



OPEN Multi-generational benefits of genetic rescue

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Genetic rescue—an increase in population fitness following the introduction of new alleles—has been proven to ameliorate inbreeding depression in small, isolated populations, yet is rarely applied as a conservation tool. A lingering question regarding genetic rescue in wildlife conservation is how long beneficial effects persist in admixed populations. Using data collected over 40 years from 1192 endangered Florida panthers (*Puma concolor coryi*) across nine generations, we show that the experimental genetic rescue implemented in 1995—via the release of eight female pumas from Texas—alleviated morphological, genetic, and demographic correlates of inbreeding depression, subsequently preventing extirpation of the population. We present unequivocal evidence, for the first time in any terrestrial vertebrate, that genetic and phenotypic benefits of genetic rescue remain in this population after five generations of admixture, which helped increase panther abundance (> fivefold) and genetic effective population size (> 20-fold). Additionally, even with extensive admixture, microsatellite allele frequencies in the population continue to support the distinctness of Florida panthers from other North American puma populations, including Texas. Although threats including habitat loss, human-wildlife conflict, and infectious diseases are challenges to many imperiled populations, our results suggest genetic rescue can serve as an effective, multi-generational tool for conservation of small, isolated populations facing extinction from inbreeding.

Keywords Endangered species conservation, Fitness, Florida panther, Genetic rescue, Inbreeding depression, *Puma concolor*

The long-term persistence of imperiled species in the wild is increasingly hampered by numerous challenges worldwide^{1,2}. Isolation and small population size, often catalyzed by anthropogenic stressors, can adversely impact population persistence. Inbreeding depression, a reduction in fitness resulting from mating among related individuals, is a conservation challenge common to imperiled populations. The synergy between small population size, isolation from conspecific populations, inbreeding depression, as well as demographic and genetic stochasticity, often results in an “extinction vortex” that can ultimately lead to extirpation of species or populations³.

Genetic rescue—an increase in population fitness following the introduction of new alleles—has been a rarely applied, yet successful management initiative for several taxa that were negatively affected by small population size, severe bottlenecks, and geographical isolation⁴. For example, genetic rescue has improved demographic performance and prevented potential extinction of wolves (*Canis lupus*) in Scandinavia⁵, prairie chickens (*Tympanuchus cupido*) in Illinois, USA^{6,7}, and wood rats (*Neotoma magister*) in Indiana, USA⁸. The endangered Florida panther (*Puma concolor coryi*) population faced similar challenges through most of the twentieth century⁹.

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Habitat loss and the impacts of unregulated take combined to reduce the population to approximately 20–30 individuals isolated at the southern end of peninsular Florida by the mid-1990s, > 1000 km from the nearest population of conspecific^{10,11}. Early studies (1981–1995) conducted by the Florida Game and Fresh Water Fish Commission (now Florida Fish and Wildlife Conservation Commission [FWC]) revealed that the panther population was characterized by low levels of genetic variation and suffered from high frequencies of phenotypic (e.g., midline dorsal cowlick of fur, kinked tails) and congenital abnormalities (e.g., atrial septal defects [ASD], cryptorchidism^{12,13}) thought to be indicative of inbreeding depression. These abnormalities, combined with poor demographic performance of the population, portended the imminent extinction of the panther and spurred wildlife managers to implement a plan for genetic rescue¹⁴ centered on the release of 8 female pumas from Texas (*P.c. stanleyana*) into South Florida in 1995. Initial assessments revealed numerous fitness benefits to panthers^{15–18}, most notably an increase in the minimum population size to 119 adults and subadults by 2015^{10,19}. The continued collection of this multi-generational data set from a wild population of large carnivores during more than 4 decades of research is unprecedented and sets the stage for a unique opportunity to track long-term consequences of genetic rescue. This knowledge could prove beneficial to the conservation of other imperiled wildlife beyond apex predators.

Although genetic rescue has been successfully applied as an effective management tool^{4,20}, the persistence of benefits accumulated from the initial influx of genetic variation into the population remains largely unknown^{21–23}. Some studies have addressed this question experimentally^{24,25}, but few have evaluated the long-term effects of genetic rescue within a wild population beyond the F2 generation⁴. We would expect “hybrid vigor”²⁶ to be associated with the F1 admixed individuals because of heterosis and subsequent improvements to population fitness incurred from genetic rescue⁴. The benefits of genetic rescue beyond the F1 generation, however, are still debated. A meta-analysis incorporating data from 156 studies and 77 species supported the benefits of outcrossing experiments often lasting beyond the F3 generation^{27,28}. However, > 80% of the data sets analyzed by Frankham^{27,28} involved short-lived invertebrates or plants, and only 30 of 156 studies examined fitness beyond F2, of which just one involved a vertebrate species (desert topminnow, *Poeciliopsis monacha*).

Here, we used morphological, biomedical, demographic, and genetic data collected from 1192 Florida panthers over 40 years—inclusive of data through the F5 generation (generation time = 4.45 years¹⁶) following a single genetic rescue event in 1995—to provide an unprecedented assessment of the persistence of benefits to the population. We examined morphological and biomedical correlates of inbreeding, genetic variation, population genetic structure, inbreeding coefficients, estimates of abundance prior to the implementation of genetic rescue in 1995, and generational changes in these characteristics post-genetic rescue. Lastly, we present the most comprehensive estimate of genetic effective population size²⁹ for Florida panthers to date, highlighting improvements following genetic rescue. Our findings clearly demonstrate the phenotypic, genetic, and demographic benefits of genetic rescue and track how these improvements persist temporally across five generations, both critical components for assessing genetic rescue as a tool for long-term management of small, isolated, and inbred populations worldwide.

Results

Sampling, microsatellite genotyping and genetic ancestry

From 1981 to 2021, we examined 1192 Florida panthers (dependent-aged kittens, subadults, and adults) to collect tissue samples and to document biomedical and morphological abnormalities. Tissue samples collected from Florida panthers were used to obtain genotypes at 16 microsatellite loci; this dataset encompassed 9 panther generations, including 4 generations prior to and 5 generations after genetic rescue (descriptive statistics on microsatellite loci are provided in *Supplemental Information [SI] Appendix 1*). Our analysis of genetic ancestry using a Bayesian clustering algorithm in program STRUCTURE³⁰—with genotype data from 904 panthers, 49 Texas pumas (including 7 of the 8 Texas female pumas introduced in 1995 [TX102 did not amplify]), and 12 additional non-Florida pumas—supported a two-cluster model ($\Delta K = 1786$ at $K = 2$, Ln likelihood_(K=2) = −31,951, SD = 0.8). From this model, we used q -values (proportion of an individual's genome [i.e., ancestry] originating from a cluster in K) to assign panthers as either canonical ($\geq 90\%$ pre-introgression ancestry) or admixed ($< 90\%$ pre-introgression ancestry) for subsequent analyses (*SI Appendix 2*). The increased level of admixture in cohorts of panthers born post-genetic rescue (Post 1–3 cohorts; see “Methods”) is apparent when compared to the pre-genetic rescue cohorts (Pre1–2; Extended Data Fig. 1; *SI Appendix 2*). Using a subset of 547 adult and subadult panthers, the mean q -value for the cluster associated with canonical panthers was at its peak in the cohort just prior to genetic rescue (Fig. 1; Pre2; mean canonical ancestry of $q = 0.849$ SE = 0.028, $n = 55$), but generations of panthers in cohorts following genetic rescue had significant admixture (Fig. 1).

Morphological and biomedical correlates of inbreeding

From 1981 to 2021, we examined 612 Florida panthers for morphological and biomedical abnormalities, including the kinked tail, dorsal cowlick of fur, ASDs, cryptorchidism, and percentage of abnormal sperm. Between the pre-genetic rescue and Post3 cohorts, the proportion of panthers with kinked tails (0.852 to 0.221), cowlicks (0.846 to 0.189), and cryptorchidism (0.553 to 0.067) all significantly declined (Fig. 2A; statistical results are presented in *SI Appendix 3*). The proportion of panthers with ASD and the percentage of abnormal sperm in males also substantially declined from pre- to post-genetic rescue periods, but these changes were not statistically significant because of small sample sizes (Fig. 2A; *SI Appendix 3*). Admixed panthers were significantly less likely to express any of the correlates of inbreeding when compared to canonical panthers (Fig. 2B; *SI Appendix 3*).

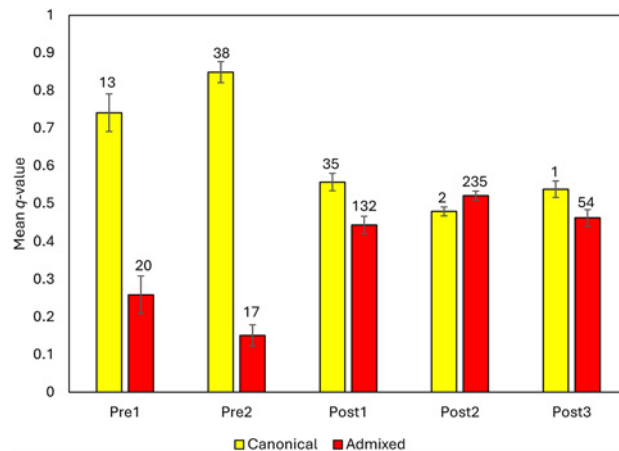


Figure 1. Ancestry of adult and subadult Florida panthers ($n = 547$) sampled 1981–2020 in Florida, USA. Ancestry was determined via a clustering analysis of microsatellite genotype data using the program STRUCTURE with $K = 2$ clusters (canonical and admixed). Cohorts include Florida panthers born during the pre-genetic rescue (Pre1 and Pre2) and post-genetic rescue (Post1–3) periods (see “Methods”). Panthers were designated as canonical if their canonical ancestry q -value was $\geq 90\%$. Mean q -values for each ancestry category are presented to demonstrate changes in the composition of population ancestry across generations of panthers pre- and post-genetic rescue. The SE values are reflective of the combined sample within each cohort, since q -values presented are proportions of that sample. Values above each bar represent the number of panthers assigned to that ancestral category in the pre- or post-genetic rescue cohort.

Measures of genetic variation

We assessed genetic diversity of the panther population based on microsatellite genotypes from 552 tissue samples of adult and subadult Florida panthers (i.e., excluding kittens) collected from 1981 to 2020 (see “Methods”). The Pre2 cohort was characterized by the lowest levels of genetic variation; all measures—including number of alleles (N_a), number of effective alleles ($N_{\text{effective}}$), allelic richness (A_r), observed (H_o), expected (H_e), and individual (H_{ind}) heterozygosity—showed substantial improvements following the implementation of genetic rescue and achieved the highest values in the Post1 or Post2 periods (Fig. 3A,B). For example, A_r increased from 3.30 for the Pre2 cohort to 4.31 in Post1 cohort, and H_o increased from 0.40 during Pre2 to 0.55 during Post2 (Fig. 3A,B; see SI Appendix 4 for pairwise contrasts). Overall, post-genetic rescue cohorts of panthers exhibited values of these metrics of genetic variation that were comparable to larger contiguous populations of pumas in the Western United States (Fig. 3A–D; SI Appendix 4, Table A4.1). Similarly, admixed panthers were genetically more diverse than canonical panthers across all measures of genetic variation (e.g., $H_e = 0.31$ and 0.57 for canonical and admixed panthers, respectively) and exhibited values more comparable to those of Western puma populations (Fig. 3C,D; SI Appendix 4, Table A4.1).

Our comparisons of H_{ind} between different cohorts of panthers and between ancestral groups showed significant improvements in the post-genetic rescue era and in admixed panthers (Fig. 3B,D; SI Appendix 5). The H_{ind} values for these same groups were statistically similar to those of Western puma populations in most cases, while canonical, Pre1 and Pre2 groups were significantly lower in most comparisons (SI Appendix 5, Tables A5.1, A5.2, and Fig. A5.1). Our evaluation of the heterozygosity-fitness correlation (HFC) between H_{ind} and several correlates of inbreeding (kinked tails, cowlicks, and cryptorchidism) showed a substantial reduction in the probability of the expression of those traits in Florida panthers across all cohorts and for both canonical and admixed panthers as H_{ind} increased (Fig. 4).

Genetically effective population size, abundance and principal coordinate analysis

By applying the linkage disequilibrium method³¹ within NeEstimator 2.1³² to our microsatellite data, we estimated genetically effective population size (N_e) for Florida panthers across a temporal scale and for ancestral groups (Extended Data Fig. 2). Prior to genetic rescue, the panther population was close to extirpation, with an $N_e = 2.7$ (95% CI 2.4–3.1). Following genetic rescue, N_e increased consistently, reaching a value of 62.1 (95% CI 40.2–115.5) during the Post3 period (Extended Data Fig. 2). Admixed panthers had substantially larger N_e than canonical panthers (Extended Data Fig. 2). The observed increase in N_e mirrors the documented population growth in our index and estimates of Florida panther abundance (Fig. 5; SI Appendix 6).

Our principal coordinate analysis (PCoA) completed in GenAlEx 6.5^{33,34} illustrates the genotypic differences between Western puma populations and panthers via ancestry and cohorts through time pre- and post-genetic rescue. The PCoA shows the historic and continued separation between panthers from Florida and Western puma populations, including Texas, even five generations post-genetic rescue (Fig. 6).

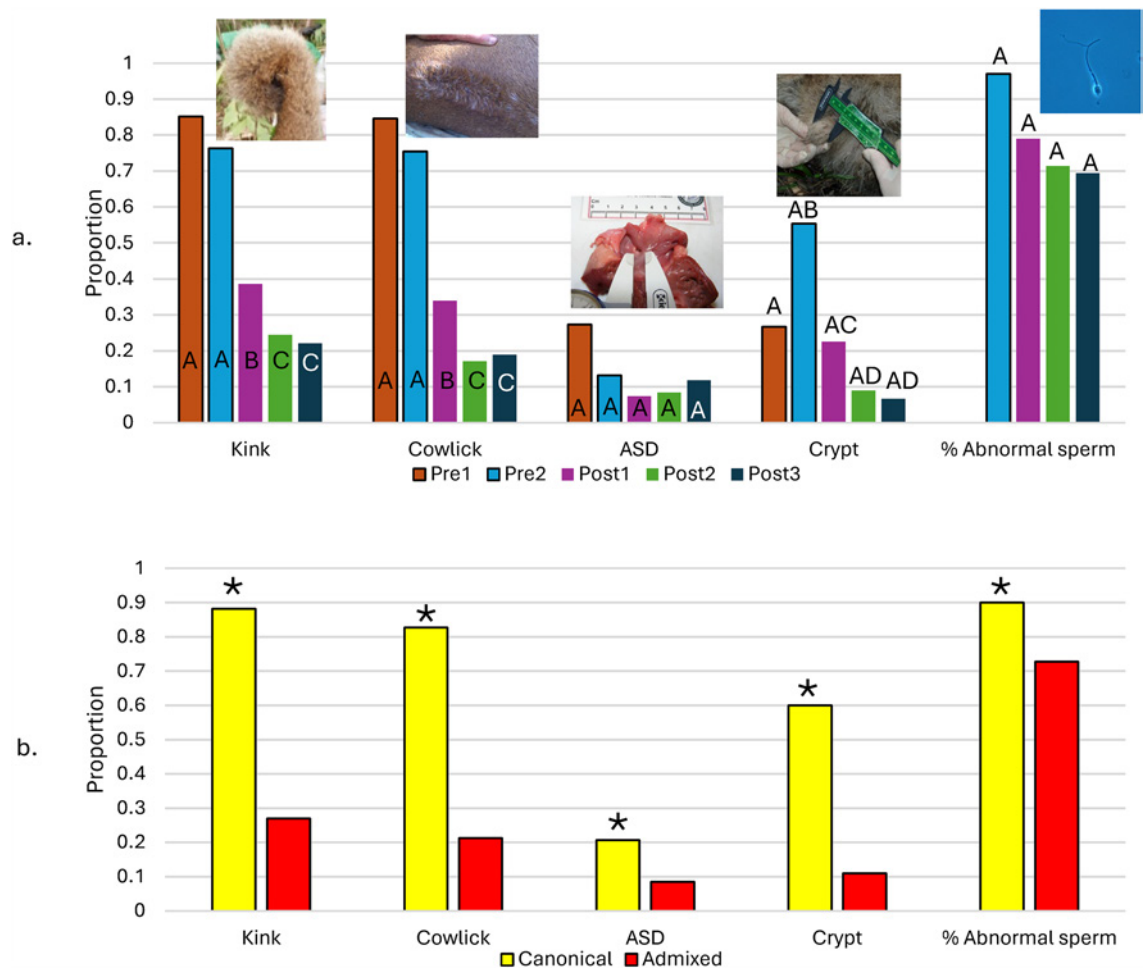


Figure 2. Proportion of Florida panthers sampled from 1981 to 2021 in Florida, USA, that exhibited correlates of inbreeding depression for morphological and physiological traits. Comparisons are made via panthers born in cohorts pre- and post-genetic rescue (a) and between defined ancestral categories (b). The percent abnormal sperm data are from Penfold et al.⁴⁶. Statistical analyses are detailed in SI Appendix 3. Different letters in Frame (a) indicate significant differences ($P < 0.05$; binomial regression analyses with post-hoc comparisons) between cohorts of panthers. The Post3 cohort includes data from 2021 for these correlates of inbreeding. We used two proportion Z-tests to determine significant comparisons ($*P < 0.05$) between ancestral categories on Frame (b) (SI Appendix 3). ASD = Atrial Septal Defect and Crypt = Cryptorchidism.

Discussion

Although initially controversial, the choice to embark on the genetic rescue of the Florida panther was a timely and wise decision that had an immediate and long-term benefit to the population. Virtually all morphological, genetic, and demographic parameters improved following this management initiative. Furthermore, our findings demonstrate the persistence of these benefits through the F_5 generation post-genetic rescue, a significant improvement in population abundance and N_e , as well as continued genetic distinctness of the admixed panthers from Western puma populations.

A goal of the genetic rescue plan was a 20% level of introgression of the Texas puma genome into the Florida population¹⁴. This was quickly surpassed, as evidenced by the 44% mean admixed ancestry immediately following genetic rescue in the Post1 (1996–2005) cohort of panthers (Fig. 1; SI Appendix 2, Table A2.1). An admixture level of 44–52% in post-genetic rescue cohorts is more than twice the level deemed adequate to forestall inbreeding¹⁴. High levels of admixture following genetic rescue can potentially result in outbreeding depression—a swamping of ancestral, locally adapted, beneficial traits in a population³⁵—and was cited as a possible concern for the F1 and F2 generations of panthers³⁶. Nevertheless, there are alternative perspectives on the levels of admixture required for successful genetic rescue attempts that avoid outbreeding depression. For instance, Harris et al.³⁷ demonstrated through model simulations how inbred Neanderthals (*Homo neanderthalensis*) benefited genetically when introgressed with modern humans (*H. sapiens*), even with the loss of a significant portion of their native ancestry. Fitzpatrick et al.³⁸ showed that genetic rescue in Trinidadian guppy (*Poecilia reticulata*) populations did not result in swamping of local adaptation but instead produced hybrids (70–80% admixed) with improved levels of fitness as well as a concomitant increase in population size post-genetic rescue. Overall, examples of outbreeding depression in mammals are rare, especially when contrasted with the well documented negative

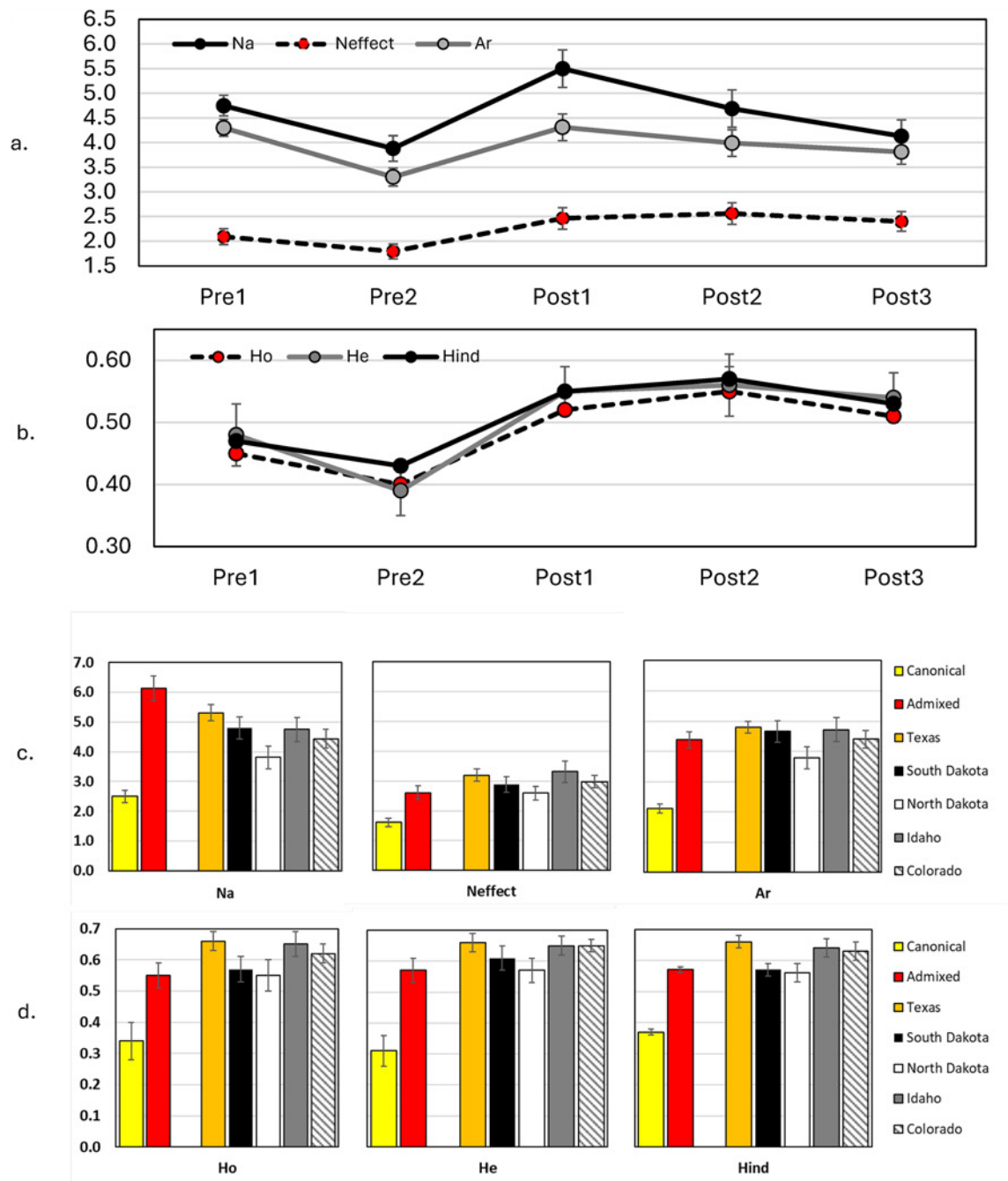


Figure 3. Metrics of genetic variation calculated using genotype data from 16 microsatellite loci in cohorts of Florida panthers pre- and post-genetic rescue (Frames **a** and **b**) and ancestral groups (Frames **c** and **d**) sampled from 1981 to 2020 in Florida, USA. Values are means and standard errors. Data from Western populations of puma are presented for comparative purposes. Metrics include number of alleles (N_a), number of effective alleles (N_{eff}), observed and expected heterozygosity (H_o , H_e), allelic richness (A_r), and individual heterozygosity (H_{ind}). Statistical analyses methods and results comparing these groups are presented in *SI Appendix 4*.

effects of inbreeding depression within imperiled populations³⁹. To date, we have not identified evidence of outbreeding depression in the panther population.

The kinked tail, cowlick, cryptorchidism, and ASDs have become infamous traits of the Florida panther population and manifestations of inbreeding depression^{13,40,41} that have been used in other wildlife populations to identify the potential presence of inbreeding^{42,43}. Our findings that morphological and biomedical correlates of inbreeding consistently declined from the pre- to post-genetic rescue cohorts provide clear evidence of the immediate and long-term benefits of genetic rescue. For example, kinked tails and cowlicks were documented in >75% of panthers sampled prior to genetic rescue but were found in fewer than 30% of panthers sampled post-genetic rescue. We note, however, that caution must be exercised when interpreting levels of inbreeding depression based only on phenotypic indicators of inbreeding (e.g., kinks and cowlicks); direct measures of fitness

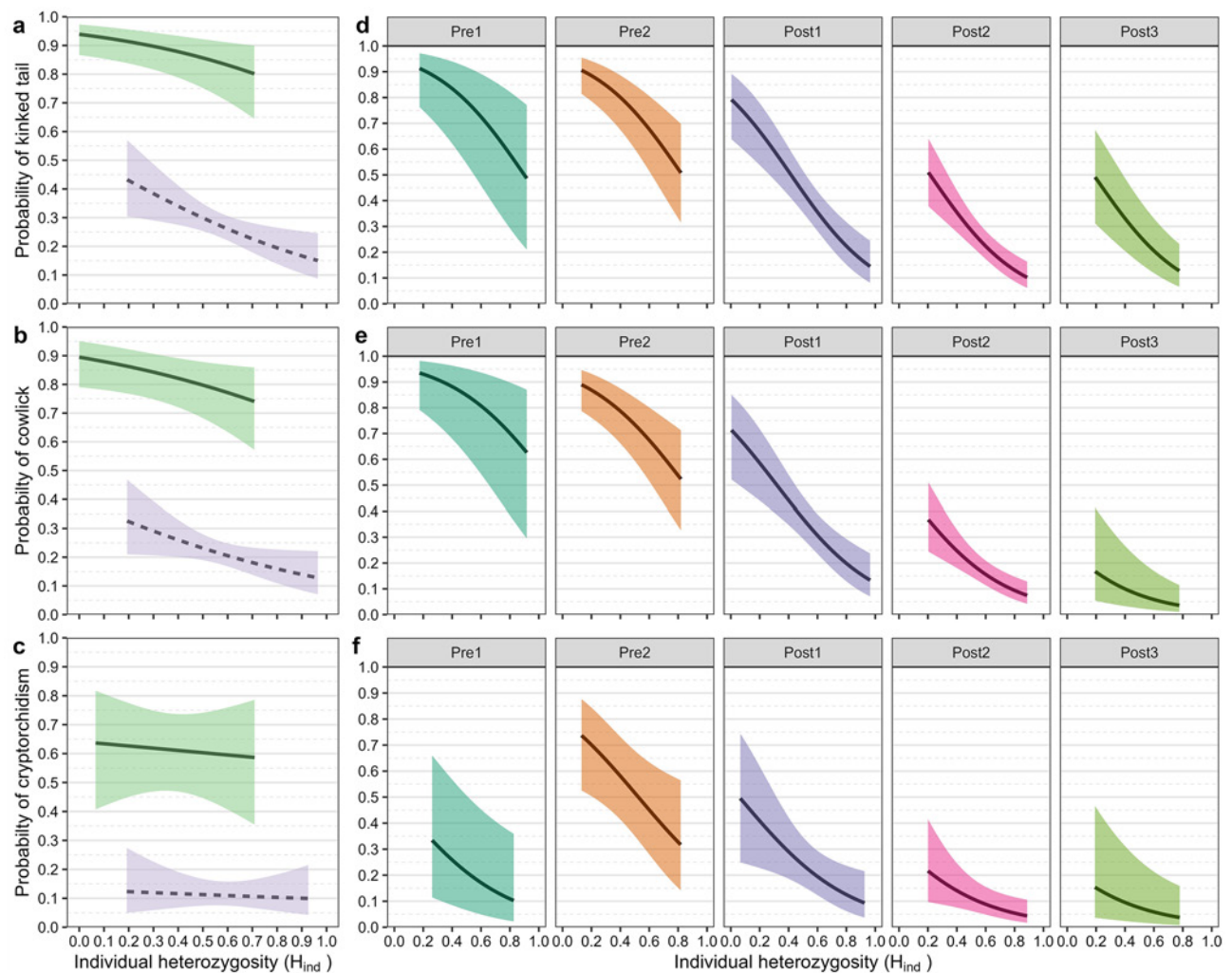


Figure 4. Assessment of the heterozygosity-fitness correlations between individual heterozygosity (H_{ind}) and the probability of presence of kinked tails, cowlicks, and cryptorchidism in Florida panthers. Frames (a–c) depict comparisons of panthers categorized as canonical (solid line) versus admixed (dashed line). Frames (d–f) represent comparisons between cohorts of panthers from the pre- and post-genetic rescue periods. Shaded areas represent 95% confidence intervals.

or its components, such as life history parameters, are preferred when available⁴⁴. That said, our previous research on panthers correlating metrics associated with inbreeding depression (e.g., heterozygosity, percent abnormal sperm) with direct traits of fitness such as survival^{17,45} and improved prospects for reproductive success⁴⁶ supports the use of indirect traits easily identified via routine examination as proxies to monitor the prevalence of inbreeding in this population.

When assessing measures of genetic variation within cohorts of panthers born pre- or post-genetic rescue, the pattern was a consistent decline in all variables from Pre1 to Pre2 and then improvements to post-genetic rescue cohorts (Fig. 3A,B; pairwise contrasts results appear in SI Appendix 4). Comparisons between these cohorts and Western pumas typically followed a trend of lower values for Pre1 and Pre2, while most contrasts with post-genetic rescue cohorts were not different (SI Appendix 4). Focusing on H_e reveals clear improvements accrued to the population during the post-genetic rescue period, with the highest level observed four generations after genetic rescue (0.56 ± 0.04 for Post2). This level of H_e approaches those calculated for Western pumas in our study (SI Appendix 4, Table A4.1) and large contiguous populations in California and New Mexico^{47,48}.

All measures of genetic variation revealed significant improvements when comparing canonical versus admixed panthers (Fig. 3C,D). Additionally, admixed panthers exhibited values for these metrics of genetic variation that were comparable to those of Western puma populations (Fig. 3C,D; SI Appendix 4, Table A4.1). For example, H_e was 0.31 ± 0.05 for canonical panthers versus 0.57 ± 0.04 for admixed panthers, which was similar to H_e for Western pumas (range: 0.57 – 0.66), highlighting the improved level of genetic diversity in admixed panthers. A puma population in the Santa Ana Mountains that is isolated between the metropolises of Los Angeles and San Diego, California, had an H_e of 0.33 ⁴⁷, which was almost as low as that for canonical panthers. Gene flow to this California population requires that periodic migrants access the Santa Ana Mountains from nearby ranges that encompass a larger metapopulation of pumas, a herculean challenge given extensive

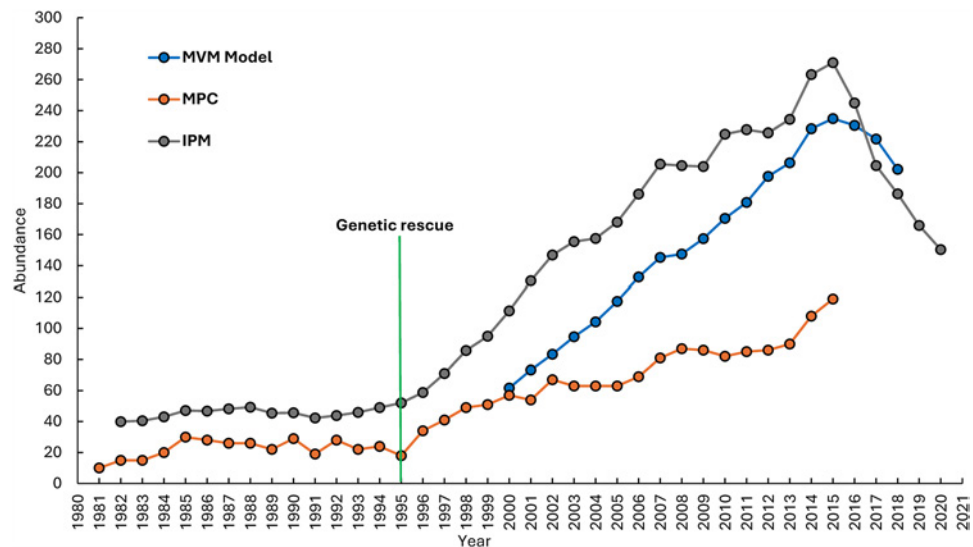


Figure 5. Estimates of the range-wide population size of adult and subadult Florida panthers from 1981 to 2020 using: (1) the 95% lower confidence interval of the motor vehicle mortality (MVM) model-averaged abundance estimate via the method of McClintock et al.⁷² plotted in blue for the period 2000–2018 (see *SI Appendix 6*); (2) the minimum population count (MPC) index of McBride et al.¹⁰ and McBride and McBride¹⁹ plotted in orange, for the period 1981–2015; (3) and the integrated population model (IPM) of Merriell⁷³ in plotted in grey, for the period 1982–2020. The year in which genetic rescue was initiated (1995) coincides with the subsequent increase in the population size that was documented by all three metrics.

development and highways separating the populations⁴⁹. Unfortunately, Florida panthers do not have a population of conspecifics within dispersal range, which impairs the population's ability to maintain genetic diversity and reduce genetic drift.

Inbreeding depression in wild populations can be evaluated using heterozygosity–fitness correlations (HFC)^{50,51}. Results of our comparisons of H_{ind} values resembled trends for other metrics of genetic variation in the population, with canonical panthers having significantly lower H_{ind} compared to admixed and all Western pumas (*SI Appendix 5*, Table A5.1 and Fig. A5.1). Our assessment of the HFC associated with H_{ind} and the probability of expressing several correlates of inbreeding (kinks, cowlicks, cryptorchidism) revealed that canonical panthers had a higher probability of expressing traits compared to admixed panthers (Fig. 4A–C), and pre-genetic rescue cohorts of panthers had a higher probability of expressing these traits than post-genetic rescue cohorts (Fig. 4D–F). Most importantly, regardless of ancestry or cohort, an increase in H_{ind} coincided with a decrease in the probability of expressing one of these correlates of inbreeding.

Historically, much of the research on HFCs has focused on populations that were not threatened or endangered⁵², yet these findings were often touted as being applicable to species of conservation concern. We identified an HFC in an imperiled population, and our results support the premise that increased H_{ind} levels are associated with a reduction in the prevalence of inbreeding correlates. Comparing results for canonical and admixed panthers allowed us to assess how HFCs are affected in a population that has undergone a severe bottleneck versus an outbred population. Previous research corroborated several HFCs in the panther population, including the positive impacts of increased heterozygosity on survival, male reproductive parameters, and population viability analyses^{16,17,45,46}. These results support the use of HFCs to identify inbreeding depression, something that has been suggested for other endangered mammals, such as the Iberian lynx (*Lynx pardinus*) and mhorr gazelle (*Nanger dama mhorr*)⁵⁰.

Our analyses of population abundance and genetic effective population size (N_e) revealed just how close Florida panthers came to extinction during the 1980s and early 1990s. Prior to genetic rescue in 1995, the panther population numbered just 10–49 individuals (Fig. 5) and had a critically low N_e of 2.7–3.2 panthers (Extended Data Fig. 2). This range for N_e for the cohorts of predominantly canonical panthers is comparable to the only other estimate of N_e based on a small data set (13 specimens; Ref.¹¹). Culver et al.'s¹¹ analyses of nuclear and mitochondrial DNA from historic (<1922) and contemporary (1980s) canonical Florida panther samples revealed that the population may have endured a bottleneck where it was as small as only 6 individuals ($N_e = 2$) at one point last century. Our Pre1 and Pre2 cohorts (77 samples), with some specimens dating back to the 1970s, further corroborate our observation that panthers were threatened by extirpation. The cohorts sampled following the genetic rescue (Post1, Post2, and Post3) exhibited a sustained increase in N_e , which was also reflected in the index and estimates of abundance that increased > fivefold from pre-genetic rescue levels to >200 panthers (Fig. 5). The most recent cohort (Post3) has an estimated N_e of 62.1 (parametric 95% CI 40.2–115.5) panthers, a remarkable 23-fold increase in N_e and a value comparable to those we calculated for several of the larger, Western puma populations (Extended Data Fig. 2). The observed increases in population abundance and N_e following genetic

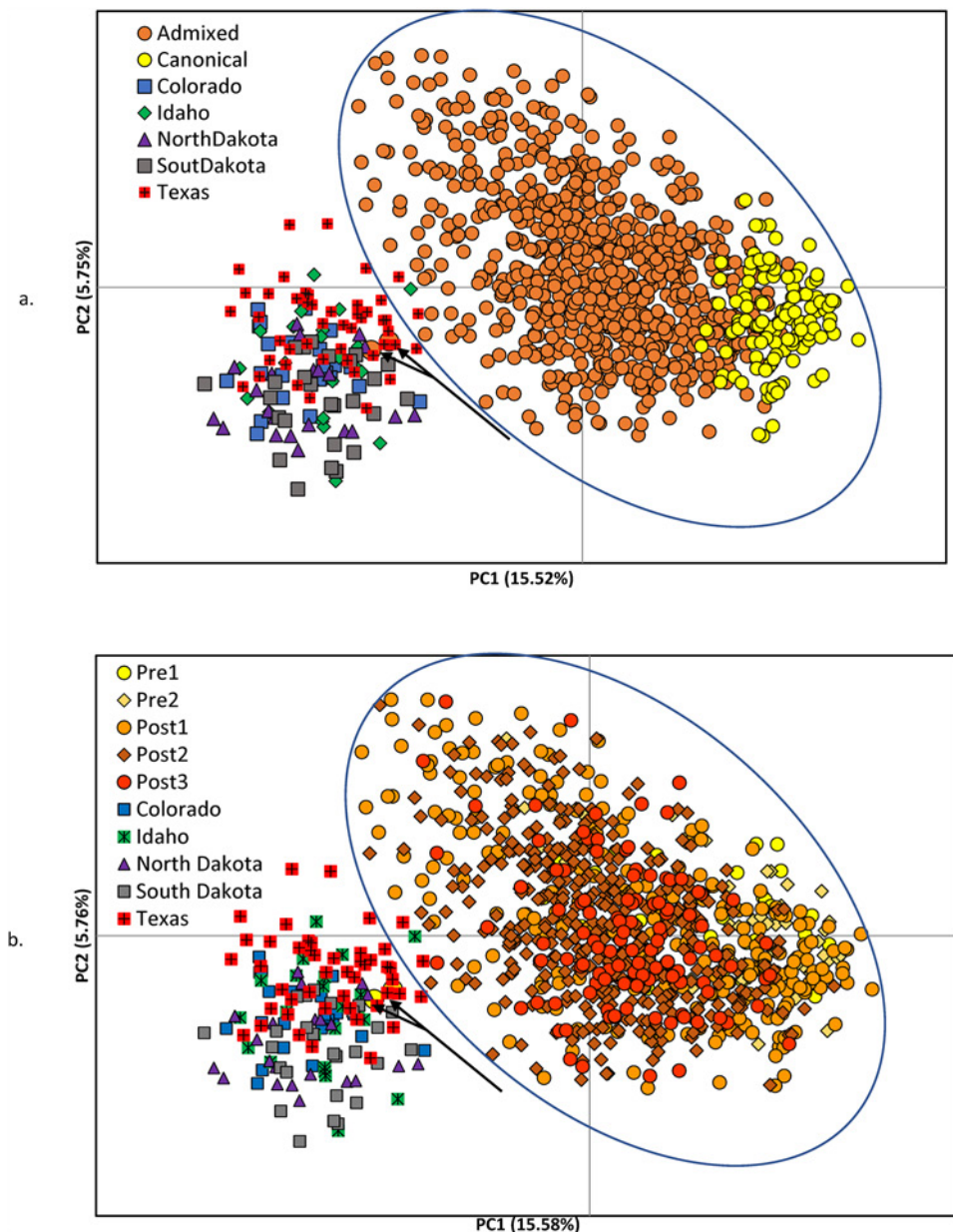


Figure 6. Principal coordinate analysis (PCoA) based on Nei's genetic distance matrices between (a) canonical and admixed Florida panthers and Western puma populations, and (b) Pre1, Pre2, Post1, Post2, and Post3 Florida panthers and Western puma populations. Ovals highlight the distinction of the cluster of Florida panther samples from the Western puma populations, including Texas. The arrows point to two unclustered Florida panther samples (UCFP010 and UCFP011; circles enlarged for clarity) collected in Palm Beach County Florida in 1983 and 1984, respectively. Both were suspected of being captive pumas that had been released or escaped into the wild. Those suspicions are further corroborated by our genetic analyses and the clustering of these two samples with non-Florida panthers. Variance explained by each PCoA axis is displayed in axis titles.

rescue are consistent with other improvements we have documented in the population, including improved demographic performance and substantial reduction in the probability of extinction^{16,45}.

Our PCoA of genotype data collected over 40 years demonstrated the continued separation of Florida panthers and western pumas—including Texas—regardless of ancestry or cohort (Fig. 6A,B). Although admixed panthers since genetic rescue have shifted the cluster of panthers toward Western pumas, this analysis still permits the identification of the geographical origin of a puma DNA sample as from a Florida or non-Florida population. These findings are further supported by: (1) our estimates of genetic structure via F_{st} between these groups that show low levels of differentiation (0.015–0.071) between all panther cohorts and ancestry groups but elevated levels of differentiation (0.105–0.316) when compared to Western pumas (SI Appendix 7, Tables A7.1 and A7.2); and (2) the ancestral cluster from our STRUCTURE $K=3$ analysis that is dominant in all Western puma populations we sampled (q -value $\bar{X}=0.951 \pm 0.008$, $N=143$) is found at very low percentages in panthers

(q -value $\bar{X} = 0.026 \pm 0.003$, $N = 545$; see Extended Data Fig. 3; SI Appendix 2), regardless of whether they were within pre- or post-genetic rescue cohorts. These data support the distinct genetic signature of Florida panthers that remains after 25 years of admixture and continues to differentiate them from other puma populations in North America.

The evidence is clear that genetic rescue has benefited one of the world's most endangered populations of large carnivores. Data presented here, whether from correlates of inbreeding, genetic variation, population abundance, or effective population size, show that genetic rescue has alleviated the immediate risk of population extinction and reduced the effects of inbreeding depression beyond the F1 generation of admixed panthers. In fact, our data confirm the improvements to this population through the F5 generation post-genetic rescue (18–22 years). However, panther population abundance has stabilized and declined in recent years (2016–2020; Fig. 5), and all measures of genetic variation slightly decreased in the most recent cohort of panthers (Post3; Fig. 3A,B) inclusive of the 5th and 6th generations post-genetic rescue. The continued isolation of this population from conspecifics ultimately means that additional genetic management will be necessary^{14,45}. Population modeling by van de Kerk et al.⁴⁵ recommended that genetic rescue be implemented every 20–40 years with 5–10 individuals from other puma populations. Although wildlife managers continue to monitor the genetic health of panthers 29 years after genetic rescue, these findings suggest the need to consider future genetic management of this population if the most recent trends continue (e.g., declining road mortality, increases in correlates of inbreeding, decreases in abundance estimates).

Similar to other large carnivores worldwide^{53,54}, Florida panthers continue to be affected by many anthropogenic stressors including habitat loss and other consequences of increased human encroachment⁹. As of 2020, Florida had over 21 million residents, a 14.6% increase from 2010 (U.S. Census Data Website 2021). Nonetheless, the recent passing of the Florida Wildlife Corridor Act (Florida Statute 259.1055 2021), designed to preserve key wildlife habitat as greenways for species movement in peninsular Florida, may help facilitate northward expansion of the breeding population within its historic range.

Our unprecedented, long-term dataset filled important gaps in our understanding of genetic rescue as a tool for wildlife conservation, specifically the persistence of benefits to a population. There is continued debate on whether there should be a paradigm shift toward increased implementation of genetic rescue in at risk populations⁵⁵ or further development of best practices for monitoring genetic rescue attempts prior to increasing their use⁵⁶. Our findings offer novel insights by showing that the beneficial effects of genetic rescue can persist in the population for at least 5 generations, thereby supporting the implementation of this effective, multi-generational management tool for the conservation and recovery of other small, inbred, and imperiled populations.

Methods

Field methods and sampling

Staff from the FWC and National Park Service captured, sampled, radiocollared, and tracked panthers in South Florida from 1981 to 2021 as described by van de Kerk et al.⁴⁵. Information regarding the study area, captures, immobilization, handling, and sampling methods for panther adults, subadults, and kittens are described elsewhere^{45,57}. We collected two 4-mm skin biopsies for DNA analysis from the external pinnae of each panther (2 mm from kittens) and stored them in a 2-ml vial with 100% EtOH. In some cases, whole blood collected at capture or tissues collected at necropsy were used for DNA analyses. Plucked hair from the abdomen of panthers served as a backup source of DNA. All live-capture and handling activities were completed under safe and humane protocols approved by the FWC and followed American Society of Mammalogists guidelines⁵⁸.

DNA extraction and microsatellite genotyping

DNA samples were processed at the National Genomics Center for Wildlife and Fish Conservation (Missoula, MT, USA). Whole genomic DNA was extracted from blood and tissue samples using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) or via a slight modification (incubation of sample overnight at 60°C on a rocker in a solution containing proteinase K and lysis buffer) of the Qiagen DNA extraction protocol to extract DNA from hair. We used 16 microsatellite loci (Fca090, Fca133, Fca243, F124, F37, Fca075, Fca559, Fca057, Fca081, Fca566, F42, Fca043, Fca161, Fca293, Fca369, and Fca668) identified by Menotti-Raymond et al.^{59,60}. Methods for the amplification, sequencing, data quality testing, and genotyping are provided in SI Appendix 1.

We assessed genotype data conformance to assumptions of Hardy–Weinberg equilibrium [HWE], linkage disequilibrium [LD], and null alleles with a subset of 161 adult and subadult radiocollared panthers to avoid over-representation of alleles from previously sampled kittens handled at maternal dens that were related to this subsample (SI Appendix 1). Tests for HWE and LD were completed in GENEPOP version 4.7⁶¹; tests for null alleles were completed in MICROCHECKER 2.2.3⁶². Similar analyses were completed on samples collected from more contiguous puma populations in Colorado, Idaho, North Dakota, South Dakota, and Texas (Western puma) for comparison (SI Appendix 1).

Assessment of genetic ancestry and delineations of panther cohorts

We assessed the effect of genetic ancestry and year-of-birth cohorts (hereafter, cohorts) on variables that are associated with genetic variation and genetic structure within panthers and among populations of Western puma. We used genotype data from the 16 microsatellite loci to implement a Bayesian clustering analysis in Program STRUCTURE version 2.3.4³⁰ to infer ancestral clusters of sampled adult and subadult panthers (see SI Appendix 2 for detailed STRUCTURE analysis methods and for STRUCTURE results from the complete data set that includes kittens). We then used q -values provided in runs for the best K (number of genetic clusters; for this analysis $K = 2$) to assign individual panthers as either canonical ($\geq 90\%$ pre-introgression ancestry) or admixed

(<90% pre-introgression ancestry) ancestry. For some analyses, we focused interpretations on subadult and adult panthers we sampled and did not include kittens handled only at maternal dens. Given the high annual mortality estimated for kittens 0.323¹⁷, it makes sense to focus population-wide changes in ancestry composition on the age groups that are more likely to contribute to subsequent generations of panthers.

To assess the temporal persistence of genetic benefits across generations, we delineated five cohorts of panthers known or estimated to have been born within a certain timeframe: pre-genetic rescue group 1 (PRE1) inclusive of panthers born < 1986; PRE2, 1986–1995; post-genetic rescue 1 (POST1), 1996–2005; POST2, 2006–2015, and POST3, 2016–2020. This permitted us to categorize panthers in groups that did not rely on ancestral designation from STRUCTURE but instead provided insight into the changes in metrics of genetic health of the panther population through time.

Morphological and biomedical correlates of inbreeding

During each panther capture or necropsy we recorded the presence or absence of correlates of inbreeding depression^{9,12} that included kinked tails resulting from a deformity of the distal caudal vertebrae; cowlicks that are a reversal of hair direction on the midline dorsum, cryptorchidism, ASDs, and percent structurally abnormal spermatozoa sperm data from Penfold et al.⁴⁶. Statistical comparisons among the proportions or mean percentage of these traits in each ancestral category or cohort were completed in Program R version 4.2.1⁶³; tests are described in detail in SI Appendix 3.

Measures of genetic variation

We analyzed genotypes from all 16 loci for a subset of adult and subadult panthers (N = 552) for analyses of genetic variation and population genetic structure within panthers and between populations of Western puma. Genotypes of panther kittens that were only sampled at maternal dens were not used in these analyses because related individuals are known to result in the over-representation of certain alleles⁶⁴. We used GenAlEx 6.5^{33,34} to determine the number of alleles (N_a), number of effective alleles ($N_{\text{effective}}$), observed heterozygosity (H_o), and expected heterozygosity (H_e). We used hp-rare⁶⁵ to estimate allelic richness (A_r) using the rarefaction technique to account for different sample sizes among groups of panthers and Western pumas. Statistical analyses to assess among-group differences for these metrics are detailed in SI Appendix 4.

Inbreeding depression in wild populations is commonly assessed using heterozygosity–fitness correlations (HFC), although their application in endangered populations is less common⁵². We estimated the heterozygosity of each individual panther by first calculating homozygosity by loci (HL), which varies between 0 (all loci heterozygous) and 1 (all loci homozygous)⁶⁶ using the Rhh package⁶⁷ in Program R. We then calculated individual heterozygosity (H_{ind}) for each panther as $1 - \text{HL}$ (see⁴⁵), thereby creating a scale where values increase as an individual approaches having a genotype that is completely heterozygous⁶⁸. We used linear regression to quantify among-group differences in mean H_{ind} separately for (a) panther ancestral groups and Western pumas, and (b) panther cohorts and Western pumas. For the HFC analysis, we used logistic regression to quantify among-group (ancestral, cohorts and Western pumas) differences in the probability of expressing a correlate of inbreeding and to estimate the influence of H_{ind} , a continuous predictor variable, on the probability of trait expression. Additional details on statistical analyses are provided in SI Appendix 5.

Genetically effective population size, abundance, and principal coordinate analysis

We calculated N_e by applying the LD method^{31,69,70} within NeEstimator 2.1³² using the random mating model and did not accept singleton alleles, since these are known to contribute to an upward bias of N_e estimates³². This method also provided 95% confidence intervals with estimates of N_e using both parametric and a jackknife over individuals methods⁷¹.

Estimating the abundance of large carnivores is difficult and has been a challenge for panther managers. Agencies involved in panther conservation have relied on minimum counts of panthers accrued via indirect field evidence (inventory of sign) and directly from captures¹⁰, but this method required extensive effort and the counts did not: (1) provide a population estimate, (2) incorporate detection coefficients, and (3) have an associated measure of error. To ameliorate those issues and provide a more statistically robust estimate, we applied the methodology of McClintock et al.⁷², with some minor adjustments (see SI Appendix 6), to estimate the population size during the post-genetic restoration period of 2000–2018. We used these estimates, the minimum population counts of McBride et al.¹⁰ for the period 1981–2015¹⁹, and the population estimate derived from an integrated population model from Merriell⁷³ for the period 1982–2020 to assess trends in the population size pre- and post-genetic rescue.

We conducted PCoA plots to visually represent the differences between allele frequencies at different sampling locations, cohorts, and ancestry and to assess the level of genetic distinctness of panthers compared to Western puma populations. PCoA is a multivariate technique that permits the plotting of patterns in a multivariate data set (e.g., multiple microsatellite loci for multiple samples). We completed two separate PCoA using GenAlEx 6.5^{33,34} with microsatellite genotypes of panthers categorized by ancestry and by cohort (Pre1, Pre2, Post 1, Post 2, Post 3) and plotted those with genotype data from Western pumas. A parallel analysis using the more traditional fixation index (F_{st}) to assess pairwise genetic structure among groups of panthers and Western pumas was also completed in GenAlEx 6.5^{33,34}.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All study data are included in the article and/or Supplemental Information.

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Author contributions

D.P.O. designed the research; D.P.O., M.W.C., M.L., M.Cr., D.S., A.J., B.C.F.C., M.E.R-P, W.E.J., M.K.O., K.L.P., and M.K.S. performed the research; D.P.O., C.P.S., B.T.M., K.L.P., and M.K.S. analyzed the data; and D.P.O., M.W.C., and M.K.O. wrote the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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