Dynamics, Persistence, and Genetic Management of the Endangered Florida Panther Population

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ABSTRACT Abundant evidence supports the benefits accrued to the Florida panther (*Puma concolor coryi*) population via the genetic introgression project implemented in South Florida, USA, in 1995. Since then, genetic diversity has improved, the frequency of morphological and biomedical correlates of inbreeding depression have declined, and the population size has increased. Nevertheless, the panther population remains small and isolated and faces substantial challenges due to deterministic and stochastic forces. Our goals were 1) to comprehensively assess the demographics of the Florida panther population using long-term (1981–2015) field data and modeling to gauge the persistence of benefits accrued via genetic introgression and 2) to evaluate the effectiveness of various potential genetic management strategies. Translocation and introduction of female pumas (*Puma concolor stanleyana*) from Texas, USA, substantially improved genetic diversity. The average individual heterozygosity of canonical (non-introgressed) panthers was 0.386 ± 0.012 (SE); for admixed panthers, it was 0.615 ± 0.007. Survival rates were strongly age-dependent (kittens had the lowest survival rates), were positively affected by individual heterozygosity, and decreased with increasing population abundance. Overall annual kitten survival was 0.32 ± 0.09; sex did not have a clear effect on kitten survival. Annual survival of subadult and adult panthers differed by sex; regardless of age, females exhibited higher survival than males. Annual survival rates of subadult, prime adult, and old adult females were 0.97 ± 0.02, 0.86 ± 0.03, and 0.78 ± 0.09, respectively. Survival rates of subadult, prime adult, and old adult males were 0.66 ± 0.06, 0.77 ± 0.05, and 0.65 ± 0.10, respectively. For panthers of all ages, genetic ancestry strongly affected survival rate, where first filial generation (F1) admixed panthers of all ages exhibited the highest rates and canonical (mostly pre-introgression panthers and their post-introgression descendants) individuals exhibited the lowest rates. The most frequently observed causes of death of radio-collared panthers were intraspecific aggression and vehicle collision. Cause-specific mortality analyses revealed that mortality rates from vehicle collision, intraspecific aggression, other causes, and unknown causes were generally similar for males and females, although males were more likely to die from intraspecific aggression than females. The probability of reproduction and the annual number of kittens produced varied by age; evidence that ancestry or abundance influenced these parameters was weak. Predicted annual probabilities of reproduction were 0.35 ± 0.08, 0.50 ± 0.05, and 0.25 ± 0.06 for subadult, prime adult, and old adult females, respectively. The number of kittens predicted to be produced annually by subadult, prime adult, and old adult females were 2.80 ± 0.75, 2.67 ± 0.43, and 2.28 ± 0.83, respectively. The stochastic annual population growth rate estimated using a matrix population model was 1.04 (95% CI = 0.72–1.41). An individual-based population model predicted that the probability that the population would fall below 10 panthers within 100 years (quasi-extinction) was 1.4% (0–0.8%) if the adverse effects of genetic erosion were ignored. However, when the effect of genetic erosion was considered, the probability of quasi-extinction within 100 years increased to 17% (0–100%). Mean times to quasi-extinction, conditioned on going quasi-extinct within 100 years, was 22 (0–75) years when the effect of genetic erosion was considered. Sensitivity analyses revealed that the probability of quasi-extinction and expected time until quasi-extinction were most sensitive to changes in kitten survival parameters. Without genetic management intervention, the Florida panther population would face a substantially increased risk of quasi-extinction. The question, therefore,

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is not whether genetic management of the Florida panther population is needed but when and how it should be implemented. Thus, we evaluated genetic and population consequences of alternative genetic introgression strategies to identify optimal management actions using individual-based simulation models. Releasing 5 pumas every 20 years would cost much less ($200,000 over 100 years) than releasing 15 pumas every 10 years ($1,200,000 over 100 years) yet would reduce the risk of quasi-extinction by comparable amount (44–59% vs. 40–58%). Generally, releasing more females per introgression attempt provided little added benefit. The positive effects of the genetic introgression project persist in the panther population after 20 years. We suggest that managers contemplate repeating genetic introgression by releasing 5–10 individuals from other puma populations every 20–40 years. We also recommend that managers continue to collect data that will permit estimation and monitoring of kitten, adult, and subadult survival. We identified these parameters via sensitivity analyses as most critical in terms of their impact on the probability of and expected times to quasi-extinction. The continuation of long-term monitoring should permit the adaptation of genetic management strategies as necessary while collecting data that have proved essential in assessing the genetic and demographic health of the population. The prospects for recovery of the panther will certainly be improved by following these guidelines. © 2019 The Authors. *Wildlife Monographs* published by Wiley Periodicals, Inc. on behalf of The Wildlife Society.

KEY WORDS Florida panther demography, genetic introgression, genetic rescue, heterosis, inbreeding depression, individual-based population models, population viability analysis, *Puma concolor coryi*.

Dinámicas, Persistencia y Manejo Genético de la Población en Peligro de Extinción de Pantera de Florida

RESUMEN La población de pantera de Florida (*Puma concolor coryi*) mejoró tras la implementación en 1995 del proyecto de introgresión genética en el sur de Florida, USA, como lo demuestran varias líneas de evidencia. Desde entonces, su diversidad genética ha mejorado, la frecuencia de índices morfológicos y biomédicos correlacionados con depresión endogámica ha disminuido, y el tamaño de la población ha aumentado. Sin embargo, la población de panteras permanece pequeña, aislada, y se enfrenta a retos sustanciales producidos por fuerzas determinísticas y estocásticas. Los objetivos de este estudio fueron 1) evaluar exhaustivamente la demografía de la población de panteras de Florida usando datos de campo (del periodo 1981–2015) y modelos con el fin de calibrar en que medida persisten los beneficios adquiridos a través de la introgresión genética y 2) evaluar la efectividad de varias estrategias de manejo genético. La diversidad genética de la población mejoró sustancialmente con la introducción de pumas hembra (*Puma concolor stanleyana*) procedentes de Texas, USA. En panteras canónicas (no procedentes de introgresión), el valor medio de heterocigosidad individual fue 0.386 ± 0.012 (SE), y en panteras mezcladas 0.615 ± 0.007. En gran medida, las tasas de supervivencia dependieron de la edad (los cachorros tenían las tasas de supervivencia más bajas), estuvieron afectadas positivamente por la heterocigosidad individual, y disminuyeron cuando la población aumentó. La tasa de supervivencia total, independientemente del sexo del cachorro, fue de 0.32 ± 0.09. La tasa de supervivencia anual de panteras adultas y subadultas varió según el sexo; independientemente de la edad, las hembras vivieron más que los machos. Las tasas anuales de supervivencia de hembras subadultas, adultas y adultas mayores fueron 0.97 ± 0.02, 0.86 ± 0.03, y 0.78 ± 0.09, respectivamente. Las tasas de supervivencia de machos subadultos, adultos, y adultos mayores fueron 0.66 ± 0.06, 0.77 ± 0.05, y 0.65 ± 0.10, respectivamente. La ascendencia genética determinó en gran medida la tasa de supervivencia de panteras de cualquier edad, siendo mayor en la primera generación filial (F1) de panteras mezcladas en todas las edades, y menor en los individuos canónicos (sobre todo panteras pre-introgresión y sus descendientes post-introgresión). En panteras con collares de radio telemetría, las causas de mortalidad más frecuentes fueron la agresión intraespecífica y la colisión con vehículos. El análisis de las causas de mortalidad reveló que en las categorías colisión con vehículos, agresión intraespecífica, otras causas y motivos desconocidos, la tasa de mortalidad de machos y hembras era similar, aunque los machos tenían más posibilidades de morir por agresión intraespecífica que las hembras. Las probabilidades de reproducción y el número anual de cachorros dependieron de la edad pero no de los ancestros o el tamaño de la población. Las probabilidades de reproducción de hembras subadultas, adultas, y adultas mayores se estimaron en 0.35 ± 0.08, 0.50 ± 0.05, y 0.25 ± 0.06, respectivamente. El número de cachorros por año y por pantera subadulta, adulta, y adulta mayor se estimó en 2.80 ± 0.75, 2.67 ± 0.43, y 2.28 ± 0.83, respectivamente. Usando un modelo demográfico matricial se estimó la tasa anual de crecimiento estocástico poblacional en 1.04 (95% CI = 0.72–1.41). Usando un modelo de población basado en el individuo e ignorando el impacto adverso de la erosión genética, se estimó la probabilidad de que la población disminuyese a menos de 10 panteras en 100 años (cuasi-extinción) en 1.4% (0–0.8%). Sin embargo, incluyendo el impacto de la erosión genética, la probabilidad de cuasi-extinción en 100 años aumentó al 17% (0–100%). El plazo medio para la cuasi-extinción, asumiendo que la cuasi-extinción ocurre en

100 años e incluyendo el impacto de la erosión genética, fue de 22 (0–75) años. Análisis de sensibilidad demostraron que la probabilidad de cuasi-extinción y el plazo hasta alcanzarla, dependían de los valores utilizados para los parámetros de supervivencia de cachorros. Sin manejo genético, la población de panteras de Florida se enfrentaría a un aumento sustancial del riesgo de cuasi-extinción. Por lo tanto, la pregunta no es si es necesario el manejo genético de la población de las panteras de Florida, sino cuándo y cómo implementarlo. Usando modelos de simulación basados en individuos, evaluamos diferentes estrategias de introgresión genética y sus posibles impactos en la población y en su genética. La reducción del riesgo de cuasi-extinción fue similar introduciendo 5 pumas cada 20 años o 15 pumas cada 10 años (44–59% vs. 40–58%), pero la primera opción resulta más económica ($200,000 en 100 años) que la segunda ($1,2000,000 en 100 años). Introducir más hembras en cada intento de introgresión no produjo beneficios adicionales. El impacto positivo del proyecto de introgresión genética persiste en la población de panteras veinte años después de su implementación. Recomendamos a los gestores que consideren repetir la introgresión genética introduciendo 5–10 individuos de otras poblaciones de puma cada 20–40 años. Adicionalmente, recomendamos que se continúe con la recopilación de datos, lo que es crucial para poder estimar y monitorizar la supervivencia de cachorros, adultos y subadultos. Estos parámetros son los más críticos para estimar la probabilidad y el periodo de cuasi-extinción, según se demostró con el análisis de sensibilidad. Se debe continuar con la monitorización de la población a largo plazo, lo que permitirá adaptar las estrategias de manejo según sea necesario, a la vez que recopilar información esencial para evaluar la salud demográfica de la población. Siguiendo estas recomendaciones, las perspectivas de recuperación de las panteras, mejorarán.

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INTRODUCTION

Promoting genetic admixture between individuals from different populations, commonly referred to as genetic introgression, has the potential to serve as a powerful management and conservation tool (Keller and Waller 2002, Tallmon et al. 2004, Whiteley et al. 2015). Genetic introgression can occur naturally through immigration of individuals into small or isolated populations (Marr et al. 2002, Vila et al. 2003, Adams et al. 2011), but it can also be implemented as a management strategy via the intentional translocation of individuals from large viable populations to small, endangered populations. Intentional genetic introgression of populations of wild animals has prevented the demise of species such as adders (*Vipera berus*; Madsen et al. 2004), prairie chickens (*Tympanuchus cupido*; Bateson et al.

2014), and bighorn sheep (*Ovis canadensis*; Madsen et al. 1999, Hogg et al. 2006). In most of these cases, genetic introgression has been effective at improving demographic performance of populations affected by inbreeding depression.

Inbreeding depression, a reduction in fitness resulting from the production of offspring by individuals related by ancestry, is a conservation challenge common to small, isolated populations. Inbreeding depression has historically been documented in captive populations (Lacy et al. 1993). The deleterious impact of inbreeding depression on wild populations was somewhat controversial (Frankham 2010a) until the seminal work of Crnokrak and Roff (1999), which verified the negative effects of inbreeding on the fitness traits of several wildlife populations including cheetahs (*Acinonyx jubatus*),

black-tailed prairie dogs (*Cynomys ludovicianus*), and white-footed mice (*Peromyscus leucopus*). Subsequently, the effect of inbreeding depression has been extensively documented in several wild animal populations, including mammals such as wolves (*Canis lupus*; Liberg et al. 2005), African lions (*Panthera leo*; Trinkel et al. 2010, 2011), and Iberian lynx (*Lynx pardinus*; Ruiz-López et al. 2012). In these examples, inbreeding depression had negative effects on a variety of fitness traits, including survival of neonates, susceptibility to disease, and male fertility. The demographic and genetic impacts of inbreeding on population growth in carnivores is highlighted best in one of the quintessential long-term conservation studies on the gray wolves of Isle Royale in northern Michigan, USA (Peterson 1977, Peterson and Page 1988, Peterson et al. 1998, Adams et al. 2011). Research on this population has proved informative for gaining a better understanding of population consequences of loss of genetic variation and impacts of intrinsic and extrinsic factors that have led to its decline to only 2 individuals as of 2017 (Hedrick et al. 2017).

Conversely, outbreeding depression is a scenario when mating between members of genetically divergent populations might result in a reduction in fitness correlated with the loss of local adaptations or genetic incompatibilities (Moritz 1999). Outbreeding depression has been hypothesized to be a possible side effect of genetic introgression (Reinert 1991, Maehr and Caddick 1995), albeit infrequently and with less and weaker empirical evidence for vertebrate species. Negative fitness responses resulting from mating between individuals from highly divergent populations of copepods were noted by Hwang et al. (2012). Other examples of outbreeding depression in varied species of plants are provided by Fenster and Gallaway (2000), Waser et al. (2000), Goto et al. (2011), and Brys and Jacquemyn (2016). In general, however, outbreeding depression is thought to be less of a concern when implementing conservation measures such as genetic introgression (Miller et al. 1999, Whiteley et al. 2015).

In the last 3 decades, conservation biologists have exponentially increased their use of molecular techniques to assist with monitoring, managing, and conserving wildlife populations (DeYoung and Honeycutt 2005). Although early research relied on methods such as restriction fragment length polymorphisms, allozyme electrophoresis, and minisatellites (Smith and Wayne 1996), the last 20 years have been dominated by the implementation of improved automated sequencing technology to provide more informative and fine-scale data from both nuclear and mitochondrial DNA sequences (e.g., mtDNA sequencing, microsatellites; DeYoung and Honeycutt 2005). Microsatellites, in particular, have become a common tool for conservation projects to assess genetic parameters, including inbreeding depression and the impacts of genetic introgression, in imperiled populations. They continue to be widely used molecular markers in conservation research.

The field of molecular ecology is changing rapidly and new technology is constantly being developed. Currently, there is interest in applying genomics or so-called whole-genome sequencing techniques to assist with conserving imperiled species. Two recent examples of the application of whole-genome sequencing to endangered species of carnivores include the work of Robinson et al. (2016) on the Channel Island fox (*Urocyon littoralis*) and Murchison et al. (2012) on the Tasmanian devil (*Sarcophilus harrisii*). For instance, Robinson et al. (2016) reported a near absence of genomic variation in Channel Island foxes from the island of San Nicolas, demonstrating an extreme reduction in heterozygosity compared to mainland populations. Similarly, Murchison et al. (2012) applied whole genome sequencing to ascertain whether the Tasmanian devil facial tumor disease originated from a male or a female devil and further used whole genome sequencing techniques to assess transmission of the disease across Tasmania. The application of whole genome sequencing in conservation biology and wildlife management will continue to increase (Whiteley et al. 2015). Although costs associated with implementing whole genome sequencing are steadily declining (Whiteley et al. 2015), other factors—such as sample preparation and the need for bioinformatic expertise to deal with voluminous amounts of data—are still an impediment to their widespread use in conservation and management of wildlife populations. Therefore, long-term data sets from more traditional molecular markers such as microsatellites will continue to have utility for providing insight into the genetic health of endangered species and for assessing impacts of management and recovery initiatives.

The identification and quantification of inbreeding depression using molecular techniques (Kardos et al. 2016), along with protocols for implementing translocations to promote genetic introgression, have been documented for varied endangered populations (Whiteley et al. 2015). Conversely, studies that provide an assessment of the longevity of benefits accrued to wild populations via genetic introgression are uncommon. Hedrick et al. (2014) provided evidence for the declining effect of genetic introgression on the Isle Royale population of wolves after only 2 or 3 generations. Whereas heterosis is expected to increase individual fitness, it can also result in outbreeding depression (Fenster and Gallaway 2000, Waser et al. 2000, Goto et al. 2011, Waller 2015, Brys and Jacquemyn 2016). Depending on the mechanisms, outbreeding depression may not become evident until generations subsequent to the first filial (F1) generation (Pickup et al. 2013, Waller 2015). For example, ancestral chromosomes and coevolved genes remain intact in the F1, but recombination may break up favorable gene complexes in F2 and later generations (Waser et al. 2000, Waller 2015, Frankham 2016). Investigating the timeframe over which the positive impacts of genetic introgression are sustained would likely necessitate long-term monitoring, but for most studies of the process, populations are typically monitored only for 1 or 2 generations following genetic introgression (Willi et al. 2007, Hedrick and Fredrickson 2009, Hedrick et al. 2014, Whiteley et al. 2015). Consequently, data are rarely gathered that would allow us to estimate the time over which populations continue to benefit from genetic introgression (Hedrick et al. 2014, Whiteley et al. 2015).

The Florida panther (*Puma concolor coryi*) provides a textbook example of how inbreeding depression in a small, isolated population can result in low levels of genetic variation that are associated with morphological and biomedical abnormalities and precipitous population decline. The timeline associated with the decline of panthers parallels that of most large carnivores in North America (Onorato et al. 2010). A robust population of panthers was thought to extend across Florida, USA, in pre-Columbian times (Fig. 1; Alvarez 1993). Subsequently, documented declines in the numbers of panthers were noted in the nineteenth and twentieth centuries, mainly as a result of
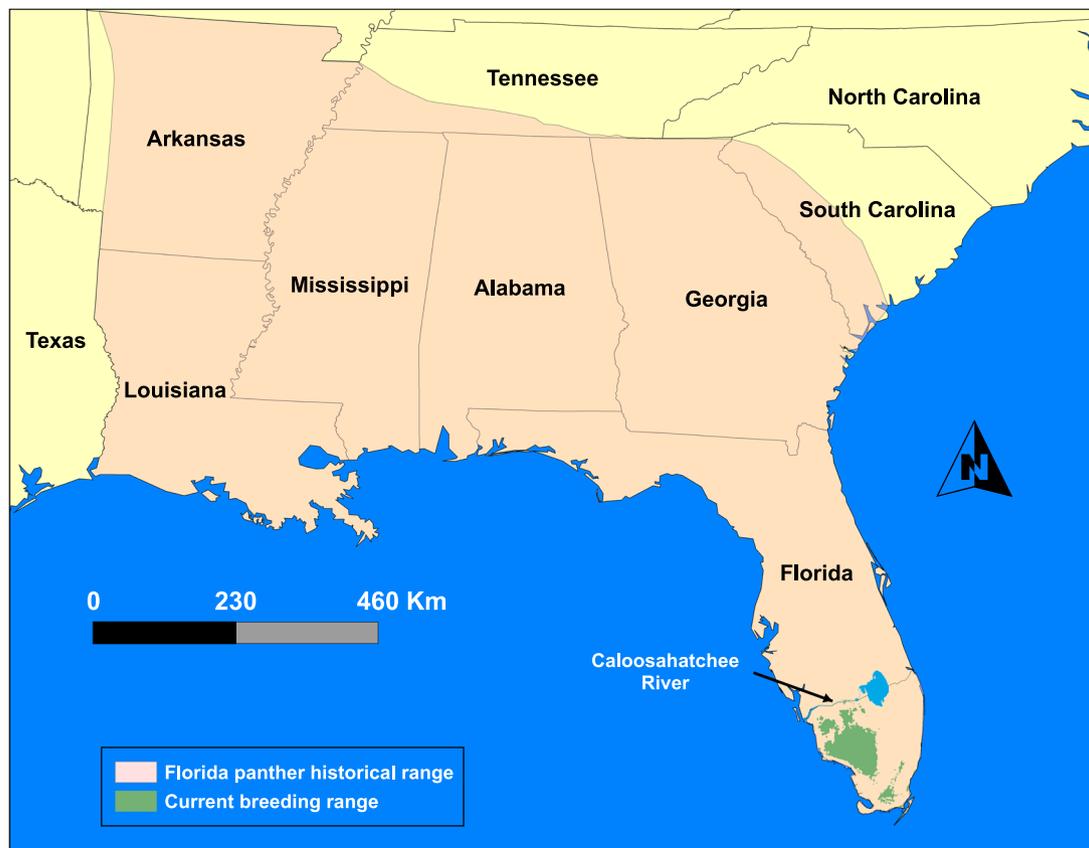
Figure 1. Historical and current (2015) breeding range of the Florida panther in the southeastern United States. Delineation of current breeding range is described in Frakes et al. (2015).

anthropogenic alteration of the landscape and unregulated hunting. As early as the 1870s, panthers were rarely encountered and considered mythical in some portions of their range (Beverly 1874). The decline in the population of panthers eventually captured the attention of lawmakers; subsequently, they received partial legal protection as a game animal in 1950 and complete legal protection in Florida by 1958 (Onorato et al. 2010). By the 1960s, the core of remaining wilderness within the Big Cypress region of South Florida, where the last group of breeding panthers was thought to persist, was further affected by the construction of Alligator Alley. This roadway, along with the Tamiami Trail, allowed access to these once inaccessible areas. All these factors ultimately led to the listing of the Florida panther as endangered on the inaugural list of endangered species on 11 March 1967 (U.S. Federal Register 1967) and subsequent protection under the Endangered Species Act of 1973 (Public Law 93-205). This designation invariably raised awareness of the plight of the panther and served as a catalyst to initiate research to avoid what appeared to be their imminent extinction (Onorato et al. 2010).

In 1973, the World Wildlife Fund initiated a survey that resulted in the capture of a single female panther and documentation of their sign in just a few locations in South Florida (Nowak and McBride 1974). The information accrued during this survey would ultimately lead to the commencement of a long-term research project on the Florida panther in 1981 by

what is now known as the Florida Fish and Wildlife Conservation Commission (FWC). Over the next decade, a multitude of studies were published that helped improve knowledge of panther reproduction (Maehr et al. 1989, Barone et al. 1994), diet (Maehr 1990), and genetics (O'Brien et al. 1990, Roelke et al. 1993), among other topics. Early research was essential for identifying challenges faced by the remaining panthers, most importantly the impacts of the continued loss of habitat, isolation of the population, and inbreeding depression (Onorato et al. 2010). In combination, these factors would force wildlife managers to contemplate the implementation of genetic introgression to avert the extinction of the panther.

By the early 1990s, the Florida panther population had declined to a minimum of 20–30 individuals (McBride et al. 2008). Several studies documented low levels of genetic diversity and expressed traits thought to be correlated with inbreeding depression (Roelke et al. 1993, Johnson et al. 2010, Onorato et al. 2010) including cowlicks (mid-dorsal pelage whorls) and kinked tails (Wilkins et al. 1997). These morphological traits probably had little direct impact on fitness of Florida panthers. More concerning were the proportion of individuals in the population in the early 1990s that were unilaterally or bilaterally cryptorchid (Roelke et al. 1993), possessed poor sperm quality (Barone et al. 1994), suffered compromised immune systems (O'Brien 1994), exhibited atrial septal defects (Roelke et al. 1993), and possessed some of the lowest levels of genetic variation observed in wild felids (Driscoll

et al. 2002). Taken together, these factors suggested that the long-term prospects for the persistence of the Florida panther were poor.

Subsequently, the United States Fish and Wildlife Service (USFWS) conducted an assessment that led to the delineation of 4 possible management options: 1) initiate a captive breeding program; 2) introduce genetic material from captive stock purported to contain both pure Florida panther and South American puma lineages; 3) translocate wild, female pumas (*Puma concolor stanleyana*) from Texas, USA, into South Florida; or 4) take no action at all (USFWS 1994). Collaborative discussions involving academics, non-governmental organizations, and state and federal wildlife agencies eventually led to the selection of option 3 and a plan for genetic introgression that involved the release of 8 female pumas from Texas into South Florida was implemented in 1995 (Seal 1994, Onorato et al. 2010).

Continued research conducted after the introgression intervention provided clear evidence of the benefits accrued to the panther population including increased levels of genetic diversity, declines in the frequency of morphological and biomedical correlates of inbreeding depression, and the substantial increase of the population size (McBride et al. 2008, Johnson et al. 2010, McClintock et al. 2015). Furthermore, Hostetler et al. (2013) showed that the post-introgression population growth was driven primarily by higher survival rates associated with admixed panthers (offspring of pairings between the female Texas pumas and male Florida panthers and subsequent generations of those progeny) following genetic introgression. The apparent success of genetic introgression in preventing the imminent extinction of the Florida panther population is encouraging, but the population remains small and isolated and continues to face substantial challenges, especially the large-scale loss of habitat.

Almost 5 panther generations (generation time = 4.5 years; Hostetler et al. 2013) have passed since the implementation of the genetic introgression program. The panther population has been monitored continuously during this time period, leading to a substantial amount of additional data and biological insights since the demographic assessment made immediately following the introgression (Hostetler et al. 2010, 2012, 2013; Benson et al. 2011). Given that the effects of genetic introgression are not permanent, it is important to discern whether the positive impacts of this management initiative are continuing or waning. Decline in the benefits of the introgression could reduce the population growth rate and probability of persistence. At the same time, panther habitat is rapidly lost to a variety of human activities (e.g., land development), and environmental changes associated with invasive species and climate change (Land et al. 2008, Dorcas et al. 2012, Frakes et al. 2015). When long-term monitoring data are available, it is prudent to periodically evaluate demographic rates, population growth, and persistence parameters, so that changes are detected, and timely management action can be implemented.

Two population modeling approaches have become popular among ecologists and wildlife managers for studying the dynamics and persistence of age- or stage-structured populations: matrix population models and individual-based models (IBMs; Brook et al. 2000, Morris and Doak 2002, Morrison et al. 2016). Matrix population models are the generalized, discrete time-version of the Lotka-Euler equation, which describes the dynamics of age- or stage-structured populations of plants, animals, and humans (Lotka 1924, Caswell 2001). They have become standard tools for modeling human and wildlife populations (Tuljapurkar 1990, Caswell 2001, Keyfitz and Caswell 2005) because 1) they are powerful and flexible, permitting adequate representation of life history of a wide variety of organisms including those with complex life histories; 2) parameters for these models can be empirically estimated using standard analytical methods such as multi-state capture-recapture methods (e.g., Williams et al. 2002); 3) it is relatively straightforward to include the effects of factors such as environmental and demographic stochasticity, and density dependence; 4) important demographic quantities such as asymptotic population growth rate, stable age- or stage distribution and generation time can be easily calculated from these models; 5) they permit prospective and retrospective perturbation analyses, which have been proven useful in wildlife conservation and management; 6) they are computer-friendly, do not require extensive programming experience, and their implementation using software packages such as R (R Core Team 2016) and MATLAB (MathWorks 2014) is straight-forward; and 7) they have sound theoretical foundation, offering analytical solutions to many aspects of population modeling. We used matrix population models for 1) calculating the aforementioned demographic quantities, 2) performing sensitivity analyses involving asymptotic population growth rate, and 3) comparative purposes to check the results obtained from equivalent simulation-based models.

Despite many advantages offered by matrix population models, it is difficult to incorporate attributes of individuals such as genetics or behavior within that framework. Agent-based or IBMs (Grimm 1999, Grimm and Railsback 2005, McLane et al. 2011, Railsback and Grimm 2011, Albeke et al. 2015) offer an alternative modeling framework with tremendous flexibility. Individual-based models are computer simulation models that rely on a bottom-up approach that begins by explicitly considering the components of a system (i.e., individuals); population-level properties emerge from the behavior of, and interactions among, discrete individuals. Using IBMs, it is possible to explicitly represent attributes of individuals such as movement, mating, or genetics (McLane et al. 2011, Railsback and Grimm 2011, Albeke et al. 2015). We used individual-based population models as the primary population modeling approach because they offered a framework for an explicit consideration of genetics both at individual and population levels.

Our overall goal was to provide a comprehensive demographic assessment of the sole extant population of the Florida panther using long-term (1981–2015) field data and novel modeling techniques. Our objectives were 1) to estimate demographic parameters for the Florida panther and investigate whether positive impacts of the 1995 genetic introgression remained in the population and if so, to what extent; 2) to estimate population growth and persistence parameters using an IBM tailored to the Florida panther's life history; and 3) to evaluate the sensitivity of population growth and persistence parameters to vital demographic rates.

The Florida panther population remains small (≤230 adults and subadults; FWC 2017) with no realistic possibility of natural gene flow, so the loss of genetic variation and concomitant increase in inbreeding-related problems are inevitable. Ensuring the long-term

persistence of the population will likely necessitate periodic genetic management interventions. Therefore, our final objective was to 4) evaluate genetic and population-dynamic consequences of varied genetic introgression strategies and, from these, identify management strategies that are affordable and effective at improving prospects of Florida panther persistence. To achieve this objective, we extended the individual-based population model to track individual genetics based on empirical allele frequencies, taking advantage of the long-term demographic and genetic data that have been collected on Florida panthers since 1981. We used individual heterozygosity to inform vital rates based on empirically estimated relationships between individual heterozygosity and demographic parameters. We then simulated population trajectories, tracked individual heterozygosity over time, and estimated quasi-extinction times and probabilities without introgression and with introgression for a range of intervals and number of immigrants per introgression event. We compared the genetic and demographic benefits of implementing alternative genetic introgression scenarios and ranked them by the level of improvement in population persistence parameters and estimated costs because funding is a persistent challenge to conservation planning.

Management of imperiled species is a complex process involving many stakeholders and plagued by layers of uncertainties (Runge 2011). It would, therefore, seem that management of threatened and endangered species would be a perfect case for adaptive resource management approach (ARM) because ARM focuses on structured decision making for recurrent decisions made under uncertainty (Williams et al. 2002, Runge 2011). Because it combines structured decision making with learning, prudent application of ARM reduces uncertainty over time and can lead to optimal management actions (Williams and Brown 2012, 2016). Key ingredients of a successful adaptive management program include stakeholder engagement, clearly defined and mutually agreed-upon objectives, alternative management actions and objective decision rules (Nichols et al. 2007; Williams and Brown 2012, 2016). Unfortunately, Florida panther stakeholders have divergent views about the state of the system (e.g., panther abundance), and it is a challenge to have mutually agreed-upon objectives, management actions, or decision rules acceptable to all or most stakeholders. Although Florida panther conservation efforts would be best served within the ARM framework in the long run, impediments to its implementation (Runge 2011, Williams and Brown 2012) are unlikely to be overcome in the near future.

STUDY AREA

The breeding population of Florida panthers mainly persists as a single population South of the Caloosahatchee River (approximately 26.7133°N latitude, 81.5566°W longitude; see Fig. 1). One exception is a female that was documented just north of the Caloosahatchee River in 2016 (and subsequently documented with kittens in 2017) by FWC, the first validation of a female panther north of the River since 1973 (FWC, unpublished data). The breeding range in South Florida is topographically flat, has a subtropical climate, and is characterized by permanent and ephemeral wetlands influenced by rains from May through October (Duever et al. 1986). The study area contains a variety of wildland land cover types, including hardwood hammocks, cypress forests, pine flatwoods, freshwater marshes, prairies, and grasslands (Davis 1943) and land characterized by human activity including citrus groves, croplands, pastureland, rock mining, and residential developments (Onorato et al. 2011). The area is intersected by a multitude of roads that fragment panther habitat. Most notable among these is Interstate 75 (Alligator Alley), which was expanded from 2 to 4 lanes in 1990, when fencing and wildlife crossings were constructed with the intention of minimizing harmful effects of road expansion on local wildlife (Foster and Humphrey 1995). A large portion of the area that comprises South Florida is under public ownership mainly as federal or state lands. Everglades National Park, Big Cypress National Preserve, Florida Panther National Wildlife Refuge, Picayune Strand State Forest, and Fakahatchee Strand Preserve State Park alone encompass 9,745 km^2, though not all of the area is considered suitable for panthers (e.g., large expanses of sawgrass marshes in the western Everglades). Frakes et al. (2015) delineated 5,579 km^2 of adult panther habitat in South Florida, 1,399 km^2 of which are in private ownership. A majority of these private lands are located in the northern extent of the breeding range. Although some private lands may be protected (e.g., conservation easements), other areas are susceptible to incompatible land uses such as rock mining or residential developments. The breeding range in South Florida is bounded to the east and west by large metropolitan areas inclusive of Miami-Fort Lauderdale-Pompano Beach and Cape Coral-Fort Myers-Naples-Marco Island-Immokalee, respectively, that are populated by >6,000,000 people (https://www.census.gov/, accessed 24 Jan 2019). These characteristics of the study area highlight the recovery challenges faced by panthers.

METHODS

Field Methods

Since 1981, Florida panthers have been captured, radiocollared, and tracked by the FWC and National Park Service staff (Table 1). Capture and handling protocols followed guidelines of the American Society of Mammalogists (Sikes 2016). Livestock Protection Company (Alpine, TX, USA) provided trained hounds and houndsmen for captures. Teams either treed or bayed panthers on the ground and then darted them with a 3-ml compressed-air dart fired from a CO_2-powered rifle. Immobilization drugs have varied during the tenure of the project, but most recently, teams immobilized panthers with a combination of ketamine HCl (10 mg/kg; Doc Lane's Veterinary Pharmacy, Lexington, KY, USA) and xylazine HCl (1 mg/kg; Doc Lane's Veterinary Pharmacy). Following immobilization, teams lowered treed panthers to the ground by a rope or caught panthers with a net; in some cases, they used a portable cushion (McCown et al. 1990) to further mitigate the impact of a fall. Capture teams administered propofol (PropoFlo™; Abbott Laboratories, Abbott Park, IL, USA) intravenously either as a bolus or continuous drip to maintain anesthesia. They administered midazolam HCl (0.03 mg/kg) intramuscularly or intravenously to supplement anesthesia in some panthers. Panthers recovered in a shaded area away from water. In some cases, capture teams reversed xylazine HCl with yohimbine HCl (Yobine®; Lloyd Inc., Shenandoah, IA, USA) at 25% its recommended dose.

Table 1. Number of Florida panthers by age, sex, and genetic ancestry categories used in subsequent demographic analyses during the study period (1981–2013) in South Florida, USA.

	Kittens[a]				Subadults and adults[b]		
	Males	Females	Total		Males	Females	Total
PIT-tagged[c]	215	180	395	Radiocollared[c]	108	101	209
Recovered	11	4	15	Subadult	72	50	122
Litter failed	47	38	85	Prime adult	65	95	160
Recaptured	25	30	55	Old adult	13	20	33
Canonical	27	20	47	Canonical	43	29	72
F1 admixed	6	12	18	F1 admixed	2	6	8
Other admixed	130	105	235	Other admixed	45	53	98

[a] Kitten samples included those that were PIT-tagged, recovered (i.e., panthers that were found dead), part of a failed litter, or recaptured as subadults or adults.

[b] Radiocollared subadult and adult panthers are presented as sample size within each age class and ancestry class.

[c] Combined number of panthers of different ancestries is less than the number radiocollared or tagged with a subcutaneous passive integrated transponder (PIT-tagged) because we did not conduct genetic sampling on all panthers. Combined number of panthers in different age classes is greater than the number of radiocollared panthers because some individuals were counted in ≥2 age classes as animals aged.

Capture teams determined sex and age of panthers, marked them with an ear tattoo and a subcutaneous passive integrated transponder (PIT-tag), and fitted them with a very high frequency (VHF) or global positioning system (GPS) radio-collar equipped with a mortality sensor. The GPS collars were programmed to attempt to collect a position at a variety of time intervals (range = 1–12 hr), depending on objectives of concurrent studies; observers routinely tracked panthers with VHF radiocollars from a fixed-wing aircraft 3 times per week. When a radio-collar transmitted a mortality signal, or when a location did not change for several days, observers investigated the site to establish the panther's fate. When observers found a dead panther, they assessed the cause of death based on an examination of the carcass and surrounding area if possible. They then transported carcasses to an experienced veterinarian for necropsy and a histopathological examination to attempt to assign the cause of death. Starting in 1995, observers continually assessed successive locations of radio-collared females to determine if they initiated denning behavior. Three to 4 fixes at the same location (VHF collars) or successive returns to the same location over a weeklong period (GPS collars) served as an indicator of a possible den. Observers then located dens more precisely via triangulation with ground telemetry. They visited dens when the dam left the site, typically to make a kill. Teams captured kittens by hand; captured kittens ranged in age from 4 to 35 days post-partum. Teams counted, sexed, weighed, dewormed, and permanently marked kittens with a subcutaneous PIT-tag.

Genetic Analysis

Teams collected blood, tissue, or hair from the majority of captured kittens, subadult, and adult Florida panthers for DNA samples. The United States Department of Agriculture Forest Service National Genomics Center for Wildlife and Fish Conservation (Missoula, MT, USA) processed samples. Technicians used Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) to extract whole genomic DNA from blood and tissue samples and used a slight modification (overnight incubation of sample in lysis buffer and proteinase K on a rocker at 60°; elution of DNA in 50 µl of buffer) of the Qiagen DNA extraction protocol (Mills et al. 2000) to extract DNA from hair samples. Technicians used a panel of 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. (1999), which had been previously applied to Florida panther samples (Johnson et al. 2010). Technicians amplified DNA samples in 10-µl reaction volumes that included 1.0 µl of DNA, 1× reaction buffer (Applied Biosystems, Life Technologies Corporation, Grand Island, NY, USA), 2.0 mM of $MgCl_2$, 200 µM of each dNTP, 1 µM of reverse primer, 1 µM of dye-labeled forward primer, 1.5 mg/ml of bovine serum albumin (BSA), and 1U Taq polymerase (Applied Biosystems). The polymerase chain reactions (PCRs) included a thermal profile of 94°C for 5 minutes followed by 36 cycles of 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 30 seconds. Technicians visualized the resulting PCR products on a LI-COR DNA analyzer (LI-COR Biotechnology, Lincoln, NE, USA). Technicians collected genotypes from hair samples ($n = 16$) using a multitube approach (Taberlet et al. 1996), error-checked genotypes using Program DROPOUT (McKelvey and Schwartz 2005), and combined them with genotypes from tissue and blood samples ($n = 487$). We evaluated all 16 loci for various descriptive statistics for the radio-collared sample of adult and subadult panthers. We excluded genotypes of kittens from that analysis because related individuals are known to result in the overrepresentation of certain alleles (Marshall et al. 1998). We determined the number of alleles, observed heterozygosity, and expected heterozygosity using GenAlEx (Peakall and Smouse 2006, 2012). We assessed allelic richness at each locus in Fstat 2.9.3 (Goudet 1995). We assessed conformance of genotype data from these 16 loci to Hardy–Weinberg assumptions, linkage disequilibrium, and null alleles (Appendix A, available online in Supporting Information). The Genomics Center also provided us with genotype data from the same 16 loci for 41 pumas from western Texas, the source population for the genetic rescue program.

Evidence of inbreeding depression in wild populations is commonly determined on the basis of heterozygosity–fitness correlations (Silva et al. 2005, Ortego et al. 2007, Mainguy et al. 2009, Johnson et al. 2011), so we also estimated the heterozygosity of each individual panther. We calculated homozygosity by loci (HL), which varies between 0 (all loci heterozygous) and 1 (all loci homozygous; Aparicio et al. 2006) using the Rhh package (Alho et al. 2010) in Program R (R Core Team 2016). A microsatellite locus has more weight in HL

when it is more informative (i.e., has more alleles) and has an even distribution of allele frequencies. For our demographic analyses, we calculated individual heterozygosity for each individual panther as 1 − HL. As do most indices of individual heterozygosity, HL takes into account the average allele frequency in the population (Aparicio et al. 2006). Thus, during simulations, the allele frequency in the population changes throughout an individual's lifetime, which causes its heterozygosity also to change. This is not biologically plausible because genetic properties are retained throughout an individual's lifetime. Therefore, for use in model simulations, we simply calculated heterozygosity for each individual as the proportion of heterozygous loci.

We also used genotype data to implement a Bayesian clustering analysis in Program STRUCTURE (Pritchard et al. 2000) to infer ancestral clusters of panthers. This method uses a Markov chain Monte Carlo (MCMC) method to determine the number of genetic clusters (K) in the sample while also providing an individual's percentage of ancestry (q values) allocated to each cluster. Runs for this analysis included a 100,000 MCMC burn-in period followed by 500,000 MCMC iterations for 1–10 ancestral genetic clusters ($K = 1$ to 10) with 10 repetitions for each K. To determine the most likely number of K genetic clusters, we used the logarithm of the probability of the data (log Pr(D)|K; Pritchard et al. 2000) and estimates of ΔK (Evanno et al. 2005) in Program STRUCTURE HARVESTER (Earl and VonHoldt 2011). We then used q values provided in runs for the best K to assign individual panthers as either canonical (in most cases ≥90% pre-introgression ancestry; see Johnson et al. 2010) or admixed (<90% pre-introgression ancestry). We recognized admixed panthers that were the offspring of matings between a Texas female puma and a canonical male panther as F1 admixed panthers for analyses.

Demographic Parameters

Survival of kittens.—Most kittens PIT-tagged at den sites were never encountered again. A small proportion of the PIT-tagged kittens were recovered dead or were recaptured as subadults or adults and radiocollared so that their fate could be monitored. We also identified instances of complete litter failure, when a female died within 9 months after giving birth (the minimum age of independence for kittens) or when a female was documented to have another litter less than 12 months after giving birth (the minimum age of independence plus a 3-month gestation period; Hostetler et al. 2010). Thus, our data set consisted of 1) live recaptures and subsequent radio-tracking of panthers of various ages that had been PIT-tagged as kittens, 2) recovery of dead panthers of various ages that had been PIT-tagged as kitten, 3) live captures and subsequent radio-tracking of other panthers, and 4) instances of complete litter failure (Table 1). We analyzed these data using Burnham's live-recapture dead-recovery modeling framework (Burnham 1993, Williams et al. 2002) to estimate survival of kittens (0–1-year old) and to test covariate effects on this parameter. We included data on individuals encountered dead or alive at an older age in our analyses because their encounter histories also provided information on kitten survival. We used a live-dead data input format coded with a 1-year time step (Cooch and White 2018).

All known litter failures occurred within the first year of the kittens' lives, so we treated all litter failures as recoveries within the first year. We treated panthers that would have died if we had not removed them to captivity as having died on the date of removal. Data preparation and analytical approach are described in more detail by Hostetler et al. (2010).

In addition to survival probability (S), the Burnham model incorporates parameters for recapture probability (p), recovery probability (r), and site fidelity (f). We set r and p to 1.0 for radio-tracked individuals because their status was known each year. We also fixed f at 1.0 for all individuals because the recapture and recovery areas were the same and encompassed the entire range of the Florida panther. Based on findings of Hostetler et al. (2010), we used a base model that 1) allowed survival to differ between kittens and older panthers, 2) allowed survival to differ between sexes and among age classes (females: ages 1 and 2, ≥3; males: ages 1, 2, and 3, ≥4), 3) constrained recapture probability to be the same for all uncollared panthers, and 4) allowed recovery rates to differ between kittens and uncollared older panthers. Even with our additional data, this model remained the best fit for a range of models for recapture, recovery, and survival of kittens, subadults, and adults of various age-class categories (Hostetler et al. 2010).

Subadult and adult survival.—To estimate survival for panthers ≥1 year of age, we analyzed radio-tracking data using a Cox proportional hazard-modeling framework (Cox 1972, Therneau and Grambsch 2000). We followed procedures outlined by Benson et al. (2011) for data preparation and analysis. Briefly, we right-censored panthers on the last day of observation before their collar failed. When an individual aged into a new age class while being tracked, we created 2 data entries, 1 ending the day it entered the new age class, the other starting on that day. We used the Fleming–Harrington method to generate survival estimates from the Cox analysis and the Huber sandwich method to estimate robust standard errors (Therneau and Grambsch 2000, Benson et al. 2011).

We considered 3 age classes: subadults (1–2.5 yr for females and 1–3.5 yr for males), prime adults (2.5–10 yr for females and 3.5–10 yr for males), and old adults (>10 yr old for both sexes), after Benson et al. (2011). We estimated overall survival using a base model that allowed survival to differ between subadult females, subadult males, prime adult females, prime adult males, and old adults of either sex (old age was additive with sex; otherwise, age and sex were interactive). This model provided the best fit to the data relative to a range of models considering differences in survival between prime-adult and older-adult age classes both additively and interactively with sex (Benson et al. 2011).

To examine whether survival rates observed in recent years differed from those observed immediately following introgression, we also estimated age-specific survival rates for 2 nearly equal periods of time; immediately post-introgression (1995–2004) and the more recent period (2005–2013). For these analyses, we separated the radio-tracking data into the 2 periods and estimated survival rates for each period separately using the base model. Similar analyses for kitten survival were not possible because of data limitations.

Probability of reproduction.—We used telemetry data obtained from radio-tracked females that reproduced at least once to

estimate annual probability of reproduction of subadult, prime-adult, and old-adult females using complementary log–log regression (Agresti 2013), following methods described in detail by Hostetler et al. (2012). Briefly, we modeled the probability of a female panther giving birth (b) as a function of covariates as:

$$b_i = 1 - \exp[-\exp(\mathbf{z}_i\boldsymbol{\gamma})],$$

where $\mathbf{z}_i$ is a row vector of covariates (see below for covariate information) for female panther i and $\boldsymbol{\gamma}$ is a column vector of model coefficients (Appendix B, Table B2). To account for differences in observation time, we included $\log(m)$ as an offset in the model, where m is the number of months a female panther was radio-tracked (Hostetler et al. 2012).

Reproductive output.—To estimate the number of kittens produced by reproductive females, we used cumulative logit regression (Min and Agresti 2005, Agresti 2013). We considered a model that includes J categories for the number of offspring, with j denoting the number of offspring ($j = 1, 2, \ldots, J$, and $J = 6$), provided that a female reproduces. We considered $J = 6$, which is greater than the largest litter size observed in our study (4 kittens) because females can produce 2 or more litters per year if litters fail. We modeled the probability that a female i produces a litter of size at most j (Y_i) as

$$\Pr(Y_i \le j) = \delta_{ij} = \begin{cases} \frac{1}{1 + e^{-(\theta_j + x_i\boldsymbol{\beta})}}, & j = 1, 2, \ldots, J-1 \\ 1, & j = J \end{cases},$$

where θ_j is the intercept for the annual number of kittens produced by a female i being at most j, $\mathbf{x}_i$ is a row vector of covariates for female panther i (see below), and $\boldsymbol{\beta}$ is a column vector of model coefficients. The probability that Y_i will be exactly j is given by

$$\Pr(Y_i = j) = \pi_{ij} = \begin{cases} \delta_{i1}, & j = 1 \\ \delta_{ij} - \delta_{i(j-1)}, & j = 2, \ldots, J \end{cases}.$$

Finally, the expected annual number of kittens produced by female i (ν_i) is given by

$$\nu_i = \sum_{j=1}^{J} j\pi_{ij}.$$

Additional details regarding the estimation and modeling of the annual number of kittens produced can be found in Hostetler et al. (2012).

Covariates.—In addition to sex and age, we tested for the effect of abundance, genetic ancestry, and individual heterozygosity on the aforementioned demographic parameters (model coefficients for kitten and adult survival are given by $\boldsymbol{\eta}$ and $\boldsymbol{\iota}$, respectively; Appendix B, Table B2). We used a minimum population count (MPC) based on radio-tracking data and field evidence of subadult and adult (i.e., independent age) panthers as an index of abundance (McBride et al. 2008). For these analyses, we did not use the population size estimates reported by McClintock et al. (2015) because unlike the minimum counts they did not cover the entire study period

(McBride et al. 2008; R. T. McBride and C. McBride, Rancher's Supply Incorporated, unpublished report; Fig. 2).

To test if demographic parameters differed depending on ancestry, we considered 3 ancestry models, dividing the panthers into 1) F1 admixed and all other panthers; 2) canonical and admixed (including F1 admixed) panthers; and 3) F1 admixed, canonical, and other admixed panthers. We quantified genetic diversity using individual heterozygosity, as described previously. We tested for the effect of genetic covariates using a subset of kittens that were genetically sampled. We calculated model-averaged estimates of model parameters on the scale in which they were estimated (Appendix B, available online in Supporting Information).

Cause-specific mortality.—We performed cause-specific mortality analysis only on dead radio-collared panthers because detection rates of uncollared panther mortalities are highly correlated with the cause of death (e.g., vehicle collision). Following Benson et al. (2011), we attributed each mortality to 1 of 4 causes: 1) vehicle collision, 2) intraspecific aggression, 3) other causes (including known causes such as diseases, injuries, and infections unrelated to the first 2 causes), and 4) unknown (i.e., mortalities for which we could not assign a cause). We then used the non-parametric cumulative incidence function estimator, a generalization of the staggered-entry Kaplan–Meier method of survival estimation, to estimate cause-specific mortality rates for male and female panthers in different age classes (Pollock et al. 1989, Heisey and Patterson 2006, Benson et al. 2011). We compared cause-specific mortality rates between sexes, age classes, and ancestries.

Statistical inference.—We used an information-theoretic approach (Akaike's information criterion [AIC]) for model selection and statistical inference (Burnham and Anderson 2002). For the analysis of kitten survival, we adjusted the AIC for overdispersion and small sample size (QAIC$_c$; details in Hostetler et al. 2010). We performed all statistical analyses in Program R (R Core Team 2016). We used Burnham's live recapture–dead
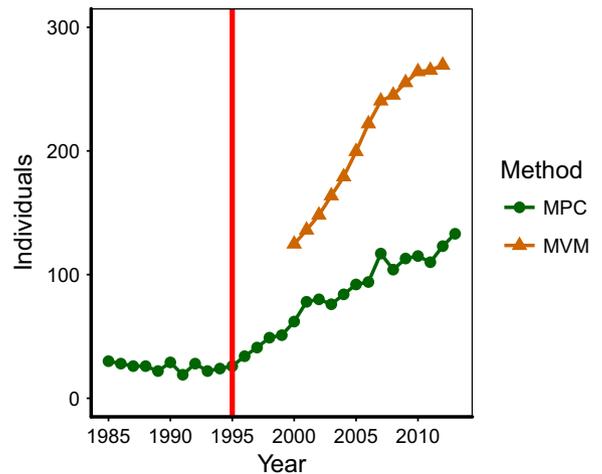
Figure 2. The Florida panther minimum population count (MPC) and population size estimated using motor vehicle collision mortalities (MVM) during our study period. Minimum population counts are from McBride et al. (2008) and R. T. McBride and C. McBride (Rancher's Supply Incorporated, unpublished report). Estimates based on MVM are from McClintock et al. (2015). The solid vertical line indicates the year of genetic introgression.

recovery model (Burnham 1993) to estimate and model kitten survival using the RMark package version 2.1.13 (Laake 2013) as an interface for Program MARK (White and Burnham 1999).

We implemented the Cox proportional hazard model for estimation and modeling of survival of subadults and adults using the R package SURVIVAL (Therneau and Grambsch 2000). We performed cause-specific mortality analyses using an R version of S-PLUS code provided by Heisey and Patterson (2006).

To account for model selection uncertainty, we calculated model-averaged parameter estimates across all models using the R package AICcmodavg (Mazerolle 2017) using AIC weights as model weights (Burnham and Anderson 2002). We used models without the categorical ancestry variables for model averaging, and estimated parameters based on the average value for the continuous covariates (individual heterozygosity and abundance index). Because only a subset of the kittens was genetically sampled, we estimated 2 kitten survival rates. First, we estimated kitten survival based on the data set that included all kittens (overall kitten survival). Second, we estimated kitten survival using a subset of the data that only included kittens that were genetically sampled (survival of genetically sampled kittens).

Matrix Population Models

We investigated Florida panther population dynamics and persistence using 2 complementary modeling frameworks: matrix population models (Caswell 2001) and IBMs (Grimm and Railsback 2005, McLane et al. 2011, Railsback and Grimm 2011, Albeke et al. 2015). Matrix population models offer a flexible and powerful framework for investigating the dynamics and persistence of age- or stage-structured populations (e.g., Caswell 2001, Robinson et al. 2008, Kohira et al. 2009, Hunter et al. 2010, Hostetler et al. 2012). With a fully developed theory behind them, these models also serve as a check for results obtained from simulation-based models such as IBMs. We used an age-structured-matrix population model for deterministic and stochastic demographic analyses, and for preliminary investigations of Florida panther population viability (Caswell 2001, Morris and Doak 2002).

For deterministic and stochastic demographic analyses, we parameterized a female-only 19×19 age-specific population projection matrix of the form:

$$\mathbf{A}(t) = \begin{bmatrix} F_1^t & F_2^t & \cdots & \cdots & F_{19}^t \\ P_1^t & 0 & \cdots & \cdots & 0 \\ 0 & P_2^t & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & P_{18}^t & 0 \end{bmatrix},$$

where F_i and P_i are the age-specific fertility and survival rates, respectively, and $\mathbf{A}(t)$ is a time-varying (or stochastic) population projection matrix. We assumed that the longevity and age of last reproduction of female Florida panthers was 18.5 years; we based this assumption on the observation that the oldest female in our study was 18.6 years old. Florida panthers reproduce year-round; thus, we used birth-flow methods to estimate F_i and P_i values following Caswell (2001) and Hostetler et al. (2013). We estimated P_i as

$$P_i = \sqrt{S_i S_{i+1}},$$

where S_i is the age-specific survival probability. We estimated F_i as

$$F_i = \sqrt{S_k} \left(\frac{m_i + P_i m_{i+1}}{2} \right),$$

where S_k is the kitten survival probability and m_i is the age-specific fecundity rate, which was estimated as

$$m_i = b_i v_i f_k,$$

where b_i is the breeding probability for a female in age class i during time step t, v_i is the annual number of kittens produced by a breeding female in age class i, and f_k is the proportion female kittens at birth (assumed to be 0.5).

We estimated the finite deterministic (λ) and stochastic annual population growth rate (λ_s), and stochastic sensitivities and elasticities following methods described in detail by Caswell (2001). To estimate λ_s, we assumed identically and independently distributed environments (Caswell 2001, Morris and Doak 2002, Haridas and Tuljapurkar 2005), and used a parametric bootstrapping approach to obtain a sequence of demographic parameters for 50,000 matrices. We then estimated $\log(\lambda_s)$ from this sequence of matrices (Tuljapurkar 1990, Caswell 2001) and exponentiated it to obtain λ_s. We calculated the sensitivity and elasticity of λ_s to changes in lower-level vital demographic rates as per Caswell (2001) and Haridas and Tuljapurkar (2005).

For population projection and population viability analysis (PVA) simulations, we expanded the population projection matrix into a 34×34 2-sex, age-structured matrix to include female and male Florida panthers (Caswell 2001, Hostetler et al. 2013). Males contribute to the population only through survival in this modeling framework. We assumed maximum longevity for males to be 14.5 years of age because the oldest male in our study was 14.4 years old. The population projection matrix was of the following form:

$$\mathbf{A}(t, \mathbf{n}) = \begin{bmatrix} F_1^t(N) & F_2^t(N) & \cdots & \cdots & F_{19}^t(N) & 0 & \cdots & \cdots & 0 \\ P_1^t(N) & 0 & \cdots & \cdots & 0 & 0 & \cdots & \cdots & 0 \\ 0 & P_2^t(N) & \ddots & \ddots & \vdots & \vdots & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & P_{18}^t(N) & 0 & 0 & \cdots & \cdots & 0 \\ M_1^t(N) & M_2^t(N) & \cdots & \cdots & M_{19}^t(N) & 0 & \cdots & \cdots & 0 \\ 0 & 0 & \cdots & \cdots & 0 & Q_1^t(N) & \ddots & \ddots & \vdots \\ \vdots & \vdots & \ddots & \ddots & \vdots & 0 & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & \cdots & 0 & 0 & \cdots & Q_{14}^t(N) & 0 \end{bmatrix}.$$

where P_i and Q_i are age-specific survival rates of female and male Florida panthers, respectively, and F_i and M_i are the rates at which female and male kittens are produced by females of age class i, respectively. Here, the population projection matrix is both time-varying and density-dependent (Caswell 2001).

We incorporated the influence of parameter uncertainty, environmental stochasticity, and density dependence on Florida panther population dynamics and persistence following the approach outlined by Hostetler et al. (2013); we used the same approach in the IBM analysis, which is described below. Unlike IBMs, matrix models do not intrinsically include demographic stochasticity, so we explicitly included the influence of

demographic stochasticity by simulating the fates of individuals as described in Caswell (2001).

Population projections using matrix models necessitate a vector of initial sex- and age-specific abundance. Because such data are currently unavailable for the Florida panther, we estimated it based on the stable sex and age distribution and MPC in 2013. Using point estimates of the demographic parameters, we estimated the stable sex and age distribution for the 2-sex, deterministic, and density-independent population projection matrix. We then multiplied the stable sex and age distribution by the MPC in 2013 to get the starting population vector, $\mathbf{n}(0)$. We projected the population over time as $\mathbf{n}(t+1) = \mathbf{A}(t,\mathbf{n})\mathbf{n}(t)$, where $\mathbf{A}(t,\mathbf{n})$ indicates the time-specific, density-dependent population projection matrix (Caswell 2001).

We had 2 indices of abundance available: the MPC (McBride et al. 2008) and population size estimated using motor vehicle collision mortalities (MVM; McClintock et al. 2015). These 2 indices are substantially different because MPC does not account for imperfect detection, whereas MVM accounts for imperfect detection and provides an estimate of population size (Fig. 2). Therefore, we examined 2 scenarios: one using density dependence based on MPC and the other using density dependence based on MVM. We ran 1,000 bootstraps of parameter values and 1,000 simulations per bootstrap for each scenario. We projected population trajectories over 200 years and then estimated population growth rates, probabilities of quasi-extinction, and time until quasi-extinction, and compared these with the results obtained from the IBM (see below). We ran scenarios with a quasi-extinction threshold of 10 and 30 panthers. We used the mean of probabilities of quasi-extinction (PQE) across simulations as the point estimate for PQE and the 5th and 95th percentiles across simulations as the 90% confidence interval. Because the confidence intervals were quantile-based but the point estimates were not, it is possible with skewed distributions for the point estimates to be outside the confidence interval. We implemented the matrix population models in the R computing environment (R Core Team 2016).

IBMs and PVA

Because of well-developed theory, ease of implementation and the modeling flexibility that they offer, matrix population models have become the model of choice for investigating the dynamics and persistence of age- and stage-structured populations (Caswell 2001). However, it is difficult to incorporate attributes of individuals such as genetics and behavior using matrix models. Quantifying the effects of genetic erosion on the Florida panther population dynamics and persistence, and evaluating benefits and costs of alternative genetic management scenarios were important goals of our study. Because IBMs rely on a bottom-up approach that begins by explicitly considering individuals (McLane et al. 2011, Railsback and Grimm 2011, Albeke et al. 2015), it is possible to represent genetic attributes of each individual, and to track genetic variation over time. We used IBMs as the primary modeling framework in this study because they allowed for explicit consideration of genetics both at individual and population levels, and population viability assessment under alternative genetic management scenarios.

We developed an IBM (Grimm 1999, Grimm and Railsback 2005, McLane et al. 2011, Railsback and Grimm 2011, Albeke et al. 2015) tailored to the life history of the Florida panther (Fig. 3). We simulated every individual from birth until death based on a set of rules describing fates (e.g., birth and death) and attributes (e.g., genetics) of individuals at each annual time step. As in other IBMs, population-level processes (e.g., population size and genetic variation) emerged from fates of, and interactions among, individuals (Grimm 1999, Railsback and Grimm 2011). We developed an overview, design concepts, and details (ODD) protocol (Grimm et al. 2006, 2010) to describe our IBM (Appendix C, available online in Supporting Information) and provide pseudocodes for individual-based simulations (Appendix D, available online in Supporting Information).
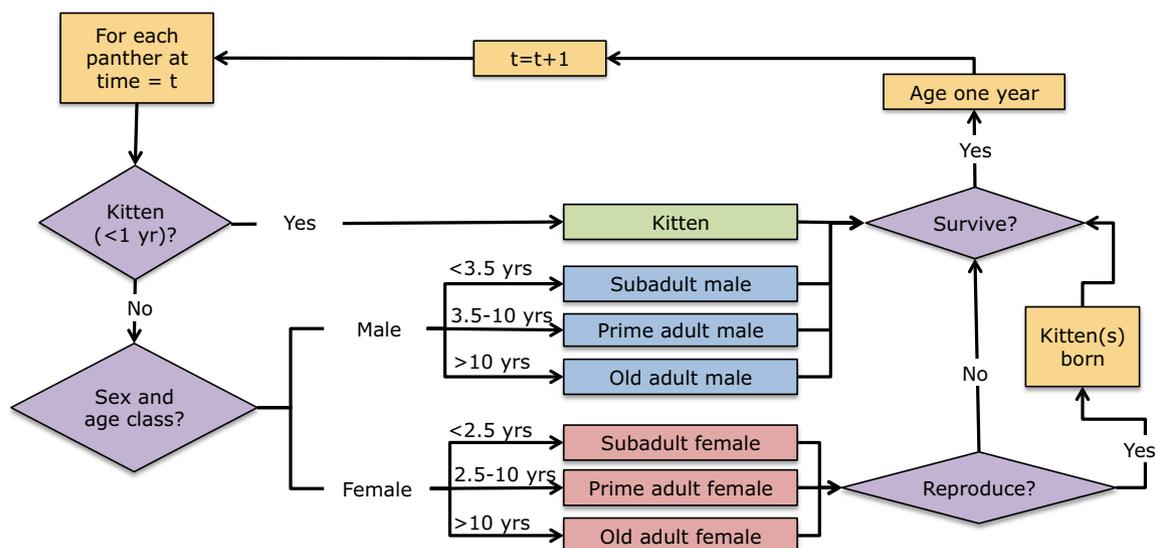
Figure 3. Schematic representation of Florida panther life history. We tailored the individual-based population model to represent the life-history pattern depicted here.

Dynamics and persistence of populations are influenced by many factors, such as demographic and environmental stochasticity and density dependence; these effects and parametric uncertainty must be considered in population models when possible (Caswell 2001, Boyce et al. 2006, Bakker et al. 2009, Hostetler et al. 2013). Because, by definition, IBMs simulate the life history of individuals in a population, they inherently incorporate the effect of demographic stochasticity. Our analyses revealed that survival of kittens, subadults, and adults and the probability of reproduction varied over time, so we incorporated environmental stochasticity in the model for these variables following Hostetler et al. (2013). Likewise, we found evidence of density-dependent effects on kitten survival. Because the MPC might be an underestimate of population size (McBride et al. 2008), we performed simulations using both the MPC and MVM abundance indices.

To account for model selection and parametric uncertainty, we used a Monte Carlo simulation approach. For each bootstrap sample, we selected a model based on the AIC (or $QAIC_c$) weights. We then sampled the intercept and slope for kitten survival (on the logit scale), subadult and adult survival (on the logit scale with log-hazard effect sizes), and probability of reproduction (on the complementary log–log scale; Appendix B) from multivariate normal distributions with mean vectors equal to the estimated parameters and variance-covariance matrices equal to the sampling variance-covariance matrices. We then transformed the results to nominal scale and converted the estimates to age-specific survival and reproduction parameters as described in Hostetler et al. (2013).

We ran 1,000 simulations for 1,000 parametric bootstraps for 200 years for the MPC and the MVM scenario for both the stochastic, 2-sex matrix-population model and the IBM. We tracked the population size at each time step and estimated quasi-extinction probabilities and times based on the population trajectory. We considered the population to be quasi-extinct if individuals of only 1 sex remained or if the population size fell below critical thresholds, set at 10 and 30 individuals. We also estimated expected time to quasi-extinction for both thresholds, defined as the mean and median time until a population becomes quasi-extinct, conditioned on quasi-extinction. We implemented the IBM using the RNetLogo package version 1.0–2 (Thiele et al. 2012, Thiele 2014) as an interface for the IBM platform NetLogo (Wilensky 1999).

Sensitivity Analysis

An important component of demographic analysis is sensitivity analysis, which is the quantification of the sensitivity of a model's outcome to changes in the input parameters (Caswell 2001, Bakker et al. 2009, Thiele et al. 2014). Using an IBM, we performed global sensitivity analyses for 2 (i.e., MPC and MVM) density-dependent scenarios to assess how sensitive quasi-extinction times and probabilities were to changes (and uncertainties) in the estimated demographic rates. We used Latin hypercube sampling to sample parameters from the entire parameter space (Marino et al. 2008, Thiele et al. 2014). We sampled intercept and slope for kitten survival (on logit scale), subadult and adult survival (on logit scale with log-hazard effect sizes), and probability of reproduction (on complementary

log–log scale) from normal distributions. We sampled the probability distribution for the number of kittens produced annually (on the real scale) from a Dirichlet distribution, the multivariate generalization of the beta distribution (Kotz et al. 2000). We sampled 1,000 different parameter sets and ran 1,000 simulations for each scenario. We calculated the probability of quasi-extinction within 200 years for each density-dependence scenario (MPC or MVM) using a quasi-extinction threshold of 10 individuals, and then calculated the partial rank correlation coefficient between each of those and each parameter transformed to the real scale (Bakker et al. 2009, Hostetler et al. 2013). Partial rank correlation coefficients quantify the sensitivity of the probability of quasi-extinction to input variables (i.e., survival and reproductive parameters), with higher absolute values indicating stronger influence of that variable on quasi-extinction probabilities.

Genetic Management

One of our goals was to estimate the benefits (improvements in genetic variation, demographic parameters, and population growth and persistence parameters) and costs (financial costs of capture, transportation, quarantine, release, and monitoring of introduced panthers) of genetic management. To achieve this objective, we extended the IBM to include a genetic component. To simulate individual- and population-level heterozygosity over time, we assigned each panther alleles for 16 microsatellite loci based on the empirical allele frequency in the population (estimated from genetic samples collected during 2008–2015) when we initialized the model (Pierson et al. 2015). At each time step, we simulated Mendelian inheritance for kittens produced such that each kitten randomly inherited alleles from its mother and from a male panther randomly chosen to have putatively sired the litter. We did not explicitly force inbreeding to occur, but the probability of inbreeding naturally increased as the population size decreased.

Introgression strategies.—We modeled genetic introgression as a result of the release of 2-year-old female pumas from Texas into the simulated Florida panther population. For the genetic introgression implemented in 1995, Texas females between 1.5 and 2.5 years of age were released because females at that age have lower mortality rates (Benson et al. 2011), higher reproductive potential, and also because it is much easier to detect with certainty the successful reproduction by females than by males. The ability to more closely monitor reproduction and the birth of kittens is an important consideration in reducing the probability of a genomic sweep of Texas alleles into the Florida panther population. Wildlife managers removed the last 2 surviving Texas females from the wild population in Florida in 2003 to reduce the possibility of genomic sweep. To simulate this strategy in the model, we removed any Texas females that were still alive 5 years after each introgression event. We assigned Texas females alleles based on the allele frequency of the Texas population (based on genotypes from 48 samples collected in west Texas that was inclusive of the pumas released in South Florida in 1995) when they entered the Florida panther population. We could not estimate demographic rates separately for the released Texas females because of small sample size (only 8 females were released in 1995); therefore, we assumed that

Table 2. Sample size (n), number of alleles (N_a), allelic richness (A_R), observed heterozygosity (H_o), and expected heterozygosity (H_e) at 16 microsatellite loci in radio-collared Florida panthers sampled from 1981 to 2013 in South Florida, USA. We calculated these values from a subset ($n = 137$) of the samples used in the analyses and they include only adult and subadult panthers. We excluded kittens because they can result in an overrepresentation of certain alleles.

Locus	n	N_a	A_R	H_o	H_e
FCA090	129	5	5.0	0.333	0.401
FCA133	136	3	3.0	0.618	0.608
FCA243	136	5	5.0	0.419	0.487
F124	137	7	6.9	0.708	0.729
F37	129	4	4.0	0.395	0.397
FCA075	131	5	5.0	0.534	0.598
FCA559	133	5	5.0	0.496	0.503
FCA057	134	6	5.9	0.679	0.624
FCA081	134	7	6.9	0.209	0.206
FCA566	130	6	6.0	0.462	0.475
F42	133	10	10.0	0.737	0.766
FCA043	136	5	4.9	0.596	0.588
FCA161	132	6	6.0	0.500	0.515
FCA293	132	4	4.0	0.614	0.624
FCA369	131	6	6.0	0.573	0.567
FCA668	133	7	7.0	0.406	0.462
Mean	132.9	5.7	5.7	0.517	0.535

survival and reproductive rates, and the effects of covariates on these rates, for the released Texas females were identical to those of female Florida panthers.

The impact of genetic introgression depends on the frequency of introgression events and the number of individuals introduced at each attempt. Thus, we defined introgression strategies based on the number of Texas females to be released (0, 5, 10, or 15) and the interval between genetic introgressions (10, 20, 40, and 80 yr), for a total of 13 strategies. We simulated each management strategy with density dependence estimated using the MPC and MVM abundance indices.

We sampled 1,000 parameter sets and for each of these sets, we ran all introgression strategies 1,000 times for 200 years. We applied the same parametric uncertainty and environmental stochasticity across all management scenarios to allow for better comparison of introgression strategies. At each time step, we recorded the projected population size and average individual heterozygosity. We calculated the heterozygosity for each individual at birth, based on the alleles it inherited. This individual heterozygosity then informed the survival rate of that individual based on the empirically estimated relationship between individual heterozygosity and age-specific survival rates. Heterozygosity did not change throughout an individual's lifetime, but its effect on survival changed as individuals aged because the effect of individual heterozygosity on survival rate differed among age classes. Survival rates of genetically sampled kittens were biased high because some kittens were sampled later in life when they had already survived for some time. To correct for this bias, we adjusted kitten survival rates predicted by the heterozygosity model proportionally. From the population trajectories, we estimated the probability of quasi-extinction (defined as the probability that the population will fall below 10 or 30 individuals or that individuals of only 1 sex will remain).

Cost-benefit analysis.—Experts estimated financial costs of introgression based on their experience with the genetic introgression executed in 1995 and we adjusted estimates to current costs to account for inflation (R. T. McBride, Rancher's Supply Incorporated; M. Cunningham and D. P. Onorato, Florida Fish and Wildlife Conservation Commission, personal communication). Costs included capturing and transporting female pumas from the Texas source population, caring for the pumas while they were in quarantine, and post-introgression monitoring. To compare the financial costs of the different scenarios, we also calculated the average costs incurred over
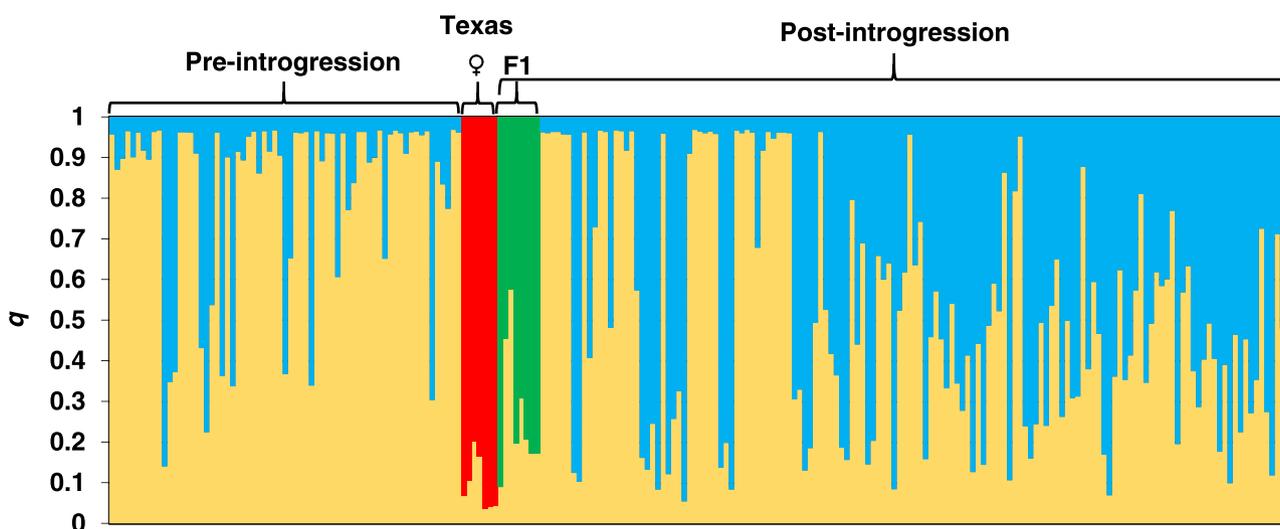
Figure 4. Proportional membership (q) of radio-collared Florida panthers ($n = 218$) and pumas from Texas, USA ($n = 7$) in 2 clusters identified by STRUCTURE. Each individual animal is represented by a separate vertical bar. Yellow indicates canonical panther ancestry and blue represents admixed ancestry. The admixed ancestry of the Texas females and F1 generation panthers that were radiocollared are highlighted red and green, respectively, to demarcate those groups. The pre-introgression period is inclusive of panthers radiocollared from 10 February 1981 to 6 March 1996. The post-introgression era, inclusive of the F1 panthers, includes animals radiocollared from 4 March 1997 to 18 February 2015 in South Florida, USA.

100 years, assuming that the population would be managed according to a particular strategy. Our goal with these exploratory analyses was to evaluate the relative costs and benefits of alternative management scenarios.

RESULTS

Genetic Variation

We assessed genetic variation at 16 microsatellite loci for 137 DNA samples collected from adult and subadult panthers (1981–2013) that were captured and subsequently radiocollared. The number of alleles at these loci ranged from 3–10 and allelic richness averaged 5.7 (Table 2). Average expected heterozygosity for this sample was 0.535 and average observed heterozygosity was 0.517 (Table 2).

We determined individual heterozygosity ($1 - HL$) and ancestry for the complete sample of panther genotypes ($n = 503$), collected from 1981 to 2015, for use as covariates in subsequent analyses. Average individual heterozygosity was 0.559 and ranged from 0–0.980. Our STRUCTURE analysis provided strong support for $K = 2$ clusters, based on the probability of the data (log $\Pr(D)|K$) and ΔK in STRUCTURE HARVESTER. Based on this result, we categorized the ancestry of each panther as either canonical ($n = 123$) or admixed ($n = 380$) using values of proportional membership (q; Fig. 4). The average individual heterozygosity of canonical panthers was 0.386 ± 0.012 (SE); for admixed panthers, it was 0.615 ± 0.007.

Survival and Cause-Specific Mortality Rates

Of the 395 kittens that were PIT-tagged and released, 55 were later encountered alive, 15 were recovered dead, and 85 were part of failed litters (Table 1). We radio-tracked 209 subadult or adult panthers for a total of 244,195 panther-days and documented 126 mortalities (Table 1).

Survival depended strongly on age and kittens had the lowest overall rate (0.32 ± 0.09; Fig. 5A; Table 3). The model-averaged kitten survival was 0.47 ± 0.09 when we included only genetically sampled individuals in the analysis (Table 3); as stated above, this estimate is biased high because many kittens were genetically sampled when they were recaptured alive or recovered dead at older ages. We found no evidence for sex-specific differences in kitten survival, but females survived better than males in all older age classes. For females, subadults had the highest survival rates, followed by prime adults and old adults. For males, prime adults had the highest survival rate, followed by subadults and old adults (Fig. 5A and Table 3).

For panthers of all ages, there was substantial evidence that ancestry affected survival, with F1 admixed panthers of all ages having the highest rates and canonical individuals having the lowest (Fig. 5B). The combined AIC weights of models that included an ancestry covariate were 0.880 for kittens and 0.992 for older panthers. The survival rates of subadult, prime-adult, and old-adult panthers in the period immediately after the introgression (1995–2004) were similar to those in more recent years (2005–2013; Fig. 5C).

After genetic introgression, individual panther heterozygosity increased (Fig. 6) and varied among ancestry categories; individual heterozygosity was the highest for F1 panthers (0.78 ± 0.01),
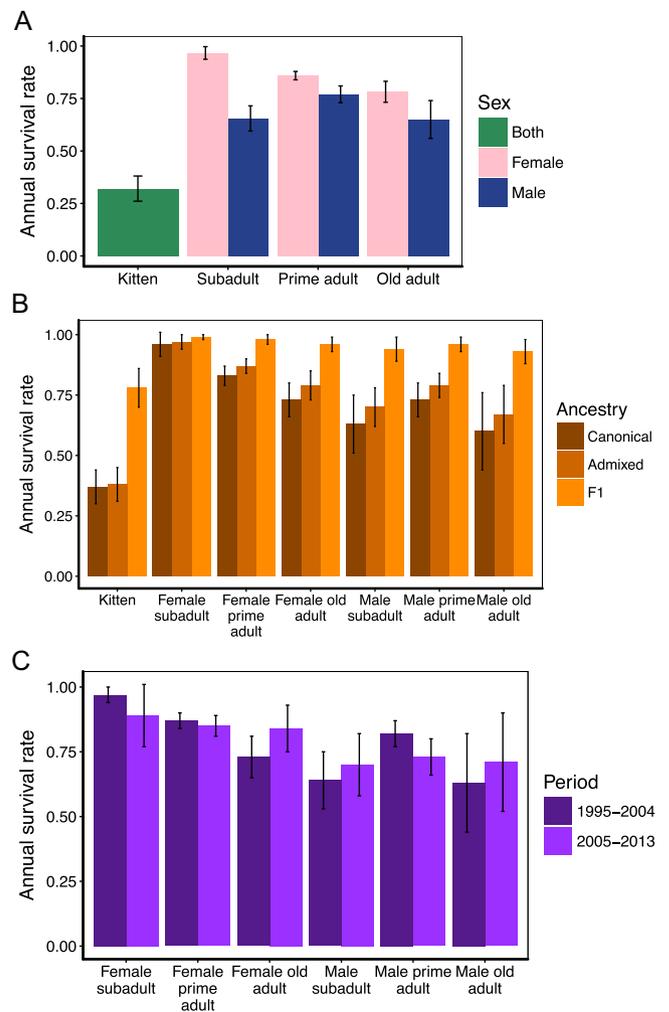
Figure 5. Overall age- and sex-specific survival rates ($\pm$SE; A), age- and sex-specific survival rates ($\pm$SE) for Florida panthers of canonical, F1 admixed, and other admixed ancestry (B), and age- and sex-specific survival estimates ($\pm$SE) for subadult and adult Florida panthers in the period immediately post-introgression (1995–2004) and more recently (2005–2013; C) in South Florida, USA.

Table 3. Model-averaged estimates ($\pm$SE) of demographic parameters for the Florida panther obtained using data collected during 1981–2013 in South Florida, USA.

Parameter	Sex	Age class	Estimate
Survival	Both	Kitten	0.32 ± 0.09[a]
	Female	Subadult	0.97 ± 0.02
		Prime adult	$0.86 + 0.03$
		Old adult	0.78 ± 0.09
	Male	Subadult	0.66 ± 0.06
		Prime adult	0.77 ± 0.05
		Old adult	0.65 ± 0.10
Probability of reproduction	Female	Subadult	0.35 ± 0.08
		Prime adult	0.50 ± 0.05
		Old adult	0.25 ± 0.06
Kittens produced annually	Female	Subadult	2.80 ± 0.05
		Prime adult	2.67 ± 0.01
		Old adult	2.28 ± 0.13

[a] Overall kitten survival (based on all kittens in our data set, including those that were not genetically sampled). Estimate of survival of genetically sampled kittens was 0.47 ± 0.09; this estimate is biased high because many kittens were genetically sampled when they were recaptured alive or recovered dead at older ages.
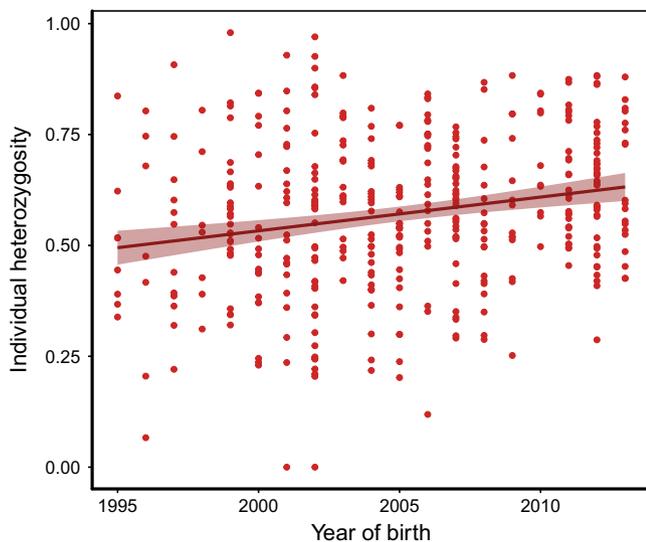
Figure 6. Temporal changes in the individual heterozygosity of Florida panthers born during the period 1995–2013 in South Florida, USA. Points are measurements, solid line is linear regression, shaded area is 95% confidence interval of slope. Slope = 0.006 ± 0.001, R^2 = 0.09.

followed by those for non-F1 admixed (0.62 ± 0.01) and canonical (0.39 ± 0.01) panthers. Individual heterozygosity positively affected survival rates of kittens (slope parameter: η = 1.455, 95% CI = 0.505–2.405). Individual heterozygosity also positively affected survival of subadult and adult panthers, but the evidence for this effect was weaker because the confidence interval for the hazard ratio overlapped 1.0 (hazard ratio, $\exp(\iota)$ = 0.311, 95% CI = 0.092–1.054; Table 4 and Fig. 7).

The MPC increased after genetic introgression (Fig. 2). This abundance index was also included in top-ranking models, indicating that population density affected survival (Table 4). Abundance reduced the survival of kittens (slope parameter: η = –0.035, 95% CI = –0.049 to –0.021); evidence was weak for the effect of abundance on survival of subadult and adult panthers (hazard ratio: $\exp(\iota)$ = 1.002, 95% CI = 0.995–1.010; Fig. 7). Regression coefficients associated with the effect of abundance and individual heterozygosity on demographic parameters are presented in Table B1 (available online in Supporting Information).

The most frequently observed causes of death for radio-collared panthers were vehicle collision and intraspecific aggression. Cause-specific mortality analyses revealed that mortality rates among possible causes were generally similar for the 2 sexes but that males were more likely to die from intraspecific aggression than were females (Fig. 8A). Male mortality from intraspecific aggression was higher for subadult and old adult panthers than for prime adults (Fig. 8B). For both males and females, canonical panthers were more likely to die from intraspecific aggression than were admixed panthers (Fig. 8C).

Reproductive Parameters

Of 101 radio-collared females, 67 reproduced at least once during our monitoring; we used these individuals to assess the annual probability of reproduction. The top-ranked model for the annual probability of reproduction included age effect only, suggesting that adding ancestry or abundance did not improve model parsimony (Table 4). Model-averaged annual probabilities of reproduction differed between female subadults (0.35 ± 0.08), prime adults (0.50 ± 0.05), and older adults (0.25 ± 0.06).

The most parsimonious model for the number of kittens produced annually by females that produced at least 1 litter (i.e., conditional on reproduction) included effects of age, ancestry, and abundance; a model that included only the effect of age was almost equally supported (Table 4). The model-averaged number of kittens produced annually, conditional on reproduction, was 2.80 ± 0.75 for subadults, 2.67 ± 0.43 for prime adults, and 2.28 ± 0.83 for old adults. Confidence intervals for the slope parameter (β) overlapped 0 for both abundance (β = 0.010, 95% CI = –0.001 to 0.021) and ancestry (β = –0.737, 95% CI = –1.637 to 0.162).

Population Dynamics and Persistence

Deterministic and stochastic demography.—The deterministic (λ) and stochastic (λ_s) annual population growth rate calculated using the matrix population model were 1.06 (5th and 95th percentiles: 0.99–1.14) and 1.04 (0.72–1.41), respectively. The generation time was 4.7 years. A female starting in age class one (kitten) was expected to live another 5.8 years and produce 10 kittens on average during her lifetime. Overall, λ_s was proportionately (elasticity) most sensitive to changes in female prime adult survival; on the absolute scale (sensitivity), however, it was most sensitive to changes in kitten survival (Fig. 9). Population trajectories, probabilities of quasi-extinction, and times until quasi-extinction estimated using the matrix population model and IBM for comparable scenarios were nearly identical for a critical quasi-extinction threshold of 10 panthers (Figs. 10 and 11) and for a critical quasi-extinction threshold of 30 panthers (Appendix E, available online in Supporting Information).

Minimum population count scenario.—Under the MPC scenario (i.e., density dependence estimated using the minimum population count data) with a critical threshold of 10 panthers, the population size reached 99 (72–104) and 100 (77–105) at 200 years (Fig. 10) as predicted by the IBM and the matrix model, respectively. The cumulative quasi-extinction probabilities within 100 years based on the MPC scenario were 1.5% (IBM; 0–4.8) and 3.0% (matrix model; 0–7.6). These probabilities increased to 2.5% (IBM; 0–11.4) and 3.8% (matrix model; 0–15.4) by year 200 (Fig. 10). The mean times until quasi-extinction were 15 years (IBM; 0–67) and 15 years (matrix model; 0–72) conditioned on going quasi-extinct within 100 years. Expected times until quasi-extinction conditioned on quasi-extinction within 200 years were higher, 34 years (IBM; 0–137) and 32 years (matrix model; 0–134; Fig. 10).

Motor vehicle mortality scenario.—Under the MVM scenario (i.e., density dependence estimated using estimates of panther population size from McClintock et al. 2015) with a critical threshold of 10 panthers, the projected population reached 188 (142–217) and 190 (143–219) at 200 years, as predicted by the IBM and the matrix model, respectively (Fig. 11). The cumulative quasi-extinction probabilities within 100 years were 1.4% (IBM; 0–0.8) and 1.3% (matrix model; 0–0.6). These probabilities increased to 2.0% (IBM; 0–1.7) and 1.9% (matrix

Table 4. Model comparison results testing for the effects of several covariates on survival of all kittens, survival of genetically sampled kittens, survival of subadult and adult Florida panthers, probability of reproduction, and kittens produced annually for Florida panthers sampled from 1981–2013 in South Florida, USA.

Model	Parameters	ΔAIC[a]	Weight
Kitten survival (all data)			
Base + F1[b] + abundance[c]	10	0.00	0.988
Base + abundance	9	10.18	0.006
Base + F1	9	10.35	0.005
Base	8	27.11	<0.001
Base + sex[d]	9	29.12	<0.001
Base + litter size[e]	9	29.14	<0.001
Kitten survival (genetically sampled kittens only)			
Base + F1 + abundance	10	0.00	0.541
Base + F1CanAdm[f] + abundance	11	0.99	0.329
Base + Het[g] + abundance	10	3.10	0.115
Base + CanAdm[h] + abundance	10	7.91	0.010
Base + abundance	9	9.44	0.005
Base + F1	9	16.38	<0.001
Base + F1CanAdm	10	18.37	<0.001
Base + Het	9	28.72	<0.001
Base	8	32.96	<0.001
Base + CanAdm	9	33.48	<0.001
Subadult and adult survival			
Base + F1CanAdm + abundance	7	0.00	0.360
Base + F1	5	0.53	0.276
Base + F1CanAdm	6	1.05	0.213
Base + F1 + abundance	6	2.22	0.119
Base + CanAdm + abundance	6	5.75	0.020
Base + CanAdm	5	9.08	0.004
Base + Het	5	9.64	0.003
Base + Het + abundance	6	9.84	0.003
Base	4	11.18	0.001
Base + abundance	5	12.78	0.001
Probability of reproduction			
Age	3	0.00	0.242
Age + CanAdm	4	0.12	0.228
Age + abundance	4	1.73	0.102
Age + F1	4	1.90	0.094
Age + CanAdm + abundance	5	2.16	0.082
Age + Het	4	2.19	0.081
Age + F1CanAdm	5	2.32	0.076
Age + F1 + abundance	5	3.72	0.038
Age + Het + abundance	5	3.99	0.033
Age + F1CanAdm + abundance	6	4.44	0.026
Kittens produced annually			
Age + F1 + abundance	5	0.00	0.194
Age	3	0.60	0.144
Age + abundance	4	0.61	0.143
Age + F1	4	0.97	0.120
Age + CanAdm	4	1.39	0.097
Age + F1CanAdm + abundance	6	1.96	0.073
Age + F1CanAdm	5	2.31	0.061
Age + CanAdm + abundance	5	2.38	0.059
Age + Het + abundance	5	2.50	0.056
Age + Het	4	2.59	0.053

[a] Akaike's information criterion (AIC) for kitten survival models was adjusted for small sample size and overdispersion (QAIC$_c$).
[b] F1 divides Florida panthers into 2 groups: F1 admixed and all other panthers.
[c] Index of Florida panther abundance.
[d] Sex of an individual Florida panther (male or female).
[e] Size of the litter in which a Florida panther kitten was born.
[f] F1CanAdm divides panthers into 3 groups: F1 admixed, canonical, and other admixed panthers.
[g] Het is the individual heterozygosity.
[h] CanAdm divides panthers into 2 groups: canonical and admixed (including F1 admixed) panthers.

model; 0–1.6) within year 200 (Fig. 11). The mean times until quasi-extinction based on the MVM scenario were 5 years (IBM; 0–61) and 6 years (matrix model; 0–64) conditioned on going quasi-extinct within 100 years. Expected times until quasi-extinction conditioned on quasi-extinction within 200 years were 11 years (IBM; 0–113) and 11 years (matrix model; 0–112; Fig. 11).

Sensitivity of extinction probability to demographic parameters.—The partial rank correlation coefficients between quasi-extinction probabilities and demographic parameters were negative; an increase in any of the demographic parameters decreased the quasi-extinction probability. Probabilities of quasi-extinction within 200 years were most sensitive to changes in kitten survival for both scenarios (Fig. 12), followed by survival of subadult females in the MVM scenarios and survival of prime adult females in the MPC scenario. The probability of reproduction of prime adult females had the third-largest partial rank correlation coefficient with quasi-extinction probability for both scenarios.

Benefits and Costs of Genetic Management

Minimum population count scenario.—The MPC scenario with a critical threshold of 10 panthers predicted that without management intervention, average individual heterozygosity would decrease, reaching 0.57 (0.49–0.64) at 100 years and 0.53 (0.42–0.62) at 200 years (Fig. 13). The population would decrease slightly, reaching an average of 79 (41–99) at 100 years and 76 (43–98) at 200 years (Fig. 14). The probabilities of quasi-extinction under the no-intervention, MPC scenario when we considered the effects of genetic erosion were 13% (0–99) at 100 years and 23% (0–100) at 200 years (Fig. 15).

When introgression was implemented every 10 years with the translocation of 5 Texas females, average individual heterozygosity under the MPC scenario increased and then stabilized at 0.68 (0.64–0.72) at 100 years and remained at that level at 200 years (Fig. 13). The population reached an average of 88 (62–104) at 100 years and stayed the same at 200 years (Fig. 14). The probabilities of quasi-extinction under this introgression strategy were 6% (0–53) within 100 years and 7% (0–82) within 200 years (Fig. 15). Results of analyses using a critical threshold of 30 panthers revealed a similar pattern of relative benefits. However, the probabilities of quasi-extinction were substantially higher (≥45%) across all scenarios (Appendix E).

Motor vehicle mortality scenario.—Without management intervention, the average individual heterozygosity predicted by the MVM scenario and a critical threshold of 10 panthers decreased to 0.60 (0.46–0.69) at 100 years and to 0.59 (0.39–0.70) at 200 years (Fig. 13). The population increased slightly and reached an equilibrium at 154 individuals (46–217) at 100 years and 157 (57–218) at 200 years (Fig. 14). The probabilities of quasi-extinction were 17% (0–100) at 100 years and 22% (0–100) at 200 years (Fig. 15).

When introgression was implemented every 10 years with 5 female pumas from Texas, average individual heterozygosity increased slightly, reaching 0.67 (0.60–0.73) at 100 years and 0.68 (0.59–0.75) at 200 years (Fig. 13). Under this strategy, the population reached an average of 164 individuals (44–226) at
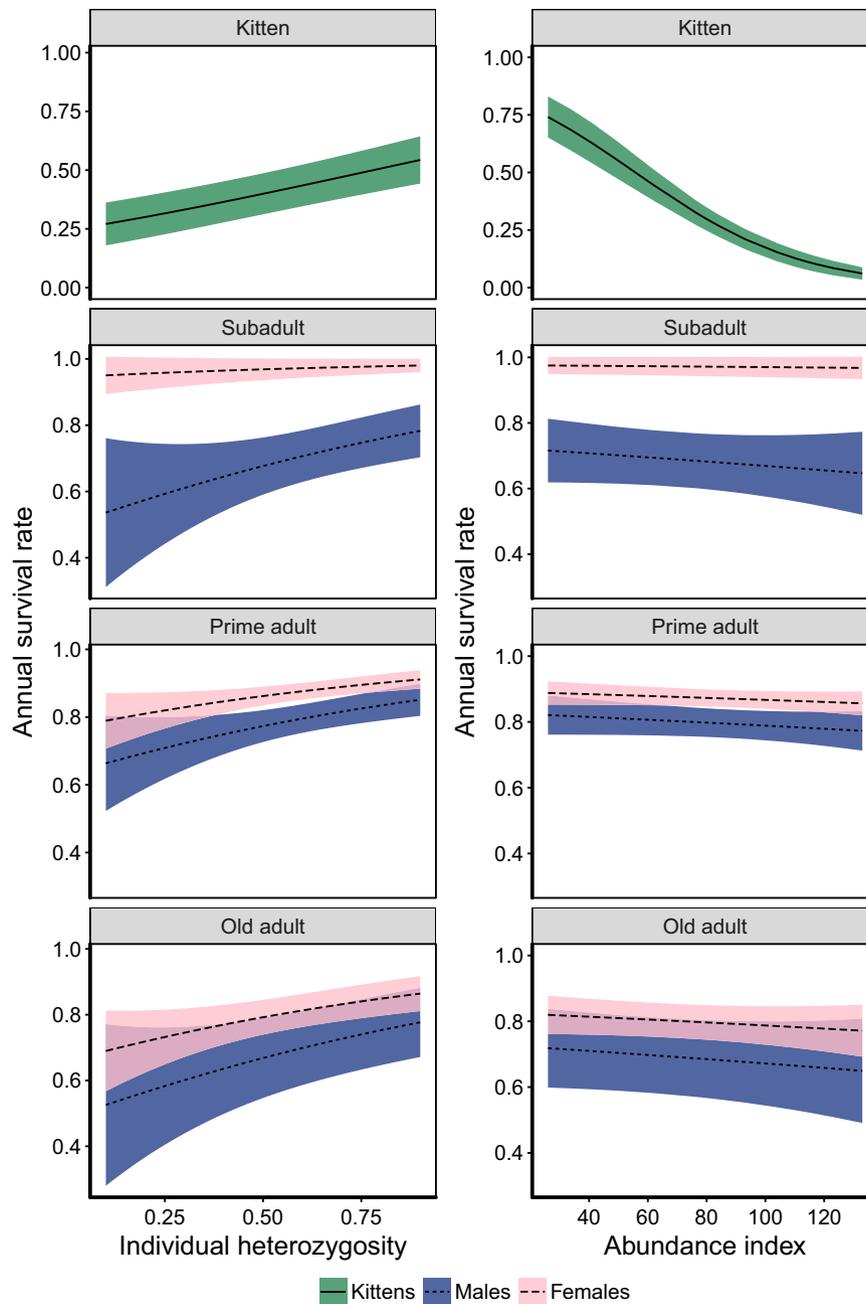
Figure 7. The effect of individual heterozygosity (left) and the abundance index (right) on Florida panther age- and sex-specific survival estimates (shaded area is SE) in South Florida, USA, 1981–2013. Abundance index data are from McBride et al. (2008) and R. T. McBride and C. McBride (Rancher's Supply Incorporated, unpublished report).

100 years and 170 (67–231) at 200 years based on the MVM scenario (Fig. 14). The probabilities of quasi-extinction under this introgression strategy were 10% (0–99) at 100 years and 12% (0–99) at 200 years (Fig. 15).

The projected population size and average individual heterozygosity reached equilibrium levels, with periodic fluctuations in these values when introgression was implemented (Figs. 16–17). Genetic introgression decreased the time until quasi-extinction across all scenarios (Fig. 18). Results of analyses using a critical threshold of 30 panthers revealed a similar pattern of relative benefits. However, the probabilities of quasi-extinction were substantially higher (≥45%) across all scenarios (Appendix E).

Addition of pumas from Texas increased both the population size and average individual heterozygosity; consequently, introgression caused periodic increases in average individual heterozygosity and population size at the interval at which the introgression occurred. Whereas average individual heterozygosity gradually decreased following introgression, density dependence caused the population size to fluctuate especially when a larger number of pumas from Texas were released. The positive effect of genetic introgression initiatives on quasi-extinction probabilities decreased as the time interval between these events increased. More frequent genetic introgressions led to greater increases in average individual heterozygosity and
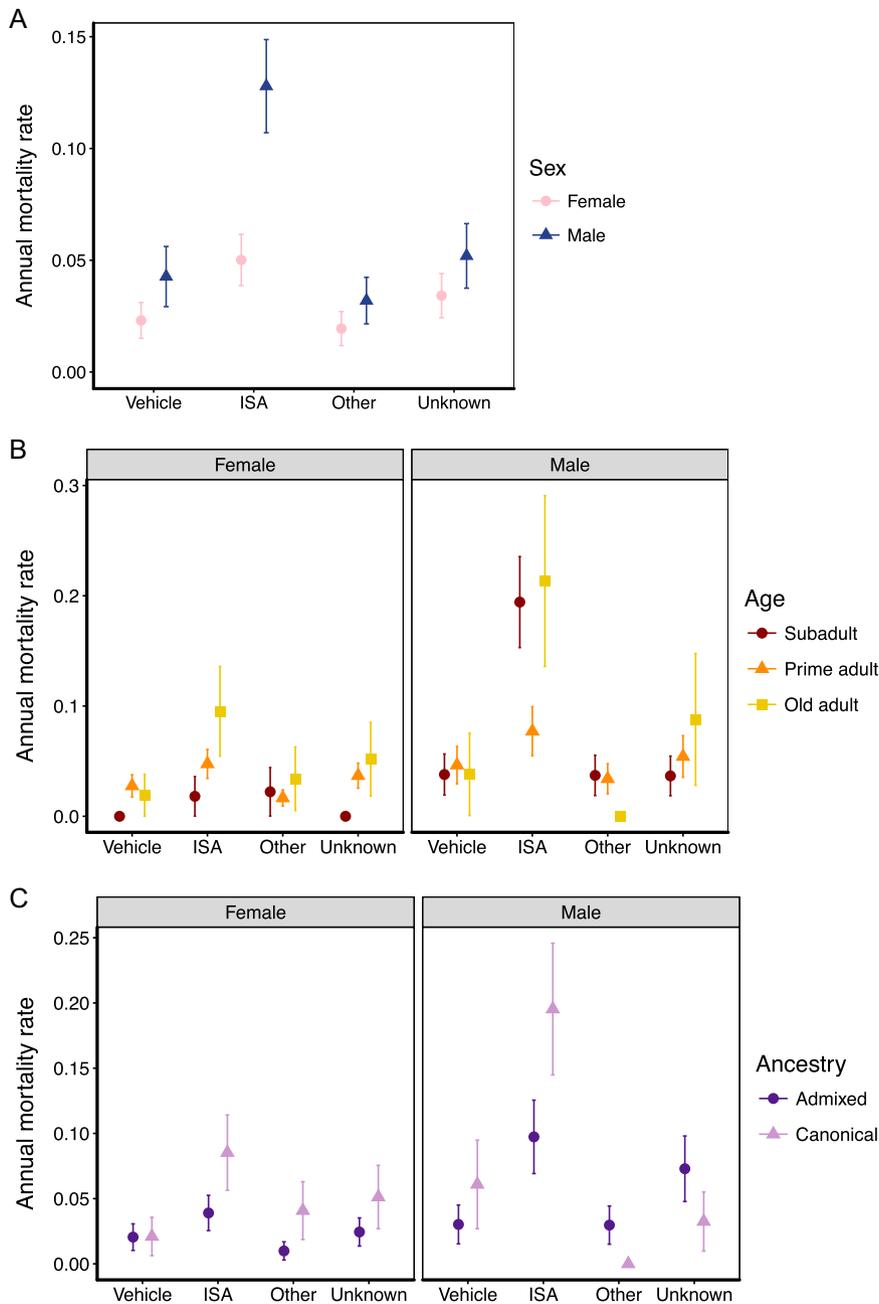
Figure 8. Cause-specific mortality rates (±SE) for male and female Florida panthers (A); subadult, prime-adult, and old-adult Florida panthers of both sexes (B); and canonical and admixed Florida panthers of both sexes (C) in South Florida, USA, 1981–2013. Causes of mortality are vehicle collision, intraspecific aggression (ISA), other causes (known causes such as diseases, injuries, and infections unrelated to the first 2 causes), and unknown cause (mortalities for which we could not assign a cause).

equilibrium population size while reducing the probability of quasi-extinction. Comparatively, performing genetic introgression less often but with more individuals per release was less effective at improving the long-term prospects for the panther population (Figs. 13–15).

Financial cost and persistence benefits.—The total estimated cost of 1 genetic introgression was $40,000, $80,000, or $120,000, for releasing 5, 10, or 15 pumas from Texas, respectively (Table 5). The total cost incurred over 100 years for each strategy ranged from $50,000 to $1.2 million (Table 6). The most expensive strategy involved the introduction of 15 pumas every 10 years; this strategy reduced the probability

of quasi-extinction by 58% and 40% under the MPC and MVM scenarios, respectively (Table 6). Introducing 5 pumas every 80 years cost the least, but improvements in population persistence under this strategy were insubstantial (Table 6). Releasing more panthers more frequently caused increased population fluctuations but did not necessarily reduce the quasi-extinction probability (Figs. 14 and 15; Table 6).

DISCUSSION

The duration (34 yr of data) and sample sizes associated with the Florida Panther Project allowed us to obtain a comprehensive understanding of genetic variation, demographic parameters,
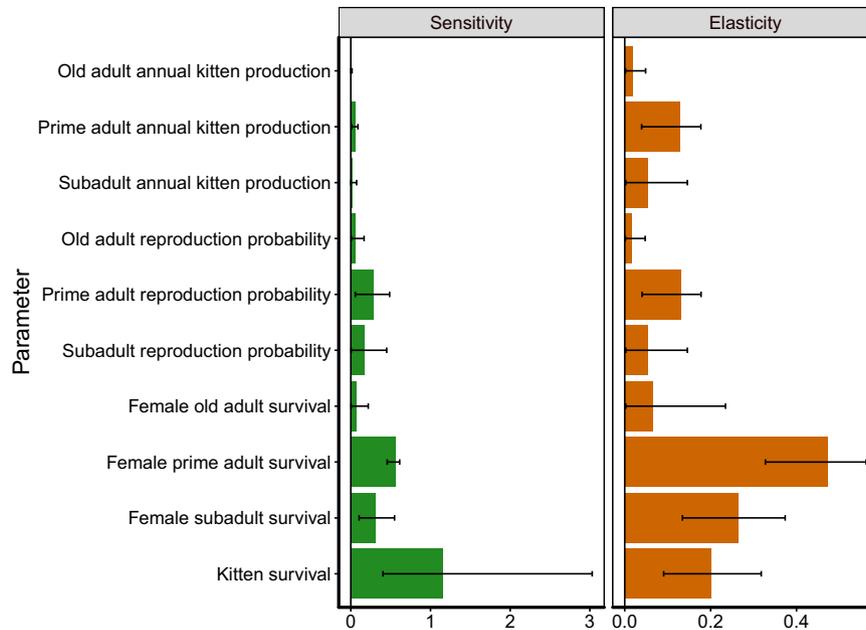
Figure 9. Sensitivity and elasticity (proportional sensitivity) of stochastic population growth rate (λ_s) of the Florida panther to vital demographic parameters in South Florida, USA, 1981–2013. Horizontal lines represent 5th and 95th percentiles.

probability of population persistence, and the duration of the positive impacts of genetic restoration on this endangered population. The Florida panther population was in dire straits in the early 1990s, and our results clearly demonstrated that the implementation of the introgression project in 1995 subsequently accrued a series of genetic and demographic improvements that have led to the change from a declining population to one that is growing and expanding its current breeding range.
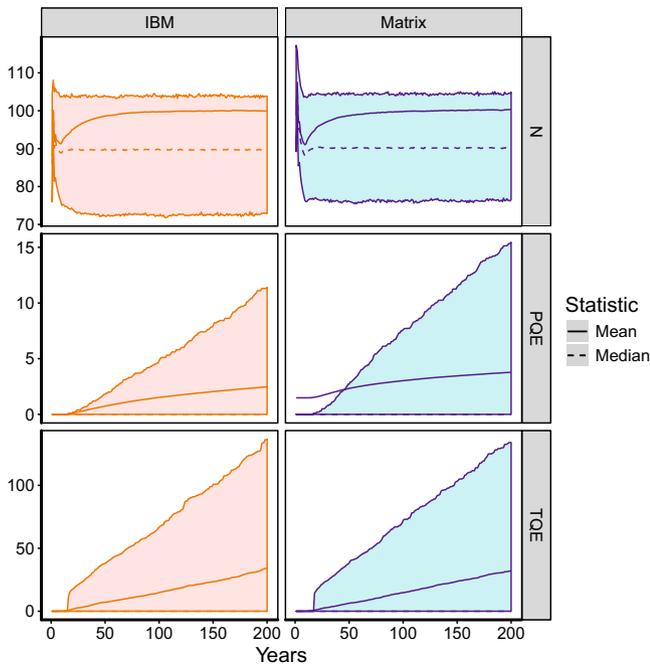
Figure 10. Mean (solid lines) and median (dashed lines) Florida panther probabilities (%) of quasi-extinction (PQE), times in years until quasi-extinction (TQE), and population trajectories (N) under the minimum population count (MPC) scenario as predicted by the individual-based model (IBM) and matrix population model. The critical threshold was 10 panthers. Shaded area indicates 5th and 95th percentiles. Based on data collected in South Florida, USA, 1981–2013.
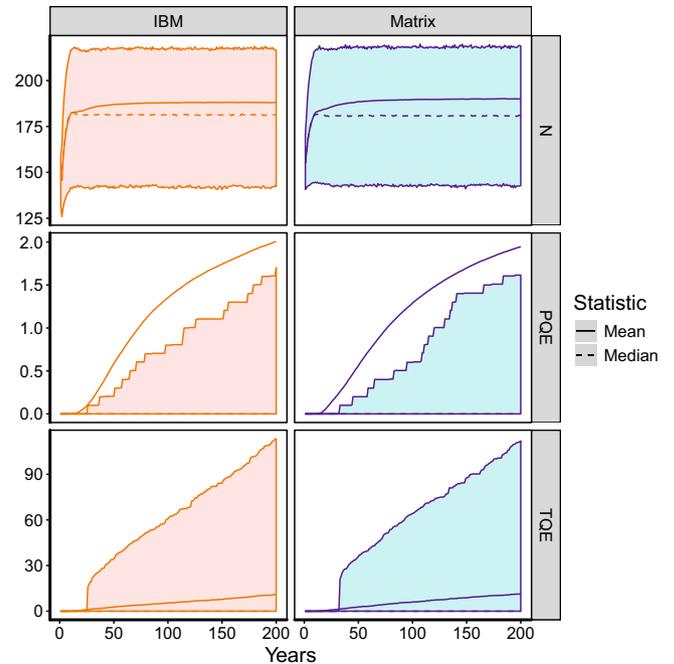
Figure 11. Mean (solid lines) and median (dashed lines) Florida panther probabilities (%) of quasi-extinction (PQE), times in years until quasi-extinction (TQE), and population trajectories (N) under the motor vehicle mortality (MVM) scenario as predicted by the individual-based model (IBM) and matrix population model. The critical threshold was 10 panthers. Shaded area indicates 5th and 95th percentiles. Based on data collected in South Florida, USA, 1981–2013.
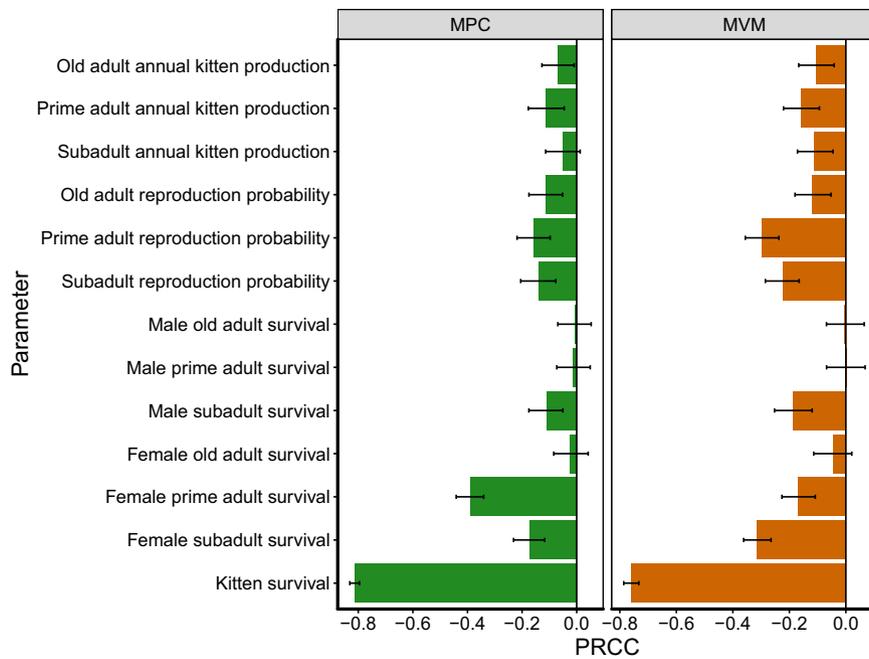
Figure 12. Partial rank correlation coefficient (PRCC) between quasi-extinction probabilities and the demographic parameters of the Florida panther for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Error bars indicate standard deviation. Based on data collected in South Florida, USA, 1981–2013.
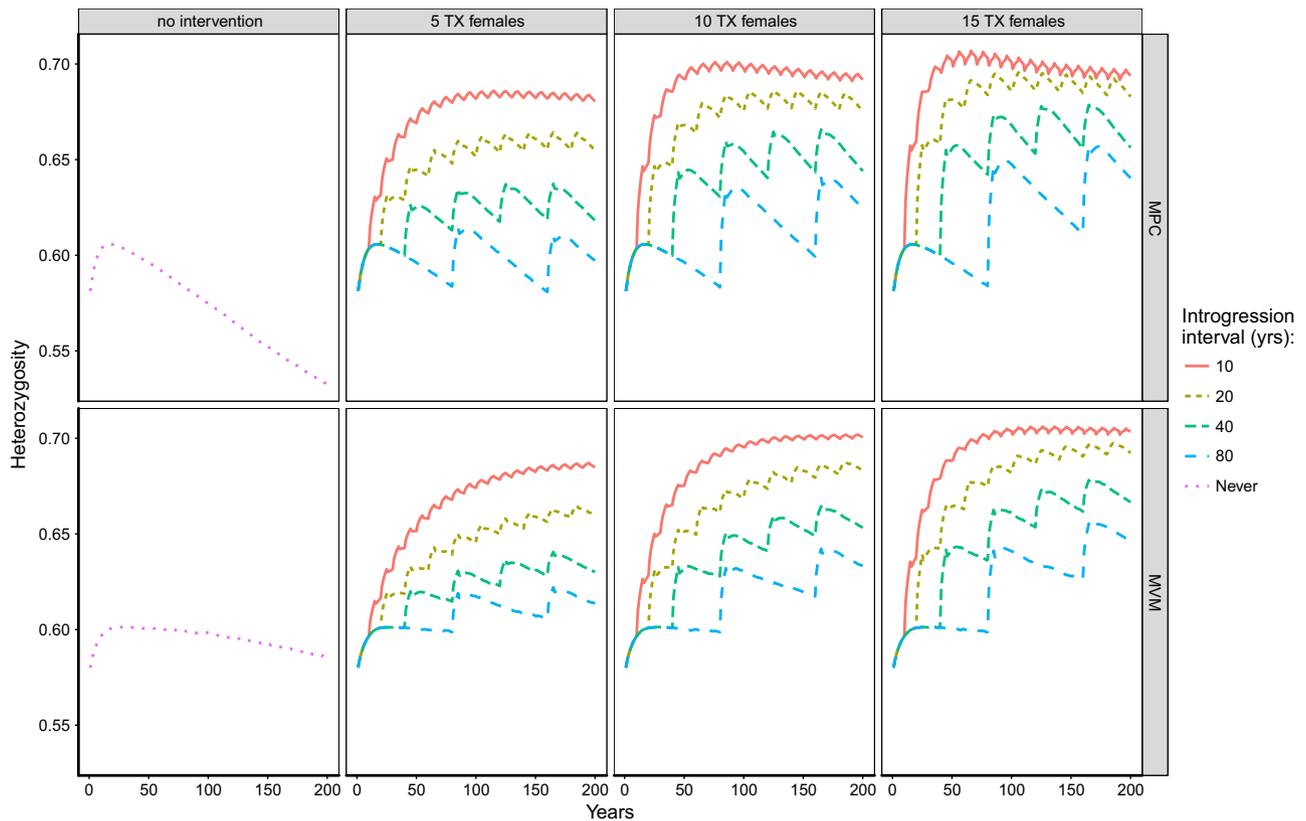
Figure 13. Average projected individual heterozygosities in the Florida panther population over 200 years without genetic management intervention and with each of the genetic introgression strategies for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Introgression strategies include the introduction of 5, 10, or 15 pumas from Texas, USA, every 10, 20, 40, or 80 years. Average individual heterozygosity peaks when pumas are added to the Florida panther population. Based on data collected in South Florida, USA, 1981–2013.
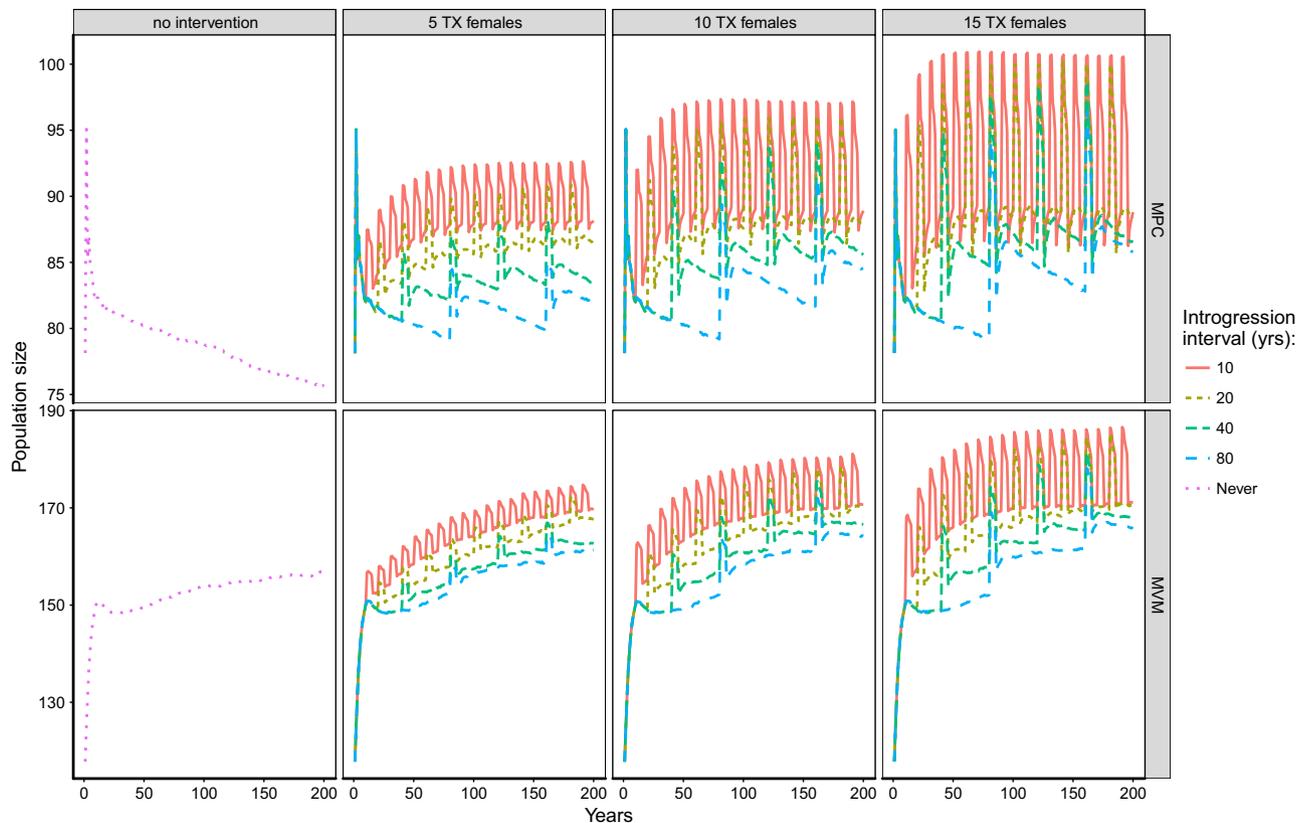
Figure 14. Average projected population sizes in the Florida panther population over 200 years without intervention and with each of the genetic introgression strategies for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Introgression strategies include the introduction of 5, 10, or 15 pumas from Texas, USA, every 10, 20, 40, or 80 years. Peaks occur when pumas are added to the Florida panther population. Based on data collected in South Florida, USA, 1981–2013.

Our research has further validated the success of genetic introgression by quantifying the reduction in the probability of extinction of the panther population because of this management initiative. These findings all bode well for continued progress toward recovery. That said, the Florida panther population remains small and isolated from other populations of puma from western North America, thereby denoting that the eventual impact of genetic drift and inbreeding may negatively affect the population in the future. Realizing this will continue to be an issue that managers will have to contemplate, we modeled the impact of varied genetic management scenarios to determine the frequency of introgression along with the number of panthers that should be released during each initiative to minimize cost and maximize benefits to the population. These results will prove invaluable to managers attempting to conserve the Florida panther.

Genetic Variation, Demographic Parameters, and Persistence of Heterotic Benefits from Genetic Introgression

Our measure of individual heterozygosity (1 – HL) was higher on average for the admixed (0.615) panthers than for those of canonical ancestry (0.386). Levels of individual heterozygosity for admixed panthers were comparable to levels observed in a sample of pumas from west Texas ($\bar{x} \pm$ SE $= 0.695 \pm 0.019$, $n = 41$), which further demonstrates the improvement in levels of genetic variation in the Florida population since 1995. The

correlation between HL and several demographic parameters has been noted in wild populations including cheetahs (Terrell et al. 2016) and several species of birds such as European shags (*Phalacrocorax aristotelis*, male and female survival probability; Velando et al. 2015) and Egyptian vulture (*Neophron percnopterus*, age at recruitment; Agudo et al. 2012). If we focus only on panthers captured and radiocollared from 2012–2015 (FP211–FP240), all of which had estimated birth years ≥2005 (≥10 yr post-introgression), those panthers had an average individual heterozygosity level of 0.618 ± 0.029, highlighting the elevated levels of genetic variation that continue to persist in cohorts of panthers 5 generations after the release of female pumas from Texas.

The proportional membership values (q) from our STRUCTURE analysis allowed us to delineate 2 clusters of ancestry for Florida panthers (Fig. 4). During the pre-introgression era, the population was dominated by panthers that retained a high percentage of the canonical ancestry, which was affected by inbreeding depression. The post-introgression era ancestry values are comprised of many admixed individuals, in essence demonstrating the success of the introgression in terms of increasing genetic variation in the population and mimicking gene flow that historically occurred with panthers and other populations of pumas (Onorato et al. 2010). A somewhat analogous situation, although abetted by natural immigration, was evident in the ancestral variation in a small population of puma intersected by a major freeway in California, USA. In
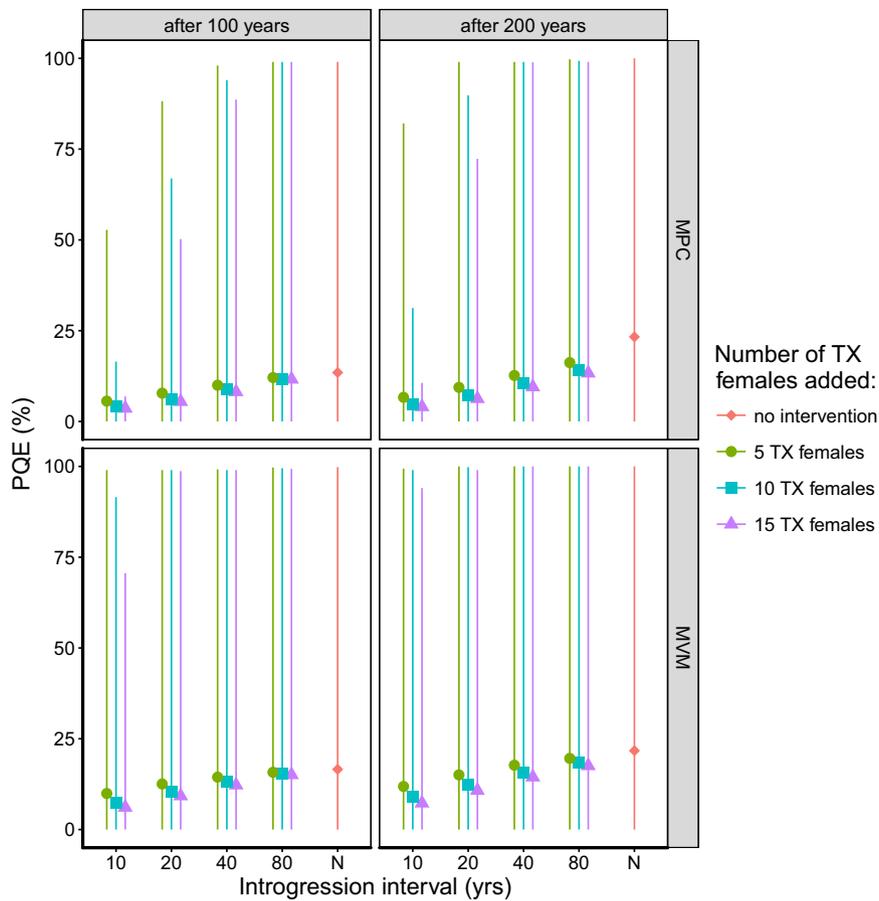
Figure 15. Average probability of quasi-extinction (PQE) of the Florida panther population within 100 years and within 200 years without genetic management intervention (N), and with each of the genetic introgression strategies for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Introgression strategies include the introduction of 5, 10, or 15 pumas from Texas, USA, every 10, 20, 40, or 80 years. The critical threshold was 10 panthers. Error bars indicate 5th and 95th percentiles. Based on data collected in South Florida, USA, 1981–2013.
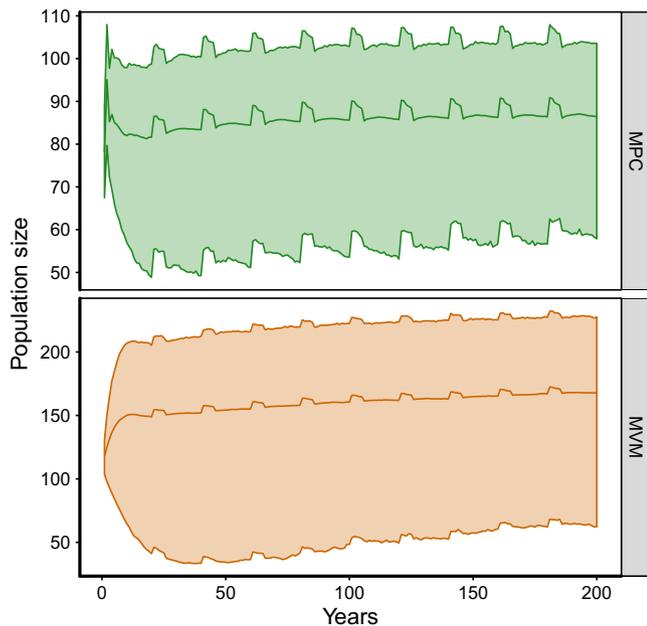
Figure 16. Average projected Florida panther population trajectories under a genetic introgression strategy involving the release of 5 female pumas from Texas, USA, every 20 years for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Shaded area indicates 5th and 95th percentiles and is representative of confidence intervals for other scenarios. Based on data collected in South Florida, USA, 1981–2013.
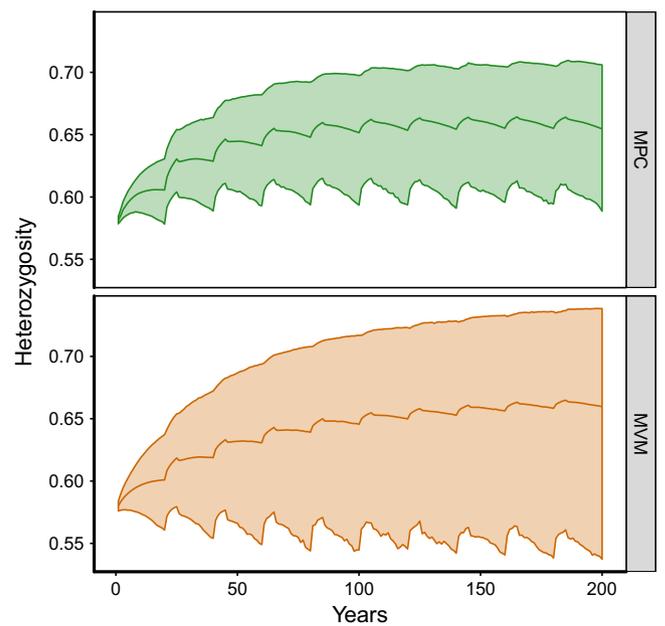
Figure 17. Average projected Florida panther population-level heterozygosities under a genetic introgression strategy involving the release of 5 female pumas from Texas, USA, every 20 years, for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Shaded area bounds the 5th and 95th percentiles and is representative of confidence intervals for other scenarios. Based on data collected in South Florida, USA, 1981–2013.
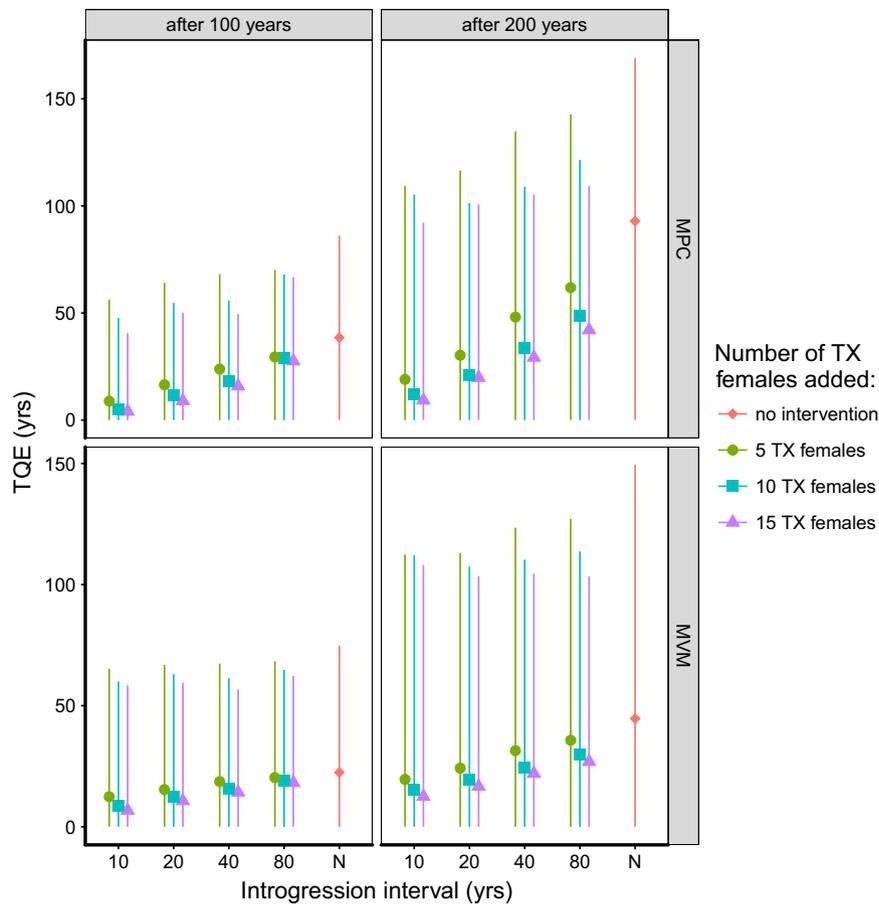
Figure 18. Average times until quasi-extinction (TQE) for the Florida panther population within 100 years and within 200 years without genetic management intervention and with each of the genetic introgression strategies for the minimum population count (MPC) and motor vehicle mortality (MVM) scenarios. Introgression strategies include the introduction of 5, 10, or 15 pumas from Texas, USA, every 10, 20, 40, or 80 years. The critical threshold was 10 panthers. Error bars indicate 5th and 95th percentiles. Based on data collected in South Florida, USA, 1981–2013.

Table 5. Average cost (2017 U.S. dollars) of introgression strategies employed over 100 years that involve the introduction of 5, 10, or 15 pumas from Texas, USA, into the Florida panther population in South Florida, USA, at an increasingly higher frequency. Costs of capture (including transportation), quarantine, and post-introgression monitoring were estimated by Roy McBride, Mark Cunningham, and Dave Onorato, respectively.

Frequency	Component	5 pumas	10 pumas	15 pumas
Once	Capture	25,000	50,000	75,000
	Quarantine	5,000	10,000	15,000
	Monitoring	10,000	20,000	30,000
	Total	40,000	80,000	120,000
Every 80 years	Capture	31,250	62,500	93,750
	Quarantine	6,250	12,500	18,750
	Monitoring	12,500	25,000	37,500
	Total	50,000	100,000	150,000
Every 40 years	Capture	62,500	125,000	187,500
	Quarantine	12,500	25,000	37,500
	Monitoring	25,000	50,000	75,000
	Total	100,000	200,000	300,000
Every 20 years	Capture	125,000	250,000	375,000
	Quarantine	25,000	50,000	75,000
	Monitoring	50,000	100,000	150,000
	Total	200,000	400,000	600,000
Every 10 years	Capture	250,000	500,000	750,000
	Quarantine	50,000	100,000	150,000
	Monitoring	100,000	200,000	300,000
	Total	400,000	800,000	1,200,000

this case, the migration of just a single male across the freeway to the south resulted in an increase in the number of individuals with admixed ancestry and substantially improved genetic diversity of the subpopulation south of the freeway (Riley et al. 2014).

Our estimate of overall kitten survival (0.321 ± 0.056) was similar to that reported by Hostetler et al. (2010), who used 13 years of post-introgression data (0.323 ± 0.071). These values are lower than kitten survival estimates derived from several puma populations in western North America (0.44–0.91; Logan and Sweanor 2001, Lambert et al. 2006, Laundre et al. 2007, Robinson et al. 2008, Ruth et al. 2011). When we included only data for individuals that had been genetically sampled, our estimate was 15% higher. The proportion of admixed kittens that were genetically sampled increased as the population expanded, which could have resulted in higher estimates of kitten survival because admixed kittens survive better than canonical kittens. Kitten survival was strongly influenced by genetic ancestry, with survival rates of F1 kittens being almost twice that of canonical kittens (Fig. 5B). Consistent with the findings of Hostetler et al. (2010), increases in heterozygosity coincided with increased kitten survival, whereas population density negatively affected kitten survival. Population density often has a strong negative impact on survival of young age classes due to infanticide and cannibalism, which has been reported from several carnivore

Table 6. Costs and benefits accumulated over 100 years for genetic introgression at different intervals and with different numbers of pumas from Texas, USA, introduced into the Florida panther population in South Florida, USA. Costs (2017 U.S. dollars) include capture and transportation, quarantine, and post-introgression monitoring. Benefits are represented as average probability of quasi-extinction (PQE, and 5th and 95th percentiles) and changes (Δ) therein under the scenario using the minimum population count (McBride et al. 2008; MPC) and the scenario using the motor vehicle mortality population estimates (McClintock et al. 2015; MVM). The table is sorted by percent change in probability of quasi-extinction under the MPC scenario.

Interval (yr)	Pumas	Cost	MPC PQE (%)	ΔMPC PQE (%)	MVM PQE (%)	ΔMVM PQE (%)
–	0	0	13 (0–99)	0	17 (0–100)	0
80	10	100,000	12 (0–99)	10 (0–58)	16 (0–100)	5 (0–38)
80	15	150,000	12 (0–99)	13 (0–65)	15 (0–100)	8 (0–56)
80	5	50,000	12 (0–99)	14 (0–73)	15 (0–99)	9 (0–41)
40	15	300,000	10 (0–98)	26 (0–104)	14 (0–99)	13 (0–60)
40	10	200,000	9 (0–94)	35 (0–183)	13 (0–99)	21 (0–95)
40	5	100,000	8 (0–89)	39 (0–211)	12 (0–99)	26 (0–124)
20	15	600,000	8 (0–88)	42 (0–257)	13 (0–99)	24 (0–123)
20	10	400,000	6 (0–67)	54 (0–318)	10 (0–99)	37 (0–217)
10	15	1,200,000	6 (0–53)	58 (0–372)	10 (0–99)	40 (0–208)
20	5	200,000	5 (0–50)	59 (0–338)	9 (0–99)	44 (0–352)
10	10	800,000	4 (0–16)	69 (0–489)	8 (0–92)	55 (0–401)
10	5	400,000	4 (0–7)	73 (0–502)	6 (0–71)	63 (0–503)

species, including polar bears (*Ursus maritimus*; Derocher and Wiig 1999), black bears (*Ursus americanus*; Garrison et al. 2007), and pumas (Logan and Sweanor 2001).

Our estimates of sex- and age-specific survival rates of subadult and adult panthers (Table 3) and the effects of covariates on these rates were similar to those reported by Benson et al. (2011) derived from data collected through 2006. Our survival rates for males (0.65–0.77) were lower than females (0.78–0.97) for all 3 age classes (subadult, prime adult, and old adult), which is the typical trend observed in most puma populations (e.g., Ruth et al. 1998, 2011). However, an unhunted puma population occupying a fragmented urban landscape in southern California exhibited lower survival (0.586 females, 0.525 males; Vickers et al. 2015), perhaps as a result of severe habitat fragmentation and the lack of extensive swaths of public land protected in perpetuity. Most populations of puma in western North America are typically subject to varied levels of management via regulated hunting, which often is biased toward the take of males (Cooley et al. 2009, Ruth et al. 2011). Consequently, annual survival estimates from hunted populations in northeast Washington (0.679–0.733 females, 0.341 males; Robinson et al. 2008) and southeastern Arizona (0.685 females, 0.584 males; Cunningham et al. 2001) were generally lower than those we estimated for Florida panthers.

Cause-specific mortality analysis showed that male panthers experienced a substantially greater mortality risk from intraspecific aggression. This differs from the pattern observed during a long-term study in Arizona where females were at a higher risk of intraspecific mortality than males (46% of male and 53% of female mortality; Logan and Sweanor 2001). Mortality associated with intraspecific strife has been documented in hunted and unhunted populations (this study; Logan and Sweanor 2001, Stoner et al. 2010) and its root cause has been difficult to determine (e.g., population density and prey population trends). That being the case, finding ways to reduce what is often a major source of mortality for puma populations continues to pose a challenge to managers.

Canonical panthers were substantially more likely to die from intraspecific aggression than were admixed panthers. This may at least partly explain the difference in survival between admixed and canonical panthers. Canonical panthers are known to be more likely to suffer from varied correlates of inbreeding depression than admixed animals, including atrial septal defects (Johnson et al. 2010) and compromised immune systems (Roelke et al. 1993). These factors have the potential to affect fitness and perhaps dominance of individuals when engaged in an intraspecific encounter. A somewhat similar scenario was observed by Hogg et al. (2006) in a population of bighorn sheep that were part of an introgression project. Admixed males in this population had a greater probability of paternity than males that were less outbred. This included coursing males with higher levels of admixture being markedly more successful at fighting males that were defending females in estrous (Hogg et al. 2006).

Heterosis resulting from genetic introgression is expected to be the strongest in F1 individuals (Shull 1908, Crow 1948, Burke and Arnold 2001, Waller 2015), and to progressively decline in subsequent generations (Pickup et al. 2013, Frankham 2015). Given that admixed (especially F1) panthers survived better than canonical panthers, and that individual heterozygosity improved survival of both sexes and all age classes, our data provide evidence for heterotic advantage, whereby individuals of mixed ancestry had higher fitness (Shull 1908, Crow 1948). Our results are consistent with findings of other studies that involved intentional genetic introgression for conservation purposes. For example, Hogg et al. (2006) detected substantial improvements in survival and reproduction of introgressed bighorn sheep, and Madsen et al. (1999, 2004) reported a dramatic increase in the population of adders after genetic introgression.

Knowledge of the persistence of benefits to a population accrued via genetic introgression is essential for determining when additional introgression may be warranted. There is a depauperate amount of information regarding this topic and the identification of factors that may influence persistence of the benefits of genetic introgression (Tallmon et al. 2004, Hedrick et al. 2014, Whiteley et al. 2015). For example, in minks (*Neovison vison*), Thirstrup et al. (2014) found that outcrossing led to larger litters in F2 and F3, but this benefit disappeared in subsequent generations. In a population of gray wolves, Hedrick et al. (2014) suggested that benefits of genetic introgression may

wane after 2 or 3 generations. The Wright–Fisher model of genetic drift predicts a geometric decline in expected heterozygosity at the rate of $(1 - 1/2N_e)$ per generation, where N_e is the effective population size (Hartl and Clark 1997, Frankham et al. 2014). Thus, it seems logical to assume that the duration of the benefits of introgression is limited, especially in a species persisting in a small population such as Florida panthers.

We expected Florida panther survival in the most recent years (2005–2013) post-introgression to differ from that observed in the decade following the introgression in 1995 because panther abundance and heterozygosity, factors known to influence panther survival (Hostetler et al. 2010, Benson et al. 2011), have changed (Figs. 2 and 6). We found that subadult and adult survival rates in recent years (2005–2013) were similar to those in the period immediately following introgression (1995–2004; Fig. 5C); overall kitten survival was similar to estimates of Hostetler et al. (2010). In fact, the pattern of covariate effects on Florida panther survival reported by Benson et al. (2011) and Hostetler et al. (2010) has barely changed. The negative effects of population density and positive effects of heterozygosity may counterbalance survival rates, reducing population fluctuations. Our result that benefits of genetic restoration have remained in the population nearly for 5 generations post-intervention was surprising but also encouraging. Possible explanations for this observation may include 1) genetic erosion occurs at slower rate than previously thought, 2) introducing 5 migrants in 1 genetic intervention may have beneficial effects similar to those from 1 migrant per generation over multiple generations, or 3) other and as yet unknown factors may reduce the rate of genetic erosion and subsequent demographic effects. A density-dependent effect on kitten survival could also explain why non-F1 admixed kittens did not survive substantially better than did canonical kittens; most kittens sampled from 2005–2013 were admixed, and their survival was lower possibly because of a density-dependent effect as the Florida panther population continuously grew during that period. Trinkel et al. (2010) also found that population density and inbreeding coefficient interacted to affect demographic parameters and growth rate of an African lion population. Likewise, population density interacted with winter weather to affect the survival and population growth rates in Soay sheep (*Ovis aries*; Milner et al. 1999, Coulson et al. 2001).

Population Dynamics and Persistence

The finite deterministic and stochastic growth rates were >1.0, reflecting that much of the data used in this study were collected following genetic introgression in 1995, during which time the population increased substantially. Because our demographic parameter estimates did not change markedly in comparison to those of Hostetler et al. (2013), the similarity between the population growth estimates from these studies was expected. But these estimates reflect population growth during the data-collection period and do not predict future growth. In fact, estimates of population size reported by McClintock et al. (2015) suggest that population growth may already be slowing (Fig. 2). A similar pattern was observed in an introduced population of African lion, which increased steadily from 1995 until 2001, then fluctuated around the presumed carrying capacity thereafter (Trinkel et al. 2010). Several other African lion populations that experienced various degrees of recovery

subsequently became relatively stable or exhibited declining trends (Bauer et al. 2015).

Our estimates of quasi-extinction probabilities were lower than those reported by Hostetler et al. (2013) using a 2-sex, age-structured matrix population model. Lower probabilities of quasi-extinction than those reported by the earlier study for comparable scenarios were likely a consequence of the fact that the estimates of abundance (McBride et al. 2008), and thus the density-dependent effects on survival of kittens and old panthers (>10 yr), have changed in recent years. Additionally, these early estimates of the probability of quasi-extinction and of time to quasi-extinction did not consider genetic erosion that will inevitably occur without management intervention.

Under the MVM scenario, the equilibrium population size was twice that attained under the MPC scenario. The MVM population estimates are approximately twice the MPCs (Fig. 2); as a result, the simulated population under the MVM scenario achieved a higher equilibrium size. Probability of quasi-extinction under the MVM scenario was generally greater than that under the MPC scenario. This result is a consequence of the fact that the estimated density dependence on kitten survival based on the MPC scenario is stronger than that for the MVM scenario (regression coefficients for abundance for the MPC scenario = –0.068 vs. –0.002 for the MVM scenario; Appendix B; Table B1). Consequently, simulated populations under the MPC scenario have a stronger tendency to move toward the equilibrium population size, which inherently reduces the quasi-extinction probability (e.g., Royama 1992).

As expected for long-lived mammals (Heppell et al. 2000, Oli and Dobson 2003), the population growth rate was proportionately most sensitive to changes in survival of prime adults, followed by that in younger age classes. Global sensitivity analysis, in contrast, showed that under all scenarios, extinction parameters were particularly sensitive to changes in survival of Florida panther kittens. This result is a consequence of the high sensitivity of the population growth rate to kitten survival (Fig. 9) and a larger standard error of kitten survival (Table 3). Results of our sensitivity analyses indicate that future research should focus on acquiring additional information on early life stages to improve the accuracy and precision of estimates of kitten survival. Our results suggest that demographic variables (or their environmental drivers) that strongly influence extinction risks are not necessarily those with the largest elasticity values. A study of island fox (Bakker et al. 2009) found that extinction risk was strongly influenced by survival modifier parameters that had low elasticity values.

Genetic Management: When and How to Intervene?

The use of genetic introgression as a management tool has always been controversial, and the probable effectiveness it might have in preventing the imminent demise of the panther population was initially debated (Creel 2006, Maehr et al. 2006, Pimm et al. 2006). These debates notwithstanding, the panther population had suffered from genetic, morphological, and biomedical correlates of inbreeding before this management intervention (Johnson et al. 2010, Onorato et al. 2010), whereas the post-introgression period has been characterized by a substantial reduction in the biomedical correlates of inbreeding and improvements in demographic vigor and abundance (McBride

et al. 2008; Hostetler et al. 2010, 2013; Johnson et al. 2010; Benson et al. 2011; McClintock et al. 2015). Nevertheless, the population remains small and isolated, habitat is still being lost, and there is no current possibility of natural gene flow between this and other North American puma populations; thus, the recurrence of inbreeding-related problems and subsequent population declines are inevitable. The question, therefore, is not whether genetic management of the Florida panther population is needed, but when and how it should be implemented.

Without genetic introgression, probabilities of quasi-extinction were substantially greater than those from models that incorporated periodic genetic introgression events (Fig. 15), highlighting the importance of genetic management in small, isolated populations. When 5 female pumas are added to the Florida panther population every 10 years, genetic variation increased substantially under the MPC and MVM scenarios. The IBM predicted that the equilibrium population size would be substantially larger when 5 pumas were released into the population every 10 years than populations without intervention (i.e., no introgression). Releasing 15 Texas females did not afford substantially greater benefit to the equilibrium population size than did releasing only 5 Texas females. Instead, adding more individuals caused increasingly large fluctuations in population size due to the numerical increases and density dependence (Fig. 14), possibly diminishing the benefits associated with increased genetic variation.

Compared with the no-intervention scenario (i.e., no genetic introgression implemented), the introduction of 5 female pumas every 10 years reduced quasi-extinction probabilities from 13% to 4% and from 17% to 6% for the MPC and MVM scenarios, respectively. The benefit of genetic-introgression strategies decreased as the interval between successive management interventions increased, and no benefit was evident once the interval reached 80 years (Fig. 15). Introgression reduced the average time until quasi-extinction (Fig. 18). This somewhat counterintuitive result can be explained by the fact that repeated genetic introgression makes the population more robust over time, which will reduce the probability of extinction. Consequently, few simulated population trajectories that fall below the critical threshold do so early, reducing the mean time to extinction. Also, density dependence has a stabilizing effect on populations (Royama 1992); populations tend toward equilibrium population sizes, which reduces the probability over time of populations falling below quasi-extinction threshold. However, time until quasi-extinction must be interpreted in conjunction with the probability of quasi-extinction, which is very low for all scenarios with genetic introgression (Fig. 15).

Cost is a significant impediment to the implementation of a genetic introgression program because captures, relocations, and monitoring efforts before and after introgression are expensive (Edmands 2007; Frankham 2010b, 2015, 2016). Costs may be acceptable to society if the management actions result in substantial fitness benefits, especially if the species of concern is popular and charismatic (Frankham 2015). We estimated the 100-year cost of introgression strategies modeled here to range from $50,000 to $1.2 million. More expensive strategies generally led to larger decreases in the probability of quasi-extinction, but cheaper strategies also substantially improved

population viability (i.e., reduced quasi-extinction probability; Table 6). One of the cheaper strategies (5 pumas released every 20 years), which cost an estimated $200,000 over 100 years, reduced the probability of quasi-extinction by 59%, and 44% for the MPC, and MVM scenarios, respectively. But these preliminary cost estimates are based on expenses incurred during the 1995 introgression and include costs of capture, quarantine, transportation, release, and post-release monitoring of females only. Although the cost for future genetic interventions would likely be higher than those reported here, the relative differences in cost between alternative intervention strategies should remain approximately the same.

Our ability to simulate genetics and link it to the viability of the Florida panther population is based on the empirically estimated relationship between individual heterozygosity and survival. Such heterozygosity and fitness correlations have been studied for decades (David 1998) but have only recently been used to predict population viability (Benson et al. 2016); none, to our knowledge, have incorporated cost into their modeling efforts. The mechanisms underlying heterozygosity and fitness correlations remain unclear (David 1998, Szulkin et al. 2010), and their significance as a proxy for inbreeding depression has been debated (Balloux et al. 2004, Miller and Coltman 2014). Nevertheless, evidence continues to mount demonstrating positive relationships between heterozygosity and survival (Coulson et al. 1998a,b; Hansson et al. 2004; Silva et al. 2005; Acevedo-Whitehouse et al. 2006; Jensen et al. 2007; Velando et al. 2015) and between heterozygosity and fecundity (Seddon et al. 2004, Charpentier et al. 2005, Agudo et al. 2012, Velando et al. 2015). Although the reported correlations are generally weak, their consequences can be substantial if population growth and persistence parameters are highly sensitive to the affected vital rates (Velando et al. 2015). Compared with scenarios that ignore genetics, incorporating the effects of inbreeding on survival increased quasi-extinction probabilities within a 100-year timeframe from 1.5% to 13% and from 1.4% to 17% for the MPC, and MVM scenarios, respectively. These results highlight the importance of incorporating genetics when analyzing the viability of small, isolated populations.

Although conservation biologists have recognized the importance of genetics in population viability, many still fail to incorporate genetic factors into PVA models (Reed et al. 2002). Explicit consideration of genetics in PVAs requires long-term genetic and demographic data. This permits the assessment of the functional relationship between genetic variability and demographic parameters. Population viability analysis software packages such as VORTEX (Lacy 1993, 2000) offer a powerful framework for individual-based simulations, but they often do not adequately capture a species' life history or genetics. Based on a thorough review of the importance of genetic factors in wildlife conservation, Frankham et al. (2014) concluded that genetic factors can strongly influence the dynamics and persistence of small and/or fragmented populations. They further noted that "Most PVA-based risk assessments ignore or inadequately model genetic factors," and recommended that "PVA should routinely include realistic inbreeding depression…" (Frankham et al. 2014:57). Our custom-built IBM permitted us to develop a model that adequately captured the Florida panther

life history, and we could parameterize the model with our long-term genetic and demographic data.

The explicit consideration of the effects of inbreeding on Florida panther population dynamics and persistence represents an important step forward. Nonetheless, our model remains imperfect. First, we neglected the possible effects of habitat loss, detrimental anthropogenic activities, climate change, the probability of immigration of pumas from western populations, disease, or catastrophes on demographic rates and population viability. Although immigration from puma populations outside Florida is possible, its probability is very low, given the extensive challenges the anthropogenically altered landscape would pose for such a migrant to make it successfully to South Florida. We omitted mutations from our model because we have no information on mutation rates, though we expect that they are low based on values reported for other mammals (Kumar and Subramanian 2002). Finally, we only considered possible effects of inbreeding on age-specific survival rates because there was no evidence that heterozygosity affected female reproduction. Loss of heterozygosity can negatively affect reproduction because inbred male panthers can suffer from cryptorchidism, which can adversely affect their fecundity. However, given the polygynous mating system, we expect the Florida panther population growth is primarily female-limited.

Although it is generally accepted that genetic introgression only temporarily relieves a population from inbreeding depression, we know of no cases in which it has been implemented more than once to conserve a threatened wildlife population. Our study provides an example of how demographic and molecular data can be used within an IBM framework to evaluate the efficacy of alternative genetic management strategies, via the Florida panther as a case study. Our model can be adjusted for and applied to other species and offers great potential as a tool for genetic management of small, isolated wildlife populations.

MANAGEMENT IMPLICATIONS

Our results and those of Hostetler et al. (2013) and Johnson et al. (2010) provide persuasive evidence that the genetic intervention implemented in 1995 prevented the demise of the Florida panther and restored demographic vigor to the population. The Florida panther population remains small and isolated from other puma populations; the closest puma population is located >1,000 km away in Texas, and the intervening landscape is highly developed and fragmented with many interstate (and other high-traffic) highways and major urban centers. Theory suggests that 1 migrant per generation is sufficient to minimize the loss of genetic diversity (e.g., Mills and Allendorf 1996). However, the possibility of successful migration of a puma to South Florida followed by successful mating, every ~5 years, is very low considering the distance between Texas and Florida (Hedrick 1995) and the many risks and barriers that dispersing pumas face (Beier 1995, Maehr et al. 2002, Thatcher et al. 2006). Consequently, the panther's long-term persistence will likely depend on subsequent timely genetic introgressions, given the near-impossibility of natural gene flow from other puma populations. To that end, our study offers considerable insights into benefits of different genetic management strategies and associated costs.

Without genetic management, the Florida panther population faces a substantial risk of quasi-extinction within 100 years (10–46%, depending on quasi-extinction threshold and scenarios). Twenty-three years have passed since genetic introgression was implemented, and the population appears healthy, as indicated by measures of genetic variation, increases in the population size, and improvements in age-specific survival rates. Our findings suggest that releasing more pumas more frequently does not necessarily improve persistence probability because such a strategy would cause larger fluctuations in population size (Fig. 14), which negatively affects persistence probability. Thus, extinction risks faced by inbred populations of large carnivores can be substantially reduced by releasing approximately 5 immigrants every 2 decades. We suggest that such a management strategy be followed only in conjunction with continued long-term monitoring of the population. Our analyses demonstrate that population growth and persistence are highly sensitive to kitten and female (adult and subadult) survival. Continuing to collect data on these demographic variables, along with biometric and genetic sampling, will remain important to panther recovery and vigilance in identifying issues associated with inbreeding depression. The collection of these monitoring data should allow managers to detect any demographic or genetic changes in a timely fashion and respond with genetic introgression or other management actions if warranted.

Recovery objectives as defined by the USFWS in its current recovery plan (USFWS 2008) delineate the need for additional populations in the historical range to downlist or delist the Florida panther. If the only extant population of Florida panthers continued to grow, it could serve as a source of panthers to be translocated to suitable habitat to seed additional populations. Maintaining current information on the genetic health of the population and precise estimates of its size will be important in assessing whether it can withstand the removal of a subset of animals for translocation. Although the 2017 documentation of a female panther with kittens north of the current breeding range is encouraging in terms of the potential for population expansion—given that no female had been recorded north of the Caloosahatchee River since 1972—the re-establishment of separate new populations will most likely require translocations by wildlife managers and a lengthy sociopolitical process.

Conservation of threatened or endangered species such as the Florida panther is inherently challenging. For panthers and other large carnivores that require vast extents of natural habitat to persist, habitat is typically the most limiting factor that will determine whether recovery efforts succeed. Although genetic management invariably plays a key role in the long-term persistence of the panther population, even a healthy population can eventually succumb to the impacts of habitat loss. The human population of Florida continues to be one of the fastest-growing in the nation; the United States Census Bureau currently ranks Florida as the third most populous. Therefore, habitat loss is going to continue to be a key issue for panthers. Diligence toward preserving panther habitat in its historical range via conservation easements or other means, and trying to keep such areas interconnected via corridors is a goal stakeholders such as government agencies, sportsmen, non-governmental organizations, ranchers,

and other private landowners must work on collaboratively to achieve.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.