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Research Proposal -II

An Estimate of Endemic Genetic Diversity and Molecular Taxonomy of
The Florida Panther
Felis concolor coryi

by

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Submitted to Florida Game and Freshwater Fish Commission
Attention: Dr. Tom Logan

BACKGROUND

The American puma (also termed mountain lion, cougar, panther and others) has a distribution which extends from northern Alberta to the southern tip of South America. Approximately 30 different subspecies have been described since the original description of the species. The different subspecies differ greatly in size with average weight ranging from 100-225 lbs. The Florida panther (Felis concolor coryi) is a relict population of less than 50 individuals which is descended from a larger population which ranged throughout the southeastern U.S. Today's Florida panthers are restricted to two small populations located in the Big Cypress Swamp ecosystem and the Everglades National Park in southern Florida.

When free-ranging populations fall to very low numbers, the occurrence of chance demographic losses in the population become very important in developing a management plan (1,2). With respect to the Florida panther, these events have been catastrophic with large percentages of the remaining panthers being destroyed by automobile traffic and illegal hunting.

A threat to small populations involves the tendency (or necessity) to breed with close relatives despite instinctive strategies which have evolved to avoid inbreeding. When inbreeding is widespread and continuous, it can produce a number of physiological impairments to survival and reproduction termed inbreeding depression (3,4). Further, populations which are derived from extensive inbreeding decrease endemic genetic diversity. When polymorphic loci which participate in resistance to infectious disease are homogenized by inbreeding the affected population becomes particularly vulnerable to future disease outbreak.

The purpose of this proposal is two-fold. First, an estimate of the extent of genetic diversity which remains in the present Florida panther population would be assessed. The amount of genetic diversity at marker genes and at loci which participate in immune defenses (namely the feline major histocompatibility complex) would be compared to several other populations of puma from different locales in the hemisphere as well as to other felid species previously studied (7,8). Second, the molecular genetic analysis of thirteen puma subspecies would be used as a basis for molecular taxonomy (Table 1). Based upon the principles of the molecular clock hypothesis, the topology or phylogeny of subspecies divergence would be estimated. An important parameter derived from these studies would be the time elapsed since the Florida panther and other subspecies have shared a common ancestor. This value would translate into management information directly by providing a measurement which relates to the efficacy of subspecies maintenance vs. managed hybridization. The answer to this question is rather difficult since puma fossils range back to the Pliocene (2-8 million years ago) and the closest feline relative to the puma (based on earlier molecular data) dates back to over 6 million years. When one considers that the five living species of great cats, Panthera, diverged in the Pleistocene within the past 1.8 million years, the puma evolutionary history is indeed a fascinating question.

PROPOSAL

1. Collect blood, tissue, and skin biopsies from 10 or more individuals from each of 13 puma subspecies (Table 1). Biopsies will be explanted into tissue culture and expanded. Genomic and mitochondrial DNA will be extracted from each. In addition soluble isozyme extracts will be prepared.
2. Genetic diversity within and between populations by three methods presently employed in our laboratory. These include:
 - a) Allozyme typing of 40-50 enzyme markers previously used to analyze the population of 10 species of felids (7,9).
 - b) Mitochondrial DNA restriction map analysis using feline mtDNA as a hybridization probe (10,11).
 - c) Restriction fragment length polymorphism (RFLP) analysis of the feline major histocompatibility complex (MHC). We will use molecular clones derived from the domestic cat in our laboratory for this purpose (12,13).
3. A table of unit characters and a matrix of molecular genetic distance between subspecies and between species will be derived using the same measurements described above: allozyme markers, mtDNA, MHC, RFLP.

- a) The molecular markers will be used as unit characters to construct minimum distance phylogenies based upon the principles of Hennigian cladistics.
 - b) The distance matrices will be used to construct evolutionary trees using phenetic methods. Further these trees can be tested for conformance to a molecular clock. In cases where such tests prove to be efficacious, the trees can be calibrated with evolutionary time and thereby employed to estimate the time of divergence nodes.
4. Results from genetic diversity and molecular taxonomy will be analyzed using appropriate evolutionary algorithms and formalized for publication in peer reviewed journals.

References

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DISTRIBUTION OF THE PUMA, *Felis concolor*

Subspecies:

1. *Felis concolor concolor*
2. *F. c. cougar*
3. *F. c. missoulensis*
4. *F. c. hippolestes*
5. *F. c. oregonensis*
6. *F. c. cancouverensis*
7. *F. c. olympus*
8. *F. c. californica*
9. *F. c. kaibabensis*
10. *F. c. browni*
11. *F. c. improcera*
12. *F. c. azteca*
13. *F. c. stanleyana*
14. *F. c. coryi*
15. *F. c. mayensis*
16. *F. c. costaricensis*
17. *F. c. bangsi*
18. *F. c. söderströmi*
19. *F. c. capricornensis*
20. *F. c. anthonyi*
21. *F. c. incarum*
22. *F. c. borbensis*
23. *F. c. osgoodi*
24. *F. c. acrocodia*
25. *F. c. greeni*
26. *F. c. puma*
27. *F. c. cuberae*
28. *F. c. pearsoni*
29. *F. c. patagonica*
30. *F. c. araucanus*

extension of *F. c. missoulensis*
to the north and neighboring areas

to uncertain limits of subspecific distribution

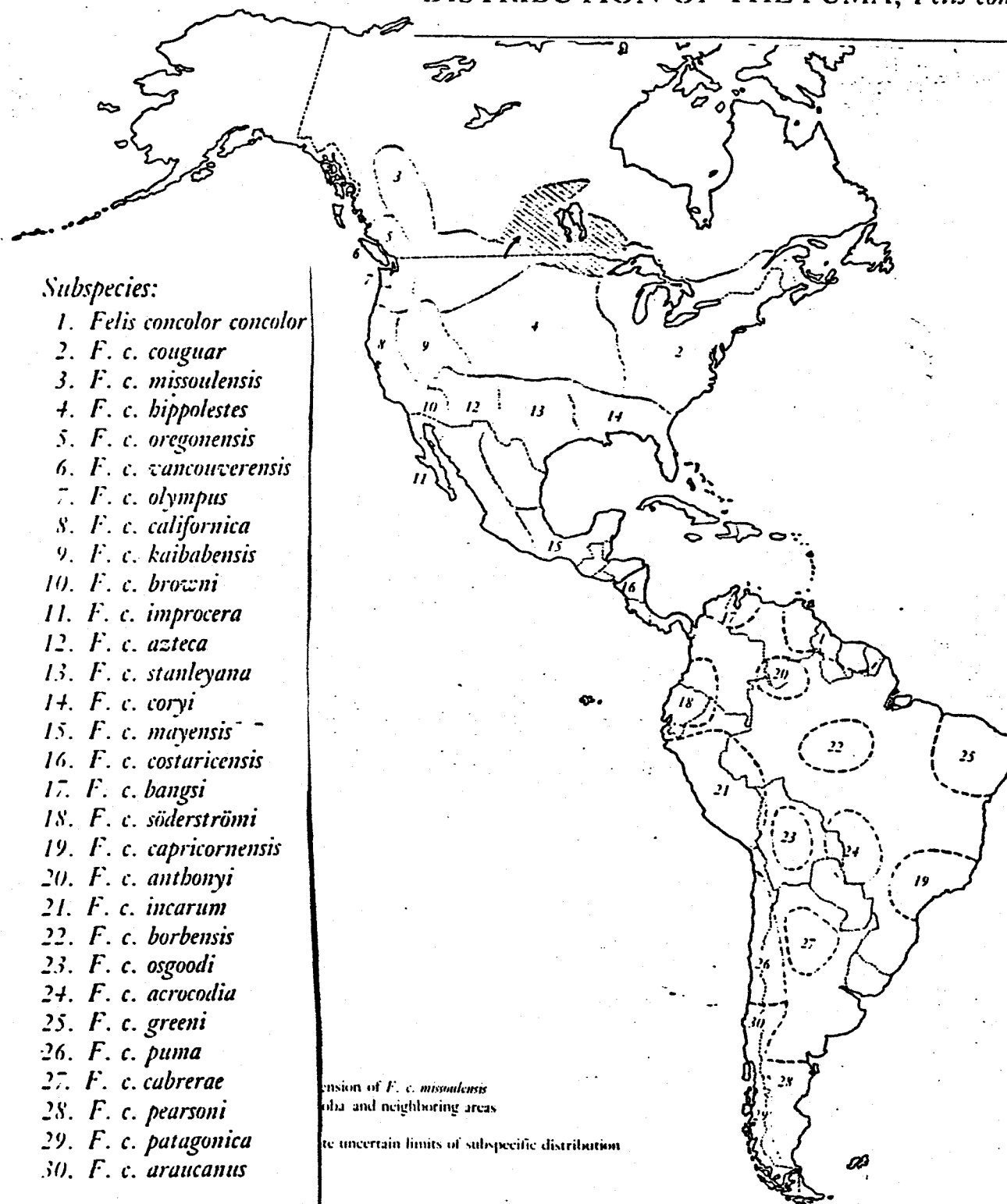


Table 1
Puma Sample Inventory at the NCI
(February, 1989)

<u>Felis concolor</u> Subspecies/Population	Locale	No. Specimens		Collaborator*
		Collected	Projected	
<u>coryi</u>	Big Cypress	25	-	D. Maehr; C. Belden
<u>coryi</u>	Everglades	8	-	S. Bass
<u>coryi</u> /Piper	Captive/Florida	7	-	L. Redfield; M. Jones
<u>stanleyana</u>	Texas	22	4	R. McBride
<u>californica</u>	California	4	8	
<u>brownii</u>	Arizona	2	5	H. Shaw; A. Fuller
<u>azteca</u>	Arizona	5	-	H. Shaw; A. Fuller
<u>kaibabensis</u>	Nevada	4	6	W. Bates; F. Lindsey
<u>hippolestes</u>	Colorado	11	-	A. Anderson
<u>oregonensis</u>	Oregon, Brit. Colum.	11	10	D. Hebert; R. Gagliuso
<u>missoulensis</u>	Alberta, Oregon	10	10	M. Henjen; W. van Dyke; R. Schmidt
<u>mayensis</u>	Belize	0	10	R. McBride
<u>greeni</u>	Brazil	5	5	H. Seuanez
<u>acrocodia</u>	Bolivia	0	20	R. McBride; F. Simone
<u>patagonica</u>	Chile	0	10	W. Franklin
Total		114	88	

*Coordinated and managed by Dr. Melody Roelke

BUDGET

I. Travel Expenses - Collection Trips

(a)	Patagonia	<u>F.c. patagonica</u>	N = 10
		Collaborators - W. Franklin, W. Johnson	
(1)	Travel for W. Johnson		\$ 2,100.00
(2)	Per diem for W. Johnson		1,900.00
(3)	Travel for M. Roelke and Assistant		4,200.00
(4)	Per diem for M. Roelke and Assistant		2,200.00
(5)	Shipping		500.00
			\$ 10,800.00
(b)	Belize	<u>F.c. mayensis</u>	N = 10
		Collaborator - R. McBride	
(1)	Transport, capture, hold free-ranging cats		\$15,910.00
(2)	Travel for M. Roelke and Assistant		1,800.00
(3)	Per diem for M. Roelke and Assistant		2,200.00
(4)	Shipping		500.00
			\$20,410.00
(c)	Bolivia/Paraguay	<u>F.c. acrocodia</u>	N = 20, 2 locales
		Collaborator - R. McBride	
(1)	Transport, capture, hold wild puma		\$18,020.00
(2)	Travel for M. Roelke and Assistant		2,400.00
(3)	Per diem for M. Roelke and Assistant		2,200.00
(4)	Supplies, Shipping		2,000.00
			\$24,620.00
(d)	North Alberta (Calgary)	<u>F.c. missoulensis</u>	N = 10
		Collaborators - R. Schmidt	
(1)	Travel for M. Roelke and Assistant		\$ 1,400.00
(2)	Per diem for M. Roelke and Assistant		900.00
(3)	Vehicle renting and air tracking		1,100.00
			\$ 3,400.00

(e) British Columbia F.c. oregonensis N = 10
Collaborators - D. Hebert

(1) Travel for M. Roelke and Assistant	1,600.00
(2) Per diem for M. Roelke and Assistant	2,200.00
(3) Dogs, houseman, vehicle	2,000.00
(4) Air tracking	500.00

\$6,300.00

I. TOTAL

\$65,130.00

II. Laboratory Supplies for DNA Analysis (190 samples)

(1) DNA extractions, kits, tubes, pipets	\$ 1,200.00
(2) Restriction enzymes	4,965.60
(3) Electrophoretic supplies	1,320.12
(4) Materials for Southern transfer	5,757.70
(5) Hybridization reagents	2,185.00
(6) Wash solutions and autoradiography	1,352.60

\$16,781.02

II. TOTAL

\$16,781.02

III. Isoenzyme Analysis 190 samples, 40 common systems
(14 samples/gel; 11 gels/system;
440 gels total)

(1) Starch, 60 g/gel 5 x 5 kg tubs	\$ 1,775.00
(2) Acrylamide (1 bottle liquid)	60.00
(3) Buffer ingredients	72.00
(4) Reagents for isozyme stains	
(a) Reagents for regular stains (substrates, dyes, cofactors)	2,482.00
(b) Radioactive substrates	3,000.00
(5) Film (2 pictures per slice) 1,320 pictures @ \$1.35 each	1,782.00

(6) Supplies	90.00
(a) pipets (2 cases @ \$45/case)	156.00
(b) pipet tips (1/animal x 40 @ \$2.60/100)	47.00
(c) filter paper (1/2 case)	

TOTAL	<u>\$ 9,464.00</u>
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III. TOTAL	<u>\$ 9,464.00</u>
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IV. Laboratory Personnel

(1) Technician/postdoctoral fellow	
Full-time/training processing materials	
24 months -- Salary + fringe	\$46,700.00

IV. TOTAL	<u>46,700.00</u>
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TOTAL REQUESTED	<u>\$138,075.02</u>
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NAME	STAIN	CAT #	SIZE	PRICE
BASIC NEEDS:				
Starch		S-4501	5 kg	\$330.00
Mineral Oil				\$3.00
Film Type 52; 20/box		wh 67800010	box	\$27.20
Agar		difco 0142-02		\$11.10
Acrylamide		enprotech		\$60.00
Reagents for regular stains:				
2-D-Phosphoglycerate	PGAM	P-0257	250 mg	\$50.50
3-Phosphoglyceric acid	ENOL	P-0259	1 g	\$29.20
4-Me-Um a-D-galactoside	a-gal	M-7633	100mg	\$58.95
4-Me-Um acetate	ES-D	M-0883	5g	\$12.25
4-Me-Um phosphate	ACP1	M-8883	100 mg	\$5.80
4-Me-Um β-glucuronide	GUS-B	M-9130	100 mg	\$34.55
4-Me-Um-β-galactoside	β-gal	M-1633	1g	\$18.40
6-Phosphogluconate	PGD	P-6888	100 mg	\$19.85
a-ketoglutarate	GOT	K-1750	25g	\$7.85
a-Naphthyl acetate	Es-a	N-8505	5g	\$5.10
a-Naphthyl acid Phosphate	Acp-2	N-7000	5g	\$19.15
Adenosine	ADA	BM 102 075	5g	\$6.50
ADP		A-6521	1g	\$36.50
Alanine		A-7627	1g	\$4.10
Ammonium Molybdate		I-0716 Baker		\$5.00
AMP		A-1877	1g	\$13.70
Ascorbic Acid		A-7631	25 g	\$5.00
Aspartic Acid		A-9256	25g	\$4.05
ATP		A-0770	500 mg	\$28.60
Bomphenol Blue powder	TF, Alb			
Cis-aconitic acid	ACON	A-3412	1G	\$12.80
D-Lactic Acid syrup	LDH	L-1375	100 ml	\$4.85
FAD		F-6625	250 mg	\$31.00
Fast Blue BB		F-3378	5g	\$17.90
Fast Red ITR		F-1375	25 g	\$3.95
Ferric Chloride		F-2877	250 g	\$11.70
Fluorescein diacetate	CA-2	F-7378	5g	\$5.50
Fructose 1,6-diphosphate	PK	F-0252	10 g	\$9.70
Fructose-6-Phosphate	GPI	F-3627	100 mg	\$6.50
G-3-P free acid	G3PD	G-5251	25mg	\$25.70
glucose (dextrose)		G-8270	100g	\$6.80
Glucose 1,6-diphosphate	PGM	G-7137	250 mg	\$315.95
Glucose-1-Phos	PGM	G-6875	5g	\$12.90
Glucose-6-Phosphate	G6PD	G-7250	5g	\$33.40
hydrogen peroxide		H-1009	100 mL	\$9.95
Inosine	NP	I-4125	5g	\$8.40
Iodine		sigma 700-2	100 mL	\$8.25
Isocitrate	IDH	I-1252	5 G	\$35.95
ITP	ITP	I-1004	500 mg	\$31.70
KCl		P-4504	250 g	\$5.65
Leu-gly	Pep B	L-9750	1g	\$28.80
Leu-tyr	Pep C	L-0501	1g	\$26.50
Malic Acid		M-1125	5g	\$10.70
Mannose-6-Phosphate	MPI	M-6876	1g	\$188.75
methyl glyoxal	GLO	M-0252	25 mL	\$11.40
MgChloride		M-0250	500 g	\$12.15

NAME	STAIN	CAT #	SIZE	PRICE
MgSulfate		I-2500 baker		\$6.50
Mn Chloride		M-87 fisher		\$3.40
MTT		M-2128		\$25.00
N-formyl-methionine	ACY-1	F-3377	1g	\$12.85
Na Arsenate		S-222 fisher		\$5.00
Na Pyrophosphate		S-390 fisher		\$5.00
Na pyruvate		BM 128 147	25 g	\$9.20
NaCl		wh 68101033	500g	\$4.42
NAD		N-7004	1g	\$18.50
NADH		BM 107 735	500 mg	\$33.25
NADP		BM 240 354	5g	\$425.25
NADPH		BM 107 824	100 mg	\$47.90
NaOH		S-5881	500 g	\$12.40
Naphthol acetyl β -D-glucosaminide	HexA	M-2133	100 mg	\$17.65
orthodianisidine		D-3252	1g	\$5.70
Oxidized Glutathione	GSR	G-4501	1g	\$33.60
PEP	PK	P-7002	25 mg	\$6.05
Phe-Pro	Pep D	P-6258	100 mg	\$7.50
Phosphocreatine	CPK	P-6502	5g	\$26.30
PMS		P-9625		\$25.00
polyvinylpyrrolidine (PVP)		P-6755	100 g	\$11.10
Potassium Ferricyanide		P-8131	100 g	\$6.85
PRPP	APRT	P-9758	1g	\$25.00
Pyridoxal-5-Phosphate		P-9255	100 mg	\$4.00
Reduced Glutathione	GLO	G-4251	5g	14.05
Rivanol	TF			
Val-Leu	Pep A	V-1625	1g	\$24.70

TOTAL: \$2,058.07

REAGENTS FOR RADIOACTIVE STAINS:

14C-Adenine	NEC-005H	1mCi	\$1,167.00
14C-Hypoxanthine	NEC-149	1mCi	\$2,325.00
		TOTAL:	\$3,492.00

ENZYMES

G6PD	G-7878	250 u	\$15.70
Hexokinase/G6PD	H-8629	200 u	\$9.45
Horseradish Peroxidase	BM 128 066	100Ku	\$95.95
Adenosine Deaminase			
Amino Acid Oxidase	BM 102 792	1mg	\$38.95
α -Glycerophosphate Dehydrogenase	G-6751	1000u	\$26.00
Glycerinaldehyde-3-Phos Dehydrogenase	BM 105 686	20 mg	\$10.60
Isocitrate Dehydrogenase	I-2002	25 mg	\$13.25
Lactate dehydrogenase	BM 106984	50 mg	\$67.15
Nucleoside phosphorylase (NP)	BM 107956	1mg	\$13.55
Phosphoglucose Isomerase (PGI)	BM 127396	2 mg	\$10.50
Xanthine Oxidase	BM 110 434	20 u	\$26.35
		TOTAL:	\$327.45

NAME	STAIN	CAT #	SIZE	PRICE
Buffer Ingredients:				
Na Acetate				\$10.00
Tris base	wh	68101048	500 g	\$16.85
Sodium Phosphate, dibasic	S-9390		500g	\$17.50
Sodium Phosphate, monobasic	S-9638		500 g	\$23.95
Citric acid	C-0759		100g	\$5.45
Boric acid	B-0252		100g	\$4.90
Histadine	H-8125		100 g	\$15.10
maleic acid	M-0375		500 g	\$9.65
EDTA				\$3.00
			TOTAL:	\$106.40
OTHER REAGENTS:				
Acetone	wh	68101000	btl	\$8.65
coomossie blue	B-0630		5g	\$6.70
DMF	D-4254		100 mL	\$4.10
DMSO	D-5879		100 ml	\$6.20
ammonium persulfate	A-9164		25 g	\$7.90
Lanthanum Chloride	L-4131		100g	\$17.75
methanol	wh68101036		btl	\$14.90
β -mercaptoethanol	M-6250		100 mL	\$8.25
Hydrochloric acid				\$5.00
acetic acid	wh	68101035		\$5.00
sulfuric acid				\$5.00
			TOTAL:	89.45
LAB SUPPLIES:				
Pipet tips	wh	66401388	1000/cs	\$21.45
Pipets	wh	66401371/66/75	500/cs	\$45.00
Microfuge tubes	PGC	500-001	1000/bag	\$35.00
			TOTAL:	101.45
GRAND TOTAL:				6606.12