

Further Studies to Determine Subspecies Identity of Panthers by Examining the NADH-5 Gene Obtained Originally From Clotted Blood and Hair for Conservation Applications

By: Marta Hubert and Marimer Gonzalez

How did the question arise?



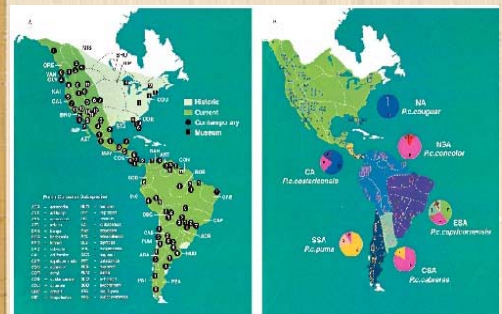
<http://www.destination360.com/south-america/ecuador/ecuador-map.php>

Sasha's Plight: South American vs. Florida Panther



Sasha, unknown feline species, possibly a Florida panther (*Puma concolor coryi*). Photo taken by Dr. Barry Barker.

Previous Research Literature: Practice in Conservation Genetics



Previous Research Literature: Practice in Conservation Genetics

Founder Effect

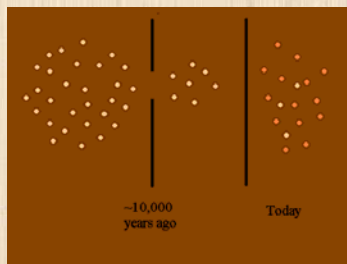


Image Courtesy of Marimer Gonzalez

Past Research: Isolating DNA from hair

Sasha's Kittens



Photo Courtesy of Joe LaFauci

How Our Research Evolved



<http://www.schoolhousevideo.org/Media/Shadow%20cat.jpg>

How Was This Done?

- SYBR Green Test



Amplifying DNA Using PCR

Agarose Gel Analysis



Sample A: Seminole

Purifying DNA

Agarose Gel Analysis

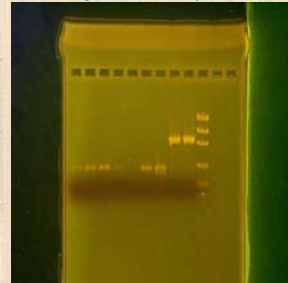
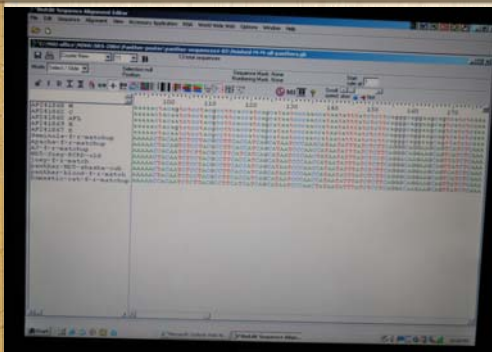


Photo courtesy of Vince Richards

Sequencing DNA



Results

Samples	Locus		
	102	170	260
M (N.America-190 cats)	G	C	A
C (Costa Rica/Panama - 2 cats)	A	C	G
AFL (Central and S. Am.-2 cats)	A	C	A
N (Washington State - 4 cats)	G	C	A
K (Brazil - 1 cat)	A	T	A
Seminole	A	C	A
Apache	G	C	
AJ	A	T	A
Joey	A	C	A
Sasha's cub	A	T	A
Sasha	A	T	A

Sasha's Origin Solved



Another Look

Samples	Locus		
	102	170	260
M	G	C	A
C	A	C	G
AFL	A	C	A
N	G	C	A
K	A	T	A
Seminole	A	C	A
Apache	G	C	
AJ	A	T	A
Joey	A	C	A
Sasha's cub	A	T	A
Sasha	A	T	A

References

- Culver M, W.E. Johnson, J. Pecon-Slattery, and S.J. O'Brien. "Genomic Ancestry of the American Puma (Puma concolor)." *Journal of Heredity* (2000): 186-196.
- "DNAzol BD Description and Protocol." [Mrcgene.com](http://www.mrcgene.com) 19 Aug. 2004. <<http://www.mrcgene.com/dnabd.htm>>.
- Rabolli, Julia. "Genetic Sampling of Hair to Identify Origin of Florida Panthers." (2004).
- Shaw, Bridgette. "Can Non-invasive Genetic Sampling Reveal the Identity of Felid Species on the Verge of Extinction?" (2003).

Photo References:

- <http://www.chem.agilent.com/cag/feature/0704/feature_graphics/BAmain.jpg>

Acknowledgements

- Dr. Barker



- Dr. Schmitt



- Dr. Sherman
- Dr. Fernandez
- Vince Richards
- Barbara and Jeff Harrod, and Aimee of Vanishing Species Wildlife
- Carlos De Jesus
- The Thermocycler