Snow Leopard Conservation Grants Program FINAL REPORT FOR 2009 PROJECT

PROJECT TITLE

POPULATION STATUS AND DISTRIBUTION OF THE SNOW LEOPARD IN CHINA

PRINCIPAL INVESTIGATORS

- Dr. Jan E. Janecka (Conservation Grants Program PI), Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843-4458, USA.
- Dr. William Murphy (Conservation Grants Program Co-PI), Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843-4458, USA
- Dr. Zhang Yuquang, Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, P.R. China, 100091
- Dr. Li Diqiang, Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, P.R. China, 100091
- Dr. B. Munkhtsog, Institute of Biology, Mongolian Academy of Sciences, & Irbis Mongolia, Jukov Avenue 77, Ulaanbaatar, 51 Mongolia.
- Dr. Rodney Jackson, Snow Leopard Conservancy, 18030 Comstock Avenue, Sonoma, CA 95476, USA.

Executive Summary

The snow leopard (*Panthera uncia*) is categorized as Endangered by the IUCN, based on low population densities, fragmented distribution, and a declining population trend. China is believed to encompass over 60% of the remaining snow leopard populations. However, detailed information on the status of this species is limited for this vast region. We conducted noninvasive genetic scat surveys to examine the distribution and genetic diversity of snow leopards in the Qinghai-Tibetan Plateau.

Scats were collected in southern and northern parts of Qinghai along wildlife trails. Genetic methods were used to obtain species, individual, and sex identification. We detected snow leopards in all of the surveyed areas, observing a total of 36 individuals. The highest proportion of snow leopard scat (88-100%) was in Nangqian (southern Qinghai), Tianjun (northern Qinghai), and Akesai (northern Qinghai). In Akesai, a more extensive survey was conducted identifying 14 snow leopards in the study site. There were 8.9 snow leopard scats/km and 2.3 individuals/km, suggesting higher snow leopard abundance than we previously estimated in the Gobi Desert of Mongolia. Additional sampling is needed to verify these observed differences.

We examined population structure using microsatellite analysis and were able to detect several important patterns. Our results suggest there are higher levels of gene flow between Tibet and southern/central Qinghai. In contrast, the Qaidam Basin appears to act as a geographic barrier based on higher levels of differentiation observed in the northern Qinghai/Gansu area. The Gobi Desert harbors the most divergent population examined thus far, implying southern Mongolia may be significantly isolated from the Qilian Mountains (northern Qinghai) due to the Alashan Plateau that separates these areas. However, our sample size was limited and so the results need to be interpreted with caution. Additional sampling is needed to test these hypotheses.

Our study provides important, preliminary information on snow leopard distribution and abundance in China, as well as population structure. We will use the information to establish long-term surveys in specific areas of China, as we continue our collaborative initiatives. Complementary to these, short surveys will also be conducted to obtain snow leopard samples distributed over broad regions for population structure analysis. These efforts will yield a solid understanding of snow leopard distribution and abundance in China, along with information on population connectivity, which will be critical for conservation efforts.

Objectives

The main objectives of our project were to: (A) obtain distribution information for snow leopards in parts of Qinghai and Sichuan, (B) compare snow leopard densities in 5 localities, (C) establish baseline information in areas that can be further monitored to examine trends in snow leopard populations, (D) examine the levels of gene flow and population structure in the Q-T Plateau, (E) develop a close working relationship with Chinese collaborators to expand an ongoing US-Chinese collaborative snow leopard study with the Chinese Academy of Forestry, and (F) facilitate the exchange of information and share ideas regarding conservation initiatives with local people and gauge the feasibility of community based conservation initiatives in Qinghai.

Methods

In 2009, the Dr. Zhang Yuquang and Dr. Li Diqiang (Chinese Academy of Forestry) led 2 snow leopard survey expeditions in Central China. The first survey (in March) occurred in northern Qinghai and Gansu and sampled 2 localities in Akesai (Figure 1). In May, a second field expedition was conducted in southern Qinghai (Zhiduo and Nangqian). The snow leopard scats were analyzed at Texas A&M University. As a result of logistical complications, Dr. Jan Janecka was not able to participate in the Qinghai surveys and so worked in Mongolia instead. In partnership with Dr. Bariusha Munkhtsog (Irbis Mongolia & Mongolian Academy of Sciences), a collaborative Mongolian Academy of Sciences-Texas A&M University noninvasive survey was conducted in the Gobi Gurvan Saikhan (Three Beauties) National Park.

The DNA from fecal material was extracted using the Qiagen Stool DNA extraction kit following manufacturer recommendations (Qiagen, Valencia, California, USA). We developed a PCR assay at Texas A&M University for inexpensive identification of snow leopard scats. This method uses snow leopard specific primers that amplify a short segment of cytochrome b. The polymerase chain reactions (PCR) were conducted in 10 μ l volume containing 1X PCR buffer, 1.5 mM MgCL₂, 0.80 mM of each dNTP, 0.40 mM forward primer, 0.40 mM reverse primer, 0.20 units of Amplitaq Gold (ABI), 0.10 μ g/ul of Bovine Serum Albumin, 1.5 μ l of extracted DNA. The PCR reaction conditions included an initial denaturing step of 95°C for 10 min followed by 50 cycles of 95°C for 1 min, 60°C for 30 sec, 72°C for 60 sec, and a final extension step of 72°C for 1 min. It is important to note that under less stringent PCR conditions (i.e., >1.5 mM MgCl₂ and/or <60°C annealing temperature) these primers will amplify in other species. The product was run on an agarose gel, and samples that yielded target amplicons of 151 bp were identified as snow leopard.

Four microsatellite loci were used for individual identification of snow leopard scats (*PUN100*, *PUN124*, *PUN225*, and *PUN229*). PCRs for microsatellites were conducted under the following conditions: 10 µl volume containing 1X PCR buffer, 2.5 mM MgCL₂, 0.20 mM of each dNTP, 0.48 mM forward primer, 0.48 mM reverse primer, 0.20 units of Amplitaq Gold (ABI), 0.10 µg/ul of Bovine Serum Albumin, 1.5 µl of extracted DNA. The PCR reaction conditions included an initial denaturing step of 95°C for 10 min followed by 50 cycles of 95°C for 1 min, 55°C for 30 sec, 72°C for 60 sec, and a final extension step of 72°C for 10 min. The four forward primers were each labeled with a different fluorescent dye (6FAM, PET, NED, or VIC). The PCR products for the four loci were pooled for each sample, and sized using an ABI 3730 automated sequencer (Department of Veterinary Integrative Biosciences, TAMU). Alleles were carefully sized in GENEMAPPER. Each sample was analyzed in triplicate and only alleles that were observed twice were used.

We conducted sex identification with primers designed to amplify a small portion of the AMELY gene in male felids (about 300 bp). The PCR amplification of the AMELY marker was done in triplicate. If the correct product appeared in 2 or more PCRs, the sample was identified as male. If the samples yielded no AMELY product, but yielded good genotypes across all 4 microsatellite loci, it was identified as female. If there was no AMELY amplification and also dropout in $\geq 2/3$ of the microsatellite genotypes, or if AMELY amplified in only 1 of the 3 PCRs, it was not assigned a sex.

We created consensus genotypes across the 4 loci and identified the sex of the individuals. We used unique genotypes to estimate allele frequencies and the probability of identity. For this purpose, because of the limited sampling in many of the sites, we grouped samples into three populations. (Please see methods below explaining the rationale used to group samples). The probability of identity for both unrelated individuals (P_{ID-unr}) and related individuals (P_{ID-rel}) was estimated in GENALEX for the combined 4 microsatellites and 1 sex marker. The P_{ID} is the chance that any two snow leopards will share the exact same genotype for all 4 microsatellites and be the same sex.

The total number of snow leopards detected was compared across 8 sites in China, one of which (Zhiduo) was sampled in both 2007 and 2009. Most of the surveys were of limited duration, which precluded direct population estimates and comparisons of density within China. A more extensive survey was conducted in Akesai (Figure 2), and we compared the results with our previous abundance estimates for the Gobi Desert (Tost Uul and Noyon Uul) to examine possible differences across the snow leopard range.

The microsatellite data was used to test for population differentiation with a subset of individuals sampled in our study sites and samples we collected in the Gobi Desert of Mongolia (Tost Uul, Noyon Uul, and Three Beauties NP, Figure 1). Three additional microsatellites (*PUN82*, *PUN132*, and *PUN327*) were genotyped in 25 snow leopards from China and 12 from Mongolia. We combined the two Tibet sites into a single Tibet population. We pooled samples from Nangqian, Zhiduo, and Dulan into a Central Qinghai population, and grouped Tianjun and Akesai into a Northern Qinghai population. The northern samples were divided from the southern samples because of the Qaidam Basin that separates them, which potentially acts as a geographic barrier for snow leopards. The Gobi samples collected near the Chinese/Mongolian border were grouped in their own, separate population. The extensive Alashan Plateau south of this region is also a potential dispersal barrier. Figure 1 shows the population groupings described above.

We examined genetic variation and structure using F_{st} estimates (GENEALEX), genotypic exact G tests (GENEPOP), genic exact G tests (GENEPOP), and population assignment tests (GENEALEX). Finally, cryptic population structure was investigated using a model-based Bayesian clustering approach (STRUCTURE). In this method, the most likely number of clusters is identified, and samples are assigned to each clusters based on linkage disequilibrium and Hardy-Weinberg equilibrium, without any influence from preconceived geographic assumptions.

4. Results and 5. Discussion

Distribution information

In 2009, we surveyed 4 sites in 3 areas. Two were in southern Qinghai (Zhiduo, n = 13; Nangqian, n = 10) and two in northern Qinghai and Gansu (Akesai, n = 89 and n = 21, Table 1). We combined them with samples collected by the Chinese Academy of Forestry in China during 2007 and 2008, for a total of 274 scats. These included scats from northern Qinghai (Dulan, n = 66; Tianjun, n = 8), the Zhiduo site surveyed in 2009 (n = 13), and Tibet (Benga, n = 29; Shenzha, n = 27). Among the 274 scats collected in China, 136 were positively identified as snow leopard (54.4%, Table 1). Snow leopards were observed in all sites surveyed. The GPS locations of the snow leopard scats are available upon request.

Snow leopard abundance in central China

In 8 of the 9 sites we did not obtain enough samples to directly estimate abundance. We therefore compared the % snow leopard scat as a rough index of abundance (Table 1). This should reflect the levels of use by snow leopards and their activity, assuming that the rate of observer misidentification is relatively constant across sites. The highest % snow leopard scat was in the two northern areas (Tianjun, 100%; Akesai, mean = 87.6%) and in Nangqian (90%). These were higher than we previously reported in Ladakh, India (54%). In turn, the lowest ratio of snow leopard scat was in Dulan (25.8%) and the two Tibet areas (Benga and Shenzha, 17.2%).

and 29.6%, respectively), comparable to our previous results from Tost Uul and Noyon Uul (33%, Gobi Desert, Mongolia).

The P_{ID-unr} ranged from 0.000326 to 0.000923, and P_{ID-rel} from 0.0213 to 0.0297, therefore, 4 microsatellites were sufficient for individual identification. We observed a total of 36 individual snow leopards (17 males, 18 females, and 1 unknown) among all the sites sampled (Table 1). All but one site (2009 sample from Zhiduo) had more than 1 individual present. The greatest number of snow leopards (6 males, 9 females) was detected in the Akesai site (Figure 2), where 89 samples were collected along 4 transects (9.2 km total, Table 2). This area had among the highest ratio of snow leopard scat (79.8%) we have observed thus far across numerous sites in Mongolia, comparable to what we have seen in the western Tsagaan Shuvuut region.

The number of snow leopard scats detected in Akesai (8.9 snow leopard scats/km, Table 2) was more than 3-fold higher than in Tost Uul (2.3 snow leopard scat/km) and Noyon Uul (1.7 snow leopard scats/km). In the Gobi Desert, red fox was the predominate species observed along transects. The number of snow leopards detected per transect in Akesai (2.3 individuals/km) was also higher than in Tost Uul (0.93 individual/km) and Noyon Uul (1.08 individual/km), suggesting higher snow leopard densities in Akesai.

Biological and environmental factors can influence the amount and distribution of sign and scat present in an area, leading to across-site variation in abundance estimators. We surveyed both regions in March, when snow leopards marking activity is at its peak, to minimize these effects. Our data suggests Akesai may have habitat more suitable for snow leopards, and less suitable for red foxes, compared to the Gobi Desert. The disparity we observed may be the result of significant ecological differences, however, the data must be interpreted with caution because of our limited sampling. More in-depth studies of snow leopards are needed in both areas to understand the differences in snow leopard ecology between the two divergent ecosystems.

Baseline information for areas to monitor snow leopard population trends

We obtained location records for 149 snow leopard samples across 8 localities of Central China. The % snow leopard scat and the minimum number of individuals detected can be used for comparison with future surveys. In Akesai, we obtained indirect abundance estimates including snow leopard scats/km, minimum number of snow leopards, and snow leopard individuals/km. We will use these results to plan additional surveys assessing the status of this felid in China.

Levels of gene flow and population structure

All seven microsatellites used in this study were variable (Table 3). The diversity of the loci in the individual identification panel we previously developed makes it useable across large parts of China. The number of alleles (A) ranged from 4 to 5 and expected heterozygosity (H_e) from 0.61 to 0.69 (Table 3). The highest A and H_e values were estimated in Northern Qinghai. However, the differences were not significant. The expected heterozygosity was lowest in the Gobi. The Tost and Noyon Uul region of the Gobi is characterized by somewhat isolated, low-altitude mountain massifs, compared to more continuous habitat distributed in many parts of China. A greater degree of isolation could explain reductions in genetic diversity, however, more sampling is needed to test this hypothesis.

Estimates of F_{st} values are commonly used to infer gene flow among populations. Values can range from 0 to 1, with F_{st} = 0 indicating one large population, and F_{st} = 1 indicating two completely isolated population with no shared genetic diversity. Typically, F_{st} below 0.025 indicate open populations, F_{st} between 0.025 and 0.050 indicate limited genetic structure (populations are somewhat isolated, but there is still occasional exchange of individuals), and F_{st} above 0.050 often indicate significant genetic structure among populations (low gene flow and present major dispersal barriers).

Among the snow leopard populations we sampled, the lowest F_{st} was observed between Tibet and Central Qinghai suggesting highest levels of gene flow (F_{st} = 0.0138, genotypic test P-value =

0.260, genic test P-value = 0.0316, Figure 1). The F_{st} between Central Qinghai and Northern Qinghai (0.0471) was higher than between Central Qinghai and Tibet, suggesting that the Qaidam basin may isolate the Qilian Mountains, while the habitat in the Tibetan region is more porous to snow leopards (Figure 1). The F_{st} between Northern Qinghai and the Gobi was nearly 3-fold higher (0.1386) than between Northern and Central Qinghai, therefore the Alashan Plateau may be a more significant barrier to gene flow than the Qaidam Basin. The reduced gene flow between southern Mongolia and Northern China indicates that Mongolia may belong to a different evolutionary significant unit. However, more sampling is required throughout the species range to test this hypothesis.

Population assignment tests showed a similar pattern of structure. These tests estimate the likelihood that an individual originated from the sampled populations, and assign the individual to the most likely population of origin. Populations with greater levels of migration have a greater proportion of individuals assigned to the "wrong" population – in other words, are genetically placed in a population different from the one in which they were sampled. These individuals are considered "misassigned" by the test. The proportion of individuals in a population that are misassigned is directly related to the level of gene flow. The highest proportion of misassigned individuals was in Tibet (67%) and Central Qinghai (38%), and the lowest in Gobi (8%), with Northern Qinghai intermediate (27%). This is consistent with the F_{st} tests, suggesting that the highest levels of gene flow are in the Central Qinghai/Tibet area, and that the Alashan Plateau is the most significant barrier in the sampled region.

The likelihood plots of pairwise population assignments indicated additional structure not detected with the F_{st} tests (Figure 3). In Figure 3A, samples from Tibet form two clusters, as do Northern Qinghai samples in Figure 3B. Therefore, there appear to be two groups of samples within these populations that have different likelihood ratios. This suggests individuals from more than one population were clumped within the groups we defined.

Cryptic structure is further suggested in the STRUCTURE analysis. In this approach, individuals are clustered into most likely groups based on allele frequencies. The program then estimates the portion of genetic variation of each individual that belong to a particular cluster (the value ranges from 0 to 1). In Figure 4, each bar represent one of the 37 individuals sampled. The colors represent the 4 significant clusters found by STRUCTURE, and indicate to which cluster a particular individual belongs. Some of the snow leopards sampled in Tibet, Central Qinghai, and Northern Qinghai are in the same cluster (green). Four samples from Shenzha are assigned to 3 different clusters. Three samples from Central Qinghai have significant mixed ancestry, with some of their variation belonging to a cluster that is primarily found in Shenzha. This provides evidence for gene flow between Tibet and Central Qinghai, and more complex patterns of structure not captured by our original population definitions. Northern Qinghai is composed of 2 clusters, also suggesting there may be multiple populations in that region.

The population structure results must be interpreted with caution because of the low number of microsatellites and samples we used. We have recently developed 32 additional microsatellites for population analysis of snow leopards, and are in the process of genotyping the samples in this study using those markers. In addition, we are working with collaborators on a phylogeography project that will include other snow leopards from Mongolia, Pakistan, Tajikistan, Nepal, and Bhutan to understand how snow leopards in China relate to other regions of Central Asia.

US-Chinese collaborative snow leopard initiatives

We have continued the collaboration between Texas A&M University and the Chinese Academy of Forestry that we began in 2006. In the spring of 2008 and 2009, problems associated with working in Central China prevented Dr. Janecka from going to Tibet and Qinghai. Dr. Zhang and Dr. Li (Chinese Academy of Forestry) therefore conducted the 2009 surveys in China. During that time, Dr. Janecka in turn was able to survey in the Gobi Desert with Dr. Munkhtsog (Mongolian Academy of Sciences) allowing for the comparisons we made between China and Mongolia. This

illustrates how close partnerships between different groups increases ability to obtain important information on snow leopards in Central China.

Exchange of information and ideas on conservation initiatives with local communities

The 2009 surveys in Qinghai were conducted by Dr. Zhang, and carried out with the help of knowledgeable local residents. By working side-by-side under difficult field conditions, a relationship was developed between biologists and the residents. In the field, Dr. Zhang and other biologist had the opportunity to educate participants about the importance of conserving ecological communities in the area. The local people shared their knowledge of the country, natural history, and wildlife that enabled the success of the surveys, yielding valuable data on snow leopards. Participation by local communities in both research and conservation efforts is critical for the success of programs.

Conclusions

Preliminary data on the distribution and abundance of snow leopards was obtained in several areas of China. Surveys confirmed the presence of snow leopards, and provided important information that can be pooled with data generated by other groups. Verified snow leopard detections are critical for defining their range in Central China. In Akesai, results suggested relatively high snow leopard abundance. The area is an excellent candidate for a long-term study site in China. Additional surveys must be carried out that will provide quantitative information, and generate population estimates for specific sites.

One of the greatest advantages of noninvasive genetic surveys, compared to other techniques such as camera trapping and GPS-telemetry, is the ability to obtain DNA samples from many sites in a relatively short amount of time. These DNA samples collected during even brief surveys can be used to examine gene flow, population structure, and landscape ecology. We were able to obtain enough samples to enable a preliminary investigation of population structure in Central Asia. Our data suggests that there are higher levels of gene flow in the central/southern portion of the Qinghai-Tibetan plateau, with evidence for more complex patterns of structure to the north. The Qaidam and Alashan Plateaus appear to isolate snow leopard populations at a regional level, with particularly high differentiation of the Gobi population in southern Mongolia. However, these hypotheses need to be further examined. Additional noninvasive sampling has the potential to answer long-standing questions on snow leopard ecology, such as how often do snow leopards move between adjacent mountain ranges, the extent of linear movement, and whether high-altitude passes act as barriers.

Despite many challenges, the noninvasive genetic approach holds promise as a valuable method for generating important information on snow leopards that will be critical for conservation efforts in China. It is important that diverse research and conservation groups coordinate their efforts and work together towards the same goal: securing the future of the endangered snow leopard and the unique mountain ecosystems it inhabits.

Photographs: SZ-1. Photograph of study site in Shenzha, Tibet from 2008 field survey. Photo Credit: Zhang Yuquang

Acknowledgements: We would like to thank the Snow Leopard Conservation Grants Program, National Geographic Society, and the Snow Leopard Conservancy for providing funds. We are also deeply grateful for kind assistance from Duo Hairui, Juan Li, Yun Ma, Hailong Yang, Nadia Mijiddorj, Gana, Bacik, Chingis, Tagwa, Dorji, Shaarav, Dustin Dwiggins, Colleen Fisher, Ali Wilkerson, Angela Love, Brian Davis, and Gang Li. Finally we would like to thank Chinese Academy of Forestry, State Forestry Administration, Irbis Mongolia, Gurvan Saikhan National Park, and the Mongolian Academy of Sciences.

	Survey	Total Scat	P. uncia Scat	% P. Uncia	Individual ID	% ID	Individuals	м	F	GPS Location	
China		274	149	54.4%	73	49.0%	36	17	18		
Qinghai		218	136	62.4%	67	49.3%	30	12	16		
Dulan	2007	66	17	25.8%	13	76.5%	3	2	1	36 03.483	96 57.658
Tianjun	2007	8	8	100.0%	5	62.5%	3	1	2	38 47.151	97 49.289
Zhiduo	2007	13	7	53.9%	2	28.6%	2	2	0	34 09.645	94 01.812
Zhiduo	2009	11	4	36.4%	0	0.0%	1*	n/a	n/a/	34 07.412	94 13.325
Nangqian	2009	10	9	90.0%	4	44.4%	3	1	2	32 06.860	96 20.565
Akesai	2009	89	71	80.0%	38	60.0%	14	6	8	38 32.493	94 59.587
Akesai	2009	21	20	95.2%	5	25.0%	5	0	3	38 53.251	95 31.552
Tibet		56	13		6		6	5	2		
Jiduo	2008	29	5	17.2%	2	40.0%	2	2	0	31 01.128	90 10.547
Shenzha	2008	27	8	29.6%	4	50.0%	4	3	2	31 12.302	88 44.599
Mean		30.444	16.6	58.7%	8.1	43.0%	4.5	2.1	2.3		

Table 1. Sampling locations, samples collected, and individuals observed.

Table 2. Transect data from the survey in Akesai, China, March 2009.

	AKS-A	%	AKS-B		AKS-C	%	AKS-D	%	Total	Mean
Transect Length	2.1 km		2.7 km		1.3 km		3.1 km		9.2	2.3
Tota Scat	21		31		22		15		89	22.25
Snow Leopard Scat	10	47.6%	27	87.1%	22	100.0%	12	80.0%	71	79.8%
Individual ID	2	20.0%	13	48.1%	18	81.8%	5	41.7%	38	53.5%
Individuals	2		7		6		4		15	4.8
м	1		1		4		3		6	2.3
E	1		6		2		1		9	2.5
S. L. scat/km	4.76		10.00		16.92		3.871			8.9
Ind/km	0.95		2.59		5.47		1.28			2.57

		Tibet	Central Qinghai	Northern Qinghai	Gobi
		n = 6	n = 8	n = 11	n = 12
	Number of	Alleles			
	PUN82	5	3	5	5
	PUN100	4	3	5	4
	PUN124	4	6	3	5
Locus	PUN132	4	5	6	3
	PUN225	4	4	4	2
	PUN229	5	4	6	6
	PUN327	4	2	5	5
	Mean	4	4	5	4
	Effective N	lumber of All			
	PUN82	3.00	2.25	4.48	2.53
	PUN100	3.60	2.98	3.23	3.13
	PUN124	2.06	2.78	2.40	3.27
Locus	PUN132	3.33	2.33	2.92	1.95
	PUN225	2.40	2.97	3.14	1.80
	PUN229	3.13	3.46	3.72	2.80
	PUN327	2.67	2.00	3.36	1.72
	Mean	2.88	2.68	3.32	2.46
	Observed I	Heterozygosi		_	
	PUN82	0.667	0.500	0.455	0.917
	PUN100	0.667	0.625	0.545	0.667
	PUN124	0.167	0.625	0.727	0.917
Locus	PUN132	0.800	0.250	0.273	0.000
	PUN225	0.333	0.857	0.273	0.500
	PUN229	0.500	0.625	0.818	0.417
	PUN327	0.833	0.500	0.455	0.333
	Mean	0.57	0.57	0.51	0.54
	Expected H	leterozygosi			
	PUN82	0.667	0.555	0.777	0.604
	PUN100	0.722	0.664	0.690	0.681
	PUN124	0.514	0.641	0.583	0.694
Locus	PUN132	0.700	0.570	0.657	0.486
	PUN225	0.583	0.663	0.682	0.444
	PUN229	0.681	0.711	0.731	0.642
	PUN327	0.625	0.500	0.702	0.420
	Mean	0.64	0.61	0.69	0.57

Table 3. Microsatellite diversity among snow leopards sampled in Central Asia.



Figure 1. Map showing the distribution of sampling sites, the number of snow leopard individuals sampled for the population structure analysis, and the four populations that were used in the genetic structure analysis. F_{st} estimates between adjacent populations are given.



Figure 2. Snow leopard individuals observed in Akesai during the March 2009 survey.



Figure 3A. Log likelihood ratios for population assignment of Tibet and Central Qinghai samples.



Figure 3B. Log likelihood ratios for population assignment of Central and Northern Qinghai samples.



Figure 3C. Log likelihood ratios for population assignment of Northern Qinghai and Gobi samples.



Figure 4. Model-based clustering of snow leopards without regard to their geographic origin. Each bar represents an individual snow leopard and the 4 colors represent genetically similar clusters identified among the samples. The y-axis is the proportion of genetic variation belonging to a particular cluster.