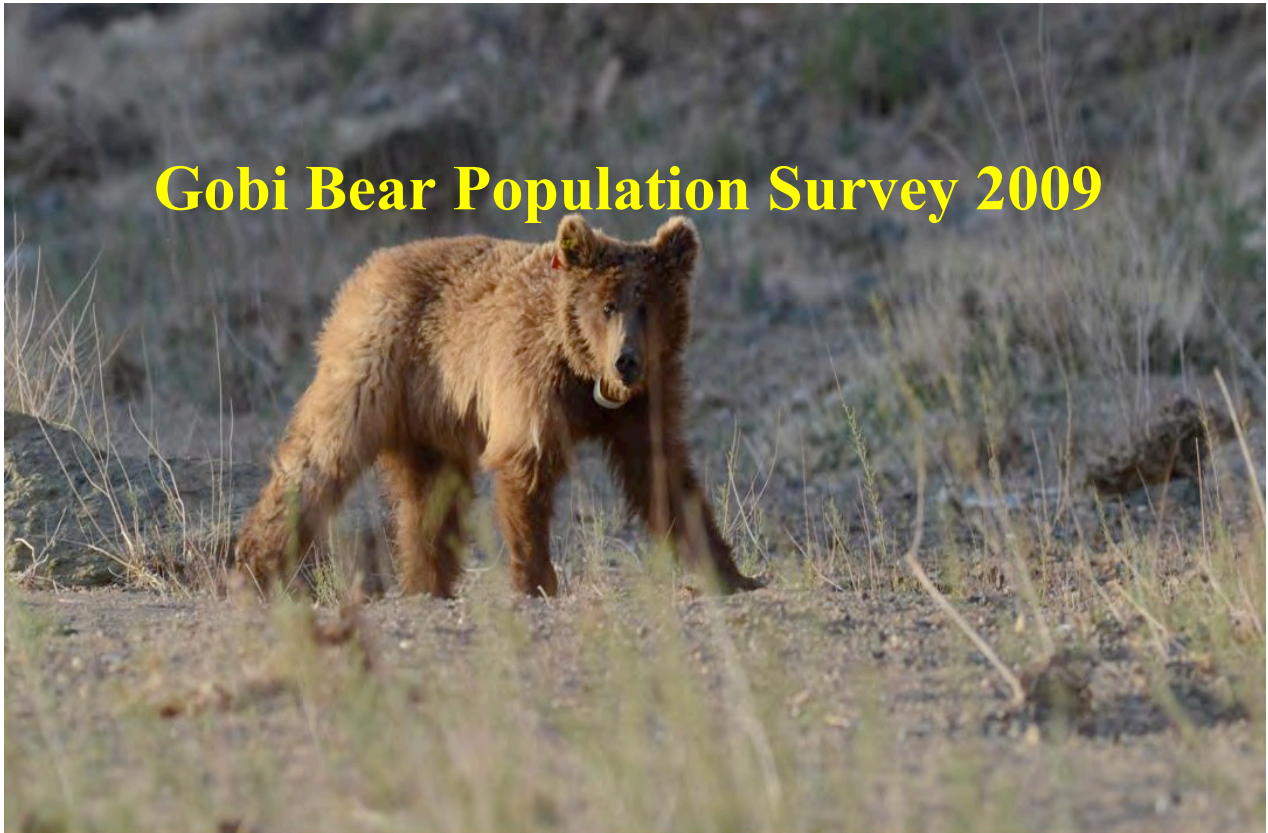


# Gobi Bear Population Survey 2009



## Final Report May 2011

**Odbayar Tumendemberel** Institute of Biology Mongolian Academy of Sciences, Jukov Ave., Ulaanbaatar 51, Mongolia

**Michael Proctor** Birchdale Ecological, & International Association for Bear Research and Management, PO Box 606, Kaslo, BC, V0G 1M0, Canada

**Harry Reynolds** Reynolds Alaska Wildlife Associates, Gobi Bear Project, & International Association for Bear Research and Management, PO Box 80843, Fairbanks, Alaska 99708, USA

**Luvsamjamba Amgalan** Institute of Biology Mongolian Academy of Sciences, Jukov Ave., Ulaanbaatar 51, Mongolia

**Tuya Tserenbataa** United Nations Development Program, Great Gobi Project, Gov. Bld. 3, Room 501B, Ministry of Nature and Environment, Ulaanbaatar, Mongolia

**Mijiddorj Batmunkh** Great Gobi Strictly Protected Area, Bayantoorai, Mongolia

**Derek Craighead** Craighead Beringia South, PO Box 147, Kelly, Wyoming 83011, USA

**Nyambayar Yanjin** Gobi Strictly Protected Area, Bayantoorai, Mongolia

**David Paetkau** Wildlife Genetics International, 200-182 Baker St. PO Box 274, Nelson British Columbia, Canada

## **ABSTRACT**

Gobi bears, (*Ursus arctos isabellinus*) survive in the Great Gobi Strictly Protected Area of southwestern Mongolia. The number of bears is thought to be low, but data-based precise estimates did not exist just a few years ago. While they were known to inhabit areas around 3 oases complexes, it is not known to what extent the bears move between these complexes, spanning a total of approximately 300 km in distance. To guide conservation efforts it is important to assess the status of this population, and a formal population survey was recommended by an international workshop organized by the Mongolian Ministry of Nature, Environment and Tourism in 2004. As part of a larger science-based conservation effort built on those recommendations and begun by the Gobi Bear Project in 2005, we carried out a DNA-based population survey (mark-recapture) in 2008 and 2009 to estimate the population, ascertain sex ratio, document inter-oases movements of individual bears, and explore genetic variability of Gobi bears. We placed barb-wire hair collection sites surrounding 13 feeders at most water sources within the 3 oases complexes Atas-Inges, Shar Khuls, and Tsagaan Bogd. Five collections occurred throughout the spring and summer by our research team and park rangers. In 2009 we collected 840 bear hair samples. We genotyped 204 of those samples at 24 microsatellite loci and identified 21 individual bears (14 males and 7 females). DNA from hair samples captured at supplemental feeders near springs enabled us to individually identify 21 bears 48 times and develop a statistically-rigorous mark-recapture population estimate of 23 bears (95% CI 22-31). Our capture probability (proportion of estimated population captured each collection session) was 0.42, indicating that our results were rigorous. We captured 1 female in our pilot survey 2008 that was not captured in 2009. In the 2009 survey, one female was sampled at 2 oases complexes. Two males were sampled at all 3 oases complexes and 5 were sampled at two complexes. The genetic variability (heterozygosity) was very low compared to other bear populations around the world. Our results indicate that this population is isolated from other bear populations as suspected and is critically endangered with less than 25-30 animals. Also of concern is the low number of females (8) we sampled. We recommend further sampling to verify the skewed sex ratio. This technique, when combined with remote photography and live capture (to determine age of individuals) may be used as a monitoring tool to assess recovery efforts.

## **Acknowledgements**

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## INTRODUCTION

The Great Gobi Strictly Protected Area (GGSPA, Fig. 1) covers approximately 45,918 km<sup>2</sup> in southwestern Mongolia and is home to a small remnant population of Gobi bears (*Ursus arctos isabellinus*), referred to as "mazaalai" by Mongolians. Gobi bears may live in one of the most extreme desert environments of any *Ursus arctos* population on earth (Schaller et al. 1993). They may hold important genetic variation from a global perspective related to their ability to survive in the harsh environmental conditions presently existing in the Gobi Desert, which may provide insight into survival in the anticipated further degradation of habitat associated with global climate change. Because of their present low population size, restricted range, and limited available habitat, further declines may be imminent. However, if effective conservation efforts are implemented, the bears have a much greater chance for recovery.

Gobi bears are confined to three oasis complexes in the GGSPA of southwestern Mongolia and utilize approximately 15,500 km<sup>2</sup> of habitat within the area. They are rarely observed and data about their biology, ecology, and behavior are sparse. There is little available information regarding the factors that limit their numbers; and efforts to promote their recovery, including re-population of any part of their former range, are problematic. Research to provide a much better understanding of Gobi bears is crucial to devising the most appropriate and timely conservation measures. Accurate assessments need to be made for population size and composition; effects of local or regional climate change and related potential affects on habitat requirements and food availability, importance and status of individual water sources within and between oasis complexes, productive capacity, including birth rate and mortality; genetic status, including level of inbreeding; and connectivity of use between oasis complexes by individual bears and by sex and age categories of bears.

To bring Gobi bears back from the brink of extinction, it is necessary to accurately know how many bears still survive and how they are distributed in the area. As part of the Gobi Bear Project, we began an effort to estimate the number of bears with the cooperation of the GGSPA staff, the Mongolian Ministry of Nature and Environment, the Mongolian Academy of Sciences, the UNDP, and the Gobi Bear Project research team. The objective was to collect enough DNA (hair) samples from bears living in the area to identify individuals and accurately estimate their numbers using mark-recapture estimation methods (Woods et al. 1999). Further objectives are to identify movements between springs and oasis complexes and estimate their genetic diversity relative to other brown bears populations of the world.

Most or all of the bears living in the area very likely frequent at least one of the supplemental feeding stations positioned at the springs that occur in each of the 3 oases complexes, Atas-Inges, Shar Khuls, and Tsaagan Bogd (Mijiddorj pers. comm., Fig. 2). Each oasis complex consists of a number of springs where surface water exists and feeder stations have been placed to supplement bear forage. These feeder stations present an excellent opportunity to collect DNA from Gobi bear hair. McCarthy et al. (2009) attempted to collect hairs from Gobi bears and used microsatellite genotypes to identify 8 individuals (successfully genotyped at 4 loci) in 2 oases complexes. Their data was insufficient to answer the intended questions of population size, sex ratio, inter-oases movements.

The Mongolian people consider the mazaalai to be a national treasure. The Gobi bear has long been listed in the Mongolian Red Book of Endangered Species, a categorization that was validated by the bear's designation as Critically Endangered (C2a(i)&D1) in the November 2005 Mongolian Biodiversity Databank Assessment Workshop (Clark et al. 2006). This workshop, organized by the Zoological Society of London and the National University of Mongolia, applied the rigorous criteria of the World Conservation Union (IUCN) in determining conservation status. This workshop was convened to devise an effective conservation program for the Gobi bear and develop recommendations for approaches that would best serve the bear's continued existence. Participants included knowledgeable Mongolian biologists and government officials, representatives of the International

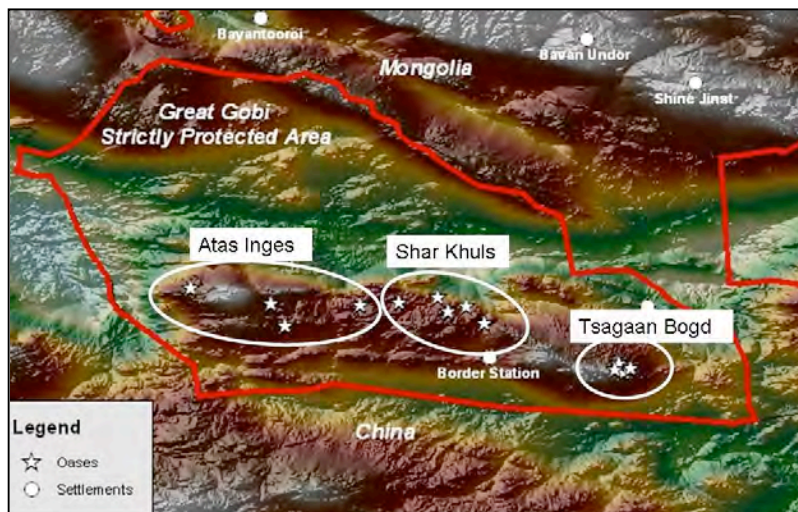
Association for Bear Research and Management, IUCN/SSC Specialist Groups, Wildlife Conservation Society and other international experts. Recommendations were made to address issues of: (1) population assessment and monitoring; (2) genetic and demographic information needs; and (3) human dimensions. Based on these recommendations, during 2005 the Gobi Bear Project was formed to initiate research that would be most effective in addressing urgent conservation needs, given the limited funding available. An essential aspect of the Project was to conduct this DNA-based survey and to estimate population size; it was specifically designed to accomplish several conservation objectives. Besides developing a rigorous estimate of the Gobi bear population size, our objectives were to learn about inter-oases movements of individual bears, ascertain the sex ratio, and develop a database of individual bear genotypes.

**Figure 1. Study area was within the Great Gobi Strictly Protected Area A (GBSPA) in southwest Mongolia.**



The results from our population assessment work will dovetail with our other efforts to understand the ecological needs of this endangered animal. Other objectives include: using GPS telemetry to understand their lifetime habitat requirements, identify critical habitats, understand what might be limiting this population, and determine if inbreeding or disease was contributing to their poor population status.

**Figure 2. Oases complexes (ovals) and springs (stars) within the Great Gobi Strictly Protected Area A (GBSPA).**



## METHODS

This survey was based on a mark-recapture method using hair sampling and DNA fingerprints of individual bears and their capture histories over several capture sessions (Woods et al. 1999, Proctor et al. 2010). Below we describe specific details of field, laboratory, and analytical methods.

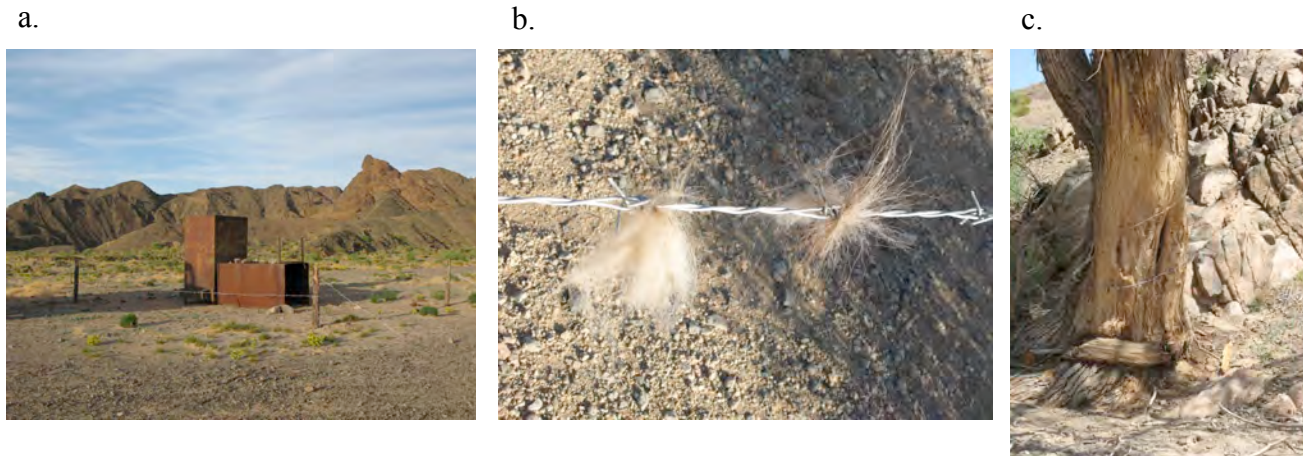
### Field Methods

We constructed a hair-snare DNA site at each of 13 springs that exist within the 3 main oases complexes (Fig. 2). A hair-snare site consists of a single strand of barb-wire surrounding a feeder station (Fig. 3a). As bears enter the wire enclosure, they leave a hair sample on the wire (Fig. 3b). In addition, it is well-known that bears in other areas choose trees to rub or scratch their backs. Therefore, we also placed barbed wire on a few trees to collect additional hair samples (Fig. 3c). Hair samples contain DNA that was used to identify individuals using microsatellite genotyping (DNA fingerprinting). Repeated hair collections were used to develop capture histories for individual bears and these data were used in a Mark-Recapture estimate of the number of bears surviving in the GGSPA.

We set up hair collections sites in 2008 and collected hairs throughout the spring and summer of that year as a pilot survey and to allow the bears to get used to the wire. In 2009 we carried out a formal mark-recapture population estimate by collecting hair in each of five collections sessions (Table 1).

Hair samples were placed in small paper envelopes for drying and storage until analysis. Relative location of samples and their adjacency to one another along the barb wire were noted to allow for sub-sampling in the lab.

**Figure 3**a) A hair snare DNA sampling site placed at each of 13 springs throughout the GGSPA; b) bear hair samples on barb-wire; and c) a rub tree fitted with barb-wire.



**Table 1.** 2009 Gobi bear hair collection sessions in the spring and summer of 2009.

Sessions 2009	Date interval
1	March 28 - 30
2	April 17 - 20
3	April 25 - May 3
4	June 4 - 8
5	July 12 - 14

## Laboratory Methods

The genetic analysis was carried out at the Wildlife Genetics International (WGI) laboratory in Nelson, British Columbia, Canada, by O. Tumendemberel (Fig. 4), while undertaking training from Dr. D. Paetkau. Samples were transported to WGI with appropriate CITES permits from Mongolia and Canada.

### Sample sub-selection

In the lab, samples were sub-sampled to minimize costs. This is a common procedure in DNA surveys (Proctor et al. 2010) because individual bears often leave many hair samples at a site per visit. Two criteria were used to exclude samples prior to extraction. First, samples with no guard hair roots and < 5 from underfur were set aside on the basis of quality. Only 6 samples containing < 10 underfur were analyzed, and the success rate for these was 0%. The second criterion for exclusion was to set a limit on the number of samples to be analyzed from a given collection time/place. The maximum was 4 extractions for collection points with < 8 samples, 5 extractions for points with 8–10 samples, and at least 50% with > 10 samples. The actual extraction rate for 2009 was 51%.

### Extraction

DNA was extracted using QIAGEN's DNeasy Tissue kits, and following the manufacturer's instructions (for details search <http://www.qiagen.com/>). We used 10 guard hair roots where available. When underfurs were used, DNA was extracted from clumps of whole underfur rather than from clipped individual roots.

**Figure 4.** Odbayar Tumendemberel at the Wildlife Genetics International (WGI) laboratory in Nelson, British Columbia, Canada. Ms. Tumendemberel received training in microsatellite genotype analysis while doing the genetic work necessary to complete this survey. Dr. David Paetkau and Renee Prive supervised and provided training at WGI. Funding for Ms. Tumendemberel's visit to Canada was provided by the Calgary Zoo (Canada), the International Association for Bear Research and Management, Reynolds Alaska Wildlife Associates, Birchdale Ecological and WGI.



### Marker selection

We expected low marker variability due to expected low genetic diversity. We therefore put considerable effort into marker selection before starting the analysis of individual identity. For this purpose we selected higher quality samples (generally extracted from  $\geq 10$  guard hair roots) that were collected at different times and places, and screened them with 26 microsatellite markers. The markers had to be run in three sets, and samples that performed particularly poorly with the first set of 10 markers were not analyzed at all 26 markers. Once we had multilocus genotypes (in various states of completion) from what we thought were 13 individuals, we reviewed marker variability and identified 13 markers that were not worthwhile to analyze because of their low variability.

In the final dataset, where we have results from at least 16 individuals per marker, the mean expected heterozygosity ( $H_E$ ) across these 13 markers was 0.03 (Table 2). Unfortunately, it was only at the end of the project that we realized the relatively higher variability of *G10O* ( $H_E = 0.51$ ), which would have made it a candidate for individual identification (ID). The 12 microsatellites that were potentially useful for individual ID had mean heterozygosity in the range of 50% in our marker selection dataset. Only *G10L* was notably less variable than the mean, but we kept it because the preliminary data suggested (incorrectly in hindsight) that this marker was relatively more variable in one oasis complex. Even with 12 markers, we were nervous because of the anticipated high level of relatedness among the study animals, so we added the amelogenin gender marker to the marker system for a total of 13 markers.

**Table 2.** Measures of marker variability, including the observed frequency of the most common allele for Gobi bears of Mongolia. Sample sizes are as per the final results (marker selection was done with smaller sample sizes). Differences between expected heterozygosity ( $H_E$ ) and observed heterozygosity ( $H_O$ ) were not significant at any marker.

Marker	N	$H_E$	$H_O$	A	Max f
G10B	22	0.54	0.59	3	0.64
G1D	22	0.75	0.82	4	0.32
D1a	22	0.62	0.68	3	0.52
I45P07	22	0.61	0.64	3	0.50
MU51	22	0.51	0.50	3	0.64
MU23	22	0.60	0.68	3	0.50
MU59	22	0.51	0.55	2	0.50
G10L	22	0.38	0.41	2	0.75
G10M	22	0.46	0.41	2	0.66
G10P	22	0.46	0.32	2	0.66
G10U	22	0.51	0.55	3	0.59
CXX110	22	0.51	0.64	2	0.55
G10O	16	0.51	0.44	2	0.53
Msut-6	20	0	0	1	1
G1A	21	0	0	1	1
G10H	19	0	0	1	1
G10C	19	0	0	1	1
CPH9	19	0	0	1	1
MU50	18	0.26	0.28	3	0.86
G10X	19	0	0	1	1
144A06	16	0	0	1	1
D123	16	0	0	1	1

Msut-2	16	0.18	0.19	2	0.91
MU26	16	0	0	1	1
CXX20	20	0	0	1	1
Mean		0.29	0.30	1.9	0.77

### **Individual microsatellite genotyping**

The 13 markers were completed in two single sequencer lanes, so the first pass involved 6 microsatellite markers, and the second, 7 markers. After the first pass we culled samples with high confidence scores for <3 of 6 markers. This cull was necessary to the efficiency and accuracy of our process, eliminating the samples with the lowest success rate and highest rate of genotyping error. Once again, samples that worked at <7 of 13 markers were culled. Any remaining samples with incomplete genotypes went on to one or more rounds of re-analysis.

### **Error checking**

Error-checking involved scrutiny of 1mis-match (MM), 2MM and 3MM pairs as detailed in Paetkau (2003). Mis-matches are pairs of genotypes that are identical except for 1 allele (1MM), 2MM (2 alleles), or more. These paired samples are potentially from the same bear and only often differ because of an error in the genotyping process. Replicating these close genotypes allows them to be verified and errors eliminated (Paetkau 2003). This process is an excellent method for minimizing or eliminating genotyping errors that would result in “false” identification of individual bears as a result of a genotyping error (see Kendall et al. 2009).

### **Population estimation**

Population estimates were generated using Program Capture (White et al. 1982) that assumes population closure during the survey. Closure violation occurs when bears leave or enter the study area during the sampling process and tends to positively bias population estimates as some bears may not be available for capture and therefore might be captured only once. Given Gobi bear distribution, movement pattern data collected from GPS collars, and the fact that no records of Gobi bear sightings or sign have been verified outside of their present distribution since the 1970’s in either China or elsewhere in Mongolia, there is strong evidence that the population is a closed one. Our population estimate represents the number of bears using the oases and springs of the presently known distribution of Gobi bears.

Program Capture consists of several estimator models that assume various forms of capture probability. The basic models are as follows. The null model (Mo) assumes all bears have an equal probability of capture. The time model (Mt) assumes that capture varies as a function of sampling session. The behavior model (Mb) assumes some bears may be trap shy (avoiding traps after one capture) or trap happy (attracted to sampling sites after one capture). The heterogeneity model (Mh) assumes each bear has its own capture probability. Models combining these assumptions are also considered.

### **Spatial analysis**

We used capture histories combined with spatial sampling locations to map the extent of individual bear movements within a Geographic Information System (GIS). This information allowed us to explore inter-oases movements of males and females and individual bears.

We also used the clustering software program Genetix (Belkhir 1999) to explore any genetic clustering due to habitat fidelity to any one oases complex. Genetix is a powerful clustering algorithm that does not rely on population specific allele frequencies and thus does not rely on high sample sizes to detect genetic similarities. Genetix uses a multidimensional Factorial Correspondence Analysis (FCA; Benzecri 1973, She et al. 1987) and provides an objective exploration into groupings of similar genotypes with no *a priori* assumptions of group membership.



## RESULTS

### Sample collection and genotyping

In 2009 we collected 840 hair samples from all 13 DNA sampling sites (Table 3 & 4). Sampling session length varied but averaged 24 days. Sampling results by spring, oases complex, and sampling session are summarized in Table 4. We determined 608 samples to be of sufficient quality for analysis from the 2009 sampling effort, 204 were successfully genotyped (Table 5). The genotyping success rate for the 2009 samples was 67% (see example genotype Fig. 5).

**Table 3.** Summary of Gobi bear hair sample collection for 2009.

#### Summary of preliminary results

Number of sites set up	13
Number of sites with hair	13
Number of hair samples collected	840
Average session length (days)	24

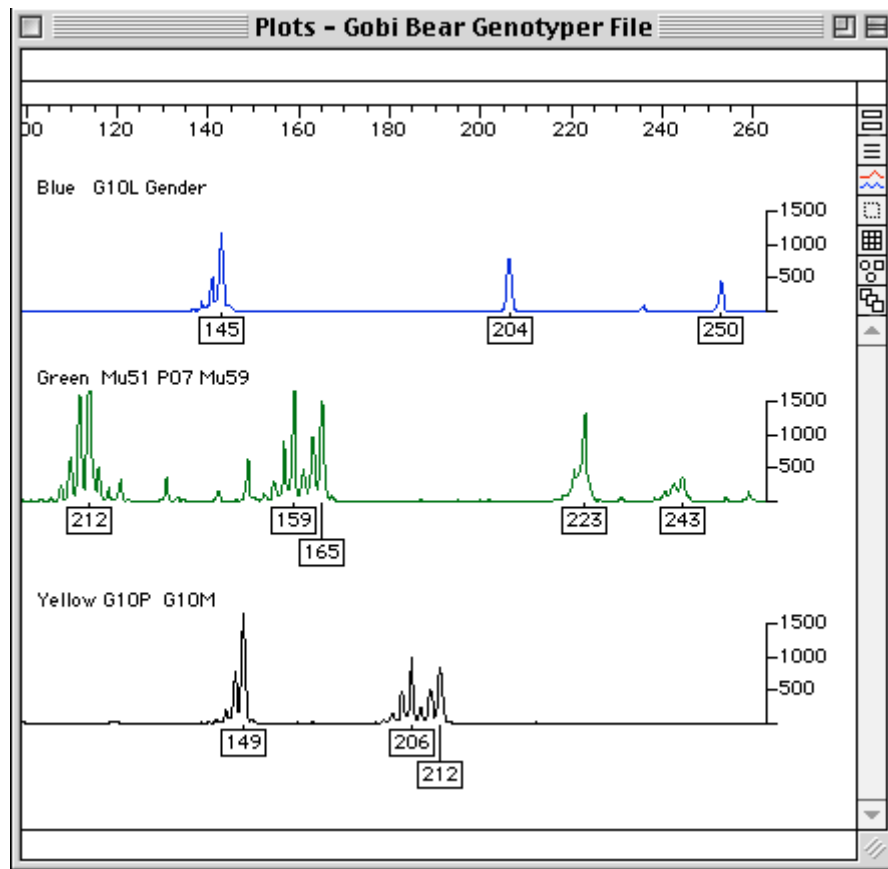
**Table 4.** Gobi bear hair sample collection results from hair snare DNA sites in the Great Gobi Strictly Protected Area from 2009.

Oases	Spring / feeder station	Sessions					Total
		28-30 March	17-20 April	15 April - 5 May	4-8 June	12-14 July	
Tsagaan Bogd	Altan Tevsh	1	32	16	10		59
	Mukhar Zadgai	5	42	22	30		99
	Khatuu Bula	2	4	4	47		57
	Suujiin Bulag	2	7	24	17		50
Shar Khuls	Khushuut				18	1	19
	Shar Khuls	3	25	37	18	6	89
	Tsagaan Burgas		23	9	21	26	79
	Tsagaan Tohoi	5	60	38	17	6	126
Atas - Inges	Khutul Us		23	27	28	19	97
	Khukh Khuls		1	1	1	18	21
	Bogts Tsagaan Ders		24		31	7	62
	Baruuntooroi		37			14	51
	Mazaalain Shand		10	2	2	17	31
	Total	18	288	180	240	114	840

**Table 5.** Summary of 2009 Gobi Bear hair samples used for microsatellite analysis. Of the 840 samples collected, 608 were deemed good enough to be taken to the Wildlife Genetics International laboratory for analysis. Sub-selected samples were removed and not genotyped because at the site and time they were collected there were many other samples. The sub-selection practice is commonly used in hair DNA surveys to reduce lab costs. Inadequate samples were either sampled mixed from 2 individuals, contained no follicles (with DNA), or yielded no result.

Sample category	Samples	% of total
total samples	608	
good samples	204	0.34
Sub-selected out	219	0.36
inadequate	185	0.30

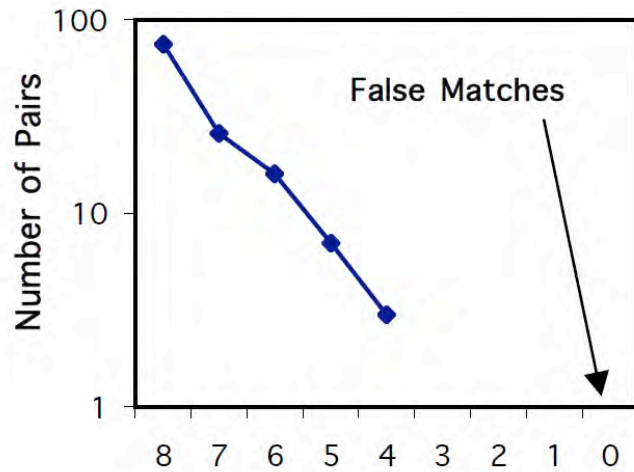
**Figure 5.** Genotype (DNA fingerprint) for 1 Gobi bear. Genotype consists of alleles at 6 microsatellite loci (genetic markers). This individual is homozygous (2 identical alleles) at the G10L, Mu51, and G10P loci and heterozygous (two different alleles) at P07, Mu59 and G10M loci.



### Error checking

After re-analyzing all paired genotypes that had 1MM, 2MM 3MM, and 4MM, our final data set contained no pairs of genotypes with less than 4MM, indicating that we likely had no “false” bears due to genotyping error (Fig. 6). Between scoring errors, loading errors, and amplification errors associated with remotely collected hair samples, 24 samples had changes recorded to genotypes that had been entered into the database as high-confidence scores.

**Figure 6.** Plot of mismatching between paired genotypes. Graph indicates that the closest two individual bears' genotypes differ at 4 alleles suggesting a very low probability two bears having an identical genotype.



The expected heterozygosity of the Gobi bear was 0.29, and this is close to the lowest values recorded for any population of *Ursus arctos* bears in the world (Table 6).

**Table 6.** Genetic variability of Gobi Bears relative to other *Ursus arctos* bear populations around the world. The Spanish Pyrenees population is also a small isolated population. The bear of Kodiak Island are isolated off the Alaska coast, but are a thriving healthy population.

Study area	Heterozygosity	Alleles / locus
Pyrenees Mts. Spain	0.25	1.7
Gobi	0.29	1.9
Kodiak Is Alaska	0.30	2.1
Pakistan	0.49	3.3
Yellowstone USA	0.55	4.4
S Selkirks Canada	0.54	4.3
Scandinavia Europe	0.66	5.8
Southern Canada	0.68	6.4
Northern Canada	0.78	7.4

**Identification of individuals**

We identified 22 individuals in 2008 and 2009. These included 8 females and 14 males, with a mean of 4.8 samples assigned to each female versus a mean of 14.1 samples per male. The 2009 samples were assigned to 21 individuals, compared to just 7 bears for the 2008 samples. The single animal that was only identified in 2008 was female, and was tied to 2 samples collected at the same time and place. Males were sampled at all 13 sites and females at 11 (Table 7).

**Table 7.** Summary of 2009 sex-specific Gobi bear identifications from DNA fingerprints by oases complex and individual spring.

Oases complex	Spring	Males	Females
Atas Inges	Baruun Tooroi	3	3
Atas Inges	Bogtstsagaanders	4	2
Atas Inges	KhukhKhuls	2	
Atas Inges	Mazaali Shands	1	1
Shar Khuls	Khoteluus	4	1
Shar Khuls	Khushuut	2	1
Shar Khuls	Shar Khuls	5	1
Shar Khuls	Tsagaan burgas	5	
Shar Khuls	Tsagaan tohoi	4	
Tsagaan Bogd	Altan Tevsh	4	1
Tsagaan Bogd	Khatuu Bulag	4	1
Tsagaan Bogd	Mukhar zadgai	5	1
Tsagaan Bogd	Suujiin bulag	3	2

### Population estimate

In the 2009 survey, we captured 21 bears a total of 48 times (Table 8) and captured bears in all 5 sessions (Table 9). The model estimates of population size were all very similar (Table 10) indicating very little model selection bias. We selected the heterogeneity estimator Mh (Chao) because it best suits individual bear capture probability variation (Boulanger et al 2002, Proctor et al. 2010) and is robust to small sample sizes as found in this project. **Therefore our estimate of the Gobi bear population size is 23 bears with a 95% confidence interval of 22-31 bears.** Other similar bear surveys with larger datasets have generally detected individual capture heterogeneity in bears and it is recommended that in small data sets it be assumed it is present (Boulanger et al. 2002, Proctor et al. 2010). Also, the Chao estimator provides a more realistic confidence interval when confronted with small sample sizes (White et al. 1982). Our per-session capture probability was 0.42 (Table 10) meaning that we captured 42% of the estimated population in each session. This capture probability rate is considered excellent in DNA surveys such as this one (Proctor et al. 2010). When the males are analyzed separately through the same process, the male-only estimate is 15 males with a 95% CI of 14-22. There were too few female captures to model a female only estimate.

**Table 8.** Recapture summary for males, females, and both sexes of Gobi bears sampled in 2009. 1x indicates the number of bears captured 1 time, 2x indicates the number of bears identified 2 times, 3x - 3 times, 4x - 4 times, 5x - 5 times.

	Males	Females	Both sexes
Bears identified	14	7	21
Captures	37	12	48
1x	2	3	5
2x	4	3	8
3x	6	1	6
4x	1	0	1
5x	1	0	1

**Table 9.** Summary of Gobi bear DNA captures by sampling session for both sexes combined in the 2009 DNA-based population survey.

<b>Both sexes</b>						
Session	1	2	3	4	5	
Animals caught	3	12	6	17	10	
Total caught	0	3	12	14	19	21
Newly caught	3	9	2	5	2	

**Table 10.** Program Capture model-based population estimates for both sexes of Gobi bears in the 2009 DNA-based population survey carried out in the GGSPA. Mo is the null model with no assumptions of capture effects by time, behavior of heterogeneity. Mh is the model that assumes individual heterogeneity (different capture probability) between individual bears. Mb assumes a behavioral response among sampled bears. Note all model estimates are very close suggesting a rigorous data set and minimal model selection bias. We selected the Mh Chao estimate as the best estimate because it most accurately reflects the biology of bears (individual capture probability variation) and is best suited for survey with small sample sizes as in this survey.

<b>Model</b>	<b>Population estimate</b>	<b>Lower confidence interval</b>	<b>Upper confidence interval</b>	<b>Capture probability within 1 session</b>
Mo	22	22	28	0.44
Mt	21	21	21	0.14 0.57 0.29 0.81 0.48
Mb	32	23	101	0.19
Mh	22	22	37	0.44
<b>Mh Chao</b>	<b>23</b>	<b>22</b>	<b>31</b>	<b>0.42</b>
Mt Chao	22	22	28	0.14 0.55 0.27 0.77 0.45
Mbh	23	22	38	
Mth Chao	24	22	35	0.12 0.50 0.25 0.71 0.42
				0.137 0.524 0.190 0.715
Mtb	22	22	35	0.324

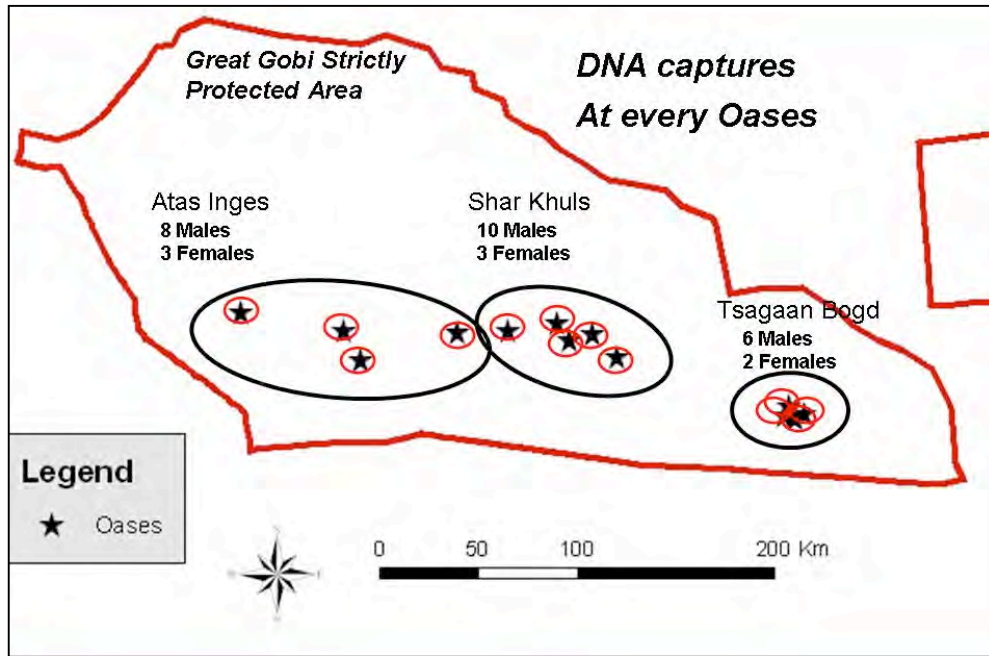
**Spatial capture results**

DNA from hair samples of Gobi bears were collected at all oases complexes and springs sampled (Fig. 7). Eight males and 3 females were sampled at the 4 springs within the Atas Inges oases complex. Ten males and 3 females were sampled at the Shar Khuls oases complex and 6 males and 2 females were sampled at the Tsagaan Bogd complex. Springs at which samples of females were collected are indicated in Fig. 8. DNA from a single individual female was captured at springs within both the Atas-Inges and Shar Khuls complexes, which confirms movement between the complexes (Fig. 9). Males were captured at all sampling sites and numbers of captures for each spring are indicated within Fig. 10.

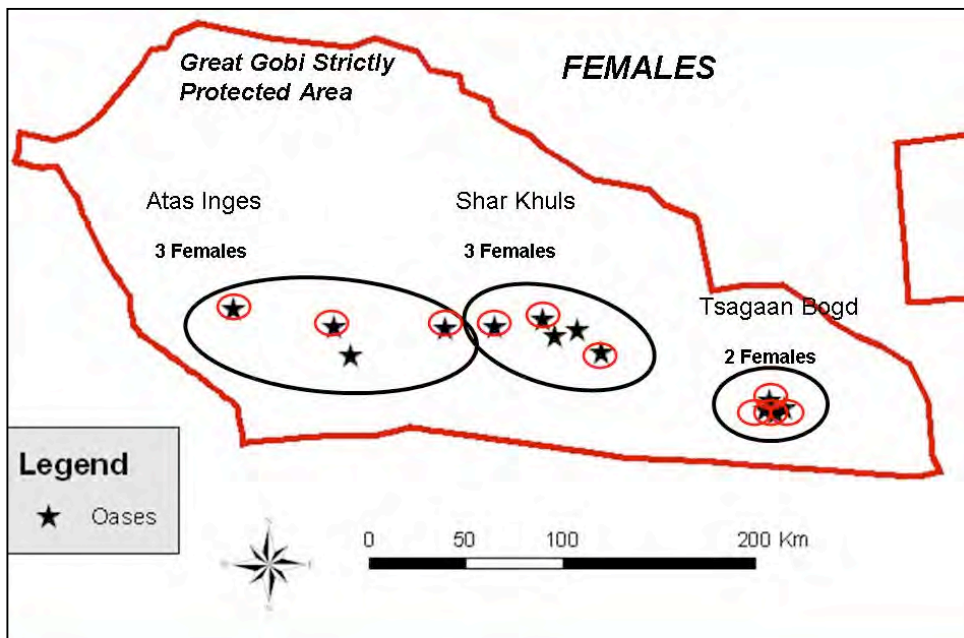
Three males were captured at both Atas-Inges and Shar Khuls oases complexes (Figs.12a, b, & c). Three males were captured at all three oases complexes (Figs.13a, b, & c). One male was captured at Shar Khuls and Tsagaan Bogd complexes (Fig.14).

We found no clustering of individuals based on capture location within oases complexes (Fig.15).

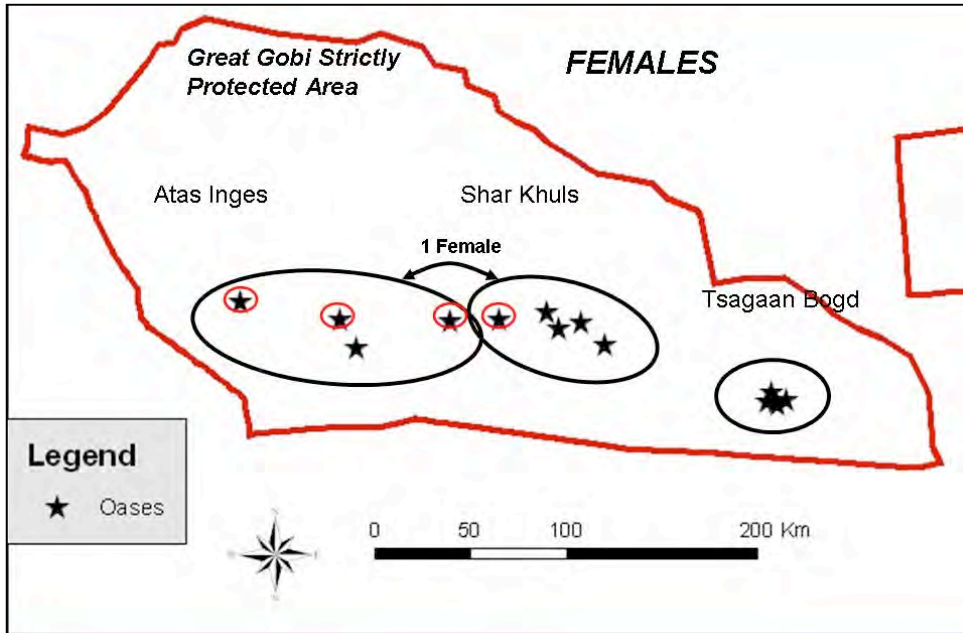
**Figure 7.** Summary of DNA captures of Gobi bears by oases complex. Red ovals indicate springs within oases complexes where Gobi bears were sampled. Note that hair samples from bears were collected at all springs at which hair traps were located.



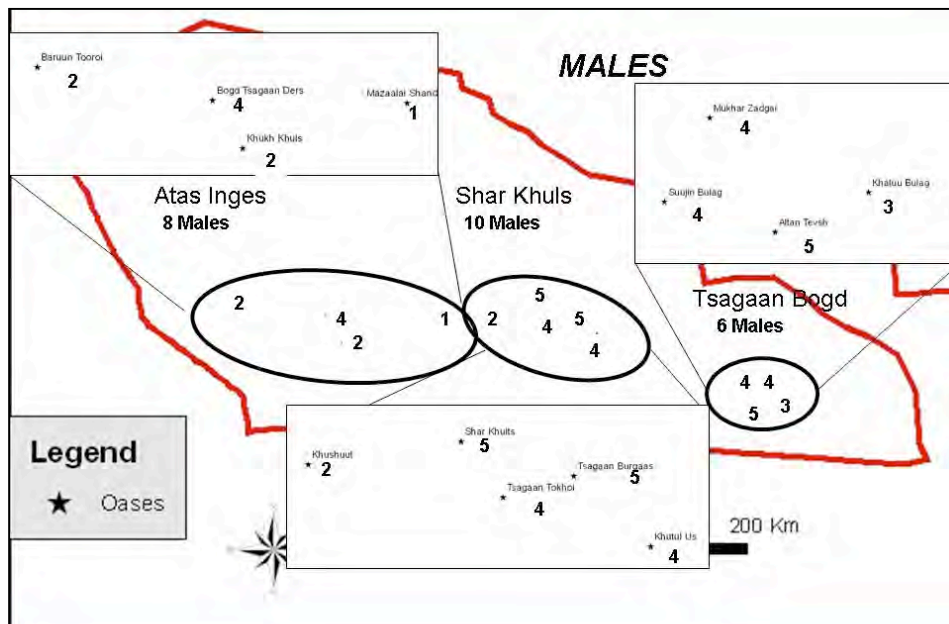
**Figure 8.** Summary of DNA captures of female Gobi bears sampled during the 2009 population survey by oases complex and spring. Red ovals indicate springs where female bears were DNA captured.



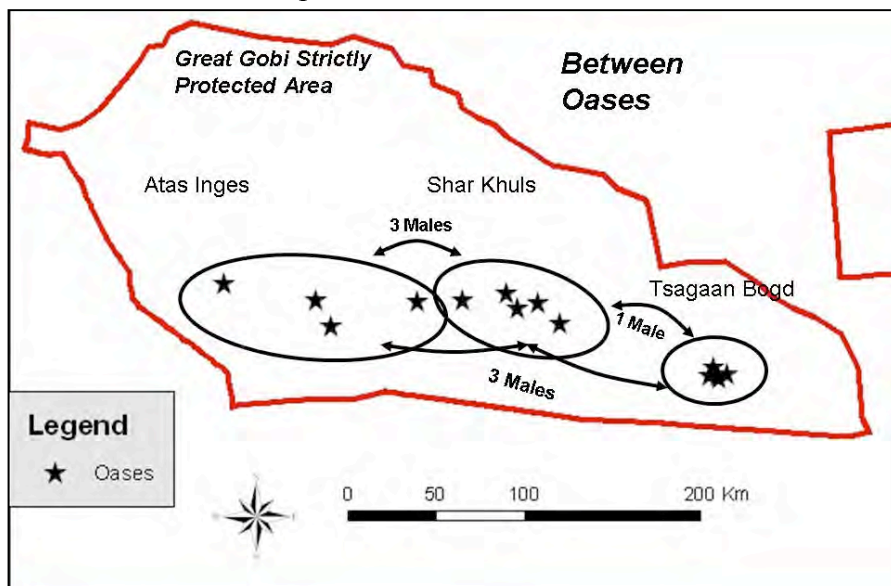
**Figure 9.** One female Gobi bear's DNA capture locations between oases complexes Shar Khuls and Atas Ingés oases complexes during the 2009 population survey. Red ovals indicate springs where 1 female bear was sampled.



**Figure 10.** Summary of male Gobi bears sampled during the 2009 population survey by oases complex and spring. Red ovals indicate springs where male bears were DNA captured. Insets provide individual spring names within each oases complex.

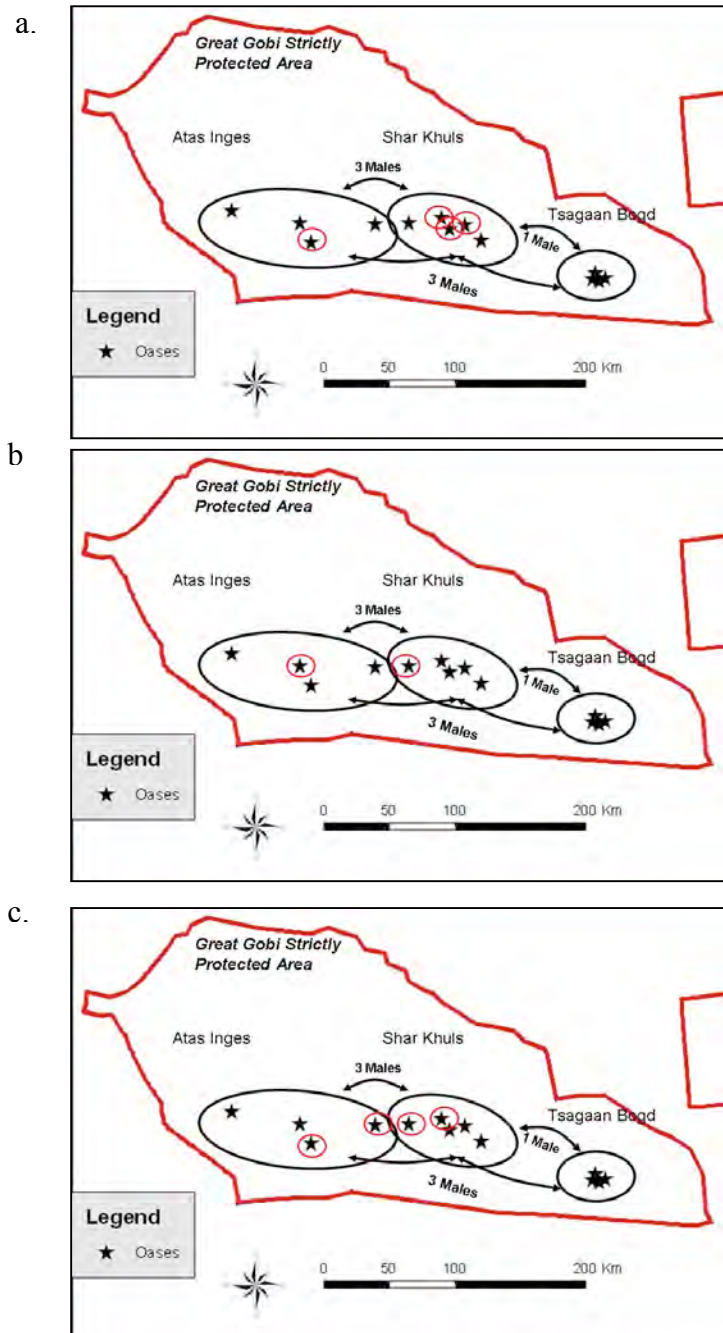


**Figure 11.** Summary of male Gobi bear recaptures between oases complexes. Arrowed lines above the oasis complexes indicate movement by males between those complexes; lines below indicate movement of 3 male bears between all 3 oasis complexes.

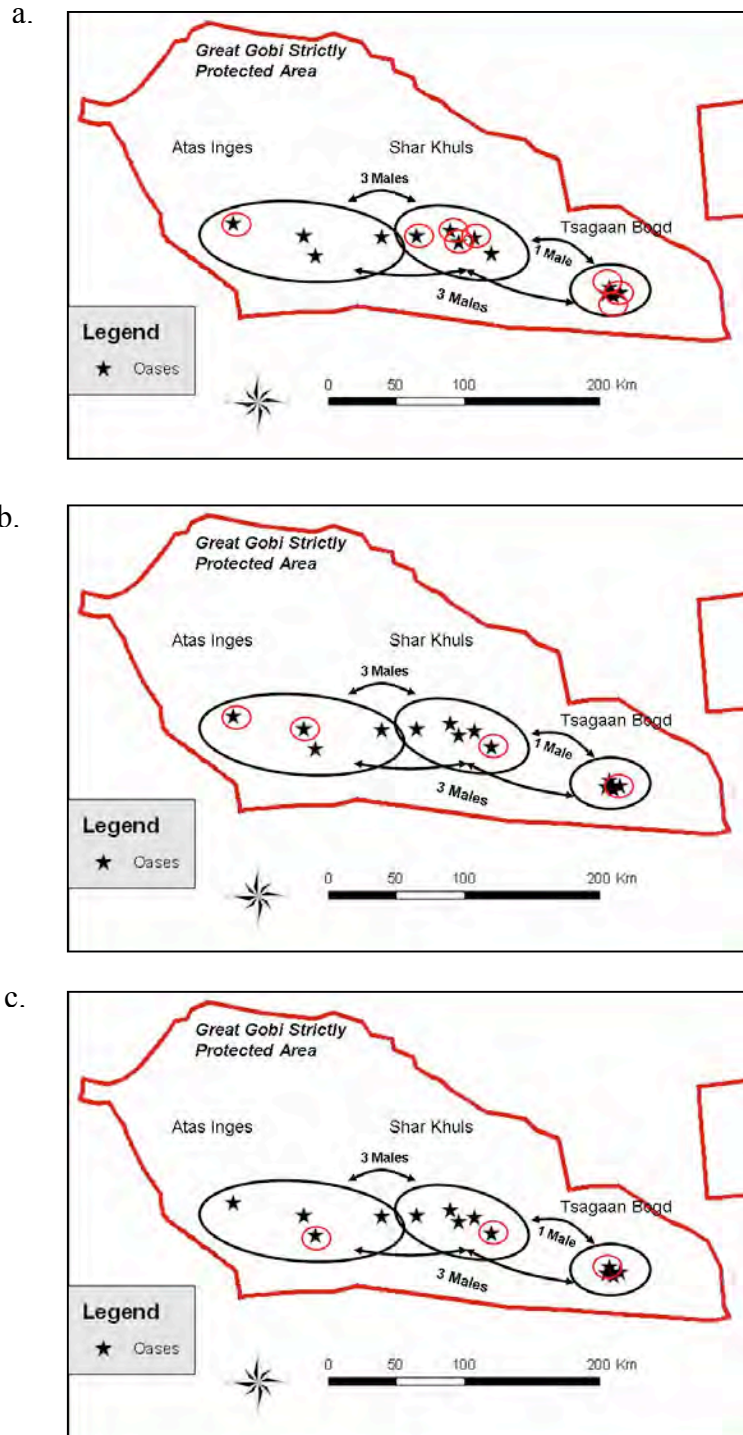




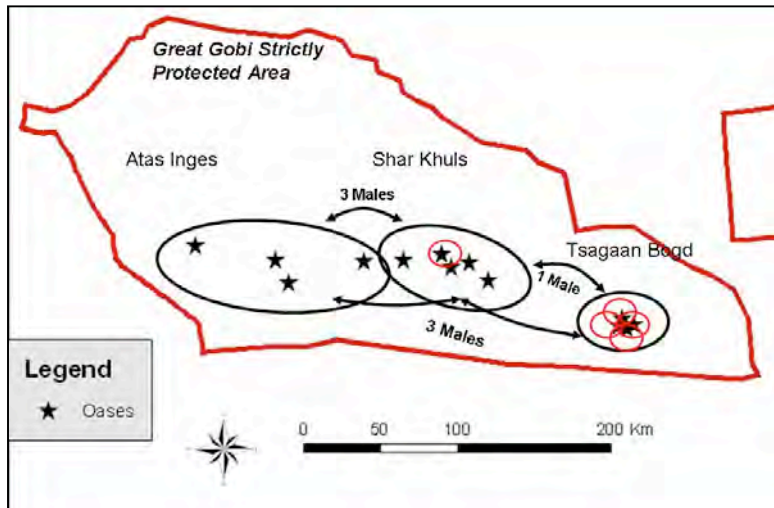
**Figure 12.** Three male Gobi bear's DNA capture locations between oases complexes in panels a, b, and c respectively, documenting movement between oases complexes during the 2009 population survey. Red ovals indicate springs where each male bear was sampled. Lines above the oasis complexes indicate movement by males between those complexes; lines below indicate movement of 3 male bears between all 3 oasis complexes.



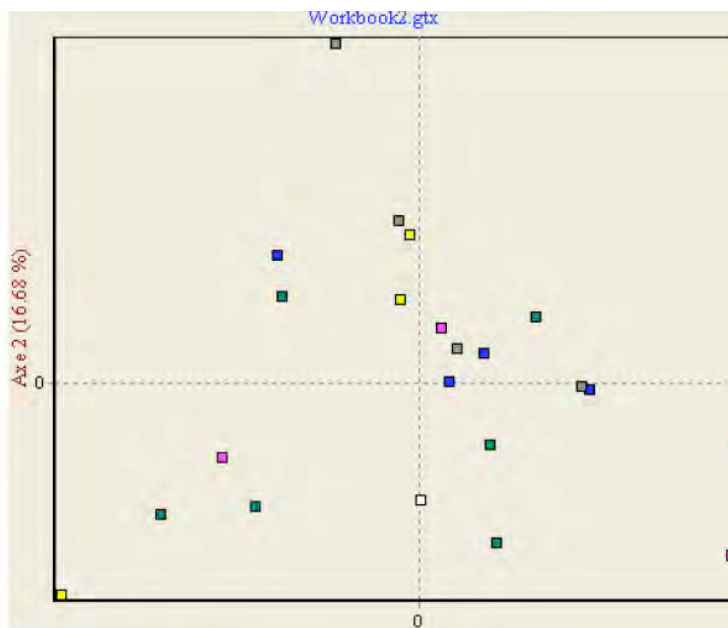
**Figure 13.** Three male Gobi bears' DNA capture locations between all three oases complexes in panels a, b, and c respectively documenting movement between oases during the 2009 population survey. Red ovals indicate springs where 1 male bear were sampled in each of the 3 panels. Lines above the oasis complexes indicate movement by males between those complexes; lines below indicate movement of 3 male bears between all 3 oasis complexes.



**Figure 14.** One Gobi male's DNA capture locations between oases complexes – Shar Khuls and Tsagaan Bogd - during the 2009 population survey. Red ovals indicate springs where 1 male bear was sampled. Lines above the oasis complexes indicate movement by males between those complexes; lines below indicate movement of 3 male bears between all 3 oasis complexes.



**Figure 15.** Genetix plot demonstrating the lack clustering between any oases complex. Plot is based on 22 individuals and 12 microsatellites. Capture locations are coded as follows: Atas - Inges grey; Shar Khuls teal; Tsagaan Bogd pink; all three oases complexes yellow; combinations of two areas blue and white.



## DISCUSSION

This survey is the first rigorous data-based population estimate of the Gobi bear. Our estimate of 22-31 bears makes the Gobi bears of Mongolia one of the smallest *Ursus arctos* populations in the world. The genetic diversity, as measured by heterozygosity, being so low, corroborates observations by GGSPA personnel that this population is totally isolated from all other *Ursus arctos* bears. The combination of very low numbers and its isolation verify that this population has a very serious conservation status and is at risk of extirpation. These realities have been presumed by the Mongolian wildlife authorities within the Ministry of Nature, Environment and Tourism (MNET), but this effort provides accurate verification of that conservation status.

Because population size is one of the most powerful predictors of persistence (Berger 1990, Shaffer et al. 2000, Reed et al. 2003) and populations with fewer than 50-100 adults are at higher risk of extirpation (IUCN [The World Conservation Union] 2003), the Gobi bears long-term prospects represent a tremendous challenge and is likely related to informed and effective management. Besides efforts to improve nutrition (through the current supplemental feeding program) there is a necessity that a long term strategy be designed to increase bear numbers either within its present range or through expansion of occupied range to its reported pre-1970's distribution.

Our high recapture rate (Table 8) is responsible for the congruity of population estimates across estimator models (Table 10) and provides confidence that there is low model selection bias and the estimate reflects the number of Gobi bears using the oases. In 2009 we captured 21 individual bears and our best estimate was 23 bears, suggesting that we captured almost the entire population that uses the sampled oases complexes. Less encouraging is that across 2008 and 2009 we captured only 8 females suggesting that there are likely fewer than 10 females in the population. Populations with this few females likely face threats from stochastic (chance) demographic events (e.g. disease, low reproductivity) as well as the potential effects of inbreeding.

We captured females at 10 of the 13 springs within each of the 3 oases complexes and the number of females frequenting each complex was similar (Table 7, Fig. 8). One female was captured at both springs within the Atas – Inges and Shar Khuls complexes that spanned approximately 120 km (Fig. 9).

We captured males at all springs within each oasis complex (Table 7, Fig. 10). Tsagaan Bogd complex had the fewest males (6; Table 7, Fig. 10) and Shar Khuls had the most males (10; Table 7, Fig. 10). Spatial distribution of male captures between oases complexes showed that 3 were captured in both Atas – Inges and Shar Khuls complexes (Fig 12) and 3 males were captured at springs within all 3 oases complexes (Figs.13). Two of these males were captured at oases that were approximately 280 km apart. Our GPS data will provide a more detail examination of home range size, but based on the pattern of DNA captures, the home ranges of several males will likely be in the range of 3000 – 6000 km<sup>2</sup>. Home ranges of this size are consistent with ecosystems of low productivity (McLoughlin et al. 2000). These movement data provide a solid base of information to explain our result of no internal genetic structure within the Gobi bears between oases complexes (Fig. 15).

*Ursus arctos* is a species characterized by long lives (20-30 years) and slow reproductive rates (Miller 1980). Therefore, there is time to implement management to resist extirpation. Several management strategies are already in place including a supplemental feeding program. We will not review recommendations here, except to mention the likely need for a long term strategy to increase the number of bears by expanding the area of suitable habitat, either by expansion of their current distribution, or establishment of a second nearby population that might be connected through occasional movements.

This project was designed to be integral to the ongoing Gobi Bear Project efforts to assess factors which may limit population dynamics of Gobi bears. Included in these efforts are data collected by GPS

telemetry, capture and examination of bears, and assessing habitat requirements. That effort has seen good success in collaring bears and can provide more detailed information on the habitat use of individual bears, diet, home range size, identification of important foraging areas, particularly those in areas between individual springs, and sources of mortality. Also, the GPS information can provide information on the relative time spent by individual bears at supplemental feeding stations, their use of habitat around the springs, and the amount of time bears go between spring visits for water intake.

### **CONCLUDING REMARKS**

This survey of the Gobi bear population using hair snagging and DNA was very successful. We captured a very high proportion of bears in the population and recaptured a good proportion of those captured bears which resulted in a rigorous estimate of population size with a 95% confidence interval. Our results provide reliable base-line data for future reference to assess the effectiveness of any management plans implemented to maintain or enhance population size, distribution, and inter-oases movements. These samples and the nuclear DNA-based analysis will also provide a start of a nuclear database with which to assess taxonomic issues surrounding the Gobi bear, by allowing inference based on more than mtDNA.

Future monitoring may take the form of a repeat DNA-based population survey at some interval (5-10 years). Note overlapping confidence intervals may preclude conclusive evidence of incremental population increase or decline, but may provide evidence of drastic changes (particularly large decreases), the assurance that the population is stable, and evidence of an increase in the number of females.

Another possibility is the combination of DNA sampling coupled with remote camera traps to monitor evidence of reproduction within the populations. It would be relatively easy to keep a data-base of all the genotypes of individual bears, and document new additions to the populations (births) through the existence of new genotypes. These can potentially be matched to mothers through parent-offspring allele sharing patterns and further documented with camera trap evidence of mothers with young. Several such monitoring efforts are underway on other small threatened populations in other locations around the world (DeBarba et al. 2010). This effort would be most efficient with GGSPA rangers monitoring camera traps and collecting hair samples. A program to genotype Gobi bears in a Mongolian lab may keep costs reasonable. An effort such as this is one reason O. Tumendemberel was trained in microsatellite genotyping in Canada during this survey.

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Appendix I. Microsatellite genotype data for 22 Gobi bears of Mongolia. Most bears were genotyped at up to 26 loci (column # loci) however all loci not presented were fixed, that is all individuals cross the population carried the same allele. We therefore do not present those data. Data were generated at the Wildlife Genetics Laboratory, Nelson BC, Canada.

Individual	Sex	# samples	# loci	<i>G10B</i>	<i>G1D</i>	<i>D1a</i>	<i>P07</i>	<i>MU51</i>	<i>MU23</i>	<i>MU59</i>	<i>G10L</i>	<i>G10M</i>	<i>G10P</i>	<i>G10U</i>	<i>CXX110</i>
235	F	2	26	140.160	174.182	169.179	159.165	212.212	195.201	243.243	145.155	206.212	149.149	175.179	147.149
256	F	9	20	140.140	180.180	181.181	165.165	212.214	195.195	243.243	145.145	206.212	149.157	159.179	147.147
619	F	3	26	140.140	174.176	169.181	159.163	212.212	195.201	223.223	145.145	206.206	149.149	175.179	149.149
646	F	2	26	140.140	174.182	179.179	163.165	212.214	195.195	223.243	145.145	206.212	149.149	175.179	149.149
699	F	1	12	140.140	174.180	179.181	159.163	210.212	195.201	223.243	145.155	206.206	149.149	175.179	147.149
824	F	9	26	140.148	176.180	179.181	159.165	212.212	195.201	223.243	145.155	206.212	149.157	175.179	147.149
835	F	10	26	140.148	176.176	181.181	165.165	212.214	201.201	223.243	145.145	206.212	149.157	175.179	147.149
836	F	2	26	140.160	176.180	181.181	159.165	212.214	187.201	223.223	145.155	206.212	149.149	175.179	149.149
20	M	14	26	140.148	180.180	169.181	165.165	214.214	195.201	243.243	145.145	212.212	157.157	175.179	147.147
149	M	30	26	140.140	174.174	169.179	163.163	212.214	195.201	223.223	145.145	206.206	149.149	175.175	149.149
255	M	59	21	140.160	174.182	169.179	159.165	212.212	187.201	223.243	145.145	206.212	149.149	179.179	147.149
273	M	20	19	140.160	174.182	181.181	165.165	210.214	195.195	243.243	145.145	206.206	149.149	175.175	147.147
463	M	2	26	140.148	176.180	181.181	159.165	212.212	195.201	223.243	145.155	206.206	157.157	175.179	147.149
465	M	8	15	160.160	180.182	169.181	159.159	212.214	187.201	223.243	155.155	212.212	149.149	175.179	149.149
483	M	5	15	148.160	176.182	169.181	159.165	212.214	201.201	223.243	145.155	206.212	149.149	175.175	147.149
555	M	5	26	140.148	174.176	169.181	159.165	212.212	195.201	243.243	145.155	206.212	149.149	179.179	147.149
595	M	9	26	140.160	174.176	169.179	165.165	212.212	187.195	223.243	145.145	212.212	149.157	179.179	147.149
657	M	12	26	140.140	174.180	169.181	163.165	214.214	201.201	223.243	145.145	206.206	157.157	179.179	147.149
696	M	9	26	140.160	176.180	169.181	159.165	212.214	187.201	223.223	145.145	206.206	157.157	179.179	147.149
759	M	2	26	140.148	176.180	169.181	159.165	210.212	195.201	223.223	145.145	206.206	149.157	175.179	147.149
793	M	12	26	140.140	174.180	169.181	159.165	212.212	201.201	223.243	145.155	206.206	149.157	179.179	147.149
808	M	10	26	140.140	180.182	181.181	159.159	212.212	195.201	223.243	145.155	206.206	149.157	179.179	147.149