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PRIMER NOTE

A select panel of polymorphic microsatellite loci for individual identification of snow leopards (*Panthera uncia*)

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Abstract

Snow leopards (*Panthera uncia*) are elusive endangered carnivores found in remote mountain regions of Central Asia. New methods for identifying and counting snow leopards are needed for conservation and management efforts. To develop molecular genetic tools for individual identification of hair and faecal samples, we screened 50 microsatellite loci developed for the domestic cat (*Felis catus*) in 19 captive snow leopards. Forty-eight loci were polymorphic with numbers of alleles per locus ranging from two to 11. The probability of observing matching genotypes for unrelated individuals (2.1×10^{-11}) and siblings (7.5×10^{-5}) using the 10 most polymorphic loci was low, suggesting that this panel would easily discriminate among individuals in the wild.

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Snow leopards (*Panthera uncia*) occupy the remote and rugged mountains of Central Asia including the Altai, Tien Shan, Kun Lun, Pamir, Hindu Kush, Karakoram, and Himalaya ranges (McCarthy & Chapron 2003). Only 3500–7000 individuals remain in the wild, and it is thought that some local populations have sharply declined over the past decade (McCarthy & Chapron 2003). Because of their cryptic nature, large home ranges, and low population densities (Jackson & Hunter 1996), snow leopards are extremely difficult to survey. Sign transect surveys (for pugmarks, scrapes, scent sprays, etc.), have been commonly used to estimate relative snow leopard abundance, but this technique is fraught with potential errors and biases. A broad range of specialists in snow leopard conservation and research recently collaborated on developing a survival strategy for the species (McCarthy & Chapron 2003) and cited an urgent need for a reliable and preferably noninvasive method to estimate leopard numbers and monitor population trends.

One technique for estimating abundance of rare species is noninvasive genetic sampling using hair and faeces. Individual identification, achieved by genotyping at multiple microsatellite loci, provides a minimum count of sampled individuals, and when coupled with mark–recapture statistics (White *et al*. 1982) can provide a population estimate. Noninvasive genetic sampling has been successfully used to obtain population estimates in other large carnivores including brown bears (*Ursus arctos*) (Poole *et al*. 2001; Bellemain *et al*. 2005), black bears (*Ursus americanus*) (Paetkau 2003), cougars (*Puma concolor*) (Ernest *et al*. 2000), wolves (*Canis lupus*) (Lucchini *et al*. 2002), and coyotes (*Canis latrans*) (Kohn *et al*. 1999).

The first step in applying this method for snow leopard population monitoring is identifying a suite of hypervariable microsattelite loci. To accomplish this, we obtained genetic samples from 19 captive snow leopards (Table 1). The region of origin is unknown for most samples. Genomic DNA was isolated using standard phenol-chloroform methods (Vardenplas *et al*. 1984) and the QIAGEN tissue kit. Amplification of 50 dinucleotide microsatellite loci (Table 2) screened from 16 of 19 domestic cat (*Felis catus*)

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Table 1 Inventory of captive snow leopards (*Panthera uncia*) that were sampled to identify polymorphic microsatellite loci for use in noninvasive genetic sampling

Sample ID	Captive facility
PUN13	Brookfield Zoo, Chicago, Illinois, USA
PUN15	Woodland Park Zoo, Seattle, Washington, USA
PUN17	Cheyenne Mountain Zoo, Colorado Springs, Colorado, USA
PUN18	Cheyenne Mountain Zoo, Colorado Springs, Colorado, USA
PUN19	Cheyenne Mountain Zoo, Colorado Springs, Colorado, USA
PUN20	Cheyenne Mountain Zoo, Colorado Springs, Colorado, USA
PUN22	Milwaukee Zoo, Milwaukee, Wisconsin, USA
PUN26	Kings Island, Cincinnati, Ohio, USA
PUN67	Baton Rouge Zoo, Baton Rouge, Louisiana, USA
PUN72	New York Zoological Park, New York, New York, USA
PUN79	Moscow Zoo, Moscow, Russia
PUN81	Moscow Zoo, Moscow, Russia
PUN82	Moscow Zoo, Moscow, Russia
PUN83	Tallinn Zoo, Tallinn, Estonia
PUN84	Tallinn Zoo, Tallinn, Estonia
990390	Woodland Park Zoo, Seattle, Washington, USA
960333	Woodland Park Zoo, Seattle, Washington, USA
200132	Woodland Park Zoo, Seattle, Washington, USA
200133	Woodland Park Zoo, Seattle, Washington, USA

chromosomes was attempted for all samples using published primers and amplification conditions (Menotti-Raymond *et al*. 1999). Amplified products were visualized and scored on an ABI 377 or ABI 3130 fluorescent detection system using associated software. Genetic diversity statistics were estimated using GIMLET (Valière 2002) and the Microsatellite toolkit (Park 2001). Hardy–Weinberg equilibrium was tested with an alpha of 0.05 (with Bonferroni correction) using GENEPOP (Raymond & Rousset 1995). Physical linkage was assessed by determining the location of each locus on the domestic cat linkage map (Menotti-Raymond *et al*. 2003), and several loci may be physically linked based on estimated pairwise distances ≤ 20 centiMorgans (Table 2).

All 50 loci amplified in snow leopards. Only loci FCA090 and FCA176 deviated from Hardy–Weinberg expectations ($P = 0.0001$). The average observed and expected heterozygosities for all loci are 0.52 (0.02 SE) and 0.58 (0.03 SE), respectively. Two loci (FCA078, FCA188) were monomorphic and seven additional loci had expected heterozygosities below 0.25 (Table 2). The number of alleles per locus at polymorphic loci ranged from 2–11 (average 4.3). Sixteen loci had expected heterozygosity levels above 0.70, but three of these loci (FCA229, FCA132, FCA075) had lower observed heterozygosities suggesting potential problems with null alleles (Table 2). However, these loci were in Hardy–Weinberg equilibrium. The expected probability of matching genotypes for unrelated (P_{ID}) individuals and siblings (P_{ID} sibs) was calculated for each locus using methods and equations described in Waits *et al*. (2001) (Table 2). Since our samples were taken from captive animals and not a single population, these diversity statistics may be elevated compared to local populations. When initiating a new study using these loci, it would be prudent to evaluate the 10–15 most polymorphic loci and recalculate diversity and probability of identity statistics.

Based on these results, we have designed a panel of 10 microsatellite loci for use in individual identification of snow leopard hair and faecal samples from China and Kyrgyzstan. To maximize efficiency and minimize costs, these loci have been combined into three polymerase chain reaction (PCR) multiplexes using fluorescently labelled primers (Group 1 — FCA 32, 100, 124; Group 2 — FCA 126, 212, 229; Group 3 — FCA 96, 132, 225, 275) using standard microsatellite amplification protocols described in the QIAGEN multiplex kit. For each reaction, we used 2 μL of DNA extract and 45 cycles. Final concentrations of PCR primers included 0.1 μM (FCA124), 0.2 μM (FCA 96, 126, 132, 212, 229, 275), 0.4 μM (FCA32), 0.6 μM (FCA100) and 0.8 μM (FCA225). Preliminary results for 53 field-collected faecal samples indicate that amplification success rates average 68%. These results are promising for future efforts to evaluate current range, population estimates, genetic diversity and structure for snow leopards.

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Table 2 Observed size range and diversity statistics for 48 polymorphic felid microsatellite loci tested on 19 captive snow leopards. Loci located on the same feline chromosome that are ≤ 20 centiMorgan units apart are delineated via identical alpha character superscripts

Locus	Size range	No. of alleles	H_E	H_O	P_{ID} (locus)	P_{ID} (cum)	P_{ID} sibs (locus)	P_{ID} sibs (cum)	Feline chromosome*
FCA126	143–163	11	0.87	0.93	4.19E-02	4.19E-02	3.41E-01	3.41E-01	B1[d]
FCA275	121–133	7	0.81	0.60	7.46E-02	3.13E-03	3.78E-01	1.29E-01	B2
FCA124	121–133	6	0.81	1.00	8.86E-02	2.79E-04	3.87E-01	4.96E-02	A2[b]
FCA100	112–120	5	0.80	0.87	8.94E-02	2.48E-05	3.86E-01	1.92E-02	A1[a]
FCA032	179–199	6	0.79	0.67	9.31E-02	2.33E-06	3.93E-01	7.50E-03	E2[i]†
FCA229	158–168	6	0.79	0.50	9.37E-02	2.16E-07	3.93E-01	2.95E-03	A1
FCA132	164–172	5	0.82	0.50	9.39E-02	2.03E-08	3.91E-01	1.16E-03	D3
FCA107	212–226	7	0.77	0.93	9.48E-02	2.01E-09	4.01E-01	4.62E-04	D2[f]
FCA212	116–130	6	0.78	0.67	9.91E-02	1.90E-10	3.98E-01	1.85E-04	B1[d]
FCA225	228–234	4	0.78	0.79	1.11E-01	2.11E-11	4.04E-01	7.49E-05	A1
FCA096	205–213	5	0.76	0.67	1.14E-01	2.42E-12	4.13E-01	3.09E-05	E2[i]
FCA088	103–113	5	0.75	0.60	1.22E-01	2.95E-13	4.18E-01	1.29E-05	B3
FCA008	134–146	4	0.76	0.67	1.24E-01	3.66E-14	4.18E-01	5.41E-06	A1[a]
FCA075	119–129	6	0.75	0.40	1.26E-01	4.63E-15	4.19E-01	2.27E-06	E2[i]
FCA211	112–118	4	0.74	0.53	1.34E-01	6.18E-16	4.25E-01	9.62E-07	B1
FCA077	141–147	4	0.73	0.60	1.41E-01	8.70E-17	4.35E-01	4.18E-07	C2
FCA208	306–316	6	0.69	0.53	1.46E-01	1.32E-17	4.56E-01	1.86E-07	A3
FCA044	111–119	5	0.71	0.67	1.50E-01	2.02E-18	4.46E-01	8.25E-08	B4
FCA187	162–170	5	0.71	0.71	1.52E-01	3.03E-19	4.44E-01	3.68E-08	B4
FCA290	220–228	4	0.71	0.80	1.52E-01	4.43E-20	4.45E-01	1.68E-08	C1
FCA171	101–109	5	0.68	0.67	1.81E-01	7.99E-21	4.66E-01	7.82E-09	A3[c]
FCA224	160–166	4	0.68	0.60	1.84E-01	1.53E-21	4.74E-01	3.67E-09	A3[c]
FCA085	128–136	4	0.65	0.47	1.88E-01	2.81E-22	4.82E-01	1.74E-09	E2[i]
FCA161	171–175	3	0.68	0.73	1.91E-01	5.67E-23	4.69E-01	8.30E-10	A3[c]
FCA069	101–107	4	0.65	0.60	2.01E-01	1.07E-23	4.85E-01	4.00E-10	B4
FCA006	197–207	3	0.67	0.60	2.02E-01	2.17E-24	4.78E-01	1.94E-10	D3
FCA176	193–215	4	0.66	0.20	2.03E-01	4.36E-25	4.84E-01	9.38E-11	A1
FCA327	178–194	5	0.61	0.60	2.07E-01	9.69E-26	5.07E-01	4.65E-11	A2
FCA045	153–159	3	0.64	0.53	2.23E-01	2.18E-26	4.96E-01	2.33E-11	D4
FCA105	206–212	4	0.61	0.73	2.23E-01	4.53E-27	5.09E-01	1.18E-11	A2[b]
FCA094	181–200	5	0.63	0.53	2.25E-01	1.01E-27	5.02E-01	6.03E-12	F2
FCA133	133–139	4	0.57	0.60	2.37E-01	2.56E-28	5.33E-01	3.14E-12	B2
FCA090	94–110	5	0.61	0.20	2.54E-01	6.57E-29	5.20E-01	1.64E-12	A1
FCA082	249–257	4	0.60	0.33	2.57E-01	1.56E-29	5.22E-01	8.72E-13	E1[h]
FCA129	179–185	4	0.55	0.36	2.72E-01	4.65E-30	5.54E-01	4.83E-13	A1
FCA080	230–246	4	0.56	0.47	2.98E-01	1.27E-30	5.53E-01	2.67E-13	A3
FCA091	136–140	3	0.55	0.67	3.27E-01	4.14E-31	5.65E-01	1.51E-13	D2[e]
FCA026	140–148	4	0.48	0.33	3.32E-01	1.37E-31	6.01E-01	9.08E-14	D3[g]
FCA262	207–215	3	0.42	0.43	3.90E-01	5.60E-32	6.45E-01	5.71E-14	D2[e]
FCA057	144–146	2	0.46	0.4	4.07E-01	2.18E-32	6.30E-01	3.68E-14	C1
FCA120	193–199	3	0.42	0.47	4.12E-01	8.98E-33	6.50E-01	2.39E-14	F1
FCA344	123–129	4	0.25	0.2	5.84E-01	5.25E-33	7.75E-01	1.85E-14	F1
FCA081	116–124	3	0.24	0.13	6.10E-01	3.24E-33	7.89E-01	1.46E-14	D2
FCA310	129–131	2	0.24	0.27	6.18E-01	1.98E-33	7.89E-01	1.16E-14	C2
FCA005	134–136	2	0.19	0.2	6.89E-01	1.36E-33	8.32E-01	9.61E-15	E1[h]
FCA249	234–236	2	0.08	0.08	8.60E-01	1.17E-33	9.28E-01	8.92E-15	D3[g]
FCA144	167–169	2	0.07	0.07	8.69E-01	1.02E-33	9.33E-01	8.32E-15	D1
FCA304	95–101	2	0.07	0.07	8.77E-01	8.94E-34	9.37E-01	7.80E-15	A2

H_E, expected heterozygosity; H_O, observed heterozygosity; P_{ID}, probability of identity unrelated; cum, cumulative; P_{ID} sibs, probability of identity siblings.

*Chromosome names were delineated via Menotti-Raymond *et al.* (2003).

†The distance in centiMorgans between FCA032 and the three other loci on feline chromosome E2 is unknown.

References

Bellemain E, Swenson J, Tallmon D, Brunberg S, Taberlet P (2005) Estimating population size from hunter-collected feces: four methods for brown bears. *Conservation Biology*, **19**, 150–161.

Ernest HB, Penedo MCT, May BP, Syvanen M, Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology*, **9**, 433–442.

Jackson RJ, Hunter DO (1996) *Snow Leopard Survey and Conservation Handbook*. International Snow Leopard Trust, Seattle, Washington. 154 pages.

Kohn MH, York EC, Kamradt DA *et al.* (1999) Estimating population size by genotyping feces. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 657–663.

Lucchini V, Fabbri E, Marucco F *et al.* (2002) Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. *Molecular Ecology*, **11**, 857–868.

McCarthy TM, Chapron G (2003) *Snow Leopard Survival Strategy*. Snow Leopard Trust and Snow Leopard Network, Seattle, Washington.

Menotti-Raymond M, David VA, Chen ZQ *et al.* (2003) Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (*Felis catus*). *Journal of Heredity*, **94**, 95–106.

Menotti-Raymond M, David VA, Lyons LA *et al.* (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics*, **57**, 9–23.

Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, **12**, 1375–1387.

Park SDE (2001) *Trypanotolerance in West African cattle and the population genetic effects of selection*. PhD Thesis, University of Dublin.

Poole KG, Mowat G, Fear DA (2001) DNA-based population estimate for grizzly bears *Ursus arctos* in northeastern British Columbia, Canada. *Wildlife Biology*, **7**, 105–115.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Vardenplas SI, Wild I, Grober-Rabi A *et al.* (1984) Blot hybridization analysis of genomic DNA. *Journal of Medical Genetics*, **21**, 164–168.

Waits LP, Luikart G, Taberlet P (2001) Estimating probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, **10**, 249–256.

White GC, Anderson DR, Burnham KP, Otis DL (1982) *Capture–Recapture and Removal Methods for Sampling Closed Populations*. Los Alamos National Laboratory, LA 8787-NERP, Los Alamos, New Mexico.