Tracking Wild Ancestry of Captive Felids: A Pilot Study

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Introduction

- Populations of many species of felids are dramatically decreasing. Reintroduction of captive felids into their native habitats could be a possible solution[1,2].
- There are many cats in captivity, but their origins are, in most cases, unknown[3].
- Scat sampling is a non-invasive method to obtain the genetic information about individuals from endangered populations without having to capture, handle, or observe the animals[4].
- To obtain ancestral information and original location of captive individuals from 6 wild cat species, we sequenced mitochondrial DNA and genotyped microsatellites of nuclear DNA, using DNA extracted from scat samples.

[1]Swanson 2003, [2]Kleiman 1989, [3]Luo et al. 2008, [4] Waits & Paetkau 2005

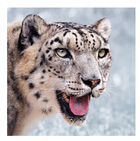
Tiger

Puma

Pallas Cat

Jaguar

Asian Golden Cat



Snow Leopard

Methods

- Genomic DNA extracted from 48 scat samples from institutions in the United States, Singapore, and Switzerland.
- 4 regions of mtDNA (ATP6, Cytochrome oxidase B, 12S, 16S) amplified through Polymerase chain reaction and sequenced on ABI 3730 machine.
- 12 microsatellites loci were genotyped through multiplex PCR.
- Analysis was carried out in the following programs: Sequencher 4.9, MacClade 4.08, GeneMapper 4.0, GeneClass 2.0, ML-Relate, Cervus 3.0.

Conclusion

- If enough genetic information is available, we can track the ancestry of captive felids to their native habitats.
- Better results could be obtained by using blood samples from captive individuals.
- For the other three species, the analysis is not complete yet.

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Results

- **Tiger samples**:

1) *mtDNA:* all 3 samples matched 100% with known wild felids from Laos, Thailand and Myanmar for 12S, 16S and CytB. The ATP6 region of the samples matched 100% with a GenBank sequence of *P. t. amoyensis* (Southern China), which is considered extinct in the wild. It also matched with one individual from Thailand and one from Laos.
2) *Genotyping*: Although the three samples are siblings, only one of the samples, (ZOO043), was assigned to Thailand ($p<0.01$). The other two where not assigned to any location. These results could be biased by small sample size.

Table 1: Population Aggregation Analysis for the tiger mtDNA sequences

Mitochondrial DNA region	12S			16S	ATP6																		CYTB									
Haplotype/AMNH ID (accession number)																							*							*		
Felix catus (NC001700)	1409	1541	1553	3013	8845	8855	8867	8878	8881	8887	8888	8889	8909	8911	8917	8941	8942	8947	8953	8955	8959	15104	15115	15223	15268	15379	15403	15409	15553	15743	15756
ALT1/4 (JF357974/JF357973)	C	T	T	C	T	A	C	T	G	C	G	T	T	G	C	C	C	C	A	A	G	G	T	G	T	G	G	A	T
AMO (NC014770)	.	.	.	.	C	.	T	C	A	T	A	G	C	A	T	T	T	T	G	G	A	A	.	.	.	.	.	.	T
COR1 (JF357971)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	A	.	.
COR2 (JF357972)	.	.	.	.	.	Y	.	.	Y	R	.	Y	A	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	A	.	Y
SUM1 (JF357969)	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	T
SUM2 (JF357970)	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	T
TIG1 (JF357967)	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T
TIG2 (JF357968)	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T
CH-001 to CH-011 (GFG001 - GFG009-11)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LA-129 (GFG016-11)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LA-181/186 (GFG026/GFG028-11)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LA-137 (GFG019-11)	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.
LA-118 (GFG015-11)	T	C	C	Y	C	.	T	C	A	T	A	G	C	A	T	T	T	T	G	G	.	.	.	.	.	.	.	.	.	.
TH-002 (GFG031-11)	.	.	.	.	C	.	T	C	A	T	A	G	C	A	T	T	T	T	G	G	A	.	.	.	.	.	A	A	.	.
TH-009 (GFG035-11)	.	.	.	.																							A	A	.	.
MM-016 (GFG030-11)				.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.				G	C	.	.	.	T
ZOO041	.	.	.	.	C	.	T	C	A	T	A	G	C	A	T	T	.	T	G	G	.	.	.	.	.	.	.	.	.	.
ZOO042	.	.	.	.	C	.	T	C	A	T	A	G	C	A	T	T	.	T	G	G	.	.	.	.	.	.	.	.	.	.
ZOO043	.	.	.	.	C	.	T	C	A	T	A	G	C	A	T	T	.	T	G	G	.	.	.	.	.	.	.	.	.	.

- **Snow Leopard samples**:

1) *mtDNA*: There were no character variations for 12S and CytB mtDNA regions. On the ATP6 region there was variability, but they were not character diagnostic. On the 16S region there was diagnostic variation. In samples AZA027 and AZA029, the variation found matched variation in samples from Tajikistan and southern Mongolia.
2) *Genotyping*: Samples AZA020, AZA021,and AZA023 were similar to Kyrgyzstan samples ($p<0.05$). Sample AZA021 was also similar to individuals from Nepal ($p<0.05$). All our captive samples were very closely related to each other. (The related values range from 0.14-0.60).

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