	1.0	1.2	0.00	0.00	0.39	0.00	0.00	0.07	0.00	0.00	0.18	#N/A	#N/A	0.64
FCA523	50	34	15	3	3	5	2.5	1.8	3.6	1.01	0.78	1.40	0.68	0.50	0.73	0.60	0.44	0.72	-0.12	-0.14	-0.02
FCA833	47	32	10	1	2	2	1.0	1.5	1.6	0.00	0.53	0.56	0.00	0.19	0.10	0.00	0.34	0.38	#N/A	0.45	0.73
FCA857	50	36	11	1	3	4	1.0	1.8	2.4	0.00	0.75	1.11	0.00	0.39	0.36	0.00	0.44	0.59	#N/A	0.12	0.38
FCA873	50	36	14	1	2	2	1.0	1.4	1.2	0.00	0.47	0.26	0.00	0.25	0.14	0.00	0.30	0.13	#N/A	0.16	-0.08
FCA1015	50	36	15	3	3	7	2.3	2.5	6.3	0.89	1.01	1.88	0.68	0.64	0.73	0.56	0.60	0.84	-0.22	-0.06	0.13
FCA1034	48	32	11	4	6	5	1.9	2.7	3.5	0.84	1.19	1.36	0.38	0.59	0.73	0.48	0.62	0.71	0.22	0.05	-0.02
Mxan	*54*	*34*	*16*	*2.7*	*3.5*	*5.0*	*1.9*	*2.4*	*3.3*	*0.617*	*0.91*	*1.22*	*0.35*	*0.51*	*0.54*	*0.35*	*0.51*	*0.61*	*n/a*	*n/a*	*n/a*

Table 5. Genetic diversity observed among 12 microsatellites located on the Y chromosome in ocelots. Abbreviations: Ca = Cameron County, Texas; Wi = Willacy County, Texas; Mx = Mexico.

	N			Allele Size			Haploype Diversity		
Locus	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**	**Ca**	**Wi**	**Mx**
DDX2	24	21	4	124	124	124	0.000	0.000	0.000
DDX11	19	11	4	141	141	141	0.000	0.000	0.000
EIF4	30	24	4	205	205	205	0.000	0.000	0.000
EIF9	26	23	4	154	154	154	0.000	0.000	0.000
SMC2	25	24	3	170, 172	170, 172	168, 170, 172	0.077	0.278	0.667
SMC7	20	13	4	173	173, 177	169, 173, 177	0.000	0.355	0.375
UBE18	27	23	4	149	149	149	0.000	0.000	0.000
USP10	18	8	1	173	173	173	0.000	0.000	0.000
USP17	26	21	5	204	204	204	0.000	0.000	0.000
USP23	26	19	4	149	149	149	0.000	0.000	0.000
USP41	24	20	4	171	171	171	0.000	0.000	0.000
ZFY1	26	24	4	136	136	136	0.000	0.000	0.000

Table 6. The pair-wise F_{st} values between individuals divided into three temporal groups for the Cameron and Willacy populations, respectively. The Mexico population is included for comparison.

	Cameron 1986-91	Cameron 1993-96	Cameron 1999-05	Willacy 1984-91	Willacy 1994-98	Willacy 2005	Mexico
Cameron 1986-91	-						
Cameron 1993-96	0.039	-					
Cameron 1999-05	0.037	0.026	-				
Willacy 1984-91	0.091	0.115	0.112	-			
Willacy 1994-98	0.084	0.099	0.107	0.029	-		
Willacy 2005	0.128	0.166	0.174	0.056	0.080	-	
Mexico 1991-05	0.130	0.162	0.158	0.064	0.081	0.110	-

Table 7. Number of variable sites, haplotypes, haplotype diversity, and nucleotide diversity for 419 bp portion of the control region for 3 extant ocelot populations.

Location	Date	N	Variable Sites	Haplo.	Haplotype Diversity	SD	Nucleotide Diversity	SD
Texas/Mexico	1986-2005	86	3	4	0.254	0.06	0.00077	0.00020
Texas	1986-2005	68	1	2	0.163	0.057	0.00039	0.00014
Cameron	1986-1989	11	0	1	0.000	0.000	0.00000	0.00000
Cameron	1996-1998	10	0	1	0.000	0.000	0.00000	0.00000
Cameron	2004-2005	5	0	1	0.000	0.000	0.00000	0.00000
Willacy	1984-1990	8	1	2	0.536	0.123	0.00128	0.00029
Willacy	1994-1998	16	1	2	0.233	0.126	0.00056	0.00023
Willacy	2005	10	0	1	0.000	0.000	0.00000	0.00000
Other areas	1990-2004	8	1	2	0.250	0.180	0.00060	0.00043
Mexico	1994-1998	10	3	4	0.733	0.012	0.00282	0.00066
Mexico	2001-2004	3	1	2	0.667	0.314	0.00319	0.00015
Other Areas	1992-1994	5	1	1	0.000	0.000	0.00000	0.00000

Table 8. Haplotype frequencies in three extant ocelot populations (1986–2005) and one historical population (1890–1956).

Haplotype	Cameron	Willacy	Tamaulipas	Texas-Historical
1	1	0.846	0.500	0.545
2	0	0.154	0.100	0.273
3	0	0	0.200	0
4	0	0	0.200	0
5	0	0	0	0.091
6	0	0	0	0.091

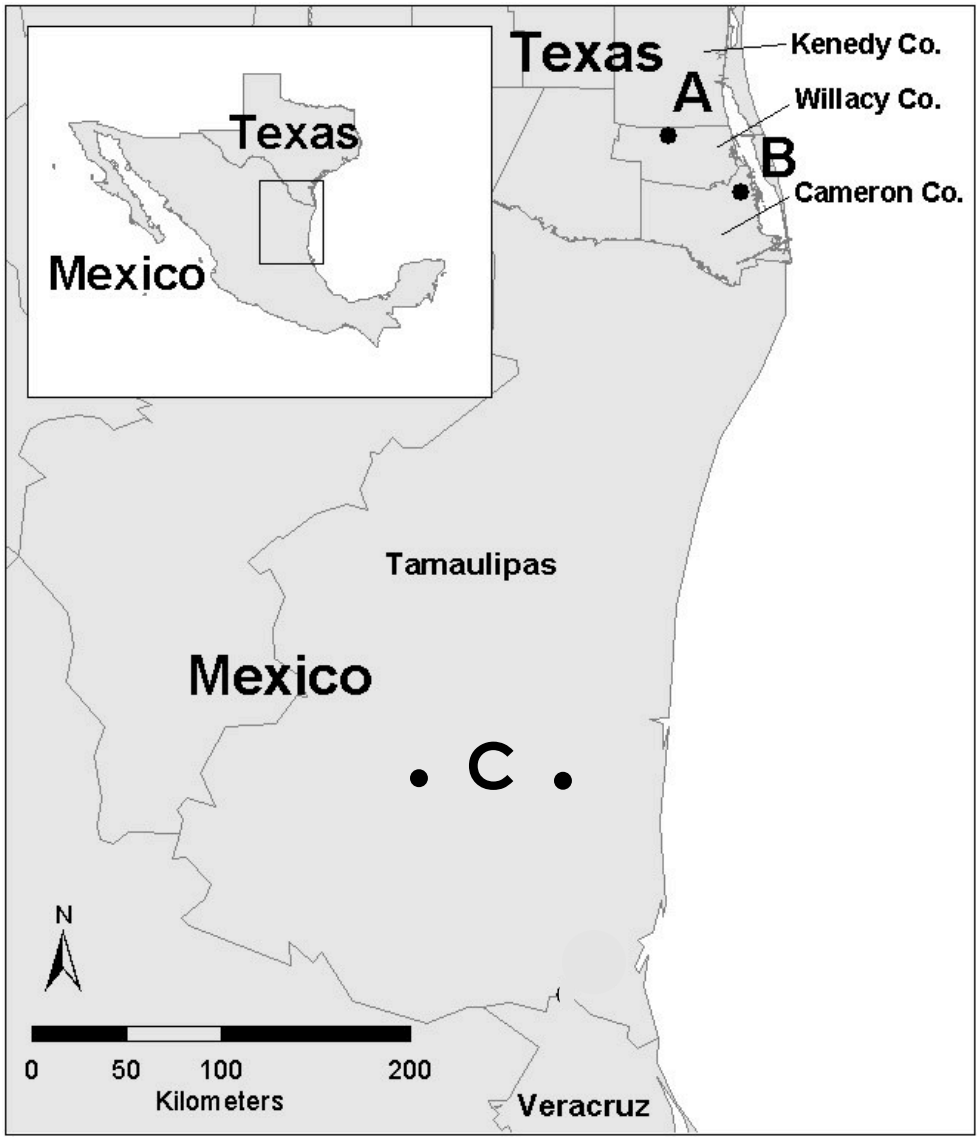
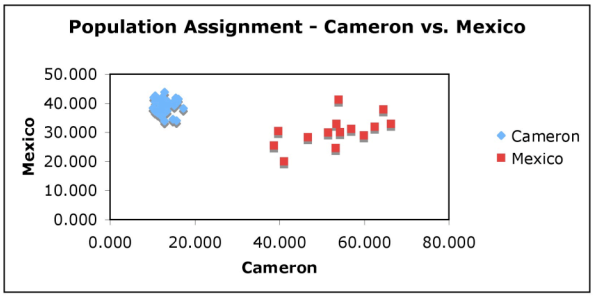
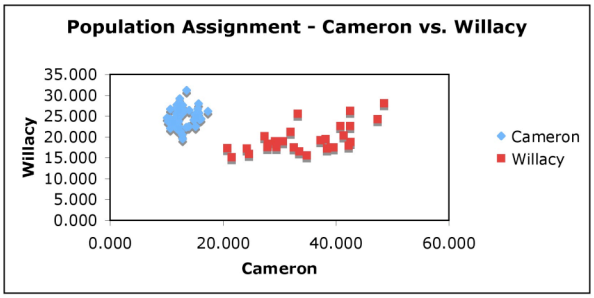


Figure 1. Study area locations. A – Private ranches in Willacy and Kenedy Counties, Texas referred to as Willacy population; B – Laguna Atascosa National Wildlife Refuge, Cameron County, Texas referred to as Cameron population; C – Tamaulipas and Veracruz State, Mexico referred to as Mexico population.

a.



b.



c.

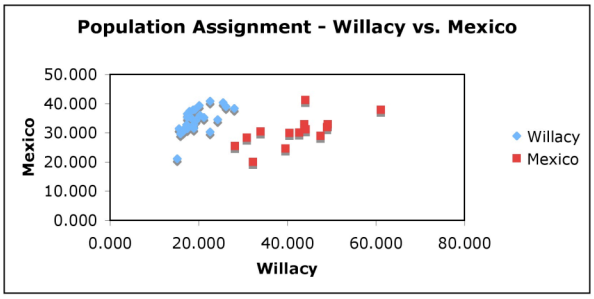


Figure 2. Population assignments calculated among the three populations. The x- and y-axis are log-likelihoods converted to positive values, and therefore the lowest values indicate the most likely population. Note that there are no miss-assignments.

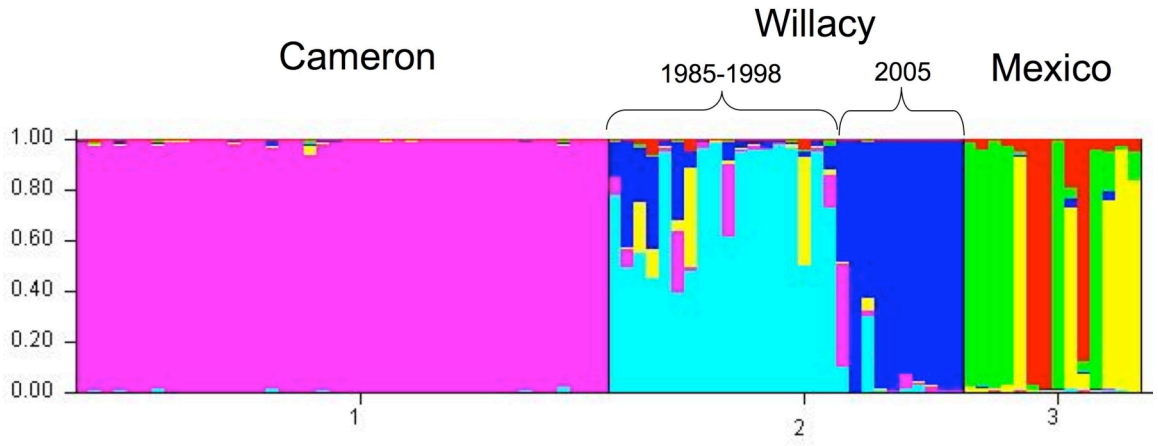


Figure 3. This figure depicts the assignments of individuals to genetic clusters defined by model-based clustering of the microsatellite data, without regard for geographic origin. The posterior probability of K = 6 clusters is 1.00. Each cluster is represented by a different color.

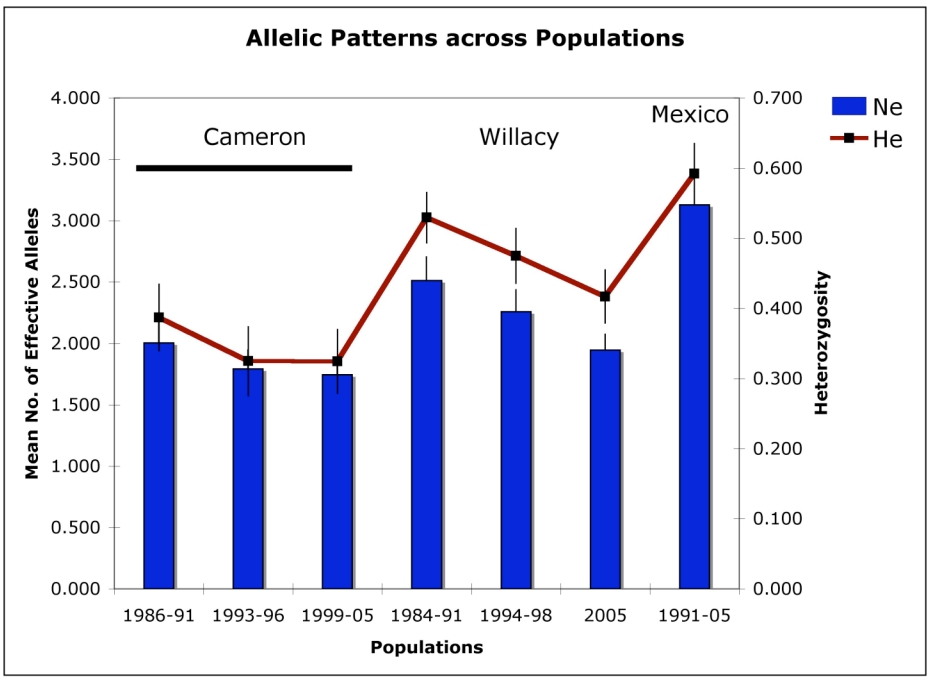


Figure 4. Trends in genetic diversity between 3 temporal groups of the Cameron and Willacy populations, respectively. The diversity in the Mexico population is shown for comparison. There were not enough samples from Mexico to divide that population into temporal groups.

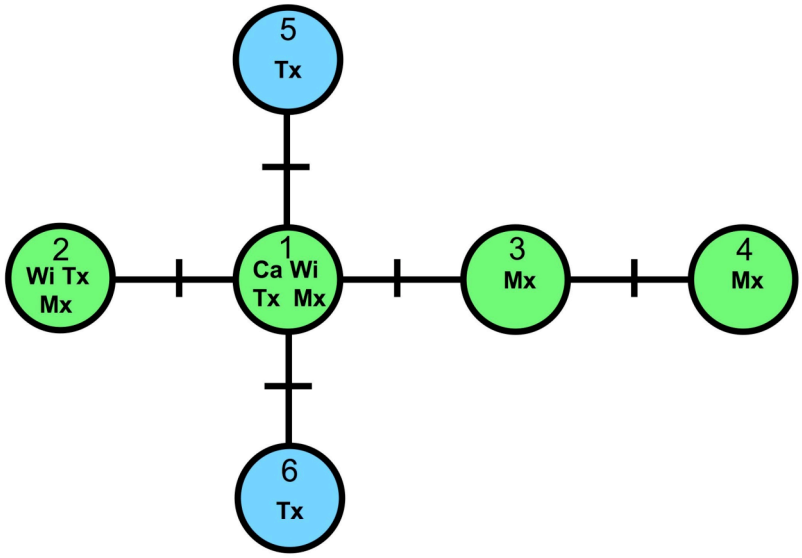


Figure 5. Minimum spanning networks representing most parsimonious mutation pathways between 6 ocelot haplotypes observed in Texas and northeastern Mexico. Each hatch mark represents a single nucleotide point mutation. Haplotype numbers correspond to Table 8. The populations in which haplotypes were observed are noted with the following abbreviations: Ca = Cameron County, Texas; Wi = Willacy County, Texas; Mx = Mexico. Haplotypes with green fill are ones observed in both extant and historic populations, haplotypes with blue fill were observed only in historical samples.