Status of the Gobi bear in Mongolia as determined by noninvasive genetic methods

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Abstract: A relict population of unique desert dwelling brown bears (*Ursus arctos*) inhabits a series of remote oases along the southern portion of the Great Gobi Strictly Protected Area in Mongolia. Little is known about these bears, which may number as few as 25 animals. We used noninvasive genetic techniques in an attempt to estimate minimum population size, determine sex ratios, and evaluate genetic diversity and degree of isolation between population centers. Between 1996 and 1998 we collected 200 hair samples using hair-traps from rub posts and attempted to amplify 6 microsatellite loci for 75 samples with 3 or more follicles. Microsatellite amplification rates were low (63%), and 3 loci were monomorphic. Complete genotypes could be obtained for only 28 samples, which provided a minimum count of 8 bears. Observed heterozygosity (0.29) and average number of alleles (2) were very low compared to other brown bear populations. Genetic data were obtained for only 2 of the 3 population centers, and sample sizes were not large enough to accurately evaluate sex ratio or levels of isolation. A 263 base-pair segment of the mitochondrial DNA control region was sequenced for 3 bears and a single control region haplotype was obtained. This haplotype was identical to a previously published haplotype for the Gobi bear, and earlier work has shown that this haplotype is closely related to brown bear haplotypes from Pakistan. Future genetic analyses that attempt to use hair or fecal samples will need to increase the number of loci to provide sufficient resolving power for individual identification and should attempt to collect fresher samples to increase success rates. The detection of very low levels of genetic diversity supports the hypothesis that this population is very small and isolated from other brown bear populations. Further studies of the Gobi bear and conservative management actions are greatly needed.

Key words: Gobi bear, Great Gobi, microsatellite, Mongolia, population estimation, *Ursus arctos*

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The range of the brown bear (*Ursus arctos*) is Holarctic and widespread, but has been substantially reduced over the past 2 centuries, and numerous insular or remnant populations now exist (Servheen 1990). Perhaps one of the least studied and most endangered populations is found in the isolated massifs of Mongolia's Great Gobi. The Gobi bear, or "mazaalai" as it is referred to by local people, is unique among bears in its use of this barren desert niche (Schaller et al. 1993), and its precarious status is evidenced by its occurrence in the International Union for the Conservation of Nature (IUCN 2008) and the Mongolian red books (Shiirevdamba et al. 1997). Protected in Mongolia, the bear's range lays almost entirely within the core area of the Great Gobi Strict Protected Area (GGSPA), which is closed to all but sanctioned research activities. Thus, human disturbance is minimal. Due to low funding and difficult access to bear habitat, management is limited to supplemental feeding each spring. Although little investigation of this secretive species has been conducted, information suggests that as few as 25 animals may remain. The harsh desert environment may make this population's continued existence precarious. Notably, its range has been reduced by half since 1970 (Schaller et al. 1993), and only 3 population centers are thought to exist (Fig. 1). These centers are separated by 60 to 100 km of open desert; thus, limited genetic interchange

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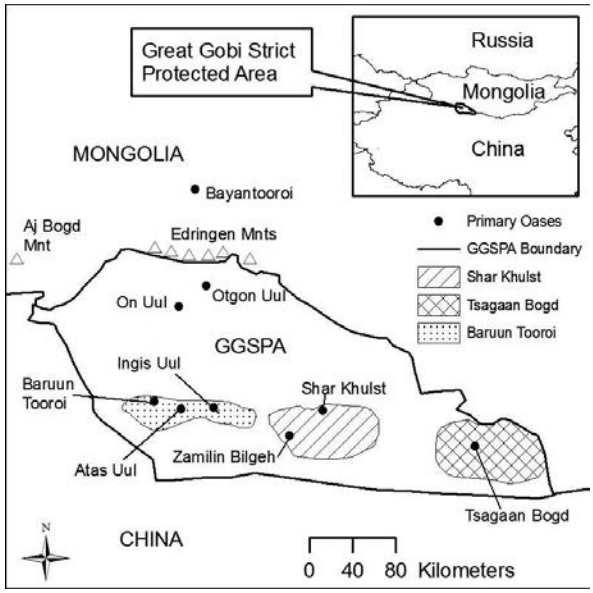

Fig. 1. Oases and oases complexes of the Great Gobi Strictly Protected Area (GGSPA), Mongolia, investigated during a study of the Gobi bear in Mongolia, 1996–98.

between sites may further reduce population viability.

The first record of an unknown bear in the Gobi came from the notes of V. Ladygin (United Nations Environmental Programme 1988). In 1900, he found tracks and diggings near Tsagaan Bogd, Tsagaan Burgasny-bulak, and Shar Khulst, sites that still support small populations of the bears. Joint Soviet and Mongolian scientific expeditions in the mid-1930s were unsuccessful in studying the bear due to its rarity, and the first confirmed observations of a Gobi bear did not occur until 1943 during an expedition of the Science Committee of the Mongolian Peoples' Republic (Bannikov 1954). With the establishment of the GGSPA in 1975, emphasis was placed on investigating Gobi bear distribution, population size, and ecology.

Bannikov (1954) suggested that the range of the Gobi bear previously extended east to the Tost-Ula Mountains, approximately 70 km east of the GGSPA border. As late as 1970, the bears ranged as far north as the Edringen mountains, which make up the northern border of the protected area, and as far west as the Aj Bogd range some 100 km outside the park. Currently, the range of the Gobi bear is thought to be restricted entirely to the southern half of GGSPA and encompasses about 15,500 km^2. Areas of bear activity center around Atas Uul, Shar Khulst, and Tsagaan Bogd mountains and associated oases. Zhirnov and Ilyinsky (1986) felt that bear home range sizes varied seasonally with food availability, but remained relatively small, and individuals rarely ventured far from oases. Despite the use of radiocollars, Schaller et al. (1993) were unable to estimate actual home range size, but calculated a minimum home range size of 650 km^2 for one male bear with straight-line movements exceeding 48 km, taking him far from the oasis around which his activity centered. At the time our study was undertaken, there had been no previous attempts to document movements of bears between activity centers, thus degree of isolation of each activity center's bear population was unknown.

After apparent declines in the 1970s, population estimates for the bear have been relatively constant over the past decades (B. Choijin, GGSPA, Bayantorooi, Mongolia, personal communication, 1996). Zhirnov and Ilyinsky (1986) estimated that 25–30 bears remained in the early 1980s. Schaller et al. (1993) believed this number was still a reasonable estimate after conducting surveys in 1990, although population parameters remain unknown.

In contrast to other brown bears, Gobi bears are relatively small with reports of adults weighing between 100 and 120 kg (United Nations Environmental Programme 1988, Schaller et al. 1993). The bear is light brown, and the head, belly, and legs can be noticeably darker than the rest of the body; light stripes or a collar are often discernible about the neck (United Nations Environmental Programme 1988). Assuming similarity to the Tibetan brown bear (*Ursus arctos pruinosus*), the Gobi bear is commonly referred to by the former's specific epithet (Mallon 1985, Zhirnov and Ilyinsky 1986). Schaller et al. (1993), observing both the Gobi bear and the Tibetan brown bear in the wild, noted distinct differences in appearance and questioned the likelihood of them being the same species or subspecies. They suggested the Gobi bear is more likely the same subspecies as the brown bears that inhabit either the nearby Tian Shan Mountains, *U. a. isabellinus*, or the Altai Mountains, *U. a. arctos*. Sokolov and Orlov (1992) established the Gobi bear as a distinct species, *U. gobiensis*. However, they based their conclusions on morphological measurements from only 3 individuals, thus leaving the new taxonomic distinction questionable. Recent mitochondrial DNA genetic analyses have evaluated the phyloge-

netic relationship of the Gobi bear to the Tibetan brown bear and Pakistan brown bear and found support for Schaller et al.'s (1993) hypothesis that the Gobi bear is closely related to *U. a. isabellinus* of Pakistan (Miller et al. 2006, Galbreath et al. 2007).

In the late 1990s the GGSPA and the Mongolian Ministry of Nature and Environment (MNE) identified the Gobi bear as a species of special concern and in need of immediate protective measures and additional research. In 1995, MNE sought funding to capture and radiocollar several Gobi bears for study and to move them between oases to facilitate genetic interchange. Given the potential risks associated with capture, the near impossibility at that time of conducting telemetry studies in the Gobi without aid of aircraft, and the lack of evidence for breeding isolates, the project received no support from donor agencies.

Recent advances in molecular methods have led to increased use of genetic techniques to address such conservation biology questions in bears (Paetkau and Strobeck 1998, Waits et al. 1998, Taberlet et al. 1999) and other species. Microsatellites, hypervariable short tandem repeats of 1–5 base pairs that are distributed throughout the nuclear genome, have been used in bear research to identify individuals (Taberlet et al. 1997, Woods et al. 1999, Bellemain et al. 2005), evaluate parent–offspring relationships (Craighead et al. 1995) and mating system (Bellemain et al. 2006), estimate movements across the landscape (Taberlet et al. 1997, Woods et al. 1999, Bellemain et al. 2005), and measure genetic variation within and between populations (Paetkau et al. 1998a,b; Waits et al. 2001).

In 1996 we initiated a field study using noninvasive genetic sampling to: (1) establish a minimum population size estimate for Gobi bears within the GGSPA; (2) determine sex ratio of the sampled population; and (3) evaluate degree of interchange between population centers within the GGSPA.

Methods

Field

Three oasis complexes were identified by GGSPA staff as bear activity centers: Baruun Tooroi, Shar Khulst, and Tsagaan Bogd (Fig. 1). Each complex contains several water holes and is separated from other complexes by roughly 60 km (Baruun Torooi–Shar Khulst) to 100 km (Shar Khulst–Tsagaan Bogd) of open desert.

To minimize disturbance of bears, we collected hair samples by use of 3 types of hair-traps. Barbed wire was strung 30–40 cm above the feed troughs of established supplemental feeding stations. Where no feeders existed we hung bait from trees and strung barbed wire around the trunk and out to an adjacent tree or stake. In areas lacking both trees and feeders, we brought in tripods of 4 m long logs. Barbed wire was strung between and wrapped around the legs of log tripods. Bait was hung from the tripod apex. We deployed 17 hair traps: 4 at feeders, 4 at tripods, and 9 at rub trees. We set an initial target of 50 hair samples from each of the oases complexes.

Collection of hair samples was initiated following den emergence in spring 1996 and continued through late summer 1998. A circuit of more than 600 km by jeep was required to reach all traps; hence, visits were opportunistic and accomplished in conjunction with other Park work. Baits were replaced on each visit.

For each hair sample we recorded type of collection device, date, location, and whether all the hairs in the sample were positively from a single animal. For example, multiple hairs pulled from around a rub tree are possibly from >1 individual, while a clump of hairs on a single barb at a wire trap are probably from one animal. Samples were collected using tweezers or sterile rubber gloves and placed in individual sealed paper envelopes.

Genetic analyses

For 158 samples collected between March 1996 and August 1997, analyses were restricted to samples with 5 or more hairs with roots. This minimum number of roots was chosen as a conservative method to provide enough DNA to avoid genotyping errors that are known to occur in microsatellite analysis of samples with low quantities of DNA (Taberlet et al. 1997, 1999; Goossens et al. 1998). Only 37 samples met this criterion and were processed. Because the initial collection of useable samples was small, a second batch of hairs was collected in summer 1998 with emphasis on multi-hair samples. Forty-two samples were acquired, of which 38 had roots and were processed, including 9 with <5 hairs. This batch of hair samples was processed at a different laboratory than the first batch using the same microsatellite primers.

All DNA extractions and polymerase chain reactions (PCRs) were performed in a room dedicated to processing bone, scat, and hair samples to

Table 1. Genotype, sex, number of appearances, source oasis, and years genotype was observed for all samples with complete data for the 3 polymorphic microsatellite loci.

	Microsatellite loci (size in bp)						Years observed		
Genotype	G10B	G10L	G1D	Sex	Obs.[a]	Oasis[b]	1996	1997	1998
A_f	136/144	143/153	177/177	F	6	BT	0	6	0
A_m	136/144	143/153	177/177	M	5	BT,SK	0	3	2
B	136/136	143/153	177/179	M	7	BT	6	0	1
C	136/136	143/143	177/179	F	3	BT	0	3	0
D	136/144	143/153	171/177	M	1	SK	0	0	1
E	136/136	143/143	171/177	M	4	SK	1	0	3
F	136/156	143/143	171/179		1	SK	0	0	1
G	136/144	143/143	173/173	F	1	BT	0	0	1

[a]Number of times genotype appeared in sample data.
[b]BT = Baruun Tooroi, SK = Shar Khulst

avoid contamination errors. DNA was extracted from all samples with visible roots using 200 µl of 5% Chelex solution (Walsh et al. 1991) and further purified with a Geneclean II Kit (Bio101, Vista, California, USA). A suite of 6 microsatellite loci (G1A, G10B, G10C, G10L, G10X, G1D) of 200 base pairs or less was used for individual identification (Paetkau et al. 1995, 1998a). Brown bears have been previously surveyed across North America (Paetkau et al. 1998a,b) and parts of Europe (Taberlet et al. 1997, Waits et al. 2001) using these loci, and a large number of alleles (5–13) per locus have been identified.

PCR reactions were carried out in 15 µL volumes in an MJ PTC-100 thermal cycler. Reaction mixes contained 2–4 µL template DNA, 1.5 µL 10x Goldtaq buffer (Sigma-Aldrich, St. Louis, Missouri, USA), 0.5 Units GoldTaq (Sigma-Aldrich), 150 µM of each dNTP, 0.15 µM of G1A, G10L, 0.2 µM of G10B, and G10X, 0.3 µM of G10C, and 0.5 µM of G1D. After an initial denaturation of 10 minutes at 95°C, 45 cycles of 30 sec at 95°C, 30 sec at 57.5°C, and 40 sec at 72°C were performed followed by a final extension of 2 min at 72°C. Primers were fluorescently labeled with dyes TET or 6-FAM or HEX. Negative controls were incorporated into all amplification and electrophoresis runs. Amplified products were evaluated using an ABI 377 (Applied Biosystems, Carlsbad, California, USA) system, according to the protocols described in Woods et al. (1999). Genotypes for each sample were determined using the Genescan and Genotyper software packages (Perkin Elmer, Applied Biosystems, Carlsbad, California, USA).

Only samples with complete genotypes at polymorphic loci were included in the results section and genetic diversity calculations. Samples with genotypes observed only 1 time in the dataset (D, F and G in Table 1) were amplified 3–11 times/locus ($\bar{x}$ = 4.8) to verify accuracy. For the 26 samples with genotypes observed multiple times (A–C, E, Table 1), 11 were amplified 2–5 times/locus ($\bar{x}$ = 2.7) to verify accuracy and estimate genotyping errors using the methods described in Broquet and Petit (2004). Probability of identity statistics were calculated using program Gimlet to evaluate the statistical power to differentiate individuals (Waits et al. 2001, Valière 2002). Genetic diversity statistics were calculated using GENEPOP 3.4 (Raymond and Rousett 1995) and FSTAT 2.9.3 (Goudet 1995).

All samples with unique genotypes were analyzed to determine gender using either the ZFX/ZFY primers described by Woods et al. (1999) or primers SE47/SE48 of the amelogenin locus (Ennis and Gallagher 1994). One primer of each pair was fluorescently labeled and PCR fragments were sized on a 5% acrylamide gel using the ABI 377 fluorescent detection system. All individuals with an X and Y fragment were scored as males, and all individuals with an X fragment only were scored as females. Amplifications were repeated 3–5 times per male and 6–8 times per female.

DNA sequencing of a short segment (263 base pairs [bp]) of the 5′ hypervariable segment of the mitochondrial DNA (mtDNA) control region was completed for 3 Gobi bears (Am, B, E, Table 1) using the L15997 and H16401 primers described in Waits et al. (1998). These sequences were compared to sequences from recently published work by aligning our sequences with previously published data from Gobi bears and other brown bears (Miller et al. 2006, Galbreath et al. 2007).

Table 2. Success rates for determining microsatellite genotypes from Gobi bear hair samples, by oasis and for all oases pooled, for a study of Gobi bears in Mongolia, 1996–98.

Oasis	Samples	Processed	Failed 6 loci	Failed 1–4 loci	Complete 6-locus genotypes
Baruun Tooroi	53	32	4	9	19
Shar Khulst	124	38	21	8	9
Tsagaan Bogd	23	5	3	2	0
All oases	200	75	28	19	28

Results

Hair samples were not collected in equal numbers from all oases due to difficulties reaching Tsagaan Bogd, and of the 200 hair samples collected only 23 came from that oasis (Table 2). Both Baruun Tooroi and Shar Khulst were well represented, although the latter was more frequently visited by rangers and thus provided a large percent of the samples (62%). Of the 200 samples collected, 75 were deemed suitable (number of hairs or presence of roots) and were processed for genetic analyses. Forty-seven (63%) of these samples provided microsatellite genotypes at 1–6 loci, but only 28 samples (37%) provided enough DNA for complete genotypes at all 6 loci. Three of the loci (G1A, G10C, G10X) were monomorphic, and 2–4 alleles per locus were detected at the remaining loci (G10B, G1D, G10L, Table 1).

Average allelic dropout rates were high, ranging from 0.12–0.35/locus, but false allele errors were rare (0–0.05/locus; Table 3). However, our error checking analyses revealed that most samples were error free, and the high error rates can be attributed to only 4 low quality samples that were error prone. All samples with genotypes at the 3 polymorphic loci were included in subsequent analyses of individual identification (Table 1) and genetic diversity (Table 4). We obtained no complete genotypes for the Tsagaan Bogd oases.

We identified 7 unique microsatellite genotypes (Table 1), and 1 genotype was observed in both a male and female bear, bringing the minimum count to 8 bears. Three of the genotypes were observed in multiple years, and 4 were only found in samples from a single year. Genetic diversity statistics were estimated from these 8 genotypes, and was very low in this population compared to other brown bear populations (Table 4). The number of alleles per locus ranged from 1 (G10X, G10C, G1A) to 4 (G1D), with an average of 2. Observed heterozygosity was 0–0.63 with a mean of 0.29. The P_{ID} and P_{IDsibs} for the 3 polymorphic loci were 0.0049 and 0.189 respectively. No genotype met the $P_{sib} < 0.05$ criterion used as a threshold for individual identification in many studies (Woods et al. 1999). Thus, our estimate of 8 bears may be an underestimate, and identical genotypes may represent more than one bear.

Sex identification was completed for 7 of the 8 unique genotypes revealing 3 female and 4 male bears. Interestingly, female bears were only detected at the Baruun Tooroi oasis. However, the sample with genotype F failed to amplify after 8 attempts, and it is possible that this individual is female since the X fragment is 56 base pairs longer than the Y fragment and was more likely to dropout and fail to amplify than the Y fragment (Table 3).

A control region sequence of 263 base pairs was obtained for 3 attempted individuals. All three sequences were an identical match to the mtDNA control region sequence previously reported for Gobi bear samples (Miller et al. 2006, Galbreath et al. 2007). This short sequence differed from published haplotypes from Pakistan by 1–2 base pairs, Tibet by 11–14 base pairs, Iran by 13 base pairs, and all other brown bear populations from Asia, Europe, and North America by 12–22 base pairs.

Table 3. Allelic drop out (ADO) and false allele (FA) microsatellite genotyping error rates for Gobi bear hair samples and X and Y fragment dropout rates estimated from male samples for a study of the Gobi bear in Mongolia, 1996–98.

Locus	ADO	FA
G10B	0.12	0
G1D	0.18	0
G10L	0.35	0.05
X	0.36	
Y	0.08	

Discussion

Our success rate for obtaining multi-locus genotypes from hair was low, and there were several potential reasons for this. The remote study area was visited infrequently and hair trap samples may have

Table 4. Genetic diversity measures for Gobi bears and other brown bear populations for a study of the Gobi bear in Mongolia, 1996–98, based on the 6 microsatellite loci used in this study.

Population	Sample size	Allelic richness	Observed heterozygosity	Citation
Gobi	8	1.9	0.29	This study
Pakistan	28	3.3	0.49	Bellemain et al. 2007
Kodiak Island, Alaska	30	2.0	0.35	Paetkau et al. 1998b
Pyrenees Mountains	6	1.7	0.25	Taberlet et al. 1997
Yellowstone, USA	57	3.3	0.50	Paetkau et al. 1998b
East Slope, Canada	41	4.2	0.60	Paetkau et al. 1998b
Kluane, Canada	50	4.4	0.77	Paetkau et al. 1998b

been exposed to the elements for 3 to 6 months, and for unknown periods on rub trees. Multiple studies using hair and fecal samples to obtain DNA have demonstrated that success rates decrease as time in the field increases (Piggot 2004, Bellemain et al. 2007, Murphy et al. 2007). The Gobi's desiccating conditions may enhance long-term preservation of the hair roots, but lengthy exposure to extreme heat and cold, ultraviolet radiation, and mechanical abrasion from sand storms, may have led to DNA degradation. The low density of bears also led to infrequent hair captures in our traps, which in turn caused us to rely more on rub trees for samples. Hairs left at rub trees were often single and were more likely to be shed without root bulbs. Future studies should attempt to improve sample quality by setting more traps, installing barbed wire on rub trees, and monitoring traps more frequently.

Two primary goals of our research were to determine a minimum population size and sex ratio for Gobi bears in the GGSPA. Prior to this study, estimates ranged from 25 to 35 animals (Zhirnov and Ilyinsky 1986, Schaller et al. 1993), with bears nearly equally distributed between the 3 activity centers (B. Choijin, personal communication, 1996). We identified a minimum of 8 bears that occurred at the Shar Khulst and Baruun Tooroi oases, or about 50% of the animals thought to use those oases at the time of this study. The overall sex ratio was nearly 50:50 with 4 males and 3 females identified, but all of the females we observed were from the Baruun Tooroi sample. Information from Tsagaan Bogd oasis is completely lacking because of poor sample quality from that area.

Detecting movement of bears between oases was also an important goal of the study. We identified 4 unique bears from Baruun Tooroi and 3 unique bears from Shar Khulst. One additional genotype (Am) appeared at both oases. Because of the high P_{sib} value and low diversity levels in this population, we can not say with a high degree of certainty that the matching genotypes represent the same individual or that they indicate movement of bears between oases. However, if interchange was frequent and widespread, we would have expected to detect more matching genotypes between the 2 oases.

Genetic diversity in the overall population is among the lowest ever observed for brown bears (Table 4), indicating high potential for inbreeding concerns. Genetic diversity levels this low have only been reported for the small isolated remnant population of brown bears in the Pyrenees Mountains at the border of France and Spain (Table 4). Genetic drift operates more rapidly in small populations such as that of the Gobi bear and increases the risk of inbreeding depression, which has been found to be more severe in highly stressful environments (Keller and Waller 2002). To what degree this might be influenced by population isolates within the reserve remains unclear.

Non-invasive genetic techniques did not provide adequate data for pedigree analyses or a mark–recapture assessment of population size. However, we were able to obtain partial answers to basic questions about minimum population size, bear movements between oases, and genetic diversity. Adding additional microsatellite loci would have provided more information; however, this was not possible because the samples yielded such low quality DNA that all DNA extract was expended for many samples when generating the current genotypes. Taberlet et al. (1999) warn that the application of noninvasive genetic sampling techniques may be most difficult for endangered species with low levels of heterozygosity; certainly, this is true in our case. Any new study should attempt new methods for improving PCR success (Piggot et al. 2004, Bellemain et al. 2005) and screen additional microsatellite loci to increase the power to identify individuals.

More frequent field collections combined with more hair traps may improve sample quality and thus reduce likelihood of genotyping errors. Funding and logistic obstacles precluded such measures in this study. Collection of feces to augment sample size is recommended for future studies; however, locating feces is far less predictable than collecting hairs at feeders and known rub trees, so we do not suggest relying completely on feces to obtain Gobi bear genetic samples. Studying and monitoring this rare animal through improved non-invasive techniques reduces the inherent risks of invasive methods such as capturing and radiocollaring bears. This is an important consideration, since the loss of any reproductive females could be catastrophic, especially if the sex ratio is already skewed toward males. At a minimum, capture and collaring of such rare animals should only be undertaken by highly skilled professionals. Such a study using advanced GPS collars was initiated in the GGSPA in 2004 and is now providing valuable data with no losses or injuries to bears (H. Reynolds, Fairbanks, Alaska, USA, personal communication, 2008). A combination of collaring and non-invasive methods may provide the data and insight needed for appropriate conservation measures to be defined and implemented.

Our mtDNA sequencing revealed one haplotype that has been observed in other Gobi bears, and we did not attempt phylogenetic analyses since this haplotype has recently been included in phylogenetic analyses of brown bears across their worldwide distribution (Miller et al. 2006, Galbreath et al. 2007). This work has revealed that while Gobi bears are distantly related to other brown bears, they share recent common ancestry with brown bears of the Deosai plains of northern Pakistan. Thus, Sokolov and Orlov's (1992) designation of the Gobi bear as a distinct species (*Ursus gobiensis*) is not justified, but the Gobi population is apparently genetically isolated from other bear populations, making it a single conservation unit (Moritz 1994, Palsboll et al. 2007).

Proactive management of the Gobi bears has been considered by the Mongolian government, including capturing and moving bears between oases to facilitate gene flow. We urge caution due to the inherent risk involved and the uncertainty that such measures would provide substantial benefits to an overall population this small, especially if some genetic exchange now occurs naturally. Fitness and growth rates of very small populations have been enhanced by the introduction of even a few outside individuals (Madsen et al. 1999, Vilà et al. 2002). Genetically similar brown bears from northern Pakistan may be the best potential candidates for such augmentation of the Gobi bear population.

The small number of bears observed in our study indicates that current low-risk management actions should be continued to help ensure the persistence of this remnant population. Management actions currently include supplemental feeding and stringent restrictions on human presence. Continued research is warranted and should include non-invasive genetic methods of assessing population status and trends.

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