HOME RANGE CHARACTERISTICS, ACTIVITY PATTERNS, AND RESOURCE SELECTION OF SYMPATRIC OCELOTS (*LEOPARDUS PARDALIS*) AND BOBCATS (*LYNX RUFUS*) AND MAJOR HISTOCOMPATIBILITY COMPLEX VARIATION IN OCELOTS

A Dissertation

by

JOHN PETER LEONARD

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

DOCTOR OF PHILOSOPHY

December 2016

Major Subject: Wildlife Science

HOME RANGE CHARACTERISTICS, ACTIVITY PATTERNS, AND RESOURCE SELECTION OF SYMPATRIC OCELOTS (*LEOPARDUS PARDALIS*) AND BOBCATS (*LYNX RUFUS*) AND MAJOR HISTOCOMPATIBILITY COMPLEX VARIATION IN OCELOTS

A Dissertation

by

JOHN PETER LEONARD

Approved as to style and content by:

Michael E. Tewes, Ph.D.
(Chairman of Committee)

Randall DeYoung, Ph.D.
(Member of Committee)

David B. Wester, Ph.D.
(Member of Committee)

Jan E. Janecka, Ph.D.
(Member of Committee)

Scott E. Henke, Ph.D.
(Head of Department)

LaVonne Fedynich, Ed.D.
(Graduate Council Representative)

George A. Rasmussen, Ph.D.
(AVP for Research and Graduate Studies)

December 2016

ABSTRACT

Home Range Characteristics, Activity Patterns, and Resource Selection of Sympatric Ocelots (*Leopardus pardalis*) and Bobcats (*Lynx rufus*) and Major Histocompatibility Complex Variation in Ocelots

(December 2016)

John Peter Leonard, B.A., University of Dallas; M.S., Texas A&M University, College Station

Chairman of Advisory Committee: Dr. Michael E. Tewes

Ocelot (*Leopardus pardalis*) and bobcat (*Lynx rufus*) are sympatric in South Texas and parts of central and northern Mexico. The ocelot once ranged extensively throughout the eastern portion of Texas, extending into parts of Oklahoma, Louisiana, and Arkansas. Due primarily to loss of native thornshrub habitat in the 19th and 20th centuries, the entire resident ocelot population in the United States is now confined to two isolated populations in southern Texas. These populations are highly vulnerable to local extinctions due to demographic factors and loss of genetic diversity. In contrast, bobcat populations are increasing throughout the United States, and bobcat populations in South Texas show greater genetic diversity than ocelots occupying the same environments. To ensure the continued existence of the ocelot in the United States and to promote its recovery, biologists need to understand whether ocelots and bobcats compete for the same resources. Additionally, understanding the effect isolation and genetic drift have on ocelot functional genetic diversity will help managers make important decisions regarding translocations.

This dissertation is divided into four chapters. Chapter I compares home range size and core area components between sympatric ocelots and bobcats on the East El Sauz Ranch and surrounding areas in Willacy County, Texas. Chapter II uses a combination of high-frequency Global Positioning Systems (GPS) location data and continuously-collected accelerometer data to compare temporal activity patterns between ocelots and bobcats with the goal of examining temporal niche partitioning between these species. Chapter III tests specific hypotheses related to habitat selection at the third order using a synoptic model of space use, and at the fourth order using a step-selection function. Chapter 4 compares levels of genetic variation within the functionally important major histocompatibility complex (MHC) between South Texas populations of ocelots and bobcats and between historical and contemporary ocelot populations.

DEDICATION

This dissertation is dedicated to my wife, Leslie, who once again followed me into the unknown so that I could accept this exciting opportunity. I could not have done this without her support and encouragement, and I truly appreciate her leaving behind a good job and a comfortable life in Seattle, Washington, to start a new life with me in Kingsville, Texas. I love you, Leslie.

ACKNOWLEDGMENTS

I would like to thank Dr. Michael Tewes for accepting me as a graduate student and for providing me with timely and insightful feedback on my proposal and dissertation, without which I would not have been able to graduate on time. Studying ocelots has been an incredible experience and I am very grateful for this opportunity. I thank Dr. David Wester for helping me with the statistical analyses involved in my project and for teaching such interesting and useful courses at Texas A&M University-Kingsville. I thank Drs. Randy DeYoung and Jan Janecka for their guidance and help in planning and carrying out the genetic sequencing project. I am grateful to everyone who helped me in the lab, including Drs. Jennifer Korn, Katherine Miller, and Damon Williford. I appreciate the people who helped with field work and made this project possible, including Dr. Arturo Caso, Wes Watts, Lauren Balderas, Shelby Carter, Justin Wied, Daniel Kunz, Dr. Poncho Ortega, Jason Lombardi, Taylor Shirley, Dr. Lon Grassman, and Jonatan Tamez. The late Anse Windham and Nikita Clark helped this project immensely by conducting telemetry flights. I would also like to thank the organizations that provided me with financial assistance. The East Foundation provided the primary financial support for my project. Additional scholarship support was provided by The Houston Safari Club, The South Texas Quail Coalition, and the Rene Barientos Scholarship Fund. Finally, I would like to thank my parents, Mike and Cindy Leonard, for taking me camping and hiking so often when I was growing up. They taught me to love wildlife and the outdoors at a young age, and it is unlikely that I would be where I am today without their support.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER I

COMPARISON OF OCELOT AND BOBCAT HOME RANGE CHARACTERISTICS

The ocelot (*Leopardus pardalis*) is a medium-sized neotropical felid with a current range that extends from South Texas to Argentina, encompassing parts of every country in Central America and South America except Chile (Fig. 1.1). In the United States, the ocelot historically ranged throughout eastern and central Texas, extending into parts of Louisiana and Arkansas (Tewes and Schmidly 1987). Due to anthropogenic impacts the geographic range of the ocelot in the United States contracted dramatically in the 20th century (Tewes and Everett 1986). The entire distribution of ocelots in the United States currently consists of 2 isolated populations in South Texas; one in and around the Laguna Atascosa National Wildlife Refuge (LANWR), the other on a group of private ranches in Willacy County, Texas (Haines et al. 2006). Fewer than 80 individuals are believed to remain between these 2 populations (M. E. Tewes, Texas A&M University-Kingsville, personal communication). In 1990, the ocelot was initially listed as "vulnerable" by the IUCN (Caso et al. 2008), but in 2002 this felid was downgraded to "least concern" because its population size was reported stable throughout most of Central and South America (Caso et al. 2008). However, the ocelot is listed as "endangered" in the United States by the U. S. Fish and Wildlife Service (Federal Register 1982).

The bobcat (*Lynx rufus*) is a nearctic felid, similar in size to the ocelot, which occurs in a variety of habitats in North America, and ranges from southern Canada to central Mexico (Lariviere and Walton 1997; Fig. 1.1). Throughout the geographic range in South Texas and much of Mexico, ocelots are sympatric with bobcats (Tewes and Schmidly 1987). Ocelots and bobcats forage primarily on birds and small mammals, are primarily active during crepuscular

Chapter 1 of this dissertation is written in the style of the *Journal of Wildlife Management*.

Figure 1.1. Geographic range of ocelots and bobcats showing the zone of overlap in the United States and Mexico. Range maps of ocelots and bobcats were obtained from the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (NatureServe and IUCN 2016a, b).

and nocturnal periods (Rolley 1987), and are similar in size (Sunquist and Sunquist 2002). According to the competitive-exclusion theory (Gause 1934), ecologically equivalent species cannot coexist. Coexistence of mammalian carnivores has been explained due to body size differences (Rosenzweig 1966), temporal, and dietary ecological partitioning (Schoener 1974). Due to ecological similarities in diet, activity, and size, it is expected that ocelots and bobcats will show differences in habitat selection or in temporal activity patterns.

Interspecific competition within carnivore guilds can impact the distribution, density, and behavior of carnivore species (Macdonald and Sillero-Zubiri 2004). Character displacement also has been found to result from interspecific competition among sympatric carnivores (Dayan and Simberloff 1998), where sympatric species of felids often display differences in body size (Rosenzweig 1966, Kiltie 1984) that lead to dietary partitioning. Additionally, interference competition between ecologically similar species may play an important role in determining species distribution, often restricting subordinate species to marginal habitats (Tannerfeldt et al. 2002, Macdonald and Sillero-Zubiri 2004, Mitchell and Banks 2005).

Throughout much of its geographic range, the ocelot occurs sympatrically with 6 felids: bobcat, jaguar (*Panthera onca*), puma (*Puma concolor*), jaguarundi (*Puma yagouaroundi*), margay (*Leopardus wiedii*), and oncilla (*Leopardus tigrinus*; Oliveira and Cassaro 2005). Coexistence of ocelots with other felid species throughout the neotropics can largely be explained due to morphological differences (Rosenzweig 1966), differences in habitat selection, and differences in diel activity patterns (Di Bitetti et al. 2010, Schoener 1974). The ocelot is several times smaller than the jaguar and puma, and although disparities in body mass suggest that jaguars and pumas may kill ocelots (Donadio and Buskirk 2006), it is unlikely that interspecific food competition between the ocelot and the larger felids limits ocelot abundance

and distribution. The ocelot averages between 2 to 4 times the body size of the jaguarundi, margay, and oncilla (Crawshaw 1995, Sunquist and Sunquist 2002), making it unlikely that these smaller felids represent significant competitors to the ocelot. Conversely, bobcats show substantial size overlap with ocelots. Male bobcats typically average 10 kg, with females averaging 7 kg, though these weights vary significantly with latitude (McCord and Cardoza 1982, Sunquist and Sunquist 2002, Hansen 2007). Ocelots range in weight from 7-10 kg in Texas (Tewes 1986).

Several studies have reported ocelots in Texas to be strongly linked to dense thornshrub communities (Tewes 1986, Harveson et al. 2004, Haines et al. 2006). However, bobcats also have been reported to select for dense thornshrub communities in Texas (Bradley and Fagre 1988, Cain et al. 2003). Horne et al. (2009) analyzed radio-telemetry data of sympatric ocelots and bobcats on LANWR to compare second and third-order selection (Johnson 1980) of the following 4 land cover categories: Closed Canopy, Mixed Canopy, Open Canopy, and Bare Ground. Ocelots showed stronger third-order selection (within home range) for closed canopy habitat types than bobcats. However, no differences were found in second-order selection (i.e., home range) for any of the land-cover types except Bare Ground, with ocelots showing a significantly stronger avoidance of this land cover type than bobcats.

The Willacy county population of ocelots is believed to represent the larger of the 2 remaining U.S. ocelot populations (M. E. Tewes, Texas A&M University-Kingsville, personal communication), yet habitat use patterns of sympatric ocelots and bobcats in Willacy County have not been as thoroughly studied as in LANWR. Additionally, there are distinct differences in the types of vegetation associations present on LANWR and on the ranches comprising the Willacy habitat. The Laguna Atascosa National Wildlife Refuge contains topography typical of

the Texas Coastal Plain, and encompasses vegetation associations such as salt flats, marshes, chaparral, and brush-grasslands (Lonard and Judd 1985). The ranches in Willacy County that provide habitat to ocelot and bobcat include many of the same vegetation associations, and additionally include evergreen forests dominated by live oak (*Quercus virginiana*) with minimal understory in some locations. To effectively manage the Willacy ocelot population, it is important to understand the intensity with which sympatric ocelots and bobcats in this area compete for habitat. The objective of this study was to compare land cover components within the home ranges of sympatric ocelot and bobcat in Willacy County to determine if spatial partitioning plays a role in allowing the coexistence of these species.

STUDY AREA

This study occurred on the East El Sauz Ranch (EESR), near Port Mansfield, Texas, in Willacy County (Fig. 1.2). The EESR is about 113 km^2 in size and managed by the East Foundation, a nonprofit, tax-exempt organization that is a legacy of landowner Robert C. East. The EESR supports about 25 ocelots (M. E. Tewes, Texas A&M University-Kingsville, unpublished data) with little interchange documented with the population on the Yturria Ranch, separated by about 8 km. The Willacy ocelot population is also separated from the population occurring on and around LANWR by about 29 km (Stasey 2012).

The EESR is located within the Tamulipan Biotic Province, a biotic region extending from South Texas to northern Mexico that is characterized by a semiarid and subtropical climate, with mean temperatures ranging from 16° C to 28° C (Jahrsdoerfer and Leslie 1988). Mean annual precipitation is 68 cm, although droughts are common, occurring with an 11% annual frequency (Lonard and Judd 1985, Haines et al. 2005).

Figure 1.2. East El Sauz Ranch (113 km^2), Willacy County, Texas, where ocelots and bobcats were trapped from 2011-2015.

Major soil associations identified in this area include: (1) Galveston-Mustang Dune Land, characterized by nearly level to gently undulating, moderately alkaline, nonsaline sandy soils, and undulating to rolling dune land, (2) Sauz, characterized by nearly level, mildly alkaline to strongly alkaline, nonsaline sandy soils, (3) Falfurrias, characterized by gently undulating, neutral, and mildly alkaline, nonsaline sandy soils, and (4) Barrada-Lalinda-Arrada, characterized by nearly level to gently sloping, mildly alkaline to strongly alkaline, saline, and nonsaline clay and loamy soils (Lonard and Judd 1985). Common woody plants occurring in this area previously associated with ocelot habitat include spiny hackberry (*Celtis pallida*), crucita (*Eupatorium odoratum*), Berlandier fiddlewood (*Citharexylum berlandieri*), honey mesquite (*Prosopis glandulosa*), desert olive (*Forestiera angustifolia*), snake-eyes (*Phaulothamnus spinescens*), colima (*Zanthoxylum fagara*), and brasil (*Condalia hookeri*; Shindle and Tewes 1998).

Preliminary camera-trapping and telemetry have shown that ocelots occur in the northwestern and southwestern areas of the EESR (M. E. Tewes, Texas A&M University-Kingsville, unpublished data). The northwestern area of the EESR is dominated by dense stands of native thornshrub, honey mesquite, and live oak. The southwestern part of the EESR contains a more open, patchy matrix of thornshrub, interspersed with open herbaceous cover, and bare ground. Large sand dunes stretch from the central part of the EESR east toward the coast, with smaller patches of dense woody vegetation found on the eastern part of the EESR.

METHODS

Capture and telemetry

From 2011 to 2015, ocelots and bobcats were trapped with single-door 108 x 55 x 40 cm Tomahawk wire box traps (Tomahawk Live Trap Co., Tomahawk, WI). Live chickens (*Gallus gallus domesticus*) and pigeons (*Columba livia*) were used as bait, housed in separate enclosures attached to the main trap and supplied regularly with food and water. Traps were checked every morning between 0830 and 1030 hrs. Trapped ocelots and bobcats were sedated with an intramuscular injection of tiletamine HCL and zolazepam, sold commercially as Telazol (Fort Dodge Laboratories, Fort Dodge, IA), at a dosage of 5 mg per kg body weight (Shindle and Tewes 2000). The weight of each captured felid was visually estimated, and the proper dosage of drugs administered with a pole syringe.

During each sedation, body temperature, heart rate, respiration, and oxygen saturation levels of ocelots and bobcats were monitored continuously. If the body temperature dropped <37.8° C, the animal was warmed with heating pads. If the body temperature increased >37.8° C, the animal was cooled by placing ice packs between its legs and adjacent to the torso. Research activities were approved by the Texas A&M University-Kingsville Institutional Animal Care and Use Committee, protocol numbers 2012-12-20B-A2, 2012-12-20B, and 2012-12-19.

Captured adult ocelots and bobcats in good condition were fitted either with very high frequency (VHF) radio collars manufactured by Advanced Telemetry Systems (ATS; Advanced Telemetry Systems Inc., Insanti, MN) or GPS collars manufactured by Sirtrack (Sirtrack Wireless, Dunedin, New Zealand), ATS, or Lotek (Lotek Wireless, Newmarket, Ontario, Canada). Animals fitted with VHF radio collars were located several times each month throughout the study period using triangulation from fixed stations (White and Garrot 1990). In

the event that a felid left the study area and could no longer be located from the ground, a flight in a fixed wing aircraft was conducted to attempt to locate the animal through homing techniques. The Sirtrack GPS collars transmitted VHF signals of sufficient strength to allow location through triangulation, allowing these collars to be treated as VHF collars throughout the study period. In contrast, ATS and Lotek GPS collars transmitted VHF radio signals that were too weak to allow triangulation.

To verify the accuracy of ground-based radio-telemetry, another researcher placed 5 VHF radio collars at various locations within the EESR, recording the coordinates of these locations with a handheld GPS unit. I subsequently recorded the bearing to each of these radio collars from 10 telemetry stations, producing 50 unique bearing estimates. I determined the accurate geographic bearings from each telemetry station to each radio collar using ArcGIS 10.1 (ESRI, Redlands, CA). I subtracted each estimated bearing from the accurate bearing to determine the angular error and calculated the standard deviation of this value. Location estimates and 95% error ellipses were created using the program Locate III (Nams 2006).

I tracked VHF-collared individuals several times each month using a 3-element yagi antenna and triangulation, striving to maintain directional readings as close to 90° apart as possible to minimize the size of the calculated error ellipses (White and Garrot 1986). I attempted to obtain 3 azimuth readings for each animal within a 30-min period. In some cases this was not possible, so the number of azimuth readings taken was reduced to 2 azimuths to perform a best biangulation estimate of location. Location estimates were taken $\geq$12 hrs. apart with an intervening nocturnal period to minimize autocorrelation. Most location estimates taken from radio collars were collected between sunrise and sunset. Estimates of habitat use and home

range size derived from VHF radio-telemetry data, as opposed to GPS location data, therefore pertain to diurnal spatial patterns only.

The Sirtrack and ATS GPS collars were store-on-board collars, meaning that it was necessary to physically recover the unit to obtain location data. The Lotek collars had a remote-communication feature, allowing researchers to download data remotely using a VHF radio signal. The Sirtrack collars were programmed to record locations every 11 hrs. The ATS and Lotek collars were programmed according to the following schedule: one location each 24-hr period at midnight (2400 hr) and at noon (1200 hr) for every calendar day, with a high-frequency track period programmed around every full moon date and every new moon date during which locations were recorded every 30 minutes. The ATS GPS collars were programmed with the high-frequency track schedule for a 72-hr period centered on each new moon and full moon night, whereas the Lotek GPS collars were programmed with the high-frequency track schedule for a 24-hr period centered on each new moon and full moon night.

For individuals initially collared with a VHF radio collar, and later recaptured and fitted with a GPS collar, I reported locations derived from the radio collar and from the GPS collar separately. In contrast, I pooled radio locations with GPS locations in the case of individuals collared with Sirtrack GPS collars, as these 2 types of locations were collected contemporaneously.

To minimize problems associated with autocorrelation, I partitioned GPS data collected with Lotek and ATS GPS collars into high-frequency locations (30-min time intervals) and low-frequency locations ($\geq$12-hr time intervals). Only the low-frequency locations were used for home range estimation. To test for differences in home range size and habitat use between

diurnal and nocturnal locations, I further subdivided the low-frequency locations into those collected by day or night.

Home range estimation

I generated bivariate-normal fixed kernel density (KDE) home range polygons (Worton 1989) at the 50% and 95% density isopleths and 95% and 100% minimum convex polygons (MCP) using the package adehabitatHR (Calenge 2006) in R (R version 3.2.3 www.r-project.org, accessed 10 Dec 2015). I initially used both the least squares cross-validation (LSCV) method and the reference bandwidth method (h_{ref}) to select a smoothing parameter (h) for each individual. I recorded for LSCV analysis whether or not the algorithm converged for each individual (Silverman 1986), and reported the h value selected for both analyses. Proper selection of smoothing parameter is one of the most challenging issues in using kernel methods for home range estimation (Worton 1995). The LSCV procedure is not recommended in cases where the algorithm fails to converge (Horne and Garton 2006), and the h_{ref} method can often result in over-smoothing when animals use several centers of activity (Calenge 2006). There is disagreement about the correct method for selecting the smoothing parameter. Some authors recommend using a subjective visual choice based on successive trials (Silverman 1986, Wand and Jones 1995).

Any study using triangulation of radio signals to estimate animal locations suffers from triangulation error (White and Garrot 1990, Withey et al. 2001); however, Moser and Garton (2007) concluded that the error for radio-telemetry was low enough that it had little effect on fixed kernel distribution. I, therefore, considered locations derived from radio-telemetry

triangulation held sufficient accuracy for the generation of home ranges and the analysis of home range land cover components.

Home range estimates derived from minimum convex polygons have been criticized for providing an unrealistic representation of animal space use, in particular for including areas that may never be visited by the animal (White and Garrott 1990). However, their reproducibility, compared to kernel methods, makes them useful for home range area comparisons. For this reason, I used 95% and 100% MCP home range estimates only for comparisons of home range areas among species, sexes, and seasons, and used KDE to examine habitat use.

Home range area comparison

I did not statistically compare KDE area among species, sexes, or seasons because the smoothing parameter can have a dramatic influence on the size of the resultant KDE area (Silverman 1986). Instead, I compared 95% and 100% MCP area among species, sexes, and seasons.

I compared 95% and 100% MCP areas among sexes, species, and seasons using the Wilcoxon rank sum test for only GPS-collared individuals and for all individuals treated together. I conducted comparisons using these 2 scenarios to determine if the inclusion of radio-telemetry locations and the partitioning of GPS data into nocturnal and diurnal locations affected the results of the tests. For seasonal comparisons, I excluded any individuals with <10 locations in a season.

To determine if nocturnal and diurnal home range areas differed for GPS-collared individuals, I conducted a Wilcoxon signed-rank test on ocelots and bobcats that were tracked with GPS collars to compare home range estimates derived from diurnal locations with those derived from nocturnal locations for the same individual.

Winter was defined as 1 October to 31 March and summer as 1 April to 30 September. This classification was selected to divide the year by dominant weather patterns, with summer generally having consistently higher diurnal and nocturnal temperatures, and winter having variable lower diurnal and nocturnal temperatures. Although this classification scheme occasionally resulted in the inclusion of warmer days into winter as well as cooler days into summer, it had the advantage of dividing the year into equally sized temporal periods, which was important for comparing home range size between climatic seasons. Attempting to define more than 2 seasons resulted in several individuals having too few relocation points per season for home range analysis.

Home range land cover components

I used the 2011 National Land Cover Database (NLCD; Homer et al. 2015) raster file as the base map for the analysis of home range land cover components. The NLCD uses a 16-class land cover classification scheme to categorize major land cover types consistently across the United States at a 30-m spatial resolution, using Landsat 5 Thematic Mapper imagery. Major land cover categories present on the EESR and surrounding areas included Evergreen Forest, Shrub or Scrub, Emergent Herbaceous Wetlands, Herbaceous, Barren Land, Woody Wetlands, Hay or Pasture, Cultivated Crops, Developed Open Space, Developed Medium Intensity, Deciduous Forest, Developed Low Intensity, Developed High Intensity, and Open Water. Visual comparison between Deciduous Forest and Evergreen Forest categories and aerial imagery revealed high similarity between these cover types. I, therefore, re-categorized classes by combining Evergreen Forest and Deciduous Forest into the category Forest. I combined Emergent Herbaceous Wetlands and Woody Wetlands into the category Wetland. Open Water

was a minor component of ocelot and bobcat home ranges and was always found closely associated with wetlands, thus I placed Open Water into the category Wetland. The categories, Barren Land, Hay or Pasture, Cultivated Crops, Developed Open Space, Developed Medium Intensity, Developed Low Intensity, and Developed High Intensity were characterized by a similar lack of cover, and were therefore re-categorized as Open. The reclassification divided the study area into the following 5 land cover types: Forest, Shrub, Herbaceous, Open, and Wetland.

To assess the accuracy of the NLCD classification, I allocated 50 random points to each of the 5 land cover types and compared the land cover classification with a 2015 digital orthophoto quarter quadrangle (DOQQ) following the procedure outlined by Congalton and Green (1999). I extracted the pixels from the re-categorized NLCD land cover file falling within each 50% and 95% KDE isopleth of each individual and calculated percent coverage of each land cover type.

I used a logratio transformation in the R package compositions (van den Boogaart et al. 2014) to transform composition data derived from the NLCD base layer prior to analysis. I used a multivariate analysis of variance (MANOVA) on the transformed data to determine whether the linear combinations of home range land cover components differed among species, sexes, seasons, or collar types.

RESULTS

Capture and telemetry

The spring 2011 trapping season occurred on the northeastern portion of the EESR from 10 March 2011 to 15 April 2011, with 525 trap-nights. During the 2011 trapping season, 4 male ocelots (E2M, E3M, E4M, and E6M), 2 female ocelots (E1F, E5F), 1 male bobcat (EB2M), and

1 female bobcat (EB1F) were captured. Ocelots E3M and E4M were collared with Sirtrack GPS collars. The other individuals were collared with VHF radio collars. The collar placed on ocelot E3M released as scheduled allowing data download. The GPS collar placed on E4M was not recovered.

The 2012 trapping season took place on the northeastern portion of the EESR from 23 March 2012 to 19 April 2012, with 578 trap-nights. During the 2012 trapping season, 3 ocelots (3F) and 4 bobcats (4F) were captured. Two of the ocelots (E5F, E7F) were collared with Sirtrack GPS collars, and the third (E1F) was collared with a VHF radio collar. Two of the ocelots (E1F and E5F) were recaptured from 2011. The GPS collars from E5F and E7F were not recovered. One of the bobcats captured (EB5F) was a juvenile and was, therefore, released without sedation. The other 3 bobcats (EB3F, EB4F, EB6F) were collared with VHF radio collars.

The spring 2013 trapping season occurred intermittently from 2 February 2013 to 15 May 2013, yielding 951 trap-nights. Trapping began on the northeastern portion of the EESR, and traps were moved to the southeastern portion on 8 April 2013. In the northeastern portion of the EESR 1 male ocelot (E8M), 3 male bobcats (EB7M, EB8M, EB11M) and 3 female bobcats (EB9F, EB10F, EB12F) were captured. Ocelot E8M was collared with a Sirtrack GPS collar, which released prematurely after 2 months of tracking. In the southeastern portion, 2 female ocelots (E9F, E10F), 3 male bobcats (EB13M, EB14M, EB16M), and 1 female bobcat (EB15F) were captured. Two of the bobcats (EB15F, EB16M) were fitted with ATS GPS collars. The other ocelots (E9F, E10F) and bobcats (EB7M, EB8M, EB9F, EB10F, EB11M, EB12F, EB13M) were collared with radio collars (Table 1.1).

In 2014, trapping activities occurred intermittently from 19 January 2014 to 16 April 2014 yielding 2,229 trap-nights located on the southeastern portion of the EESR. During the 2014 trapping season, 1 male ocelot (Y12M), 1 female ocelot (E10F), and 1 female bobcat (EB17F) were captured. The female ocelot (E10F) had been previously captured and collared on the EESR and the male ocelot (Y12M) had been previously captured and collared on the Yturria Ranch on 6 March 2007. Both ocelots were fitted with Lotek GPS collars. The female bobcat (EB17F) was fitted with an ATS GPS collar. The collar placed on EB17F was not recovered.

The spring 2015 trapping season lasted from 9 February 2015 to 13 May 2015, with 1,886 trap-nights occurring on the northeastern portion of the EESR. During the 2015 trapping season, 2 male ocelots (E6M, E13M), 1 female ocelot (E12F), and 1 male bobcat (EB8M) were captured. Bobcat EB8M and ocelot E6M were recaptured felids. The 3 ocelots and 1 bobcat were collared with Lotek GPS collars.

For the test beacons placed throughout the study area, mean angular error was not significantly different from 0 ($P > 0.05$), indicating lack of bias, and standard deviation of the angular error was 8.75°. Mean error ellipse area was 0.921 km^2. Although >50% of the error ellipses were of sufficiently small size to allow unambiguous determination of habitat use, I believed that inclusion of only these locations in a point-based habitat analysis would likely result in a biased estimate of habitat use. I, therefore, used radio-locations only for generation of home ranges.

Table 1.1. Capture date, date of last relocation (end date), number of very high frequency locations, and number of low-frequency global positioning systems locations collected on ocelots and bobcats captured on the East El Sauz Ranch from 2011 to 2015.

ID	Species	Sex	Capture Date	End Date	VHF Locations	GPS Locations
E1F	Ocelot	F	3/11/2011	3/28/2013	105	0
E2M	Ocelot	M	3/15/2011	8/3/2012	70	0
E3M	Ocelot	M	3/9/2011	12/6/2011	35	259
E4M	Ocelot	M	3/18/2011	6/16/2012	46	0
E5F	Ocelot	F	4/5/2011	3/8/2013	59	0
E6M	Ocelot	M	4/5/2011	5/15/2011	46	0
E7F	Ocelot	F	3/19/2012	10/1/2012	31	0
E8M	Ocelot	M	2/24/2013	4/16/2013	12	26
E9F	Ocelot	F	4/23/2013	4/30/2014	93	0
E10F	Ocelot	F	4/26/2013	3/8/2014	91	0
E10F*	Ocelot	F	3/1/2014	7/25/2014	0	171
E12F	Ocelot	F	3/20/2015	11/7/2015	0	104
Y12M	Ocelot	M	3/3/2014	7/27/2014	0	180
E6M*	Ocelot	M	4/22/2015	1/28/2016	0	359
EB1F	Bobcat	F	3/13/2011	4/19/2012	41	0
EB2M	Bobcat	M	3/14/2011	7/28/2011	28	0
EB3F	Bobcat	F	3/16/2012	6/26/2013	104	0
EB4F	Bobcat	F	3/26/2012	8/6/2013	100	0

Table 1.1. (continued)

ID	Species	Sex	Capture Date	End Date	VHF Locations	GPS Locations
EB7M	Bobcat	M	2/10/2013	7/20/2014	111	0
EB8M	Bobcat	M	2/11/2013	2/12/2014	101	0
EB9F	Bobcat	F	2/13/2013	7/20/2014	105	0
EB10F	Bobcat	F	2/25/2013	2/12/2014	103	0
EB11M	Bobcat	M	3/5/2013	2/12/2014	95	0
EB12F	Bobcat	F	4/3/2013	9/8/2013	58	0
EB13M	Bobcat	M	4/16/2013	7/20/2014	63	0
EB14M	Bobcat	M	4/16/2013	7/20/2014	90	0
EB15F	Bobcat	F	4/27/2013	10/20/2013	0	339
EB16M	Bobcat	M	5/8/2013	10/16/2013	0	314
EB8M*	Bobcat	M	3/22/2015	7/14/2015	0	159

* Individual that was originally captured and fitted with a VHF radio collar but was later recaptured and fitted with a GPS collar.

Home range area comparison

Ocelot home range area (km^2) ranged from 0.56 to 14.41 (mean = 6.55, SD = 4.48) at the 95% MCP level and from 1.12 to 29.37 (mean = 10.62, SD = 7.79) at the 100% MCP level. Bobcat EB13M was likely a disperser, as it was located by fixed-wing aircraft west of Highway 77, about 30 km from its capture site. Bobcat EB16M used a drainage canal to move 12 km from its capture site, but was considered a transient, rather than a disperser, because it returned to its area of capture during the study period. I excluded EB13M and EB16M from all comparisons of home range area. With dispersers and transients removed from analysis, bobcat home range area ranged from 1.34 to 20.54 (mean = 5.66, SD = 5.52) at the 95% MCP level, and from 2.22 to 31.76 (mean = 9.72, SD = 9.79) at the 100% MCP level (Table 1.2).

Minimum convex polygon areas appeared to be non-normally distributed, thus I used only non-parametric statistical tests for comparisons. For all GPS and radio-collared individuals, I failed to reject the null hypothesis of home range equality between nocturnal and diurnal locations for 95% MCP ($P = 0.623$) and 100% MCP ($P = 0.983$) areas. I, therefore, concluded that MCP areas did not differ based on whether points were collected at night or during the day.

For GPS-collared individuals, ocelots were observed to have larger 95% MCP ($P = 0.058$) and 100% MCP home range areas ($P = 0.020$) than bobcats. For radio-collared individuals, I observed no difference in home range area between species for either 95% MCP areas ($P = 0.395$) or 100% MCP areas ($P = 0.492$). Among ocelots, I observed no difference in home range area due to sex either at the 95% MCP level ($P = 0.667$) or at the 100% MCP level ($P = 0.730$). Among bobcats, I observed no difference in home range area due to sex both at the 95% ($P = 0.121$) and 100% ($P = 0.281$) MCP levels.

Table 1.2. Home range area (km^2) of ocelots and bobcats, calculated using 95% and 100% minimum convex polygons (MCP), and 50% and 95% bivariate-normal fixed kernel density home ranges (KDE). Individuals identified as dispersers or transients were removed prior to analysis.

	95% MCP		100% MCP		50% KDE		95% KDE	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male Ocelots	6.55	4.48	11.38	7.84	3.56	1.88	15.12	7.36
Female Ocelots	5.82	4.54	9.86	8.13	3.60	2.83	14.49	10.74
Diurnal Ocelot	6.21	4.40	10.39	8.35	2.97	2.09	12.96	8.79
Nocturnal Ocelot	8.34	4.95	11.41	6.37	4.70	2.36	18.32	8.25
Combined Ocelots	6.55	4.48	10.62	7.79	3.58	2.29	14.84	8.79
Male Bobcats	8.07	6.65	13.88	11.65	3.24	2.54	16.12	12.13
Female Bobcats	2.90	1.71	4.98	4.01	1.41	0.89	6.08	4.21
Diurnal Bobcat	6.22	5.74	10.86	10.06	2.58	2.21	12.61	10.71
Nocturnal Bobcat	1.99	0.92	2.31	0.95	1.13	0.47	3.79	1.20
Combined Bobcats	5.66	5.52	9.72	9.79	2.38	2.11	11.43	10.39

I observed no significant differences in home range area between radio-collared and GPS-collared individuals. Using the Wilcoxon signed-rank test, I observed no difference in 100% MCP area between GPS and radio-collared individuals for ocelots alone ($P = 0.628$), for bobcats alone ($P = 0.396$), or for all individuals treated together ($P = 0.375$). I observed no difference in home range area due to locations being collected nocturnally or diurnally for 95% MCP area ($P = 0.195$) or 100% MCP area ($P = 0.844$). For individuals that were tracked >1 season, I observed no difference in 100% MCP home range area between winter and summer using the Wilcoxon signed-rank test, for all individuals ($P = 0.838$), for ocelots only ($P = 0.320$) (Table 1.5), or for bobcats only ($P = 0.275$; Table 1.6).

Kernel home range generation

The LSCV algorithm failed to converge for 9 of 38 home range polygons generated. All failures to converge occurred with GPS-collared individuals, which had higher numbers of locations than radio-collared individuals. Rather than further subset GPS location estimates in an effort to force convergence of the LSCV algorithm, I used the h_{ref} method for home range generation in subsequent analyses. The *h* value selected using this method ranged from 427 to 702 (mean = 172, SD = 146.24) for ocelots, and from 164 to 669 (mean = 340, SD = 153.76) for bobcats. Although there was substantial variation in the *h* value selected for each individual, visual inspection of each home range revealed that this method produced home range contours that were relatively uniform in extent and smoothness.

Area (km^2) of home range core areas (50% KDE) ranged from 0.46 to 7.34 (mean = 3.58, SD = 2.29) for ocelots (Table 1.4), and from 0.58 to 8.57 (mean = 2.38, SD = 2.11) for bobcats (Table 1.3). Area (km^2) of home range 95% KDE contours ranged from 2.03 to 27.83 (mean =

Table 1.3. Area (km^2) and percent coverage of Forest, Shrub, Herbaceous (Herb), Open, and Wetland land cover types within 50% kernel home ranges created for ocelots, using h_{ref} bandwidth selection method. Home range estimates derived from nocturnal locations are shaded in gray.

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
E6M	GPS	167	1.50	35	34	19	12	0	281
E3M	GPS	115	6.00	19	61	15	5	0	569
E8M	GPS	19	4.70	38	39	16	6	0	616
Y12M	GPS	87	4.54	32	34	32	1	1	500
E2M	Radio	48	2.23	18	45	32	4	1	385
E4M	Radio	45	0.62	13	50	32	4	0	211
E6M	Radio	45	2.20	51	33	7	5	4	354
E6M	GPS	192	2.67	34	35	21	11	0	341
E3M	GPS	179	5.98	15	64	17	5	0	493
E8M	GPS	19	3.20	41	35	14	9	0	512
Y12M	GPS	93	5.48	25	38	34	2	2	496
E12F	GPS	54	0.72	28	32	24	16	0	252
E10F	Radio	89	3.88	10	28	47	8	7	494
E1F	Radio	102	2.12	28	48	21	3	0	349
E5F	Radio	57	2.70	12	32	51	3	2	374
E7F	Radio	30	0.46	41	43	6	10	0	172
E9F	Radio	92	6.97	9	32	44	1	14	567

Table 1.3 (continued)

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
E10F	Radio	56	7.34	8	36	43	4	10	702
E10F	GPS	115	7.06	9	29	47	6	10	580
E12F	GPS	48	1.19	41	33	15	11	0	289

Table 1.4. Area (km^2) and percent coverage of Forest, Shrub, Herbaceous (Herb), Open, and Wetland land cover types within 50% kernel home ranges created for bobcats, using h_{ref} bandwidth selection method. Home range estimates derived from nocturnal locations are shaded in gray.

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
EB16M[1]	GPS	153	17.18	2	31	21	36	10	1040
EB8M	GPS	59	1.47	27	49	18	6	0	292
EB2M	Radio	27	4.04	28	48	20	2	2	543
EB10F	Radio	101	1.05	12	69	10	9	0	246
EB11M	Radio	93	4.28	25	50	16	9	0	482
EB13M[2]	Radio	61	42.44	3	27	41	5	24	2534
EB14M	Radio	88	2.17	0	45	44	0	11	385
EB7M	Radio	108	3.52	23	22	40	14	1	500
EB8M	Radio	99	8.57	21	59	17	3	0	669
EB16M[1]	GPS	161	19.04	2	30	21	37	11	1079
EB8M	GPS	100	0.80	9	51	32	7	1	201
EB15F	GPS	163	1.05	0	41	51	2	6	205
EB1F	Radio	40	0.86	17	30	51	1	2	232
EB3F	Radio	103	0.88	37	36	15	13	0	200
EB4F	Radio	98	0.58	24	45	26	5	0	164
EB12F	Radio	57	3.21	32	48	16	2	1	429
EB9F	Radio	103	1.80	5	24	58	3	10	339

Table 1.4 (continued)

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
EB15F	GPS	176	1.46	0	36	52	0	12	218

1 Transient individual that was removed from home range area comparisons but included in multivariate land cover comparisons.

2 Disperser individual that was removed from home range area comparisons and multivariate land cover comparisons.

Table 1.5. Area (km^2) and number of locations taken (in parentheses) of 95% minimum convex polygon home ranges for ocelots. Time periods during which an individual was not tracked are denoted with -. Home range estimates derived from nocturnal locations are shaded in gray.

ID	Winter 2011	Summer 2011	Winter 2012	Summer 2012	Winter 2013	Summer 2013	Winter 2014	Summer 2014	Summer 2015	Winter 2016
E10F	-	-	-	-	-	6.99 (52)	7.37 (59)	4.52 (34)	-	-
E10F	-	-	-	-	-	-	6.81 (23)	11.12 (92)	-	-
E12F	-	-	-	-	-	-	-	-	0.85 (50)	-
E12F	-	-	-	-	-	-	-	-	1.23 (42)	-
E1F	0.77 (11)	0.87 (29)	-	1.60 (25)	2.71 (31)	-	-	-	-	-
E2M	-	1.39 (22)	0.94 (12)	-	-	-	-	-	-	-

Table 1.5 (continued)

ID	Winter 2011	Summer 2011	Winter 2012	Summer 2012	Winter 2013	Summer 2013	Winter 2014	Summer 2014	Summer 2015	Winter 2016
E3M	17.84 (12)	9.34 (79)	6.10 (24)	-	-	-	-	-	-	-
E3M	4.62 (12)	12.82 (140)	5.89 (27)	-	-	-	-	-	-	-
E4M	-	0.64 (42)	0.63 (13)	-	-	-	-	-	-	-
E5F	-	2.06 (22)	2.13 (18)	1.54 (11)	-	-	-	-	-	-
E6M	-	2.27 (24)	3.82 (83)	-	-	-	-	-	3.49 (83)	3.53 (84)
E6M	-	-	-	-	-	-	-	-	9.66 (101)	5.21 (91)
E7M	-	-	-	0.37 (24)	-	-	-	-	-	-

Table 1.6. Area (km^2) and number of locations taken (in parentheses) of 95% minimum convex polygon home ranges for bobcats. Time periods during which an individual was not tracked are denoted with -. Home range estimates derived from nocturnal locations are shaded in gray.

ID	Summer 2011	Summer 2012	Winter 2013	Summer 2013	Winter 2014	Summer 2014	Winter 2015	Summer 2015
EB15F	-	-	-	1.83 (143)	1.00 (20)	-	-	-
EB15F	-	-	-	2.57 (156)	2.03 (20)	-	-	-
EB16M[1]	-	-	-	34.11 (138)	6.56 (15)	-	-	-
EB16M[1]	-	-	-	33.06 (145)	5.88 (16)	-	-	-
EB1F	1.06 (27)	-	-	-	-	-	-	-
EB2M	4.48 (19)	-	-	-	-	-	-	-
EB3F	-	0.97 (28)	1.51 (47)	1.10 (20)	-	-	-	-
EB4F	-	0.87 (27)	0.59 (26)	1.11 (44)	-	-	-	-

Table 1.6 (continued)

ID	Summer 2011	Summer 2012	Winter 2013	Summer 2013	Winter 2014	Summer 2014	Winter 2015	Summer 2015
EB4F	-	0.87 (27)	0.59 (26)	1.11 (44)	-	-	-	-
EB8M	-	-	1.29 (14)	11.30 (63)	6.49 (22)	-	-	1.17 (54)
EB8M	-	-	-	-	-	-	-	1.31 (91)
EB10F	-	-	-	3.50 (65)	1.22 (27)	-	-	-
EB11M	-	-	-	7.24 (67)	9.67 (20)	-	-	-
EB12F	-	-	-	5.77 (57)	-	-	-	-
EB13M[2]	-	-	-	26.87 (52)	-	-	-	-
EB14M	-	-	-	5.15 (56)	2.93 (31)	-	-	-
EB7M	-	-	-	7.07 (66)	7.20 (31)	-	-	-
EB9F	-	-	2.01 (12)	3.61 (62)	1.53 (28)	-	-	-

1 Transient individual that was removed from seasonal home range area comparisons.

2 Disperser individual that was removed from seasonal home range area comparisons.

14.84, SD = 8.79) for ocelots (Table 1.6), and from 2.64 to 39.80 (mean = 11.43, SD = 10.39) for bobcats (Table 1.5).

Home range land cover components

Within the EESR boundaries, pixel counts and percent coverage for each land cover type were: Forest = 8,024 (6.4%), Shrub = 17,291 (13.8%), Herbaceous = 63,466 (50.5%), Open = 13,250 (10.5%), Wetland = 23,652 (18.8%). Overall classification accuracy was 85.2%.

Bobcat EB13M was excluded from home range land cover comparisons, as it was a disperser and lacked a clear home range area. Although Bobcat EB16M was likely a transient, it was included in the analysis of home range land cover components as it showed frequent use of the same areas around a drainage canal, allowing home range contours to be delineated around areas of high use.

The first set of home range land cover comparisons included individuals collared with VHF and GPS collars. With VHF-and GPS-collared individuals included, I found no significant difference in 95% KDE land cover components due to species ($P = 0.497$), collar type ($P = 0.620$), ocelot gender ($P = 0.146$), or bobcat gender ($P = 0.221$). I failed to find a difference in 50% KDE land cover components due to species ($P = 0.223$), collar type ($P = 0.584$) or bobcat gender ($P = 0.110$). At the $\alpha = 0.05$ significance level, I identified a difference in 50% KDE land cover components between male and female ocelots ($P = 0.032$); male ocelot home ranges contained a mean of 29.18% Forest and 45.55% Shrub and female ocelot home ranges contained a mean of 20.67% Forest and 34.78% Shrub.

The second set of home range landcover comparisons included only GPS-collared individuals. I identified differences in home range land cover components between ocelots and bobcats at the 50% ($P = 0.084$) and 95% ($P = 0.016$) KDE levels. I identified differences in

Table 1.7. Area (km^2) and percent coverage of Forest, Shrub, Herbaceous (Herb), Open, and Wetland land cover types within 95% kernel home ranges created for ocelots, using h_{ref} bandwidth selection method. Home range estimates derived from nocturnal locations are shaded in gray .

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
E6M	GPS	167	7.17	37	32	22	8	2	281
E3M	GPS	115	26.99	30	43	19	7	1	569
E8M	GPS	19	18.62	31	38	21	9	2	616
Y12M	GPS	87	21.70	23	40	27	5	5	500
E2M	Radio	48	9.61	25	47	24	2	2	385
E4M	Radio	45	3.51	15	57	26	2	0	211
E6M	Radio	45	9.37	43	34	15	6	2	354
E6M	GPS	192	13.76	36	29	24	9	1	341
E3M	GPS	179	22.45	34	46	13	6	0	493
E8M	GPS	19	13.10	34	33	20	12	1	512
Y12M	GPS	93	20.08	22	42	27	4	5	496
E12F	GPS	54	3.74	39	36	13	11	1	252
E10F	Radio	89	19.63	6	31	47	4	12	494
E1F	Radio	102	8.77	25	46	23	5	2	349
E5F	Radio	57	10.21	15	39	42	3	2	374
E7F	Radio	30	2.03	42	42	7	9	0	172
E9F	Radio	92	27.17	4	38	29	17	12	567

Table 1.7 (continued)

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
E10F	Radio	56	27.83	7	36	37	9	10	702
E10F	GPS	115	26.32	6	36	38	7	12	580
E12F	GPS	48	4.68	39	37	13	10	2	289

Table 1.8. Area (km^2) and percent coverage of Forest, Shrub, Herbaceous (Herb), Open, and Wetland land cover types within 95% kernel home ranges created for bobcats, using h_{ref} bandwidth selection method. Home range estimates derived from nocturnal locations are shaded in gray.

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
EB15F	GPS	163	3.60	0	52	32	3	12	205
EB1F	Radio	40	3.79	17	39	36	4	5	232
EB3F	Radio	103	3.83	39	36	17	7	1	200
EB4F	Radio	98	2.64	19	43	31	5	2	164
EB12F	Radio	57	13.65	23	39	25	11	2	429
EB9F	Radio	103	10.41	22	38	36	2	3	339
EB15F	GPS	176	4.64	0	49	33	3	15	218
EB16M[1]	GPS	153	67.17	2	24	15	48	12	1040
EB2M	Radio	27	17.65	26	45	22	6	1	543
EB8M	GPS	59	5.02	26	51	19	3	1	292
EB10F	Radio	101	6.21	32	55	8	5	0	246
EB11M	Radio	93	20.96	31	37	22	9	1	482
EB13M[2]	Radio	61	310.23	5	28	35	15	17	2534
EB14M	Radio	88	13.72	2	45	29	3	20	385
EB7M	Radio	108	22.64	26	25	34	12	3	500
EB8M	Radio	99	39.80	32	43	19	5	1	669

Table 1.8 (continued)

ID	Collar	Points	Area	Forest	Shrub	Herb	Open	Wetland	h_{ref}
EB16M[1]	GPS	161	74.69	2	24	15	47	12	1079
EB8M	GPS	100	2.94	22	48	23	4	2	201

1 Transient individual that was removed from home range area comparisons but included in multivariate land cover comparisons.

2 Disperser individual that was removed from home range area comparisons and multivariate land cover comparisons.

landcover components between male and female ocelots at the 95% KDE contour ($P = 0.079$). At the 50% KDE contour, I found no difference due to sex among GPS-collared ocelots ($P = 0.133$). I identified differences in home range landcover components between male and female bobcats at the 50% ($P = 0.036$; Tables 1.3-1.4) and 95% ($P = 0.010$; Tables 1.7-1.8) density contours. Within the 50% KDE contour, ocelot home ranges contained a mean of 27.01% Forest, 39.02% Shrub, 24.75% Herbaceous, 7.25% Open, and 1.97% Wetland. Bobcat 50% KDE contours contained a mean of 6.68% Forest, 39.48% Shrub, 34.42% Herbaceous, 14.68% Open, and 6.74% Wetland. Ocelot 95% KDE contours contained 28.24% Forest, 37.22% Shrub, 22.91% Herbaceous, 8.03% Open, and 3.60% Wetland. Bobcat 95% KDE contours contained 8.65% Forest, 41.29% Shrub, 22.86% Herbaceous, 18.06% Open, and 9.13% Wetland.

DISCUSSION

The largest 100% MCP area observed was for male bobcat EB13M. However, this individual was a disperser, and was, therefore, excluded from home range area comparisons. The second largest 100% MCP area observed was for male bobcat EB16M, which was likely a transient, as it used a drainage canal for travel to and from the EESR, yet showed no signs of establishing a clearly defined home range. This individual also was excluded from home range area comparisons. With these individuals removed from the analysis, ocelots displayed a significantly larger home range area than bobcats when only GPS-collared individuals were considered. When VHF locations were included, however, there were no significant differences in MCP area between ocelots and bobcats. The larger mean home range size observed in GPS-collared ocelots may have been an artifact of small sample size, as removal of EB16M left only 2 GPS-collared bobcats (EB8M and EB15F) in the analysis.

No seasonal differences were observed in 100% MCP areas for ocelots or bobcats. This contrasts with Tewes (1986) that found ocelot home ranges contracted during hot summers and expanded during milder winters. No gender differences were observed in home range area for either ocelots or bobcats. This contrasts with other studies that have reported home ranges of male bobcats are larger than those of female bobcats (Hall and Newsom 1978, Kitchings and Story 1979, Litvaitis et al. 1986).

Using MANOVA to compare land cover components of ocelot and bobcat home ranges, I detected significant differences between groups only when radio-telemetry derived KDE estimates were removed from the analysis. This may be due to lower power resulting from fewer locations obtained by VHF radiotelemetry than GPS radiotelemetry. By considering GPS-collared individuals alone, I detected a strong significant difference in land cover components of ocelot and bobcat home ranges at the 50% and 95% KDE contours. Within 50% and 95% KDE contours, ocelot home ranges included a higher proportion of Forest, a lower proportion of Shrub, a lower proportion of Herbaceous, a lower proportion of Open, and a lower proportion of Wetland than bobcat home ranges (Fig. 1.3). Differences in home range landcover components due to gender were observed in ocelots at the 50% and 95% KDE contours for GPS-collared individuals, and at the 50% KDE contour for GPS and VHF-collared individuals. Male ocelot home range core areas (50% KDE contours) contained higher proportions of Forest and Shrub than those of female ocelots.

Core areas are those parts of the home range that are used more frequently than other areas (Kaufmann 1962). These are regarded as important areas as core areas may contain den sites, refugia, and dependable food sources (Samuel et al. 1985). That male and female ocelots showed greater differentiation in home range land cover components at the 50% KDE contour

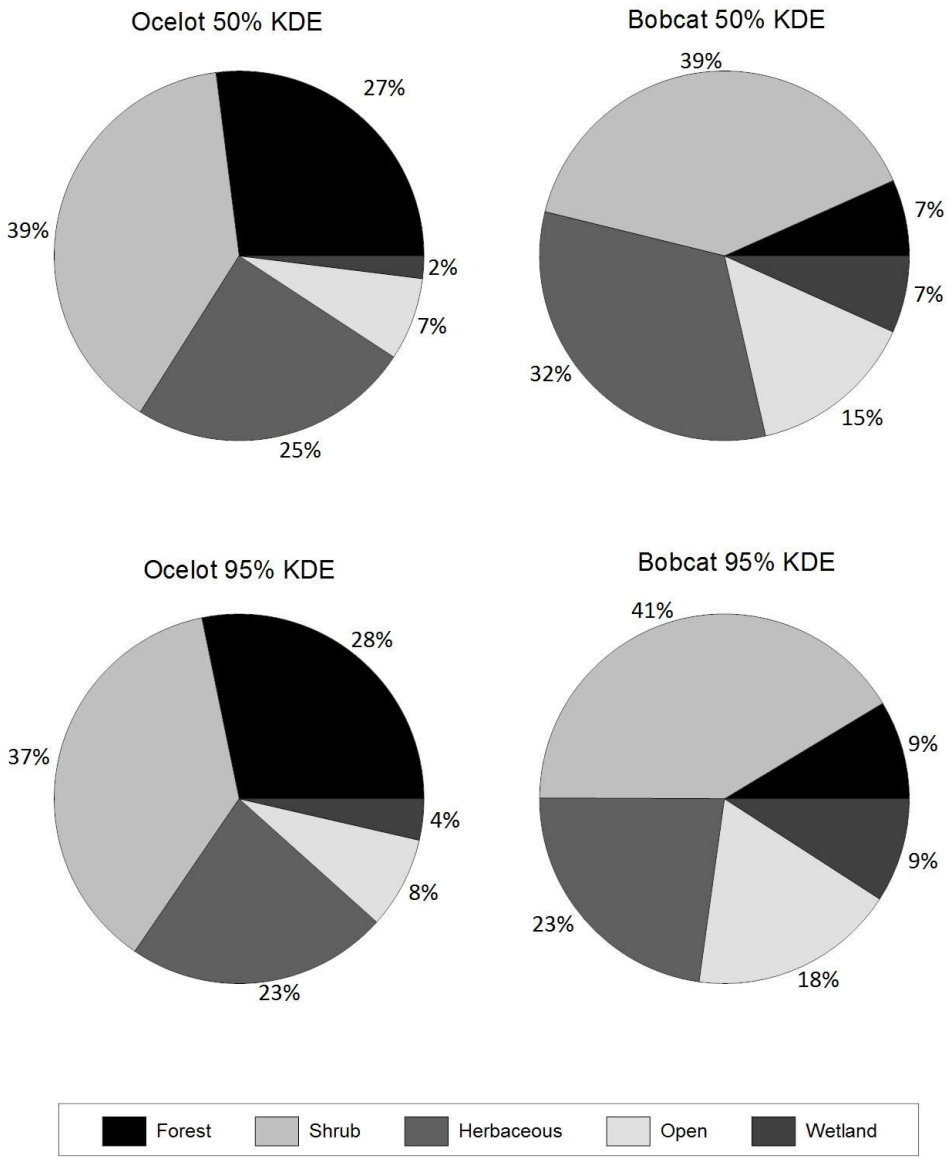

Figure 1.3. Relative proportions of land cover components within GPS-collared ocelot and bobcat 50% and 95% kernel density contours. Kernel density contours were generated using adehabitatHR, with the h_{ref} method of bandwidth selection. Landcover components were derived from recategorized National Land Cover Database raster file.

may indicate gender-related differences in habitat requirements, with male core areas located in areas with high percentages of forest and shrub cover. Alternatively, female ocelots may be prevented from establishing home range core areas in optimal locations (i.e., those with high forest and shrub cover) due to the presence of male ocelots.

The differences observed in home range landcover components, at the 50% and 95% KDE contours, between ocelots and bobcats suggests habitat-related niche partitioning, with ocelots centering home ranges in areas with abundant forest cover and bobcats showing greater preference to the more open shrub cover type. The areas classified as Shrub were characterized by small patches of woody vegetation interspersed with open areas. Tree height and canopy density were lower in areas classified as Shrub than in those classified as Forest. Areas classified as Forest were characterized by a closed canopy of live oak, whereas those classified as Shrub were characterized by a more open canopy of honey mesquite, huisache, and granjeno (*Celtis pallida*). Areas classified as Forest also contained a strong shrub component at the mid-canopy level, with patches of dense shrub cover existing underneath a closed oak canopy.

Ocelots are widely reported to prefer native thornshrub habitats in Texas (Tewes 1986, Laack 1991, Harveson et al. 2004), and Navarro-Lopez (1985) reported ocelots in Texas to be also associated with live oak forests. This study found ocelot home range areas differed from bobcat home range areas primarily in the relative abundance of forested habitat. For the Willacy population of ocelots, the oak forest habitat occurring primarily on the northwestern portion of the EESR may be a more important land cover type than the more open Shrub habitat occurring in the southwestern portion of the EESR.

Horne et al. (2009) found evidence for habitat-related niche partitioning between sympatric ocelots and bobcats on LANWR, with ocelots selecting areas with >75% canopy

cover, and bobcats selecting areas with <75% canopy cover. Areas of dense canopy cover preferred by ocelots were documented by Shindle and Tewes (1998) as dominated by granjeno, crucita, Berlandier fiddlewood, honey mesquite, and desert olive. My study also found ocelots select areas with closed canopy cover. However, the land cover types available on the EESR differed from those on LANWR due to the presence of closed canopy live oak forests on the EESR. Both the EESR and LANWR fall within the Tamaulipan Biotic Province (Jahrsdoerfer and Leslie 1988), and the live oak forests occurring in the northwestern portion of the EESR can be considered Tamaulipan thornshrub with an emergent oak canopy.

Significant differences between landcover components of ocelot and bobcat home ranges were only detected when VHF locations were removed. This may be due to biased sampling inherent in radio telemetry. The Willacy ocelot population is located entirely on private lands and I had access only to the EESR. Sometimes, I failed to locate an ocelot or a bobcat through ground-based telemetry due to that individual occurring outside the EESR. I attempted to locate these individuals using aerial surveys. However, it is likely that locations falling within the boundaries of the EESR were over-represented compared to those located far from the EESR. This inability to consistently monitor individuals could have biased my home range estimates based on VHF data for ocelots and bobcats.

Ocelot habitat management should include conservation and expansion of closed-canopy oak habitat and Tamaulipan thornshrub. The intensity with which bobcats compete with ocelots for limited habitat is unclear. This study found similarities in the land cover types comprising ocelot and bobcat home ranges, yet there was evidence of spatial niche partitioning, with ocelots more strongly associated with forested land-cover types. Future studies should examine whether

fine scale spatial, dietary, and temporal niche partitioning between sympatric ocelots and bobcats occurs, and determine if bobcat removal could increase ocelot populations.

LITERATURE CITED

Bradley, L. C., and D. B. Fagre. 1988. Movements and habitat use by coyotes and bobcats on a ranch in southern Texas. Proceedings of the Annual Conference of the Southeast Association of Fish and Widlife Agencies 42:411-430.

Cain, A. T., V. R. Tuovila, D. G. Hewitt, and M. E. Tewes. 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189-197.

Calenge, C. 2006. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. Ecological Modelling 197:516-519.

Caso, A., C. Lopez-Gonzalez, E. Payan, E. Eizirik, T. de Oliveira, R. Leite-Pitman, M. Kelly, and C. Valderrama. 2008. *Leopardus pardalis*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>. Accessed 12 May 2016.

Congalton, R. G., and K. Green. 1999. Assessing the accuracy of remotely sensed data: principles and practices. Lewis, New York, USA.

Crawshaw, P. G., Jr. 1995. Comparative ecology of ocelot Felis pardalis and Jaguar Panthera onca in a protected subtropical forest in Brazil and Argentina. PhD Dissertation, University of Florida, USA.

Dayan, T., and D. Simberloff. 1998. Size patterns among competitors: ecological character displacement and character release in mammals, with special reference to island populations. Mammal Review 28:99-124.

Di Bitetti, M. S., C. D. De Angelo, Y. E. Di Blanco, and A. Paviolo. 2010. Niche partitioning and species coexistence in a Neotropical felid assemblage. Acta Oecologica 36:403-412.

Donadio, E., and S. W. Buskirk. 2006. Diet, morphology, and interspecific killing in Carnivora. American Naturalist 167:524-536.

Federal Register. 1982. CFR Part 17. Vol 47, No. 140.

Gause, G. F. 1934. The struggle for existence. Hafner, New York, USA.

Hall, E. R., and J. D. Newsom. 1978. Summer home ranges and movement patterns of the bobcat in bottomland hardwoods of southern Louisiana. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 30:427-436.

Hansen, K. 2007. Bobcat: master of survival. Oxford University Press, New York, New York, USA.

Haines, A. M., M. E. Tewes, L. L. Laack, W. E. Grant, and J. H. Young. 2005. Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States. Biological Conservation 126:512–522.

Haines, A. M., M. E. Tewes, L. L. Laack, W. E. Grant, and J. H. Young. 2006. A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. Biological Conservation 132:424-436.

Harveson, P. M., M. E. Tewes, G. L. Anderson, and L. L. Laack. 2004. Habitat use by ocelots in South Texas: implications for restoration. Wildlife Society Bulletin 32:948–954.

Homer, C. G., J. A. Dewitz , L. Yang, S. Jin, P. Danielson, G. Xian, J. Coulston, N. D. Herold, J. D. Wickham, and K. Megown. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. Photogrammetric Engineering and Remote Sensing 81:345-354.

Horne, J. S., and E. O. Garton. 2006. Likelihood cross-validation versus least squares cross-validation for choosing the smoothing parameter in kernel home-range analysis. Journal of Wildlife Management 70:641-648.

Horne, J. S., A. M. Haines, M. E. Tewes, and L. L. Laack. 2009. Habitat partitioning by sympatric ocelots and bobcats: implications for recovery of ocelots in southern Texas. The Southwestern Naturalist 54:119–126.

Jahrsdoerfer, S. E., and D. M. Leslie, Jr. 1988. Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: description, human impacts, and management options. United States Fish and Wildlife Service, Biological Report 88: 1-63.

Janecka, J. E., T. L. Blankenship, D. H. Hirth, C. W. Kilpatrick, M. E. Tewes, and L. I. Grassman, Jr. 2007. Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. Wildlife Biology 13:38-47.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61:65-71.

Kaufmann, J. H. 1962. Ecology and social behavior of the coati, *Nasua nirica*, on Barro Colorado Island Panama. University of California Publications in Zoology 60:95-222.

Kiltie, R. A. 1984. Size ratios among sympatric neotropical cats. Oecologia 61:411-416.

Kitchings, J. T., and J. D. Story. 1979. Home range and diet of bobcats in eastern Tennessee. Pages 47-52 *in* L. Blum and P.C. Escherich, editors. Proceedings of the 1979 bobcat research conference: current research on biology and management of Lynx rufus. Scientific and Technical Series 6. National Wildlife Federation, Washington, D.C., USA.

Laack, L. L. 1991. Ecology of the ocelot (*Felis pardalis*) in south Texas. Thesis, Texas A&I University, Kingsville, Texas, USA.

Lariviere, S., and L. R. Walton. 1997. Lynx rufus. Mammalian Species 563:1-8.

Litvaitis, J. A., J. A. Sherburne, and J. A. Bissonette. 1986. Bobcat habitat use and home range size in relation to prey density. The Journal of Wildlife Management 50:110-117.

Macdonald, D.W., and C. Sillero-Zubiri. 2004. The biology and conservation of wild canids. Oxford University Press, Oxford, United Kingdom.

McCord, C. M., and J. E. Cardoza. 1982. Bobcat and lynx. Pages 728-766 *in* J.A. Chapman and G.A. Feldhammer, editors. Wild mammals of North America. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Mitchell, B.D., and P.B. Banks. 2005. Do wild dogs exclude foxes? Evidence for competition from dietary and spatial overlaps. Australian Ecology 30:581-591.

Moser, B. W., and E. O. Garton. 2007. Effects of telemetry location error on space-use estimates using a fixed-kernel density estimator. Journal of Wildlife Management 71:2421-2426.

Nams, V. O. 2006. Locate III User's Guide. Pacer Computer Software. Tatamagouche, Nova Scotia, Canada.

NatureServe and International Union for Conservation of Nature (IUCN). 2016a. *Leopardus pardalis*. The IUCN Red List of Threatened Species. Version 2016.1. http://www.iucnredlist.org. Downloaded on 11 June 2016.

NatureServe and International Union for Conservation of Nature (IUCN). 2016b. *Lynx rufus*. The IUCN Red List of Threatened Species. Version 2016.1. http://www.iucnredlist.org. Downloaded on 11 June 2016.

Navarro-Lopez, D. 1985. Status and distribution of the ocelot (*Felis pardalis*) in south Texas. Thesis, Texas A&I University, Kingsville, Texas, USA.

Oliveira, T. G., and K. Cassaro. 2005. Guia de Campo dos Felinos do Brasil. São Paulo, Instituto Pró-Carnívoros, Fundação Parque Zoológico de São Paulo, São Paulo, Brazil.

Rolley, R. E. 1987. Bobcat. Pages 671-681 *in* M. Novack, J. A. Baker, M. E. Obbard, and B. Malloch, editors. Wild furbearer management and conservation in North America. Ministry of Natural Resources, Toronto, Ontario, Canada.

Rosenzweig, M. L. 1966. Community structure in sympatric Carnivora. Journal of Mammalogy 47:602-612.

Samuel, M. D., D. J. Pierce, and E. O. Garton. 1985. Identifying areas of concentrated use within the home range. Journal of Animal Ecology 54:711-719.

Schoener, T. W. 1974. Resource Partitioning in Ecological Communities. Science 185:27-39.

Shindle, D. B., and M. E. Tewes. 1998. Woody species composition of habitats used by ocelots (*Leopardus pardalis*) in the Tamaulipan Biotic Province. The Southwestern Naturalist 43:273–279.

Shindle, D. B., and M. E. Tewes. 2000. Immobilization of wild ocelots with tiletamine and zolazepam in southern Texas. Journal of Wildlife Disease 36:546–550.

Silverman, B. W. 1986. Density estimation for statistics and data analysis. Chapman & Hall, London, United Kingdom.

Stasey, W. C. 2012. Evaluating translocation strategies for ocelot in the Tamaulipan Biotic Province. Dissertation. Texas A&M University-Kingsville, Kingsville, USA.

Sunquist, M. E., and F. Sunquist. 2002. Wild cats of the world. University of Chicago Press, Chicago, Illinois.

Tannerfeldt, M., B. Elmhagen, and A. Angerbjorn. 2002. Exclusion by interference competition? The relationship between red and arctic foxes. Oecologia 132:213-220.

Tewes, M. E. 1986. Ecological and behavioral correlates of ocelot spatial patterns. Dissertation, University of Idaho, Moscow, USA.

Tewes, M. E., and D. D. Everett. 1986. Status and distribution of the endangered ocelot and jaguarundi in Texas. Pages 147-158 *in* S. D. Miller and D. D. Everett, editors. Cats of the world: biology, conservation, and management. National Wildlife Federation, Washington, D.C., USA.

Tewes, M. E., and D. J. Schmidly. 1987. The Neotropical felids: jaguar, ocelot, margay, and jaguarondi. In Wild furbearer management and conservation in North America. Ontario Ministry of Resources. Ottawa, Canada.

van den Boogaart, K. G., R. Tolosana, and M. Bren. 2014. Compositions: Compositional Data Analysis. R package version 1.40-1.https://CRAN.R-project.org/package=compositions.

Wand, M., and M. Jones. 1995. Kernel smoothing. Chapman and Hall/CRC, USA.

White, G. C., and R. A. Garrott. 1986. Effects of biotelemetry triangulation error on detecting habitat selection. Journal of Wildlife Management 50:509-513.

White, G. C., and R. A. Garrott. 1990. Analysis of wildlife radio-tracking data. Academic Press, San Diego, USA.

Withey, J. C., T. Bloxton, and J. M. Marzluff. 2001. Effects of tagging and location error in wildlife radiotelemetry studies. Pages 43-75 *in* Millspaugh, J. J., and J. M. Marzluff, editors. Radio tracking and animal populations. Academic Press, San Diego, USA.

Worton, B. J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70:164-168.

Worton, B. J. 1995. Using Monte Carlo simulation to evaluate kernel-based home range estimators. Journal of Wildlife Management 59:794-800.

CHAPTER II

COMPARISON OF OCELOT AND BOBCAT TEMPORAL ACTIVITY PATTERNS

The ocelot (*Leopardus pardalis*) is a federally endangered species in the United States (Federal Register 1982). Its range has declined since the 1800s due primarily to anthropogenic land conversion of native thornshrub habitat (Tewes and Everett 1986). The ocelot is now confined to two isolated populations in South Texas, one on the Laguna Atascosa National Wildlife Refuge (LANWR) and the other on private ranches in Willacy County, Texas (Haines et al. 2006). The number of individuals believed to be remaining in these populations is <80 (M. E. Tewes, Texas A&M University-Kingsville, personal communication, August 2016). The primary reason for this decline is believed to be absence of habitat.

In South Texas, ocelots have been widely reported to be dependent on dense stands of native thornshrub (Navarro-Lopez 1985; Tewes 1986; Caso 1994; Shindle and Tewes 1998; Shinn 2002; Harveson et al. 2004; Connolly 2009; Horne et al. 2009; Stasey 2012). These studies characterized the ocelot as a habitat specialist, highly vulnerable to habitat loss due to anthropogenic land clearing. Elsewhere throughout its range, the ocelot has been recorded in mangrove forests, coastal marshes, savanna grasslands, thorn scrubs, tropical forest, and subtropical forest (Emmons 1988; Emmons et al. 1989; Sunquist and Sunquist 2002).

Throughout its range, the ocelot shows a higher level of body-size overlap with the bobcat than with any other sympatric felid, with bobcat weights averaging 7.3 kg for females and 10 kg for males (McCord and Cardoza 1982; Sunquist and Sunquist 2002) and ocelot weights ranging from 6.6 - 18.6 kg (Oliveira et al. 2010). Ocelot and bobcat geographic ranges overlap across an area extending from northern and central Mexico to southern Texas.

Chapter 2 of this dissertation is written in the style of the *Journal of Mammalogy*.

In contrast to the federally endangered ocelot, the bobcat is the most widely distributed and abundant native felid in North America, with populations that are believed to be increasing (Roberts and Crimmins 2010). Additionally, bobcat populations in southern Texas and northern Mexico have been reported to have significantly higher genetic diversity than ocelot populations occupying the same geographic region (Janecka et al. 2016). This difference is likely due to larger population sizes of bobcats than ocelots in this region and to the greater dispersal ability of bobcats due to their more general habitat preferences (Sunquist and Sunquist 2002). Species that demonstrate habitat specialization, such as the ocelot, are predicted to be more sensitive to the effects of fragmentation than are species that use a wider variety of habitat types (Didham 2010; Buchi and Vuilleumier 2014). Understanding the level to which ocelot and bobcat compete for the same limiting resources is important for ocelot conservation and management. If sufficient evidence exists that bobcats limit ocelot population size or distribution, then bobcat removal may be warranted to increase the sizes of remaining ocelot populations.

Interspecific competition among members of an ecological guild is expected to be particularly strong for carnivores such as felids. Guilds of sympatric carnivores are often used to test hypotheses relating to the evolutionary and ecological consequences of competitive interactions (Schoener 1974). The competitive exclusion hypothesis (Gause 1934) states that ecologically equivalent species cannot coexist. Schoener (1974) described three types of ecological partitioning that could allow for coexistence of similar species, including habitat partitioning, dietary partitioning, and temporal partitioning. There is evidence that intraguild competition and killing are important selective factors in carnivore assemblages (Palomares and Caro 1999; Donadio and Buskirk 2006).

Bobcats occur in a variety of habitats in the United States, including boreal and coniferous forests, bottomland hardwood forest, coastal swamp, desert, and scrubland (Kelly et al. 2008). In Mexico, bobcats have been found to use dry scrub, grassland, and tropical dry forest habitats (Monroy-Vilchis and Velazquez 2003). Although bobcats can occupy a wider variety of habitats than ocelots, in Texas they have often been found closely associated with the same dense thornshrub communities used by ocelots (Bradley and Fagre 1988; Cain et al. 2003). At the home range level, Horne et al. (2009) found few significant differences in the habitat used by ocelots and bobcats within LANWR, although evidence of spatial niche partitioning was observed at a microhabitat scale.

Ocelots primarily feed on small mammals (e.g., *Neotoma* spp., *Peromyscus* spp., *Lyomys* spp., *Reithrodontomys* spp., *Baiomys* spp., *Sigmodon* spp., *Agouti* spp., and *Sylvilagus* spp.), although they have also been reported to eat young white-tailed deer (*Odocoileus virginianus*), small reptiles, birds, and fish (Emmons 1988). Although bobcats consume prey as large as adult white-tailed deer (Litvaitis et al. 1986; Labisky and Boulay 1998), they feed primarily on small mammals and birds, similar in size to prey preferred by ocelots (Litvaitis 1981; Blankenship 2000; Baker et al. 2001; Luna-Soria and Lopez-Gonzalez 2005). Consequently, there is potential for dietary overlap between sympatric ocelots and bobcats.

High level of spatial, dietary, and size overlap between ocelot and bobcat makes it likely that sympatric ocelots and bobcats will show temporal niche partitioning and concentrate activity at different times of the diel. Though bobcats are commonly described as nocturnal or crepuscular, bobcat eyes are proportionally smaller than those of strictly nocturnal cats (Buie et al. 1979; McCord and Cardoza 1982; Kitchener 1991), suggesting that bobcats may be less dependent on nocturnal hunting than other felids. Studies have found bobcats are crepuscular,

with highest movement rates occurring at dusk (Marshall and Jenkins 1966; Hall and Newsom 1976; Zezulak and Schwab 1980; Zezulak 1981). Ocelots also have been described as nocturnal or crepuscular, with activity peaks occurring at dawn and dusk (Emmons 1988; Nowell and Jackson 1996; Sunquist and Sunquist 2002). However, ocelots occasionally travel during the daytime. Di Bitetti et al. (2010) recorded photographs of ocelots throughout the diel, in a camera-trap study of a neotropical felid assemblage, indicating that ocelots occasionally make movements during daylight. This pattern was in contrast to the margay (*Leopardus wiedii*), which was never photographed during daylight hours (Di Bitetti et al. 2010). Crawshaw and Quigley (1989) found ocelot activity to peak between 1700 hr and 2200 hr in Brazil.

Less studied than diel activity patterns are the activity patterns of mammalian carnivores based on lunar illumination. Cyclic changes in lunar illumination may play an important role in determining the activity patterns of mammalian carnivores such as the ocelot and bobcat as prey species have been found to vary their movement and foraging patterns based on lunar illumination (Clarke 1983; Brown et al. 1988; Daly et al. 1992; Bouskila 1995; Griffin et al. 2005). Predators will alter habitat use and movement patterns to maximize hunting success (Zielinski 1986; Zielinski 1988). Reduced activity during periods of high lunar intensity has been well documented in bats, and has been termed “lunar phobia” (Morrison 1978; Saldana-Vasquez and Munguia-Rosas 2013).

Using global positioning systems (GPS)-collar data, Rockhill et al. (2013) found movement rates greater for bobcats during crepuscular and daytime periods than at night, with nocturnal movements highest during periods of lunar illumination. The highest movement rates for bobcats occurred during late morning and from mid-afternoon through dusk. Bobcats were significantly more active on full moon nights than on new moon nights. No comparable study of

ocelot movement patterns using high-frequency GPS telemetry data has been undertaken, however, Emmons et al. (1989) found ocelots alter hunting patterns according to the amount of ambient moonlight in Peru by hunting in more densely vegetated areas during periods of high lunar illumination.

Temporal activity patterns of animals are often inferred from camera trap data (e.g., Di Bitetti et al. 2010) based upon the assumption that probability of photographing an animal during a particular time period is proportional to animal activity during that time period. Such studies have provided insight into the overall temporal activity patterns of elusive carnivore species including ocelots, however, the conclusions drawn by such studies are often based on relatively few photographic captures (e.g., Di Bitetti et al. 2010). Modern GPS collars allow the collection of high-frequency location data which can be used to collect movement data at a fine temporal scale. However, high-frequency track schedules often entail a reduction in battery life, and researchers must compromise between track schedule intensity and duration.

In contrast to GPS fixes, which entail a substantial cost in battery life, accelerometer data can be recorded at high rates with virtually no reduction in usable battery life. Accelerometers consist of cylinders containing small spheres, which record the number of times the spheres hit the edges of the cylinders during a specific time period. Accelerometers have been used for studying topics such as foraging, reproduction, activity, energy budgets and locomotion (Shepard et al. 2008; Brown et al. 2013; Zhang et al. 2015). When used in conjunction with GPS location data, accelerometers can be a powerful tool for studying animal movement and activity patterns.

The goal of this study was to investigate the effect of lunar illumination and time of day on movement and activity patterns of sympatric ocelots and bobcats using a combination of high-frequency GPS telemetry data and continuously collected accelerometer data. An additional goal

was to determine whether accelerometer data can be used to predict movement distance and velocity for ocelots and bobcats.

MATERIALS AND METHODS

Study area. — The study was conducted on the East El Sauz Ranch (EESR), a 113 km^2 privately owned ranch located near Port Mansfield, Willacy County, Texas. Climate of the EESR is semiarid and subtropical, with mean temperatures ranging from 16° C to 28° C. Mean annual precipitation is 68 cm, although droughts are common (Lonard and Judd 1985; Haines et al. 2005).

The EESR supports about 25 ocelots, which mainly occupy the oak-thornshrub vegetation community in the northwest portion of the ranch. The EESR is separated by about 8 km from the Yturria Ranch, and ocelots originally identified from the Yturria Ranch have occasionally been observed on the EESR, indicating some level of connectivity between these two populations.

Capture and telemetry — From 2013 to 2015, ocelots and bobcats were trapped with single-door 108 x 55 x 40 cm wire box traps (Tomahawk Trap Co., Tomahawk, WI). Live chickens and pigeons were used as attractants, and were maintained in separate enclosures attached to the main trap and supplied *ad libitum* with food and water. Trapped adult ocelots and bobcats were sedated with a mixture of tiletamine HCL and zolazepam (Telazol, Fort Dodge Laboratories, Fort Dodge, Iowa), at a dosage of 5 mg per kg body weight (Shindle and Tewes 2000). Animal weight was visually estimated, and the drugs administered with a pole syringe. Captures were conducted in compliance with the Texas A&M University-Kingsville Institutional

Animal Care and Use Committee protocol numbers 2012-12-20B-A2, 2012-12-20B, and 2012-12-19.

I used GPS collars manufactured by either Advanced Telemetry Systems (ATS, Insanti, MN, USA) or Lotek (Lotek Wireless, New Market, Ontario, Canada) to track ocelot and bobcat movements from 2013 to 2015. I deployed the ATS collars on ocelots and bobcats captured in 2013, programming these collars according to the following track schedule: one location each 24-hr at noon (1200 hr), and a second location at midnight (2400 hr). High-frequency monitoring with locations recorded every 30 minutes for a 72-hr period was centered around each full moon and each new moon night. Lotek GPS collars were used in 2014 and 2015. To optimize battery life, I reduced the 72-hr high-frequency monitoring to a 24-hr schedule centered on each full moon and each new moon night, but otherwise programmed the collars according to the same schedule.

Accelerometer data.— In addition to recording GPS location data, the Lotek GPS collars contained biaxial accelerometers which measured activity 4 times each second simultaneously on the horizontal (ActivityX) and vertical (ActivityY) axes throughout the entire period the animals were collared (Lotek Wireless Inc. 2013). Activity values were reported on each axis as the difference in acceleration between 2 consecutive measurements and were recorded as a value between 0 and 255. Readings for ActivityX and ActivityY were averaged and reported according to a user-specified, 5-min, time interval.

Movement data. — I partitioned GPS location data to include only fixes obtained during high-frequency track periods, and calculated distance (m) moved between consecutive locations. Analysis required the removal of the first location recorded during high-frequency monitoring as it was impossible to calculate distance traveled to these points. I censored locations with a

horizontal dilution of precision (HDOP) >5 to avoid including unreliable location estimates (Dussault et al. 2001). I removed locations for which travel distance could not be collected due to the previous location being censored. I calculated average velocity traveled (m/hr) by dividing the total distance moved between two consecutive points by the total time lag in hours between those points.

Defining time periods — I used the R package "oce" (Kelley and Richards 2015) to calculate solar altitude, moon altitude, and moon illuminated fraction for each date and time combination in the GPS location and activity datasets. Solar altitude and moon altitude were reported as degrees above (positive) or below (negative) the horizon. Moon illuminated fraction reported the proportion of the moon that was illuminated, and ranged from 0 to 1.

Solar altitude was reclassified into the following categorical periods: Night, Day, and Crepuscular. I defined Night as those time periods during which solar altitude was >13.5° below the horizon (i.e., solar altitude < -13.5°). Day was defined as those time periods during which solar altitude was >13.5° above the horizon (i.e., solar altitude >13.5°). I defined Crepuscular as those time periods during which solar angle was between 13.5° below the horizon and 13.5° above the horizon (i.e., -13.5° ≤ solar altitude ≤ 13.5°). These values were selected as the threshold for defining the crepuscular period as they roughly corresponded to 1 hour before and after the official sunrise and sunset times. I believed solar angle provided a more ecologically meaningful estimate of diel periods than local time because sunrise and sunset times, as well as twilight duration, vary through the year.

For the accelerometer dataset, I reclassified moon illuminated percent into the following categories: High, Mid and Low. The High category was defined as those periods during which lunar illuminated fraction was >50%. I defined Mid as those periods during which moon

illuminated percent was between 10% and 50% (i.e., 10% ≤ moon illuminated percent ≤ 50%). The Low category was defined as those time periods during which moon illuminated percent was <10%. For the GPS location dataset, high-frequency locations were collected only during full moon and new moon. Therefore, moon phase was categorized as either Full or New for this dataset.

For the activity dataset, I combined periods based on solar altitude with those based on moon phase into 9 groupings (i.e., Low Moon Day, Low Moon Night, Low Moon Crepuscular, Mid Moon Day, Mid Moon Night, Mid Moon Crepuscular, High Moon Day, High Moon Night, and High Moon Crepuscular). I termed this new categorical variable Lunar-Diel. For the GPS location dataset, I used a similar classification scheme, but separated Lunar-Diel into 6 categories (i.e., New Moon Day, New Moon Night, New Moon Crepuscular, Full Moon Day, Full Moon Night, Full Moon Crepuscular).

Data analysis. — I calculated a Pearson's correlation coefficient between ActivityX and ActivityY to determine whether these variables could be treated as independent. I used principal component analysis to decompose the two activity values into a single activity index. I added a constant term to the activity index to remove negative values and performed a log transformation. I termed the resulting variable Activity Index and used it as the measure of accelerometer-based activity readings for subsequent analyses.

Animal movement and activity values collected at high frequencies provide useful information about animal behavior, yet these measures often contain substantial autocorrelation which can violate the assumptions of traditional hypothesis testing (Dray et al. 2010). Autocorrelation is a property of random variables such that values calculated from samples taken near each other, either in time or space, tend to be either more or less similar, for positive or

negative autocorrelation respectively, than expected under an independent arrangement (Dray et al. 2010). Although autocorrelation is an intrinsic component of ecological data, it is often treated as a nuisance because it violates assumptions of independence, thus complicating statistical hypothesis testing (Legendre 1993). To avoid problems associated with autocorrelation, Turchin (1998) recommended subsampling highly autocorrelated datasets prior to statistical analysis. Such a subsampling approach effectively eliminates the problem of autocorrelation, yet can result in a loss of valuable data, potentially obscuring ecologically meaningful processes (Dray et al. 2010).

To investigate autocorrelation in the datasets I applied an autocorrelation function (ACF) to the Activity Index dataset and the velocity dataset. The ACF is a tool for the analysis of time series data, which plots autocorrelation values measured for different time lags against these corresponding time lags (Diggle 1990). I used the function "acf" in program R (R version 3.2.3 www.r-project.org, accessed 10 Dec 2015) to compute and plot estimates of the autocorrelation function for Activity Index and velocity datasets.

Studies of animal activity patterns often use movement distance between consecutive GPS fixes as a measure of activity (e.g., Rockhill et al. 2013). Accelerometers provide a different measure of activity by recording high values during times of rapid acceleration that are not always the result of travel. Time periods during which the animal moves great distances are likely to have high corresponding activity values, yet not all time periods showing high accelerometer-derived activity values will be indicative of long-distance travel. I used two techniques to test whether accelerometer values could be used to predict movement velocity. The first technique used linear regression with velocity as a continuous variable, whereas the second

technique used the machine learning algorithm, random forests (Breiman 2001), and treated velocity as a categorical variable.

Accelerometer data were reported at 5-min intervals and GPS fixes were obtained at 30-min intervals, thus there were 6 activity readings corresponding to each step between 2 consecutive GPS locations. For each 30-minute time step, I averaged the 6 preceding Activity Index values obtained from the activity dataset to obtain the variable "Mean Activity Index" for each GPS relocation.

I performed a simple linear regression using velocity as the dependent variable and Mean Activity Index, individual, and interaction between individual and Activity Index as the independent variables. Individual and the interactive term were included as random effects.

Using the Random Forests machine learning algorithm (Breiman 2001) required movement velocity to be categorized into discrete, non-overlapping classes. This involved delineating thresholds in movement velocity. Thresholds for classifying movement velocity into discrete categories were based upon ecological considerations and the need to optimize prediction accuracy. Preliminary analysis of velocity data revealed 3 categories of ocelot and bobcat movement velocity. The movement velocity category Low consisted of periods of inactivity or low activity, during which the individual was stationary or moved short distances. The movement velocity category Mid consisted of periods of intermediate activity during which the individual was actively traveling throughout its range. The movement velocity category High consisted of infrequent periods of rapid travel. This category, by design, was the most infrequently observed movement type, yet it was included to determine if accelerometer data could accurately predict periods of rapid travel for ocelots and bobcats.

The thresholds were varied from 100 m/hr to 500 m/hr for the Low category, and from 600 m/hr to 1,000 m/hr for the High category, with the Mid category representing the intermediate values. Prediction accuracy was tested for each of these classification schemes, before settling on the following classification which optimized prediction accuracy: Low (velocity < 300 m/hr), Mid (300 m/hr ≤ velocity ≤ 900 m/hr.), and High (velocity > 900 m/hr). I used the Random Forests algorithm in the R package "randomForest" (Liaw and Wiener 2002) to predict movement velocity based upon the preceding 6 ActivityX and ActivityY values recorded in the 30 minutes prior to each GPS fix. Individual identity was also included as a predictor variable. Random Forests is a relatively new machine learning approach that works well for complex ecological data that are not easily fitted with linear methods (Cutler et al. 2007). I implemented the algorithm using 5,000 trees, with a maximum of 6 variables allowed at each split and recorded the out-of-bag classification error estimate for each of the 3 velocity categories. The out-of-bag classification error provides a measure of prediction error for machine learning models that use bootstrap aggregating by reporting the mean prediction error on each training sample.

To account for the temporal autocorrelation in this dataset I used a generalized least squares model in the R package "nlme" (Pinheiro et al. 2016), including a first-order autoregressive pattern as the correlation structure of the model. Predictor variables included in the model were species, Lunar-Diel, and an interaction term. I performed identical analyses for the accelerometer-derived Activity Index data and for the location-derived velocity data. To determine if the model accounted for the observed autocorrelation in the dataset I applied an ACF to the normalized residuals from the model and compared this with the ACF of the original Activity Index values.

I conducted a series of Tukey pairwise comparisons between relevant combinations of species, diel period, and moon phase. I expected to find similar patterns with the accelerometer-derived Activity Index data and the location-derived velocity data. Specific hypotheses tested were:

1) Bobcats will be more active during the day than ocelots.

2) Bobcats will be more active during the crepuscular period than ocelots.

3) Ocelots will be more active at night than bobcats.

4) Ocelots will be more active at night than at any other period

5) Ocelots will be more active on nights with low lunar illumination (i.e., new moon nights).

6) Bobcats will be more active on nights with high lunar illumination (i.e., full moon nights).

The overall hypothesis was that ambient light, either from the sun or the moon, was the primary basis for ocelot-bobcat temporal niche partitioning, with ocelots specializing in hunting during periods of low light, and bobcats specializing on periods of intermediate light levels. I expected both felids to show lower activity during the day.

RESULTS

Capture and Telemetry. — In 2013, I captured and collared 1 male bobcat (EB16M) and 1 female bobcat (EB15F) bobcat with ATS GPS collars programmed to obtain location fixes every 30 minutes for a 72-hr period centered around each full moon and each new moon. Between 2014 and 2015, I captured and collared 2 male ocelots (E6M, Y12M), 2 female ocelots (E10F, E12F), and 1 male bobcat (EB8M) with Lotek minitrack GPS collars programmed to

obtain location fixes every 30 minutes for a 24-hr period centered around each full moon night and each new moon night.

Between 2013 and 2015, I obtained 5,080 high frequency GPS fixes (1,416 ocelot; 3,664 bobcat), 1,193 low frequency GPS fixes (495 ocelot; 698 bobcat), and 259,525 accelerometer-based activity readings (217,709 ocelot; 41,816 bobcat). The ATS collars placed on bobcats EB15F and EB16M in 2013 did not record continuous activity data, in contrast to the Lotek collars (Table 2.1). Therefore, these individuals were excluded from analyses involving accelerometer-derived activity data but were included in analyses of GPS-derived velocity data.

Summary movement velocity data — Across all individuals, movement velocity (m/hr) ranged from 0.53 to 1,715.00 (mean = 215.7, SD = 299.7). For individual ocelots, velocity values (mean $\pm$ SD) were 238.63 $\pm$ 294.98 for E10F, 114.88 $\pm$ 173.33 for E12F, 337.65 $\pm$ 387.06 for E6M, and 241.07 $\pm$ 330.76 for Y12M. For individual bobcats, velocity values (mean $\pm$ SD) were 166.95 $\pm$ 245.91 for EB8M, 164.15 $\pm$ 245.30 for EB15F, 189.14 $\pm$ 631.44 for EB16M. Mean velocity was observed to vary by individual ($P < 2.2 \times 10^{-16}$).

Ocelot E10F showed strong nocturnal movement patterns, with the highest average velocity occurring at 2100 hr on new moon nights, and little movement occurring from 0800 hr to 1700 hr (Fig. 2.1). Ocelot E12F showed high movement rates between 2000 hr and 2300 hr during full moon nights, but also showed an unexpected peak in movement at 1300 hr (Fig. 2.2). Ocelot E6M showed strong nocturnal movement patterns, with little movement occurring between 0800 hr and 1600 hr, and high movement rates from 1900 hr to 0600 hr (Fig. 2.3). Ocelot Y12M did not show as clear a nocturnal pattern as ocelots E10F and E6M, with low movement rates only between 1200 hr and 1600 hr, and relatively high rates throughout the remaining diel (Fig. 2.4). Bobcat EB8M showed crepuscular movement patterns, with

Table 2.1. — Location and activity data collected on ocelots and bobcats. Start date indicates the date on which the collar was deployed; end date indicates the date of the last successful GPS location or activity value; low-frequency GPS provides the cumulative low-frequency GPS locations (>12 hr interval) collected for that individual; high-frequency GPS provides the cumulative high-frequency GPS locations (i.e., 30-min interval) collected for that individual; activity readings shows the number of accelerometer-based activity observations collected for that individual.

ID	Species	Sex	Collar Brand	Start Date	End Date	Low-Frequency GPS	High-Frequency GPS	Activity Readings
EB15F	Bobcat	F	ATS	4/27/13	10/20/13	265	1,738	0
EB16M	Bobcat	M	ATS	5/8/13	10/16/13	286	1,590	0
E10F	Ocelot	F	Lotek	2/28/14	7/25/14	148	365	41,784
E12F	Ocelot	F	Lotek	3/20/15	9/25/15	98	338	53,748
E6M	Ocelot	M	Lotek	4/20/15	1/28/16	96	235	80,452
Y12M	Ocelot	M	Lotek	3/2/14	7/27/14	153	478	41,725
EB8M	Bobcat	M	Lotek	3/4/14	8/15/15	147	336	41,816

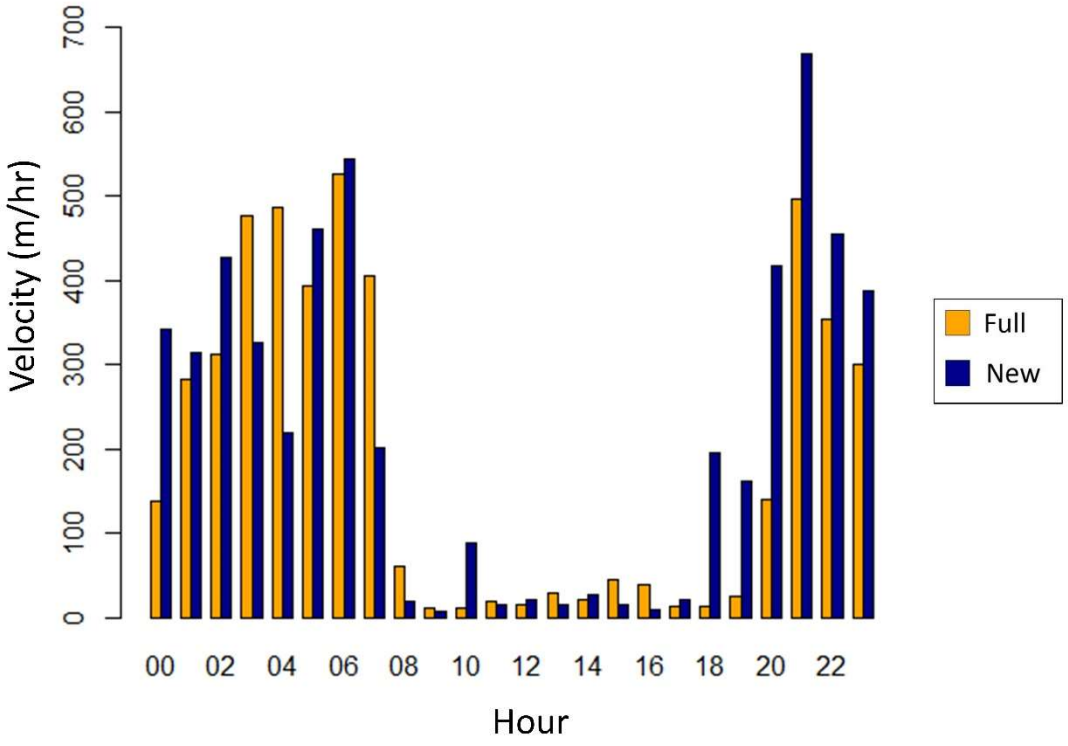

Figure 2.1. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for ocelot E10F. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

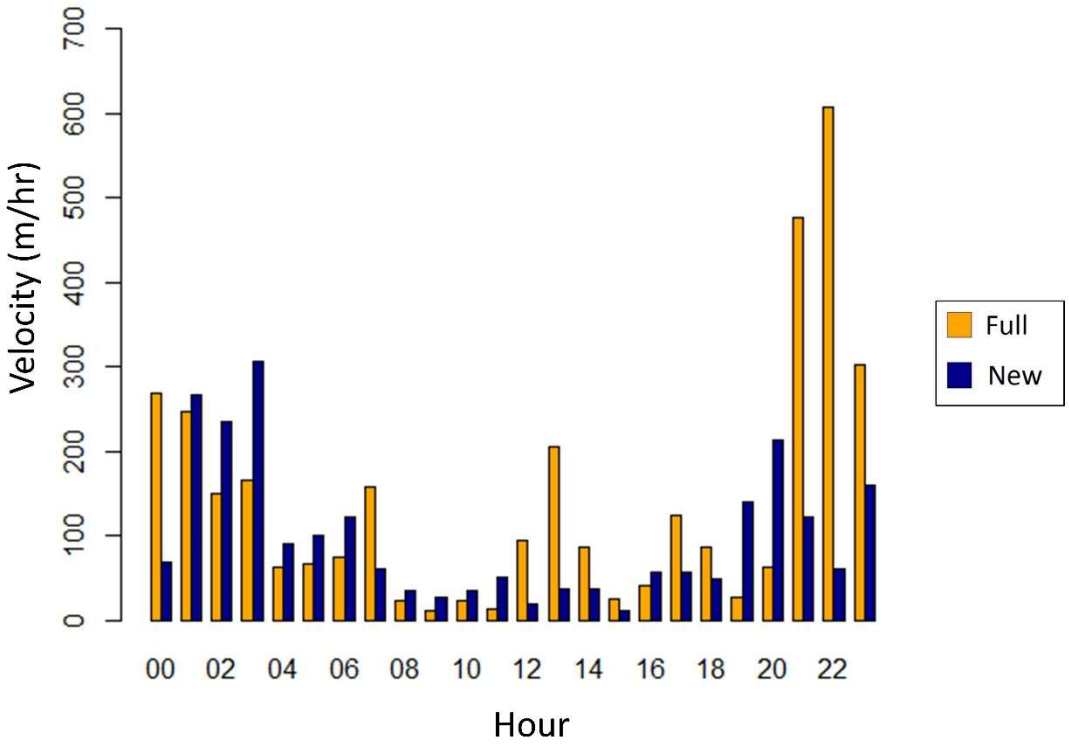

Figure 2.2. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for ocelot E12F. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

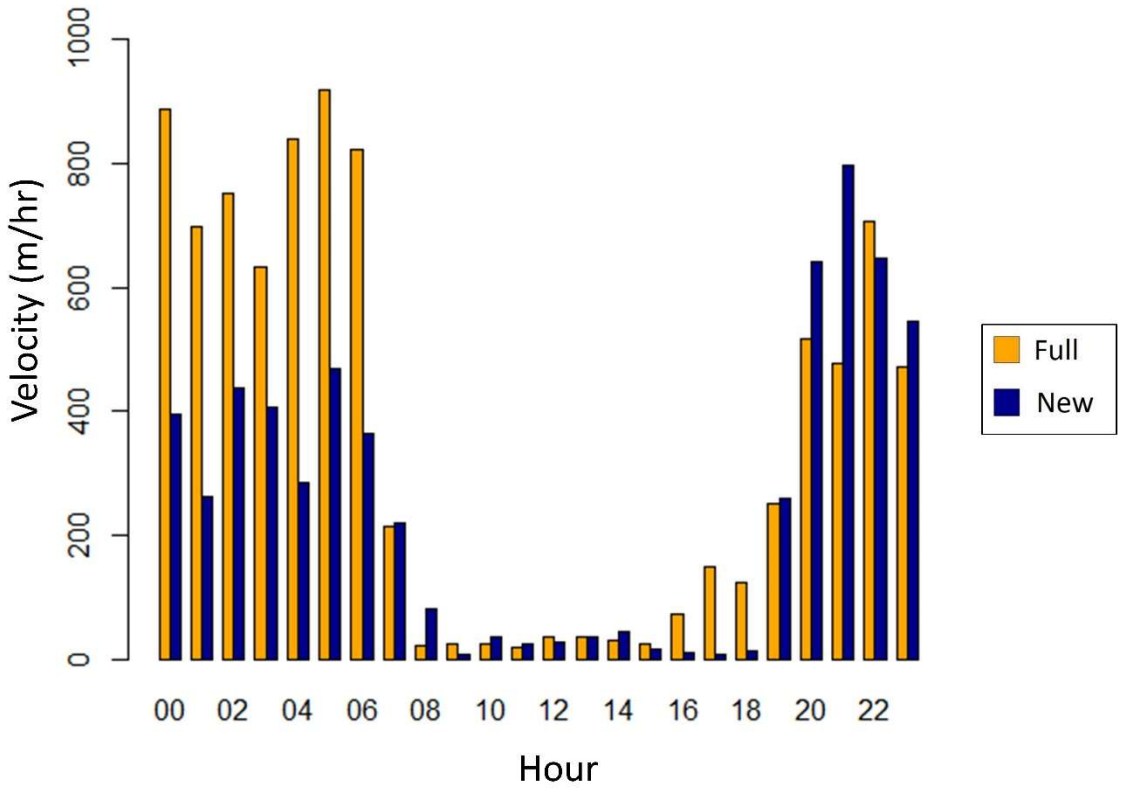

Figure 2.3. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for ocelot E6M. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

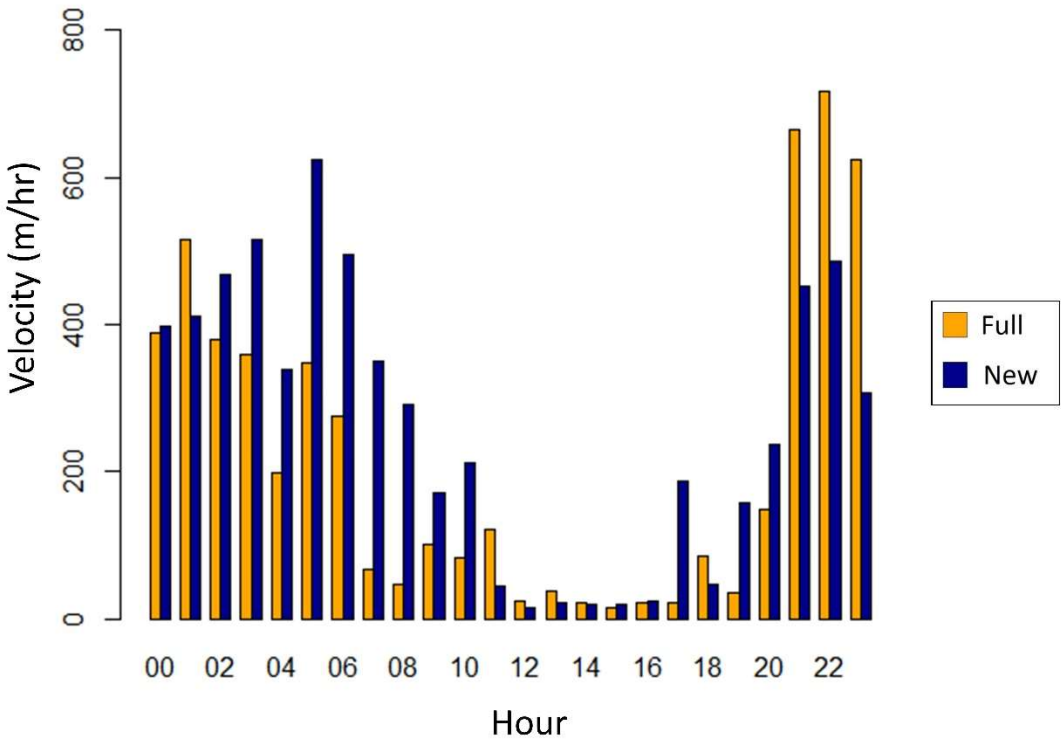

Figure 2.4. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for ocelot Y12M. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

movement peaks between 0500 hr and 1000 hr and between 1700 hr and 2100 hr, and activity during full moon periods higher than during new moon periods (Fig. 2.5). Bobcat EB15F showed a pronounced peak in movement around midnight (2400 hr), and reduced movement rates from 1000 hr to 1500 hr (Fig. 2.6). Bobcat EB16M showed a nocturnal movement pattern similar to those of ocelots E10F and E6M, with low movement rates from 0600 hr to 1800 hr and relatively high movement times throughout the night (Fig 2.7).

Summary activity data. —For all individuals, Activity Index ranged from -1.32 to 5.88 (mean = 1.76, SD = 2.74). Individual Activity Index values (mean $\pm$ SD) were 1.62 $\pm$ 2.72 for E10F, 1.81 $\pm$ 2.65 for E12F, 1.78 $\pm$ 2.83 for E6M, 1.54 $\pm$ 2.73 for EB8M, and 2.03 $\pm$ 2.72 for Y12M. Overall activity patterns by moon phase and time of day were similar when using Activity Index or movement velocity to characterize activity. The strong crepuscular pattern observed in bobcat EB8M was consistent with Activity Index data (Fig. 2.12). However, ocelot E12F (Fig. 2.9) showed a diel activity pattern much more similar to that displayed by ocelots E10F (Fig. 2.8), E6M (Fig. 2.10), and Y12M (Fig. 2.11) when examining Activity Index data rather than velocity data. The unusual activity pattern seen with the E12F velocity dataset (Fig 2.2) may be an artifact of too few location points recorded for that individual.

Values for ActivityX and ActivityY ranged from 0 to 255. The first principal component accounted for 98.3% of the variation in the 2 activity values and was, therefore, retained. Boxplots showed the activity data strongly skewed to the right (Fig. 2.13) primarily because of the large number of records with activity values of zero. Although removal of these records resulted in a more normal distribution, records with activity values of zero represented ecologically meaningful information, likely corresponding to periods of inactivity (e.g., Zhang et

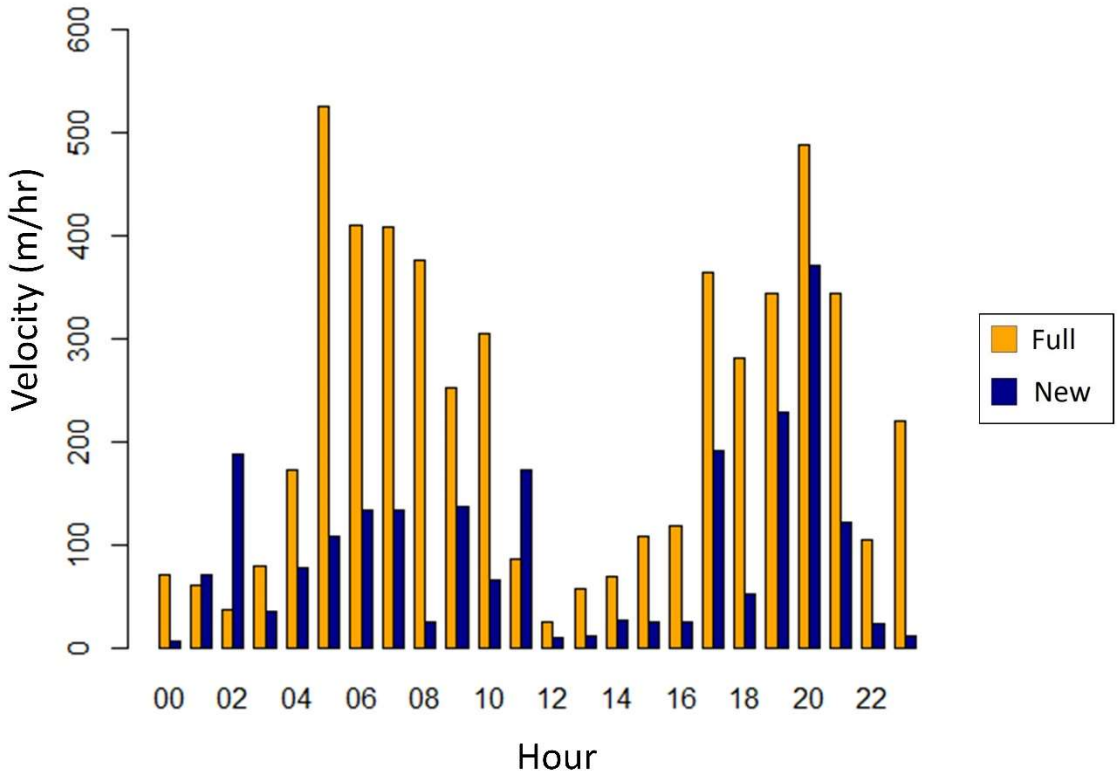

Figure 2.5. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for bobcat EB8M. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

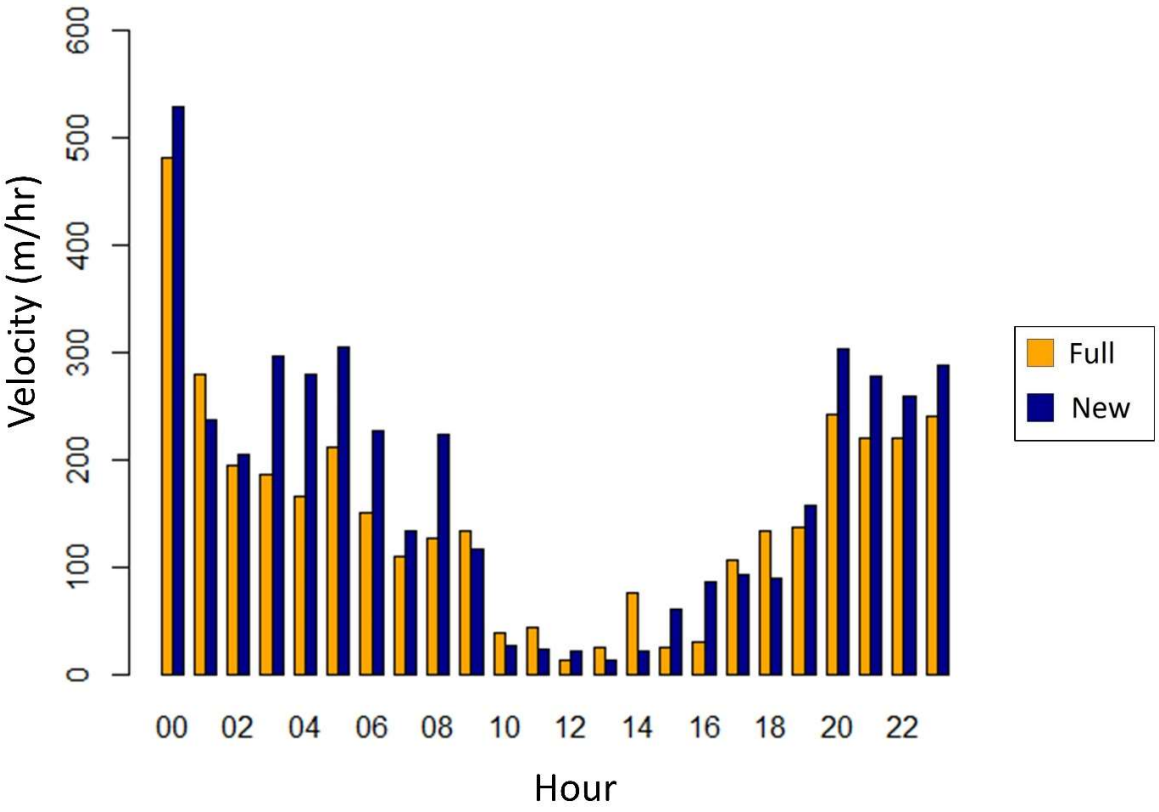

Figure 2.6. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for bobcat EB15F. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

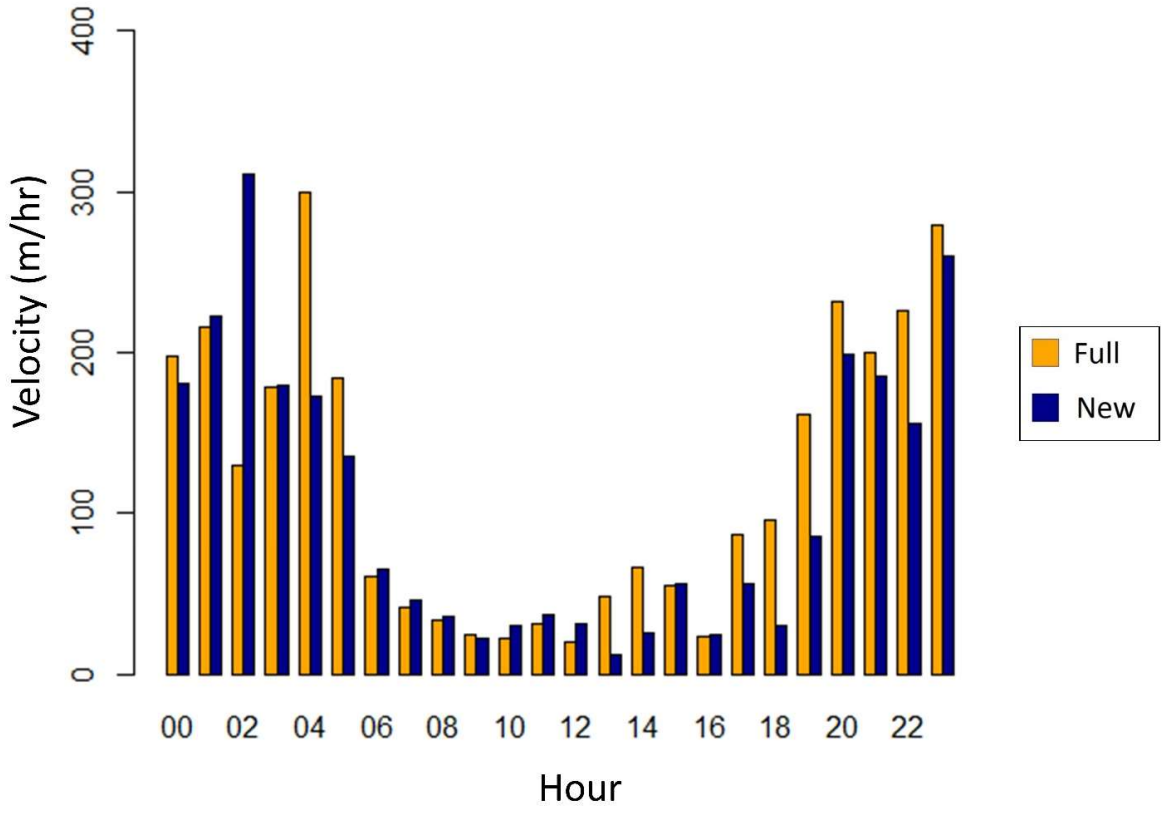

Figure 2.7. — Global positioning systems (GPS)-derived velocity values were averaged by hour and moon phase for bobcat EB16M. During each 30-min interval between high-frequency GPS points, travel distance was calculated in meters and velocity was determined by dividing travel distance (m) by time period (hr).

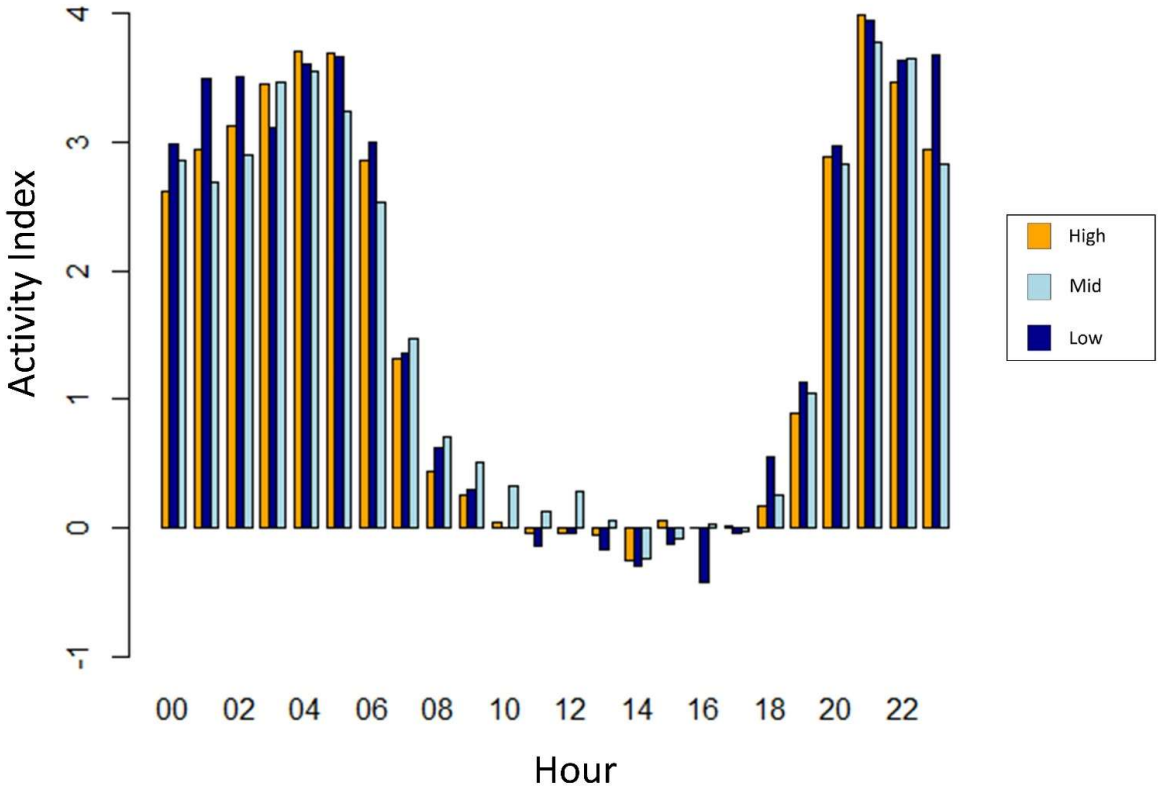

Figure 2.8. — Activity Index value averaged by hour and moon phase for ocelot E10F. Activity Index was derived from accelerometers inside GPS collars and was recorded continuously and grouped at 5-min intervals throughout the study period. Moon phase was separated into High for high lunar illumination, Mid for intermediate levels, and Low for low lunar illumination.

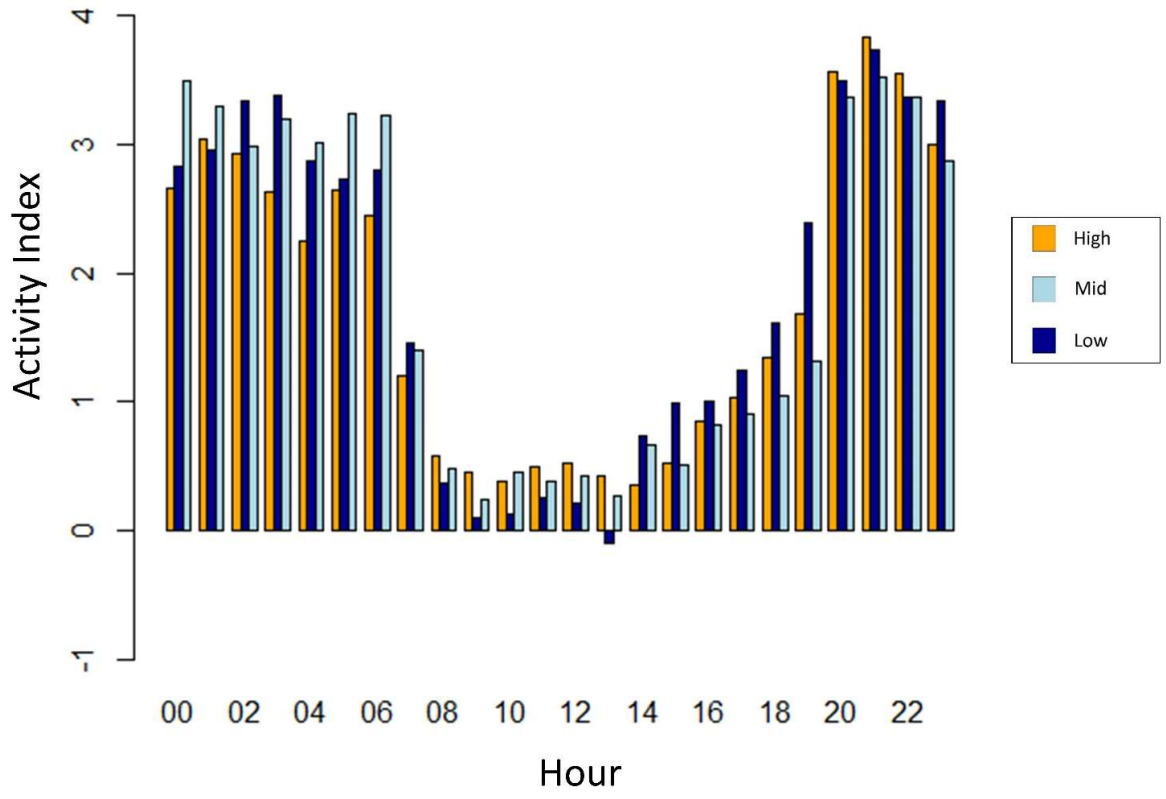

Figure 2.9. — Activity Index value averaged by hour and moon phase for ocelot E12F. Activity Index was derived from accelerometers inside GPS collars and was recorded continuously and grouped at 5-min intervals throughout the study period. Moon phase was separated into High for high lunar illumination, Mid for intermediate levels, and Low for low lunar illumination.

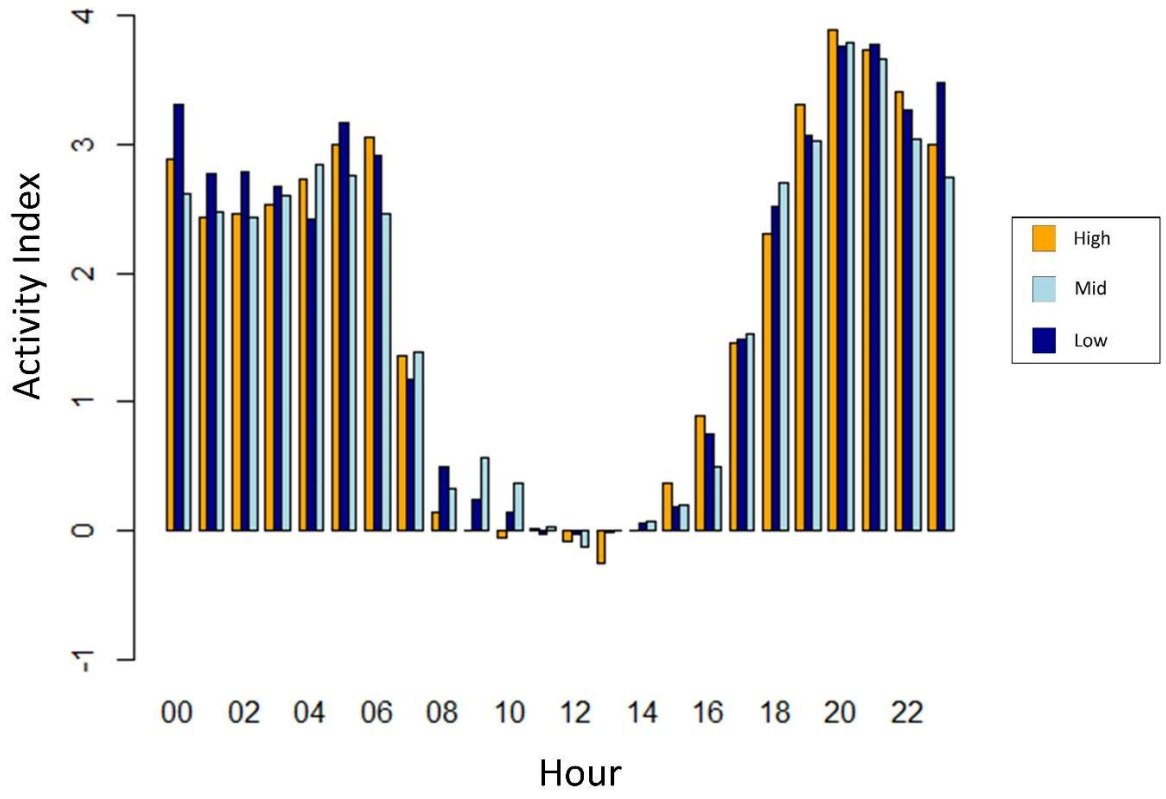

Figure 2.10. — Activity Index value averaged by hour and moon phase for ocelot E6M. Activity Index was derived from accelerometers inside GPS collars and was recorded continuously and grouped at 5-min intervals throughout the study period. Moon phase was separated into High for high lunar illumination, Mid for intermediate levels, and Low for low lunar illumination.

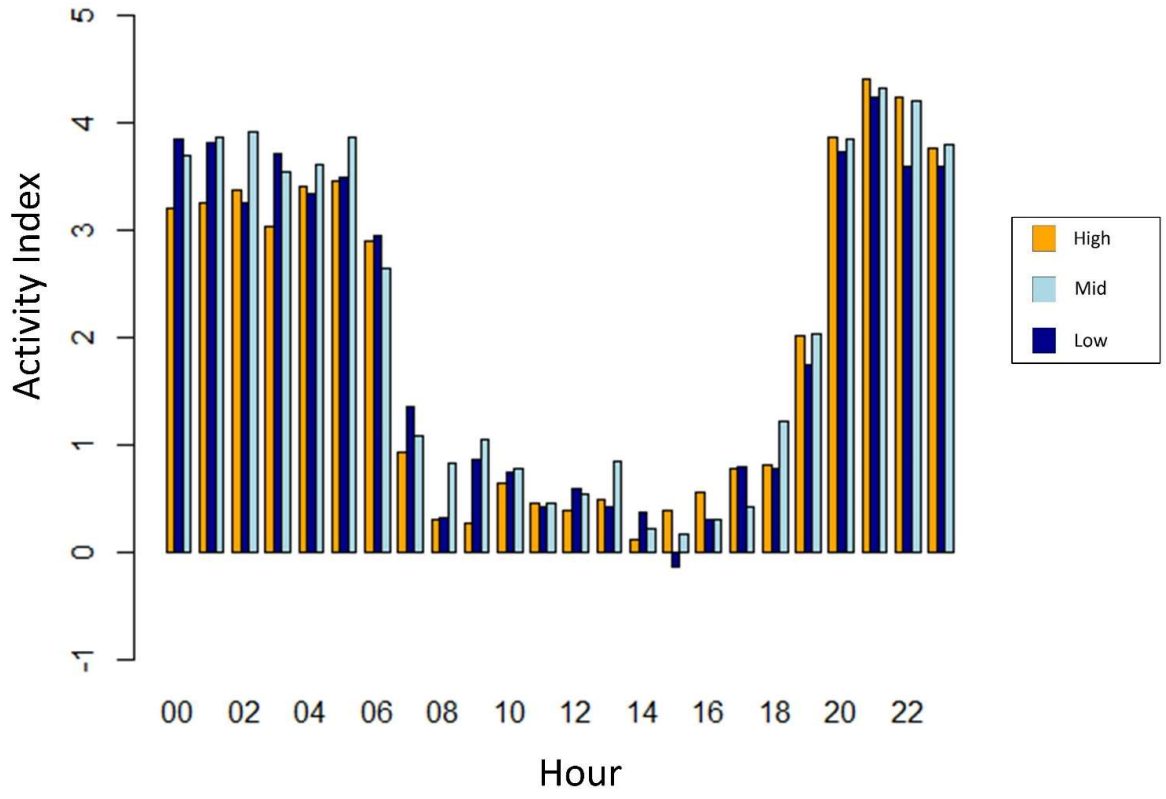

Figure 2.11. — Activity Index value averaged by hour and moon phase for ocelot Y12M. Activity Index was derived from accelerometers inside GPS collars and was recorded continuously and grouped at 5-min intervals throughout the study period. Moon phase was separated into High for high lunar illumination, Mid for intermediate levels, and Low for low lunar illumination.

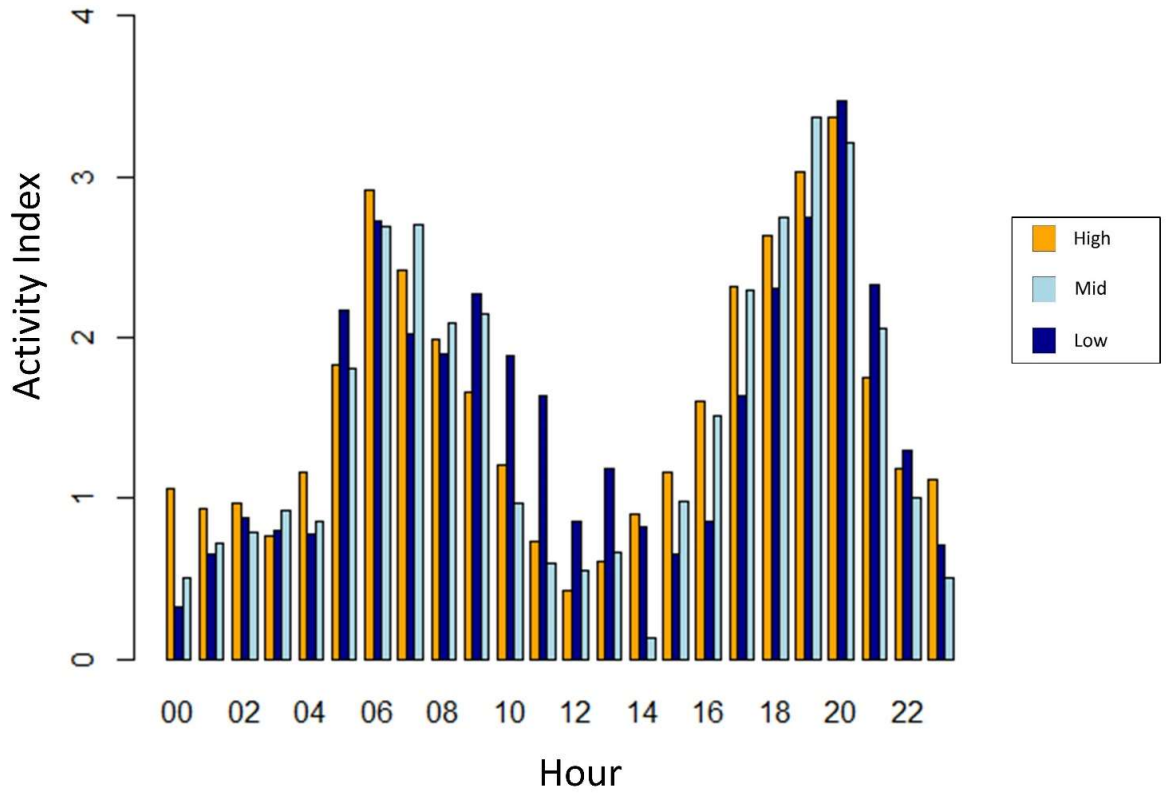

Figure 2.12. — Activity Index value averaged by hour and moon phase for bobcat EB8M. Activity Index was derived from accelerometers inside GPS collars and was recorded continuously and grouped at 5-min intervals throughout the study period. Moon phase was separated into High for high lunar illumination, Mid for intermediate levels, and Low for low lunar illumination.

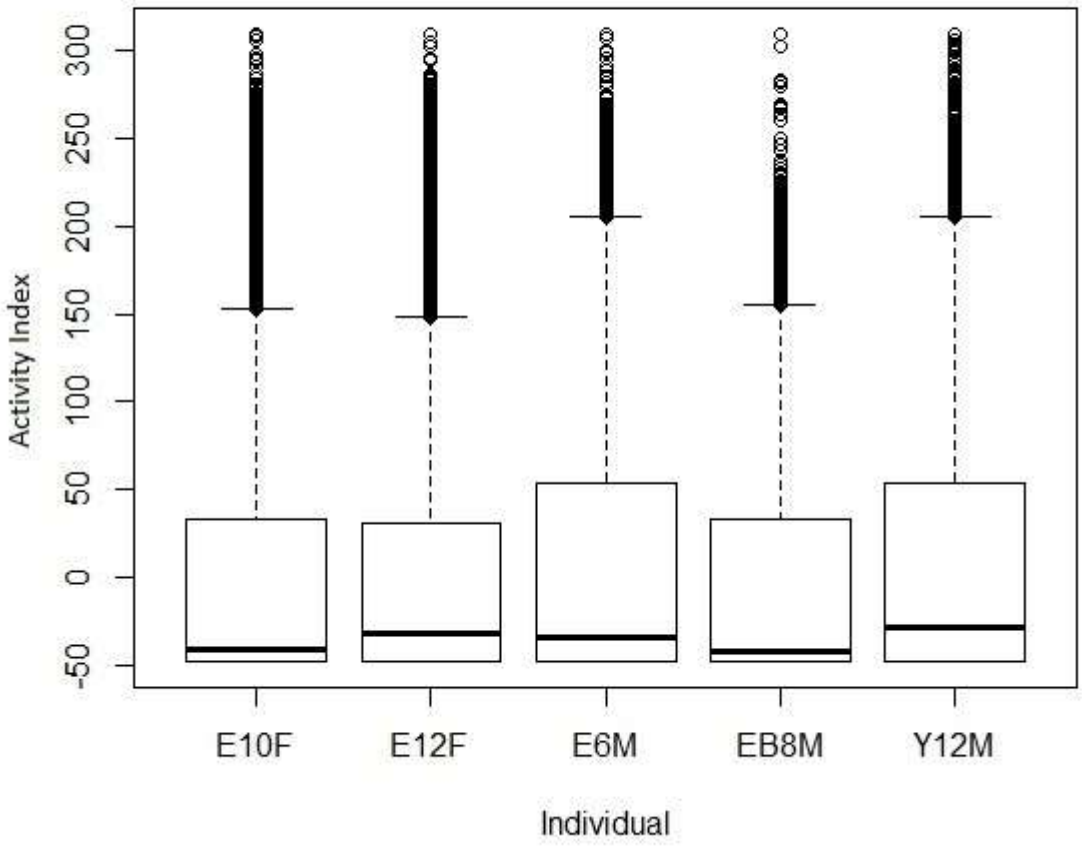

Figure 2.13. — Boxplot of Activity Index values for felids captured from 2013 to 2015 on the East El Sauz Ranch, Willacy County, Texas, prior to log-transformation. Non-log transformed Activity Index values showed a strongly skewed structure necessitating log transformation.

al. 2015), and were, therefore, included in the dataset. Log transformation of the Activity Index produced boxplots that showed an approximately normal distribution, sufficient for parametric analysis (Fig. 2.14). ActivityX and ActivityY showed strong positive correlation (corr = 0.962) and this relationship did not vary by individual (Fig. 2.15).

Predicting movement velocity from accelerometer data. —The linear regression between movement velocity (m/hr) and mean Activity Index for the preceding 6 activity periods resulted in an R-squared of 0.6008 and an adjusted R-squared of 0.5987. Both Activity Index ($P < 2.2 \times 10^{-16}$) and individual ($P < 2.2 \times 10^{-16}$) had a significant effect. Each individual showed a positive relationship between Activity Index and movement velocity, although the slope of the least squares regression line differed for each individual (Fig. 2.16).

For the Random Forest analysis, the overall out-of-bag estimate of error rate was 15.3%. High velocity values were misclassified 20% of the time, Mid values were misclassified 29.9% of the time, and Low values were misclassified 0.8% of the time.

Model fitting. — For the Activity Index dataset, autocorrelation values were highest at a time lag of 1, decreasing with each subsequent time lag in a pattern analogous to exponential decay, indicating that a first-order autoregressive process would likely be the best fit (Fig. 2.17). I found a similar pattern with the movement velocity dataset, although in this dataset a time lag of 1 corresponded to a 30-min time lag rather than a 5-min time lag (Fig. 2.18). I obtained a Durbin-Watson test statistic of 0.311 for the Activity Index values. A Durbin-Watson test statistic <2 is generally considered to be indicative of positive serial correlation, so the value calculated for the Activity Index data strongly suggests autocorrelation. The ACF plot for velocity showed substantially less autocorrelation than the ACF plot for activity, with a correlation of 0.214 between consecutive observations (30-min time lag), that declined to

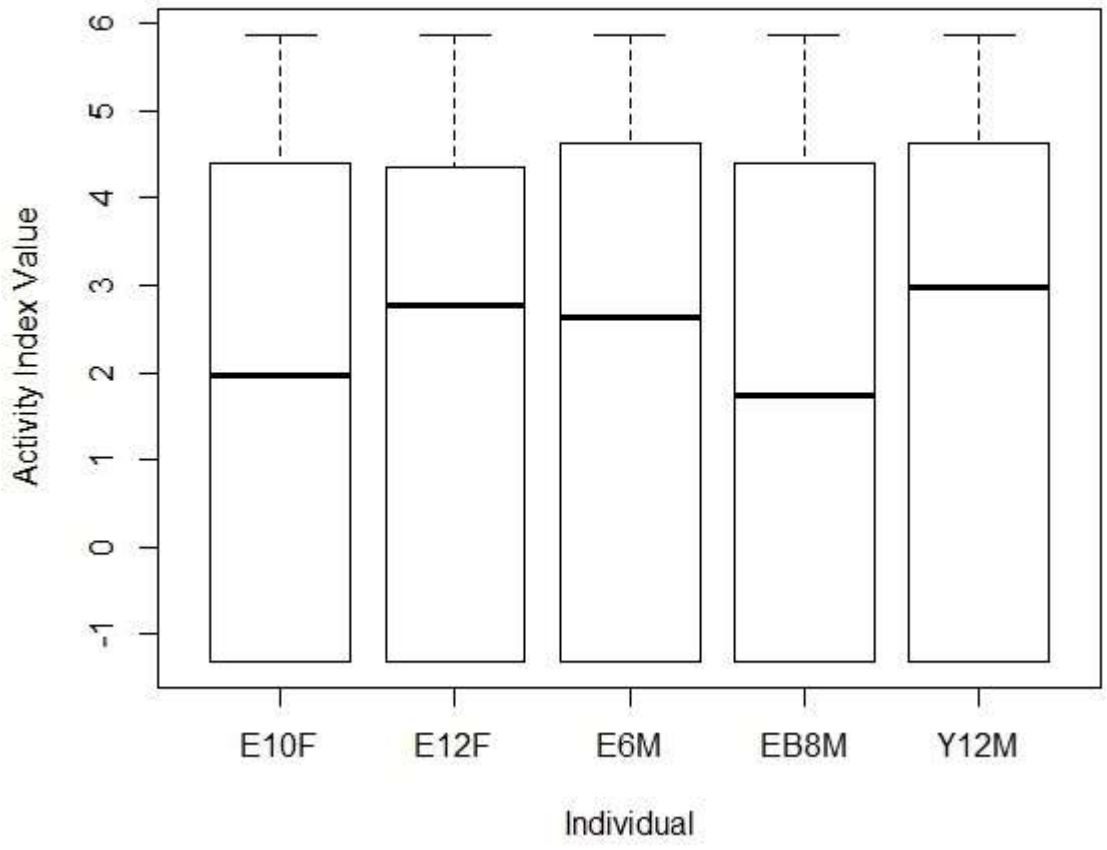

Figure 2.14. — Boxplot of Activity Index values for felids captured from 2013 to 2015 on the East El Sauz Ranch, Willacy County, Texas, after log-transformation.

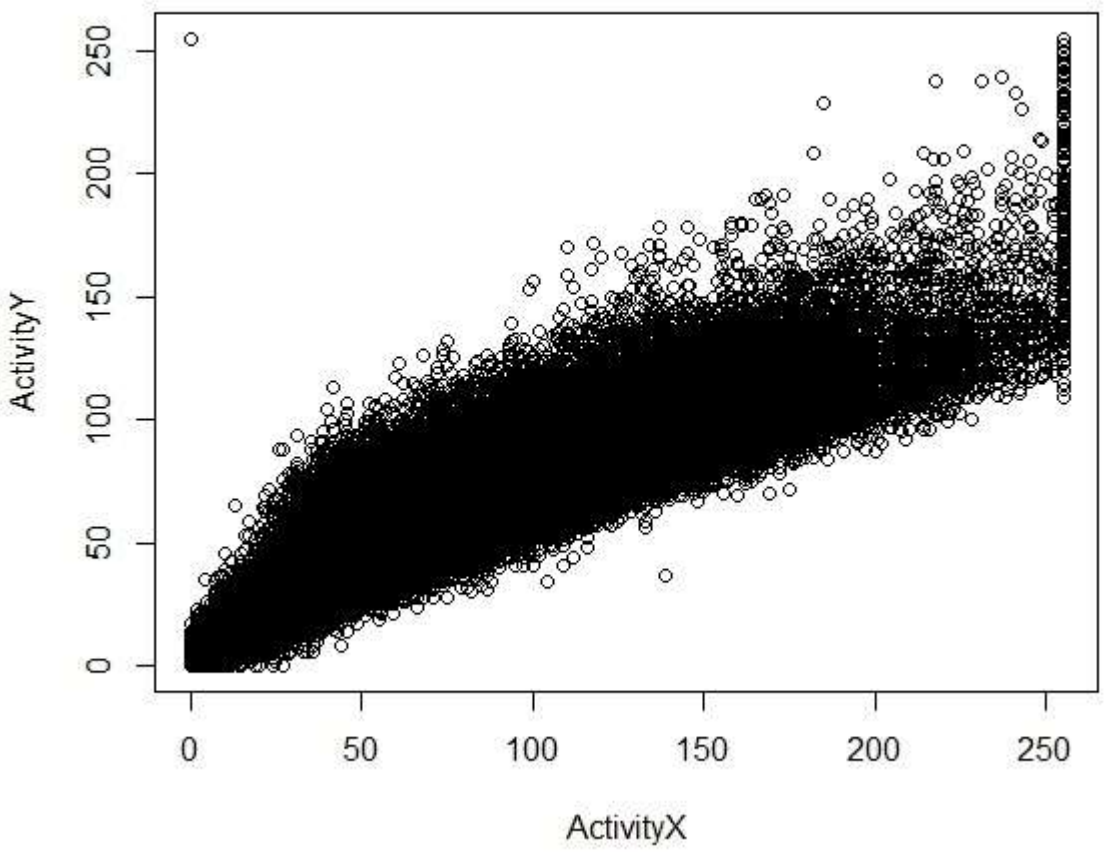

Figure 2.15. — Scatterplot of non-transformed ActivityX and ActivityY accelerometer values showing the strong positive correlation between these values. Data represents ocelots and bobcats collared with Lotek GPS collars on the East El Sauz Ranch from 2013 to 2015.

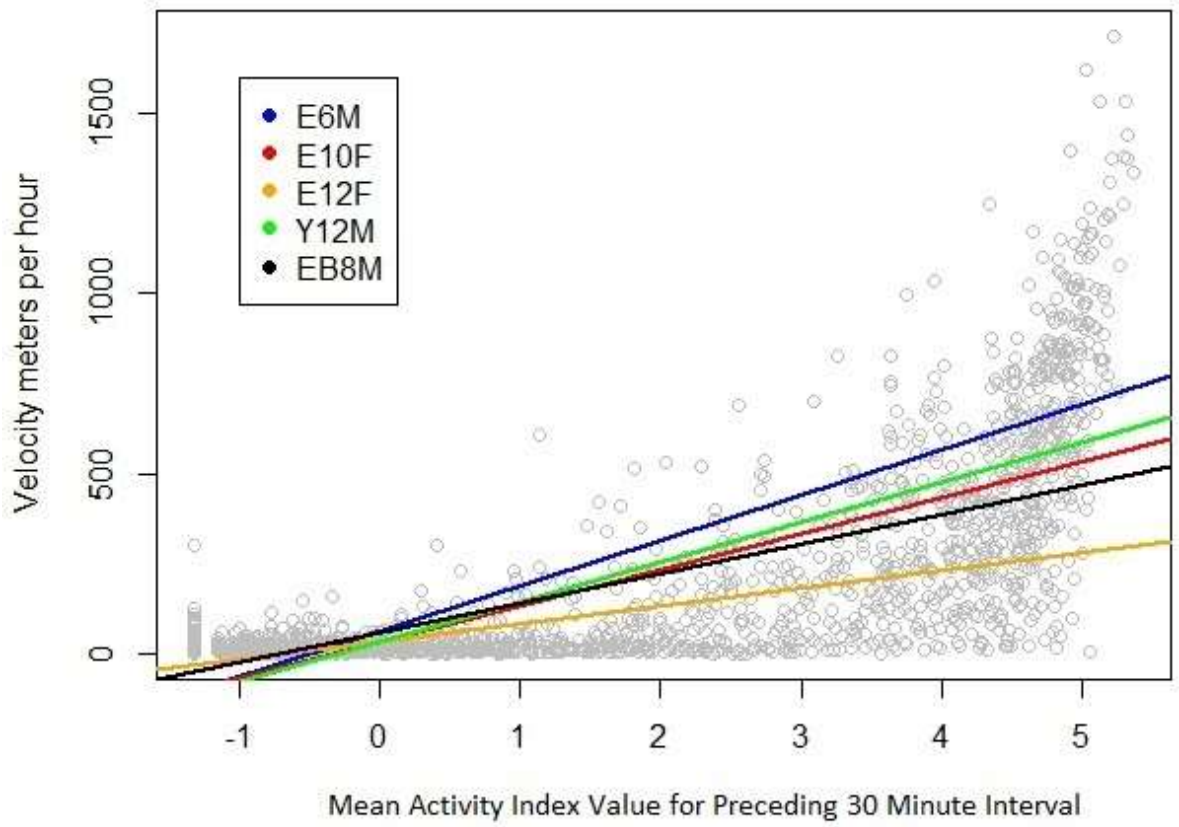

Figure 2.16. — Scatterplot of Velocity (m/hr) and Mean Activity Index Value for preceding 30-min interval with least-squares regression lines drawn separately for each felid. Felids were captured on the East El Sauz Ranch from 2013 to 2015 and collared with Lotek GPS collars. Inferences and *P* values are approximate given the heteroscedasticity.

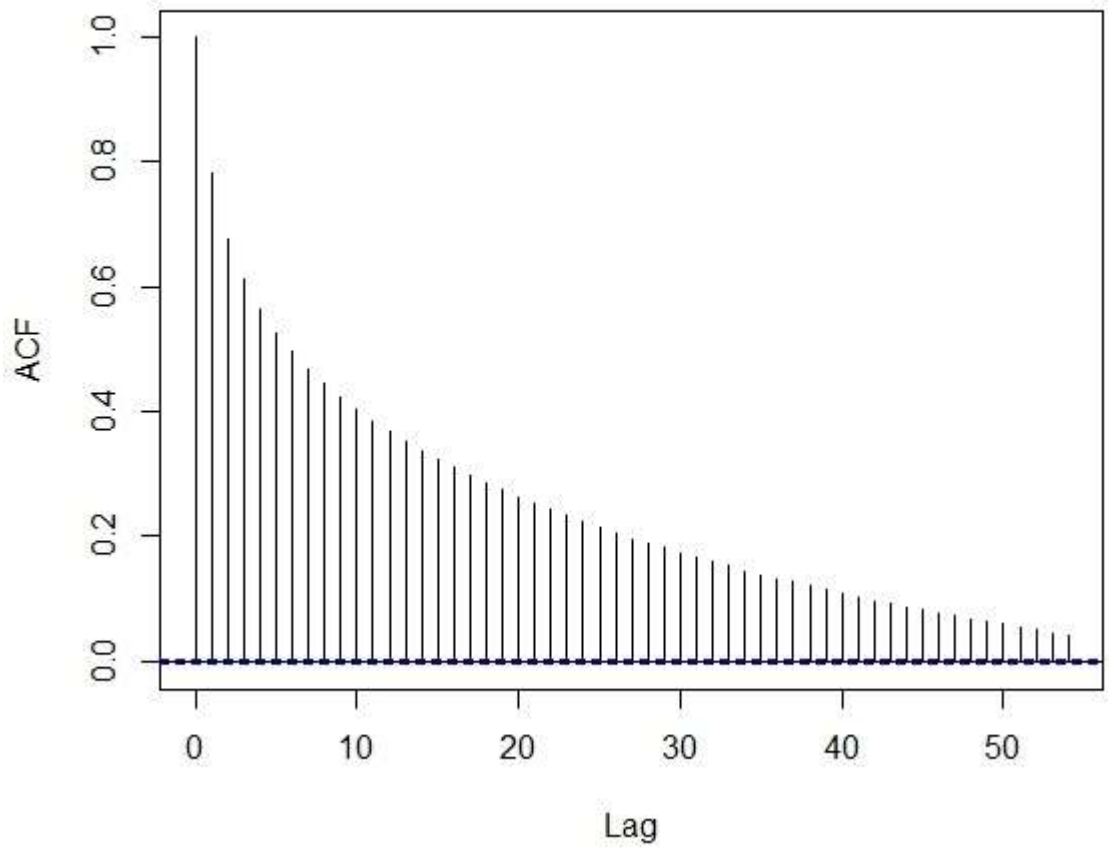

Figure 2.17. — Results of autocorrelation function (ACF) on log-transformed Activity Index for ocelots and bobcats collared with Lotek GPS collars on the East El Sauz Ranch between 2013 and 2015. The autocorrelation function estimates correlation between observations separated by different time lags. This plot shows strong autocorrelative structure between subsequent Activity Index values. A lag value of 1 represents a 5-min time lag for the Activity Index dataset.

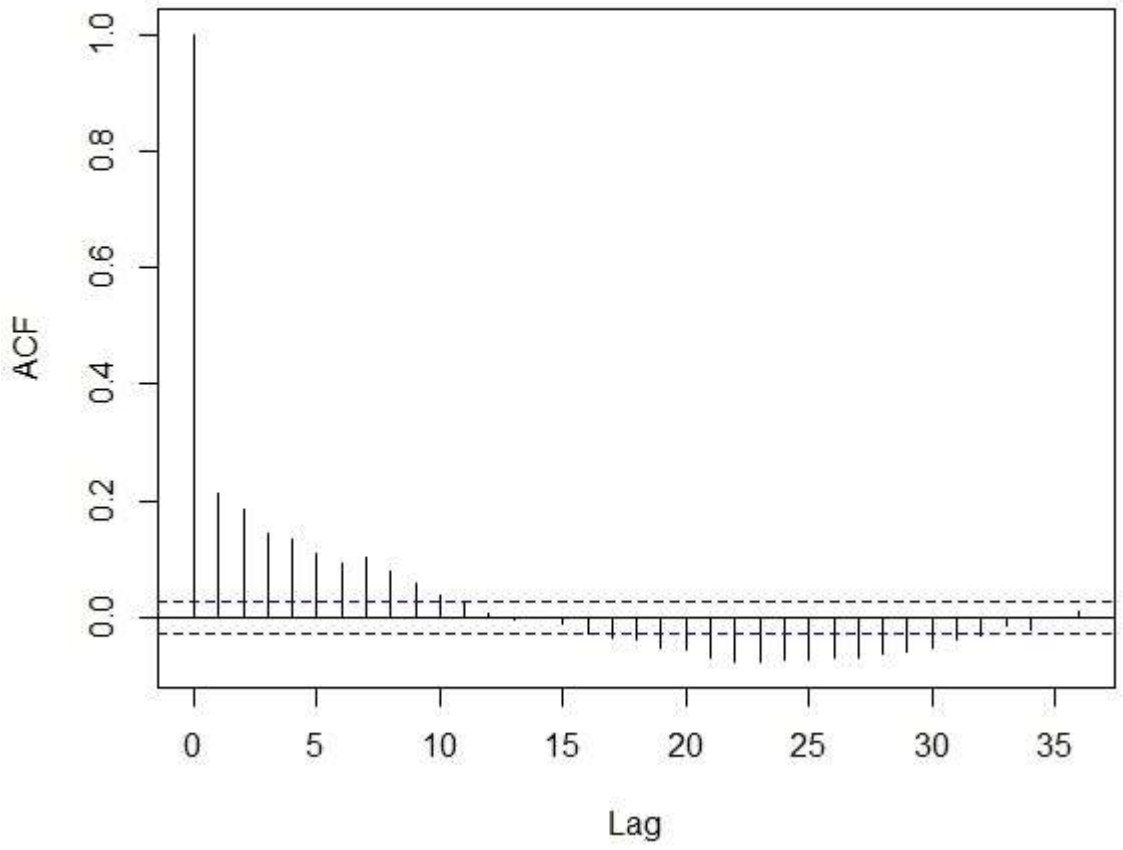

Figure 2.18. — Results of autocorrelation function (ACF) on GPS-derived Velocity values for ocelots and bobcats collared with ATS or Lotek GPS collars on the East El Sauz Ranch from 2013 to 2015. The autocorrelation function estimates correlation between observations separated by different time lags. This plot shows strong positive autocorrelative structure between subsequent velocity values. A lag value of 1 represents a 30-min time lag for the velocity dataset.

approximately zero with a time lag of 12 (6-hr time lag), but became -0.076 with a time lag of 22 (11-hr time lag).

During crepuscular time periods, regardless of lunar illumination, I found no difference in ocelot and bobcat Activity Index values ($P = 0.653$). During the daytime periods, regardless of lunar illumination, I found mean bobcat Activity Index higher than mean ocelot Activity Index ($P < 0.01$; Table 2.2). During the night, regardless of lunar illumination, I found mean ocelot Activity Index higher than mean bobcat Activity Index ($P < 0.01$) (Table 2.2). Using the GPS-derived movement velocity dataset, I failed to find differences between ocelot and bobcat velocity patterns for any of the periods examined (Table 2.3).

Bobcat Activity Index values were higher at night than during the day ($P < 0.01$), higher during the crepuscular period than during the day ($P < 0.01$), and higher during the crepuscular period than at night ($P < 0.01$). These patterns did not vary due to moon phase. I found no differences in activity values in any diel-based time period (Crepuscular, Night, Day) due to moon phase. These results support the hypothesis that bobcats are primarily crepuscular.

Ocelot Activity Index values were higher at night than during the day ($P < 0.01$), higher during the crepuscular period than during the day ($P < 0.01$), and higher at night than during the crepuscular period ($P < 0.01$). These patterns did not vary due to moon phase. I found no differences in activity values in any of the 3 diel periods (Crepuscular, Night, Day) due to moon phase. These results support the hypothesis that ocelots are primarily nocturnal.

Table 2.2. —Tukey pairwise comparisons between ocelot and bobcat Activity Index values during 3 diel periods (Crepuscular, Day, Night) and 3 levels of lunar illumination (High, Mid, Low). Estimate refers to the estimated difference between ocelot and bobcat (ocelot-bobcat) Activity Index values. SE is the standard error for the estimated difference in Activity Index value. *Z* value gives the *z* score corresponding to the null hypothesis that the difference between ocelot and bobcat Activity Index value is equal to 0. *P* gives the significance for the hypothesis test that mean ocelot Activity Index is equal to mean bobcat Activity Index.

Diel Period	Lunar Illumination	Estimate	SE	Z value	P
Crepuscular	High	-0.09471	0.08523	-1.111	1
	Mid	-0.02836	0.11126	-0.255	1
	Low	0.091645	0.13497	0.679	1
Day	High	-0.95259	0.06504	-14.647	<0.01
	Mid	-0.8234	0.08448	-9.747	<0.01
	Low	-0.95019	0.10358	-9.174	<0.01
Night	High	1.692375	0.07059	23.975	<0.01
	Mid	1.908233	0.09133	20.894	<0.01
	Low	1.981153	0.11302	17.53	<0.01

Table 2.3. —Results for Tukey pairwise comparisons between ocelot and bobcat Velocity values during three diel periods (Crepuscular, Day, Night) and 2 moon phases (Full, New). Estimate refers to the estimated difference between ocelot and bobcat (ocelot-bobcat) movement velocity values. SE is the standard error for the estimated difference in movement velocity. *Z* value gives the *z* score corresponding to the null hypothesis that the difference between ocelot and bobcat movement velocity is equal to 0. *P* gives the significance for the hypothesis test that mean ocelot movement velocity equal to mean bobcat movement velocity.

Diel Period	Moon Phase	Estimate	SE	z value	P
Crepuscular	Full	93.563	31.002	3.018	>0.99
	New	122.554	31.549	3.885	>0.99
Day	Full	-7.893	23.596	-0.335	>0.99
	New	11.981	24.053	0.498	>0.99
Night	Full	201.295	23.932	8.411	>0.99
	New	128.608	24.57	5.234	>0.99

DISCUSSION

Ocelot mean Activity Index value was greater on new moon nights than on full moon nights. The pattern was opposite for bobcats where mean Activity Index value was greater on full moon nights than on new moon nights. However, neither of these relationships was statistically significant. Larger sample sizes may be necessary to find differences in ocelot and bobcat activity patterns due to moon phase.

Although I did not find any differences in ocelot and bobcat movement and activity pattern due to lunar illumination, the diel movement patterns discovered suggest temporal niche partitioning between ocelot and bobcat. Ocelots and bobcats have been found to use similar environments on the EESR, with some interspecific home range overlap occurring between individuals. Both ocelots and bobcats are more active at night than during the day; however, bobcat activity peaked during the crepuscular period, whereas ocelot activity was highest at night. The lack of an effect on ocelot and bobcat activity due to lunar illumination contrasts with the finding by Rockhill et al. (2013) that bobcats had higher daytime movement rates during periods of low lunar illumination and higher nighttime movement rates during periods of high lunar illumination.

Tukey comparisons between ocelots and bobcats during the same diel-moon phase combinations revealed ocelots more active than bobcats at night and bobcats more active than ocelots during the day. No differences in activity were observed between ocelots and bobcats during the crepuscular period. The crepuscular period may be a period during which ocelots and bobcats compete for the same resources. Alternatively, ocelots and bobcats, although both active during the crepuscular period, may be engaged in different activities or occupy different habitats during this period.

The movement distances found in this study were higher than reported by Caso (1994) that found mean total distance traveled by ocelots in a 24-hr period was 1,560 m, with hourly movement ranging from 100 to 364 m. In contrast, this study found that ocelots occasionally traveled distances >600 m in a 30-min time interval. This discrepancy may be due to the increase in sampling intensity and location accuracy possible with GPS collars. Caso (1994) used triangulation from fixed receiver stations to monitor individuals hourly for 24-hr periods. Konecny et al. (1989) found ocelot hourly movement distances in Belize ranged from 0 - 2,809 m with an average hourly distance of 329 m. This study monitored 4 ocelots and 3 bobcats at 30-min intervals twice each month. A more accurate representation of the occasional long-distance movements made by ocelots is possible with GPS collars programmed with high-frequency sampling regimes than with VHF radio-telemetry.

Caso (1994) found 2 peaks in ocelot activity at 2100 hr and 0500 hr, indicating a crepuscular activity pattern. Ludlow and Sunquist (1986) reported that activity of ocelots in the Venezuelan Llanos were higher at night than during the day, but that distinct activity peaks occurred around sunrise and sunset. Konecny et al. (1989) also found that ocelot activity peaked in early morning and late evening periods. In South Texas, Laack (1991) reported that ocelots were more active at night than during the day, but did not report the crepuscular activity peak found by other studies. This study also found ocelot activity was high during early morning and late evening hours, but activity patterns were higher at night than during the crepuscular period. The results of this study support the hypothesis that ocelots are primarily nocturnal with some crepuscular activity, whereas bobcats are primarily crepuscular.

Significant differences found between ocelot and bobcat activity patterns were observed using the accelerometer-based Activity Index dataset rather than the GPS-derived velocity

dataset. This result may indicate that accelerometers, which collect data continuously, may be a more powerful tool for studying fine-scale animal activity patterns.

Using a random forests algorithm to predict movement velocity from accelerometer values resulted in relatively high misclassification rates for High and Intermediate velocity values. This highlights the difficulty in predicting animal movement from accelerometer data. In general, high accelerometer values relate to high movement rates. However, there may be other types of activity, aside from locomotion, that could yield high accelerometer readings even when movement velocity is low. Individual variation in stride length, movement path, or other movement metrics may make it difficult to directly infer movement velocity from accelerometer data. In addition, felids may be active in a localized area without moving over long distances. However, the random forests algorithm produced low misclassification rates for the Low velocity category, indicating that accelerometers can accurately predict periods of inactivity (i.e., no movement) in ocelots and bobcats.

Although 3 bobcats were captured and tracked with GPS collars, only one of these collars recorded continuous accelerometer-based activity estimates. Due to this lack of replication, any inferences drawn from the Activity Index dataset about bobcat temporal activity patterns should be treated with caution. However, the GPS-derived velocity values that were collected on bobcats EB15F and EB16M support the hypothesis that bobcats are more active than ocelots during the crepuscular and daytime periods. Future research should attempt to characterize ocelot and bobcat activities and examine high-resolution spatial patterns during periods of activity overlap.

Future research on ocelot and bobcat activity patterns using accelerometer data should attempt to classify active periods according to behavior. Weller and Bennett (2001) conducted an

activity budget study of captive ocelots, characterizing ocelot activities such as walking, running, climbing, jumping, spraying, and scraping. Although behavior of captive animals is likely to differ from that of wild individuals, the accelerometer-based signatures resulting from basic movements could be characterized using captive ocelots and bobcats. These signatures could then be used to predict behavior patterns in free ranging individuals. Wang et al. (2015) used data from tri-axial accelerometers to predict movement, resting, and attack behaviors of wild pumas. A similar study conducted on sympatric ocelots and bobcats may reveal different temporal patterns in specific activities, such as pouncing or walking, that may be obscured by only considering broad measures of activity.

LITERATURE CITED

BAKER, L. A., R. J. WARREN, D. R. DIEFENBACH, AND W. E. JAMES. 2001. Prey selection by reintroduced bobcats (*Lynx rufus*) on Cumberland Island, Georgia. American Midland Naturalist 145: 80–93.

BLANKENSHIP, T. L. 2000. Ecological response of bobcats to fluctuating prey populations on the Welder Wildlife Foundation Refuge. Ph.D. dissertation. Texas A&M University-Kingsville, Kingsville, United States.

BOUSKILA, A. 1995. Interactions between Predation Risk and Competition: A Field Study of Kangaroo Rats and Snakes. Ecology 76: 165–178.

BRADLEY, L. C., AND D. B. FAGRE. 1988. Movements and habitat use by coyotes and bobcats on a ranch in southern Texas. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 42: 411–430.

BREIMAN, L. 2001. Random Forests. Machine Learning 45: 5–32.

BROWN, J. S, B. P. KOTLER, R. J. SMITH, AND W. O. I. WIRTZ. 1988. The effects of owl predation on the foraging behavior of heteromyid rodents. Oecologica 76: 408–415.

BROWN, D. D., R. KAYS, M. WIKELSKI, R. WILSON, AND A. P. KLIMLEY. 2013. Observing the unwatchable through acceleration logging of animal behavior. Animal Biotelemetry 1: 20.

BUCHI, L. AND S. VUILLEUMIER. 2014. Coexistence of specialist and generalist species is shaped by dispersal and environmental factors. American Naturalist 183: 612-624.

BUIE, D. E., T. T. FENDLEY, AND H. MCNAB. 1979. Fall and winter home ranges of adult bobcats on the Savannah River Plant, South Carolina. Pp. 42-46 *in* Proceedings of the 1979 bobcat research conference: current research on biology and management of Lynx rufus. (L. Blum and P. C. Escherich, eds.). Scientific and Technical Series 6. National Wildlife Federation, Washington, D.C., United States.

CAIN, A. T., V. R. TUOVILA, D. G. HEWITT, AND M. E. TEWES. 2003. Effects of a highway and mitigation projects on bobcats in southern Texas. Biological Conservation 114: 189–197.

CASO, A. 1994. Home range and habitat use of three neotropical carnivores in northeast Mexico. M.S. thesis, Texas A&M University-Kingsville, Kingsville, United States.

CLARKE, J. A. 1983. Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). Behavioral Ecology and Sociobiology 13: 205–209.

CONNOLLY, A. R. 2009. Defining habitat for the recovery of ocelots (*Leopardus pardalis*) in the United States. M.S. thesis. Texas State University-San Marcos, San Marcos, United States.

CRAWSHAW JR., P. G. AND H. B. QUIGLEY. 1989. Notes on ocelot movement and activity in the Pantanal region, Brazil. Biotropica 21: 377-379.

CUTLER, D. R., T. C. EDWARDS JR., K. H. BEARD, A. CUTLER, K. T. HESS, J. GIBSON, J. J. LAWLER. 2007. Random forests for classification in Ecology. Ecology 88: 2783-2792.

DALY, M., P. R. BEHRENDS, M. I. WILDON, AND L. F. JACOBS. 1992. Behavioural modulation of predation risk: moonlight avoidance and crepuscular compensation in a nocturnal desert rodent, Dipodomys merriami. Animal Behaviour 44: 1–9.

DI BITETTI, M. S., C. D. DE ANGELO, Y. E. DI BLANCO, AND A. PAVIOLO. 2010. Niche partitioning and species coexistence in a Neotropical felid assemblage. Acta Oecologica 36: 403-412

DIDHAM, R. K. 2010. Ecological consequences of habitat fragmentation. *in* (R. Janson, ed.) Encyclopedia of life sciences. Wiley, Chichester. doi:10.1002/9780470015902.a0021904.

DIGGLE, P. 1990. Time series: a biostatistical introduction. Oxford University Press, Oxford, United Kingdom.

DONADIO, E. AND S. W. BUSKIRK. 2006. Diet, morphology, and interspecific killing in Carnivora. American Naturalist 167: 524-536.

DRAY, S., M. ROYER-CARENZI, AND C. CALENGE. 2010. The exploratory analysis of autocorrelation in animal-movement studies. Ecological Research 25: 673-681.

DUSSAULT, C., R. COURTOIS, J. OUELLET, AND J. HUOT. 2001. Influence of satellite geometry and differential correction on GPS location accuracy. Wildlife Society Bulletin 29: 171-179.

EMMONS, L. H. 1988. A field study of ocelots (*Felis pardalis*) in Peru. Revue D Ecologie-La Terre Et La Vie 43: 133-157.

EMMONS, L. H., P. SHERMAN, D. BOLSTER, A. GOLDIZEN, AND J. TERBORGH. 1989. Ocelot behavior in moonlight. Pp. 233-242 *in* (K. H. Redford and J. F. Eisenberg, eds.) Advances in Neotropical Mammalogy. Sandhill Crane Press, Gainesville, United States.

FEDERAL REGISTER. 1982. CFR Part 17. Vol 47, No. 140.

GAUSE, G. F. 1934. The struggle for existence. Hafner, New York, United States.

GRIFFIN, P. C., S. C. GRIFFIN, C. WAROQUIERS, AND L. S. MILLS. 2005. Mortality by moonlight: predation risk and the snowshoe hare. Behavioral Ecology 16: 938–944.

HAINES, A. M., M. E. TEWES, L. L. LAACK, W. E. GRANT, AND J. H. YOUNG. 2005. Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States. Biological Conservation 126: 512–522.

HAINES, A. M., M. E. TEWES, L. L. LAACK, J. S. HORNE, AND J. H. YOUNG. 2006. A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. Biological Conservation 132: 424–436.

HALL, H. T. AND J. D. NEWSOM. 1976. Summer home ranges and movements of bobcats in bottomland hardwoods of southern Louisiana. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 30: 422–436.

HARVESON, P. M., M. E. TEWES, G. L. ANDERSON, AND L. L. LAACK. 2004. Habitat use by ocelots in South Texas: implications for restoration. Wildlife Society Bulletin 32: 948–954.

HORNE, J. S., A. M. HAINES, M. E. TEWES, AND L. L. LAACK. 2009. Habitat partitioning by sympatric ocelots and bobcats: implications for recovery of ocelots in southern Texas. The Southwestern Naturalist 54: 119–126.

JANECKA, J. E., M. E. TEWES, I. A. DAVIS, A. M. HAINES, A. CASO, T. L. BLANKENSHIP, AND R. L. HONEYCUTT. 2016. Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot. Conservation Genetics DOI 10.1007/s10592-016-0846-1.

KELLEY, D. AND C. RICHARDS. 2015. Oce: Analysis of Oceanographic Data. R package version 0.9-17. https://CRAN.R-project.org/package=oce.

KELLY, M., A. CASO, AND C. LOPEZ GONZALES. 2008. *Lynx rufus*. The IUCN Red List of Threatened Species 2008:e.T12521A3352506. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T12521A3352506.en. Downloaded on 10 May 2016.

KITCHENER, A. 1991. The natural history of the wild cats. Cornell University Press, Ithaca, New York, United States.

KONECNY, M. J. 1989. Movement patterns and food habits of four sympatric carnivore species in Belize, Central America. Pp. 243-264 *in* (K.H. Redford and J. F. Eisenberg, eds.) Advances in Neotropical mammalogy. Sandhill Crane Press, Gainesville, United States.

LAACK, L. L. 1991. Ecology of the Ocelot (Felis pardalis) in South Texas. M.S. Thesis, University of Wisconsin-Stevens Point, Stevens Point, United States.

LABISKY, R. F. AND M. C. BOULAY. 1998. Behaviors of bobcats preying on white-tailed deer in the Everglades. The American Midland Naturalist 139: 275-281.

LEGENDRE, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology 74: 1659-1673.

LIAW, A., AND M. WIENER. 2002. Classification and Regression by randomForest. R News 2: 18-22.

LITVAITIS, J. A, A. G. CLARK, AND J. H. HUNT. 1986. Prey selection and fat deposits of bobcats (*Felis rufus*) during autumn and winter in Maine. Journal of Mammalogy 67: 389-392

LITVAITIS, J. A. 1981. A Comparison of coyote and bobcat food habits in the Wichita Mountains, Oklahoma. Proceedings of the Oklahoma Academy of Sciences 61: 81-82.

LONARD, R. I., AND F. W. JUDD. 1985. Effects of a severe freeze on native woody plants in the Lower Rio Grande Valley, Texas. Southwestern Naturalist 30: 397-403.

LOTEK WIRELESS INC. 2013. PS4400 / 4500 / 6000 / 7000 / 8000 / IridiumTrack / GlobalstarTack / Wildcell / MiniTrack. User's Manual.

LUDLOW, M. E., AND M. E. SUNQUIST. 1987. Ecology and behavior of ocelots in Venezuela. National Geographic Research 3:447-461.

LUNA-SORIA, H., AND C. A. LOPEZ-GONZALEZ. 2005. Abundance and food habits of cougars and bobcats in the Sierra San Luis, Sonora, Mexico. USDA Forest Service 36: 416-420.

MARSHALL, A. D. AND J. H. JENKINS.1966. Movements and home ranges of bobcats as determined by radio-tracking in the upper coastal plain of west-central South Carolina.

Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissioners 20: 206–214.

MCCORD, C. M., AND J. E. CARDOZA. 1982. Bobcat and lynx. Pp. 728-766 *in* (J.A. Chapman and G. A. Feldhammer, eds.) Wild mammals of North America. The Johns Hopkins University Press, Baltimore, Maryland, United States.

MONROY-VILCHIS, O. AND A. VELAZQUEZ. 2003. Regional distribution and abundance of bobcats (*Lynx rufus escuinape*) and coyotes (*Canis latrans cagottis*), as measured by scent stations: a spatial approach. Ciencias Naturales y Agropecuarias 9: 293–300.

MORRISON, D. 1978. Lunar phobia in a neotropical fruit bat, Artibeus jamaicensis (Chiroptera:Phyllostomidae). Animal Behavior 26: 852–855.

NAVARRO-LOPEZ, D. 1985. Status and distribution of the ocelot (*Felis pardalis*) in south Texas. M. S. thesis, Texas A&I University, Kingsville, Texas, United States.

NOWELL, K. AND P. JACKSON. 1996. Wild Cats. Status Survey and Conservation Action Plan. IUCN/SSC Cat Specialist Group, Gland, Switzerland and Cambridge, United Kingdom.

OLIVEIRA, T. G., M. A. TORTATO, L. SILVEIRA, C. B. KASPER, F. D. MAZIM, M. LUCHERINI, A. T. JÁCOMO, J. B. G. SOARES, R. V. MARQUES, AND M. SUNQUIST. 2010. Ocelot ecology and its effect in the small-felid guild in the lowland Neotropics. Pp. 563-584 *in* (D.W. Macdonald and A. Loveridge, eds.) Biology and Conservation of Wild Felids. Oxford University Press, Oxford, United Kingdom.

PALOMARES, F., AND T. M. CARO, 1999. Interspecific killing among mammalian carnivores. American Naturalist 153: 492–508.

PINHEIRO, J., D. BATES, S. DEBROY, D. SARKAR, AND R CORE TEAM. 2016. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.

ROBERTS, N. M. AND S. W. CRIMMINS. 2010. Bobcat population status and management in North America: Evidence of large-scale population increase. Journal of Fish and Wildlife Management 1: 169–174.

ROCKHILL, A. P., C. S. DEPERNO, AND R. A. POWELL. 2013. The effect of illumination and time of day on movements of bobcats (*Lynx rufus*). PLoS ONE 8(7):e69213. doi:10.1371/journal.pone.0069213.

SALDANA-VAZQUEZ, R. A., AND M. A. MUNGUIA-ROSAS. 2013. Lunar phobia in bats and its ecological correlates: A meta-analysis. Mammalian Biology 78: 216–219.

SCHOENER, T.W. 1974. Resource Partitioning in Ecological Communities. Science 185: 27–39.

SHEPARD, E. L. C., R. P. WILSON, F. QUINTANA, A. G. LAICH, N. LIEBSCH, D. A. ALBAREDA, L. G. HALSEY, A. GLEISS, D. T. MORGAN, A. E. MYERS, C. NEWMAN, AND D. W. MACDONALD. 2008. Identification of animal movement patterns using tri-axial accelerometry. Endangered Species Research 10: 47–60.

SHINDLE, D. B., AND M. E. TEWES. 1998. Woody species composition of habitats used by ocelots (*Leopardus pardalis*) in the Tamaulipan Biotic Province. The Southwestern Naturalist 43: 273–279.

SHINDLE, D. B. AND M. E. TEWES. 2000. Immobilization of wild ocelots with tiletamine and zolazepam in southern Texas. Journal of Wildlife Disease 36: 546–550.

SHINN, K. J. 2002. Ocelot distribution in the Lower Rio Grande Valley National Wildlife Refuge. M. S. thesis. University of Texas-Pan American, Edinburg, United States.

STASEY, W. C. 2012. Evaluating translocation strategies for ocelot in the Tamaulipan Biotic Province. Ph.D. dissertation. Texas A&M University-Kingsville, Kingsville, United States.

SUNQUIST, M. AND F. SUNQUIST. 2002. Wild Cats of the World. University of Chicago Press, Chicago, United States.

TEWES, M. E., AND D. D. EVERETT. 1986. Status and distribution of the endangered ocelot and jaguarondi in Texas. Pp. 147-158 in Cats of the world: biology, conservation, and management. (S. D. Miller and D.D. Everett, eds.) National Wildlife Federation, Washington, D.C., United States.

TEWES, M. E. 1986. Ecological and behavioral correlates of ocelot spatial patterns. Ph.D. dissertation, University of Idaho, Moscow, United States.

TURCHIN, P. 1998. Quantitative analysis of movement: measuring and modeling population redistribution in plants and animals. Sinauer Associates, Sunderland, United States.

WANG, Y., B. NICKEL, M. RUTISHAUSER, C. M. BRYCE, T. M. WILLIAMS, G. ELKAIM, AND C. C. WILMERS. 2015. Movement, resting, and attack behaviors of wild pumas are revealed by tri-axial accelerometer measurements. Movement Ecology 3:2.

WELLER, S. H., AND C. L. BENNETT. 2001. Twenty-four hour activity budgets and patterns of behavior in captive ocelots (*Leopardus pardalis*). Applied Animal Behaviour Science 71: 67-79.

ZEZULAK, D. S. AND R. G. SCHWAB. 1980. Bobcat biology in a Mojave Desert community. California Department of Fish and Game, Sacramento. Federal Aid in Wildlife Restoration Project W-54-R-12, Job IV-4. 25pp.

ZEZULAK, D. S. 1981. Northeastern California bobcat study. California Department of Fish and Game, Sacramento. Federal Aid in Wildlife Restoration Project W-54-R-12, Job IV-3. 10pp.

ZHANG, J., V. HULL, J. HUANG, S. ZHOU, W. XU, H. YANG, W. J. MCCONNELL, R. LI, D. LIU, Y. HUANG, Z. OUYANG, H. ZHANG, AND J. LIU. 2015. Activity patterns of the giant panda (*Ailuropoda melanoleuca*). Journal of Mammalogy 96: 1116–1127.

ZIELINSKI, W. J. 1986. Circadian rhythms of small carnivores and the effects of restricted feeding on daily activity. Physiology and Behavior 38: 613–620.

ZIELINSKI, W. J. 1988. The influence of daily variation in foraging cost on the activity of small carnivores. Animal Behaviour 36: 239–249.

CHAPTER III

ESTIMATING THIRD AND FOURTH ORDER RESOURCE SELECTION FOR SYMPATRIC OCELOTS AND BOBCATS

Manly et al. (2002) defined resource selection as the disproportionate use of resources relative to their availability. Understanding how animals use resources selectively is crucial for wildlife management and conservation and for understanding basic ecology (Boyce and McDonald 1999). Resource selection functions (RSF) are often employed to estimate the probability of an animal using various areas given specific attributes of those areas (Manly et al. 2002). The traditional approach for estimating animal resource selection involves first defining the area utilized by the animal using a home range estimator (e.g., kernel density; Worton 1987) and comparing the distribution of resources within the home range boundary to the general availability throughout the landscape (e.g., Neu et al. 1974, Aebischer et al. 1993). In such studies, defining the extent of the area considered available can have a dramatic impact on the outcome of the resource selection study (McClean et al. 1998, Beyer et al. 2010). Additionally, delineating the boundaries of home ranges can be problematic, as many methods (e.g., minimum convex polygon, kernel density) can lead to the inclusion of large areas that are never visited by the individual (Moser and Garton 2007).

Johnson (1980) described habitat selection as a hierarchical process involving four orders of selection, with first order describing the geographic range of a species, second order determining the home range of individuals, third order describing the use of different habitat components within the home range, and fourth order describing the actual selection of resource

Chapter 3 of this dissertation is written in the style of the *Journal of Wildlife Management*.

units available at each site. Properly defining the relevant order of selection is an essential component of animal resource selection studies, and patterns observed at one scale of selection may not apply to other scales (Mayor et al. 2009).

The synoptic model of space use allows simultaneous estimation of animal home range and resource selection by evaluating both the spatial distribution of relocation points and the relevant spatial attributes occurring at those points (Horne et al. 2008). The synoptic model of space use can provide a more realistic estimate of the utilization distribution (UD) than more traditional methods, such as kernel-based approaches. In most cases, the synoptic model is equivalent to the third order of selection (Johnson 1980), as probabilities of use are assigned to individual resource units within the home range of an individual, allowing estimates of differential selection of habitat types within home range boundaries. This allows estimation of the internal anatomy of home range (Adams and Davis 1967). However, with the synoptic model, availability is not explicitly defined for each individual at the home range level. Rather, the synoptic model uses a weighted parametric spatial distribution to define home range and resource selection. This attribute has the effect of modeling availability as an exponential decay function, gradually decreasing with increased distance from the home range center.

The advent of low-weight global positioning systems (GPS) collars has given researchers an unprecedented insight into the movement and behavior of wild animals (Cagnacci et al. 2010). Modern GPS collars can be programmed to record locations at extremely high frequencies, allowing the entire movement paths of individuals to be visualized in detail. Whereas such high-frequency datasets contain significant autocorrelative structure that can make traditional habitat-selection studies difficult (Fieberg et al. 2010), such datasets allow estimation of fourth-order selection (Johnson 1980) by allowing researchers to explicitly define availability at each step. A

relatively new framework for conducting such analyses is the step selection function (SSF; Fortin et al. 2005, Thurfjell et al. 2014). The SSF is a special application of the RSF (Thurfgell et al. 2014). Habitat availability is estimated at each relocation point with the SSF using a high-frequency GPS telemetry dataset by generating a series of potential (i.e., available) steps that could be traveled by the animal from each actual relocation point. Relevant spatial attributes are extracted along the potential movement path and at the end point of the path. The available steps are compared with the actual steps taken by the animal, often using a case-controlled logistic regression (e.g., Fortin et al. 2005, Coulon et al. 2008, Leblond et al. 2010) to determine if specific spatial attributes are selected at each step disproportionately to their availability.

The ocelot (*Leopardus pardalis*) is a federally endangered felid (Federal Register 1982) with its range in the United States declining dramatically in the 19th and 20th centuries (Navaro-Lopez 1985, Tewes and Everett 1986). Ocelots and bobcats (*Lynx rufus*) are sympatric from South Texas through central Mexico (Tewes and Schmidly 1987). Studies of ocelot and bobcat habitat selection have shown both felids select for dense thornshrub communities in Texas (Tewes 1986, Bradley and Fagre 1988, Cain et al. 2003, Harveson et al. 2004, Haines et al. 2006). Horne et al. (2009) estimated habitat selection of sympatric ocelots and bobcats on the Laguna Atascosa National Wildlife Refuge (LANWR) at the second and third orders (Johnson 1980), finding no differences in second-order habitat selection, yet significantly stronger selection of closed canopy habitat by ocelots than by bobcats at the third order of selection.

Ocelots and bobcats are similar in size (Sunquist and Sunquist 2002), feed primarily on small mammals and birds, and show similar temporal activity patterns (Rolley 1987). Species that are ecologically equivalent cannot coexist according to the competitive-exclusion theory (Gause 1934). Therefore, it is possible that ocelots and bobcats show a finer scale of temporal

and spatial niche partitioning than can readily be observed using radio-telemetry to evaluate second order (Johnson 1980) habitat selection. In contrast to the ocelot, bobcat populations are increasing in North America (Roberts and Crimmins 2010). The extent to which bobcats compete with ocelots for limited resources and constrain ocelot distribution and population sizes is unknown. The purpose of this study was to compare ocelot and bobcat resource selection at the third order (Johnson 1980) using a synoptic model of space use (Horne et al. 2008) and at the fourth order (Johnson 1980) using an SSF.

STUDY AREA

This study was conducted on the East El Sauz Ranch (EESR), an approximately 113 km^2 ranch located near Port Mansfield, Willacy County, Texas. The EESR occurs within the Tamaulipan biotic province, and has a semiarid, subtropical climate, with mean temperatures ranging from 16° C to 28° C, and a mean annual precipitation of 68 cm (Lonard and Judd 1985, Haines et al. 2005). Common woody plants found on the EESR previously associated with ocelot use include spiny hackberry (*Celtis pallida*), crucita (*Eupatorium odoratum*), honey mesquite (*Prosopis glandulosa*), desert olive (*Forestiera angustifolia*), colima (*Zanthoxylum fagara*), and brasil (*Condalia hookeri*; Shindle and Tewes 1998).

METHODS

Capture and telemetry

Between 2011 and 2015 researchers captured ocelots and bobcats using single-door 108 x 55 x 40 cm wire box traps (Tomahawk™ Trap Co., Tomahawk, WI). Traps were baited with live chickens or pigeons, which were housed in separate enclosures attached to the main trap and

supplied regularly with food and water. Adult ocelots and bobcats were sedated with an intramuscular injection of Telazol™ (Fort Dodge Laboratories, Fort Dodge, Iowa) delivered with a pole syringe, at a dosage of 5 mg per kg body weight (Shindle and Tewes 2000). Captures were conducted in compliance with the Texas A&M University-Kingsville Institutional Animal Care and Use Committee protocol numbers 2012-12-20B-A2, 2012-12-20B and 2012-12-19.

Adult ocelots and bobcats were collared with GPS collars manufactured by either Sirtrack (Sirtrack Wireless, Dunedin, New Zealand), Advanced Telemetry Systems (ATS; Advanced Telemetry Systems Inc, Insanti, MN), or Lotek (Lotek Wireless, Newmarket, Ontario, Canada). The Sirtrack GPS collars were used during the 2011, 2012, and 2013 trapping seasons and were programmed to record locations every 11 hr. The ATS collars were used in 2013 and 2014 and were programmed to record one location each 24 hr period at midnight (2400 hr) and one location each 24 hr period at noon (1200 hr), with an additional high-frequency track schedule programmed at 30-min intervals for a 72-hr period centered on each new moon and each full moon night. The Lotek GPS collars were used in 2014 and 2015 and were programmed with a similar fix schedule as the ATS collars, but with a 24-hr high-frequency track period centered on each new moon and each full moon night.

I partitioned data collected by Sirtrack collars into nocturnal and diurnal locations. I partitioned data collected by Lotek and ATS collars into high-frequency locations (i.e., locations collected at 30-min intervals) and low-frequency locations (i.e., locations collected either at noon or midnight each 24-hr period). I further partitioned low-frequency locations into diurnal and nocturnal locations for each individual. High-frequency locations were used for SSF analysis and low-frequency locations were used for synoptic model analysis.

Defining seasons

I split each year into 2 seasons based on general temperature and weather patterns. I defined winter as 1 October to 31 March and summer as 1 April to 30 September. I selected these months to define seasons to separate each year based on the dominant weather patterns occurring in South Texas. Weather patterns in South Texas also show substantial inter-annual variability, oscillating between prolonged drought and wet conditions. However, the period defined in this study as winter was dominated by cooler, more variable conditions than the period identified as summer, with generally consistently hot temperatures. I elected to separate each year into 2 seasons because South Texas lacks the traditional 4 seasons found in more northerly regions. In addition, any attempts to separate years into more than 2 seasons would yield an insufficient number of locations for each individual. I treated each combination of individual ID, season, and diel period as a unique study unit in my analysis.

Model development

I used a synoptic model of space use (Horne et al. 2008) to simultaneously estimate home range size and within home range habitat selection, in a design similar to Johnson's (1980) third-order selection for the GPS-collared ocelots and bobcats captured on the EESR. The synoptic model is based on a weighted distribution that is used to model an animal's probability of use across the availability grid (Lele and Keim 2006, Horne et al. 2008). The spatial distribution of each individual was modeled using equation 3.1,

$$f_u(x) = \frac{w(x) \times f_0(x)}{\int [w(x) \times f_0(x)]}$$ **Equation 3.1.**

where $f_0(x)$ is the null model of space use which does not include habitat selection, $f_u(x)$ is the probability density at location x, and $w(x)$ is a function that introduces weights based on specific spatial attributes to the null model. The integral in the denominator of equation 3.1 is approximated by the availability grid, which includes areas potentially available to the study animals, along with relevant spatial attributes occurring at those points. The synoptic model is nearly analogous to the resource selection function described by Manly et al. (2002); however, it differs by including a null model of space use, thus preventing all units within the study area from being treated as equally available to the animal.

For this study, I defined the null model as a bivariate normal distribution. This model includes parameters defining the means and variances of the x and y coordinates and an additional parameter defining the correlation between these coordinates. I considered this an appropriate null model for ocelots and bobcats as the bivariate normal distribution links the utilization distribution (UD) to a central place (Horne et al. 2008), which is appropriate for non-migratory territorial species such as ocelots and bobcats.

I defined the selection function using equation 3.2,

$$w(x) = Exp[H(x)'\beta] \qquad \textbf{Equation 3.2.}$$

where $H(x)$ is the vector of covariate values describing habitat attributes at each location x, and β is a vector of selection coefficients (i.e., parameters) to be estimated.

Maximum likelihood was used to estimate the parameters defining the null models and selection coefficients for the competing models and competing models were ranked for each individual using information theoretic methods (Burnham and Anderson 2002). For each

individual, partitioned by diel period and season, I selected the top model using Akaike's information criterion corrected for small sample sizes (AICc; Burnham & Anderson 2002, Horne et al. 2008). I considered the top model for each individual to be the one with the lowest AICc value; however, any models that were within 2 units of the lowest AICc value were considered competitive and reported as potential alternatives to the top synoptic model. In such cases, I used model averaging to combine coefficients from the top 2 or 3 models per individual. The new averaged model was considered to be the best model in subsequent analyses.

Model averaging was also used for the top models per individual across all ocelots and bobcats, separated by diel period and season. I calculated strength of coefficient for each parameter by creating 85% confidence intervals (Arnold 2010). A positive 85% confidence interval for a model-averaged coefficient was considered indicative of selection for that habitat attribute, whereas a negative 85% confidence interval for the coefficient indicated avoidance of that attribute. A confidence interval overlapping zero to indicated use in proportion to availability for that habitat attribute.

I averaged all competing models for each individual-diel period-season combination to obtain composite models. I again calculated the strength of coefficient for each parameter by creating 85% confidence intervals (Arnold 2010). I counted the number of individuals with 85% confidence intervals falling into each of these categories by season and species.

Construction of availability file

To define the area considered to be available to all animals, and approximate the integral in equation 3.1, I constructed an availability grid, consisting of a 30-m grid of points encompassing a rectangular 20,940 m x 22,350 m area covering all bobcat and ocelot location points recorded

throughout the study period. Defining availability is a crucial step in studies of animal resource selection, and studies of resource selection may be biased either by including areas not truly available to the study animal, or by excluding areas that are available (McClean et al. 1998, Beyer et al. 2010). Deciding where to draw the boundaries of the area considered available can have a dramatic influence on resulting selection coefficients when using methods such as those described by Neu et al. (1974). With the synoptic model; however, all locations within the availability grid are not treated as being equally available to each animal since the center of each home range is included explicitly in the model. Thus, determining extent of the availability grid is less crucial for constructing a realistic RSF, as even availability grids that are unrealistically large have little influence on the model parameters.

Creation of base layers

For classification of land cover types, I used the Coastal Change Analysis Program (C-CAP) land cover map developed by the National Oceanic and Atmospheric Administration (NOAA) Coastal Services Center. The C-CAP land cover data were developed to meet a target accuracy of 85%, as verified through ground assessment. Major land cover classifications found within the EESR and surrounding areas include: Low Intensity Developed, Pasture or Hay, Grassland, Deciduous Forest, Evergreen Forest, Mixed Forest, Grassland, Scrub or Shrub, Palustrine Scrub or Shrub Wetland, Palustrine Emergent Wetland, Bare Land, Palustrine Aquatic Bed, Unconsolidated Shore, and Palustrine Emergent Wetland.

Inclusion of habitat categories representing a small proportion of the available area in a synoptic model of space use often yields covariates equal to fixed negative infinity (-Inf) which can make interpretation of model covariates difficult. To reduce the number of available land

cover types, I recategorized all C-CAP land cover types into the following 5 categories: Wetland, Shrub, Grassland, Forest, and Open. The categories Deciduous Forest, Mixed Forest, and Evergreen Forest primarily occurred in the northwestern portion of the EESR, and were characterized by a closed layer of emergent live oak (*Quercus virginiana*) with a mid-story shrub layer of varying density. Visual comparison of C-CAP data to aerial imagery revealed that Deciduous Forest, Mixed Forest, and Evergreen Forest occurred within similar oak-dominated environments on the EESR, allowing these classes to be combined into the single category Forest. The Grassland classification was taken directly from the C-CAP category without modification, and the Shrub classification was renamed from the C-CAP Shrub or Scrub category. The Open classification was created by combining the following C-CAP land cover types: Cultivated, Developed Open Space, Bare Land, Low Intensity Developed, Pasture or Hay, and Unconsolidated Shore. All land cover types reclassified as Open were characterized by a similar lack of vegetation cover. The Wetland classification was created by combining the following C-CAP land cover types: Palustrine Aquatic Bed, Palustrine Emergent Wetland, Palustrine Scrub or Shrub Wetland, and Open Water. I allocated 50 random points to each of the 5 reclassified NLCD land cover categories, and visually verified classification accuracy by aligning points with a 2014 digital orthophoto quarter quadrangle (DOQQ) image, following the procedure outlined by Congalton and Green (1999).

To assess the importance of canopy cover for ocelot and bobcat habitat selection, I included tree canopy values obtained from a 2011 NLCD tree canopy raster file. This raster file reports percent tree canopy cover for the United States at 30-m resolution, and ranges in value from 0 to 100. The dataset was produced by the USDA Forest Service Remote Sensing Applications Center (RSAC) using a random forests (Breiman 2001) regression algorithm.

Visual comparison of the C-CAP raster layer and the Canopy raster layer indicated that areas with >0% canopy coverage, as indicated by the Canopy layer, coincided with C-CAP land cover categories Shrub and Forest.

Fresh water availability on the EESR and surrounding areas varies dramatically with rainfall. A large number of ephemeral wetlands cover the southwestern portion of the EESR, yet these are often completely dry during drought periods. Artificial water sources, such as water tanks and ponds, can increase abundance of small mammal communities in arid environments (Switalski 2013), which may lead to preferential use of these areas by predators. Additionally, Blankenship (2000) found bobcats show close association with wetlands and increase predation of waterfowl during periods of low mammalian prey abundance. Distance to perennial fresh water source was therefore included as a covariate in the model. I hypothesized that such perennial fresh water sources might serve to attract ocelots and bobcats, particularly during drought periods and hotter summer months. I obtained a map of all perennial fresh water sources (i.e., lakes, streams, canals, and water tanks) in Kennedy and Willacy counties from the USGS Geographic Names Information System (USGS 2014). Water source locations were plotted on a GIS map of the study area, and a raster layer showing the Euclidean distance in meters to the nearest perennial water was developed for each point in the study area.

For each point in the availability grid and each relocation point, I extracted the value of the underlying basemap (i.e., Land Cover, Canopy, and Water Distance) using a raster extract function in ArcGIS 10.1 (ESRI, Redlands, CA). I recoded the categorical Land Cover attribute as 5 binary attributes representing the 5 reclassified land cover types (i.e., Wetland, Shrub, Grassland, Forest, and Open), entering a 1 if that land cover type overlapped a point and 0 if it did not. The values extracted from the water-distance raster layer were given the covariate name

Waterdist in the availability grid and the locations file. Values obtained from the NLCD tree canopy layer were included as the covariate Canopy in the availability grid and locations file (Table 3.1).

Creation of a priori models

I constructed 5 competing *a priori* synoptic models including various covariate combinations. Model 1 was considered the null model, consisting of a bivariate normal distribution in which the means and standard errors for the x and y coordinates, along with the correlation between these coordinates, entirely describe the UD of individuals. Models 2 through 5 used data from relevant spatial attributes to assign weights to this underlying distribution. Model 2 contained Canopy. Model 3 contained Waterdist and Canopy. Model 4 contained the 5 binary land cover variables (Wetland, Shrub, Grassland, Forest, and Open). Model 5 contained the 5 binary land cover variables, and Waterdist and Canopy (Table 3.2). I used an information-theoretic approach to rank these models for each individual partitioned by day and season. The model with the lowest AICc was selected, although models within 2 units of the top model were considered competitive (Burnham and Anderson 2002).

Creating utilization distributions

I used the top synoptic model for each individual to create UDs for each individual by assigning probability densities to each point in the availability grid and truncating the grid at the 95% cumulative probability level. This method produced a unique grid of points for each individual that showed the outer boundaries of the home range and the relative probabilities of use for all locations within the home range. I calculated the areas of 95% UDs produced using

Table 3.1. Covariates included in synoptic models to describe ocelot and bobcat resource selection in Willacy County, Texas.

Variable	Description
Wetland	Binary. Derived from CCAP raster layer. Includes Palustrine Aquatic Bed, Palustrine Emergent Wetland, Palustrine Scrub/Shrub Wetland, and Open Water.
Shrub	Binary. Derived from CCAP raster layer. Includes only Shrub/Scrub classification.
Grassland	Binary. Derived from CCAP raster layer. Includes only Grassland classification.
Forest	Binary. Derived from CCAP raster layer. Includes Evergreen Forest, Deciduous Forest, and Mixed Forest.
Open	Binary. Derived from CCAP raster layer. Includes Cultivated, Developed Open Space, Bare Land, Low Intensity Developed, Pasture/Hay, and Unconsolidated Shore.
Waterdist	Distance to nearest perennial freshwater source in meters.
Canopy	Percent canopy cover. Derived from NLCD 2011 USFS Tree Canopy raster layer.

Table 3.2. List of competing synoptic models with covariates included. Null model (BVN) includes parameters mean of *x*, mean of *y*, standard deviation of *x*, standard deviation of *y*, and correlation. All models include BVN.

Model Number	Parameters Included
1	BVN
2	BVN+Canopy
3	BVN+Waterdist+Canopy
4	BVN+Wetland+Shrub+Grassland+Forest+Open
5	BVN+Wetland+Shrub+Grassland+Forest+Open+Waterdist+Canopy

the synoptic model and compared these to estimates derived using a 95% fixed kernel density estimates (KDE) and 95% minimum convex polygon (MCP) estimates.

Step-selection function

To estimate fourth-order selection of ocelot and bobcat, I used a SSF following the approach developed by Fortin et al. (2005). For this analysis, I used only the high-frequency GPS data collected for ocelots and bobcats using ATS and Lotek collars. At each GPS relocation point, I generated 10 random points sampled from the observed distribution of step lengths and turn angles for all individuals (Fig. 3.1). At each random point, I extracted the value of the NLCD Tree Canopy layer, assigning this variable the name Canopy. Additionally, I calculated the mean value of the Canopy layer occurring along a straight line segment connecting each random or observed point with the previous observed GPS relocation point, giving this covariate the name Linear_Cnpy. This covariate was included to incorporate the theoretical movement path of the animal between subsequent points. This does not assume that the animal followed a straight path between subsequent relocation points, but rather that the habitat attributes of its actual movement path correlate with those occurring along a straight line segment connecting consecutive relocation points.

A major component of animal space use is the tendency to remain linked to a fixed geographic area (i.e., home range). To account for this tendency, I included as a coefficient the straight-line distance (m) to the centroid of each animal spatial distribution, assigning to this covariate the name Centerdist. I used the R package oce (Kelley and Richards 2016) to extract solar altitude for each date and time combination reported in the dataset, naming this covariate Sun_alt. This function reported the angle of the sun either above (positive) or below

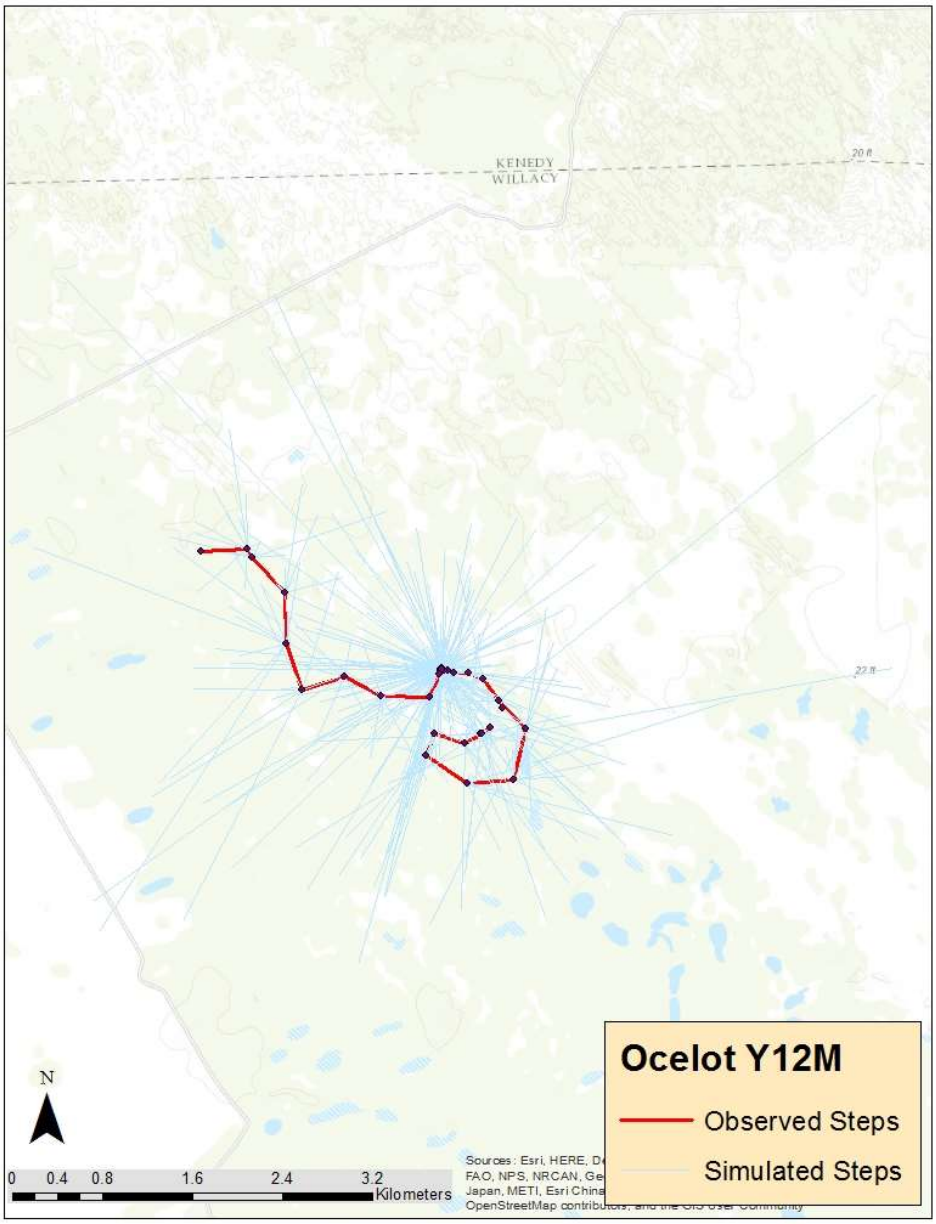

Figure 3.1. Example of simulated steps (light blue) and observed steps (red) used in generating a step selection function for ocelot Y12M. Simulated steps were drawn from the distribution of step lengths and turn angles observed in the entire dataset of observed steps. Environmental covariates were extracted along observed and simulated steps for use in a case-controlled logistic regression.

(negative) the horizon. To simplify analysis, I added a constant to all sun altitude measurements so that the lowest possible value of Sun_alt was 0, and all other values were positive. Additionally, I included lunar illuminated fraction for each date and time combination, giving this covariate the name Moon_illum (Table 3.3). I conducted identical operations at each selected step between actual relocation points and between each actual relocation point and the set of subsequent random points.

I created 8 competing SSF additive and interactive models and tested model fit independently for each individual (Table 3.4). I used a case control logistic regression (i.e., conditional logistic regression) to model the probability of selection for each point given the various combinations of covariates included in the models and ranked competing models according to AIC value (Burnham and Anderson 2002). I used parameter averaging to create composite SSF models for each individual.

Hypotheses

Previous studies have found ocelots in South Texas to be strongly associated with areas of dense woody canopy, particularly thornshrub communities (Tewes 1986, Harveson et al. 2004, Jackson et al. 2005, Haines et al. 2006, Horne et al. 2009). I, therefore, expected ocelots to show strong positive selection for land cover types classified as Forest or Shrub and to avoid areas classified as Bare or Grassland. Additionally, I expected ocelots to show strong positive association with Canopy. Bobcats are habitat generalists, using plant communities with open canopies as well as closed canopies (Bradley and Fagre 1988, Cain et al. 2003, Horne et al. 2009). I, therefore, expected the confidence intervals for bobcat selection coefficients to be more variable compared to ocelots. In addition, I expected bobcats to show weaker selection of Forest and Shrub

Table 3.3. List of covariates included in competing step-selection functions with descriptions.

Variable	Description
Canopy	Percent canopy cover. Derived from NLCE 2011 Tree Canopy raster layer.
Centerdist	Straight line distance (m) to the centroid of an individual's GPS relocations.
Linear_Cnpy	Mean canopy value extracted along a straight line connecting two consecutive points.
Sun_alt	Solar altitude in degrees above (positive) or below (negative) the horizon.
Moon_illum	Lunar illuminated fraction. Given as a proportion ranging from 0 to 1.

Table 3.4. List of competing step selection models that were developed and compared for each individual.

Model Number	Parameters Included
1	Canopy
2	Centerdist
3	Canopy+Centerdist
4	Linear_Cnpy
5	Canopy+Linear_Cnpy
6	Canopy+Linear_Cnpy+Centerdist
7	Canopy*Sun_alt
8	Canopy*Moon_illum

cover types and weaker association with Canopy compared to ocelots. I expected both felids to show negative association with Waterdist during the warmer summer months and during drought periods, as this would indicate selection for areas near perennial water sources.

I expected ocelots and bobcats to show similar patterns of selection at the third-order (synoptic model) and fourth-order (SSF). I expected to find strong negative parameters for Centerdist for all individuals, indicating a declining probability of use farther from the home range center. Additionally, I expected to find positive covariates for the interaction between Canopy and Sun_alt, indicating that ocelots are more strongly tied to closed canopy habitats during the day than at night.

RESULTS

Capture and telemetry

In 2011, male ocelot E3M was collared with a Sirtrack GPS collar. In 2013, male ocelot E8M was collared with a Sirtrack GPS collar and bobcats EB15F and EB16M were collared with ATS GPS collars. In 2014, ocelots E10F and Y12M were collared with Lotek GPS collars. In 2015, ocelots E6M and E12F and bobcat EB8M were collared with Lotek GPS collars. Between 2011 and 2016, I collected GPS location data on 6 ocelots (4M, 2F) and 3 bobcats (2M, 1F), collecting 5,074 high frequency GPS locations and 1,483 low frequency GPS locations (Table 3.5).

Comparison of synoptic and kernel UD areas

Area (km^2) of synoptic model UDs, truncated at the 95% cumulative probability contour, ranged in size from 1.43 to 10.73 (mean = 5.79, SD = 2.46). For comparison, I also reported home

Table 3.5. Summary of high frequency and low frequency GPS data collected on ocelots and bobcats from 2011 to 2016.

ID	Species	Sex	Capture Date	End Date	High Frequency GPS	Low Frequency GPS
E3M	Ocelot	M	3/9/2011	12/6/2011	0	259
E8M	Ocelot	M	2/24/2013	4/16/2013	0	26
E10F	Ocelot	F	3/1/2014	7/25/2014	365	148
E12F	Ocelot	F	3/20/2015	11/7/2015	332	103
Y12M	Ocelot	M	3/3/2014	7/27/2014	478	153
E6M	Ocelot	M	4/22/2015	1/28/2016	235	96
EB15F	Bobcat	F	4/27/2013	10/20/2013	1,738	265
EB16M	Bobcat	M	5/8/2013	10/16/2013	1,590	286
EB8M	Bobcat	M	3/22/2015	7/14/2015	336	147

range estimates using 95% KDE and 95% minimum convex polygon (95% MCP; Chapter I). Area (km^2) of 95% KDE home range estimates ranged from 3.00 to 78.48 (mean = 21.17, SD = 19.47). Area (km^2) of 95% MCP home range estimates ranged from 0.85 to 34.11 (mean = 7.27, SD = 7.63). In all cases, the UD created using the synoptic model was smaller than that created using KDE (Table 3.6). Plotting synoptic model UDs on aerial images of the study along with kernel UDs and MCP UDs revealed that the synoptic model UDs appeared to more closely conform to the boundaries of vegetation types, whereas KDE and MCP UDs often included extensive areas of the Open cover type and other cover types that were not frequently used (Fig. 3.2).

Model averaged parameters

Partitioning GPS locations by diel period and by season resulted in 31 unique groups of points for which competing synoptic models were tested. The top model was model 5 for 16 groups, model 2 for 7 groups, and model 3 for 9 groups (Tables 3.7-3.11). For 11 of the groups, 2 or more models had AICc values within 2 units of each other, indicating uncertainty in selecting the top model (Burnham and Anderson 2002). For individuals with no clear top model, I reported all models within 2 AICc units of the top model along with model-averaged parameter estimates for the composite model (Tables 3.7-3.11). In some cases, selection coefficients for a land cover covariate could not be estimated because the individual never occurred in that land cover type during the study. In these cases, a selection parameter of negative infinity (-Inf) was reported.

The covariate Canopy had the most consistently positive covariates, with ocelots and bobcats showing positive selection for this variable regardless of season. The covariate Waterdist

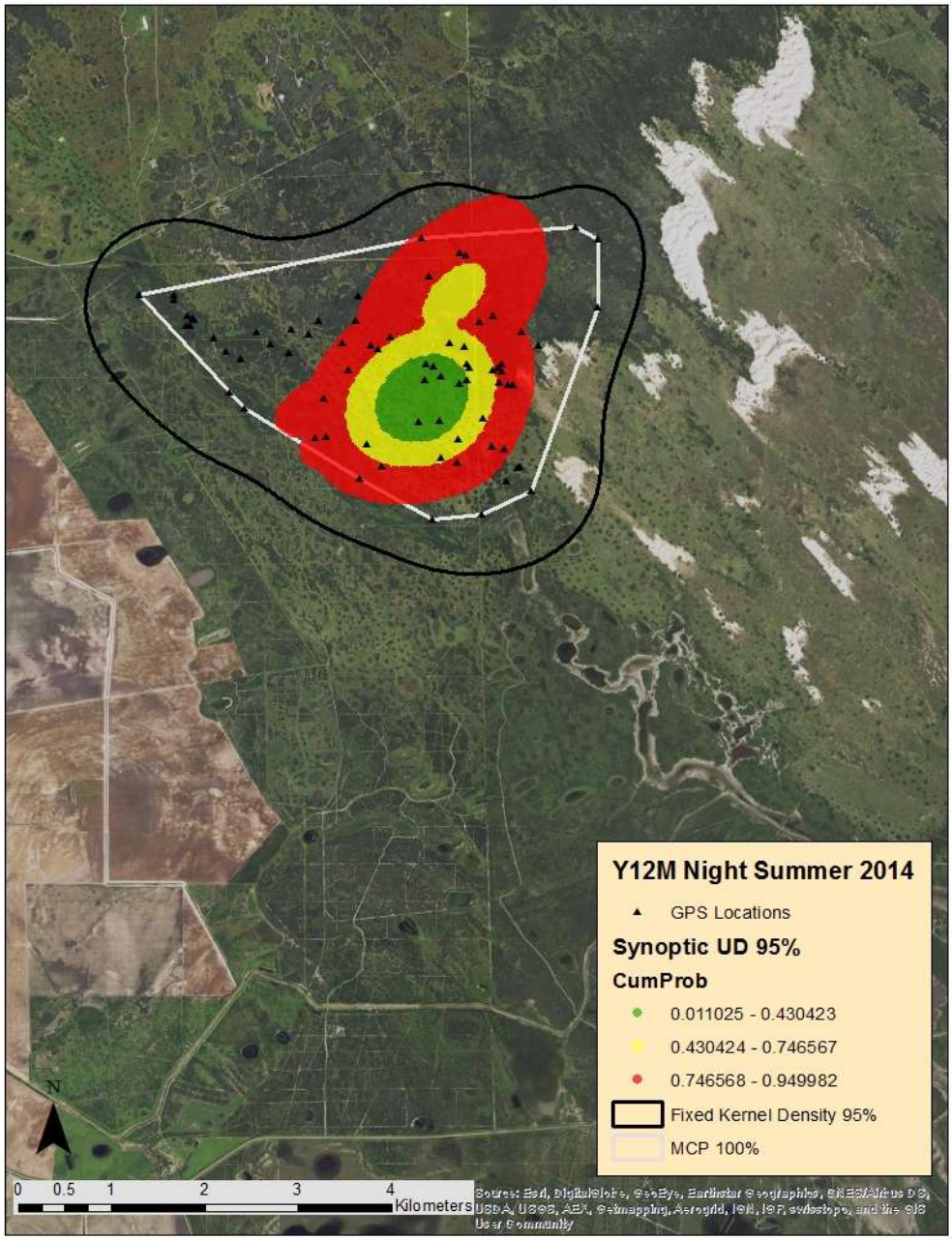

Figure 3.2. Example utilization distribution created using synoptic model compared to 100% minimum convex polygon (MCP 100%) and KDE home range estimates on the East El Sauz Ranch. Utilization distributions were created for ocelot Y12M nocturnal locations collected in summer 2014.

Table 3.6. Comparison of utilization distribution (UD) areas constructed using 95% fixed kernel density (95% KDE), 95% minimum convex polygon (95% MCP) and the synoptic model utilization distribution truncated at the 95% cumulative probability contour (95% Synoptic). Utilization distributions created using nocturnal location data are shaded in gray.

Year	Season	Species	ID	95% KDE	95% MCP	95% Synoptic
2011	Summer	Ocelot	E3M	20.71	9.34	7.03
		Ocelot	E3M	22.58	12.82	8.47
	Winter	Ocelot	E3M	58.84	10.99	9.34
		Ocelot	E3M	30.14	4.62	6.45
2012	Winter	Ocelot	E3M	16.98	6.10	9.13
		Ocelot	E3M	19.12	5.89	8.74
2013	Summer	Bobcat	EB15F	3.59	1.83	3.25
		Bobcat	EB16M	70.47	34.11	4.80
		Bobcat	EB15F	4.68	2.57	4.64
		Bobcat	EB16M	78.48	33.06	3.71
	Winter	Ocelot	E8M	14.85	4.22	7.10
		Ocelot	E8M	NA	3.40	6.17
2014	Summer	Ocelot	E10F	24.62	4.52	4.51
		Ocelot	Y12M	19.20	9.50	5.26
		Ocelot	E10F	25.07	11.12	7.09
		Ocelot	Y12M	17.90	9.06	5.91

Table 3.6 (continued)

Year	Season	Species	ID	95% KDE	95% MCP	95% Synoptic
	Winter	Ocelot	E10F	25.20	6.14	3.80
		Ocelot	Y12M	19.98	6.79	10.17
		Ocelot	E10F	26.28	6.81	5.18
		Ocelot	Y12M	20.88	7.20	8.05
		Bobcat	EB15F	3.19	1.00	2.72
		Bobcat	EB16M	48.97	6.56	3.12
		Bobcat	EB15F	4.61	2.03	4.55
		Bobcat	EB16M	NA	5.88	1.43
2015	Summer	Ocelot	E12F	3.25	0.85	2.42
		Ocelot	E6M	7.11	3.49	5.98
		Ocelot	E12F	4.33	1.23	3.25
		Ocelot	E6M	16.01	9.66	10.73
		Bobcat	EB8M	5.17	1.67	4.40
		Bobcat	EB8M	3.00	1.31	2.64
2016	Winter	Ocelot	E6M	8.24	3.53	6.93
		Ocelot	E6M	11.56	5.21	8.19

Table 3.7. Top model selected by individual for each time period from 2011 to 2012. In cases where 1 or more competing models was within 2 AICc values of the top selected model all competitive models are reported, along with the averaged model (Avg). The fields Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy contain model-averaged parameter estimates (β) with associated standard errors in parentheses (SE). Values obtained from nocturnal data are shaded in gray. Parameter values of -Inf indicate fixed negative infinity resulting from too few observations in a given habitat type for parameter estimation

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
Summer 2011	E3M	5	-12057.6	0.3(0.8)	0.3(0.3)	-1(0.4)	-0.3 (0.4)	0.7(0.9)	7.3(1)	300(0.0)
	E3M	5	-18373.2	0.8(0.4)	0.1(0.2)	-0.6(0.2)	-0.3(0.2)	-Inf (NA)	4.4(1)	300(0.0)
Winter 2011	E3M	5	-511.7	-Inf(NA)	-0.3(0.5)	0.6(0.5)	-Inf(NA)	-0.4(0.7)	18.7(3.7)	300(0.1)
	E3M	3	-1772.4	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	8.3(3.1)	300(0.0)
Winter 2012	E3M	3	-3424.2	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	7.9(2.9)	300(0.0)
		5	-3423.6	-Inf (NA)	0.5(0.3)	-0.8(0.5)	0.3(0.4)	-Inf(NA)	7.9(3.0)	300(0.0)
		Avg		-Inf (NA)	0.5(0.3)	-0.8(0.5)	0.3(0.4)	-Inf(NA)	7.9(3.0)	300(0.0)
	E3M	5	-4911.3	-Inf (NA)	0.7(0.4)	-0.5(0.6)	-1(0.6)	0.8 (0.9)	0.0(2.6)	300(0.0)
		2	-4909.8	NA (NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	NA (NA)	300(0)
		Avg		-Inf (NA)	0.7(0.4)	-0.5(0.6)	-1(0.6)	0.8 (0.9)	0.0(2.6)	300(0.0)

Table 3.8. Top model selected by individual for each time period in 2013. In cases where 1 or more competing models were within 2 AICc values of the top selected model, all competitive models were reported, along with the averaged model (avg). The fields Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy contain model-averaged parameter estimates (β) with associated standard errors in parentheses (SE). Values obtained from nocturnal data are shaded in gray. Parameter values of "-Inf" indicate fixed negative infinity resulting from too few observations in a given habitat type for parameter estimation.

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
Summer 2013	EB15F	5	-9201.3	-Inf(NA)	0.3(0.1)	-0.3(0.1)	-Inf(NA)	-Inf (NA)	6.0(2.2)	300(0.1)
	EB16M	3	3592.7	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	-24.8(2.53)	300(0.0)
	EB15F	5	-6851.9	-0.7(0.3)	0.3(0.2)	0.5(0.2)	-Inf(NA)	-Inf (NA)	-0.1(2.2)	300(0.1)
	EB16M	3	6906.2	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	-33.2(3.2)	300(0.0)
Winter 2013	E8M	2	-2316.9	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	NA(NA)	300(0.0)
		5	-2316.5	-Inf(NA)	0.1(0.3)	-Inf(NA)	-0.1(0.3)	-Inf(NA)	0.0(3.8)	300(0.0)
		3	-2315.0	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0.0(3.8)	300(0.0)

Table 3.8 (continued)

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
		Avg		-Inf(NA)	0.1(0.3)	-Inf(NA)	-0.1(0.3)	-Inf(NA)	-0.0(3.8)	300(0.01)
	E8M	5	-2459.1	-Inf(NA)	0.8(0.6)	-0.6(0.7)	-0.16 (0.64)	-Inf (NA)	1.5(3.2)	300(119.4)
		2	-2458.0	NA(NA)	NA(NA)	NA(NA)	NA (NA)	NA (NA)	NA (NA)	300(104.3)
		Avg		-Inf(NA)	0.8(0.6)	-0.6(0.7)	-0.2(0.6)	-Inf(NA)	1.5(3.2)	300(114.2)

Table 3.9. Top model selected by individual for each time period in summer 2014. In cases where 1 or more competing models were within 2 AICc values of the top selected model all competitive models were reported, along with the averaged model (avg). The fields Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy contain model-averaged parameter estimates (β) with associated standard errors in parentheses (*SE*). Values obtained from nocturnal data are shaded in gray. Parameter values of -Inf indicate fixed negative infinity resulting from too few observations in a given habitat type for parameter estimation.

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
Summer 2014	E10F	5	-6521.4	-Inf(NA)	-2.2 (0.8)	-0.3(0.5)	2.5(0.4)	-Inf(NA)	-10.1(2.7)	300(0)
	Y12M	5	-8802.3	-Inf(NA)	-1.3(0.3)	-1.0(0.3)	0.3(0.3)	2.0(0.6)	-1.5(1.7)	300(0)
	E10F	5	-3404.2	-1.7(0.8)	0.1(0.3)	-0.2(0.3)	0.8(0.3)	0.8(0.6)	-7.1(1.6)	300(0)
		2	702.6	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	300(0)
		3	704.5	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0(2.5)	300(0)
		Avg		-1.7(0.8)	0.2(0.3)	-0.2(0.3)	0.8(0.3)	0.8(0.6)	-7.1(1.6)	300(0)
	Y12M	3	-10073.9	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	-11.9 (1.5)	300(0)

Table 3.10. Top model selected by individual for each time period in winter 2014. In cases where 1 or more competing models was within 2 AICc values of the top selected model all competitive models are reported, along with the averaged model (avg). The fields Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy contain model-averaged parameter estimates (β) with associated standard errors in parentheses (*SE*). Values obtained from nocturnal data are shaded in gray. Parameter values of Inf indicate fixed negative infinity resulting from too few observations in a given habitat type for parameter estimation.

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
Winter 2014	EB15F	5	-235.0	-Inf(NA)	-1.3(0.5)	-0.2(0.5)	-Inf(NA)	1.5(0.7)	5.5(7.1)	300(0)
	EB16M	2	167.1	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	300(0)
		3	169.0	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0(4.1)	300(0.1)
		Avg		NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0(4.1)	300(0)
	E10F	5	-1355.2	-Inf(NA)	-0.6(0.3)	-1.3(0.5)	1.9 (0.4)	-Inf(NA)	-12.7(2.8)	300(0.1)
	Y12M	3	-2155.1	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	-17.8(2.8)	300(0.1)
	EB15F	2	-835.5	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	300(0)

Table 3.10 (continued)

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
		5	-835.1	-Inf(NA)	-0.2(0.2)	0.2(0.2)	-Inf(NA)	-Inf(NA)	3.4(6.4)	300(0)
		Avg		-Inf(NA)	-0.2(0.2)	0.18 (0.24)	-Inf(NA)	-Inf(NA)	3.4(6.4)	300(0)
	EB16M	2	710.1	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	300(0)
		3	712.0	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0(3.4)	300(0)
		Avg		NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	0(3.4)	300(0)
	Y12M	3	-2465.4	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	-7.7(2.3)	300(0)

Table 3.11. Top model selected by individual for each time period from 2015 to 2016. In cases where 1 or more competing models was within 2 AICc values of the top selected model all competitive models are reported, along with the averaged model (avg). The fields Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy contain model-averaged parameter estimates (β) with associated standard errors in parentheses (*SE*). Values obtained from nocturnal data are shaded in gray. Parameter values of -Inf indicate fixed negative infinity resulting from too few observations in a given habitat type for parameter estimation.

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
Summer 2015	EB8M	5	-6352.4	-Inf(NA)	0.3(0.2)	-0.9(0.3)	0.6(0.2)	-Inf(NA)	2.3(2.4)	300(0.2)
	E12F	5	-4745.7	-Inf(NA)	0.5(0.3)	1.1(0.3)	-1.7(0.4)	-Inf(NA)	-4.9(2.9)	300 (0)
	E6M	5	-10389.0	-0.7(0.8)	0.4(0.3)	-0.3(0.3)	0.5(0.3)	-Inf(NA)	-9.5(2.3)	300(0.1)
	EB8M	2	-624.5	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	300(0)
		3	-622.7	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	1.3(3.1)	300(0)
		Avg		NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	1.3(3.1)	300(0)
	E12F	2	-7244.8	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA (NA)	300(0.1)

Table 3.11 (continued)

Season	ID	Model	AICc	Wetland β (SE)	Shrub β (SE)	Grassland β (SE)	Forest β (SE)	Open β (SE)	Waterdist β (SE)	Canopy β (SE)
		3	-7244.1	NA(NA)	NA(NA)	NA(NA)	NA (NA)	NA(NA)	-4.2(3.2)	300(0.7)
		5	-7243.1	-Inf(NA)	0.7(0.4)	-0.2(0.5)	0.1(0.4)	-0.7(0.8)	-4.0(3.4)	300(0)
		Avg		-Inf(NA)	0.7(0.4)	-0.2(0.5)	0.1(0.4)	-0.7(0.8)	-4.1(3.3)	300(0.4)
	E6M	3	-8370.0	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	-2.5(1.0)	300(44.2)
		5	-8368.4	0.3(0.5)	0.4(0.2)	-0.2(0.2)	0.2(0.2)	-0.7(0.4)	-3.1(1.0)	300(42.7)
		Avg		0.3(0.5)	0.4(0.2)	-0.2(0.2)	0.1(0.2)	-0.7(0.4)	-2.7(1.0)	300(43.8)
Winter 2016	E6M	5	-11849.2	-Inf(NA)	1.2(0.3)	-0.3(0.3)	0.4(0.3)	-1.5(0.8)	-4.6(2.2)	300(0)
	E6M	3	-7384.1	NA(NA)	NA(NA)	NA(NA)	NA(NA)	NA(NA)	-2.5(1.0)	300 (46.6)

was positively selected by 2 bobcats and negatively selected by 2 bobcats in the summer. Waterdist was negatively selected by 6 ocelots in the summer. During the winter, 2 bobcats showed negative association with Waterdist, and during the winter, 6 ocelots showed negative association with Waterdist and 3 ocelots showed positive association with Waterdist. More individuals showed a negative association with Waterdist, indicating that ocelots and bobcats may avoid areas that are far from perennial water sources. Of the binary land cover covariates, Forest was the land cover type most positively selected by ocelots. In the summer, 3 bobcats showed positive selection for Forest, although no bobcats showed positive selection for Forest in the winter (Table 3.12).

Step-selection function results

For each individual, I considered the best SSF model to be the one with the lowest AIC value. There was no clear top model across all individuals. Model 1 was selected as top model for 1 individual, model 6 was the top model for two individuals, model 3 was the top model for 1 individual, model 4 was the top model for 1 individual, and model 8 was the top model for 2 individuals.

Composite SSF models created for each individual revealed positive association with Canopy by 3 ocelots, and negative association with Centerdist by 2 ocelots and 2 bobcats. I found negative association with Linear_Cnpy by 1 ocelot, and positive association with Linear_Cnpy by 2 bobcats. Composite models revealed positive association with the interactive term Canopy*Sun_alt by 1 ocelot, negative association with the interactive term Canopy*Moon_illum by 1 ocelot and 1 bobcat, and positive association with the interactive term Canopy*Moon_illum by one ocelot and one bobcat (Table 3.13).

Table 3.12. Counts of individuals (separated by diel period and season) with model averaged parameter 85% confidence intervals (85% CI) overlapping zero (0), greater than zero (+), and less than zero (-). The fields, Wetland, Shrub, Grassland, Forest, Open, Waterdist, and Canopy provide of counts of individuals with 85% CI falling within the categories 0, -, and +. Model parameters with 85% CI overlapping zero were considered to be used in proportion to their availability, those that were entirely negative indicated avoidance, and those that were entirely positive indicated positive selection. Bobcats are shaded in gray; ocelots are unshaded.

Season	85% CI	Wetland	Shrub	Grassland	Forest	Open	Waterdist	Canopy
Summer	0	9	11	12	10	10	12	0
	-	1	2	3	1	0	4	0
	+	0	3	1	3	0	2	18
Summer	0	4	7	8	6	4	6	0
	-	2	3	4	2	1	7	0
	+	0	3	1	5	2	0	13
Winter	0	6	8	9	8	3	10	0
	-	0	2	3	1	2	2	0
	+	1	3	2	0	1	2	14
Winter	0	3	12	13	14	5	10	0
	-	1	2	6	2	1	7	0
	+	0	6	0	3	1	3	20

Table 3.13. Model averaged coefficients (β) and standard errors (SE) for each individual. Coefficients for which 95% CI overlaps a positive number are marked with a single asterisk (*). Those for which the 95% CI overlaps a negative number are marked with a double asterisk (**). All others have 95% CI overlapping zero.

ID	Canopy		Centerdist		Linear_Cnpy		Canopy*Sun_alt		Canopy*Moon_illum	
	β	SE	β	SE	β	SE	β	SE	β	SE
E10F	0.021*	0.004	-0.001	0.001	-0.001	0.008	0.000	0.000	0.000	0.007
E12F	0.022*	0.004	-0.002**	0.001	-0.021**	0.006	0.000	0.000	-0.002	0.007
E6M	0.012*	0.002	-0.002**	0.000	0.001	0.004	0.000*	0.000	-0.012**	0.004
EB15F	0.002	0.002	-0.001**	0.000	0.014*	0.003	0.000	0.000	0.004	0.003
EB16M	-0.003	0.002	0.000	0.000	0.014*	0.004	0.000	0.000	-0.011**	0.004
EB8M	-0.006	0.01	-0.001**	0.001	-0.01	0.006	0.000	0.000	0.022*	0.008
Y12M	0.006	0.005	-0.001	0.000	0.003	0.005	0.000	0.000	0.019*	0.006

DISCUSSION

Home range areas produced using KDE were larger than those produced using the synoptic model. This may indicate that the synoptic model provides a more realistic depiction of space use than kernel methods, which can include areas not used by an individual. Synoptic model UDs may also help explain ocelot and bobcat coexistence on the EESR. Ocelots and bobcats on the EESR show substantial home range overlap at the 95% KDE contours; however, this overlap decreases substantially with synoptic model UDs truncated at the 95% cumulative probability contour.

The same number of ocelots avoided the Shrub land cover type as selected for it. On the EESR, the Shrub land cover type is characterized by patches of woody vegetation interspersed with more open ground cover. Previous studies have found ocelots closely associated with thornshrub communities (Tewes 1986, Bradley and Fagre 1988, Cain et al. 2003, Harveson et al. 2004, Haines et al. 2006). The results of this study do not indicate that Tamaulipan thornshrub is unimportant to ocelots on the EESR. The Forest land cover type, occurring primarily in the northwestern portion of the EESR, contained a substantial thornshrub component, and can be considered Tamaulipan thornshrub with an emergent oak canopy.

The Forest land cover type appeared to be more important to ocelots than any other cover type. On the EESR, this land cover type consists primarily of dense stands of live oak with a mid-level shrub layer of varying densities. Compared to the Shrub land cover type, areas classified as Forest had higher tree heights and a more closed canopy. Horne et al. (2009) found closed canopy habitats to be important to ocelots, with ocelots selecting areas with >75% canopy cover, and bobcats selecting areas with <75% canopy cover on LANWR. The Laguna Atascosa National Wildlife Refuge lacks the extensive stands of live oak occurring in the northwestern

portion of the EESR, and areas with >75% canopy cover in LANWR were dominated by shrub species including granjeno, crucita, Berlandier fiddlewood (*Citharexylum berlandieri*), honey mesquite, and desert olive. Horne et al. (2009) suggested that areas of dense thornshrub on LANWR may be substantially denser than areas reported as dense elsewhere. This discrepancy may account for the variable selection by ocelots of the Shrub cover type on the EESR, as this cover type may be more open than that on LANWR. Alternatively, areas with a mature oak canopy may provide better habitat to ocelots than thornshrub areas lacking this emergent oak layer. Future ocelot conservation efforts should include management and conservation of Forest and Shrub land cover types.

Whereas the Canopy covariate was universally positively selected by ocelots and bobcats at the third order of selection, only ocelots showed a positive association with Canopy at the fourth order of selection. This result highlights the importance of determining scale when conducting studies of animal resource selection. One possible explanation for this discrepancy is that both ocelots and bobcats associate closely with closed canopy habitats at the home range level, using these areas in preference to more open land cover types in the vicinity, with ocelots more dependent than bobcats on dense canopy cover during travel periods.

I expected ocelots to show strong positive selection of the Linear_Cnpy covariate, yet this was not the case. This result may suggest that current GPS sampling regimes (i.e., 30-min intervals) are too far apart to accurately determine the actual movement paths of ocelots and bobcats, and the composition of vegetation types along the true movement path correlates only weakly with the composition of vegetation types along a straight-line path connecting 2 consecutive GPS points.

The covariate Centerdist was introduced in the models to account for the tendency of individuals to remain tied to a specific geographic area. I expected individuals to show a negative relationship with this covariate, with probability of using a location inversely proportional to distance from the home range center. Two ocelots and 2 bobcats showed 95% CIs for this covariate that were negative, seemingly confirming this hypothesis. However, 3 other individuals (2 ocelots, 1 bobcat) had 95% CIs overlapping zero, indicating that distance from home range center had no effect on probability of use.

LITERATURE CITED

Adams, L. and S.D. Davis. 1967. The internal anatomy of home range. Journal of Mammalogy 48:529-536.

Aebischer, N. J., P. A. Robertson, and R. E. Kenward. 1993. Compositional analysis of habitat use from animal radio-tracking data. Ecology 74:1313-1325.

Arnold, T. 2010. Uninformative parameters and model selection using Akaike's information criterion. Journal of Wildlife Management 74:1175-1178.

Beyer, H. L., D. T. Haydon, J. M. Morales, J. L Frair, M. Hebblewhite, M. Mitchell, J. Matthiopoulos. 2010. The interpretation of habitat preference metrics under use-availability designs. Philosophical Transactions of the Royal Society B 365: 2245-2254.

Blankenship, T. L. 2000. Ecological response of bobcats to fluctuating prey populations on the Welder Wildlife Foundation Refuge. Doctoral Dissertation. Texas A&M University-Kingsville, USA.

Boyce, M., and L. McDonald. 1999. Relating populations to habitats using resource selection function. Trends in Ecology and Evolution 14:268-272.

Bradley, L. C., and D. B. Fagre. 1988. Movements and habitat use by coyotes and bobcats on a ranch in southern Texas. Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies 42:411-430.

Breiman, L. 2001. Random Forests. Machine Learning 45:5-32.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. Springer, New York, NY, USA.

Cagnacci, F., L. Boitani, R. A. Powell, and M. S. Boyce. 2010. Animal ecology meets GPS-based radiotelemetry: a perfect storm of opportunities and challenges. Philosophical Transactions of the Royal Society B 365:2157-2162.

Cain, A. T., V. R. Tuovila, D. G. Hewitt, and M. E. Tewes. 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189-197.

Congalton, R. G. and K. Green. 1999. Assessing the Accuracy of Remotely Sensed Data: Principles and Practices. Taylor & Francis Group, Boca Raton, United States.

Coulon, A., N. Morellet, M. Goulard, B. Cargnelutti, J. M. Angibault, and A. J. M. Hewison. 2008. Inferring the effects of landscape structure on roe deer (*Capreolus capreolus*) movements using a step selection function. Landscape Ecology 23:603-614.

Federal Register. 1982. CFR Part 17. Vol 47, No. 140.

Fieberg J., J. Matthiopoulos, M. Hebblewhite , M. S. Boyce , and J. L. Frair. 2010 Correlation and studies of habitat selection: problem, red herring, or opportunity? Philosophical Transactions of the Royal Society B. 365:2233–2244.

Fortin, D., H. L. Beyer, M. S. Boyce, D. W. Smith, T. Duchesne, and J. S. Mao. 2005. Wolves influence elk movements: behavior shapes a trophic cascade in Yellowstone National Park. Ecology 86:1320-1330.

Gause, G. F. 1934. The struggle for existence. Hafner, New York, USA.

Haines, A. M., M. E. Tewes, L. L. Laack, W. E. Grant, and J. H. Young. 2005. Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States. Biological Conservation 126:512–522.

Haines, A. M., M. E. Tewes, L. L. Laack, W. E. Grant, and J. H. Young. 2006. A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. Biological Conservation 132:424-436.

Harveson, P. M., M. E. Tewes, G. L. Anderson, and L. L. Laack. 2004. Habitat use by ocelots in South Texas: implications for restoration. Wildlife Society Bulletin 32:948–954.

Horne, J. S., E. O. Garton, and J. L. Rachlow. 2008. A synoptic model of animal space use: simultaneous estimation of home range, habitat selection, and inter/intra-specific relationships. Ecological Modeling 214:338-348.

Horne, J. S., A. M. Haines, M. E. Tewes, and L. L. Laack. 2009. Habitat partitioning by sympatric ocelots and bobcats: implications for recovery of ocelots in southern Texas. The Southwestern Naturalist 54:119–126.

Jackson, V. L., L. L. Laack, and E. G. Zimmerman. 2005. Landscape metrics associated with habitat use by ocelots in South Texas. Journal of Wildlife Management 69:733-738.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61:65-71.

Kelley, D., and C. Richards. 2016. oce: Analysis of Oceanographic Data. R package version 0.9-19. https://CRAN.R-project.org/package=oce

Leblond, M., C. Dussault, and J. P. Ouellet. 2010. What drives fine-scale movements of large herbivores? A case study using moose. Ecography 33:1102–1112.

Lele, S. R., and J. L. Keim. 2006. Weighted distributions and estimation of resource selection probability functions. Ecology 87:3021-3028.

Lonard, R. I., and F. W. Judd. 1985. Effects of a severe freeze on native woody plants in the Lower Rio Grande Valley, Texas. Southwestern Naturalist 30:397-403.

Manly, B. F. J., L. L. McDonald, D. L. Thomas, T. L. McDonald, and W. P. Erickson. 2002. Resource selection by animals: statistical design and analysis for field studies. Kluwer Academic Press, Boston, MA, USA.

Mayor, S. J., D. C. Schneider, J. A. Schaefer, and S. P. Mahoney. 2009. Habitat selection at multiple scales. Ecoscience 16:238-247.

McClean, S. A., M. A. Rumble, R. M. King, and W. L. Baker. 1998. Evaluation of resource selection methods with different definitions of availability. Journal of Wildlife Management 62:793-801.

Moser, B. W., and E. O. Garton. 2007. Effects of telemetry error on space use estimates using a fixed-kernel density estimator. Journal of Wildlife Management 71:2421-2426.

Navarro-Lopez, D. 1985. Status and distribution of the ocelot (*Felis pardalis*) in south Texas. Thesis, Texas A&I University, Kingsville, Texas, USA.

Neu, C. W., C. R. Byers, and J. M. Peek. 1974. A technique for analysis of utilization-availability data. Journal of Wildlife Management 38:541-545.

Roberts, N. M., and S. W. Crimmins. 2010. Bobcat population status and management in North America: evidence of large-scale population increase. Journal of Fish and Wildlife Management 1:169-174.

Rolley, R. E. 1987. Bobcat. Pages 671-681 *in* M. Novack, J. A. Baker, M. E. Obbard, and B. Malloch, editors. Wild furbearer management and conservation in North America. Ministry of Natural Resources, Toronto, Ontario, Canada.

Shindle, D. B., and M. E. Tewes. 1998. Woody species composition of habitats used by ocelots (*Leopardus pardalis*) in the Tamaulipan Biotic Province. The Southwestern Naturalist 43:273-279.

Shindle, D. B., and M. E. Tewes. 2000. Immobilization of wild ocelots with tiletamine and zolazepam in southern Texas. Journal of Wildlife Disease 36:546–550.

Sunquist, M., and F. Sunquist. 2002. Wild Cats of the World. University of Chicago Press, Chicago, USA.

Switalski, A. 2013. The effects of artificial water sources on small mammal communities. M.S. Thesis. Arizona State University, Tempe, USA.

Tewes, M. E., 1986. Ecological and behavioral correlates of ocelot spatial patterns. Dissertation, University of Idaho, Moscow, USA.

Tewes, M. E., and D. D. Everett. 1986. Status and distribution of the endangered ocelot and jaguarundi in Texas. Pages 147-158 *in* Miller, S. D., and D. D. Everett, editors. Cats of the world: biology, conservation, and management. National Wildlife Federation, Washington, DC, USA.

Tewes, M. E., and D. J. Schmidley. 1987. The Neotropical felids: jaguar, ocelot, margay, and jaguarondi. In Wild furbearer management and conservation in North America. Ontario Ministry of Resources. Ottawa, Canada.

Thurfjell, H., S. Ciuti, and M. S. Boyce. 2014. Applications of step-selection functions in ecology and conservation. Movement Ecology 2:4.

USGS and U.S. Board of Geographic Names. 2014. GNIS Non-Populated Places. <http://geonames.usgs.gov> accessed May 5, 2014.

Worton, B. J. 1987. A review of models of home range for animal movement. Ecological Modelling 38:277-298.

CHAPTER IV

CHARACTERIZATION OF MAJOR HISTOCOMPATIBILITY COMPLEX DIVERSITY AMONG TAMAULIPAN OCELOT POPULATIONS

INTRODUCTION

Small, isolated populations are vulnerable to local extinctions because of demographic factors and environmental stochasticity (Lacy 1987). In addition to these threats, the loss of genetic variation due to genetic drift and inbreeding can reduce the fitness of small populations and decrease time until extinction (Brook et al. 2002). Declining populations may enter a positive feedback loop, known as an extinction vortex, where genetic drift leads to losses of genetic variation, leading to further reductions in survival and reproductive rates (Blomqvist et al. 2010). Although genetic drift can overwhelm natural selection in small populations, balancing selection can maintain variation at functionally important loci (Aguilar et al. 2004).

Studies of genetic variation in wildlife populations often use neutral genetic markers, such as microsatellites and mitochondrial sequences (Kirk and Freeland 2011) to index variation. Commonly used surrogates for overall genetic variation, such as heterozygosity, population size, and quantitative genetic variation, have been found to correlate with population fitness (Reed and Frankham 2003). However, recent studies have found higher levels of genetic variation among certain regions of the genome, most notably the major histocompatibility complex (MHC), than would otherwise be predicted solely through the use of neutral markers (Hedrick et al. 2000; Goda et al. 2010; Ujvari and Belov 2011).

The MHC is a large genomic region occurring in all jawed vertebrates that encodes genes which play a role in the adaptive immune response and are critical for self/nonself

discrimination (Klein and Figueroa 1986). The MHC is associated with infectious disease immunity in vertebrates (Hedrick and Kim 1999) and is one of the most variable regions in the genome, with over 1,000 alleles found in certain loci of the human MHC (Ujvari and Belov 2011). The MHC is divided into classes I, II, and III. Class I MHC molecules are found on the surfaces of all nucleated cells in the body, and present cytosol-derived peptide fragments from intracellular pathogens on cell surfaces to T cells (Cresswell 2005). Class II MHC molecules are expressed on the surfaces of macrophages, dendritic cells, and B cells, and present peptides from extracellular pathogens to T cells (Delves et al. 2006). Class III of the MHC encodes a variety of proteins involved in the immune response that do not play a direct role in antigen presentation (Cresswell 2005).

The highest levels of variation in the MHC are seen in antigen binding sites within classes I and II (Mayer and Brunner 2007). Genes within classes I and II of the MHC often exist as multiple repeats, which likely arose through gene duplication events (Ota and Nei 1994; Dawkins et al. 1999; Kulski et al. 2002). The class I and II genes are simultaneously expressed on the surfaces of antigen-presenting cells (Kumanovics et al. 2003). The number of unique MHC loci varies among species, populations, and individuals (e.g., Llaurens et al. 2012; Lighten et al. 2014b).

The MHC is exposed to balancing selection within populations which tends to maintain high levels of polymorphism at MHC loci (Clarke and Kirby 1966; Apanius et al. 1997; Penn and Potts 1999). High allelic diversity at MHC loci, particularly those coding for antigen binding sites, is correlated with the immunological fitness of populations (Doherty and Zingernagel 1975; O'Brien and Evermann 1988, Sommer 2005).

The ocelot (*Leopardus pardalis*) has suffered a dramatic reduction in distribution and population size in the United States since the 1800s, primarily due to anthropogenic removal of native Tamaulipan thornshrub throughout much of its former range (Jahrsdoerfer and Leslie 1988; Schmidly 2002; Schmidly 2004). There are currently 2 known breeding populations of ocelots in the United States: 1 in and around the Laguna Atascosa National Wildlife Refuge (LANWR) in Cameron County, Texas, and the other on a series of private ranches in Willacy County, Texas (Willacy; Navarro-Lopez 1985; Tewes and Everett 1986; Laack 1991; Haines et al. 2006). Though these populations are separated by <30 km, no dispersals between the 2 populations have been observed in over 30 years of monitoring (Tewes 1986; Laack 1991; Haines et al. 2005; Laack et al. 2005; Haines et al. 2006). Conservation concerns identified through long-term monitoring of ocelots in Texas are removal and fragmentation of thornshrub habitat (Tewes and Everett 1986; Jahrsdoefer and Leslie 1988; Haines et al. 2006), road mortality (Haines et al. 2005), inbreeding (Janecka et. al 2008), and loss of genetic diversity (Walker 1997; Janecka et al. 2011).

Though the ocelot occurs in a variety of habitats throughout Central and South America, including tropical forest, mangrove forests, coastal marshes, and savanna grasslands (Emmons 1988; Emmons et al. 1989; Sunquist and Sunquist 2002), it is widely considered a habitat specialist in South Texas, highly dependent on dense woody communities with ≥85% canopy cover (Navarro-Lopez 1985; Tewes 1986; Laack 1991; Horne et al. 2009).

Effects of habitat fragmentation are predicted to be more severe for habitat specialists, such as ocelots, than for generalists, with specialists showing a marked reduction in dispersal and gene flow at relatively lower levels of habitat degradation than generalists (Didham 2010). The landscape between the 2 remnant ocelot populations in South Texas is dominated by

extensive cotton and sorghum fields with greatly reduced native plant cover (Janecka et al. 2011), making natural ocelot dispersals between these 2 populations difficult. Additionally, both the LANWR and Willacy ocelot populations are ≥150 km from the nearest known ocelot populations in Tamaulipas, Mexico (Caso 1994; Janecka et al. 2007), and the intervening landscape is one of the most heavily human-influenced regions in the world (Sanderson et al. 2002). Ocelot populations in Texas seem isolated from those in Mexico, and the only suspected case of dispersal from Mexico to Texas in >30 years of monitoring remains a single individual captured on the Santa Ana National Wildlife Refuge, Texas, along the Rio Grande River (Janecka et al. 2011).

Several studies have found low levels of neutral genetic variation within the remaining ocelot populations in the United States (Walker 1997; Janecka et al. 2008; Janecka et al. 2011; Korn 2013; Janecka et al. 2014). Using autosomal microsatellites, Janecka et al. (2011) found a reduction in mean heterozygosity in Texas ocelots compared to ocelots sampled in Tamaulipas, Mexico. Heterozygosity values were highest in ocelot populations from Tamaulipas, Mexico, lowest in the LANWR population, with intermediate values in the Willacy population (Janecka et al. 2011). Additionally, ocelots from Mexico had higher levels of mitochondrial diversity than those in Willacy and LANWR (Janecka et al. 2011).

Effective population sizes (N_E) were low for the Willacy and LANWR populations with a lower effective population size in Willacy (N_E = 2.9-3.1) than in LANWR (N_E = 8.0-13.9; Janecka et al. 2008). The lower effective population size in Willacy, despite overall higher levels of heterozygosity and allelic richness in Willacy than in LANWR, was attributed to a sharp recent reduction in genetic diversity, possibly due to a bottleneck, suffered in Willacy (Janecka et al. 2008). Evidence supports that this loss of variation occurred during the 20th

century as a result of anthropogenic factors (Janecka et al. 2014). Effective population size is inversely proportional to rates of allelic loss due to drift (Charlesworth 2009), and the values calculated by Janecka et al. (2008) strongly suggest that these populations will only continue to lose genetic diversity over time.

Critically low levels of genetic variation, combined with a complete lack of dispersals between ocelot populations in the United States, suggest that translocations could be a viable option for ensuring the continued existence of this felid in the United States. In 2008, the Ocelot Translocation Team was formed by the U.S. and Wildlife Service as a subcommittee of the Ocelot Recovery Team, with the purpose of assessing the feasibility of translocating ocelots into Texas from northern Mexico or reciprocally between the two Texas populations. Translocations hold potential for improving the genetic fitness of isolated and declining wildlife populations and even low levels of introgression can mimic the effects of natural migrations and increase genetic diversity (Whiteley et al. 2015). Most studies in conservation genetics have used neutral genetic markers, such as microsatellites and mitochondrial DNA, to estimate overall genetic variation, study demographic history, and make decisions related to translocations (Kirk and Freeland 2011). Ujvari and Belov (2011) argued that conservation programs should make use of as many genetic markers as possible, including functionally important MHC genes.

Previous studies of ocelot genetic diversity have examined neutral variation, using either microsatellite markers or mitochondrial sequencing (e.g., Walker 1997; Janecka et al. 2008; Janecka et al. 2011; Korn 2013; Janecka et al. 2014). Measuring ocelot genetic variation at functionally important MHC loci has the potential to guide translocation efforts and offer an improved understanding of the evolutionary forces influencing these populations (Funk et al.

2012). The primary goal of this study was to measure historical and contemporary genetic variation at exon 2 of the DRB, a functionally important portion of the MHC, for the LANWR and Willacy ocelot populations in the United States and to compare these results to levels of variation in Tamaulipas, Mexico. Specific objectives were to (1) identify private alleles occurring in each population, (2) test for the historical signature of positive selection on DRB molecules, (3) compare measures of neutral diversity to MHC diversity at the population and individual level, (4) estimate structural similarity of MHC protein products through supertype analysis, and (5) determine if alleles or supertypes have been lost over time due to drift.

MATERIALS AND METHODS

Study area

Study areas consisted of three locations within southern Texas, USA, and one location in Tamaulipas Mexico (Fig. 4.1). Within Willacy County, Texas, ocelots have been documented on two private ranches: the Yturria Ranch and the East El Sauz Ranch (EESR). Pedigree analysis (Korn 2013) has confirmed that the ocelots occurring on the Yturria Ranch and EESR are part of the same population (Willacy), and are, therefore, treated as such in this study. Ocelots on the Yturria Ranch were captured near a 2 km^2 conservation easement located northeast of Raymondville, Texas, USA. Ocelots on the EESR were captured either in the northern habitat patch, which is dominated by live oak (*Quercus virginiana*) and dense thornshrub, or in the southern habitat patch, which consists of a more open environment, with patches of honey mesquite (*Prosopis glandulosa*), huisache (*Acacia farnesiana*), and granjeno (*Celtis pallida*).

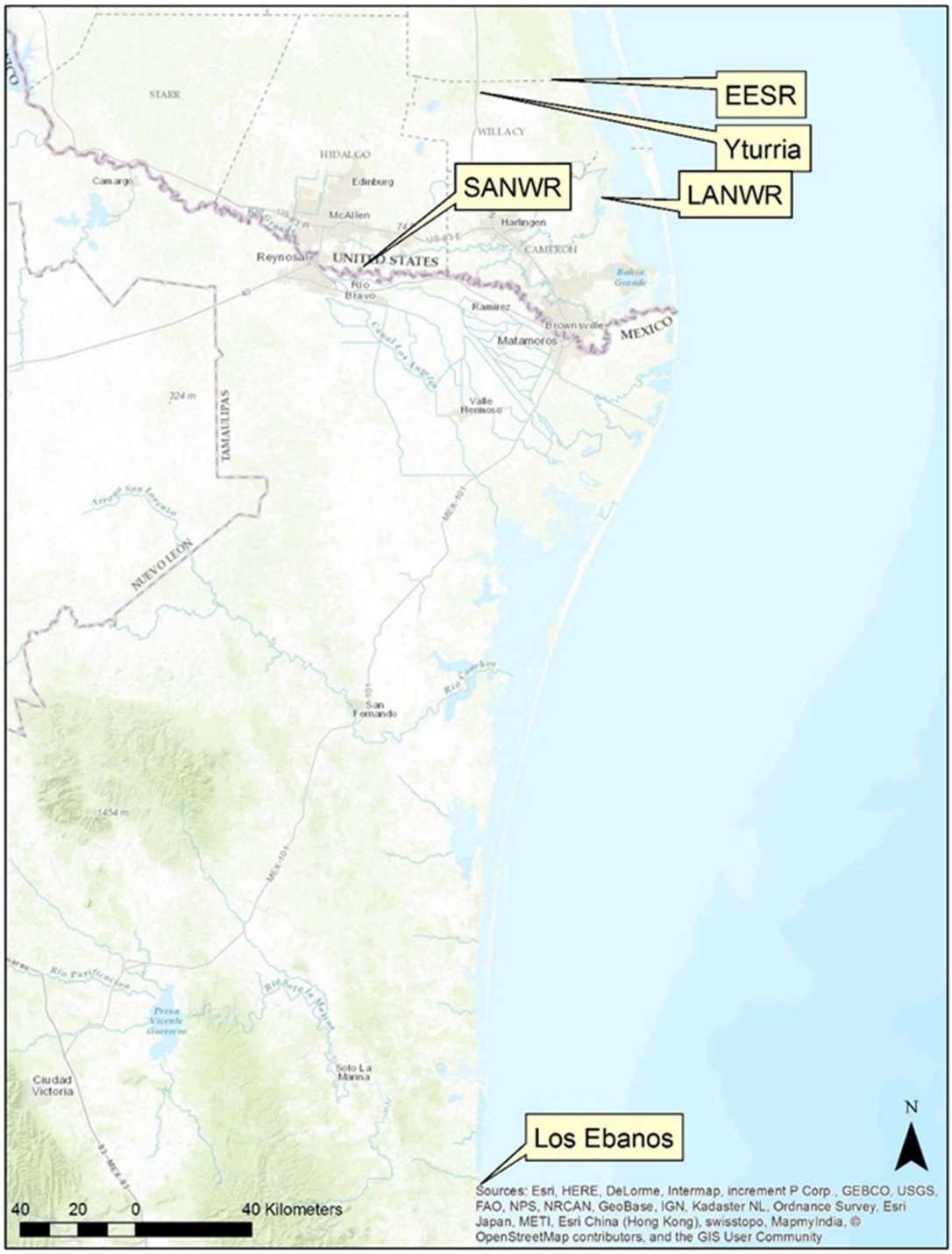
EESR
Yturria
LANWR
SANWR
Los Ebanos
STARR
WILLACY
HIDALGO
CAMERON
Camargo
Edinburg
McAllen
Harlingen
Reynosa
UNITED STATES
Rio Bravo
Ramirez
Brownsville
Matamoros
MEXICO
Valle Hermoso
TAMAULIPAS
NUEVO LEON
San Fernando
Ciudad Victoria
Soto La Marina
Presa Vicente Guerrero
Rio Purificacion
Rio Soto la Marina
Rio Conchos
Canal los Angeles
Bahia Grande
324 m
1454 m
MEX-101
MEX-40
MEX-97
N
40 20 0 40 Kilometers
Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 4.1. Locations from which samples were collected. Sampling locations were: East El Sauz Ranch (EESR), Willacy County, Texas, USA; Yturria Ranch (Yturria), Willacy County, Texas, USA; Santa Ana National Wildlife Refuge (SANWR), Hidalgo County, Texas, USA; Laguna Atascosa National Wildlife Refuge (LANWR), Cameron County, Texas, USA; Los Ebanos Ranch (Los Ebanos), Tamaulipas, Mexico.

Within Cameron County, the primary portion of the known ocelot population occurs on LANWR, a 190 km^2 wildlife refuge northeast of Los Fresnos, Texas, USA. This population is considered to be entirely separate from the population occurring in Willacy as no dispersals have been documented between the LANWR and Willacy ocelot populations in >30 years of monitoring.

To compare MHC variation of ocelots occurring in the United States with those in Mexico, I included in my analysis ocelots captured in the 1990s on the Los Ebanos Ranch, in southern Tamaulipas, Mexico (Caso 2013). An ocelot captured on the Santa Ana National Wildlife Refuge (SANWR) was grouped with the Mexican ocelots based on previous microsatellite analysis which identified this individual as a disperser from Mexico (Janecka et al. 2011).

Sample collection

Genetic samples used in this study were collected during several radio-telemetry and GPS-telemetry studies dating from 1984 to 2015 (e.g., Tewes 1986; Laack 1991; Caso 1994; Shindle and Tewes 1998; Haines et al. 2005). Live trapping was carried out using wire Tomahawk (Tomahawk Live Trap Co., Tomahawk, WI) box traps with attachments for live bait. Live trapping followed the standard capture and sedation protocols (Tewes 1986; Beltran and Tewes 1995; Shindle and Tewes 2000), approved by the University Institutional Animal Care and Use Committee (2009-12-17A, 2012-12-20B). All trapping conducted in Mexico was approved by the Mexican Ministry of Environmental and Natural Resources (SEMARNAT; permit DGVS-10022-2004). Blood was collected and placed in Longmire's lysis buffer (Longmire et al. 1997) and stored at room temperature until DNA extraction. Tissue samples

were collected from road-killed individuals and frozen at -20° C until DNA extraction. Genetic material was extracted from blood and tissue using a Qiagen DNA extraction kit (Qiagen DNeasy, Valencia, California, USA).

Samples included 36 individuals from LANWR (14 contemporary, 22 historical), 4 individuals from Los Ebanos Ranch, near Soto la Marina, Tamaulipas, Mexico, 1 individual from Santa Ana National Wildlife Refuge, and 24 individuals from Willacy (8 contemporary, 16 historical). Korn (2013) divided LANWR and Willacy samples into 3 time-groups (1991-1998, 1999-2005, and 2006-2013), finding differences in Allelic Richness and heterozygosity between time-groups. Due to fewer samples in my analysis, I separated ocelot populations only into historical and contemporary, using the year 2000 as a threshold between time-groups. Samples collected prior to the year 2000 were considered historical. Those collected during or after the year 2000 were considered contemporary. Each sample was assigned to 1 of 5 groups: LANWR Historical, LANWR Contemporary, Willacy Historical, Willacy Contemporary, and Mexico.

PCR amplification

I amplified a 238 bp fragment of exon 2 of the ocelot DRB using the degenerate forward primer: DRB219m: 5'-CCACACAGCACGTTTC(C/T)T-3' and the reverse primer: DRB61a: 5'-CCGCTGCACTGTGAAGCT-3'. These primers have been used to amplify exon 2 of the DRB in domestic cats (*Felis catus*; Yuhki and O'Brien 1997), Eurasian Lynx (*Lynx lynx*; Wang et al. 2009), cheetahs (*Acinonyx jubatus*; Drake et al. 2004), Bengal tigers (*Panthera tigris tigris*; Pokorny et al. 2010), clouded leopard (*Neofelis nebulosa*), leopard (*Panthera pardus*), and Amur tiger (*Panthera tigris altaica*; Wang et al. 2008). To allow

multiplexing of all individuals on a single MiSeq (Illumina, San Diego, CA) sequencing run, forward and reverse primers were modified with 7 unique, 10-bp multiplex identifier (MID) sequences (Roche Diagnostics Technical Bulletin TCB No.005-2009). Additionally, unmodified forward and reverse primers were included and treated as unique barcodes.

Because the samples used in this study were collected by different researchers, over >30 years, using different extraction and storage protocols, nucleic acid concentration and quality varied widely between individuals. I attempted to separate samples into pools of similar individuals based on DNA concentration and extraction date. I optimized polymerase chain reaction (PCR) for each pool by varying concentrations of template DNA and primers.

Amplification was carried out in 25-µL reactions, containing 5 µL Phusion Hot Start Flex 2X Master Mix (New England Biolabs, Ipswich, MA), 0.5 µL forward primer (10 µM concentration), 0.5 µL reverse primer (10 µM concentration), between 0.5 and 0.75 µL template DNA (various concentrations), and 0.75 µL Dimethyl sulfoxide (DMSO; 100% concentration). I used a touchdown PCR protocol that consisted of a 30-sec denaturation at 98° C, 10 touchdown cycles, 25 regular PCR cycles, and a hold at 4° C. The 10 touchdown cycles consisted of 10 sec at 98° C, a 20-sec annealing phase that started at 70° C and dropped by 1° C each cycle, and a 15-sec extension at 72° C. The 25 regular PCR cycles consisted of 10-sec at 98° C, 20-sec at 61.8° C, and 15-sec at 72° C.

I ran all PCR products on a 2% agarose gel, pre-loaded with BioStar 6X loading dye (BioStar Lifetech, Bangalore, India) to verify PCR success. On samples showing bands of correct length, I performed a bead clean-up using AMPure XP beads (Beckman Coulter, Beverly, Massachusetts) at a volumetric ratio of 1:1. Any samples that showed nonspecific

bands that could not be removed by adjusting reaction mixture were excluded from the analysis.

Library preparation and sequencing

I quantitated all amplicons with a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices, CA, USA). I combined PCR amplicons into equimolar pools containing only samples that were amplified with unique MID combinations. Each pool contained PCR products from ≤64 individuals, yet, because of the MID barcoding scheme used, each pool could be treated as a single sample in subsequent library preparation steps. I used the Illumina TruSeq PCR-Free HT Library Prep Kit (Illumina Technical Bulletin No. 770-2013-001) to ligate index sequences and Illumina P5 and P7 adaptors. Each sample, therefore, contained 2 levels of barcoding, which were used to demultiplex samples and identify individuals: Roche 10-bp MID sequences attached to primers, and Illumina index sequences which were included in the TruSeq adapters. Sequencing was performed using 250 bp paired-end sequencing on an Illumina MiSeq instrument.

Bioinformatics and genotyping

All sequencing reads were delivered as FASTQ files, demultiplexed according to the Illumina TruSeq index used. I assembled paired-end sequencing products based upon the region of overlap using FLASH (Magdoc and Salzberg 2011). I assessed per-base quality scores using PRINSEQ (Schmeider and Edwards 2011), and used AmpliCLEAN (Sebastian et al. 2015) to filter reads by mean quality score, retaining sequences with at least a mean of Q.30 (99.99% base call accuracy). I used AmpliSAS (Sebastian et al. 2015) to demultiplex files by

individual according to unique MID combinations and obtained counts of unique sequences occurring in each individual.

Yuhki et al. (2008) found the domestic cat (*Felis silvestris catus*) MHC to contain 3 functional DRB genes and 1 DRB pseudogene, which would allow a maximum of 8 unique DRB sequences in an individual. I conservatively allowed for the presence of $\leq$8 ocelot DRB loci, thus allowing a maximum of 16 unique DRB sequences per individual. This allowed for the possibility of a duplication of a large MHC region, spanning all feline DRB loci. Next-generation sequencing allows more sensitive detection of alleles in an individual than traditional approaches (i.e., cloning and Sanger sequencing; Lighten et al. 2014b), yet the higher error rates combined with greater sequencing depth increases the risk of misidentifying artifacts as true alleles (Lighten et al. 2014b). Therefore, it is necessary to implement strict allele validation thresholds to separate true alleles from PCR or sequencing artifacts (Lighten et al. 2014b). I used the degree of change (*DOC*) method described by Lighten et al. (2014a) to demarcate break points between true alleles and artifacts and genotype individuals.

For each individual, I calculated the total read depth of the 16 most sequenced variants. I estimated A_i, the number of putative alleles for each individual *i*, by calculating the observed sequencing depths (O_i) of the 16 most common variants n(O_{in}). I calculated the cumulative sequencing depth of each of the 16 most sequenced variants using equation 4.1. For each of the 16 most sequenced variants I calculated the rate of change (first derivative) in cumulative sequencing depth (ROC_{in}) between each variant *n* of individual *i*. I calculated the degree of change (*DOC*, second derivative), for each individual by dividing *ROC* of each *n* variant by *ROC* of the *n+2* variant using equation 4.2. I calculated standardized degree of change (*SDOC*)

by dividing *DOC* by the total change among all variants and transforming these values into percentages using equation 4.3.

$C_{in} = \sum_n O_{in}$ **Equation 4.1.**

$DOC_{i1} = (ROC_{i1} / ROC_{i2})$ **Equation 4.2.**

$SDOC_{i1} = \mathrm{DOC}_{i1} / \sum DOC_{i1\text{-}i9} \; x \; 100.$ **Equation 4.3.**

All calculations necessary for genotyping individuals were carried out using a custom Excel macro (Microsoft, Redmond, WA) developed by Lighten et al. (2014a). I plotted the cumulative read depth across the 16 most sequenced variants using a point and line graph, rejecting any samples that lacked a clear inflection point (Fig. 4.2). For any samples that showed a clear inflection point, I assigned putative allele status to any variants occurring on or before the inflection point. Any variants occurring after the inflection point were assumed to be artifacts.

I calculated allele frequency as the percentage of individuals in a population possessing each allele and compared allele presence and absence across populations to identify private alleles and determine if MHC alleles had been lost over time in either Willacy or LANWR.

MHC sequence analysis

All putative ocelot MHC nucleotide sequences were aligned using MUSCLE v3 (Edgar 2004). I used MEGA 7 (Kumar et al. 2016) to translate all sequences into protein products using the second reading frame, which has been used in previous feline DRB studies (e.g., Yuhki and O'Brien 1997; Wang et al. 2009; Drake et al. 2004) and which was the only reading

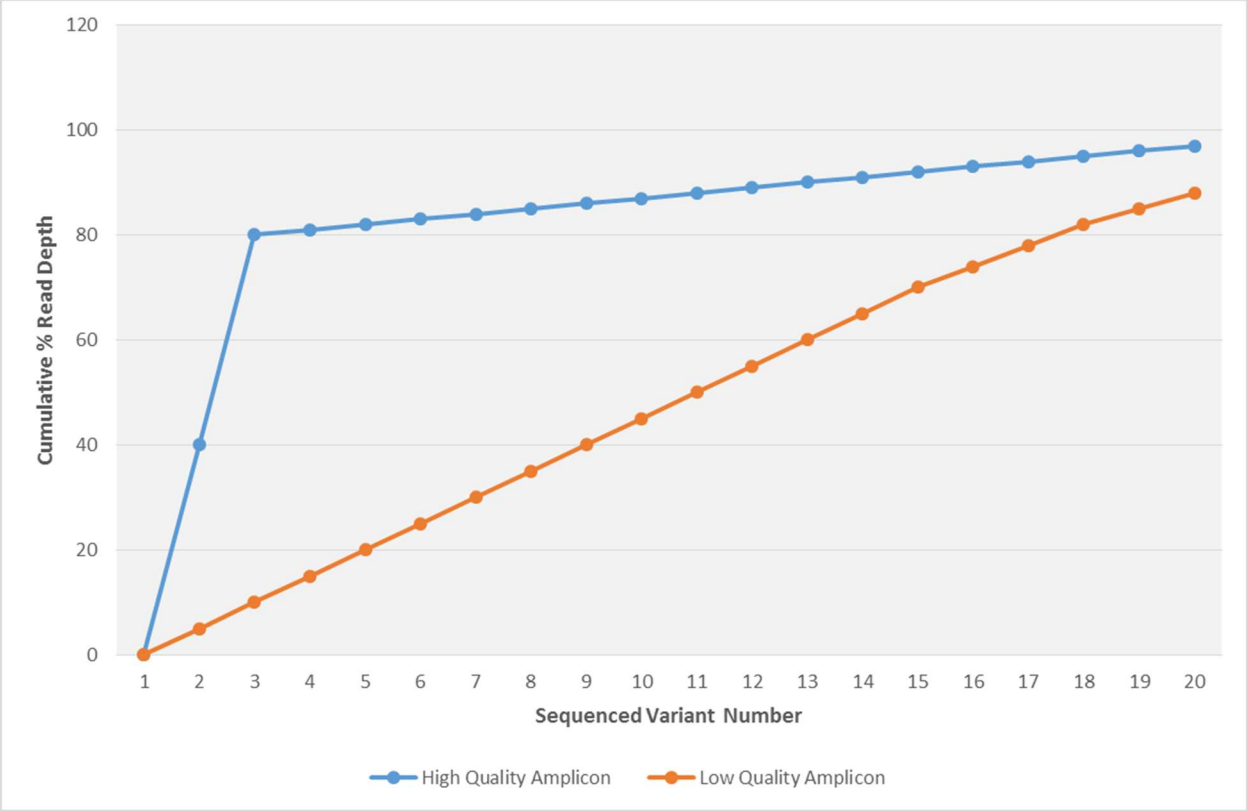

Figure 4.2. Plot of cumulative read depth for a high quality amplicon (blue) and a low quality amplicon (red). The high quality amplicon shows a clear breakpoint, allowing true alleles to be identified as those occurring before the breakpoint. The low quality amplicon shows a gradual increase in cumulative sequencing depth, making it impossible to differentiate between true alleles and artifacts.

frame to produce full-length protein products. I calculate pairwise uncorrected genetic distances and pairwise number of differences using MEGA 7 (Kumar et al. 2016). I also used MEGA 7 (Kumar et al. 2016) to examine nucleotide and amino acid differences across the exon, identifying conserved and variable sites, and calculating the estimated transition/transversion bias.

Phylogenetic analysis

I used Maximum Likelihood to rank models of nucleotide substitution using MEGA 7 (Kumar et al. 2016), and used the highest-ranked model for phylogenetic analysis. The top model of nucleotide substitution was Jukes Cantor (Jukes and Cantor 1969) with a discreet gamma distribution (BIC = 1184.952, AICc=982.098). I constructed a phylogenetic tree to infer evolutionary history of the identified nucleotide sequences using the Maximum Likelihood method based on the Jukes Cantor model with a discrete gamma distributed rate heterogeneity. I used 500 bootstrap replicates to create a consensus tree (Felsenstein 1985), using the second reading frame to determine all codon positions. The phylogenetic analysis was implemented in MEGA7 (Kumar et al. 2016).

Testing for selection

I used 2 methods to test for positive selection using the ratio of nonsynonymous to synonymous (*dN/dS*) base-pair substitutions. First, I used a codon-based Z-test of selection, implemented in MEGA7 (Kumar et al. 2016), to test the null hypothesis of neutrality (*dN=dS*), averaging the analysis over all sequence pairs. I computed variance of the difference using 500

bootstrap replicates, using the Nei-Gojobori method with a JukesCantor correction (Nei and Gojbori 1986).

Secondly, I used the HyPhy package (Pond et al. 2005a), implemented using the Datamonky server (Delport et al. 2010) to test for recombination and detect codon sites under selection. I used the GARD (Pond et al. 2006) algorithm to test for genetic recombination within the exon 2 DRB sequence. To test for codon-specific positive selection, I used 3 methods: single likelihood ancestor counting (SLAC; Pond and Frost 2005b), random-effects likelihood approach (REL; Pond and Frost 2005b), and mixed-effects model of evolution (MEME; Murrell et al. 2012). For all analyses, I used the Jukes Cantor model of substitution with gamma distributed rate heterogeneity, which was the best-fitting model of nucleotide substitution. I considered any amino acid site with *P*-value $\leq$0.1 (SLAC and MEME) or Bayes Factor $\geq$50 (REL) to be potentially under positive selection. Amino acid sites identified by 2 or more methods as being under positive selection were considered to be potential antigen binding sites.

Identification of DRB supertypes

Major histocompatibility complex loci are extremely polymorphic, yet many MHC alleles show overlapping protein binding capabilities and have been shown to be near functional-equivalents to each other, based on the chemistry of the antigen binding sites (Sette and Sidney 1998). These groupings of MHC alleles with similar binding specificities are termed supertypes (Sidney et al. 1995). There is evidence that rare MHC supertypes give individuals resistance to common infectious diseases (Trachtenberg et al. 2003), making supertype analysis potentially important for wildlife conservation (Sommer 2005).

I selected only amino acid residues identified by 2 or more methods as being under positive selection for supertype analysis (Doytchinova and Flower 2005). At each positively selected site, I characterized each amino acid residue according to the 5 physiochemical descriptors described by Sandberg et al. (1998): z1 (hydrophibicity), z2 (steric bulk), z3 (polarity), and z4 and z5 (electronic effects). I used discriminant analysis of principle components (DAPC) in the R package adegenet (Jombart 2008) to identify DRB clusters potentially representing different functional groups. This approach uses a k-means clustering algorithm based on Bayesian information criterion (BIC) to identify optimal number of clusters and performs a discriminant analysis on the retained principal components to assign DRB alleles to supertype.

Comparing neutral and functional genetic diversity

To estimate individual MHC diversity, I calculated nucleotide diversity (π) and Tajima's D for all DRB sequences found in an individual using the pegas package (Paradis 2010) in R. An additional measure of individual MHC diversity was MHC allelic richness, which was simply the number of MHC alleles possessed by an individual. To estimate individual neutral variation, I calculated multilocus microsatellite heterozygosity by dividing the number of heterozygous loci per individual by the total number of genotyped loci for that individual. Microsatellite data used for this analysis consisted of 16 unlinked microsatellite loci taken from Korn (2013). To determine whether microsatellite variation influences MHC variation, I performed simple linear regression on (1) multilocus microsatellite heterozygosity and MHC allelic richness, (2) multilocus microsatellite heterozygosity and nucleotide diversity, and (3) multilocus microsatellite heterozygosity and Tajima's D (*P*-value). Using the

microsatellite data from Korn (2013) I calculated rarefied allelic richness and observed heterozygosity (*Ho*) using the R package hierfstat.

Unequal sample sizes can bias estimates of allelic richness, as large samples are expected to show higher levels of allelic richness than smaller samples (Kalinowski 2004). Due to differences in the number of individuals genotyped in each population, comparing simple allelic richness between populations could lead to misleading results. To account for differences in sample sizes between populations, I used randomization tests, implemented in R, to estimate the total number of unique MHC alleles that could be expected in each population given equal sample sizes. For each U.S. population (i.e., Willacy Historical, Willacy Contemporary, LANWR Historical, and LANWR Contemporary) I drew a random sample of 5 individuals, recording the total number of unique alleles present in all 5 individuals. I excluded the Mexico samples from this analysis due to an insufficient number of individuals. I repeated this for 5,000 iterations to create a distribution of estimated allelic richness per population. I performed Wilcoxon rank-sum tests to compare simulated allele frequencies between historical and contemporary U.S. ocelot populations. Additionally, I reported the mean and standard errors of the simulated allele counts as an additional estimate of population-level MHC gene diversity.

RESULTS

Bioinformatics and pre-processing

Sequencing yielded 9,789,106 forward reads and 8,258,246 reverse reads. Paired-end merging resulted in 8,126,085 reads. Mean quality score for all samples was >Q.30 for the entire amplicon, and >Q.35 for all but the first and last 10 bases of each amplicon. Filtering by

quality score reduced the total number of sequences to 6,987,424. Sequencing depth per individual ranged from 525 to 151,332 (mean = 21,520, SE = 1,706.31). Prior to genotyping, the number of unique sequences per individual ranged from 146 to 20,451 (mean = 2,033.31, SE = 176.46). Most (>98%) of these reads were low-level sequencing artifacts that differed by ≤2 bp from true alleles, and individually comprised <1% of total read depth of each amplicon.

Genotyping and MHC sequence analysis

Genotyping revealed 20 unique alleles among all 65 individuals. Sixteen alleles were identical to previously published ocelot DRB sequences, whereas 4 were unique (Fig. 4.3). The number of alleles per individual ranged from 1 to 8 (mean = 4.92, SE = 0.24). The newly discovered DRB alleles were given the temporary names PA 1, PA 2, PA 3, and PA 4, indicating putative allele. Each of the 4 newly discovered alleles showed high (>98%) sequence similarity to previously published ocelot DRB sequences. All translated sequences coded for 79 amino-acid protein products, and did not contain insertions, deletions, or stop codons. Of the 238 nucleotides in the DRB sequences, 83 sites were variable and 155 were conserved. There were 70 parsimony-informative sites, 13 singleton sites, 139 zero-fold degenerate sites, 17 two-fold degenerate sites, and 31 four-fold degenerate sites. For the translated protein products, 44 amino acid residues were variable, and 33 were conserved. On the aligned protein products, variable sites occurred at amino acid positions 1-3, 5, 9-10, 17-18, 20, 22-23, 26-27, 29-31, 34-35, 39, 43, 49, 51-53, 55-56, 58-60, 62-63, 65-67, 69-70, and 78-79.

The estimated transition/transversion bias (*R*) was 0.69. Nucleotide frequencies across all alleles were A = 21.20%, T = 20.82%, C = 19.30%, and G = 38.67%. The overall nucleotide

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Lepa-DRB*0202	F	L	G	K	T	E	C	H	F	T	N	G	T	E	R	V	R	F	L	D	R	Y	F	Y	N	R	E	E	L	A	R	F	D	S	E	V	G	E	Y	R
Lepa-DRB*0203	*	*	E	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	Y	V	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0204	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Y	V	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*0211	H	M	W	*	S	*	*	*	*	I	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0213	N	M	W	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*022102	V	M	*	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0232	T	M	W	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*
Lepa-DRB*0237	T	M	W	*	F	*	*	*	Y	P	*	*	*	*	*	*	*	Y	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	F	*
Lepa-DRB*0247	T	M	W	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	S	*	*	N	*	*	*	*	*	*
Lepa-DRB*024902	T	M	W	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	F	*
Lepa-DRB*0252	T	M	W	*	F	*	*	*	*	*	*	*	*	*	*	*	*	Y	*	V	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0307	N	M	W	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	E	*	H	Y	*	*	G	*	*	Y	V	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*031402	N	M	W	*	S	*	*	*	*	*	*	*	*	*	*	*	W	Y	*	N	*	H	Y	*	*	G	*	*	Y	V	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*031602	H	M	W	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	E	*	H	Y	*	*	G	K	*	N	L	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*031604	H	M	W	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	E	*	H	Y	*	*	G	K	*	N	L	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*0504	*	*	*	*	A	*	*	*	Y	P	*	*	*	*	*	*	*	L	*	*	*	*	*	*	*	G	*	*	Y	V	S	*	*	*	*	*	*	*	F	*
PA_1	V	M	*	*	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
PA_2	V	M	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
PA_3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PA_4	V	M	*	*	C	*	*	*	*	*	*	*	*	E	*	*	*	Y	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Q	*	*	*	F	*

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
Lepa-DRB*0202	A	V	T	E	L	G	R	P	I	A	K	Y	L	N	G	L	K	D	Y	M	E	Q	G	R	T	A	V	D	W	Y	C	R	H	N	Y	G	V	V	D
Lepa-DRB*0203	*	*	*	*	*	*	*	*	T	*	*	*	W	*	*	*	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Lepa-DRB*0204	*	*	*	*	*	*	*	*	T	*	*	*	W	*	*	*	*	*	F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0211	*	*	*	*	*	*	*	*	D	*	E	*	W	*	*	*	*	*	F	*	*	E	T	*	*	E	*	*	R	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*0213	*	*	*	*	*	*	*	*	D	*	E	*	W	*	*	*	*	*	*	*	*	E	R	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	E
Lepa-DRB*022102	*	*	*	*	*	*	*	*	T	*	*	Q	W	*	*	Q	*	*	F	*	*	*	E	*	*	*	*	*	*	F	*	*	*	*	*	*	*	G	E
Lepa-DRB*0232	*	*	S	*	*	*	*	*	D	*	*	*	W	*	*	Q	*	*	H	L	*	*	E	*	*	*	*	*	R	F	*	*	*	*	*	*	*	G	E
Lepa-DRB*0237	*	*	A	*	*	*	*	*	D	*	*	*	W	*	*	Q	*	E	V	L	*	R	K	*	A	E	*	*	T	V	*	*	*	*	*	*	*	G	E
Lepa-DRB*0247	*	*	S	*	*	*	*	*	D	*	*	*	W	*	*	Q	*	E	V	L	*	*	R	*	A	E	*	*	T	F	*	*	*	*	*	*	*	*	E
Lepa-DRB*024902	*	*	S	*	*	*	*	*	A	*	*	*	W	*	*	Q	*	E	V	L	*	R	K	*	A	E	G	*	T	V	*	*	*	*	*	*	*	F	*
Lepa-DRB*0252	*	*	A	*	*	*	*	*	D	*	*	*	W	*	*	Q	*	*	F	*	*	R	K	*	*	*	*	*	*	L	*	*	*	*	*	*	*	F	*
Lepa-DRB*0307	*	*	*	*	*	*	*	*	D	*	E	*	W	*	*	*	*	*	F	*	*	E	T	*	*	E	*	*	R	*	*	*	*	*	*	*	*	F	*
Lepa-DRB*031402	*	*	*	*	*	*	*	*	D	*	E	*	W	*	*	Q	*	E	L	L	*	R	K	*	*	*	*	*	*	L	*	*	*	*	*	*	*	G	E
Lepa-DRB*031602	*	*	*	*	*	*	*	*	E	*	*	S	W	*	*	Q	*	E	F	L	*	R	K	*	*	*	*	*	*	L	*	*	*	*	*	*	*	*	E
Lepa-DRB*031604	*	*	*	*	*	*	*	*	E	*	*	S	W	*	*	Q	*	E	F	L	*	R	K	*	*	*	*	*	*	L	*	*	*	*	*	*	*	G	E
Lepa-DRB*0504	*	*	R	*	*	*	*	*	D	*	*	*	W	*	*	*	*	*	F	*	*	*	K	*	A	E	*	*	T	V	*	*	*	*	*	*	*	F	*
PA_1	*	*	*	*	*	*	*	*	T	*	*	Q	W	*	*	Q	*	E	V	L	*	R	K	*	A	E	*	*	T	V	*	*	*	*	*	*	*	G	E
PA_2	*	*	*	*	*	*	*	*	D	*	*	*	W	*	E	Q	*	*	*	*	*	*	*	*	*	*	*	*	R	L	*	*	*	*	*	*	*	F	*
PA_3	*	*	*	*	*	*	*	*	E	*	*	S	W	*	*	Q	*	E	F	L	*	R	K	*	*	*	*	*	*	L	*	*	*	*	*	*	*	G	E
PA_4	*	*	*	*	*	*	*	*	*	*	*	Q	W	N	*	Q	K	*	*	*	*	*	K	*	*	*	*	*	*	*	*	*	*	*	*	*	*	F	*

Figure 4.3. Alignment of translated ocelot DRB protein products. Amino acid names follow International Union of Pure and Applied Chemistry (IAUPAC) nomenclature. Amino acid residues identical to the consensus sequence (top allele) amino acid residue are marked with an asterisk.

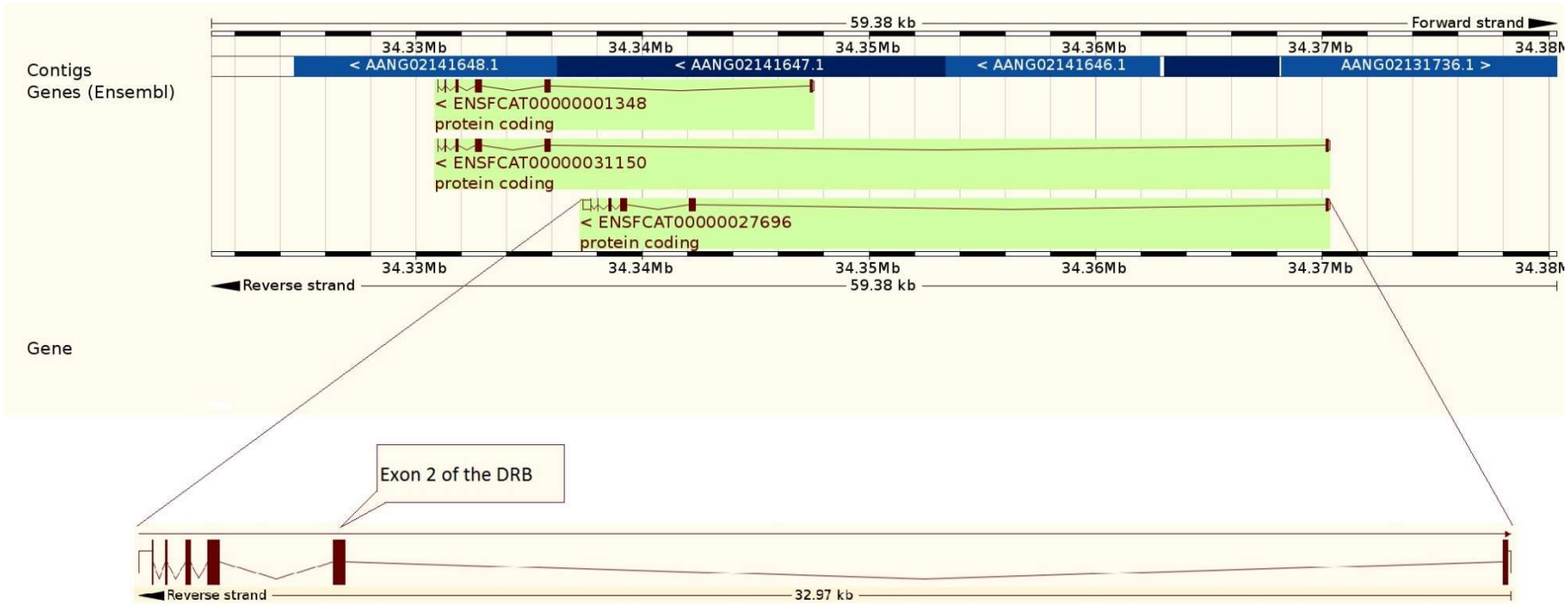

Figure 4.4. Diagram of the domestic cat DRB sequence, taken from the Ensemble cat genome browser (Yates et al. 2016). The DRB sequence consists of 6 exons and 5 introns. Exon 2 of the DRB molecule is identified.

p-distance calculated for all sequences using 500 bootstrap replicates was 0.124 (SE = 0.023). The number of nucleotide differences between pairs of alleles ranged from 1 to 16 (mean = 9.78, SE = 3.04). Across all codons *dN* ranged from 0 to 6.21 (mean = 1.02, SE = 1.48), and *dS* ranged from 0 to 112.42 (mean = 2.40, SE = 12.67).

Allele frequencies across populations

The Mexico population had one allele, Lepa-DRB*0307, that was missing in all other populations. Among contemporary ocelot populations in the U.S., LANWR Contemporary had 2 alleles not shared by Willacy Contemporary: Lepa-DRB*0213 and Lepa-DRB*031402. Willacy Contemporary had 4 alleles not shared by LANWR Contemporary: Lepa-DRB*0204, Lepa-DRB*0504, PA 1, and PA 2. LANWR Historical had 7 alleles no longer present in LANWR Contemporary: Lepa-DRB*0204, Lepa-DRB*0232, Lepa-DRB*0252, Lepa-DRB*0504, PA 2, PA 3, and PA 4. Willacy Historical had 3 alleles no longer present in Willacy Contemporary: Lepa-DRB*0213, Lepa-DRB*0252, and Lepa-DRB*031402 (Table 4.1.).

Phylogenetic analysis

The phylogenetic analysis identified 4 distinct lineages for the DRB alleles (Fig. 4.5), with PA 3 falling outside the four lineages. The first lineage included Lepa-DRB*0211, Lepa-DRB*0307, Lepa-DRB*0213, Lepa-DRB*031402, Lepa-DRB*031602, and Lepa-DRB*031604. The second lineage included Lepa-DRB*0252, Lepa-DRB*0232, Lepa-DRB*0247, Lepa-DRB*0237, and Lepa-DRB*024902. The third lineage included Lepa-

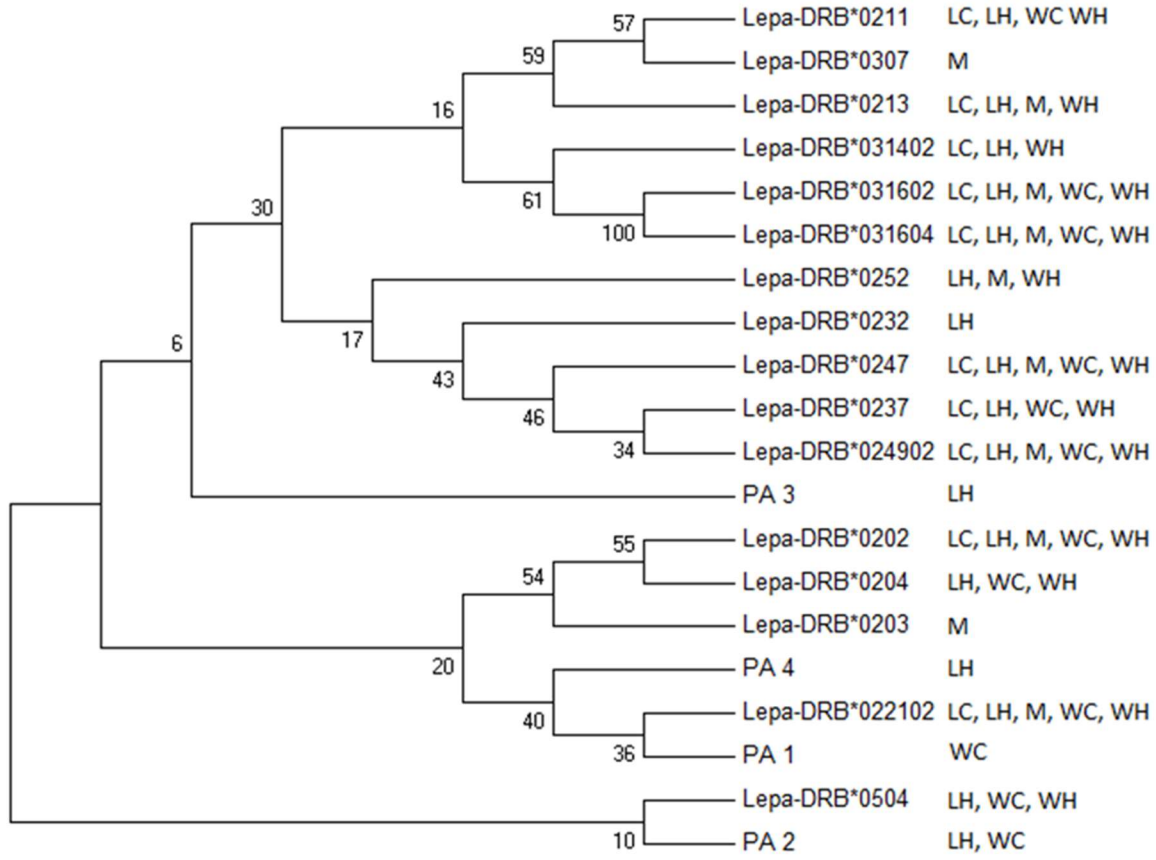

Figure 4.5. Molecular Phylogenetic analysis by Maximum Likelihood method based on the Jukes Cantor model. The bootstrap consensus tree inferred from 500 bootstrap replicates is taken to represent the evolutionary history of the ocelot DRB alleles. All branches correspond to partitions reproduced in ≥50% bootstrap replicates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate

differences among sites. The analysis involved 20 nucleotide sequences. Codon positions included were second. There were a total of 79 positions in the final dataset. Text to the right of allele names refers to ocelot populations in which that DRB allele was found. LC = LANWR Contemporary; LH = LANWR Historical; M = Tamaulipas, Mexico; WC = Willacy Contemporary; WH = Willacy Historical.

Table 4.1. Number and percent (%) of individuals displaying each of the 20 ocelot DRB alleles in the 5 populations. Percent refers to the percent of individuals in a population possessing that allele.

DRB Alleles	LANWR Contemp.	LANWR Historical	Mexico	Willacy Contemp.	Willacy Historical
Lepa-DRB*0202	7(50%)	11(50%)	3(60%)	8(100%)	10 (62.5%)
Lepa-DRB*0203	0(0%)	0(0%)	2(40%)	0(0%)	0 (0%)
Lepa-DRB*0204	0(0%)	1(4.6%)	0(0%)	2(25%)	1 (6.25%)
Lepa-DRB*0211	1(7.1%)	2(9.1%)	0(0%)	1(12.5%)	4 (25%)
Lepa-DRB*0213	1(7.1%)	3(13.6%)	1(20%)	0(0%)	2 (12.5%)
Lepa-DRB*022102	11(78.6%)	19(86.3%)	1(20%)	5(62.5%)	8 (50%)
Lepa-DRB*0232	0(0%)	2(9.1%)	0(0%)	0(0%)	0 (0%)
Lepa-DRB*0237	9(64.3%)	15(68.2%)	0(0%)	4(50%)	6 (37.5%)
Lepa-DRB*0247	7(50%)	11(50%)	4(80%)	4(50%)	11(68.8%)
Lepa-DRB*024902	7(50%)	12(54.6%)	5(100%)	5(62.5%)	11(68.8%)
Lepa-DRB*0252	0(0%)	1(4.6%)	1(20%)	0 (0%)	1 (6.25%)
Lepa-DRB*0307	0(0%)	0(0%)	2(40%)	0 (0%)	0 (0%)
Lepa-DRB*031402	1(7.14%)	2(9.1%)	0(0%)	0 (0%)	4 (25%)

Table 4.1 (continued)

DRB Alleles	LANWR Contemp.	LANWR Historical	Mexico	Willacy Contemp.	Willacy Historical
Lepa-DRB*031602	4(28.6%)	8(36.4%)	3(60%)	2(25%)	8(50%)
Lepa-DRB*031604	11(78.6%)	13(59.1%)	1(20%)	4(50%)	11(68.8%)
Lepa-DRB*0504	0(0%)	4(18.2%)	0(0%)	1(12.5%)	5(31.3%)
PA_1	0(0%)	0(0%)	0(0%)	1(12.5%)	0(0%)
PA_2	0(0%)	1(4.6%)	0(0%)	2(25%)	1(6.3%)
PA_3	0(0%)	1(4.6%)	0(0%)	0(0%)	0(0%)
PA_4	0(0%)	1(4.6%)	0(0%)	0(0%)	0(0%)

DRB*0202, Lepa-DRB*0204, Lepa-DRB*0203, PA 4, Lepa-DRB*022102, and PA 1. The fourth lineage included Lepa-DRB*0504 and PA 2. The first 3 lineages were found in all 5 populations, but the fourth lineage only occurred in LANWR Historical, Willacy Contemporary, and Willacy Historical. Allele PA 3 was found only in LANWR Historical.

Testing for selection

Using the codon-based Z-test of selection I failed to reject the null hypothesis of strict neutrality ($P = 0.337$), indicating that there was no evidence for positive or negative selection acting across the entire sequence.

I found no evidence of recombination within the DRB sequences using GARD. Using SLAC, I identified 4 amino acid sites with evidence for positive selection and 9 sites with evidence for negative selection. Using MEME, I found 9 amino acid sites with evidence of episodic diversifying selection. Using REL, I found 6 amino acid sites with evidence for positive selection and 16 sites with evidence for negative selection. Six amino acid sites were identified by 2 or more methods as being under positive selection: 1, 62, 63, 69,70, and 78 (Table 4.2).

Comparing neutral and functional genetic diversity

I found no linear relationship between individual microsatellite multilocus heterozygosity and individual nucleotide diversity (Adjusted R-squared = 0.007, $P = 0.251$), between individual microsatellite multilocus heterozygosity and individual MHC allelic richness (Adjusted R-squared = -0.017, $P = 0.838$), and between individual microsatellite

Table 4.2. Summary of results from likelihood models of amino acid sites under selection. The model of nucleotide substitution a Jukes Cantor model with a discrete gamma distributed rate heterogeneity. Amino acid sites found by 2 or more models to be under positive selection are reported in bold. Positive values for normalized dN-dS, E[dN-dS], and beta2 are evidence for positive selection. Negative values for normalized dN-dS, E[dN-dS], and beta2 are evidence for negative selection.

	SLAC		**REL**		**MEME**	
Amino Acid Site	Normalized dN-dS	P-value	E[dN-dS]	Bayes Factor	beta2	P-value
1			**2.71**	**56.41**	**18.34**	**0.04**
7	-6.47	0.02	-3.12	165595.00		
9	-5.69	0.06	-1.42	2696.85		
13	-3.09	0.04	-2.97	228145.32		
20					7.81	0.06
22	-8.23	0.03	-1.00	57.05		
23			-2.01	1534.00		
29					6.16	0.1
32	-6.47	0.01	-2.11	1061220.86		
37			-2.54	1140.82		
40			-2.22	1951.79		
42			-1.99	24535.67		
44			-1.99	1359.67		
48	-4.12	0.01	-2.31	137210.16		

Table 4.2 (continued)

SLAC			REL		MEME	
Amino Acid Site	Normalized dN-dS	P-value	E[dN-dS]	Bayes Factor	beta2	P-value
49			1.35	56.00		
53	-114.54	0.01	-1.51	215.54		
54	-6.473	0.011	-2.025	487618.000		
59					7.087	0.055
61			-1.973	611.528		
62	**3.649**	**0.097**			**9.781**	**0.057**
63	**4.776**	**0.098**	**2.690**	**1142.350**	**14.973**	**0.053**
64	-2.344	0.085	-1.966	2813.620		
65			-0.996	69.782		
69			**2.426**	**523.412**	**14.132**	**0.072**
70	**5.341**	**0.082**	**2.965**	**3162.220**	**4.930**	**0.035**
78	**6.389**	**0.042**	**3.309**	**409924.000**	**44.777**	**0.000**

multilocus heterozygosity and the *P*-value for Tajima's D test (Adjusted R-squared = -0.022, *P* = 0.738).

Comparison of simulated allele counts between historical and contemporary Willacy revealed significantly higher allelic richness, corrected for population size differences, in Willacy Historical than in Willacy Contemporary ($P < 2.2 \times 10^{16}$). In LANWR, simulated allele counts were significantly higher in LANWR Historical than in LANWR Contemporary ($P < 2.2 \times 10^{16}$) (Table 4.2).

Identification of DRB supertypes

The clustering analysis in adegenet (Jombart 2008) revealed 3 distinct supertypes based on a Δ BIC $\leq$2 (Fig. 4.6). The first 6 principal components explained >95% of the variance in the data, and were, therefore, retained for discriminant analysis. Supertype 1 included the following alleles: Lepa-DRB*031402, Lepa-DRB*031602, Lepa-DRB*031604, and PA 3. Supertype 2 included the following alleles: Lepa-DRB*0237, Lepa-DRB*024902, Lepa-DRB*0252, Lepa-DRB*0504, PA 1, and PA 2. Supertype 3 included Lepa-DRB*0202, Lepa-DRB*0203, Lepa-DRB*0204, Lepa-DRB*0211, Lepa-DRB*0213, Lepa-DRB*022102, Lepa-DRB*0232, Lepa-DRB*0247, Lepa-DRB*0307, and PA 4. Supertypes 1 and 2 were found in all populations studied, and supertype 3 was found in all populations except Willacy Contemporary (Table 4.4).

DISCUSSION

Although differences in sample size between populations makes it difficult to directly compare allele frequencies, the results suggest that both LANWR and Willacy have lost MHC

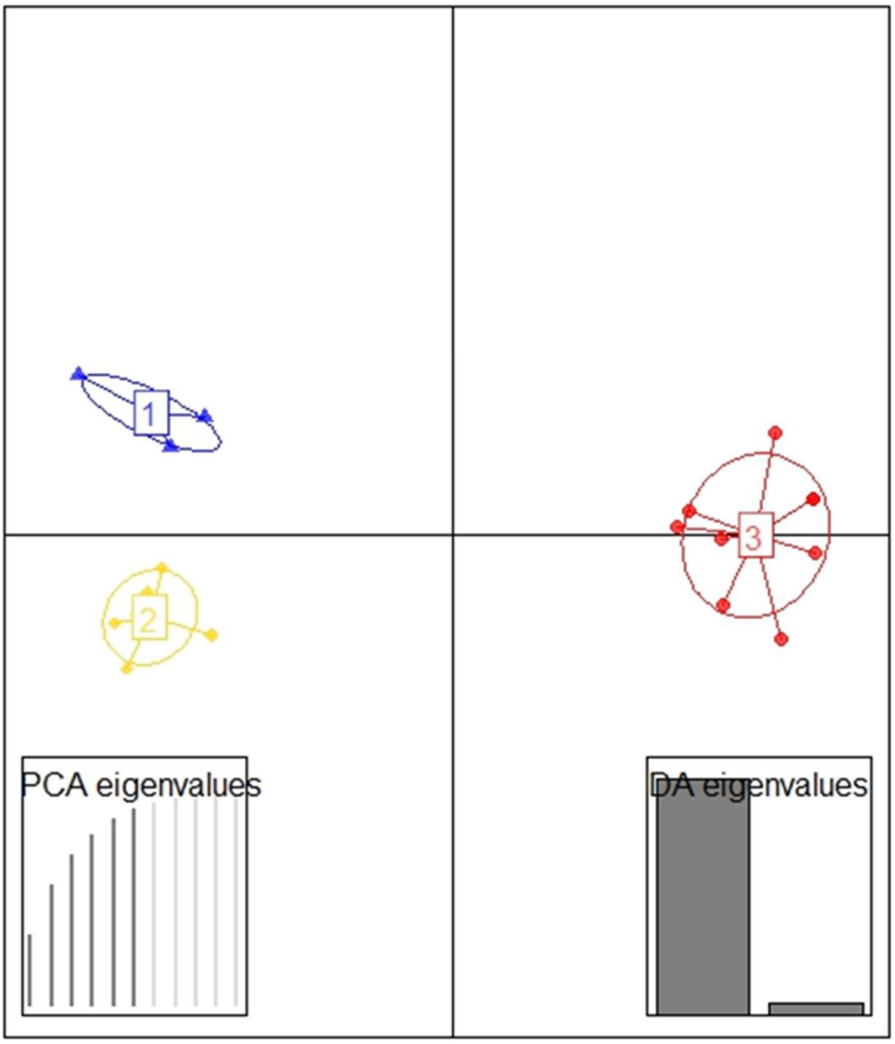

Figure 4.6. Discriminant analysis of principal components (DAPC) for defining *Leopardus pardalis* MHC DRB supertypes based on physiochemical descriptors of sites under positive selection. Eigenvalues of the principle components analysis are displayed in the lower left and the eigenvalues of the discriminant analysis are displayed on the lower right.

Table 4.3. Comparison of neutral and functional genetic variation. Microsatellite data were taken from Korn (2013). Microsatellite data were not available for the samples from Mexico. Rarefied allelic richness (Rarefied AR) and observed heterozygosity (Ho) were calculated using the R package hierfstat. Tajima's D (*p*-value) and nucleotide diversity (Nuc. Div.) were calculated for all MHC sequences appearing in a population using the R package pegas. Population allelic richness (Population AR) reports the number of unique MHC alleles occurring in a population. Individual allelic richness (Indiv. AR) reports the mean number of MHC alleles per individual. Simulated allelic richness (Simulated AR) reports the allelic richness obtained from performing 5000 simulated random samples (n = 5) from each population and counting the number of unique alleles in each simulation. Standard errors are given in parentheses.

	LANWR Historical	LANWR Contemp.	Willacy Historical	Willacy Contemp.	Mexico
Microsatellites					
Sample size (n)	22	14	14	7	NA
Rarefied AR	1.64(0.04)	1.62 (0.03)	1.65 (0.03)	1.65 (0.04)	NA
Ho	0.58 (0.05)	0.59 (0.06)	0.68 (0.06)	0.60 (0.07)	NA
MHC					
Sample size (n)	22	14	16	8	4
Tajima's D (*p-value*)	0.31	0.37	0.30	0.31	0.14
Population AR	17	10	14	11	10
Simulated AR	10.66 (0.03)	8.00 (0.02)	10.87 (0.03)	10.01 (0.01)	NA
Nuc. Div.	0.14 (0.01)	0.13 (0.01)	0.14 (0.01)	0.13 (0.01)	0.13 (0.01)
Indiv. AR	5.04 (0.47)	4.35 (0.56)	5.38 (0.40)	5.00 (0.53)	4.40 (1.03)

Table 4.4. Number and percent (%) of individuals displaying each of the 3 ocelot DRB supertypes in the 5 populations. Percent refers to the percent of individuals in a population possessing that DRB supertype.

DRB Supertype	LANWR Contemp.	LANWR Historical	Mexico	Willacy Contemp.	Willacy Historical
1	11 (78.57%)	19 (86.36%)	3 (60%)	6 (75%)	15 (93.75%)
2	13 (92.86%)	18 (81.82%)	5 (100%)	7 (87.5%)	15 (93.75%)
3	2 (14.29%)	1 (4.55%)	1 (20%)	0 (0%)	1 (6.25%)

alleles over time, probably due to drift. LANWR Historical had 7 alleles that were not found in LANWR Contemporary, and Willacy Historical had 3 alleles no longer present in Willacy Contemporary. The simulated allelic richness values, which used a randomization procedure to correct for different sample sizes, were significantly higher in historical than in contemporary populations.

Despite the loss observed in MHC alleles over time, no relationship was observed at the individual level between multilocus microsatellite heterozygosity and measures of MHC diversity. This could indicate that although MHC alleles have been lost in both Willacy and LANWR due to drift, individual heterozygosity at MHC loci may be retained through balancing selection. Over time, loss of MHC alleles due to drift may compromise the immunological fitness of these ocelot populations. Private alleles were observed in all populations, suggesting that translocations either reciprocally between LANWR and Willacy, or between Mexico and the United States, have the potential to restore and maintain ocelot MHC diversity.

I observed a very low value of *dN-dS* at amino acid position 53, and all analyses indicated a high rate of negative selection at this site. However, this low observed value was likely caused by a G to T substitution at nucleotide position 159 combined with a G to A nucleotide substitution at nucleotide position 160, both occurring in a single allele (Lepa-DRB*0202). This substitution produces a leucine at amino acid position 53, whereas a tryptophan occurs at this site in all other alleles. It is unclear what effect this substitution might have on the functionality of the resulting protein product, but it is important to note that the low *dN-dS* value observed at this site was not due to an excess of synonymous substitutions, but rather to a single allele in which 2 separate substitutions altered the amino acid product.

The Lepa-DRB*0202 allele was found in all populations studied including the historical and contemporary groups. Removal of this allele from the analysis yielded normalized *dN-dS* values ranging from -7.84 to 6.16 (mean = -0.03, SE = 2.35) across the exon. The low *dN-dS* value observed at amino acid position 53 was removed, but tests of positive selection at all other sites yielded similar results.

Ocelots were found to have between 1 and 8 alleles per individual, with no pseudogenes detected. This is consistent with previous studies that have found domestic cats to have 4 DRB loci (Yuhki et al. 2008). I allowed for the possibility of a large gene duplication event in ocelots which could increase the number of alleles per individual as high as 16, but found no evidence that such a duplication occurred. The variable regions found in translated DRB protein products in this study partially coincided with areas of variability found by Kennedy et al. (2002) in domestic cat sequences. Kennedy et al. (2002) identified areas of hyper-variability at amino acid positions 8-16, 26-38, and 56-74.

A comparison of MHC supertype frequency across populations revealed supertype 3 to be the least common MHC supertype. It was found to be missing from Willacy Contemporary. Although several MHC alleles were found in historical samples that were missing from contemporary samples, this was the only instance of a MHC supertype disappearing from a population. Supertypes are assigned based on inferred amino acid structure at functionally important antigen binding sites (Sidney et al. 1995). The loss of a MHC supertype from an ocelot population may be cause for concern as it could represent a loss of functional genetic variation in this population.

It is important to note that putative antigen binding sites were identified solely through tests for positive selection. Although this technique has been used in previous studies (e.g.,

Lillie et al. 2015), it is impossible to determine with certainty whether these sites code for antigen binding portions of the DRB molecule without directly examining the structure of the DRB molecule itself. Brown et al. (1993) studied the structure of the orthologous human HLA molecule using X-ray crystallography, and found amino acid residues influencing the shape and function of the ABS at the following codon positions: 3, 5, 20, 22, 29, 30, 39, 52, 53, 60, 62, 63, 66, and 69. I identified only 6 sites likely involved in antigen binding using tests of positive selection. Kennedy et al. (2002) identified 3 portions of the domestic cat DRB molecule with high dN/dS ratio at amino acid positions 11-16, 26-38, and 56-75. However, the analysis by Kennedy et al. (2002) did not statistically test these sites for positive selection. The approach taken by this study is more conservative in identifying positively selected sites, and may underestimate the actual number of amino acids involved in antigen binding.

Whereas it is generally accepted that pathogen mediated balancing selection contributes to the high levels of polymorphism seen at MHC loci (Clarke and Kirby 1966), there is disagreement about the mechanisms behind this selection. The two most prominent hypotheses relating to the maintenance of MHC polymorphism are the overdominance or heterozygote-advantage hypothesis and the frequency-dependent selection hypothesis (Sommer 2005; Piertney and Oliver 2006). The overdominance hypothesis posits that individuals that are heterozygous at key MHC loci will show lower levels of infection and greater pathogen resistance than individuals that are homozygous (Doherty and Zinkernagel 1975). Evidence supporting the overdominance hypothesis was found in Chinook salmon (*Oncorhynchus tshawytscha*), with MHC homozygotes showing significantly higher rates of infection than MHC heterozygotes (Evans and Neff 2009). In contrast, in giant pandas (*Ailuropoda melanoleuca*), individual MHC heterozygosity was uncorrelated with parasite burden, yet

MHC alleles showed strong evidence of balancing selection (Zhang et al. 2015). Frequency-dependent selection posits that specific MHC alleles, particularly those that are rare in a population during a specified period, will confer immunity to individuals during local disease outbreaks (Takahata and Nei 1990). Rare MHC alleles will be prevented from disappearing due to drift, and high polymorphism of MHC alleles will be maintained in the population. Investigating the selective mechanisms and magnitude of selection behind MHC polymorphism in ocelots is beyond the scope of this study, however, the extreme isolation and low population sizes of the relict U.S. ocelot populations may prevent either selective mechanism from maintaining MHC polymorphism in the future due to the strength of drift.

Using pedigree analysis, Korn (2013) found several instances of inbreeding in the Willacy and LANWR populations. If overdominance at MHC loci is the primary mechanism that maintains MHC variation in ocelot populations, continued inbreeding will likely increase the proportion of MHC homozygotes. Conversely, if frequency dependent selection is the primary mechanism behind the maintenance of MHC polymorphism in ocelot populations, the low population sizes found in Willacy, and especially LANWR, could reduce the frequency of rare yet beneficial MHC alleles to the point that genetic drift overwhelms the ability of selection to maintain these alleles in the population. Over time it is likely that both the number of MHC alleles per individual and the number of alleles per population will decline in Willacy and LANWR. This result could drastically impact ocelot immunological fitness and chances of persisting in the United States.

The remaining ocelots in the United States have reduced genetic diversity based on microsatellite analysis and sequencing of mitochondrial DNA (Walker 1997; Janecka et al. 2008; Janecka et al. 2011; Korn 2013; Janecka et al. 2014). Due the apparent lack of dispersal

between Willacy and LANWR and between Mexico and either of these populations, a continued loss of genetic diversity in Willacy and LANWR is a certainty. Translocations are needed to mimic natural connectivity and reduce genetic drift and inbreeding.

My study found private MHC alleles occurring in Willacy, LANWR, and Mexico, indicating that translocations have the potential to restore missing MHC alleles to Willacy and LANWR. Additionally, Willacy Contemporary was found to be missing a DRB supertype present in all other populations. This may be an artifact of sample size, or it may indicate that this population has already lost functional genetic variation in the MHC.

LITERATURE CITED

Aguilar A, Roemer G, Debenham S, Binns M, Garcelon D, Wayne RK (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. Proceedings of the National Academy of Science 101:3490-3494

Apanius V, Penn D, Slev PR, Ruff LR, Potts WK (1997) The nature of selection on the major histocompatibility complex. Critical Reviews in Immunology 17:179-224

Beltran JF, Tewes ME (1995) Immobilization of ocelots and bobcats with ketamine hydrochloride and xylazine hydrochloride. Journal of Wildlife Diseases 31:43-48

Blomqvist D, Paulina A, Larsson M, Flodin LA (2010) Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. BMC Evolutionary Biology 10:33

Brook BW, Tonkyn DW, O'Grady JJ, Frankham R (2002) Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology 6:1-16

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strongminger JL (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 365:33-39

Caso A (1994) Home range and habitat use of three neotropical carnivores in northeast Mexico. Thesis, Texas A&M University-Kingsville

Caso A (2013) Spatial differences and local avoidance of ocelot (*Leopardus pardalis*) and jaguarundi (*Puma yagouaroundi*) in northeast Mexico. Dissertation, Texas A&M University-Kingsville

Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10:195-205

Clarke B, Kirby DRS (1966) Maintenance of histocompatibility polymorphisms. Nature 211:999-1000

Cresswell P (2005) Antigen processing and presentation. Immunological Review 207:5–7

Dawkins R, Leelayuwat C, Gaudieri S, Tay G, Hui J, Cattley S, Martinez P, Kulski J (1999) Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. Immunological Reviews 167:275-304

Delport W, Poon AFY, Frost SDW, Pond SLK (2010) Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. Bioinformatics 26:2455-2457

Delves P, Martin SJ, Burton DR, Roitt IM (2006) Roitt's Essential Immunology, 11th edn. Blackwell Publishing, London

Didham RK (2010) Ecological consequences of habitat fragmentation. In: Janson R (ed) Encyclopedia of life sciences. Wiley, Chichester. doi:10.1002/9780470015902.a0021904

Doherty PC, Zinkernagel RM (1975) A biological role for the major histocompatibility antigens. Lancet 1:1406–1409

Drake GJC, Kennedy LJ, Auty HK, Ryvar R, Ollier WER, Kitchener AC, Freeman AR, Radford AD (2004) The use of reference strand mediated conformational analysis for the study of cheetah (Acinonyx jubatus) feline leucocyte antigen class II DRB polymorphisms. Molecular Ecology 13:221–229

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792-1797.

Emmons LH (1988) A field study of ocelots (*Felis pardalis*) in Peru. Revue D Ecologie-La Terre Et La Vie 43:133-157

Emmons LH, Sherman P, Bolster D, Goldizen A, Terborgh J (1989) Ocelot behavior in moonlight. In: Redford KH, Eisenberg JF (eds) Advances in Neotropical Mammalogy, Sandhill Crane Press. Gainesville, United States, pp 233-242

Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology 18:4716–4729

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends in Ecology and Evolution 27:489-496.

Goda N, Mano T, Kosintev P, Vorobiev A, Masuda R (2010) Allelic diversity of the MHC class II DRB genes in brown bears (*Ursus arctos*) and a comparison of DRB sequences within the family Ursidae. Tissue Antigens 76:404–410

Haines AM, Tewes ME, Laack LL (2005) Survival and cause-specific mortality of ocelots in southern Texas. Journal of Wildlife Management 69:255-263

Haines AM, Tewes ME, Laack LL, Horne JS, Young JH (2006) A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. Biological Conservation 132:424-436

Hedrick PW, Lee RN, Parker KM (2000) Major histocompatibility complex (MHC) variation in the endangered Mexican wolf and related canids. Heredity 85:617–624

Hedrick PW, Kim TJ (1999) Genetics of complex polymorphisms: Parasites and maintenance of the major histocompatibility complex variation. In: Singh RS, Krimbas CB (eds) Evolutionary genetics: from molecules to morphology. Cambridge University Press, Cambridge, United Kingdom, pp 204-234

Horne JS, Haines AM, Tewes ME, Laack LL (2009) Habitat partitioning by sympatric ocelots and bobcats: implications for recovery of ocelots in southern Texas. Southwestern Naturalist 54:119–126

Jahrsdoerfer SE, Leslie DM (1988). Tamaulipan brushland of the Lower Rio Grande Valley of south Texas: description, human impacts, and management options. US Fish and Wildlife Service, Biological Report 88:1-62

Janecka JE, Walker CW, Tewes ME, Caso A, Laack LL, Honeycutt RL (2007) Phylogenetic relationships of ocelot (*Leopardus pardalis albescens*) populations from the Tamaulipan biotic province and implications for recovery. The Southwestern Naturalist 52:89-96

Janecka JE, Tewes ME, Laack LL, Grassman LI, Haines AM, Honeycutt RL (2008) Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States. Conservation Genetics 9:869–878

Janecka JE, Tewes ME, Laack LL, Caso A, Grassman LI, Haines AM, Shindle DB, Davis BW, Murphy WJ, Honeycutt RL (2011) Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas. Animal Conservation 14:608–619

Janecka JE, Tewes ME, Laack L, Caso A, Grassman LI, Honeycutt RL (2014) Loss of genetic diversity among ocelots in the United States during the 20th century linked to human induced population reductions. PLoS ONE doi:10.1371/journal.pone.0089384

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian Protein Metabolism. Academic Press, New York, United States, pp 21-132

Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conservation Genetics 5:539-543

Kennedy LJ, Ryvar R, Gaskell RM, Addie DD, Willoughby K, Carter SD, Thomson W, Ollier WER, Radford AD (2002) Immunogenetics 54:348-352

Kirk H, Freeland JR (2011) Applications and implications of neutral versus non-neutral markers in molecular ecology. International Journal of Molecular Sciences 12:3966-3988

Klein J, Figueroa F (1986) Evolution of the major histocompatibility complex. Immunogenetics 6: 295-386

Korn JM (2013) Genetic pedigree and prey dynamics of ocelot and fine-scale movement patterns of bobcat in south Texas. Dissertation, Texas A&M University-Kingsville

Kulski JK, Shiina T, Anzai T, Kohara S, Inoko H (2002) Comparative genomic analysis of the MHC: the evolution of class I duplication blocks, diversity and complexity from shark to man. Immunological Reviews 190:95-122

Kumanovics A, Takada T, Lindahl KF (2003) Genomic organization of the mammalian MHC. Annual Review of Immunology 21:629-657

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874

Laack LL (1991) Ecology of the ocelot (*Felis pardalis*) in south Texas. Thesis, Texas A&I University

Laack LL, Tewes ME, Haines AH, Rappole J (2005) Reproductive life history of ocelots *Leopardus pardalis* in southern Texas. Acta Theriologica 50:505-514

Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. Conservation Biology 1:143-158

Lighten J, van Oosterhout C, Paterson IG, McMullan M, Bentzen P (2014a) Ultra-deep Illumina sequencing accurately identifies MHC class IIb alleles and provides evidence for copy number variation in the guppy (*Poecilia reticulata*). Molecular Ecology Resources 14:753-767

Lighten J, van Oosterhout C, Bentzen P (2014b) Critical review of NGS analyses for de novo genotyping multigene families. Molecular Ecology 16:3957-3972

Lillie M, Grueber CE, Sutton JT, Howitt R, Bishop PJ, Gleeson D, Belov K (2015) Selection on MHC class II supertypes in the New Zealand Hochstetter's frog. BMC Evolutionary Biology 15:63

Llaurens V, McMullan M, van Oosterhout C (2012) Cryptic MHC polymorphism revealed but not explained by selection on the class IIb peptide-binding region. Molecular Biology and Evolution 29:1631-1644

Longmire JL, Maltbie M, Baker RJ (1997) Use of "lysis buffer" in isolation and its implications for museum collections. Occasional Papers, The Museum of Texas Tech University 163, 1-3

Magdoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957-2963

Mayer F, Brunner A (2007) Non-neutral evolution of the major histocompatibility complex class II gene DRB1 in the sac-winged bat Saccopteryx bilineata. Heredity 99, 257-264

Murrell B, Wertheim JO, Moola S, Weighill T, Scheffler K, Pond SLK (2012) Detecting individual sites subject to episodic diversifying selection. PLoS Genetics doi: e1002764

Navarro-Lopez D (1985) Status and distribution of the ocelot (*Felis pardalis*) in South Texas. Thesis, Texas A&I University

Nei M, Gojobori T (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous substitutions. Molecular Biology and Evolution 3:418-426

O'Brien SJ, Evermann JF (1988) Interactive influence of infectious disease and genetic diversity in natural populations. Trends in Ecology and Evolution 3:254–259

Ota T, Nei M (1994) Divergent evolution and evolution by the birth-and-death process in the immunoglobulin V-H gene family. Molecular Biology and Evolution 11:469-482

Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26:419-420.

Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. American Naturalist 153:145-164

Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. Heredity 96:7–21

Pokorny L, Sharma R, Goyal SP, Mishra S, Tiedemann R (2010) MHC class I and MHC class II DRB gene variability in wild and captive Bengal tigers (*Panthera tigris tigris*). Immunogenetics 62:667–679

Pond SLK, Frost SDW, Muse SV (2005a) HyPhy: hypothesis testing using phylogenies. Bioinformatics 21:676-9

Pond SLK, Frost SDW (2005b) Not so different after all: a comparison of methods for detecting amino acid sites under selection. Molecular Biology and Evolution 22:1208-1222

Pond SLK, Posada D, Gravenor MB, Woelk CH, Frost SDW (2006) GARD: a genetic algorithm for recombination detection. Bioinformatics 22:3096-3098

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.

Sandberg M, Eriksson L, Jonsson J, Sjostrom M, Wold S (1998) Journal of Medical Chemistry 41:2481-2491

Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV, Woolmer G (2002) The human footprint and the last of the wild. Bioscience 52:891–904

Schmeider R, Edwards R (2011) Quality control and preprocessing of metagenomics datasets. Bioinformatics 27:863-864

Schmidly DJ (2002) Texas natural history: a century of change. Texas Tech University Press, Lubbock, United States

Schmidly DJ (2004) The mammals of Texas. University of Texas Press, Austin, United States

Sebastian A, Herdegen M, Migalska M, Radwan J (2015) AmpliSAS: a web server for multilocus genotyping using next-generation amplicon sequencing data. Molecular Ecology Resources doi:10.1111/1755-0998.12453

Shindle DB, Tewes ME (1998) Woody species composition of habitats used by ocelots (*Leopardus pardalis*) in the Tamaulipan biotic province. Southwestern Naturalist 43:273–279

Shindle DB, Tewes ME (2000) Immobilization of wild ocelots with tiletamine and zolazepam in southern Texas. Journal of Wildlife Diseases 36:546-550

Sidney J, del Guercio MF, Southwood S, Engelhard VH, Appella E, Rammensee HG, Falk K, Rotzschke O, Takiguchi M, Kubo RT (1995) Several HLA alleles share overalapping peptide specificities. Journal of Immunology 154:247-259

Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Frontiers in Zoology 2:16

Sunquist M, Sunquist F (2002) Wild cats of the World. University of Chicago Press, Chicago

Takahata N, Nei M (1990) Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics 124:967-978

Tewes ME, Everett DD (1986) Status and distribution of the endangered ocelot and jaguarundi in Texas. In: Miller SD, Everett DD (eds) Cats of the world: biology, conservation, and management. National Wildlife Federation, Washington, DC, pp. 147-158

Tewes ME (1986) Ecological and behavioral correlates of ocelot spatial patterns. Dissertation, University of Idaho

Trachtenberg E, Korber B, Sollars C, Kepler TB, et al (2004) Advantage of rare HLS supertypes in HIV disease progression. Nature Medicine 9:928-935

Ujvari B, Belov K (2011) Major Histocompatibility Complex (MHC) markers in conservation biology. International Journal of Molecular Science 12:5168-5186

Walker CW (1997) Patterns of genetic variation in ocelot (*Leopardus pardalis*) populations for south Texas and northern Mexico. Unpublished Ph.D. dissertation, Texas A&M University and Texas A&M University-Kingsville

Wang Q, Xiaobing W, Pen Y, Sheng S (2008) Sequence variability analysis on major histocompatibility complex class II DRB alleles in three felines. Frontiers of Biology in China 3:55-62

Wang X, Wei K, Zhang Z, Xu X, Zhang W, Shen F, Zhang L, Yue B (2009) Major histocompatibility complex class II DRB exon-2 diversity of the Eurasian lynx (Lynx lynx) in China. Journal of Natural History 43:245–257

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. Trends in Ecology and Evolution 3:43-49

Yates A, Akanni W, Amode MR, Barrell D, Billis K, et al (2016) Ensembl 2016. Nucleic Acids Research 2016 44, Database issue: D7 10-6.

Yuhki N, O'Brien SJ (1997) Nature and origin of polymorphism in feline MHC class II DRA and DRB genes. Journal of Immunology. 158:2822-2833

Yuhki H, Mullikin J, Beck T, Stephens R, O'Brien SJ (2008) Sequences, annotations and single nucleotide polymorphism of the major histocompatibility complex in the domestic cat. PLOS One 3:1-17

Zhang L, Wu Q, Hu Y, Wu H, Wei F (2015) Major histocompatibility complex alleles associated with parasite susceptibility in wild giant pandas. Heredity 114:85-93

VITA

Name: John Peter Leonard

Education: B.A., Biology with an Environmental Science Concentration, University of Dallas

M.S., Wildlife and Fisheries Sciences, Texas A&M University

Address: 955 N Lantana Dr., Kingsville, Texas 78363

Professional Experience

2013-present	Doctoral Research Assistant, Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, Texas
2011-2013	Advanced Math and Science Tutor, ATAC Inc. Seattle, WA
2012	Sea Sampler, International Pacific Halibut Commission, Seattle, WA
2010-2011	National Marine Fisheries Observer, Saltwater Incorporated, Anchorage, AK
2008-2010	Environmental Resources Management Promoter, U.S. Peace Corps, Namuaimada, Fiji Islands
2004	Field Technician, University of Georgia, Athens, GA
2003-2004	Lab Assistant, University of Dallas, Irving, Texas