frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 16 November 2026
DOI 10.3389/fevo.2026.2014455

Check for updates

OPEN ACCESS

EDITED BY
Lenin Arias Rodriguez,
Universidad Juárez Autónoma de Tabasco, Mexico

REVIEWED BY
Juan De Dios Valdez Leal,
Universidad Juárez Autónoma de Tabasco, Mexico
Lizzie Croose,
British Small Animal Veterinary Association, United Kingdom

*CORRESPONDENCE
Kathan Bandyopadhyay
kathan.bandyopadhyay@tamuk.edu

RECEIVED 29 September 2026
REVISED 15 October 2026
ACCEPTED 19 October 2026
PUBLISHED 16 November 2026

CITATION
Tewes ME, Taylor DR, DeYoung RW, Campbell TA, Brookover E and Bandyopadhyay K (2026) Contrasting road-based scat detections of sympatric bobcats and ocelots in South Texas. *Front. Ecol. Evol.* 14:2014455. doi: 10.3389/fevo.2026.2014455

COPYRIGHT
© 2026 Tewes, Taylor, DeYoung, Campbell, Brookover and Bandyopadhyay. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Contrasting road-based scat detections of sympatric bobcats and ocelots in South Texas

Michael E. Tewes[1], Daniel R. Taylor[1], Randy W. DeYoung[1], Tyler A. Campbell[2], Emma Brookover[1] and Kathan Bandyopadhyay[1]*

[1]Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, TX, United States, [2]East Foundation, San Antonio, TX, United States

Noninvasive genetic surveys are widely used to monitor elusive carnivores, but their effectiveness depends not only on the recovery of DNA but also on where animals choose to leave biological material in the landscape. We evaluated road-based scat sampling for sympatric bobcats (*Lynx rufus*) and ocelots (*Leopardus pardalis*) on private rangelands in southern Texas. We surveyed 34 km of non-paved ranch roads for 14 consecutive days during spring 2016 and 10 consecutive days during winter 2017, using mitochondrial DNA sequencing to identify species and microsatellite genotyping to identify individuals. We identified 72 bobcat scats but no ocelot scats. Concurrent camera stations (n = 28) recorded six ocelot detections during the scat-sampling periods, confirming that ocelots were present in the sampled landscape. Failed amplification of bobcat microsatellite loci declined from 63.3% in 2016 to 22.6% in 2017 after samples were preserved in DETs buffer rather than frozen. From 39 bobcat scats collected in 2017, we identified 20 individuals, including 12 females and eight males. Road transects yielded an average of 3.5 bobcat scats per day, approximately twice the number of bobcat photographic detections per day from the camera array. Road-based scat sampling therefore provided an efficient source of individual-level genetic information for bobcats, but the same approach failed to detect ocelots despite independent confirmation of their presence. This contrast may reflect differences in abundance and detection, but it also fits species-specific patterns of space use and fecal deposition. Our findings highlight an often-overlooked feature of noninvasive genetic monitoring: survey performance depends on the behavior that determines where biological samples become available to observers.

KEYWORDS
gene flow, genetic monitoring, microsatellite genotyping, mitochondrial DNA sequencing, noninvasive

1 Introduction

Biodiversity is declining worldwide as open-natural ecosystems (ONEs) are altered by land conversion, habitat fragmentation, infrastructure development, exploitation, and other human pressures (Dirzo et al., 2014; McGill et al., 2015; Bandyopadhyay et al., 2024). Mammalian carnivores are particularly vulnerable to these changes because many occur at naturally low densities, use large areas, and come into frequent conflict with people as landscapes become increasingly modified (Ripple et al., 2014; Jhala et al., 2025; Cristescu

et al., 2013). Fragmentation can further isolate populations and reduce the amount of suitable habitat available to terrestrial mammals, increasing extinction risk even where some habitat remains (Crooks et al., 2017). For small or geographically isolated populations, maintaining genetic variation and connectivity is also important as it influences the capacity of populations to persist and respond to environmental change (Frankham, 2010). These concerns are especially pronounced for carnivores whose remaining populations are small, fragmented, or difficult to monitor (Veals et al., 2022, 2023; Bandyopadhyay et al., 2026).

Monitoring carnivore populations is often constrained by low densities, large home ranges, and elusive behavior, making repeated demographic and genetic sampling difficult (Waits and Paetkau, 2005; Jhala et al., 2019; Oliveira et al., 2026; de Oliveira et al., 2026). Live capture provides high-quality biological samples but is costly, labor-intensive, and difficult to apply across large areas. Noninvasive genetic sampling offers an alternative by recovering DNA without handling animals (Höss et al., 1992; Taberlet et al., 1999; Ruell and Crooks, 2007). Scat is particularly useful because it can provide information on species, sex, life history, individual identity, genetic diversity, and population structure (Kohn et al., 1999; Schwartz et al., 2007). Its effectiveness, however, depends not only on DNA quality but also on whether samples can be reliably located in the field (Kolipakam et al., 2019).

Southern Texas provides an important natural setting to investigate this current conservation issue (Lombardi et al., 2023; Sergeyev et al., 2023a). Bobcats (*Lynx* rufus) and ocelots (Leopardus pardalis) occur sympatrically and are broadly similar in body size, activity period, and prey use, yet differ markedly in how they use the landscape (Horne et al., 2009; Leonard et al., 2020; Emmons, 1987; Leonard, 2017; Branney et al., 2026; Maharjan et al., 2026). Bobcats are habitat generalists that occupy a range of woody and open vegetation types (Sergeyev et al., 2023a; Maharjan et al., 2026). Unlike ocelots, bobcats remain widely distributed across North America, ranging from southern Canada to Mexico and occurring throughout Texas (Lariviére and Walton, 1997). Recent work in South Texas has shown that bobcat resource selection responds to landscape configuration at fine spatial scales, with animals selecting areas associated with woody cover and edge while still using heterogeneous working landscapes (Branney et al., 2024). Ocelots, in contrast, remain strongly associated with dense woody vegetation, long-term analyses spanning several decades have shown consistent selection for woody cover by Texas ocelots, while much of the remaining high-quality habitat occurs on privately owned lands (Veals et al., 2022). More recent telemetry studies similarly found ocelots strongly tied to woody cover, whereas bobcats and coyotes (*Canis latrans*) used a wider range of vegetation conditions (Sergeyev et al., 2023a). Behavioral-state analyses further suggest that the three carnivores partition their shared landscape through differences in when and where they rest, forage, and patrol territories (Sergeyev et al., 2023b).

These differences have direct consequences for endangered ocelot conservation (U.S. Department of Interior, Fish, and Wildlife Service, 1982). The ocelot historically occurred across a much broader portion of the southern United States, but habitat loss and fragmentation reduced its distribution (Murray and Gardner, 1997; Tewes and Schmidly, 1987; Woodward, 1980) to two remaining breeding populations in southern Texas (Tewes and Everett, 1986; Janečka et al., 2008, 2011; Leslie, 2016; Smith et al., 2025). Dense woody vegetation remains a defining feature of occupied habitat, and recent spatial capture–recapture work has shown that private ranchlands can support relatively high local densities where suitable vegetation persists (Lombardi et al., 2022). At the same time, landscape connectivity between occupied areas remains limited by habitat configuration and roads (Haines et al., 2005; Tewes, 2017), making protection and restoration of connective habitat an important component of recovery (Veals et al., 2023). Genetic information is therefore valuable for tracking individuals, assessing connectivity, and evaluating the consequences (Schwartz et al., 2006) of continued isolation (Smith et al., 2025; Weeks et al., 2011). Recent development of a chromosome-level reference genome for the Texas ocelot also provides a foundation for increasingly detailed genomic monitoring of this population (Foley et al., 2026). These advances do not remove the need for field-based genetic sampling; rather, they increase the value of obtaining biological material repeatedly and with minimal disturbance.

Roads and trails can provide accessible locations for collecting carnivore scat and obtaining DNA from otherwise difficult-to-survey habitats (MacDonald, 1980; Kohn et al., 1999). This is particularly relevant in South Texas, where ocelots are closely associated with dense thornscrub, while bobcats use a broader range of habitats and travel routes (Shindle and Tewes, 1998; Horne, 1998; Horne et al., 2009; Veals et al., 2022). Ocelots may also concentrate fecal deposition at latrine sites rather than along roads (Moreno et al., 2006; King et al., 2017). These differences in space use and marking behavior may therefore influence how effectively road-based scat surveys detect the two species (Sergeyev et al., 2023b; Maharjan et al., 2026).

Our study was based on the premise that existing ranch roads could provide a practical sampling network for collecting noninvasive genetic material from sympatric bobcats and ocelots. We therefore sought to (1) determine the feasibility of systematic fecal collection along ranch roads for detecting bobcat and ocelot presence, (2) use mitochondrial sequencing and microsatellite genotyping to identify species and individuals and estimate minimum abundance from recovered samples, and (3) evaluate the additional information provided by noninvasive scat sampling relative to concurrent camera and live-capture monitoring. More broadly, we asked whether a single road-based sampling approach provides comparable genetic information for two ecologically similar felids that use the same landscape in different ways.

2 Methods

2.1 Study area

The study was conducted on the East Foundation's El Sauz Ranch, located on 110 km^2 in Willacy and Kenedy Counties, Texas (Figure 1). The property was operated as a cattle ranch and venue for wildlife research, education, and outreach to support wildlife conservation and other public benefits of ranching and private land stewardship (www.eastfoundation.net). The eastern border of the property was the coastline of the Laguna Madre of the Gulf of



FIGURE 1
The East Foundation's El Sauz Ranch located on 110 km^2 in Willacy and Kenedy Counties, Texas, USA, where scats were collected for bobcat and ocelot research during 2016 and 2017. Source: Prepared using Environmental Systems Research Institute (Esri). (2017). ArcGIS Desktop [GIS software]. Redlands, CA, USA: Environmental Systems Research Institute. https://www.esri.com. Used under license.

Mexico, and the western portion of the property included known ocelot habitat. The study area was located in southern Texas within the Tamaulipan Biotic Provence (Blair, 1950). This region had hot summers, with daily high temperatures in the upper 30s (°C) and mild winters (Haines et al., 2006). Annual precipitation averaged 64 cm, with high variation across years (Laack, 1991). The major soil associations were sandy, with sand dunes in the central part of the ranch adding topography to an otherwise flat landscape. The Tamaulipan thornscrub ecosystem is unique to southern Texas and northeast Mexico, and is characterized by dense thorny brush (Blair, 1950; Fulbright and Bryant, 2002). Woody habitat that provided canopy cover preferred by ocelots (Shindle and Tewes, 1998; Horne et al., 2009) and bobcats (Cain et al., 2003) was dominated by native thornscrub, honey mesquite *(Prosopis glandulosa)*, and live oak *(Quercus virginiana)* communities. The predominant land uses across the region included cattle ranching, agriculture, energy development, and urbanization.

2.2 Sample collection

We established 34 km of scat transects on existing non-paved ranch roads in the northwest and southwest areas of the ranch, where bobcats & ocelots were known to occur (Figure 2). Road substrate consisted of either crushed caliche stone or unimproved sandy 2-track. These transects followed accessible ranch roads suitable for repeated daily surveys rather than encompassing the entire ranch road network. We estimated effective sampling area in ArcGIS by placing a 2 km^2 buffer around the transects, which is the smallest ocelot home-range size reported for a female ocelot in southern Texas (Navarro-Lopez, 1985; Tewes, 1986; Laack, 1991). We removed carnivore scats from the transects one day prior to the sampling period to avoid collecting scat that was not deposited during the sampling period. Because scats of sympatric small carnivores can overlap in morphological characteristics, distinguishing species reliably in the field is challenging (Morin et al., 2016; Elbroch, 2003). Therefore, we collected suspected felid scats, including samples of uncertain identity, and subsequently confirmed species identity through mitochondrial DNA sequencing. Sampling occasions began every morning at sunrise the following day after clearing. We collected carnivore scats that were potentially from a felid, and collected scats if identification was uncertain to avoid missing detections. To minimize effort devoted to species identification, we did not collect raccoon (*Procyon lotor*) scats, which were readily distinguishable in the field because they often contained conspicuous undigested plant material, particularly fruit remains and seeds (Osaki et al., 2019). Collections were repeated for 14 consecutive days in April to May 2016 and 10 consecutive days in January 2017. The sampling periods were selected to balance repeated opportunities for detecting freshly deposited scats, accounting for species-specific movement and territorial behavior, with seasonal field conditions and the availability of personnel and logistical resources (Waits and Paetkau, 2005; Horne et al., 2009). We photographed and recorded GPS location for each scat collected.

The DNA in scat degrades rapidly over time, so it was important to collect and quickly preserve samples (Foran et al., 1997). We used two distinct methods of scat preservation, flash-freezing and a storage buffer. During 2016, we collected whole scats by inverting a 532 mL whirl-pak (Nasco, Stamford, CT) and added 1–5 g absorbent packets of silica gel (MiniPax®, Sigma-Aldrich, St. Louis, MO) to remove moisture and prevent molding and degradation of the sample. Samples were kept in a shaded compartment in the vehicle and transported to a cooler with dry ice ≤4 hrs from the time of collection. Samples were kept in the cooler with dry ice for 2–8 days before being transported to a −20 °C freezer. Samples

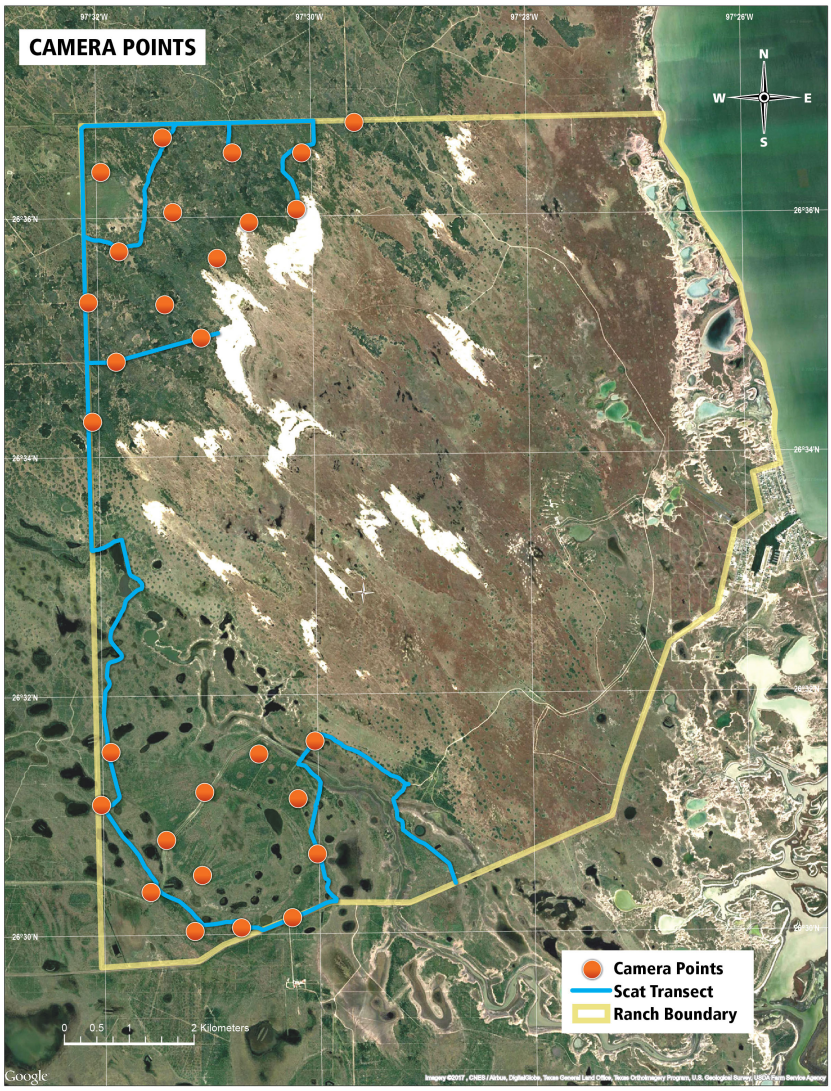


FIGURE 2
The East Foundation's El Sauz Ranch in Kenedy and Willacy Counties, Texas, USA. Scat collection transects (34 km) were established in 2016 and 2017 on existing ranch roads in the northwest and southwest areas of the ranch, where ocelots and bobcats are known to occur. A camera grid, 28 stations with 2 cameras per station, was placed near game trails or roads and spaced about 1 km apart. Source: Prepared using Environmental Systems Research Institute (Esri). (2017). ArcGIS Desktop [GIS software]. Redlands, CA, USA: Environmental Systems Research Institute. https://www.esri.com. Used under license.

were kept at −20 °C until extraction. During 2017, we placed 0.4 mL of the outer surface of each partially liquified scat into 2 mL screw top tubes containing 1.4 mL of DETs (DMSO/EDTA/Tris/salt) buffer (Frantzen et al., 1998). The DETs buffer consists of 20% dimethyl sulfoxide, 0.25 M sodium-EDTA, 100 mM Tris, pH 7.5, and NaCl to saturation (Seutin et al., 1991). Samples were kept in a portable cooler while in the field and stored at 4 °C ≤8 hrs of collection. We wore disposable latex gloves and replaced gloves between samples.

2.3 DNA extraction

We performed DNA extractions under a fume-hood in the Molecular Ecology Laboratory at Texas A&M University-Kingsville. Each frozen sample was taken individually from the -20 °C storage and placed on a sterile disposable petri dish in the fume-hood. Forceps were cleaned with 95% ethanol prior to use for handling each sample. The surface of each frozen scat was shaved with a new razor blade to target epithelial cells remaining on the surface from the colon wall of the defecating animal (Reed et al., 2004; Rutledge et al., 2009). Enough liquified scat was used to displace 400 µL of lysis buffer. For the DETs method, we began by homogenizing the samples in the 2 mL collection tubes of DETs buffer for 3 min using a vortex mixer. We pipetted 200 µL of homogenized scat into the tube of lysis buffer by cutting the end of a 1,000-µL filtered pipette tip. For both methods, we used the QIAamp® DNA Stool Mini Kit and the protocol recommended by the manufacturer (Qiagen, Hilden, Germany), with the exception that we performed the final elution step using 120 µL of storage buffer after incubation for 5 min. We performed extractions in sets of 12, including 11 samples and one extraction negative of reagents for quality control (Waits and Paetkau, 2005). We stored DNA extracts at 4°C until further use.

2.4 Species identification

We sequenced the mitochondrial DNA ATP6 gene to identify species for each scat sample. We chose primers recommended by Chaves et al. (2012) due to their ability to distinguish between 66 species of carnivores, including 12 felids such as bobcats and ocelots, without amplifying non-carnivore (prey) species. The fragments were 172 base-pairs in size, with 126 base-pairs excluding the forward and reverse primers: ATP6-DF3 (5′–AACGAAAATCTATTCGCCTCT–3′; Haag et al., 2009), and ATP6-DR1 (5′–CCAGTATTTGTTTTGATGTTAGTTG–3′; Trigo et al., 2008). We amplified extracts from scat via the polymerase chain reaction (PCR) in 25-μL volumes comprised of 12.5 μL of AmpliTaq Gold® 360 PCR master mix (*Taq* DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers; Applied Biosystems), 8.3 μL double-deionized H_2O, 1 μL bovine serum albumin (BSA; 2 mg/mL; Thermo Scientific, Waltham, MA), 10 pmol of each forward and reverse primer, and 3 μL DNA extract. Thermocycling conditions were an initial denaturation at 94 °C for 3 min, followed by 10 cycles at 94 °C for 45 sec, a touchdown annealing temperature of 60 °C for 45 sec that decreased 1 °C each cycle to 50 °C, 72 °C for 1 min 30 sec. Next, 30 cycles of 94 °C for 45 sec, 50 °C for 45 sec, and 72 °C for 1 min 30 sec were followed by a final extension at 72 °C for 5 min. We chose to use 3 min initial denaturation instead of 10 min to allow the enzyme activity from the built-in hot start polymerase to increase over cycles as the amount of template increased (Lorenz, 2012; Applied Biosystems).

We visualized PCR products via electrophoresis on a 1% agarose gel containing ethidium bromide and a 100 base-pair DNA ladder. Samples that did not show a band of the predicted size were removed from further analysis. We removed unincorporated primers and dNTPs from successful amplifications using an enzymatic method (ExoSAP-IT®, Affymetrix Inc., Santa Clara, CA). We cycle-sequenced forward strands using the BigDye® Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems), and removed dye terminators using the DyeEx® 2.0 spin kit (Qiagen). We loaded sequencing reaction products onto a 3130*xl* Genetic Analyzer for separation and detection (Applied Biosystems). We edited and aligned sequences using the computer program Geneious® V10 (Kearse et al., 2012). Each round of PCR included positive and negative controls to confirm amplification and guard against contamination.

We also confirmed the ability of the primers to discriminate among bobcat, ocelot, jaguarundi *(Puma yagouaroundi)*, and mountain lion *(Puma concolor)* using high-quality DNA extracted from blood collected in the Tewes Laboratory. Ocelot blood samples were collected according to local and institute guidelines under Tewes' USFWS federal permits. We included jaguarundi and mountain lion because they have been historically documented in the area. We determined the number of variable sites between sequences of 20 bobcats, five ocelots, one jaguarundi, and two mountain lions and constructed a percent identity matrix to quantify variability among sequences. We compared the sequences to references available in Genbank using the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990). We determined species using a consensus of the top three results.

2.5 Individual identification

Individual bobcats and ocelots were identified using microsatellite genotyping. Our empirically optimized protocol for 10 μL PCR volumes contained 5 μL of AmpliTaq Gold® PCR master mix (Applied Biosystems), 1.8 μL double-deionized H_2O, 0.2 μL BSA (20 mg/μL), 5 pmol of forward and reverse primer, and 2 μL DNA extract. Thermocycling conditions for were an initial denaturation at 94 °C for 10 min, followed by 10 cycles at 94 °C for 30 sec, a within-cycle decreasing annealing temperature of 62 °C for 30 sec, 61 °C for 30 sec, 60 °C for 30 sec, and an extension of 72 °C for 60 sec. Next, 40 cycles at 94 °C for 30 sec, 55 °C for 90 sec, and 72 °C for 60 sec were followed by a final extension of 72 °C for 30 min.

We amplified 11 microsatellite loci in separate reactions; the reaction products were then combined into three panels for separation and detection. The loci were originally described by Menotti-Raymond et al. (1999), and these 11 loci were chosen based on previous studies of bobcats and ocelots in Texas (Janečka et al., 2008, 2016; Davis, 2015). Panel One was a mixture containing PCR products of FCA008 (2 μL), FCA043 (1 μL), FCA082 (1 μL), FCA090 (2 μL) and 5 μL double-deionized H_2O. Panel Two was a mixture containing PCR products of FCA035 (2 μL), FCA096 (2 μL), FCA132 (1 μL), FCA133 (1 μL), and 5 μL double-deionized H_2O. Panel Three was a mixture of 1 μL each PCR products of FCA045, FCA077, FCA176 and 5 μL double-deionized H_2O, anddenatured 1.1 μL of each PCR product mixture with 0.5 μL GeneScan ROX500 size standard and 11 μL Hi-Di formamide (Applied Biosystems). The resulting product was loaded onto an Applied Biosystems 3130*xl* genetic analyzer for fragment separation and detection. We scored alleles and merged into consensus genotypes using the computer program Genemapper® (Applied Biosystems).

We calculated failed amplification rates instead of success rates for nuclear DNA analyses because amplification "success" can be misleading in the presence of genotyping errors such as allelic dropout or false alleles. A failed amplification is defined as the number of individual PCRs with the absence of a scored allele within the correct size range for each locus divided by the number of individual PCRs attempted at each locus (Panasci et al., 2011). The 11 loci were amplified twice for each sample, and the sample was removed if the two rounds yielded greater than 50% failure of the 22 total amplifications. We re-extracted and re-analyzed six randomly chosen samples that failed to amplify to determine if re-extraction would yield better results.

To estimate the minimum number of markers needed for distinguishing between individuals, we calculated the probability of identity ($P_{(ID)unbiased}$; Paetkau et al., 1998), and the probability of identity among siblings with codominant loci ($P_{(ID)sib}$; Evett and Weir, 1998) using Cervus 3.0 (Kalinowski et al., 2007). We accepted the minimum number of markers needed to achieve a $P_{(ID)sib}$ of <0.001 for analyses (Waits et al., 2001). We removed two loci with high rates of failed amplification, and assembled consensus genotypes of the remaining nine loci for all individuals. We followed the multiple-tube approach (Taberlet et al., 1996), and accepted two matching heterozygote and three matching homozygote alleles at each locus, where alleles were confirmed in replicate PCR reactions.

Each round of PCR included negative controls to monitor for contamination. We quantified genotyping errors from loci with consensus genotypes by calculating mean allelic dropout and false allele rates (Broquet and Petit, 2004). We retained a genotype for analysis if at least five of nine loci were assigned a consensus allele score.

We identified sex of each sample by analyzing Y-chromosome deletions in the zinc-finger region (Pilgrim et al., 2005; Ruell et al., 2009). This method provides a positive for both sexes and minimizes interference with prey DNA because Y-chromosome deletions are absent in the prey species of wild felids (Pilgrim et al., 2005). We used PCR and the following primer sequences resulting in Y- and X-chromosome products for males 163 and 166 base-pairs long and 166 base-pairs for females: Zn-finger forward (5′-AAGTTTACACAACCACCTGG-3′) and Zn-finger reverse (5′-CACAGAATTTACACTTGTGCA-3′; Pilgrim et al., 2005). The forward primer was fluorescently labeled for detection on an automated sequencer. We amplified the zinc-finger region in 10 μL reactions containing 5 μL of AmpliTaq Gold® PCR master mix (Applied Biosystems, Waltham, MA), 1.8 μL double-deionized H_2O, 0.2 μL BSA (20 mg/mL), 5 pmol of each forward and reverse primer, and 2 μL DNA extract. The PCR profile consisted of an initial denaturation at 94°C for 10 min, followed by 45 cycles at 94°C for 30 sec, 56°C for 90 sec, 72°C for 1 min, followed by a final extension at 72 °C for 30 min. Each round of PCR contained two male and two female bobcats extracted from blood positive controls, and one PCR negative of reagents for quality control.

2.6 Comparison of noninvasive genetic data to camera traps

We found matching genotypes using the Excel Microsatellite Toolkit (Park, 2001). We evaluated a plot of mismatching alleles before and after additional rounds of PCR (Paetkau, 2004). We accepted closely matching genotypes if they contained up to two mismatching alleles to avoid adding "ghost" individuals to the population because of errors associated with genotyping noninvasive sources of DNA (Creel et al., 2003). We recorded capture histories of each individual for the 10 sampling occasions, all from 2017 due to failure to obtain individual genotypes from the 2016 samples, after identifying matching genotypes. We determined abundance using the number of unique genotypes identified (Burnham and Overton, 1979; Chao, 1988).

TABLE 1 A percent identity matrix constructed from mitochondrial DNA sequences of the ATP6 gene from 20 bobcats, 5 ocelots, 1 jaguarundi, and 1 mountain lion. Bobcat and ocelot samples were collected from southern Texas, USA, 2012–2017, the mountain lion from West Texas, USA, 2010, and the jaguarundi from Tamaulipas, Mexico, 2004.

	Bobcat	Ocelot	Jaguarundi	Mt Lion
Bobcat		89.7%	92.6%	92.6%
Ocelot	89.7%		91.2%	91.2%
Jaguarundi	92.6%	91.2%		94.1%
Mt Lion	92.6%	91.2%	94.1%	

ATP6, mitochondrial ATP synthase subunit 6; DNA, deoxyribonucleic acid; Mt Lion, mountain lion (*Puma concolor*); USA, United States of America.

We used an existing grid of infrared cameras to both verify the presence of wild felids and to compare indices of abundance derived from cameras to abundance estimated from fecal genotyping. The camera grid consisted of 28 stations with two cameras per station, placed near game trails, and spaced 1 km apart (Figure 2). The cameras were set for continuous detection with a 30-sec delay between images. We recorded the number of independent detections of bobcats and ocelots during the scat collection periods. We estimated minimum abundance from individual identifications over a similar 3-month period.

Finally, we evaluated the feasibility of genetic monitoring using noninvasive sampling based on time, scale, and effort required to perform monitoring activities. We determined average daily search time, and average search time transect for a per felid-positive detection. The scale of this study was demonstrated through the total transect distance and estimated effective sampling area. Effort was determined based on the number of observers, method of transportation, and other technical considerations.

3 Results

3.1 Species identification

We collected 172 carnivore scats and identified 89.5% to species ($n = 154$); 18 samples failed to amplify. We identified zero ocelot, 72 bobcat, and 81 coyote scat samples. No sequences of non-target prey DNA amplified from scat samples. With DNA extracted from blood samples, we identified four variable sites with three haplotypes in the 20 bobcat sequences, and one haplotype in the five ocelot sequences, resulting in 15 variable sites with 89.7% sequence identity between bobcats and ocelots (Table 1). We identified 22 variable sites among bobcats, ocelots, jaguarundi, and mountain lion sequences acquired from blood samples.

3.2 Individual identification

Failed amplification rate of frozen bobcat samples ($n = 33$) using 11 loci was 66.9% ($SD = 13.9\%$; Table 2). The FCA035 and FCA096 loci were removed from analysis after failed amplification rates of 79.6% and 88.7%, respectively. With the remaining nine loci, failed amplification rate of frozen bobcat samples was 63.3% ($SD = 12.6\%$; Table 2). Of the frozen bobcat samples, eight were female, zero male, and 25 failed to amplify from the zinc-finger region. Because only two bobcat scats met genotyping requirements for frozen samples, samples collected during the 14 days in 2016 were removed from further analysis.

Failed amplification rate of DETs-preserved bobcat samples ($n = 39$) using 11 loci was 28.3% ($SD = 9.9\%$; Table 2). As before, the FCA035 and FCA096 loci were removed from analysis after failed amplification rates of 36.9% and 47.9%, respectively. With the remaining nine loci, failed amplification rate of DETs bobcat samples was 22.6% ($SD = 7.2\%$). Of the DETs-preserved bobcat samples, there were 22 female, 11 male, and six that failed to amplify from the zinc-finger region. Twenty-six bobcat scats met genotyping requirements for DETs samples and were included in analysis of

TABLE 2 Genetic data from noninvasive sampling of bobcat scat in Kenedy and Willacy Counties, Texas, USA, during 2016–2017 preserved frozen or in a DMSO/EDTA/Tris/salt (DETs) storage buffer. The DETs buffer performed better than frozen storage for preserving DNA for genetic analysis.

	Method	Bobcat Scats	Failed Amp. Rate	*SD*	Male Scats	Female Scats	Unknown Sex Scats	Consensus genotypes	*N*	No. Males	No. Females
2016–11 loci	Frozen	33	66.86%	13.91%	0	8	25	2	2	0	2
2016 9 loci			63.29%	12.58%							
2017–11 loci	DETs	39	28.27%	9.86%	11	22	6	26	20	8	12
2017 9 loci			22.60%	7.24%							

Amp., amplification; DETs, dimethyl sulfoxide/ethylenediaminetetraacetic acid/Tris/salt buffer; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; N, number of genetically identified individuals; No., number; SD, standard deviation; Tris, tris(hydroxymethyl)aminomethane.

individual identification and population size. The average consensus genotype was 8.23 of 9 loci (*SD* = 1.27). Of these, 20 individual bobcats were identified, including 12 females and eight males (Figure 3). Observed $P_{(ID)unbiased}$ was 7.2×10^{-10}, and $P_{(ID)sib}$ was 2.7×10^{-4} at nine loci. There were no difference between original and re-extracted samples in amplification rates (n = 6, 71.9% vs. 72.2%). Mean allelic dropout rate was 13.8% (*SD* = 7.5%), and false allele rate was 2.8% (*SD* = 3.1%). Three sampling occasions were impacted by rain, and we were only able to produce consensus genotypes from 36% of bobcat scats compared to 79% of scats collected in fair weather. The rain impacted the 7th, 8th, and 9th sampling occasions, which likely contributed to the low frequency of recaptures.

3.3 Population size estimation

We identified a minimum population size of 20 individual bobcats (12 female, 8 male) from 26 genotypes collected in the 10-day period in 2017. We assigned a match to two genotypes that differed by one mismatching allele after multiple rounds of replication amplified one of the consensus heterozygote alleles, likely indicating allelic dropout. The 6 other comparisons had exact matches. There were four female and two male recaptured bobcat samples. These consisted of three individual females (two caught twice and one caught three times) and one male (caught three times). No individual bobcat was collected twice in the same sampling occasion. Effective sampling area was 111.1 km^2. Using only the minimum population size of the 20 unique bobcats, minimum bobcat density was estimated at 0.18 bobcat per km^2, or 5.56 km^2 per individual bobcat.

Camera stations recorded six ocelot, 36 bobcat, 61 coyote, and zero American badger (*Taxidea taxus*) detections during the same collection periods. Therefore, ocelots were confirmed to occupy the study area during scat sampling periods. Image quality and lack of distinctive spotting patterns in bobcats made individual identification via camera difficult. Nonetheless, 3 months of camera trapping detected a minimum bobcat abundance of 12 individuals and a minimum ocelot abundance of five individuals. We identified twice as many bobcat scats as we did camera detections. Average transect sampling time was 3 hr 55 min per day with one observer and utility vehicle. The frequency of encounter for bobcat scat was 9.32 km per scat and about one observer transect search hour per bobcat scat.

4 Discussion

Road-based scat sampling produced markedly different results for the two sympatric felids. We identified species from nearly 90% of all carnivore scats collected, including 72 bobcat scats, but did not detect ocelot DNA in any sample. This contrast was not simply a consequence of ocelot absence: camera traps detected ocelots during the sampling periods and documented at least five individuals within the surrounding 3-month period. Scat sampling was particularly effective for bobcats, with 42% of collected samples assigned to the species and 20 individuals identified from samples collected in 2017. Coyotes accounted for many of the remaining samples, illustrating the difficulty of assigning carnivore scat to species based on appearance alone. Together, these results show that molecular identification worked well, but the availability of scat along roads differed sharply among species.

The lack of ocelot scat differs from work in Belize, where fecal sampling along trails successfully detected ocelots (Wultsch et al., 2014). Lower abundance may partly explain this difference, but where animals deposit scat is also likely important. Ocelots use scent for territorial and social communication and frequently defecate at repeatedly used latrine sites (Emmons, 1988; Moreno et al., 2006; King et al., 2017). In Panama, most ocelot scats were associated with latrines rather than trails (Moreno et al., 2006). Such sites in South Texas may occur within dense woody cover, where they would be unlikely to intersect road-based surveys. Recent telemetry studies reinforce the strong association of Texas ocelots with woody vegetation and show that ocelots and bobcats partition the same landscape through differences in habitat use and behavioral state (Sergeyev et al., 2023a, 2023b; Sergeyev et al., 2024). Bobcats are less restricted to dense cover and make greater use of heterogeneous and open portions of South Texas rangelands (Branney et al., 2024). More recent work also shows that bobcat movement behavior changes near roads and highways, with individuals regularly traveling and patrolling within these modified landscapes (Maharjan et al., 2026; Litvaitis et al., 2015). Roads may therefore intersect the movements and fecal

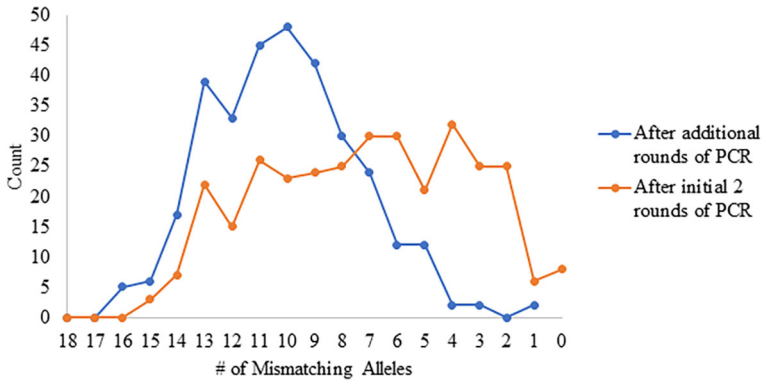


FIGURE 3
Mismatch distribution for 11-locus microsatellite DNA genotypes from noninvasive samples of bobcats collected in southern Texas, USA, during 2016–2017. Plots depict frequency of mismatching alleles before and after verification using ≥2 independent PCRs. We evaluated a plot of mismatching alleles before and after additional rounds of PCRs from consensus bobcat genotypes consisting of 11 microsatellite loci.

deposition of bobcats more often than those of ocelots (Yamashita et al., 2026). Our data cannot distinguish routine defecation from deliberate scent marking, but they show that road-based collection encountered sign from the two felids at very different rates. Thus, differences in habitat use may concentrate ocelot scat within dense thornscrub and bobcat scat along open ranch roads, contributing to contrasting detection rates (Horne et al., 2009).

Noninvasive sampling also provided information on individual bobcats that was difficult to obtain from cameras alone. We identified a minimum of 20 individuals—12 females and eight males; from 26 usable genotypes collected during the 10-day sampling period in 2017, compared with a minimum of 12 bobcats identified from three months of camera trapping. Individual differences in scat detection are expected because movement, home-range size, territorial behavior, and marking rates can all affect the probability that an individual's scat is encountered (Janečka et al., 2006; Ruell et al., 2009; Lonsinger et al., 2016). Female scats were approximately twice as common as male scats among samples for which sex could be determined. Female bobcats generally occupy smaller home ranges than males, whereas males range more widely and overlap with several female territories (Bailey, 1974; Lariviére and Walton, 1997). Recent work in South Texas further shows sex-specific differences in bobcat movement near roads (Maharjan et al., 2026), while long-term observations suggest that bobcat spatial and social relationships may be more flexible than the traditional view of a strictly solitary felid (Branney et al., 2026). We therefore view the observed sex ratio cautiously: it may reflect differences in local abundance, space use, road use, or marking behavior rather than any single mechanism.

Sample preservation strongly affected the amount of individual-level information that could be recovered. Mitochondrial DNA was sufficiently robust for species identification under both preservation protocols, but microsatellite amplification improved substantially when scat was stored in DETs buffer rather than frozen. Failed amplification declined from 63.3% for the nine retained loci in 2016 to 22.6% in 2017. The poorer performance of frozen samples was likely associated with variable temperatures and repeated freeze–thaw exposure before long-term storage. Rain also reduced genotyping success, with considerably fewer consensus genotypes recovered from samples collected during wet conditions. These patterns are consistent with the broader experience of scat-based genetic studies, where preservation, weather, sample age, and DNA degradation strongly influence amplification success (Waits and Paetkau, 2005; Oliveira et al., 2026; de Oliveira et al., 2026). Thus, field preservation was as important as laboratory genotyping for determining whether a collected scat ultimately yielded an individual identification.

The contrast between bobcats and ocelots has a practical implication for carnivore monitoring in South Texas. Scat DNA can provide species, sex, and individual identity from a sample that requires no capture or handling, and our results show that road surveys can recover this information efficiently for bobcats. The same sampling design, however, should not be assumed to work equally well for an ecologically similar species occupying the same landscape. Recent studies continue to show strong dependence of Texas ocelots on woody vegetation, sex-specific variation in their space use, and behavioral responses to landscape structure (Veals et al., 2022; Sergeyev et al., 2023a, 2023b; Smith et al., 2025). For ocelots, searches directed toward latrines or other frequently used locations—potentially aided by detection dogs—may therefore be more productive than road transects alone (Morini et al., 2024). Combining such sampling with cameras would also provide independent evidence of presence when scat detection is low.

Noninvasive genetic sampling remains particularly valuable for the small and isolated ocelot populations of South Texas, where repeated capture is difficult and access to private lands can limit intensive monitoring. Our results show, however, that a sampling method effective for one carnivore may perform poorly for another occupying the same landscape. Road-based scat surveys yielded abundant genetic information for bobcats but failed to detect ocelots despite their confirmed presence. Monitoring programs should therefore account for species-specific habitat use and fecal deposition when designing noninvasive surveys. For ocelots, targeted searches of latrines and dense woody cover, combined with camera traps or detection dogs, may provide a more effective source of genetic material than road surveys alone. Matching sampling design to species behavior will be essential if noninvasive genetics is to contribute reliably to long-term monitoring and recovery of ocelots in South Texas.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ME: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TC:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. KB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The authors declare financial support was received for the research, authorship, and/or publication of this article. Collaborative funding was provided by the Rob and Bessie Welder Wildlife Foundation, East Foundation, the Feline Research Program at the Caesar Kleberg Wildlife Research Institute, the Brown Foundation, the Tim and Karen Hixon Foundation, and the United States Fish and Wildlife Service.

Acknowledgments

We thank L. Balderas, S. Carter, J. Lombardi, T. Shirley, J. Wied, and P. Ortega, Jr., for assistance in the field and D. Williford for assistance in the laboratory. This is manuscript 26–137 of the Caesar Kleberg Wildlife Research Institute and 033 of the East Foundation.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. Generative AI was used to assist with language editing and to improve the clarity and readability of the manuscript. All scientific content, analyses, interpretations, and conclusions were developed and verified by the authors.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi: 10.1016/S0022-2836(05)80360-2

Bailey, T. N. (1974). Social organization in a bobcat population. *J.Wildl. Manage.* 38, 435–446. doi: 10.2307/3800874

Bandyopadhyay, K., Banerjee, K., Mazzamuto, M., Koley, S., Koprowski, J. L., Qureshi, Q., et al. (2024). Review of small cat ecology and status within India. *Mammal Rev.* 54, 341–356. doi: 10.1111/mam.12348

Bandyopadhyay, K., Cristescu, B., Anjaria, S., Verschueren, S., Koprowski, J. L., Holbrook, J. D., et al. (2026). Potential vs. realized large carnivore density: The

influence of livestock on carnivore carrying capacity estimation. *Anim. Conserv.*, 1–12. doi: 10.1111/acv.70079

Blair, W. F. (1950). The biotic provinces of Texas. *Tex. J. Sci.* 2, 93–117.

Branney, A. B., Dutt, A. M. V., Wardle, Z. M., Tanner, E. P., Tewes, M. E., and Cherry, M. J. (2024). Scale of effect of landscape patterns on resource selection by bobcats (Lynx rufus) in a multi-use rangeland system. *Landsc. Ecol.* 39 (8), 147.

Branney, A. B., Yamashita, T. J., Petracca, L. S., Lombardi, J. V., Scognamillo, D. G., Blankenship, T. L., et al. (2026). Rethinking solitude: 5 decades of data reveal social tolerance in a traditionally solitary felid. *J. Mammal.* 00, 1–12. doi: 10.1093/jmammal/gyag058

Broquet, T., and Petit, E. (2004). Quantifying genotyping errors in noninvasive population genetics. *Mol. Ecol.* 13, 3601–3608. doi: 10.1111/j.1365-294X.2004.02352.x

Burnham, K. P., and Overton, W. S. (1979). Robust estimation of population size when capture probabilities vary among individuals. *Ecol.* 60, 927–936. doi: 10.2307/1936861

Cain, A. T., Tuovila, V. R., Hewitt, D. G., and Tewes, M. E. (2003). Effects of a highway and mitigation projects on bobcats in southern Texas. *Biol. Conserv.* 114, 189–197. doi: 10.1016/S0006-3207(03)00023-5

Chao, A. (1988). Estimating animal abundance with capture frequency data. *J. Wildl. Manage.* 52, 295–300. doi: 10.2307/3801237

Chaves, P. B., Graeff, V. G., Lion, M. B., Oliveira, L. R., and Eizirik, E. (2012). DNA barcoding meets molecular scatology: Short mtDNA sequences for standardized species assignment of carnivore noninvasive samples. *Mol. Ecol. Res.* 12, 18–35. doi: 10.1111/j.1755-0998.2011.03056.x

Creel, S., Spong, G., Sands, J. L., Rotella, J., Zeigle, J., Joe, L., et al. (2003). Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Mol. Ecol.* 12, 2003–2009. doi: 10.1046/j.1365-294X.2003.01868.x

Cristescu, B., Bernard, R. T., and Krause, J. (2013). Partitioning of space, habitat, and timing of activity by large felids in an enclosed South African system. *J. Ethol.* 31, 285–298. doi: 10.1007/s10164-013-0376-y

Crooks, K. R., Burdett, C. L., Theobald, D. M., King, S. R., Di Marco, M., Rondinini, C., et al. (2017). Quantification of habitat fragmentation reveals extinction risk in terrestrial mammals. *Proc. Nat. Acad. Sci. U.S.A.* 114, 7635–7640. doi: 10.1073/pnas.1705769114

Davis, I. A. (2015). *Landscape Genetics of Bobcats (Lynx Rufus) Across Western Texas* (Canyon, USA: West Texas A&M University). Thesis.

de Oliveira, M. E., Gonçalves, F., and Galetti, J. P. M. (2026). Scat DNA applications for low-density carnivore survey: Techniques, efficiency and future directions. *Mammal Rev.* 56, e70007. doi: 10.1111/mam.70007

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J., and Collen, B. (2014). Defaunation in the anthropocene. *Science* 345, 401–406. doi: 10.1111/mam.70007

Elbroch, M. (2003). *Mammal Tracks and Sign: A Guide to North American Species. 1st ed.* (Mechanicsburg, Pennsylvania, USA: Stackpole Books).

Emmons, L. H. (1987). Comparative feeding ecology of felids in a Neotropical rainforest. *Behav. Ecol. Sociobiol.* 20, 271–283. doi: 10.1007/BF00292180

Emmons, L. H. (1988). A field study of ocelots (*Felis pardalis*) in Peru. *Rev. d'Ecol. (La Terre la Vie).* 43, 133–157. doi: 10.3406/revec.1988.5418

Evett, I. W., and Weir, B. S. (1998). *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists* (Sunderland, Massachusetts, USA: Sinauer).

Foley, N. M., Brookover, E., Tewes, M. E., and Murphy, W. J. (2026). A chromosome-level genome assembly of the Texas ocelot (*Leopardus pardalis albescens*). *J. Hered.* 117, 267–271. doi: 10.1093/jhered/esaf095

Foran, D. R., Crooks, K. R., and Minta, S. T. (1997). Species identification from scat: An unambiguous genetic method. *Wildl. Soc Bull.* 25, 835–839. Available online at: http://www.jstor.org/stable/3783732 (Accessed November 8, 2017).

Frankham, R. (2010). Challenges and opportunities of genetic approaches to biological conservation. *Biol. Conserve.* 143, 1919–1927. doi: 10.1016/j.biocon.2010.05.011

Frantzen, M. A. J., Silk, J. B., Ferguson, J. W., Wayne, R. K., and Kohn, M. H. (1998). Empirical evaluation of preservation methods for fecal DNA. *Mol. Ecol.* 7, 1423–1428. doi: 10.1046/j.1365-294x.1998.00449.x

Fulbright, T. E., and Bryant, F. C. (2002). *The last great habitat* (Kingsville, TX, USA: Caesar Kleberg Wildlife Research Institute). Special Publication Number 1.

Haag, T., Santos, A. S., De Angelo, C., Srbek-Araujo, A. C., Sana, D. A., Morato, R. G., et al. (2009). Development and testing of an optimized method for DNA-based identification of jaguar (*Panthera onca*) and puma (*Puma concolor*) faecal samples for use in ecological and genetic studies. *Genetica* 136, 505–512. doi: 10.1007/s10709-008-9347-6

Haines, A. M., Janečka, J. E., Tewes, M. E., Grassman, L. I. Jr., and Morton, P. (2006). The importance of private lands for ocelot (*Leopardus pardalis*) conservation in the United States. *Oryx* 40, 90–94. doi: 10.1017/S0030605306000044

Haines, A. M., Tewes, M. E., Laack, L. L., Grant, W. E., and Young, J. (2005). Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States. *Biol. Conserv.* 126, 512–522. doi: 10.1016/J.BIOCON.2005.06.032

Horne, J. S. (1998). *Habitat Partitioning of Sympatric Ocelot and Bobcat in Southern Texas* (Kingsville, USA: Texas A&M University-Kingsville). Thesis.

Horne, J. S., Haines, A. M., Tewes, M. E., and Laack, L. L. (2009). Habitat partitioning by sympatric ocelots and bobcats: Implications for recovery of ocelots in southern Texas. *Southw. Nat.* 54, 119–126. doi: 10.1894/PS-49.1

Höss, M., Kohn, M., Pääbo, S., Knauer, F., and Schröder, W. (1992). Excrement analysis by PCR. *Nature* 359, 199. doi: 10.1038/359199a0/

Janečka, J. E., Blankenship, T. L., Hirth, D. H., Tewes, M. E., Kilpatrick, C. W., Grassman, J. Jr., et al. (2006). Kinship and social structure of bobcats (*Lynx rufus*) inferred from microsatellite and radio-telemetry data. *J. Zool.* 269, 494–501. doi: 10.1111/j.1469-7998.2006.00099.x

Janečka, J. E., Tewes, M. E., Davis, I. A., Haines, A., Caso, A., Blankenship, T., et al. (2016). Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot. *Conserv. Genet.* 17, 1093–1108. doi: 10.1007/s10592-016-0846-1

Janečka, J. E., Tewes, M. E., Laack, L. L., Caso, A., Grassman, L. I.Jr., Haines, A. M., et al. (2011). Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas. *Anim. Conserv.* 14, 608–619. doi: 10.1111/j.1469-1795.2011.00475.x

Janečka, J. E., Tewes, M. E., Laack, L. L., Grassma, L. I. Jr., Haines, A. M., and Honeycutt, R. L. (2008). Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States. *Conserv. Genet.* 9, 869–878. doi: 10.1007/S10592-007-9412-1

Jhala, Y. V., Banerjee, K., Chakrabarti, S., Basu, P., Singh, K., Dave, C., et al. (2019). Asiatic lion: Ecology, economics, and politics of conservation. *Front. Ecol. Evol.* 7, 312. doi: 10.3389/fevo.2019.00312

Jhala, Y. V., Mungi, N. A., Gopal, R., and Qureshi, Q. (2025). Tiger recovery amid people and poverty. *Science* 387, 505–510. doi: 10.1126/science.adk4827

Kalinowski, S. T., Taper, M. L., and Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16, 1099–1106. doi: 10.1111/j.1365-294X.2007.03089.x

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinf.* 28, 1647–1649. doi: 10.1093/bioinformatics/bts199

King, T. W., Salom-Perez, R., Shipley, L. A., Quigley, H. B., and Thornton, D. H. (2017). Ocelot latrines: Communication centers for Neotropical mammals. *J. Mamm.* 98, 106–113. doi: 10.1093/jmammal/gyw174

Kohn, M. H., York, E. C., Kamradt, D. A., Haught, G., Sauvajot, R. M., and Wayne, R. K. (1999). Estimating population size by genotyping faeces. *Proc. R. Soc London Ser. B.* 266, 657–663. doi: 10.1098/rspb.1999.0686

Kolipakam, V., Singh, S., Pant, B., Qureshi, Q., and Jhala, Y. V. (2019). Genetic structure of tigers (*Panthera tigris tigris*) in India and its implications for conservation. *Glob. Ecol. Conserv.* 20, e00710. doi: 10.1016/j.gecco.2019.e00710

Laack, L. L. (1991). *Ecology of the Ocelot (Felis Pardalis) in South Texas* (Kingsville, USA: Texas A&I University). Thesis.

Lariviére, S., and Walton, L. R. (1997). Lynx rufus. *Mamm. Species* 24, 1–8. doi: 10.2307/3504533

Leonard, J. P. (2017). *Home Range Characteristics, Activity Patterns, and Resource Selection of Sympatric Ocelots (Leopardus Pardalis) and Bobcats (Lynx Rufus) and Major Histocompatibility Complex Variation in Ocelots* (Kingsville, USA: Texas A&M University-Kingsville). Dissertation.

Leonard, J. P., Tewes, M. E., Lombardi, J. V., Wester, D. W., and Campbell, T. A. (2020). Effects of sun angle, lunar illumination, and diurnal temperature on temporal movement rates of sympatric ocelots and bobcats in South Texas. *PLoS One* 15 (4), e0231732.

Leslie, D. M. Jr. (2016). *An international borderland of concern: Conservation of biodiversity in the Lower Rio Grande Valley* (U.S. Geological Survey Scientific Investigations) (Accessed November 8, 2017). Report 2016-5078.

Litvaitis, J. A., Reed, G. C., Carroll, R. P., Litvaitis, M. K., Tash, J., Mahard, T., et al. (2015). Bobcats (Lynx rufus) as a model organism to investigate the effects of roads on wide-ranging carnivores. *J. Environ. Manage.* 55, 1366–1376. doi: 10.1007/s00267-015-0468-2

Lombardi, J. V., Sergeyev, M., Tewes, M. E., Schofield, L. R., and Wilkins, R. N. (2022). Spatial capture-recapture and LiDAR-derived vegetation metrics reveal high densities of ocelots on Texas ranchlands. *Front. Conserv. Sci.* 3, 1003044. doi: 10.3389/fcosc.2022.1003044

Lombardi, J. V., Yamashita, T. J., Blackburn, A., Young, J. H. Jr., Tewes, M. E., and Anderson, C. J. (2023). Examining the spatial structure of woody cover within a highway road effect zone for ocelots in Texas. *Urban Ecosyst.* 26, 1057–1069. doi: 10.1007/s11252-023-01350-y

Lonsinger, R. C., Gese, E. M., Knight, R. N., Johnson, T. R., and Waits, L. P. (2016). Quantifying and correcting for scat removal in noninvasive carnivore scat surveys. *Wildl. Biol.* 22, 45–54. doi: 10.2981/wlb.00179

Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *J. Vis. Exp.* 63, 3998. doi: 10.3791/3998

MacDonald, D. W. (1980). Patterns of scent marking with urine and faeces amongst carnivore communities. *Symp. Zool. Soc Lond.* 45, 107–139.

Maharjan, R., Kiernan, S., Towson, J. G., Saldo, E. A., Langschied, T. M., Brookover, E. K., et al. (2026). Understanding sex-specific behavioral states of bobcats in response to highway proximity in South Texas. *Ecol. Evol.* 16, e74079. doi: 10.1002/ece3.74079

McGill, B. J., Dornelas, M., Gotelli, N. J., and Magurran, A. E. (2015). Fifteen forms of biodiversity trend in the Anthropocene. *Trends Ecol. Evol.* 30, 104–113. doi: 10.1016/j.tree.2014.11.006

Menotti-Raymond, M., David, V. A., Lyons, L. A., Schaffer, A. A., Tomlin, J. F., Hutton, M. K., et al. (1999). A genetic linkage map of microsatellites in the domestic cat (Felis catus). *Genomics* 57, 9–23. doi: 10.1006/geno.1999.5743

Moreno, R. S., Kays, R. W., and Samudio, R. Jr. (2006). Competitive release in diets of ocelot (*Leopardus pardalis*) and puma (*Puma concolor*) after jaguar (*Panthera onca*) decline. *J. Mamm.* 87, 808–816. doi: 10.1644/05-MAMM-A-360R2.1

Morin, D. J., Higdon, S. D., Holub, J. L., Montague, D. M., Fies, M. L., Waits, L. P., et al. (2016). Bias in carnivore diet analysis resulting from misclassification of predator scats based on field identification. *Wildl. Soc Bull.* 40, 669–677. doi: 10.1002/wsb.723

Morini, R. F. S., Grotta-Neto, F., and Duarte, J. M. B. (2024). Faecal standing crop with real time correction using scat detection dogs to estimate population density. *J. Appl. Ecol.* 61, 1460–1468. doi: 10.1111/1365-2664.14658

Murray, J. L., and Gardner, G. L. (1997). Leopardus pardalis. *Mamm. Species* 548, 1–10. doi: 10.2307/3504082

Navarro-Lopez, D. (1985). *Status and Distribution of the Ocelot (Felis Pardalis) in South Texas* (Kingsville, USA: Texas A&I University). Thesis.

Oliveira, B. F., Bertrand, R., Comte, L., Lenoir, J., Grenouillet, G., Lancaster, L. T., et al (2026). Genetic diversity impacts climate-induced species range shifts. *Ecol. Lett.* 29 (4), e70345.

Osaki, A., Sashika, M., Abe, G., Shinjo, K., Fujimoto, A., Nakai, M., et al (2019). Comparison of feeding habits and habitat use between invasive raccoons and native raccoon dogs in Hokkaido, Japan. *BMC Ecol.* 19 (1), 35.

Paetkau, D. (2004). The optimal number of markers in genetic capture-mark-recapture studies. *J. Wildl. Manage.* 68, 449–452. doi: 10.2193/0022-541X(2004)068[0449:TONOMI]2.0.CO;2

Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R., et al. (1998). Variation in genetic diversity across the range of North American brown bears. *Conserv. Biol.* 12, 418–429. doi: 10.1111/j.1523-1739.1998.96457.x

Panasci, M., Ballard, W. B., Breck, S., Rodriguez, D., Densmore, L. D. III, Wester, D. B., et al. (2011). Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. *J. Wildl. Manage.* 75, 1616–1624. doi: 10.1002/jwmg.221

Park, S. (2001). *The Excel Microsatellite Toolkit* (Dublin, Ireland: University of Dublin).

Pilgrim, K. L., McKelvey, K. S., Riddle, A. E., and Schwartz, M. K. (2005). Felid sex identification based on noninvasive genetic samples. *Mol. Ecol. Notes* 5, 60–61. doi: 10.1111/j.1471-8286.2004.00831.x

Reed, J. E., Baker, R. J., Ballard, W. B., and Kelly, B. T. (2004). Differentiating Mexican gray wolf and coyote scats using DNA analysis. *Wildl. Soc Bull.* 32, 685–692. doi: 10.2193/0091-7648(2006)34[1127:DOFMGW]2.0.CO;2

Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M., et al. (2014). Status and ecological effects of the world's largest carnivores. *Science* 343, 1241484. doi: 10.1126/science.1241484

Ruell, E. W., and Crooks, K. R. (2007). Evaluation of noninvasive genetic sampling methods for felid and canid populations. *J. Wildl. Manage.* 71, 1690–1694. doi: 10.2193/2006-061

Ruell, E. W., Riley, S. P. D., Douglas, M. R., Pollinger, J. P., and Crooks, K. R. (2009). Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture-recapture sampling. *J. Mamm.* 90, 129–135. doi: 10.1644/07-MAMM-A-249.1

Rutledge, L. Y., Holloway, J. J., Patterson, B. R., and White, B. N. (2009). An improved field method to obtain DNA for individual identification from wolf scat. *J. Wildl. Manage.* 73, 1430–1435. doi: 10.2193/2008-492

Schwartz, M. K., Luikart, G., and Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Tren. Ecol. Evol.* 22, 25–33. doi: 10.1016/j.tree.2006.08.009

Sergeyev, M., Crawford, D. A., Holbrook, J. D., Lombardi, J. V., Tewes, M. E., and Campbell, T. A. (2024). Selection in the third dimension: using LiDAR-derived canopy metrics to assess individual and population-level habitat partitioning of ocelots, bobcats, and coyotes. *Remote Sens. Ecol. Conserv.* 10 (2), 264–278.

Sergeyev, M., Lombardi, J. V., Tewes, M. E., and Campbell, T. A. (2023a). Ocelots in the moonlight: influence of lunar phase on habitat selection and movement of two sympatric felids. *PLoS One* 18, e0286393. doi: 10.1371/journal.pone.0286393

Sergeyev, M., Tanner, E. P., Cherry, M. J., Lombardi, J. V., Tewes, M. E., and Campbell, T. A. (2023b). Influence of abiotic factors on habitat selection of sympatric ocelots and bobcats: testing the interactive range-limit theory. *Front. Ecol. Evol.* 11, 1166184. doi: 10.3389/fevo.2023.1166184

Seutin, G., White, B. N., and Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analysis. *Canad. J. Zool.* 69, 82–90. doi: 10.1139/z91-013

Shindle, D. B., and Tewes, M. E. (1998). Woody species composition of habitats used by ocelots (Leopardus pardalis) in the Tamaulipan Biotic Province. *Southw. Nat.* 43, 273–279. Available online at: https://www.jstor.org/stable/30055366 (Accessed November 8, 2017).

Smith, M. M., Veals Dutt, A. M., Lombardi, J. V., Branney, A. B., Reeves, A. M., Scognamillo, D. G., et al. (2025). Sex-specific resource strategies mediate home range sizes of an endangered carnivore across multiple scales. *Sci. Rep.* 15, 34190. doi: 10.1038/s41598-025-15493-9

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., et al. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res.* 24, 3189–3194. doi: 10.1093/nar/24.16.3189

Taberlet, P., and Luikart, G. (1999). Non-invasive genetic sampling and individual identification. *Biol. J. Linn. Soc.* 68 (1–2), 41–55.

Tewes, M. E. (1986). *Ecological and Behavioral Correlates of Ocelot Spatial Patterns* (Moscow, USA: University of Idaho). Dissertation.

Tewes, M. E. (2017). Clinging to survival in the borderlands: ocelots face dwindling habitat and growing isolation. *Wildl. Prof.* 11, 26–29.

Tewes, M. E., and Everett, D. D. (1986). "Status and distribution of the endangered ocelot and jaguarundi in Texas," in *Cats of the World: Biology, Conservation, and Management*. Eds. S. D. Miller and D. D. Everett (National Wildlife Federation, Washington, D.C. USA), 147–158.

Tewes, M. E., and Schmidly, D. J. (1987). "The Neotropical felids: jaguar, ocelot, margay, and jaguarundi," in *Wild Furbearer Management and Conservation in North America*. Eds. M. Novak and J. Baker (Ontario Ministry of Resources, Ottawa, Canada), 697–711.

Trigo, T. C., Freitas, T. R. O., Kunzler, G., Cardoso, L., Silva, J. C. R., Johnson, W. E., et al. (2008). Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L. geoffroyi* and *L. tigrinus* in southern Brazil. *Mol. Ecol.* 17, 4317–4333. doi: 10.1111/j.1365-294x.2008.03919.x

U.S. Department of Interior, Fish, and Wildlife Service (1982). *Endangered and Threatened Wildlife and Plants; Endangered Status for U. s. Population of the Ocelot: Federal Register*, Vol. 47. Washington, D.C., USA: U.S. Department of the Interior, Fish and Wildlife Service, 31670–31672.

Veals, A. M., Holbrook, J. D., Blackburn, A., Anderson, C. J., DeYoung, R. W., Campbell, T. A., et al. (2022). Multiscale habitat relationships of a habitat specialist over time: The case of ocelots in Texas from 1982 to 2017. *Ecosphere* 13, e4204. doi: 10.1002/ecs2.4204

Veals, A. M., Holbrook, J. D., Cherry, M. J., Campbell, T. A., Young, J. H. Jr., and Tewes, M. E. (2023). Landscape connectivity for an endangered carnivore: habitat conservation and road mitigation for ocelots in the US. *Landscape Ecol.* 38, 363–381. doi: 10.1007/s10980-022-01569-8

Waits, L. P., Luikart, G., and Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.* 10, 249–256. doi: 10.1046/j.1365-294x.2001.01185.x

Waits, L. P., and Paetkau, D. (2005). Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *J. Wildl. Manage.* 69, 1419–1433. doi: 10.2193/0022-541X(2005)69[1419]2.0.CO;2

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., et al. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol. Appl.* 4, 709–725. doi: 10.1111/j.1752-4571.2011.00192.x

Woodward, D. W. (1980). *The Ocelot – Selected Vertebrate Endangered Species of the Seacoast of the United States. FWS/OBS/01.9* (Washington, DC, USA: U.S. Fish and Wildlife Service).

Wultsch, C., Waits, L. P., and Kelly, M. J. (2014). Noninvasive individual and specific identification of jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) in Belize, Central America using cross-species microsatellites and faecal DNA. *Mol. Ecol. Res.* 14, 1171–1182. doi: 10.1111/1755-0998.12266

Yamashita, T. J., Tanner, A. M., Tanner, E. P., Scognamillo, D. G., Tewes, M. E., Young, J. H., et al. (2026). At the intersection of soundscapes and roads: Quantifying anthrophony's influence on wildlife crossing structure use. *Ecol. Appl.* 36, e70192. doi: 10.1002/eap.70192