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[1] National Centre for Biological Sciences, TIFR, Bangalore, India 560065
*<laura.bertola@gmail.com>
[2] The Endangered Wildlife Trust, Conservation Science Unit, Johannesburg, South Africa
[3] Wildlife Conservation Research Unit, Recanati, Kaplan Centre, Department of Zoology, University of Oxford, Oxford, UK
[4] Department of Biology, Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium
[5] Department of Zoology, Entomology and Fisheries Sciences, College of Natural Sciences, Makerere University, Kampala, Uganda
[6] Wildlife Research and Training Institute, Naivasha, Kenya
[7] Institute of Environmental Sciences CML, Leiden University, The Netherlands
[8] Sustainable Research Institute, School of Earth and Environment, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK
[9] IUCN SSC Cat Specialist Group, c/o Foundation KORA, Ittigen, Switzerland

WILLIAM F. SWANSON[1*], LAURA CARPENTER[2], JASON V. LOMBARDI[3], LISANNE S. PETRACCA[4], RANDALL W. DEYOUNG[4], TYLER A. BOSTWICK[4], ASHLEY REEVES[5] AND LINDSAY A. MARTINEZ[5]

Ex-situ propagation, wilding and reintroduction of ocelots in South Texas, USA

The ocelot *Leopardus pardalis* is the most imperilled wild cat species native to the United States, with as few as 100–120 individuals residing in two isolated populations in South Texas. Recent genetic analysis confirmed that both populations have low genetic diversity and high levels of inbreeding relative to U.S. zoo-based ocelots. To support ocelot recovery efforts in the United States, a coalition of private landowners, academic institutions, state and federal governments, and zoological entities established a programme (Recover Texas Ocelots; www.RecoverTexasOcelots.org) in 2021 to reintroduce a wild population of ocelots to a part of their historic range in southern Texas. An initial ocelot reintroduction site, comprising 350 km² of private Texas ranchland, was identified and a Programmatic Safe Harbor Agreement was established between private landowners and the U.S. Fish and Wildlife Service. An Ocelot Conservation Facility, composed of a veterinary science building, breeding enclosures and wilding enclosures, is currently under construction at Texas A&M University-Kingsville to enable ocelot propagation, behavioural development and monitoring prior to in-situ release. Ocelots from U.S. zoos will serve as the source population for introgression with wild Texas ocelots through natural breeding and/or assisted reproduction to ensure increased genetic diversity in the reintroduced population. Over the next 10 to 20 years, our goal is to establish a new, sustainable and ecologically viable ocelot population to support Texas ocelot recovery.

History of wild ocelots in Texas and their current population status

The ocelot is the rarest of wild felids native to the United States and is listed as federally endangered (USFWS 2016). Although the ocelot as a species is classified as Least Concern on the IUCN Red List of Threatened Species™ (Paviolo et al. 2015), global populations are decreasing and conservation of the imperilled population in the United States remains a national priority (USFWS 2016). Breeding populations of ocelots in the United States persist in two isolated populations (Fig. 1) in Tamaulipan mixed deciduous thornscrub found in three coastal counties in southern Texas (Lombardi et al. 2021). Until their mid-20th century decline, ocelots historically ranged throughout much of Texas, and into other southern U.S. states, but are now believed to number less than 120 known wild individuals. As these ocelots live primarily on privately owned ranches, cooperation with Texas's private landowners is key to ocelot conservation (Haines et al. 2006, Lombardi et al. 2022).

Threats to ocelots in Texas include high road mortality (Blackburn et al. 2021) and expanding agricultural and urban zones in the Lower Rio Grande Valley of South Texas (Lombardi

et al. 2020). Additionally, localised natural disasters or disease outbreaks could extirpate existing populations in Texas. Finally, Texas's ocelots have inbreeding and low genetic diversity, in part caused by isolation from ocelot populations in northern Mexico (Janečka et al. 2011, 2014, Bostwick 2025).

To follow up on earlier ocelot genetic studies, we used whole genome sequencing data from blood samples collected from 85 ocelots (44 wild, 41 U.S. zoo-based) to assess current genetic diversity, inbreeding, and genetic divergence in zoo-based and wild Texas ocelot populations (Bostwick 2025). For all individuals, single nucleotide polymorphisms (SNPs) were used to calculate nucleotide diversity (a metric of genomic diversity) and runs of homozygosity were identified to assess inbreeding severity and timing. We found that the genetic diversity of the two wild ocelot populations in Texas is nearly half of that present in the zoo-based populations (Bostwick 2025). Additionally, the wild Texas ocelot populations exhibit severe signals of inbreeding, with close inbreeding occurring within the last two generations (Bostwick 2025).

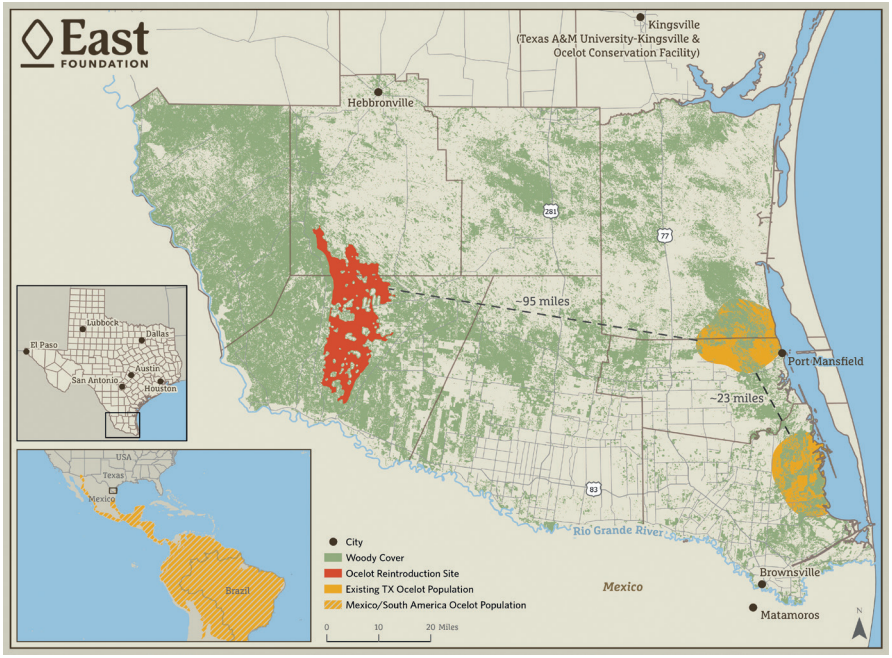

Fig. 1. The current range of ocelots in Texas and the Americas (inset), including the proposed reintroduction site located on the East Foundation's San Antonio Viejo Ranch and adjacent properties.

Federal recovery plans and zoo involvement

Passage of the Endangered Species Act ESA in the United States in 1973 led to the U.S. Fish and Wildlife Service (USFWS) listing ocelots as endangered but only for non-U.S. populations; this oversight was corrected with revision of the Act in 1982. The initial USFWS recovery plan for U.S. ocelots was completed in 1990, with an emphasis on conducting ecological studies of wild populations and preserving their declining habitat (USFWS 1990). Revision of the recovery plan in 2016 expanded the focus to enable ex-situ management of ocelot populations, incorporation of biobanking and assisted reproduction, and reintroduction of ocelots into their historic range in Texas (USFWS 2016).

Ocelots have been maintained in U.S. zoos since the 1890's, with monitoring via a regional studbook beginning in 1994 and ex-situ management overseen by a Species Survival Plan (SSP) established in 2001. Most of the original SSP population was derived from Generic ocelots (i.e., unknown subspecific origins) entering zoos in the early 1970's following ESA passage and donation of pet ocelots, with subsequent genetic augmentation from importation of Brazilian ocelots (*L.p. mitis*) in the early 2000's. The zoo-based population currently consists of 55 cats at 33 institutions, comprising offspring derived from both Generic and Brazilian ocelot founders. The most recent Ocelot SSP includes breeding recommendations for 16 pairings, and 13 kittens have been born in U.S. zoos over the last five years (Carpenter et al. 2025). With initiation of the recovery programme for Texas ocelots, a Saving Animals From Extinction (SAFE) programme was established for ocelots under the auspices of the American Zoo & Aquarium Association (AZA). The two objectives are: 1) propagating ocelots through natural breeding and/or assisted reproduction to support wilding/reintroduction in Texas and 2) increasing awareness and education about ocelots within human communities in Texas and other southern U.S. states. Thus far, eight U.S. zoos that house ocelots have committed to become SAFE Ocelot partners, providing in kind and financial resources for SAFE activities in support of the recovery programme, and more zoos are expected to join SAFE as the programme develops.

Assisted reproduction for ocelot propagation

Development of assisted reproductive technology (ART) in zoo-based ocelots – including semen collection and cryopreservation, artificial insemination (AI), in-vitro fertilisation (IVF), and embryo freezing and transfer (ET) – began in the early 1990's, with 14 pregnancies and more than 20 offspring produced by both AI and ET, including in combination with semen and embryo freezing (Swanson et al. 1996, Swanson 2019). This progress in zoos led to investigation of ART as an approach to mitigate the low genetic diversity of wild Texas ocelot populations. Best practices were identified in the field for wild ocelot semen collection (Reeves et al. 2024) and cryopreservation for long-term storage (Reeves et al. 2025, in review) and, since 2019, semen has been collected and banked from 21 wild Texas ocelots for use in ART procedures (Fig. 2A).

Since 2020, researchers have been attempting to use frozen semen from wild Texas ocelots for AI and IVF with zoo-housed ocelots (Reeves et al. 2024). As of October 2025, 13 laparoscopic oviductal AI procedures have been performed in zoo-based females using frozen Texas ocelot semen (Fig. 2B). No pregnancies have been produced from AI using wild male semen thus far, with low semen quality in wild males (Reeves et al. 2024) likely impacting freezing tolerance and fertilisation success in vivo. In 2024, four IVF procedures were conducted with oocytes collected laparoscopically from zoo-housed cats using frozen semen from wild males for insemination; however, no embryos were produced, likely due in part to poor quality of oocytes from donor females. Future ART efforts in ocelots will focus on improving semen cryopreservation techniques and recovery of higher-quality oocytes, in conjunction with additional AI and IVF/ET procedures. Ex-situ ocelot propagation success likely will im-

Fig. 2. Assisted reproduction in ocelots: A) collection of semen from a wild Texas ocelot on the East Foundation's El Sauz Ranch in South Texas in January 2025, and B) laparoscopic oviductal AI of a female ocelot at a U.S. zoo in 2024 (Photos: a) Jonathon Vail; b) Aidan Branney).

prove following construction of a dedicated ocelot breeding facility in Texas, allowing multiple ocelots to be housed at a single location, reducing the need to conduct advanced reproductive procedures while traveling between distant zoological institutions, and creating more opportunities for natural breeding and/or application of ART.

Texas Ocelot Recovery Program

Building a collaborative network

Starting in 2021, we began a programme to lay the groundwork to recover federally endangered ocelots by reintroducing them into historic habitat in southern Texas. The coalition "Recover Texas Ocelots" is composed of scientists from a diversity of disciplines and representing multiple partner organisations, including: the East Foundation (an Agricultural Research Organization and private landowner; Campbell et al. 2025), Texas A&M University Natural Resources Institute, Caesar Kleberg Wildlife Research Institute at Texas A&M University-Kingsville, U.S. Fish and Wildlife Service, Lindner Center for Conservation and Research of Endangered Wildlife at the Cincinnati Zoo & Botanical Garden, Duquesne University, and Texas Parks and Wildlife Department.

The goal of Recover Texas Ocelots is to reintroduce a wild population of ocelots to a part of their historic range in southern Texas where ocelots have been absent for ca. 75 years. Creating this new population will increase the number of wild ocelots within Texas, broaden their current geographic range, expand ocelot genetic diversity in the wild, and provide a safeguard against extirpation of ocelots from the United States. The aim is to help recover ocelots from their endangered status in the United States, in accordance with the USFWS Recovery Plan (USFWS 2016). In support of these efforts, the U.S. federal government has provided contract funding to programme partners to conduct ecological and genetic studies of wild ocelots, preserve biological resources from wild cats, and improve propagation of zoo-based ocelots as a source population for reintroduction.

Reintroduction site selection & Safe Harbor Agreement

Capitalising on previous research on ocelot habitat use in Texas (Lombardi et al. 2021, Sergeyev et al. 2023), programme partners identified a preferential ocelot reintroduction location in Texas (Martinez et al. 2024b) using Geographic Information Systems (GIS) and remote sensing imagery (Fig. 1). GIS was used to complete a habitat suitability assessment, identifying areas within ocelots' historic range in Texas that: retain large patches of woody canopy cover with dense understory vegetation, are remote from likely threats to ocelots (including coastal storms, high-traffic highways, and suburban development), and have low ownership fragmentation. The selected habitat spans over 350 km^2 of woody cover and exists mostly on a handful of remote, privately-owned ranchlands located about 100 km inland from the existing ocelot populations in Texas (Martinez et al. 2024b). One programme partner, the East Foundation, owns a large private cattle ranch (>600 km^2) within this habitat and offered access to their property (the San Antonio Viejo ranch) as the primary ocelot release site. This ranch is known to support populations of bobcats *Lynx rufus* and coyotes *Canis latrans*, as well as various prey species. The defined mission of the East Foundation is to protect this property in perpetuity for cattle ranching, wildlife conservation and research. This assures long-term site sustainment and security for the reintroduction programme.

While ocelot conservation has proven compatible with cattle ranching in Texas, endangered species conservation on private lands in the United States is often complicated by Endangered Species Act regulations that expose landowners to legal liabilities. To overcome this possible barrier, the East Foundation and the USFWS developed a Programmatic Safe Harbor Agreement that will give private landowners in the ocelot reintroduction area legal assurances of the continued freedom to operate their properties as working ranches without increased regulatory burdens related to ocelots (Martinez et al. 2025).

Breeding, wilding and reintroduction guidelines

Programme partners plan to secure a source stock of ocelots for reintroduction to the selected in-situ site by developing a managed breeding programme as an alternative to attempted translocation of ocelots from the two small extant wild populations in Texas or acquired from other countries. Zoological, ecological and veterinary experts involved in Recover Texas Ocelots have collaboratively developed an initial set of protocols for propagating ocelots in an ex-situ conservation breeding programme, providing behavioural development or wilding of offspring in preparation for release, releasing individuals into the wild, and conducting long-term monitoring post-release (Ocelot Reintroduction Study Captive Propagation Team 2023). Protocols were informed by conservation efforts for other wild species worldwide, especially the breeding and reintroduction programme for Iberian lynx *Lynx pardinus* (Vargas et al. 2009, Serra et al. 2024). Initial protocols will guide implementation of the ocelot breeding and reintroduction programme in Texas with periodic updates and modifications as new knowledge is acquired.

Ocelot Conservation Facility – planning and construction

Ocelot breeding and wilding activities will take place at the Ocelot Conservation Facility (OCF), located on a six-acre portion of the wildlife research park of the Caesar Kleberg Wildlife Research Institute at Texas A&M University-Kingsville. The OCF was designed collaboratively by programme partners and informed by Iberian lynx breeding centres (Vargas et al. 2009) and ocelot breeding en-

closures and habitats in zoos. The OCF will contain endocrinology and gamete biology laboratories, veterinary treatment and surgical suites, quarantine space, 16 indoor-outdoor ocelot breeding enclosures, and four large outdoor wilding enclosures for entrainment of natural behaviours in offspring, including hunting of live prey and navigating complex habitats with minimal disruption from humans (Fig. 3). OCF construction began in early 2025, with funding provided entirely by private donors, and a projected opening date in late 2026.

Fig. 3. The Ocelot Conservation Facility, under construction at Texas A&M University – Kingsville and expected to open in the second half of 2026, will consist of 1) a veterinary science building, 2) ocelot breeding enclosures and 3) semi-natural wilding enclosures.

Ocelot sourcing for OCF and genetic considerations

The OCF's breeding programme will utilise both natural and assisted reproduction to create the source stock for reintroduction. While genetic material has been banked from the existing Texas ocelot population (Reeves et al. 2024), Texas ocelots alone would provide a poor basis for creating a genetically robust new population because of their low genetic diversity and documented inbreeding (Janečka et al. 2014, Bostwick 2025). Instead, Texas ocelots should be outbred to unrelated ocelots to create the source stock. Accordingly, Texas ocelots, or other wild individuals from the *L. p. pardalis* ocelot subspecies occurring in Central and North America (which we define as the "northern" ocelot subspecies, Kitchener et al. 2017), will be crossed with zoo-born ocelots. To avoid overwhelming the extant Texas ocelot ancestry, we are currently planning for a maximum of 25% of the genetic makeup of reintroduced ocelots to be derived from zoo-based ocelots. To obtain the targeted 75% northern/25% zoo genetic ratio, zoo-born and wild Texas ocelots will be bred to one another, and then the resulting offspring will be backcrossed to other wild northern individuals. This approach will allow for introgression of novel genetics from zoo-based ocelots into the Texas population to avoid inbreeding and possibly promote in-situ adaptability by reintroducing a more genetically diverse source stock.

Zoo-based ocelots in the U.S. have been assessed genetically for potential contribution to the ex-situ propagation programme (Bostwick 2025). Zoo populations (Generic and Brazilian) showed little to no inbreeding, plus had higher genetic diversity compared to wild Texas ocelots. Comparing the two zoo stocks, Generic ocelots had on average slightly higher levels of inbreeding and lower conserved genetic diversity than Brazilian zoo-based ocelots, although the Generics had on average lower genetic distance from wild Texas ocelots compared to Brazilian zoo-based ocelots. We concluded that either Generic or Brazilian populations would be suitable for captive propagation for conservation due to much higher level of diversity compared to Texas ocelots, and that consideration of individual variation when selecting founding individuals is important, as some individuals in the zoo-based population demonstrated higher levels of inbreeding and lower diversity than others (Bostwick 2025).

Through the AZA's Ocelot SSP and SAFE Ocelot programmes, the U.S. zoo community has committed to providing the initial zoo-sourced ocelots for breeding in the Ocelot Conservation Facility and exchanging ocelots periodically with the OCF as needed to optimise genetic variability for breeding and reintroduction. Demographic and genetic management of ocelots within U.S. zoos over the past 25 years under SSP guidance has enabled the zoo population to retain at least 90% of its original founder heterozygosity. To ensure future sustainability of the ex-situ population while supporting recovery in-situ, the SSP and SAFE will continue to actively manage breeding of zoo-based ocelots, grow the number of ocelot-holding institutions, and explore importation of new ocelot founders from other regional zoo associations.

Challenges and inspiration

As with many other wildlife reintroduction programmes, establishing an ecologically viable, sustainable reintroduced ocelot population is expected to require decades of hard work and investment of substantial financial resources. Ocelots mature slowly and produce average litters of one or two kittens annually, so we anticipate that breeding and back-crossing individuals to produce the initial in-situ founders and preparing them for life in the wild will require at least five years. Further, our viability analyses suggest that building a stable reintroduced population will take a minimum of ten years of releasing at least two to four OCF-bred ocelots every year (Martinez et al. 2024a). In developing this program, we have been inspired by the extraordinary recovery efforts for the Iberian lynx, and many of our plans and activities have been guided by knowledge gained from that project. We hope that by documenting the progress, challenges, failures and successes associated with ocelot breeding and reintroduction, we can provide the world with another inspirational model for felid reintroduction and conservation.

For more information about this programme and collaborators, or to sign up for updates, visit www.RecoverTexasOcelots.org.

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[1] Centre for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220, USA
*<william.swanson@cincinnatizoo.org>

[2] Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220, USA

[3] Lombardi Research, Folsom, CA 95630, USA

[4] Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, TX 78363, USA

[5] East Foundation, San Antonio, TX 78216, USA