

Does genetic introgression improve female reproductive performance? A test on the endangered Florida panther

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Abstract Genetic introgression has been suggested as a management tool for mitigating detrimental effects of inbreeding depression, but the role of introgression in species conservation has been controversial, partly because population-level impacts of genetic introgressions are not

well understood. Concerns about potential inbreeding depression in the endangered Florida panther (*Puma concolor coryi*) led to the release of eight female Texas pumas (*P. c. stanleyana*) into the Florida panther population in 1995. We used long-term reproductive data (1995–2008) collected from 61 female Florida panthers to estimate and model reproduction probability (probability of producing a litter) and litter size, and to investigate the influence of intentional genetic introgression on these parameters. Overall, 6-month probability of reproduction ($\pm 1\text{SE}$) was 0.232 ± 0.021 and average litter size was 2.60 ± 0.09 . Although F_1 admixed females had a lower reproduction probability than females with other ancestries, this was most likely because kittens born to F_1 females survive better; consequently, these females are unavailable for breeding until kittens are independent. There was no evidence for the effect of ancestry on litter size or of heterozygosity on probability of reproduction or litter size. In contrast, earlier studies have shown that genetic introgression positively affected Florida panther survival. Our results, along with those of earlier studies, clearly suggest that genetic introgression can have differential effects on components of fitness and highlight the importance of examining multiple demographic parameters when evaluating the effects of management actions.

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Introduction

The loss of genetic diversity, and the resulting potential for inbreeding depression, is well-established as a contributing threat to small, isolated populations. Specifically,

inbreeding depression has been shown to influence many fitness traits, including age of first reproduction (Charpentier et al. 2008), litter or clutch size (Ortego et al. 2007), survival (Hostetler et al. 2010; Keller et al. 1994), lifetime reproductive success and other measures of individual fitness (Ferreira and Amos 2006; Slate et al. 2000), and population persistence (Brook et al. 2002; Ebert et al. 2002). However, outbreeding depression is also a risk when intentional hybridization is undertaken to mitigate the effects of inbreeding depression (Edmunds 2007).

The Florida panther (*Puma concolor coryi*), an endangered puma subspecies, is a textbook example of a small and isolated population. Once distributed across the southeastern United States, their number was reduced to fewer than 30 individuals by the 1980s (McBride et al. 2008). The population was severely inbred and characterized by extremely low genetic diversity (Culver et al. 2000; Johnson et al. 2010; Roelke et al. 1993). Many phenotypic traits characteristic of inbreeding were reported from this population, including very low sperm quality and high incidences of cryptorchidism, atrial septal defects, cowlicks, and kinked tails (Barone et al. 1994; Mansfield and Land 2002; Roelke et al. 1993). Due to concerns about inbreeding depression, 8 female Texas pumas (*P. c. stanleyana*) were released into the Florida panther population in 1995. Since this genetic introgression, the population has increased (McBride et al. 2008), although the processes that led to this increase continue to be debated (Creel 2006; Maehr et al. 2006; Pimm et al. 2006). The influence of genetic introgression on age-specific survival of Florida panthers has been explored (Benson et al. 2011; Hostetler et al. 2010; Johnson et al. 2010), but the potential effect of introgression on the panther's reproductive performance remains unclear (but see Pimm et al. 2006 for a qualitative comparison). Furthermore, the Scientific Review Team, commissioned by the US Fish and Wildlife Service and the Florida Fish and Wildlife Conservation Commission (FWC) partly in order to make recommendations for continuing Florida panther research, recommended a reanalysis of existing data to rigorously estimate reproductive parameters and an evaluation of the potential influence of introgression on demographic variables (Beier et al. 2003).

We used long-term field and genetic data (1995–2008) to address the following questions: (1) Do female probability of reproduction or litter size differ among age classes? (2) Do probability of reproduction or litter size vary seasonally? (3) Are probability of reproduction or litter size affected by population size (through negative or positive density-dependent effects)? (4) Are probability of reproduction or litter size affected by genetic diversity (as measured by heterozygosity)? (5) Are reproductive parameters positively influenced by genetic introgression as predicted by the hybrid vigor theory?

Materials and methods

Study area

Our study area was the entire current breeding range of the Florida panther, encompassing Big Cypress National Preserve, Everglades National Park, Florida Panther National Wildlife Refuge, Okaloacoochee Slough Wildlife Management Area and State Forest, Fakahatchee Strand Preserve State Park, Picayune Strand State Forest, Big Cypress Seminole Indian Reservation, Corkscrew Regional Ecosystem Watershed Wildlife Management Area, and adjoining private lands in south Florida. Although transient males tend to roam widely, no females have been verified north of Caloosahatchee River since 1972 (Land et al. 2008). The climate of south Florida is tropical (Henry et al. 1994), with a wet season extending roughly from June to November and a dry season from December to May.

Field methods

We located and treed Florida panthers using trained hounds and houndsmen (supplied by Livestock Protection Company, Alpine, Texas). Immobilization and capture protocols are described in detail elsewhere (FWC 2008; Land et al. 2008; McCown et al. 1990; Taylor et al. 2002). Age of captured panthers (if unknown) was estimated using a combination of tooth wear, pelage characteristics, size, and developmental stage of teats and reproductive organs. Blood and tissue samples were collected for health assessment and genetic analysis.

We affixed radio collars on captured adults, sub-adults, and older kittens. Collars contained mortality switches that altered the pulse rate of transmitters when a panther did not move for a period of time. We located radio-collared panthers using aerial telemetry, generally three times per week. When mortality signals were detected, the locations were investigated on the ground to confirm the death of the panther.

Successive locations of females were continually assessed to determine the commencement of denning behavior; lack of movement between 3 or 4 fixes was taken as evidence of possible denning (Land et al. 1998). We visited dens 4–35 days post-partum (approximate median = 14 days). Kittens in dens were counted, sexed, sampled for genetic material, and implanted with passive integrated transponder (PIT) tags. Florida panther gestation length averages 3 months and litter sizes range from 1 to 4 (Maehr 1992).

Genetic ancestry and heterozygosity

We estimated the heterozygosity and genetic ancestry of individuals from genetic samples using techniques detailed

elsewhere (Benson et al. 2011; Hostetler et al. 2010; Johnson et al. 2010). We used results from the genetic ancestry analysis along with pedigree information and field evidence to assign panthers to three groups that reflect the genetic makeup of the current Florida panther population (pre-introgression type panthers, F_1 admixed panthers, and other admixed panthers; Benson et al. 2011).

Pre-introgression type panthers represent the genotypes present on the landscape prior to the introgression program and include mostly canonical Florida panthers that showed no evidence of non-Florida genetic admixture (no direct non-Florida relatives or <10% non-Florida genetic contribution). Two pre-introgression type panthers in the sample of individuals used to investigate the influence of genetic ancestry on reproductive rates ($n = 58$) were descendants of panthers from the Everglades genetic population with documented genetic links to Central America (Culver et al. 2000; O'Brien et al. 1990). Admixed panthers were mostly the descendants of the introduced female Texas pumas released in 1995 (exceptions were apparently also descendants of western North America pumas; Benson et al. 2011; Johnson et al. 2010).

Differences in fitness between classes of admixed panthers are possible and hybrid vigor is predicted to be strongest in the F_1 generation (Arnold and Hodges 1995; Burke and Arnold 2001), so we included two classes of admixed panthers in our analyses: F_1 and other admixed. We defined F_1 admixed panthers as any offspring produced by matings between a Texas female and a pre-introgression type male.

In summary, all individuals in our radio-collared sample were placed into 1 of 3 categories: pre-introgression type panthers ($n = 24$), F_1 admixed panthers ($n = 6$), and other admixed panthers ($n = 28$). We were able to further classify most individuals from the other admixed category into 3 finer categories: backcrossed to Florida admixed panthers ($n = 6$), backcrossed to Everglades admixed panthers ($n = 3$), and backcrossed to Texas admixed panthers ($n = 11$). We considered potential differences in reproductive rates between pre-introgression type, F_1 admixed, and these finer admixture distinctions (see Appendix A).

Index of abundance

To examine the potential effects of panther density on reproductive rates, we used the minimum population counts as an index of abundance (McBride et al. 2008). These minimum counts were based on radio-tracking and field evidence of sub-adult and adult panthers (tracks, scat, kills, and scrapes) collected for calendar years. In contrast, we analyzed reproductive rates based on years from June to May. We applied the calendar year count for year t to the June $t - \text{May } t + 1$ year. In 2004, the panther survey was incomplete, so the minimum count is unknown. We

followed McBride et al. (2008) and interpolated it halfway between the 2003 and 2005 counts.

Estimating age classes

We knew the ages (to within a few days) for panthers handled as kittens at natal dens that were subsequently recaptured and radio-collared ($n = 29$, 46.8% of total). These kittens were handled <35 days after birth as estimated by the date when the mother was first located at the den site and by the physical characteristics of the kittens. Age of panthers not marked as kittens was estimated in the field at time of first capture using tooth wear and pelage characteristics. We separated female panthers into 4 age classes: kittens (0–1 years old), sub-adults (1–2.5 years old), prime-adults (2.5–10 years old), and older-adults (≥ 10 years old). Since panther kittens do not reproduce, we limited analyses to sub-adults, prime-adults, and older-adults. The sub-adult age class was the period of a panther's life after independence from its mother until the approximate ages when females establish home ranges. We estimated age of independence at just over 1 year (mean = 397 days, SD = 74 days, $n = 32$; FWC and US National Park Service, unpublished data) from known-age kittens that were radio-tracked simultaneously with their mothers (before and after independence). For simplicity, we standardized the beginning of the sub-adult age class to 1 year.

Modeling probability of reproduction

We used radio-tracking and reproductive histories of female panthers monitored between June 1995 and May 2008 (13 years) in the present analyses. We organized the data into 6-month intervals (June–November and December–May). We recorded the following information for each panther in each 6-month interval: number of months tracked, number of kittens produced, number of kittens produced that survived to be PIT-tagged in the den, age class, heterozygosity, and ancestry category. If a female advanced to an older age class within a 6-month interval, two records were created, each with one of the age classes and the appropriate number of months tracked.

Since no female panther was recorded giving birth twice within 1 season, we modeled probability of a female panther giving birth during a season (p) using binomial regression. The parameter p can be modeled as a function of covariates. We modeled p using a complementary log–log link:

$$p_i = 1 - \exp[-\exp(\mathbf{Z}_i\boldsymbol{\gamma})] \quad (1)$$

where \mathbf{Z}_i is the matrix of covariates for panther i and $\boldsymbol{\gamma}$ is the vector of binomial model's coefficients.

Often, panthers were not tracked for entire seasons (e.g., due to radio failure before the end of a season or radio-collaring after the start of a season). To account for the

effect of the number of months a panther was tracked in a season on the probability that it gives birth while being tracked, we used $\log(m)$ as an offset, where m is the number of months a panther was tracked during a season. The combination of this offset and the complementary log–log link for p were chosen to generate the property:

$$\Pr(b = 0|M = m) = \Pr(b = 0|M = 1)^m \quad (2)$$

where b is a binary variable indicating whether a panther reproduced or not in an interval. We assumed an exponential model for reproduction; therefore, the probability that a panther does not reproduce in m months is the same as the probability that a panther does not reproduce in 1 month, taken to the m power. We used the `glm` function in the `stats` package in R (version 2.11.1; R Development Core Team 2010; code available on request) to fit the model.

Modeling litter size

We estimated and modeled litter sizes of female panthers, conditional on their giving birth, using cumulative logit regression. Although this model is traditionally used for ordered categorical data (Agresti 2007), it can also be used for count data (Min and Agresti 2005). The model includes J categories for number of offspring, with N_j being the number of offspring represented by category j (because panthers can have 1 to 4 offspring, $J = 4$ and $N_j = j$ in our case). The probability that the litter size will be at most N_j for individual i (Y_i) is represented by δ_{ij}

$$P(Y_i \leq N_j) = \delta_{ij} = \left\{ \begin{array}{ll} \frac{1}{1 + \exp(-\theta_j + \beta \mathbf{X}_i)} & j = 1, 2, \dots, J-1 \\ 1 & j = J \end{array} \right\} \quad (3)$$

where θ_j is the intercept for litter size being at most N_j , \mathbf{X}_i is the matrix of covariates for panther i , and β is the vector of the model's coefficients. In equation 3, the intercept θ varies by offspring count (as denoted by its subscript j) but β is independent of offspring count. This model therefore represents the underlying structure of the data in a flexible fashion but assumes the effects of \mathbf{X} are identical for all $J - 1$ cumulative logit models. If $\beta_k > 0$, the k th covariate increases the probability of a larger litter size. The probability that Y_i will be exactly N_j is

$$P(Y_i = j) = \pi_{ij} = \left\{ \begin{array}{ll} \delta_{i1} & j = 1 \\ \delta_{ij} - \delta_{i(j-1)} & j = 2, \dots, J \end{array} \right\} \quad (4)$$

The expected average litter size for individual i is

$$\mu_i = \sum_{j=1}^J N_j \pi_{ij}. \quad (5)$$

We implemented the cumulative logit model using the `clm` function in the `ordinal` package (Christensen 2010) in

R (version 2.11.1; R Development Core Team 2010; code available on request).

Modeling approach

We used an information-theoretic approach (Akaike's Information Criterion; AIC) for model selection and statistical inference (Burnham and Anderson 2002). We calculated AIC values, Akaike differences (ΔAIC_i , difference between AIC value of the i th model and the top-ranked model) and Akaike weights (w_i , the weight of evidence that the i th model is actually the best predictive model of the models being considered given the data) as in Burnham and Anderson (2002). The sum of the weights of models including a given variable can be interpreted as a measure of its importance, relative to other variables (Burnham and Anderson 2002). We calculated model-averaged estimates of reproductive outcomes (p probability of reproduction and μ average litter size) and unconditional variances for each level of categorical variables and across continuous covariates (Burnham and Anderson 2002). To generate these model-averaged estimates, we used all models included in the model selection process for a given analysis, weighted by their Akaike weights. Models with no effects of the covariates being presented were included in the averages (as having the same reproductive outcome values for all values of the covariate); the model averages therefore represent unconditional estimates of reproductive outcomes (Burnham and Anderson 2002).

We used a step-wise approach to examine the effects of various covariates on reproductive parameters. We used this approach so that we could minimize the number of models tested and focus on specific questions (which were about individual covariates), while still incorporating observed patterns in covariates tested earlier. First, we examined the effects of age class on reproductive parameters (i.e., reproduction probability and litter size). We considered 3 age-structures: Age1, which separates female panthers into 3 age classes (sub-adults, prime-adults, and older-adults); Age2, which separates females into 2 age classes: older-adult and young-adult (sub-adults and prime-adults combined) panthers; and Age3, which also divides female panthers into 2 age classes: sub-adult and adult (prime-adults and older-adults combined) age classes. We looked for the effect of all 3 age-structures on p , but did not test for the effect of Age1 or Age2 on μ , because these models separate out older-adults and sample size was inadequate for a separate analysis of litters produced by older-adult panthers. We chose the minimum AIC model from each age class analysis as a base model to examine the additive effect of season (wet: June–November vs. dry: December–May) on p and μ . The minimum AIC models from these analyses were used as base models for all subsequent analyses.

We next looked for the effects of age class and season on reproductive rates, excluding older-adults. We did this to check the robustness of our estimates of reproductive parameters for sub-adults and prime-adults. Excluding the older-adults had very little effect on estimates (Appendix B).

We used the minimum AIC models from the seasonal analyses as base models, and looked for linear and quadratic effects of abundance index on reproductive parameters; the quadratic term was used to test for the possibility of both negative and positive density-dependence in components of reproduction. Effects of abundance on both p and μ were tested additively with the base models.

Next, we looked for the additive effect of ancestry on p and μ . We excluded 1 panther with unknown ancestry from these analyses. We tested three models of ancestry: Ancest1, which divides females into 3 ancestry categories (pre-introgression type, other admixed, and F_1 admixed); Ancest2, which combines the other admixed with the pre-introgression type females; and Ancest3, which combines the other admixed with the F_1 admixed females. There were no older-adult female admixed panthers in our dataset, so we wanted to examine the robustness of statistical inference and parameter estimates regarding the influence of ancestry to the inclusion of the pre-introgression type older-adults. Excluding older-adults had very little effect on estimates or inference (Appendix B). Finally, we tested for the effects of heterozygosity on reproductive parameters. We excluded 1 panther with unknown heterozygosity from these analyses. We tested for the additive effect of heterozygosity on both p and μ .

Appendix C contains results of analyses performed on an annual time scale, rather than 6-month, for comparison with other studies and for use in population models. For these models, kittens were only counted (included as reproduction events and in litter counts) if they received PIT tags in the den, and estimates of temporal variance in reproductive parameters were generated (Appendix C). We examine the effects of individual and temporal variation on inference in Appendix D.

Estimating average age at first reproduction and inter-birth interval

We made an ad hoc estimate of average age at first reproduction from the estimated ages at first recorded litters of female panthers that were radio-tracked consistently from age 21 months (or earlier) to that litter date. These restrictions were imposed so that panthers that first gave birth while not being radio-tracked did not have their second litter recorded as their first. This estimate may be biased low, however, because panthers that first reproduced later in life are less likely to be included. We report overall

average age at first reproduction and averages for the three ancestry groups.

We also obtained an ad hoc estimate of average inter-birth interval from the observed gaps between successive reproductive events for female panthers that had at least two recorded births with continuous radio-tracking between them. This estimate may also be biased low, because short inter-birth intervals are more likely to be included in the time the panthers were continuously radio-tracked than long ones. Therefore, we also estimated average inter-birth interval (I) in years from the 6-month probability of reproduction (p) as:

$$I = \frac{1}{2p}. \quad (6)$$

We estimated I for all female panthers, for young-adult panthers, and for young-adult panthers by ancestry group.

Results

We tracked 61 female panthers for a total of 2,414 panther-months and recorded 94 litters. Estimated ages of dams when they produced litters ranged from 21 months to 11.45 years. There was only one recorded litter produced by 9 older-adult (≥ 10 years) females, which were tracked for a total of 313 months; by contrast, 28 sub-adults produced 13 litters in 379 months and 57 prime-adults produced 80 litters in 1,722 months. Mean litter size was 2.60 and the modal litter size was 3 (Fig. 1). A naïve estimate of probability of reproduction during a 6-month period for all female panthers (number of litters/number of months tracked \times 6) was 0.234. These correspond closely to the model-based estimates for all female panthers (mean \pm 1 SE litter size = 2.60 ± 0.09 , probability of reproduction = 0.232 ± 0.021). Plots of reproductive events and litter sizes as functions of age class, season, abundance, ancestry, and heterozygosity are presented in Appendix E.

Probability of reproduction

There was strong evidence that reproduction probability varied among age classes; the Δ AIC of the constant parameter model was about 20 (Table 1A). Older-adults had a lower probability of reproduction than younger females, but there was little evidence that reproduction probabilities differed between sub-adult and prime-adult females (Fig. 2a). Furthermore, there was evidence that probability of reproduction varied seasonally (Model 1, Table 1B), with model-averaged probability of reproduction being higher during the dry season (Fig. 2b). The lowest AIC model for abundance included a positive

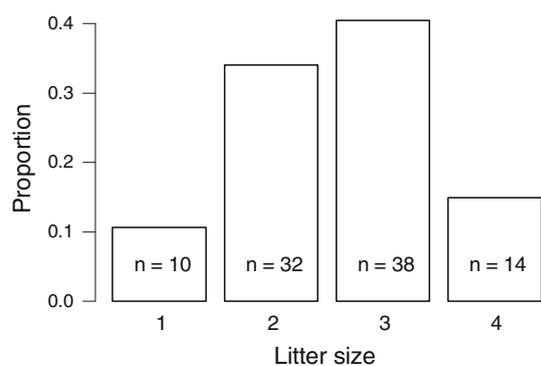


Fig. 1 Frequency distribution of litter sizes produced by radio-collared female Florida panther (*Puma concolor coryi*) (1995–2008). Numbers inside bars indicate number of litters of that size

relationship between index of abundance and reproduction probability (Model 1, Table 1C; Fig. 2c).

The lowest AIC model evaluating the effect of ancestry on reproductive parameters indicated a difference between F_1 panthers and those with other ancestry (Model 1, Table 1D). Model-averaged probability of reproduction was lower for F_1 s than pre-introgression type and marginally higher for other admixed than pre-introgression type (Fig. 3a). The most parsimonious heterozygosity model suggested that heterozygosity had no effect on probability of reproduction (Model 1, Table 1E). Model-averaged probability of reproduction declined somewhat with increasing heterozygosity (Fig. 3b).

Litter size

There was evidence for age-specific variation in litter size; sub-adult females produced somewhat larger litters than females of other age classes (Table 2A; Fig. 2d). Older adult females produced only 1 litter during this study, and we were unable to test for differences in litter size between older-adults and younger females. There was some evidence for seasonal variation in litter size (Model 1, Table 2B), with larger litters during the dry season (Fig. 2e). However, there was no evidence that litter size was affected by abundance (Model 1, Table 2C; Fig. 2f).

The most parsimonious model evaluating the effect of ancestry revealed no evidence that ancestry influenced litter size (Model 1, Table 2D), although admixed females produced slightly larger litters than pre-introgression type females (Fig. 3c). The most parsimonious heterozygosity model suggested that heterozygosity had no effect on litter size (Model 1, Table 2E; Fig. 3f).

Average age at first reproduction and inter-birth interval

Observed ages of first reproduction ranged from 21 months to 6.88 years. The ad-hoc estimate of average age of first

reproduction was 2.62 ± 0.25 years ($n = 20$). For pre-introgression type females, the average age of first reproduction was 2.59 ± 0.23 years ($n = 6$); it was 3.56 ± 1.13 years ($n = 4$) and 2.27 ± 0.15 years ($n = 10$) for F_1 admixed and other admixed females, respectively.

The observed inter-birth intervals ranged from 4.14 months to 4.24 years, with an ad-hoc estimate of the mean at 1.30 ± 0.11 years ($n = 43$). The average inter-birth interval estimated from reproduction probabilities was 2.16 ± 0.19 years, or 1.90 ± 0.17 years excluding older-adults. The ad-hoc average inter-birth intervals by ancestry were 1.32 ± 0.13 years ($n = 19$), 2.73 ± 1.51 years ($n = 2$), and 1.14 ± 0.13 years ($n = 22$) for pre-introgression type, F_1 admixed, and other admixed female panthers, respectively. The average inter-birth intervals by ancestry estimated from reproduction probabilities (excluding older-adults) were 1.74 ± 0.16 years for non- F_1 panthers and 2.16 ± 0.19 years for F_1 panthers.

Discussion

The case of the Florida panther demonstrates how loss and degradation of habitat, persecution, rapid urbanization and other anthropogenic influences can threaten biodiversity. Indeed, the plight of the panther has attracted the attention of ecologists, conservation organizations, state and Federal agencies, and various interest groups (<http://www.floridapanther.org/>; <http://floridapanther.org/>; Fergus 1998; Gross 2005). Various conservation measures have been undertaken to ensure long-term persistence of this population; perhaps the most drastic among these efforts was the genetic introgression project implemented in 1995 (Seal 1994). Although the prevalence of phenotypic traits indicative of inbreeding (e.g., heart defects, susceptibility to heavy parasite loads, poor sperm quality and cryptorchidism) has declined (Johnson et al. 2010; Mansfield and Land 2002; Onorato et al. 2010) and the panther population has increased (McBride et al. 2008), some aspects of the reproductive ecology of panthers, especially as they relate to genetic introgression, remain poorly understood.

The overall 6-month probability of reproduction was 0.232 ± 0.021 , which translates into annual cumulative probability of reproduction of 0.410 ± 0.034 (Appendix C). This annual estimate is within the range reported for western North America puma populations (0.24–0.45; Cooley et al. 2009; Stoner et al. 2006). However, our annual estimate of reproduction probability was lower than that used by previous demographic analyses of Florida panthers, based on earlier datasets (0.5; Maehr et al. 2002; Root 2004). Reproduction probability varied substantially among age classes; older females (≥ 10 years) had a lower probability of reproduction compared to younger females

Table 1 Model selection table evaluating the effects of (A) age class, (B) season, (C) abundance index, (D) ancestry, and (E) heterozygosity on probability of reproduction (p) by Florida panther (*Puma concolor coryi*)

Model		K	ΔAIC	w_i
(A) Age models				
1	Age2^a	2	0.00	0.562
2	Age1 ^b	3	0.50	0.438
3	Constant ^c	1	19.94	0.000
4	Age3 ^d	2	21.58	0.000
(B) Season models				
1	Age2 + Season	3	0.00	0.743
2	Age2	2	2.12	0.257
(C) Abundance index models				
1	Age2 + Season + Abundance	4	0.00	0.433
2	Age2 + Season + Abundance + Abundance ²	5	0.78	0.293
3	Age2 + Season	3	0.91	0.274
(D) Ancestry models				
1	Age2 + Season + Ancest2^e	4	0.00	0.627
2	Age2 + Season + Ancest1 ^f	5	1.58	0.284
3	Age2 + Season	3	4.54	0.065
4	Age2 + Season + Ancest3 ^g	4	6.48	0.025
(E) Heterozygosity models				
1	Age2 + Season	3	0.00	0.610
2	Age2 + Season + Het ^h	4	0.89	0.390

The top ranked model for age class (part A, model 1, in bold) was used as a base model to test for the effects of season; the top ranked model for season (part B, model 1, in bold) was used as a base model to test for the effects of abundance, ancestry, and heterozygosity. For each model, we present the number of parameters (K), the difference in Akaike's Information Criterion from the best ranked model (ΔAIC), and the Akaike weight (w_i). The ancestry and heterozygosity analyses were performed on a subset of the data (excluding 1 panther with unknown ancestry and heterozygosity)

^a Age2 divides panthers into young-adults and older-adults

^b Age1 divides panthers into sub-adults, prime-adults, and older-adults

^c No predictor variables

^d Age3 divides panthers into sub-adults and adults

^e Ancest2 divides panthers into 2 ancestry categories: F_1 admixed, and other admixed and pre-introgression type combined

^f Ancest1 divides panthers into 3 ancestry categories: pre-introgression type, other admixed, and F_1 admixed

^g Ancest3 divides panthers into 2 ancestry categories: pre-introgression type and admixed

^h Heterozygosity

but the reproduction probability of sub-adult females (1–2.5 years) did not differ from that of prime adults (2.5–10 years). We expected probability of reproduction to be higher for prime-adults than for the other two age classes because they should have a defined home range that allows them to maximize their energetic allocations towards reproduction. Our results provide strong evidence of reproductive senescence (manifested as a lower probability of reproduction) in older Florida panthers, but suggest that age-specific differences in reproduction prior to senescence are minor.

The overall average litter size was 2.60 ± 0.09 ; this estimate, although slightly higher than previous estimates for Florida panthers (Lotz et al. 2005: 2.4; Maehr and Caddick 1995: 2.25), is within the range of litter size

estimates reported for western populations of pumas (1.7–3.0; Cooley et al. 2009; Lambert et al. 2006; Landré et al. 2007; Logan and Sweanor 2001; Robinson et al. 2008; Stoner et al. 2006). Sub-adult females produced somewhat larger litters. This result was to some extent surprising because we expected prime-aged females to produce larger litters than sub-adult or older females. Effects of age and/or breeding experience on litter size have previously been shown for other large carnivores (Garrison et al. 2007; Zedrosser et al. 2009), although not for pumas (Logan and Sweanor 2001).

Our estimates of litter size are likely underestimates due to undetected kitten mortality that may have occurred before den visits, although if remains of a kitten were detected in the den, that kitten was counted in the reproductive analyses

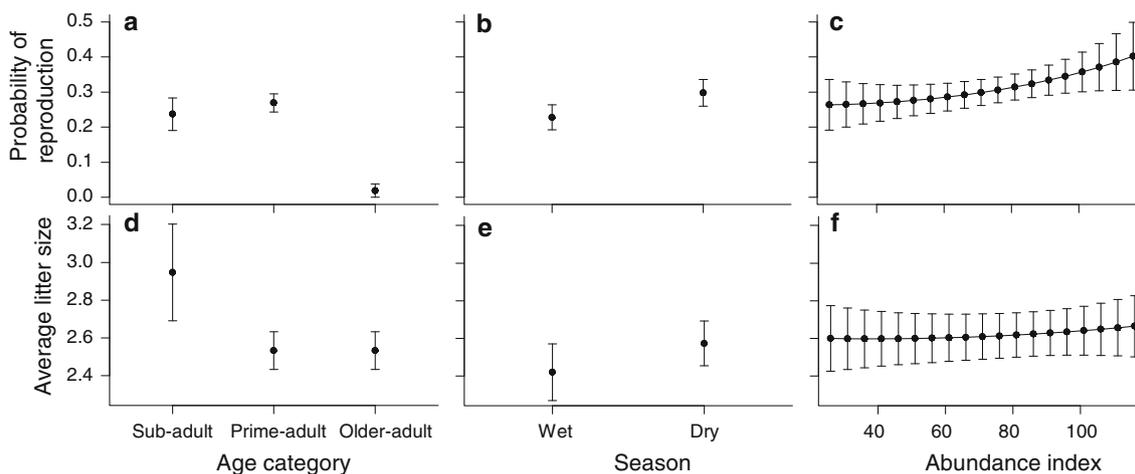


Fig. 2 Model-averaged reproductive rates (\pm unconditional standard errors) by (a, d) age class, (b, e) season, and (c, f) abundance index. Sub-adults are defined as ages 1–2.5 years old, prime-adults ages 2.5–10, and older-adults ages 10 and older. We present (a–c) 6-month

probability of reproduction, and (d–f) average litter size. b is presented for young-adult panthers (<10 years old), c for young-adult panthers in the dry season, e for adult panthers (\geq 2.5 years old), and f for adult panthers in the dry season

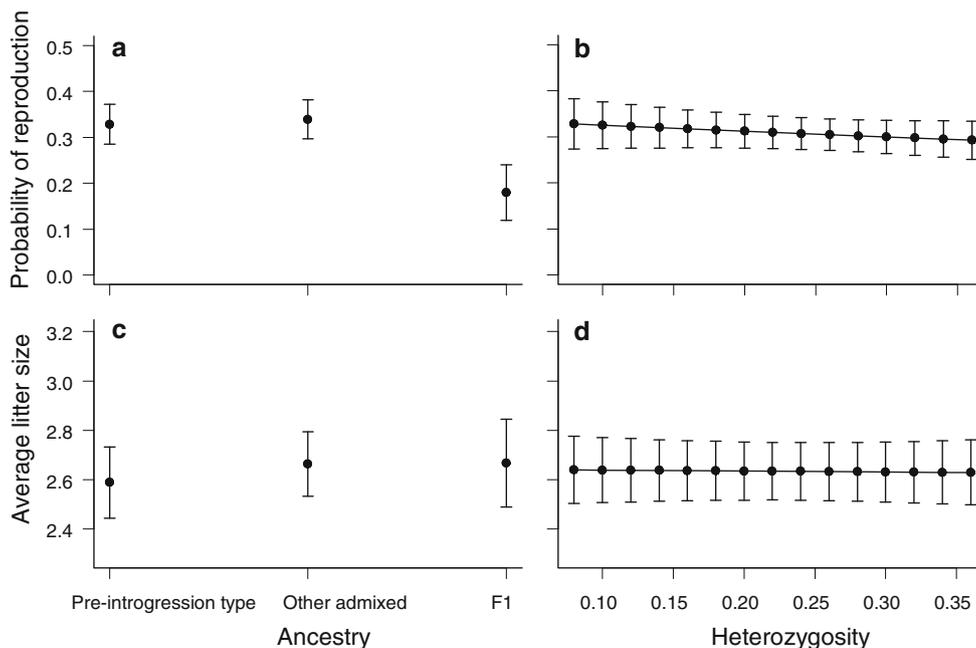


Fig. 3 Model-averaged reproductive rates (\pm unconditional standard errors) by (a, c) ancestry category and (b, d) heterozygosity. We present (a, b) 6-month probability of reproduction for young-adult

panthers (<10 years old) in the dry season and (c, d) average litter size, for adult panthers (\geq 2.5 years old) in the dry season

presented in the main text. Since puma mortality is high during the first 3 months of life (Hostetler et al. 2010; Logan and Sweanor 2001), delays in visiting dens could lead to systematic undercounts. In fact, there is some evidence that the observed litter sizes are negatively related to the estimated number of days between parturition and the den visit (J. A. Hostetler, unpublished analysis).

Probability of reproduction was higher in the dry season than in the wet, but litter sizes were similar. Previous

examinations of raw number of litters per month suggest that the actual birth peak is March–June (Lotz et al. 2005), which is supported by our results. Dry season coincides with the peak white-tailed deer (*Odocoileus virginianus*) fawning season in south Florida (February–March; Fleming 1994; Richter and Labisky 1985; Schortemeyer et al. 1991) and peak feral hog (*Sus scrofa*) farrowing (parturition) season (Belden and Frankenberger 1990), which may allow females to make caloric gains necessary to achieve a

Table 2 Model selection table evaluating the effects of (A) age class, (B) season, (C) abundance index, (D) ancestry, and (E) heterozygosity on litter size (μ)

Model		K	ΔAIC	w_i
(A) Age models				
1	Age3^a	4	0.00	0.794
2	Constant ^b	3	2.70	0.206
(B) Season models				
1	Age3 + Season	5	0.00	0.551
2	Age3	4	0.41	0.449
(C) Abundance index models				
1	Age3 + Season	5	0.00	0.619
2	Age3 + Season + Abundance	6	1.74	0.260
3	Age3 + Season + Abundance + Abundance ²	7	3.26	0.121
(D) Ancestry models				
1	Age3 + Season	5	0.00	0.443
2	Age3 + Season + Ancest3 ^c	6	0.87	0.286
3	Age3 + Season + Ancest2 ^d	6	1.98	0.165
4	Age3 + Season + Ancest1 ^e	7	2.86	0.106
(E) Heterozygosity models				
1	Age3 + Season	5	0.00	0.729
2	Age3 + Season + Het ^f	6	1.98	0.271

The top ranked model for age class (part A, model 1, in bold) was used as a base model to test for the effects of season; the top ranked model for season (part B, model 1, in bold) was used as a base model to test for the effects of abundance, ancestry, and heterozygosity. For each model, we present the number of parameters (K), the difference in Akaike's Information Criterion (ΔAIC), and the Akaike weight (w_i). The ancestry and heterozygosity analyses were performed on a subset of the data (excluding 1 panther with unknown ancestry and heterozygosity)

^a Age3 divides panthers into sub-adults and adults

^b No predictor variables

^c Ancest3 divides panthers into 2 ancestry categories: pre-introgression type and admixed

^d Ancest2 divides panthers into 2 ancestry categories: F₁ admixed, and other admixed and pre-introgression type combined

^e Ancest1 divides panthers into 3 ancestry categories: pre-introgression type, other admixed, and F₁ admixed

^f Heterozygosity

successful pregnancy by preying on vulnerable young fawns and piglets.

We found that abundance index positively influenced reproduction probability. This may be due to a mate-finding Allee effect (Gascoigne et al. 2009). However, there was also a decrease in kitten survival with increasing abundance index, possibly due to infanticide by male panthers (Hostetler et al. 2010). Female panthers are unlikely to copulate while they have dependent offspring; in fact, more than one instance of parturition within a 12-month period is indicative of the failure of the initial litter (Hostetler et al. 2010). Therefore, the increase in probability of reproduction may be caused by the decrease in kitten survival. Abundance index also increased fairly steadily over time during our study period (1995–2008; McBride et al. 2008). The probability of reproduction may have increased over time due to some cause other than, or in addition to, increasing population size. One intriguing possibility is that reproductive vigor of the male panthers has increased since 1995, due to the reduction in

inbreeding depression in male reproductive traits (Johnson et al. 2010).

In earlier studies, we found that F₁ admixed panthers of all ages survived substantially better than panthers in other ancestry categories (Benson et al. 2011; Hostetler et al. 2010). Based on these results and predictions of hybrid vigor theory (Crow 1948), we expected higher probability of reproduction and larger litters for F₁ female panthers. Thus, our results that reproduction probabilities were lower for F₁ (and backcrossed to Texas; Appendix A) female panthers than for other admixed and pre-introgression type female panthers were unexpected. Although outbreeding depression (due to loss of local adaptations and/or co-adapted gene sequences; Edmands 2007; Hallerman 2003) cannot be ruled out as a possibility, the relative lack of genetic differentiation within North American pumas (Culver et al. 2000) and the former adjacency and presumed interbreeding between *P. c. coryi* and *P. c. stanleyana* make outbreeding depression less likely. An alternative, and perhaps more plausible, explanation is that

F₁ panthers bred less often because their offspring were more likely to survive to independence (Hostetler et al. 2010). In addition, the high mortality of kittens (particularly of pre-introgression types) may have provided a survival filter, selecting the highest quality pre-introgression type female panthers to survive and to potentially reproduce. Finally, the sample of radio-collared F₁ female Florida panthers was small ($n = 6$) and it is possible that these individuals reproduced less often than other panthers due to chance alone (or for reasons other than genetic ancestry). Thus, although our results provide strong evidence that genetic introgression did not positively influence probability of reproduction for Florida panthers, we cannot conclude that introgression negatively affected this reproductive parameter.

Interestingly, litter size did not vary among females with various ancestry categories. Additionally, we found no evidence that heterozygosity influenced reproduction probability or litter size. These results were surprising because heterozygosity has been shown to influence litter or clutch size (Ortego et al. 2007), lifetime reproductive success and other measures of individual fitness (Ferreira and Amos 2006; Slate et al. 2000), and increase age-specific survival probabilities of Florida panthers (Benson et al. 2011; Hostetler et al. 2010). Our study extends the work of Pimm et al. (2006), who graphically examined the lifetime kitten production of 24 female panthers and found no evidence of a difference between pre-introgression type and admixed panthers. Our analyses involved a larger sample size, more detailed ancestry categories, and separated two components of reproduction (reproduction probability and litter size) using reliable statistical methods (including a novel method for modeling litter size).

It is possible that differences in environmental conditions faced by F₁ female panthers and their pre-introgression type counterparts affected their probabilities of reproduction or litter sizes. Temporal variation may be unlikely; for all but the first 2 years of our study, panthers of all three ancestry classes were tracked on the landscape, and including year of the study in analyses had little effect on inference regarding the influence of genetic factors on reproductive parameters (Appendix D). Furthermore, radio-tracked females of all ancestry classes were predominantly found on public lands. However, differences in panther density or habitat conditions experienced by admixed females may have altered their reproductive output (Benson et al. 2011). In particular, half the F₁ females lived in Everglades National Park, which had a lower adult male panther density; this may also have contributed to the lower probability of reproduction found for this group.

Our results clearly demonstrate that one intrinsic or extrinsic factor can have different effects on different

demographic rates or fitness components. For example, prior to this study, we had concluded that genetic introgression positively influences Florida panthers' demographic traits, whereas population density can negatively influence them (Benson et al. 2011; Hostetler et al. 2010). The present study shows that neither of these conclusions hold for Florida panther reproductive parameters. These contrasts highlight the importance of simultaneously examining the effect of management actions on all components of fitness. Examining the total direct fitness implications of the introgression (either through estimating lifetime output of surviving kittens or estimating population growth rate of hypothetical single ancestry populations) is a goal of future research.

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