

INTROGRESSION LEVEL ACHIEVED THROUGH FLORIDA PANTHER GENETIC RESTORATION

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D Abstract: Florida panthers (*Puma concolor coryi*) exist today as a small isolated population of 60-70 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 pressures. 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 8 Texas puma (*P. c. stanleyana*) in 1995. Our goal was to achieve a 20% representation of Texas puma genes in the panther population. To date, 4 of the 8 cougars are still alive and have produced a minimum of 35 descendants, 25 of which are thought to still be alive. Based on our pedigree knowledge, we calculate that the panther population has 18-24% representation of Texas puma genes as the result of genetic restoration.

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 60-70 individuals exists on approximately 8810 km² (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 and Goldman 1946). This gene flow occurred as individuals dispersed widely and bred but human settlement of the eastern United States resulted in local extirpations of *Puma* thereby eliminating dispersal mechanisms from functioning. 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 and Soule 1986). Even with adequate habitat protection, these genetic concerns could lead to panther extinction.

Florida panthers exhibit reduced genetic variability when compared to western cougars (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, ed.). This plan called for the release of 8 female Texas cougars into areas occupied by Florida panthers to mimic the 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 any 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 cougars 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 cats.

STUDY AREA

The Florida study area encompassed most of interior Florida south of Orlando (28.3°N) and 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 savannahs, 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 and 137 cm respectively (Henry et al. 1994). The Texas cougars used for genetic restoration originated from west Texas, primarily in Pecos, Presidio, and Brewster counties.

METHODS

Florida panthers and Texas cougars were captured using trained hounds, anesthetized using a combination of Ketaset® (ketamine hydrochloride) and Telazol® (tiletamine Hcl and zolazepam Hcl) (McCown et. al 1990) and fitted with radiocollars (Telonics Inc., Mesa, AZ 84401). 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* > 4 months of age were vaccinated for feline viral rhinotracheitis, feline calicivirus, feline panleukopenia, and rabies. All animals were ear tattooed and had subcutaneous transponder chips implanted between the shoulder blades. Neonate kittens were handled following Land et al. (1998).

Criteria for selecting appropriate Texas cougars for release in Florida were identified by Seal, ed. (1994). The 8 cougars were quarantined for a minimum of 30 days to screen for possible pathogens and were released at 5 sites throughout areas occupied by Florida panthers (Johnson 1996).

All radiocollared *Puma* were monitored from fixed-winged aircraft 3 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 Board-certified

pathologists typically within 24 hours.

Panther population size was estimated at 60-70 individuals based on the sum of all extant radiocollared panthers (35) plus their known offspring (12) and all known uncollared panthers detected through intensive capture and survey efforts over the past year (9) (D. Land, unpublished data). In addition to these known animals, we added 5-10 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-five intercross animals are known to have been produced, and 26 of these may still survive in the south Florida population (see Appendix). Two were recovered after colliding with vehicles, 7 are strongly suspected of dying based either on tracking evidence or their dam's behavior. Evidence of the fates of another five intercross offspring has not been found, three of which would be independent from their dams. Thus, probably 21 to 26 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 14 April 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 grand-offspring. 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 status presently unknown ('?' status animals in the Appendix). The last column shows additional contributions that may be likely if the TX females are allowed to continued breeding. TX101 was contracepted with melanogesterol acetate for two years prior to her death in March 2000. TX107 has been recommended for such an implant, but at this time she has not yet been contracepted. The other TX females are all 8 or 9 years old, and each may be expected to produce about one more litter. The last column of the table assumes that each of four 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 already have dispersed. TX105 has recently shown denning behavior, but no litter has yet been found. 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. F₁ 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 that it could 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 F₁ cats and perhaps four second-generation intercross cats evaded detection.

maybe?

Table 1.

ID	Maximum Descendants ^a	Contribution	Contrib., less likely deaths	Contrib., less unknown fates	Potential future contribution
TX101 (deceased)	4 F ₁ (0) ^b 10 B-FL (6) 3 B-TX (0) 7 F ₂ (1)	6.5	5.75	5.00	+0.0
TX105	1 F ₁ (0) Another litter? ^c	0.5	0.5	0.0	+1.0 ^d
TX106	4 F ₁ (2)	2.0	1.0	1.0	+1.0
TX107	2 F ₁ (0) 3 B-TX (0) 7 F ₂ (1)	4.25	4.25	2.75	+1.0 ^e
TX108	3 F ₁ (1) 1 F ₂ (0)	1.75	1.25	0.75	+1.0 ^{de}
Contribution		15.0	12.75	9.5	13.5
% of population		21.4%	18.2%	13.6%	19.3%

^a 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 yet been found

^d No males known to be in area currently

^e To be contracepted when next captured

Generations:

TX = female translocated from Texas

F₁ = intercross between TX female and FP male

F₂ = progeny of F₁ x F₁ mating

B-TX = progeny of F₁ male x TX backcross

B-FL = progeny of F₁ x FL backcross

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 F₁, F₂, 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 14% to 18% if the aging Texas puma females are excluded, or about 19% to 24% if those TX females are included. With a projection of four 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 about 19%. 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-FL) 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, increasing representation by up to 7.1%. Thus, accounting for these possible intercross litters, the plausible range of representation of Texas puma genes in the population is from 13.6% to 28.5%.

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 of about 20%. 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 FL 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. After 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. *why*

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. As much as 50% 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 reduces the genetic diversity inserted into the population. Although five 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 sufficiently 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 F_1 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 (ref.) 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 FL and 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 March 2000, about 5 years [?] after the release of 8

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.

Literature Cited D

Davis, J.H. 1943. The natural features of southern Florida. Florida Geological Survey Bulletin 25. Tallahassee, Fl. 311 pp.

Gilpin, M.E. and M.E. Soule. 1986. Minimum viable populations: Processes of species extinction. pp. 19-34 in Conservation Biology: The science of scarcity and diversity. Sinauer Associates, Sunderland, MA.

Henry, J.A., K.M. Portier and J. Coyne. 1994. The climate and weather of Florida. Pineapple Press, Inc. Sarasota, Fl. 279pp.

Johnson, K.A., E.D. Land and M.A. Lotz. 1998. Florida panther genetic restoration. Annual performance report, Fl. Game and Fresh Water Fish Commission, Tallahassee, FL. 56pp.

Lacy, R.C. 1989. Analysis of founder representation in pedigrees: Founder equivalents and founder genome equivalents. Zoo Biology 8:111-124.

Land, E.D., D.R. Garman and G.A. Holt. 1998. Monitoring female Florida panthers via cellular telephone. Wildlife Society Bulletin 26:29-31.

McCown, J.W., D.S. Maehr, and J. Roboski. 1990. A portable cushion as wildlife capture aid. Wildlife Society Bulletin 18:34-36.

Maehr, D.S. 1990. The Florida panther and private lands. Conservation Biology 4:167-170.

Roelke, M.E., J.S. Martenson, and S.J. O'Brien. 1993. The consequences of demographic reduction in the endangered Florida panther. Current Biology 3:340-350.

Seal, U.S., ed. 1994. A plan for genetic restoration and management of the Florida panther (*Felis concolor coryi*). Report to the Fl. Game and Fresh Water Fish Commission. Conservation Breeding Specialist Group, Apple Valley, MN. 22pp.

U.S. Fish and Wildlife Service. 1987. Florida panther (*Felis concolor coryi*) recovery plan. Prepared by the Florida Panther Interagency Committee for the U.S. Fish and Wildlife

Service, Atlanta, GA. 75pp.

Young, S.P. and E.A. Goldman. 1946. The puma - mysterious American cat. Dover Publications, NY. 358pp.

I will add some reference to the concept of "effective population size"

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Appendix.. Texas pumas and known intercross *Puma concolor* in the south Florida population.

ID	Sex	Dam	Sire ¹	Birth	Gen.	Status ²	Notes	Collared	Genetic Samples
TX101	F			1991±2y	TX	D	Contracepted 11/97 Died 3/00	-	Y
FP65	M	TX101	FP45	12/96	F ₁	A		Y	Y
FP66	F	TX101	FP45	12/96	F ₁	A	With 2 kittens	Y	Y
K52	M	FP66	FP54	9/98	B-FL	d	Not observed after handled at den. Dam re-bred early.	-	N
K53	F	FP66	FP54	9/98	B-FL	d		-	N
K54	M	FP66	FP54	9/98	B-FL	d		-	N
K76	M	FP66	FP60	12/99	B-FL	D	Roadkill (2/00)	-	Y
K77	F	FP66	FP60	12/99	B-FL	a	Still with dam	N	Y
K78	F	FP66	FP60	12/99	B-FL	a		N	Y
FP73	F	TX101	FPx	9/95	F ₁	A	Litters in 98 and 99	Y	Y
FP74	M	FP73	FPx	6/97	B-FL	D	Roadkill (9/99). May have had littermates.	-	Y
FP84	M	FP73	Fpx	2/99	B-FL	D	May have littermates Died 4/00	-	Y
FP79	M	TX101	FPx	9/95	F ₁	A	Sired 12 known offspring	Y	Y
FP87	F	FP55	FP79	4/99	B-FL	A	Independent from dam	Y	Y
K61	M	FP55	FP79	4/99	B-FL	a	Not observed after independence	N	N
TX102	F			1991±2y	TX	D	Pregnant when hit by car (9/95)	-	Y
TX103	F			1991±2y	TX	D	Pregnant when died (3/99)	-	Y
TX104	F			1991±2y	TX	D	Not known ever to have bred. Died 4/98	-	Y
TX105	F			1991±2y	TX	A	May have a recent unrecorded litter	Y	Y
K34	F	TX105	FP16	9/96	F ₁	?	Not observed after independence	N	N
TX106	F			1991±2y	TX	A	With 1 kitten	Y	Y
K23	F	TX106	FP51	11/95	F ₁	?	Not observed after independence	N	N
K47	M	TX106	FP51	2/98	F ₁	d	Disappeared after male	-	N

ID	Sex	Dam	Sire ¹	Birth	Gen.	Status ²	Notes	Collared	Genetic Samples
							entered area		
K62	F	TX106	FP54	6/99	F ₁	d	No longer with dam	-	Y
FP83	F	TX106	FP54	6/99	F ₁	A	Still with dam	Y	Y
TX107	F			1992-2y	TX	a	Contraptured 4/00	Y	Y
FP70	F	TX107	FPx	5/97	F ₁	A	With 3 kittens	Y ³	Y
FP92	M	FP70	FP79	6/99	F ₂	a	Still with dam	Y	Y
FP88	F	FP70	FP79	6/99	F ₂	a		Y	Y
FP91	F	FP70	FP79	6/99	F ₂	a		Y	Y
FP71	F	TX107	FPx	5/97	F ₁	A	With 3 kittens	Y	N
K69	M	FP71	FP79	6/99	F ₂	d	Still with dam	N	Y
K70	F	FP71	FP79	6/99	F ₂	a	K69 presumed dead based on field sign	N	Y
FP86	F	FP71	FP79	6/99	F ₂	a		Y ³	Y
FP90	M	FP71	FP79	6/99	F ₂	a		Y	Y
K56	F	TX107	FP79	2/99	B-TX	?	Monitoring hampered by failure of dam's collar 11/99; dam recaptured 4/00	N	N
K57	M	TX107	FP79	2/99	B-TX	a		N	N
FP93	F	TX107	FP79	2/99	B-TX	A		Y	Y
TX108	F			1992-2y	TX	A		Y	Y
FP61	F	TX108	FP16	7/96	F ₁	A	With 1 kitten	Y	Y
FP85	M	FP61	FP16	3/99	B-TX	A	Inbred	Y	Y
K45	F	TX108	FP16	1/98	F ₁	a/d	One of K45 and K46 with dam prior to independence; other likely dead.	N	N
K46	M	TX108	FP16	1/98	F ₁	a/d		N	N

¹ FPx indicates that the sire was unknown, but temporal and/or spatial circumstances make it likely that it was a FP male, rather than an F₁ or other intercross.

² Status codes:

A = Radio-collared; monitored regularly

a = Presumed alive; observed recently or signs of continued presence with dam

? = Fate unknown; not collared and not recently observed

d = Presumed dead; disappeared under circumstances that suggest mortality is likely

D = Known to be dead

³ Radiotransmitter failed

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Florida(0%)	19	19 x 0.00 = 0.00	kittens 4 x 0.00 = 0.00			
F1(50%)	9	9 x 0.50 = 4.50				
FlxF1(50%)	5	5 x 0.50 = 2.50				
FlxFP(25%)	3	3 x 0.25 = 0.75	17 x 0.25 = 4.25			
FlxTX(75%)	1	1 x 0.75 = 0.75				
Total	37	8.50	21	4.25	Total 58	12.75
TX(100%)	4	4 x 1.00 = 4.00			62	16.75

Proportion of Texas genes in 37 collared cats
excluding 4 Texas females: $8.50 / 37 = 0.230 = 23.0\%$
including known kittens: $12.75 / 58 = 0.220 = 22.0\%$
(assuming all known Fx kittens average out to 25%)

including known kittens and 1 more litter from each of the 3 Texas
females without implants (6 F1 kittens), the proportion would be:
 $15.75 / 64 = 0.246 = 24.6\%$

Excluding the 6 probable nonbreeding FP adults (and excluding TX females):
(excluding known kittens) $8.50 / 31 = 0.274 = 27.4\%$
(including known kittens) $12.75 / 52 = 0.245 = 24.5\%$

including 1 more litter from each of the 3 Texas females
without implants (6 F1 kittens), the proportion would be:
 $15.75 / 58 = 0.272 = 27.2\%$

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If subregions were considered, proportions of Texas genes would be
locally higher in ENP and BCNP south of I-75.

ENP: Five collared panthers: TX105, TX108, 85Fx(25), 61F1, 94F1

Florida(0%)	0	0 x 0.00 = 0.00
F1(50%)	2	2 x 0.50 = 1.00
FlxF1(50%)	0	0 x 0.50 = 0.00
FlxFP(25%)	1	1 x 0.25 = 0.25
FlxTX(75%)	0	0 x 0.75 = 0.00
Texas(100%)	2	2 x 1.00 = 2.00
Total	5	3.25

Proportion of Texas genes in the 5 collared cats in ENP
including 2 Texas females: $3.25 / 5 = 0.65 = 65\%$

(excluding 2 Texas females)

Proportion of Texas genes in the 3 other collared cats:
 $1.25 / 3 = 0.417 = 41.7\%$