Short Communication

Mitochondrial DNA variation and population structure of the Critically Endangered saiga antelope *Saiga tatarica*

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Abstract We analysed the mtDNA control region (HV1) of 93 tissue samples from all five populations of the saiga antelope *Saiga tatarica*. The results show a slight but clear distinction between *S. t. mongolica* and *S. t. tatarica*, supporting the current designation of *S. t. mongolica* as a subspecies rather than a separate species. Levels of genetic diversity were low in *S. t. mongolica*, consistent with the small size of its population and long isolation. Although populations of *S. t. tatarica* have reasonable levels of genetic diversity, their conservation status is perilous. The Kalmykian and Kazakhstan samples each contained unique haplotypes, although the species as a whole appears polyphyletic, consistent with recent fragmentation and rapid population decline. An understanding of the population genetics of this species is an essential prerequisite for conservation action.

Keywords Control region, genetic diversity, Kalmykia, Kazakhstan, Mongolia, *Saiga tatarica*.

The saiga antelope *Saiga tatarica* has two population groups that differ in some aspects of morphology and are classified as separate subspecies. *S. t. tatarica* has three populations in Kazakhstan and one in the Republic of Kalmykia, Russia. Between 1980 and 1994 this group was numerous, averaging 950,000 individuals (Milner-Gulland *et al.*, 2001). Censuses have been patchy following the break-up of the Soviet Union, but from 1998 a sharp decline was observed. The best estimates of the total population size in 2003 were an order of magnitude lower, at *c*. 36,000 animals, and in 2003 the subspecies was categorized as Critically Endangered on the IUCN Red List (IUCN, 2004). The Mongolian subspecies *S. t. mongolica*, categorized in 2003 as Vulnerable on the basis that the population was small but stable, is much less numerous, with the maximum recorded group size since 1980 being 5,000 animals in 2000 (Lushchekina *et al.*, 1999; L. Amgalan, unpubl. data). Numbers declined sharply following the harsh winter of 2001/2, and in 2003 the population size was estimated to be only 750.

Although the Mongolian saiga is considered a subspecies this has been disputed, with some authorities recognizing it as a separate species on the basis of morphological and ecological differences with other groups (Lushchekina *et al.*, 1999). There has also been discussion of the extent to which the Kalmykian population of *S. t. tatarica* is distinct from saiga in Kazakhstan (Sokolov *et al.*, 1998). These discussions are now more important, with the possible imminent demise of one of the Kazakhstan populations, the precarious position of the Mongolian population, and the relative stability of the Kalmykian population. Captive breeding is successfully underway in Kalmykia, and there is discussion about extending this to other areas. With limited funds conservation prioritization is necessary, but if this is to be effective in conserving the species some understanding of genetic diversity between and within populations is required. Here we report the results of analyses of saiga mitochondrial DNA (mtDNA) that clarify the phylogenetic status of the populations. Analysis of mtDNA offers several advantages for studies of this sort. It is present in relatively high copy number in cells, and can often be found when nuclear DNA has degraded, improving the likelihood of successful detection from a variety

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Received 8 December 2004. Revision requested 20 April 2005. Accepted 8 June 2005.

of tissue sources. A number of primer sequences have been described for it, and there is good understanding of the rate of evolutionary change in mtDNA sequences, making it particularly attractive for determining phylogeny (Walker *et al.*, 1995; Avise, 2000).

Tissue samples were obtained from a total of 93 animals representing all five saiga populations (Fig. 1) over the period 1998–2000 (38 from Kalmykia, 40 from Kazakhstan of which 12 were from Ural, 13 from Ustiurt, 15 from Betpak-Dala, and 15 from the Shargyn Gobi region of Mongolia). The majority of the samples were non-invasively obtained from museum specimens, hair and faeces collected in the field, or from saiga found dead from road-kills or poaching. Twenty samples were from saiga shot under scientific licence for other purposes by the Kalmykian Department of Hunting Management.

For DNA extraction we used Instagene or Chelex 100 resin (Bio-Rad Laboratories) according to the manufacturer's protocols with some modifications (adding the enzymes proteinase K and RNAase A and changing the duration and temperature of incubation to 65°C for 45–60 min). A standard lysis buffer containing proteinase K was followed by phenol/chloroform extraction (Sambrook *et al.*, 1989) for muscle and liver samples that had been frozen or preserved in ethanol. The best results for the museum samples were obtained using the kit ExtraGene Diatom prep (Biocom, Shaikhaev, Russia) based on the method of Hoss & Paabo (1993).

For PCR amplification for the analysis of intraspecific genetic diversity we used primers for the first hypervariable fragment (HV1) of the mtDNA control Pro (L 15376)-5′-CAC TAT CAA CAC CCA AAG CTG AAG-3′ and Dlc (H 16498)-5′-ATG GCC CTG AAG AAA GAA CAA GAT-3′, both designed at the Center for Reproduction of Endangered Species, San Diego, USA. PCR reactions were performed in a total volume of 30 µl containing 1.5 mM $MgCl_2$, 200 µm each dNTP, 0.5 µm each primer, PCR buffer and 0.5 µl (2.5 units) *Taq* Polymerase (SibEnzim, Russia). The thermal cycling programme consisted of predenaturation at 94°C for 3 min, 40 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 6 min. PCR-products were purified on columns (QIAGEN, QIAquick, Germany) and sequenced using dye terminator automatic sequencing with ABI PRISM 373 or 377 in accordance with the ABI PRIZM User's manual (Applied Biosystems, Foster City, California, USA).

Bioedit (Hall, 1999) was used to obtain a preliminary multiple alignment, which was then manually corrected. Genetic diversity between populations was evaluated using a 408 bp fragment of the 5′ peripheral domain of the control region (HV1), one of the most variable

Fig. 1 The current range of the saiga antelope, showing the approximate range area of each of the populations. 1, Kalmykia; 2, Ural; 3, Ustiurt; 4, Betpak-dala (all *S. t. tatarica*); 5, Mongolia (*S. t. mongolica*; 5a, Shargyn Gobi population; 5b, Mankhan population).

https://doi.org/10.1017/S0030605306000135 Published online by Cambridge University Press

fragments of mtDNA in mammals (Taberlet, 1996; Avise, 2000). Pairwise genetic distances between populations were estimated using Kimura's two-parameter method (Kimura, 1980). Mega 2.1 (Saitou & Nei, 1987; Kumar *et al.*, 2001) and Arlequin version 2.000 (Schneider *et al.*, 2000) were used for statistical calculations. The neighbour-joining method in Mega 2.1 was used for inferring the phylogenetic relationships between the control region haplotypes (i.e. for estimating the relatedness of individuals based on the similarity of their DNA sequences). The deciding criterion used for definition of the phylogenetic status of different saiga populations with respect to their gene tree was the relationship between maximum distances within either population (max d_{AA} or max d_{BB}) versus minimum distance between two populations (min d_{AB}), as proposed by Neigel & Avise (1986). We also produced a Bayesian phylogeny model using the programme MrBayes (Huelsenbeck & Ronquist, 2001), which gave qualitatively the same results as the neighbour-joining tree, thus cross-validating our findings.

Analysis of the mtDNA control region showed a clear distinction between the Mongolian and other saiga groups. The Mongolian group had a high proportion of unique haplotypes but low divergence between them (Table 1), as would be expected from long-term isolation and small population size (Lushchekina *et al.*, 1999). The results would also be consistent with the population having gone through a genetic bottleneck. The neighbour-joining tree shows how clearly distinct the Mongolian clade is (Fig. 2), despite the small genetic differences from the other populations.

In historical times the populations of *S. t. tatarica* could have mixed. For example, there is evidence that during particularly severe winters saiga are able to cross the Volga river to forage (Sokolov & Zhirnov, 1998). However, in the last century land use changes have separated their ranges, and movement between populations is now much less likely (Bekenov *et al.*, 1998). Our study concurs with the suggestion that these populations were not completely separated in the past, because they are not distinct on the neighbour-joining tree (Fig. 2). However, we did not find common haplotypes among animals from the west (Kalmykian) and east (Kazakhstan) banks of the Volga. In contrast, saiga from the three Kazakhstan populations shared some haplotypes (Table 2).

The maximum intrapopulation genetic distance within the Kalmykian and Kazakhstan groups was 6.8%, considerably higher than the minimum distance between the two groups (0.5%). Similarly, the maximum intrapopulation genetic distance for *S. t. mongolica* (2.5%) and *S. t. tatarica* (7.6%) was substantially higher than the minimum interpopulation distance (1.0%). This suggests that the species is polyphyletic, such that some haplotypes in each population are genealogically closer to heterospecific than to homospecific haplotypes (Neigel & Avise, 1986; Avise, 1994).

The data support the interpretation that all extant *S. t. tatarica* populations originated from one highly polymorphic ancestral population in the not too distant past, as would be expected from the broad geographic range and nomadic life-history of the species. Generalized polyphyly together with distinct haplotypes being found in different geographical areas is consistent with a recent rapid decline in saiga numbers and the fragmentation into separate populations of a previously well-mixed species.

The threatened status of the species makes collection of large samples for genetic analysis difficult. Our results are preliminary because of the small sample sizes that were available to us; however, this is the first time that genetic analysis has been carried out across all saiga populations. The subspecies status of the Mongolian population has been controversial. Our study provides further evidence for the designation of this group as a subspecies rather than a separate species, and also suggests that setting up a captive breeding programme for the Mongolian saiga should be a conservation priority so that its unique genetic diversity can be preserved.

Compared to other bovids (Arctander *et al.*, 1996a, 1996b; Simonsen *et al.*, 1998; Birungi & Arctander, 2000) the level of genetic diversity and population differentiation of *S. t. tatarica* are not exceptional. However, the current rapid decline of all *S. t. tatarica* populations is

Table 1 Genetic diversity of saiga populations (see text for details), based on a 408 bp segment of the mtDNA control region. The mean nucleotide diversity was calculated in Mega2.1 using the Kimura 2-parameter method (Kimura, 1980). Nucleotide differences are mean number of pairwise differences.

Population	N	Haplotypes	Unique haplotypes	Mean nucleotide diversity ± SE	Variable sites	Parsimoniously -informative sites	Singletons	Mean nucleotide differences ± SE
Kalmykia	38	20	13	0.031 ± 0.006	39	28	11	12.05 ± 5.57
Ural	12	5	2	0.028 ± 0.005	27	21	6	11.15 ± 5.42
Ustiurt	13	11	9	0.0036 ± 0.005	51	31	20	14.27 ± 6.85
Betpak-dala	15	10	7	0.039 ± 0.005	59	31	28	15.48 ± 7.33
Mongolia	15	10	7	0.008 ± 0.003	12	7	5	3.39 ± 1.84

https://doi.org/10.1017/S0030605306000135 Published online by Cambridge University Press

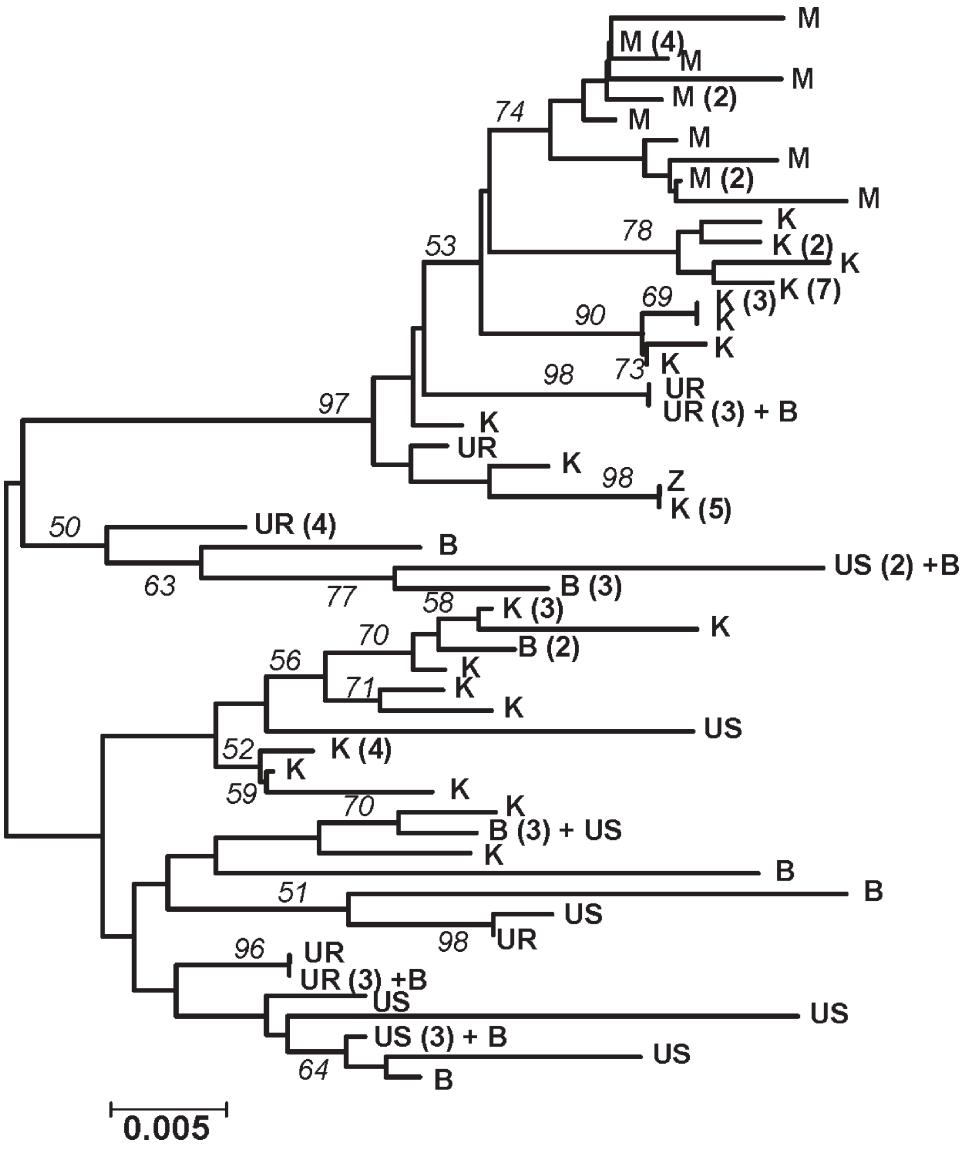

Fig. 2 Neighbour-joining tree based on Kimura's 2-parameter distances among mtDNA control region haplotypes of saiga antelopes from different populations, obtained by successive clustering of pairwise comparisons to show phylogenetic distances. Bootstrap values at the nodes are based on 500 replicates; only values above 50% are shown. Populations: M, Mongolia; K, Kalmykia; US, Ustiurt; UR, Ural; B, Betpak-dala, Z, animal in San Diego zoo, thought to be from Kalmykia. Numbers in brackets are the number of samples with each sequence (if > 1). If individuals from two populations have the same sequence, both symbols are given.

cause for great concern. As the populations all contain unique haplotypes, their management as evolutionarily distinct units will most effectively conserve their genetic diversity.

Table 2 Common haplotypes in the five saiga populations. On the diagonal, total number of haplotypes/number of samples. Below the diagonal, number of common haplotypes between two populations.

	Kalmykia	Ural	Ustiurt	Betpak-dala	Mongolia
Kalmykia	20/38				
Ural	0	5/12			
Ustiurt	0	1	11/13		
Betpak-dala	0	2	2	10/15	
Mongolia	0	0	0	0	10/15

Acknowledgements

We gratefully acknowledge the financial support of INTAS (Project 97-11197), the Russian Foundation for Basic Research (Project 03-04-48457) and the Zoological Society of San Diego. We thank our collaborators A. Blizniuk, A. Bondarev, M. Lundervold, B. Nyambayar and P.A. Sorokin for their help. We thank Carlos Fernandes for helpful comments. Correspondence and requests for materials should be addressed to Marina Kholodova at mvkholod@hotmail.com

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https://doi.org/10.1017/S0030605306000135 Published online by Cambridge University Press

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Biographical sketches

Marina Kholodova carried out the genetic analyses for this study, and is Head of the A.N. Severtsov Institute of Ecology and Evolution's Molecular Diagnostics Centre. E.J. Milner-Gulland has a long-standing interest in the ecology and conservation of saiga antelopes, and was coordinator of the INTAS project carrying out this work. Andrew Easton is a virologist with an interest in the phylogenetic analysis of nucleotide sequences. Luvsanjamba Amgalan, Yuri Arylov, Amankul Bekenov, Yuri Grachev and Anna Lushchekina are experts on the ecology and conservation of the saiga, and were responsible for the field-based data collection for this study. Oliver A. Ryder holds the Kleberg Genetics Chair in Genetics at the San Diego Zoo's Arnold and Mabel Beckman Center for Conservation Research. Conservation genetics studies on a wide variety of threatened species have been conducted in his laboratories.

https://doi.org/10.1017/S0030605306000135 Published online by Cambridge University Press