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Research Proposal - I

Molecular Genetic Analysis of Free Ranging Florida Panther Populations

by

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Submitted to Florida Game and Fresh Water Fish Commission

Attention: Dr. Tom Logan

BACKGROUND

The Florida panther (Felis concolor coryi) consists of a restricted population, of approximately 50 animals which are descended from a widely dispersed population which spanned the southeastern U. S. The present population is an endangered subspecies which has been awarded protection under the Endangered Species Act by the U. S. Fish and Wildlife Service (USFWS) of the Department of Interior, the National Park Service, and the Florida Game and Freshwater Fish Commission. A long term study of the free ranging panthers was initiated in 1976 in an effort to learn about, and to stabilize the ecosystem occupied by the panther.

The remaining population of Florida panthers has been subdivided by natural phenomena into at least two apparently isolated populations; one in the Big Cypress Swamp ecosystem (BCS) and the second centered in the Everglades National Park (ENP). These populations are separated by a 50 km area termed Shark River Slough, a remnant of a shallow "river of grass" which historically flowed from Lake Okeechobee to the southern tip of Florida. Today's ENP population is small, ≤ 10 animals, and the remaining panthers occupy the Big Cypress area. ←

There have recently been some surprising phenotypic observations which tend to stratify the two populations. BCS animals generally display a midthoracic cowlick and kinked tail (phenotypes which have developed as an often mentioned diagnostic phenotype for coryi), while most of the ENP panthers lack these two markers. Further, a novel allele of the *APRT* biochemical genetic locus was recently documented in all panthers in ENP, but was found in only 1% of 21 BCS specimens. These traits raise the possibility, but do not prove, that the ENP and BCS animals may have distinct genealogical backgrounds. Further, the captive "Piper" population, which is said to be descended from coryi, and has been ←

rumored to have been mixed with other non-coryi subspecies, shares some of the phenotypes found in ENP. These observations raise the possibility that the ENP population may contain genetic materials from non-coryi individuals released into the Everglades. However, it is also possible that the ENP and BCS population show genetic differences due to genetic drift; a real possibility in small populations of this size. ←

In order to discriminate between the introgression vs genetic drift hypotheses, it is important to understand that each scenario makes distinct predictions, particularly if the putative introgressor is the Piper captive animals. The prediction of the genetic drift hypothesis is that ENP or BCS specific genotypes which are shared with Piper would be equally distributed in the two free ranging populations. Conversely, the introgression hypothesis would predict that population specific phenotypes shared with Piper would be non-randomly dispersed in the introgressed population (e.g., ENP). The present data discovered only three informative traits. To be rigorous, more would be required. ← That is the object of this proposal: to increase the number of informative genetic traits which discriminate the two populations so that a definitive conclusion can be drawn.

PROPOSAL

1. Collect blood, tissue, and skin biopsies from several individuals from the three populations. Biopsies will be explanted into tissue culture and expanded. Genomic and mitochondrial DNA will be extracted from each (1, 2).
2. Genetic diversity within and between populations will be assessed by Southern Blot analysis following digestion with restriction enzymes. The individual methods employed include:
 - a) Mitochondrial DNA restriction map analysis using feline mtDNA as a hybridization probe (3-6).
 - b) Restriction fragment length polymorphism (RFLP) analysis of the feline major histocompatibility complex. We will use molecular clones derived from domestic cat in this laboratory for this purpose (7, 8).
 - c) DNA fingerprints of hypervariable genes in felids. Our laboratory has identified three molecular clones also from the domestic cat which have been shown to be effective for such measurements (9, 10).
3. The results will be analyzed using the principles of maximum parsimony as well as cladistic analysis to test the hypothesis (11-13).
4. DNA and previously collected isozyme results will be formalized for publication once the solution is apparent.

5. These data will form the beginning of using molecular data to approach the important question of the relationship between F. concolor coryi and the other 20+ subspecies of Puma. This analysis will be part of a larger long term analysis which will estimate the time interval which has elapsed since coryi and other puma "subspecies" shared a common ancestor. This is not a trivial question with an obvious answer; especially in light of recent phylogenetic studies which show the numerous or rather ancient feline lineage which diverged from other cat species 5-6 million years ago (14-16).

Will Paper state in Paper collection be used?

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Proposed Budget

I. Laboratory Supplies for DNA Analysis (40 samples)

(1) DNA extractions, kits, tubes, <u>pipets</u>	\$ 339.30
(2) Restriction enzymes	1,644.00
(3) Electrophoretic supplies	379.40
(4) Materials for Southern transfer	1,554.75
(5) Hybridization reagents	1,635.00
(6) Wash solutions and autoradiography	355.45

Total	\$ 5,907.90
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II. Personnel

(1) Technician: full-time/training, processing materials, organize results (12 months)	\$22,490.00
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III. Travel

(1) Collection trip to Florida 2 trips @ \$750.00	\$ 1,500.00
(2) Publication costs	\$ 500.00

Total requested	\$30,397.90
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Materials for DNA Analysis

January 10, 1989

Florida Panthers collected:	23 from Big Cypress
	+7 from Everglades National Park
	+1 from Corbett Management Area
	<u>31</u>
	+6 Piper stock
TOTAL:	<u>40</u>

For the following cost estimates the number 40 was used.

I. DNA extractions		
a. extraction reagent kit: 20 samples per kit @ \$150/kit	300.00	
b. polypropylene tubes 6/sample = 1/2 case @ \$50/cs	25.00	
c. disposable pipets 1 box of 50	14.30	
		339.30
II. Restriction enzyme digests		
a. microfuge tubes: 37 enzymes x 40 = 1480 @ \$12/500	36.00	
b. enzymes		
1. for MHC: 5 enzymes @ 20U x 40	38.60	
2. for MtDNA: 30 enzymes @ 10U x 40	1,225.00	
3. for DNAfp: 2 enzymes @ 80U x 40	256.00	
c. pipet tips: 45/gel x 76 = 3420 @ \$2.60/100	88.40	
		1,644.00
III. Electrophoresis		
a. agarose: 76 gels x 3 gm ea. = 228 gm @ \$310/500 gm bottle	155.00	
b. TAE buffer: 3.3 liters/gel x 76 = 251 l. @ \$.43/liter	108.00	
c. Ethidium bromide: 10 gm.	10.00	
d. Polaroid film: 76 gels @ \$1.40/gel	106.40	
		379.40
IV. Southern Blots		
a. denaturation: 500ml/gel x 76 @ \$.63/liter	24.00	
b. neutralization: 500ml/gel x 76 @ \$4.95/liter	188.00	
c. membrane: 15 filter/roll @ \$120.00/roll = 5 rolls	600.00	
d. 10X SSC: 1 liter/gel x 76 @ \$6.50/liter	494.00	
e. Whatman paper: 2.5 sheets/gel x 76 @ \$95.00/100 = 2 bx	190.00	
f. paper towels: 1 pk/gel x 76 @ 16pks/cs = 5 cs @ \$11.75/cs	58.75	
		1,554.75

V. Hybridization		
a. probes: 1 MHC, 1 MtDNA, 2 DNAfp; 4 x \$300 plasmid prep	1200.00	
b. labelling: 1 labelling reaction will do four filters		
1. ³² P - for 20 labellings need 2 mCi @ \$92.00/mCi	184.00	
2. Sephadex columns 20 x \$2.2044.00		
3. random prime kit for 20 reactions	125.00	
c. bags: 2 filters/bag, 20 bags/roll @\$3.00/roll	6.00	
d. hybridization solution: 25ml/filter x 764 = 2 L @ \$38.00/l	76.00	
		1,635.00

VI. Washes and autoradiography		
a. 250ml per wash step/filter		
1. 2 washes 2XSSC+1%SDS = 500ml x 76 @ \$3.20/liter	121.60	
2. 1 wash 1XSSC+1%SDS = 250ml x 76 @ \$2.65/liter	50.35	
3. 1 wash .2XSSC+1%SDS = 250ml x 76 @ \$2.13/liter	40.50	
b. autorad film		
1. XAR-5 - 76 @ \$45.00/50 sheets	68.00	
2. XRP-5 - 76 @ \$25.00/50 sheets x 2	75.00	
		355.45

GRAND TOTAL - - - - - \$5907.90

Other numbers used in calculations:

- For MHC 5 enzymes; 2 gels to run through 40 samples for total of 10 gels
- For MtDNA 30 enzymes; 2 gels to run through 40 samples for total of 60 gels
- For DNAfp 2 enzymes; 3 gels to run through 40 samples for total of 6 gels
- 76 total gels/filters