

Introgression Level Achieved through Florida Panther Genetic Restoration

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Abstract

Florida panthers (*Puma concolor coryi*) exist today as a small isolated population of 60 to 70 individuals in southern Florida after two centuries of habitat loss and persecution eliminated them from much of the southeastern United States. Many observed phenotypic traits such as cryptorchidism, kinked tails, cowlicks, and atrial septal defects are assumed to be manifestations of inbreeding. Dispersal mechanisms can no longer function to maintain genetic diversity within the small population. A plan to restore genetic diversity within the panther population to levels comparable to western puma was initiated with the release of eight Texas puma (*P. c. stanleyana*) in 1995. The goal was to achieve a 20% representation of Texas puma genes in the panther population. To date, four of the eight Texas pumas are still alive and have produced a minimum of 36 descendants, 25 of which are thought to still be alive. Based on our pedigree knowledge, we calculate that the panther population has 18% to 22% representation of Texas puma genes as the result of genetic restoration.

Introduction

Florida panthers (*Puma concolor coryi*) are endangered by a combination of population and habitat factors (USFWS 1987). Loss and fragmentation of habitat and unregulated killing over the past two centuries have reduced and isolated *Puma* populations in the eastern United States to the point where only one population estimated to number between 60 to 70 individuals exists on approximately 8,810 square kilometers (2.2 million acres) of habitat in south Florida (Maehr 1990). Small population size and geographic isolation increase the chance for extinction of Florida panthers due to demographic instability inherent in small numbers and erosion of genetic diversity from restricted gene flow and inbreeding. Maintaining genetic diversity is key for production of

fit individuals as well as providing population elasticity in order to respond to changing environmental and habitat conditions.

Genetic diversity within the panther population would have been maintained at higher levels when the population was greater in size. Furthermore, natural exchange of genetic material occurred historically among the Florida panther population in the southeastern United States and contiguous populations of *P. c. cougar* to the north, *P. c. hipolestes* to the northwest and *P. c. stanleyana* to the west (Young & Goldman 1946). Gene flow occurred as individuals dispersed widely and bred, however, human settlement of the eastern United States resulted in local extirpations of *Puma*, thereby eliminating this exchange. With limits to dispersal and decreasing

population size, breeding among close relatives occurs and can lead to inbreeding depression, loss of genetic variation, declining health, reduced survivability, and eventual extinction (Gilpin & Soulé 1986). Even with adequate habitat protection, these genetic concerns could lead to panther extinction.

Florida panthers exhibit reduced genetic variability when compared to western pumas (Roelke et al. 1993), and panther traits such as kinked tails, cowlicks, atrial septal defects, cryptorchidism, and poor sperm characteristics may be manifestations of inbreeding. Concern that the predicted downward trend in panther population viability may have begun led to the development and implementation of a "Plan for Genetic Restoration and Management of Florida Panthers"

(Seal 1994). This plan called for the release of eight female Texas pumas into areas occupied by Florida panthers to mimic the former natural exchange of individuals among these populations. The resultant *Puma* population in Florida after several generations of intercrossed offspring have been assimilated was expected to trace 20% of its genome to material from the Texas population. This level of genetic restoration was deemed adequate to forestall negative impacts of inbreeding and to raise the panther population genetic diversity to levels documented in western North American *Puma* populations. Periodic releases of *Puma* into Florida would be necessary to maintain the desired levels of genetic variation within the panther population over time.

The objectives of this study are to document the productivity of the Texas pumas that were released in 1995 and their subsequent offspring and to calculate the percentage of the panther population's genome that originated from these translocated Texas (TX) cats.

Methods

The study area encompassed most of interior Florida south of Orlando (28.3°N), extending to southern Everglades National Park. Approximately 50% of panther habitat is in public ownership and includes areas such as Big Cypress National Preserve, Florida Panther National Wildlife Refuge, Fakahatchee Strand State Preserve, and Everglades National Park. Major vegetation communities are pine forests and savannas, cypress and mixed hardwood swamps, hardwood hammocks, and open marshlands (Davis 1943). Climate is sub-

tropical with average annual temperature and precipitation of 74° F (23 C) and 137 centimeters respectively (Henry et al. 1994). The TX pumas used for genetic restoration originated from west Texas, primarily in Pecos, Presidio, and Brewster counties.

Florida panthers (FP) and TX pumas were captured using trained hounds, anesthetized following McCown et al. (1990), and fitted with radiocollars. Vital signs were monitored during anesthesia and all animals underwent a complete physical examination to assess general health condition. Samples taken included whole blood and skin biopsies, and *Puma* greater than four months of age were vaccinated for feline viral rhinotracheitis, feline calicivirus, feline panleukopenia, and rabies. All animals were tattooed on the ear and had subcutaneous transponder chips implanted between the shoulder blades. Neonate kittens were handled following Land et al. (1998).

Criteria for selecting appropriate TX pumas for release in Florida were identified by Seal (1994). The 8 pumas were quarantined for a minimum of 30 days to screen for possible pathogens and were released at five sites throughout areas occupied by Florida panthers (Johnson et al. 1998).

All radiocollared *Puma* were monitored from fixed-winged aircraft three times weekly and locations plotted on 1:24000 USGS Topological Maps. Associations among radiocollared cats were noted during each flight. Universal Transverse Mercator coordinates were obtained from the maps and stored in electronic databases. Radiocollars were equipped with mortality sensors

and all carcasses detected were recovered and subjected to full necropsies by certified pathologists typically within 24 hours.

Panther population size was estimated at 60 to 70 individuals based on the sum of all extant radio-collared panthers (35), plus their known offspring (12) and all known uncollared panthers detected through intensive capture and survey efforts over the past year (nine) (D. Land, unpublished data). In addition to these known animals, we added five to ten individuals to our estimate to reflect the percentage of the population that remains undetected. Each year we encounter previously unknown individuals that are discovered through collisions with motor vehicles or are captured during routine surveys.

Results

Thirty-six intercross animals are known to have been produced, and 25 of these may still survive in the south Florida population (see Appendix). Two were recovered after colliding with vehicles, one died of unknown causes, and eight are strongly suspected of dying based either on tracking evidence or their dams' behavior. Evidence of the fates for another three intercross offspring has not been found subsequent to independence from their dams. Thus, probably 22 to 25 intercross cats exist presently within a total population of about 70 *Puma concolor*.

Table 1 shows the known contributions of each TX female to the south Florida population of *Puma concolor* as of August 2000. The numbers of descendants of each TX female are tallied by the type of intercross, with a dashed line separating each female's own offspring from her

Table 1. Known contributions of Texas pumas to the population of *Puma concolor* in south Florida as of May 2000.

TX101 (deceased)	4 F ₁ (-0) ^b 10 B-FP (-6) ^b 3 B-TX (-1) ^b 7 F ₂ (-1) ^b	6.25	5.00	4.75	+0.0
TX105	2 F ₁ (-0) ^b	1.00	1.00	0.50	+1.0 ^d
TX106	4 F ₁ (-2) ^b	2.00	1.00	0.50	+1.0
TX107	2 F ₁ (-0) ^b 3 B-TX (-1) ^b 7 F ₂ (-1) ^b	4.25	3.50	3.50	+0.0
TX108	3 F ₁ (-1) ^b 1 B-FP (-0) ^b	1.75	1.25	1.25	+1.0 ^{c,d}
% of living population	37.1%	21.8%	16.8%	15.0%	+4.3%
Notes: * Some descendants show up under two TX females from which they descend ^b Known or suspected deaths ^c Has recently shown denning behavior, but no litter has been found ^d No males known to be in area currently Generations: TX = female translocated from Texas F ₁ = intercross between TX female and FP male F ₂ = progeny of F ₁ x F ₁ mating B-TX = progeny of F ₁ male x TX backcross B-FP = progeny of F ₁ x FP backcross					

grand-offspring. The genetic contribution of a TX female is expressed as the number of copies of her genome that are represented in her descendants. Thus, each offspring contributes 0.5, and each grand-offspring contributes 0.25. The contributions of each female are given first for all descendants not known to have died. Subsequent columns show the reduced contributions obtained after omitting all animals that are likely to have died ('d' status animals in the Appendix), and then after omitting also the animals whose present status is unknown ('?' status animals in the Appendix). The last column shows additional contributions that may be likely if the TX fe-

males are allowed to continue breeding. TX101 was contracepted with melanogesterol acetate for two years prior to her death in March 2000 and TX107 was contracepted in April 2000. The other TX females are all eight or nine years old, and each may be expected to produce about one more litter. The last column of the table assumes that each of three TX females will produce two more offspring. At the bottom of the table are the total contributions of the TX lineages to the south Florida population and the percent contributions out of an assumed population of 70 animals for the subset of intercross descendants defined for each column.

It is possible that there were some undocumented intercross

animals in addition to those listed in the Appendix and tallied in Table 1. FP74 and FP84 were discovered as presumed offspring of F₁ female FP73 at ages that allow for the possibility that unobserved littermates could have already dispersed. TX105 exhibited denning behavior in August 1999, but no litter was found. In May 2000, we captured a female kitten from this litter (FP94) and found no sign of other littermates. The two male F₁ cats that are old enough to reproduce may have mated with unknown females: FP79 is suspected of siring four litters and other uncollared females are known to occur in his home range; FP65 was not the most mature, resident male in the

vicinity of any known breeding females, but he too may have sired unobserved litters. F_1 females K23 and K34 have not been observed since they would have become independent from their dams, but they may be alive and would be old enough to be breeding. One of TX108's litter of K45 and K46 is believed to have lived to at least the time of independence from its dam. If it is still alive, it would be old enough to have recently produced a litter. Other than these cases, it is unlikely that any undocumented intercross litters survive. The other TX and intercross cats have all been monitored sufficiently closely so that rearing of an unobserved litter would be unlikely, have had known litters at intervals that make it unlikely that additional litters could have been produced, or are still too young to have reared a litter.

Recognizing the above possibilities of undocumented intercross cats, it is likely that at least a few additional intercross cats exist. However, it would be unlikely that more than about four F_1 cats and perhaps four second-generation intercross cats evaded detection.

Discussion

The number of *Puma concolor* in Florida containing some Texas puma ancestry is not precisely known. However, the tabulation presented here is probably fairly complete, with perhaps several F_1 , F_2 , or backcross litters having been undetected. Similarly, it is not known precisely how many total *Puma concolor* are presently in the population. Assuming that most of the population is collared or otherwise known (such as kittens observed recently), it is likely that the total population

size is approximately 70 cats.

The likely representation of Texas puma genes in the south Florida population is about 15% to 16.8% if the aging Texas puma females are excluded, or about 19.6% to 21.3% if those TX females are included. With a projection of three more F_1 litters to be produced in the next few years before all the TX females become post-reproductive, we estimate that the ultimate representation of TX genes in the population would be 19.3% (15.0% now, plus 4.3% in future progeny of TX cats). This is perhaps fortuitously close to the original genetic restoration program goal of 20% representation.

It is possible that an additional one or two F_1 litters and perhaps up to 6 backcross (B-FP) litters were undetected. If these litters do exist, and each produced two surviving kittens, the TX contribution could be as much as 5.0 higher. In this case, representation would be increased by up to 7.1%. Thus, accounting for these possible intercross litters, the plausible range of current representation of TX puma genes in the population is from 15.0% to 28.9%.

If there are no further genetic manipulations, and if future breeding success is unrelated to the ancestry of cats, then the expectation is that the percent of TX genes in the population would remain near the current level. If animals containing more TX ancestry are more successful as breeders, then the representation of TX genes will gradually increase. The reverse will occur if natural selection favors the ancestral FP genes in the population. Most likely, however, change in the frequency of TX genes will occur primarily due to chance, at least during the early generations of intercrossing. Af-

ter a few more generations, almost all animals will likely contain representation from both ancestral sources, and the range of TX representation among animals will narrow.

Although the average representation of Texas puma genes is probably close to the 20% goal of genetic restoration, most of the TX genes are derived from only a few of the Texas cats. More than 40% of the TX genes are derived from TX101, and much of the remaining TX genes come from TX107. The unequal representations of the Texas pumas in the intercross descendants reduce the genetic diversity inserted into the population. Although five

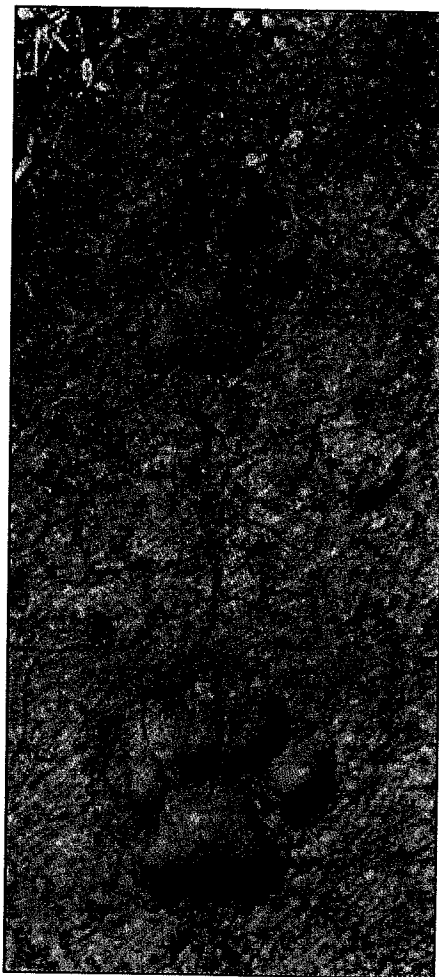


Figure 1. Florida panther prints. Photo courtesy of Friends of the Florida Panther Refuge.

Texas pumas have contributed some descendants, the diversity contributed by those five is the equivalent of about three "effective founders" (founders that have contributed equally to the population: Lacy 1989). As a result, while the genetic restoration will have considerably reversed prior inbreeding, that may be a relatively short-term benefit.

The population of *Puma concolor* in south Florida is still so small that inbreeding will likely become common again among the intercrossed descendants within the next few generations. Already, one intercross animal (FP85) is thought to be an inbred offspring of a mating of an F₁ female to her FP father. Although there are an estimated 70 animals in the population, perhaps only half are breeders of the current generation. (Many are

still kittens, and some females and many males may not be successful breeders.) The genetically effective population size would be still smaller. To counter a resumption of inbreeding and loss of genetic diversity, further releases of non-local cats may be considered as part of ongoing management of the genetic restoration. The effects of any future releases on the representation of TX ancestry achieved in this initial genetic restoration will need to be assessed.

Our analysis of the representation of Texas puma genes that has been achieved in the genetic restoration program for the Florida panther has been based on the pedigree as it is known from field observations through August 2000, about five years after the release of eight Texas females. This pedigree analysis will need

to be regularly updated and refined as field monitoring continues. In addition, molecular genetic analyses are underway that will likely provide confirmation and/or refinement of the pedigree, as well as information about the likely ancestry of previously unknown cats. These data on the ancestry of the cats in the population will then allow analysis of the effects of the genetic restoration on the morphological traits (including cowlicks, kinked tails, cryptorchidism, and atrial-septal defects), reproductive performance, survivorship, and population viability.

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Figure 2. Florida panther kitten. Photo courtesy of Friends of the Florida Panther Refuge.

