# GENOMIC INSIGHTS INTO ENDANGERED OCELOT (*LEOPARDUS PARDALIS*) POPULATIONS TO INFORM SPECIES RECOVERY

A Thesis

by

TYLER A. BOSTWICK

Submitted to the College of Graduate Studies
Texas A&M University-Kingsville
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2025

Major Subject: Range and Wildlife Management

# GENOMIC INSIGHTS INTO ENDANGERED OCELOT (*LEOPARDUS PARDALIS*) POPULATIONS TO INFORM SPECIES RECOVERY

A Thesis

by

#### TYLER BOSTWICK

Approved as to style and content by:

Lisanne Petracca, Ph.D. Committee Chair

Randy W. DeYoung, Ph.D. Committee Member

Alynn Martin, Ph.D. Committee Member

Ashley Reeves, Ph.D. Committee Member

Bart Ballard, Ph.D. Department Chair

Darin T. Hoskisson, Ph.D. Associate Vice President for Academic Affairs and Dean, College of Graduate Studies

August 2025

#### **ABSTRACT**

Genomic Insights into Endangered Ocelot (*Leopardus pardalis*)

Populations to Inform Species Recovery

August 2025

Tyler Bostwick, B.S. Wildlife Conservation and Management
California State Polytechnic University, Humboldt
Chair of Advisory Committee: Dr. Lisanne Petracca

With habitat and species loss occurring at unprecedented rates, conservation action is needed to preserve threatened and endangered populations. Habitat loss and fragmentation isolate populations, which can result in increased susceptibility to stochastic events such as severe weather or disease. Small populations are also vulnerable to loss of genetic diversity due to genetic drift and inbreeding, both of which can reduce fitness. Reintroduction can increase genetic diversity and mitigate impacts of environmental stochasticity for target species. However, the genetic provenance of stock animals used in reintroductions can have lasting effects and needs careful consideration. Ocelots (Leopardus pardalis) are a target species for reintroduction in the United States, given that there are likely fewer than 100 individuals remaining in two isolated populations in South Texas. Reintroduction efforts have begun, with the official ground breaking of the ex-situ breeding center in 2024. To maximize the success of ex-situ breeding and reintroduction efforts, I: 1) assessed genetic diversity and inbreeding of wild ocelots and 2) analyzed genetic divergence between wild and zoo-sourced ocelots to inform the selection of ex-situ breeding candidates. Using whole genome sequencing, I found that wild ocelots had significantly lower nucleotide diversity (Ranch: 0.0017, Refuge: 0.0018, Generic:

0.0032, Brazilian: 0.0033) and higher inbreeding (wild: 43.23-50.67% genome in run of homozygosity, zoo-based: 1.13-3.57% genome in run of homozygosity) than zoo-based ocelots. In addition, I identified evidence of dispersal (n=5) and gene flow (n=2 first-generation offspring) between the wild South Texas populations. These are the first dispersers documented between populations since at least 1991. The offspring of dispersers had higher genetic diversity than either parental population, which demonstrates how gene flow can ameliorate the loss of genetic diversity, even among inbred populations. I evaluated the potential of zoo-based stocks (Brazilian and Generic) for the ex-situ breeding in reference to genetic diversity as well as genetic distance and divergence from wild South Texas stocks. Brazilian ocelots had the highest genetic diversity, with > 32,000 private alleles, while Generic ocelots had > 14,000 private alleles, and were less genetically divergent from wild ocelots. Overall, zoo-based stocks had higher greater nucleotide diversity and fewer ROH than wild stocks. Either stock would be suitable for ex-situ breeding, but consideration of individual variation in the selection of founding individuals is necessary, as ROH ranged from 0% to > 15% of the genome. This research is the first to show gene flow between wild ocelot populations in the United States and adds to the growing literature on ex-situ breeding considerations to inform species reintroductions, ultimately providing critical information to support ocelot reintroduction and recovery.

#### **DEDICATION**

This thesis is dedicated to my parents. Starting from when we moved to Montana so that I could grow up surrounded by nature, my parents have always fostered and supported my love for wildlife. I am incredibly lucky that my life has been so surrounded by the animals I love, from watching elk in our yard to raising fox kits for rehabilitation. All of these experiences have had and will have a lasting impact on my life. Pam and Dave, you have set the foundation for my love of animals and science, and for that I am eternally grateful. Your continuous love and support for my work and passions has allowed me to reach all of my dreams and I truly would not be where I am today without your support.

I am also, at heart, a cat lover, and this dedication wouldn't be complete without my two cats Orion and Jupiter. The constant companionship from them proved to be the best way to destress after a long day, and for that they are a part of this thesis as well.

I love you all dearly and thank you for your unwavering support and companionship.

iv

#### **ACKNOWLEDGEMENTS**

I thank my committee chair, Dr. Petracca, for taking a chance on me and this project and allowing me to sculpt it into my dream master's. I appreciate your trust and willingness to work with me to create not only something that I am passionate about, but has the chance to improve ocelot conservation. Thank you for your continued support and guidance throughout my time in South Texas.

I thank my committee members, Drs. DeYoung, Martin, and Reeves, for their time, work, and support throughout the course of this research. Dr. DeYoung was instrumental in helping me to understand genetics, and setting the foundation for everything I have learned through the course of this thesis. Dr. Matt Smith and Michael Barret in the last year of my thesis were also instrumental in helping me to decipher code, and providing a safe space for me to bounce around ideas.

I express thanks to all of the collaborators on this project. This work would not have been possible without the continued support from the United States Fish and Wildlife Service, Laguna Atascosa National Wildlife Refuge, the Center for Conservation and Research of Endangered Wildlife, and the East Foundation. The collaboration of all of these organizations allowed for the acquisition and analysis of both wild and zoo-based ocelot blood samples. The inclusion of photographic identification of ocelots from Laguna Atascosa National Wildlife Refuge was only possible due to the efforts of Refuge staff, volunteers, and biologists, notably Andy Grunwald, Brandon Jones, and Laura de la Garza. The long photographic capture history of individuals would not have been possible without all of the hours spent cataloging and identifying individuals, and for that I am extremely grateful.

I thank my lab-mates in the Spatial Population Ecology of Carnivores Lab (Alexandria Hiott, Chloe Nouzille, James Helferich, Katie McDaniel, and Tori Locke), as without their continued support, humor, and companionship I would not be where I am today. Ali Dart, our program manager, deserves a special thanks for her continuous support and encouragement. Tori's passion for ocelots and her identification of individuals provided critical frame of reference for parts of my results, and I'll never forget the frantic late-night analyses and phone calls with her and Alexandria as we found results that none of us expected. The family I have found here is incredibly dear to me. I love you all and I can't wait to see where you go after this.

#### CONTRIBUTORS AND FUNDING SOURCES

#### **Contributors**

This work was supervised by a thesis committee consisting of Drs. Lisanne Petracca, Randy DeYoung, and Alynn Martin of the Department of Range and Wildlife Management, Texas A&M University – Kingsville, and Dr. Ashley Reeves of the East Foundation.

Samples analyzed for this thesis were collected in partnership with the Caesar Kleberg Wildlife Research Institute, East Foundation, and the Center for Conservation and Research of Endangered Wildlife (CREW).

All other work conducted for the thesis was completed by the student independently.

### **Funding Sources**

Graduate study and research were supported by Customs and Border Protection through the United States Fish and Wildlife Service under Contract Number 140F0S23C0008. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of Customs and Border Protection or the United States Fish and Wildlife Service.

# **TABLE OF CONTENTS**

	Page
ABSTRACT	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	V
CONTRIBUTORS AND FUNDING SOURCES	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	
LIST OF TABLES	xiii
CHAPTER 1. GENOMIC EFFECTS OF RARE GENE FLOW BETWEEN INBRED	
POPULATIONS OF OCELOTS ( <i>LEOPARDUS PARDALIS</i> ) IN THE UNITED STATES	1
Introduction	
Study Area	5
Methods	6
Captures and DNA extraction	6
Sequencing and variant calling	7
Population structure and admixture	8
Genomic Diversity and runs of homozygosity	9
Results	11
Population structure and diversity	11
Runs of homozygosity	12
Discussion	13
Management implications	18

Figures and Tables	20
References	30
CHAPTER 2. GENOMIC EVALUATION OF SOURCE STOCKS FOR EX-SITU BREEDIN	NG
OF ENDANGERED OCELOTS ( <i>LEOPARDUS PARDALIS</i> ) IN SOUTH	
TEXAS	34
Introduction	34
Study Area	39
Methods	40
Sample acquisition and DNA extraction	40
Sequencing and variant calling	41
Genomic diversity and inbreeding	41
Outlier loci and adaptive divergence	42
Results	43
Genomic diversity and inbreeding	43
Divergence	44
Discussion	45
Management implications	48
Figures and Tables	50
References	61
Management Implications	65
Appendices	67
VITA	71

# LIST OF FIGURES

Figure 1.1 Historic and current range of ocelots in the United States, adapted from Haines et. al.
(2006). Ocelots previously ranged through Oklahoma, Arkansas, Louisiana, and Texas. Ocelot
range in the United States was greatly reduced to 2 known breeding populations in Texas as of
1982
Figure 1.2 Timeline of ocelot population decline and range contraction in the United States. In
the Pleistocene, ocelots ranged across the United States, with fossil records in Florida dating
from 115-130 kya (Kurten 1965). Noticeable range contractions began in 1853, with the type
specimen for the Texas ocelot collected in Arkansas, and fewer records of ocelots coming out of
the Hill Country in Central Texas (Navarro Lopez 1985). By 1981, experts in South Texas
believed they no longer remained until their subsequent capture in 1982 (Tewes 2019). Ocelots
gained federal protection the same year and are currently a candidate species for ex-situ breeding
and reintroduction into their historic range.
<b>Figure 1.3</b> Breeding populations of ocelots sampled from 2012 to 2024 in South Texas, USA.
The known Ranch population consists of a conservation easement with the United States Fish
and Wildlife Service on the Yturria Ranch and the East Foundation's El Sauz Ranch in Willacy
County, with ocelots using the surrounding habitat. The Refuge population is found on Laguna
Atascosa National Wildlife Refuge in Cameron County
Figure 1.4 Admixture of wild ocelots in South Texas from 2012 to 2024, grouped by their
population of residence based on 12,146,279 single nucleotide polymorphisms (SNPs)
distributed throughout the genome. $K = 2$ genetic clusters had the lowest CV error (0.34) and
was selected as the best fit out of $K = 1-10$ . Color was assigned via the proportion of ancestry

that assigns to a given population. Three individuals, E29M, LO03M and OM331, who were captured in the Refuge population assigned to the Ranch. Two individuals, E35M and LO01F, assigned at nearly 50% to both populations, indicating they are first-generation offspring of the dispersing individuals. E35M was first captured in the Ranch population, but ancestry and photo evidence indicate he was born in the Refuge population and dispersed to the Ranch......23 Figure 1.5 Principal components analysis (PCA) of wild ocelots in South Texas captured between 2012 and 2025, with 32.2% variance explained by the first 2 PC based on single nucleotide polymorphism (SNP) loci. Individuals are represented by their population of residence. The two distinct clusters formed along PC1, with some substructure along PC2. Three individuals captured in the Refuge populations clustered with the Ranch population, indicating they originated in the Ranch and dispersed to the Refuge. Two individuals displayed in the dashed box, E35M and LO01F, were directly in between the two population clusters, lending evidence to their status as first-generation offspring. 24 Figure 1.6 Kinship estimates for wild ocelots in South Texas captured between 2012 and 2024 based on single nucleotide polymorphism (SNP) loci. Kinship was calculated using the KINGrobust estimator. Kinship values were binned into putative relationships: 1<sup>st</sup> degree relationships (parent-offspring of full sibling) have expected values of 0.25, 2<sup>nd</sup> degree relationships (grandparent-offspring, half sibling) have expected values of 0.125, 3<sup>rd</sup> degree relationships (1<sup>st</sup> cousins) have expected values of 0.0625. Putative relationship values were calibrated using known parent-offspring relationships. LO01F has a putatively first-degree relationship with LO03M and E35M, one of the dispersing male ocelots from the Ranch population and the other first-generation offspring in the Refuge population, respectively. All dispersing male ocelots (OM331, LO03M, and E29M) had higher kinship with the other individuals from the Ranch

population, evidence of their natal population being that of the Ranch. Aside from firstgeneration offspring and dispersing individuals, Ranch and Refuge individuals had no kinship values indicative of 3<sup>rd</sup> degree or higher relationships, demonstrating the lack of admixture between populations. 25 Figure 1.7 Distribution of runs of homozygosity (ROH) lengths in megabase pairs (Mb) in wild ocelot population in South Texas from 2012 to 2024 based on single nucleotide polymorphism (SNP) loci. The number of expected generations (7 year in ocelots) since an individual's maternal and paternal lineages shared a common ancestor was calculated from the recombination rate of the domestic cat (1.1 cM per Mb) and the length of a ROH in Mb. ROH of 22.87 Mb indicate inbreeding within the last 2 generations, while ROH of 0.5 Mb are due to inbreeding over 80 generations ago or genetic drift. Peak historical inbreeding occurred between 60-80 generations, or 420-560 years ago. Recent inbreeding in the last 100 years is present in both populations, with more ROH in the Refuge population.... 27 Figure 1.8 Percentage of the ocelot genome held within runs of homozygosity (Froh), stretches of the genome where all loci are homozygous, in the 2 wild populations in South Texas between 2012 and 2024 based on single nucleotide polymorphism (SNP) loci. Mean  $\pm$  2 SD are shown in black, with individual F<sub>ROH</sub> percentages displayed as points. Both populations showed individual variation, with the Refuge population on average having higher F<sub>ROH</sub>..... 28 **Figure 1.9** Karyotype plot with runs of homozygosity (ROH) on chromosome 10 from ocelot individuals (n=3) from South Texas captured between 2012 and 2024 based on single nucleotide polymorphism (SNP) loci. ROH are represented as red boxes, and areas not in ROH are displayed in gray. The individuals are as follows: (a) Male ocelot from the Refuge population (b) LO01F, first-generation offspring from the Refuge population, (c) Male ocelot (LO03M) from

the Ranch population that dispersed to the Refuge population and is the putative father of
LO01F. The individual from the Refuge population (a) has nearly the entire chromosome within
ROH. The Ranch male (b) still had long runs present in this chromosome, but they were shorter
in length. LO01F, this individual's offspring (c), had no long runs, and the location of the longest
run in the Ranch male (b) was broken into smaller runs.
Figure 2.1 The ocelot's current and historic range. The <i>Leopardis pardalis pardalis</i> subspecies
ranges from Mexico through Central America, and historically though Texas, Oklahoma,
Arkansas, and Louisiana. The Leopardus pardalis mitis subspecies ranges from the Panama
Canal through South America. 50
Figure 2.2 Current ocelot populations in the United States and the proposed reintroduction site.
The reintroduction site is on the East Foundation's San Antonio Viejo Ranch, along with habitat
on surrounding private ranches. The Ranch and the Refuge are about 30 km apart. The
reintroduction site is 170 km inland
Figure 2.3 Nucleotide diversity (mean $\pm$ 2 SD) in both zoo-based ocelots and wild ocelots in
South Texas from 2012 to 2024. Zoo-based ocelots were split into their origins: Brazilian and
Generics. Wild ocelots are separated by populations of residence: the Ranch population and the
Refuge. Zoo-based ocelot exhibited much higher nucleotide diversity than wild ocelots;
however, there was individual variation in all groups.
Figure 2.4 Private alleles in zoo-based ocelots and wild ocelot populations in South Texas from
2012 to 2024. Wild ocelots, here in yellow and orange for their respective populations, each had
just over 1,000 alleles not present in any other group. Of the zoo-based ocelots, here in blue and
green for the respective origins, Brazilians had the highest level of diversity not shared with
other groups, at 32 432 private alleles

Figure 2.5 Percent genome in runs of homozygosity ( $F_{ROH}$ ) (mean $\pm 2$ SD) of zoo-based ocelots
in the United States. Ocelots were separated by origin, with those of Brazilian origin in green and
of Generic origin in orange. Individual FROH are shown as points. Brazilian ocelots exhibited
lower FROH than Generics. Brazilian mean FROH was 1.31% (SD = 2.26). Generic ocelots had
a mean FROH of 3.57% (SD = 4.98).
Figure 2.6 Principal components analysis (PCA) of zoo-based and wild ocelots in South Texas
from 2012 to 2024 with 45.54% variance explained by the first 2 PC based on single nucleotide
polymorphism (SNP) loci. Wild ocelots are represented by circles and colored by population
(purple for Ranch and pink for Refuge). Wild ocelot populations clustered tightly, while zoo-
based ocelots grouped out by origin
Figure 2.7 Neighbor-joining trees based on Nei's (1972) genetic distance for zoo-based and wild
ocelots in South Texas from 2012 to 2024. Trees were constructed using a) outlier $(n = 69,243)$
and b) neutral (n=3,551,533) single nucleotide polymorphism (SNP) loci. The tip of each branch
represents an individual, and is colored by their population of origin. Using outlier loci, the wild
populations showed great genetic distance between them, likely due to drift rather than true
adaptation. Regarding the zoo-based ocelots, the Brazilian stock as a whole displayed greater
genetic distance from the wild ocelots than the Generics. Some individuals within the generic
origin had greater genetic distance from the wild populations. Using neutral loci, ocelots grouped
based on population and origin. Zoo-based ocelots had the greatest genetic distance between
individuals, indicative of their higher neutral diversity than wild ocelots
Figure 2.8 Pairwise comparison of zoo-based and wild ocelots in South Texas from 2012 to
2024. Neighbor-joining trees were constructed from Nei's (1972) genetic distance calculated
with outlier single nucleotide polymorphism (SNP) loci. (a) Comparison between Generics and

the Refuge, constructed from 4,188 outlier loci. (b) Comparison between Generic and the Rand	en,
constructed from 8,348 outlier loci	59
Figure 2.9 Pairwise comparison of zoo-based and wild ocelots in South Texas from 2012 to	
2024 based on single nucleotide polymorphism (SNP) loci. Neighbor-joining trees were	
constructed from Nei's (1972) genetic distance calculated with outlier loci. (a) Comparison	
between Brazilians and the Refuge, constructed from 18,786 outlier loci. (b) Comparison	
between Brazilians and the Ranch, constructed from 29,452 outlier loci	60
Figure S1 Kinship estimates for zoo-based ocelots in the United States. Kinship was calculate	d
using the KING-robust estimator. Kinship values were binned into putative relationships: 1st	
degree relationships (parent-offspring of full sibling) have expected values of 0.25, 2 <sup>nd</sup> degree	
relationships (grandparent-offspring, half sibling) have expected values of 0.125, 3 <sup>rd</sup> degree	
relationships (1st cousins) have expected values of 0.0625. Putative relationship values were	
calibrated using known pedigree relationships	71

# LIST OF TABLES

Page

<b>Table 1.1</b> Diversity metrics by population for wild ocelots in South Texas from 2012 to 2024
based on single nucleotide polymorphism (SNP) loci. Metric calculated for each population were
nucleotide diversity ( $\pi$ ), percent genome in runs of homozygosity ( $F_{ROH}$ ), inbreeding coefficient
(F <sub>is</sub> ), expected heterozygosity (H <sub>e</sub> ) and observed heterozygosity (H <sub>o</sub> ). The Refuge population had
higher F <sub>ROH</sub> , which indicated more recent and severe inbreeding. The Refuge also had an excess
of heterozygotes, likely due to recent dispersing individuals from the Ranch population or a
historic bottleneck event. 26
Table 1.2 Runs of homozygosity (ROH) identified in both populations of wild ocelots in South
Texas from 2012 to 2024 based on single nucleotide polymorphism (SNP) loci. Average counts
of ROH segments per individual were normalized to allow for direct comparison with different
sample sizes from the two populations. The Refuge population had more ROH of longer lengths
(>1Mb) than the Ranch population, indicating more recent and severe inbreeding that led to the
accumulation of long homozygous regions in the genome
Table 2.1 Diversity metrics of both wild and zoo-based ocelots in the United States based on
single nucleotide polymorphism (SNP) loci. Metrics assessed for all groups included nucleotide
diversity $(\pi)$ , percent genome in run of homozygosity $(F_{ROH})$ , inbreeding coefficient $(F_{is})$ ,
expected heterozygosity (H <sub>e</sub> ), and observed heterozygosity (H <sub>o</sub> ). Zoo-based ocelots had higher
genetic diversity and lower mean F <sub>ROH</sub> than wild ocelots
<b>Table 2.2</b> Weighted F <sub>ST</sub> between the 2 wild populations of ocelots in South Texas and the 2 zoo-
based ocelot stocks based on single nucleotide polymorphism (SNP) loci . Zoo-based ocelots
displayed significant differentiation from wild ocelot populations. Notably, the wild populations

Table S1 Parameters used in the software PLINK to call runs of homozygosity (ROH) in both wild and zoo-based ocelots based on single nucleotide polymorphism (SNP) loci. The parameters homzyg-gap and homozyg-window-snp were chosen to reduce the breaking long ROH by allowing tolerance for regions of reduced coverage and isolated sequencing errors. The remaining parameters were selected for the robustness of the ROH calls while balancing the need to reduce artificial breaking of long runs. This can occur, especially in very dense sequencing data, where there is more opportunity to genotyping error or spurious heterozygous alleles to reach the maximums set by the parameter and thus cause the non-reporting of biologically meaningful ROH.

Table S2 Population of origin, % of genome in run of homozygosity (F<sub>ROH</sub>) and observed heterozygosity (H<sub>O</sub>) for all individuals included in the study based on single nucleotide polymorphism (SNP) loci. Wild ocelots were assigned Ranch or Refuge based on their capture location. Ocelots captured on the Ranch property were given the designation Ranch, and individuals captured on the Refuge property were given the designation Refuge. Zoo-based ocelots were assigned via their origin, Brazilian or Generic, based on their designation from the Association of Zoos and Aquariums (AZA).

# CHAPTER 1. Genomic effects of rare gene flow between inbred populations of ocelots (*Leopardus pardalis*) in the United States

### INTRODUCTION<sup>1</sup>

Loss of habitat connectivity due to rapid urbanization imperils wildlife populations (Taylor et al. 1993, Elmqvist et al. 2015). Populations isolated by habitat loss and fragmentation are more vulnerable to extirpation via stochastic events (Willoughby et al. 2015). As a result, the expanding urban footprint has led to species range contractions and, in some cases, species extinctions. Intact mammal species assemblages are present in only 24% of the world's ecosystems (Ripple et al. 2014, Wolf and Ripple 2017). Overall, the global loss of genetically and ecologically distinct populations has demonstrated the importance and need for genetic management in the conservation of wildlife populations (Lacy 1997).

Population history and demographic factors have significant effects on the amount of genetic diversity in a population. The number of breeding individuals and degree of gene flow directly influence the amount of genetic variation present (Mayr 1954). Thus, small populations are disproportionately impacted by the random differences in survival and reproduction of individuals, termed genetic drift, which can result in the loss of genetic variation and accumulation of deleterious alleles (Wright 1931). Additionally, genetic diversity tends to be lower in populations at the edge of their range, in contrast to those at the core (Antonovics 1976, Brussard 1984, Hardie and Hutchings 2010). This is known as the central-marginal hypothesis, and occurs because those populations tend to be historically smaller and more susceptible to stochastic events and geographic isolation (Antonovics 1976, Hardie and Hutchings 2010).

<sup>&</sup>lt;sup>1</sup> This thesis is written in the style of the Journal of Wildlife Management