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Tracing genetic differentiation of Chinese Mongolian sheep using microsatellites

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Summary

The genetic consequences of population differentiation and isolation have been the subject of conservation biology. In this study, we analysed the genetic diversity and structure of Mongolian sheep in China. These animals belong to a traditional local breed with high production, extensive adaption, early maturity and roughage resistance. For this purpose, 26 microsatellites were genotyped for five Mongolian sheep populations. The Bayesian clustering indicated five clusters as the most probable genetic structure of the populations investigated. In addition, a clear genetic structure was revealed in three populations distributed at large geographical scales, while the other cluster encompassed UQ and HLBR sheep that displayed no clear differentiation, probably due to their close and small geographical distributions. Overall, our results are helpful in understanding the interplay of population dynamics in these close genetic lineages of Mongolian sheep.

Keywords Chinese Mongolian sheep, genetic differentiation, genetic structure, microsatellite.

According to the traditional classification, Chinese indigenous sheep are separated into three groups: Mongolian group, Tibetan group and Kazakh group (Zheng 1988). In China, the total population of Mongolian sheep is estimated at around 20 million (National Consultative Committee 2004). Mongolian sheep are distributed in pastoral and agricultural areas extensively in northern China. They are fat-tailed, produce quality meat and carpet wool, and have sound body conformation, strong walking ability and admirable adaptation to very different ecological conditions. With long-term natural and human selection, habitat change and cross-breeding, some local varieties have been formed and are distributed in Inner Mongolia, Gansu, Ningxia, Shanxi, Shandong and Zhejiang provinces in China. Under the impact of invasion of exotic breeds and cross-breeding between these and traditional breeds, over 15% of Chinese local sheep breeds are classified as in danger of extinction (Ma *et al.* 2002), including several Mongolian sheep populations, such as Lanzhou Large-Tailed sheep, Tong sheep and Hanzhong sheep (Ma *et al.* 2002). Although these sheep breeds originated from the Mongolian group, they display different phenotypes and genetic characters. Recently, studies of Chinese sheep have indicated that the majority of Chinese local sheep breeds have high genetic diversity and population (sub)structure (Ma *et al.* 2006; Yuan *et al.* 2006; Sun *et al.* 2007, 2010; Zhong *et al.* 2010). However, these reports do not provide in-depth information about the genetic differentiation of Mongolian sheep populations.

To assess the potential (sub)structure of Chinese Mongolian sheep, the current study investigated the genetic differentiation of five Mongolian sheep populations, along with one introduced breed as reference, using 26 microsatellites (Table S1), of which 19 were from the panel recommended by ISAG/FAO for sheep diversity studies (Hoffmann *et al.* 2004) and seven were from ILRI datasets (Luo *et al.* 2009). Unrelated sheep of both sexes were randomly sampled from five local conservation farms, 48 individuals per population [Ujumqin sheep (UQ) and Hulunbeier sheep (HLBR) from Inner Mongolia, Tan sheep (TAN) from Ningxia, Hu sheep (HU) from Zhejiang and Small-tailed Han sheep (STH) from Shandong]; one exotic population from Australia but sampled in China was also

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used [48 Poll Dorset (PD)]. The laboratory work was carried out in the framework of a FAO/IAEA coordinated research project, and the experiment was performed as described by Zhong *et al.* (2010).

Genotypic linkage disequilibrium and deviations from Hardy–Weinberg equilibrium for each locus in each population were performed using GENEPOP v.3.4 (Raymond & Rousset 1995). Diversity analysis was implemented in The Excel Microsatellite Toolkit version 3.1.1 (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/index.php) and GDA v1.1 (http://en.bio-soft.net/dna/gda.html), and the diversity information is shown in Table 1. The mean number of alleles (MNA) was more than 6.8 and the H_O values ranged from 0.661 (STH) to 0.698 (TAN) in the five local Mongolian sheep populations, indicating their high diversity.

Because these five local populations belong to the Mongolian sheep group, sharing a common genetic background, the tree-based hierarchical clustering of individuals to define clusters of genetically similar populations was constructed with a distance matrix derived from either *Dps* or *Dkf* using MICROSAT v1.5d (http://hpgl.stanford.edu/projects/microsat/). The PHYLIP program package version 3.5 (http://cmgm.stanford.edu/phylip/#1) was used to construct the neighbour-joining tree from the distance matrix. The result file was entered into TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) to find a suitable graphic display. However, there were no obvious (sub)clusters present in these Chinese Mongolian sheep populations based on either the *Dps* or the *Dkf* distance matrix (Fig. S1).

The Bayesian model clustering was carried out using STRUCTURE program version 2.0 (Pritchard *et al.* 2000). STRUCTURE's posterior probabilities of *K*, a log-likelihood of the microsatellite data on 288 individuals given *K* clusters and the corresponding ΔK statistic (Evanno *et al.* 2005), all showed a peak at $K = 5$, indicating that five genetic clusters were the best solution for the dataset. The estimated individual membership coefficients were graphically visualized using a computer program DISTRUCT version 1.1 (Rosenberg 2004). The potential structure was displayed in Fig. 1. In the first round of STRUCTURE at $K = 2$, the defined clusters appeared along plausible lineage origins. A clear distinction was identified between the Mongolian group and the exotic breed (PD). At $K = 3$, the TAN population was separated first from the other four populations. Subsequently, the HU and STH populations were identified as independent clusters at $K = 4$ and 5, respectively, corresponding very well to their different geographical distributions. It was noted that no further cluster was detected at $K = 6$. The clustering pattern at $K = 5$ elucidated a clear genetic structure among the Mongolian sheep group, which was consistent with the geographical partitioning of these populations, and therefore, we believe that the results at $K = 5$ give the solution that captures most of the underlying genetic differentiation among these five Mongolian sheep populations.

Table 1 Descriptive statistics of 26 microsatellite loci in six sheep populations and their immigration rates estimated by BAYESASS.

	No. of samples						Populations					
Populations	Female	Male	MNA	H_O	$\hat{H}$	NPA	UQ	TAN	HU	HLBR	STH	PD
Ujumqin (UQ)	28	20	8.73 (3.09)	0.666 (0.014)	0.694 (0.030)	11	**0.674** (0.007)					
Tan (TAN)	18	30	8.04 (2.99)	0.698 (0.013)	0.714 (0.026)	10	0.310 (0.014)	**0.927** (0.028)				
Hu (HU)	23	25	6.85 (2.13)	0.682 (0.014)	0.692 (0.025)	8	0.006 (0.009)	0.060 (0.027)	**0.993** (0.007)	0.308 (0.014)		
Hulunbeier (HLBR)	17	31	8.92 (3.20)	0.690 (0.014)	0.713 (0.032)	15				**0.677** (0.009)	0.308 (0.015)	
STH	22	26	7.84 (2.85)	0.661 (0.014)	0.703 (0.024)	10					**0.673** (0.007)	
Poll Dorset (PD)	24	24	5.12 (1.63)	0.652 (0.014)	0.624 (0.028)	2						**0.993** (0.007)

MNA, mean number of alleles; H_O, observed heterozygosity; $\hat{H}$, Nei's unbiased gene diversity; NPA, number of private alleles; STH, small-tailed han.

Bold letters along the right diagonal are proportions of non-migrants. Standard deviation is given in parentheses. Empty cells represent mean proportions of lower than 0.050.

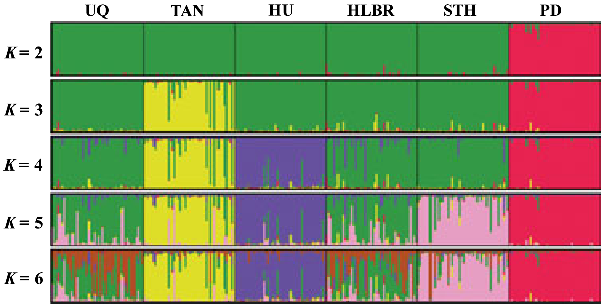

Figure 1 Population structure of six sheep populations displayed with individual Q-matrix.

To investigate whether immigration rates were consistent with measures of population structure, we used a genetic assignment method implemented in BAYESASS+ v1.3 (Wilson & Rannala 2003). As shown in Table 1, high homogeneities were revealed in the TAN and HU populations, while the other three populations (UQ, HLBR and STH) contained some level of unique Mongolian sheep genetic background. This was in agreement with a previous study (Zheng 1988), which reported that these populations shared the same genetic origin despite their different morphological characteristics.

Our study has demonstrated that natural and human selection have contributed to the present high genetic diversity and differentiation among the dispersed Mongolian sheep populations in China. To conserve the current genetic pool of these unique genetic resources, introgression and cross-breeding must be prohibited on the conservation farms.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Neighbour-joining (NJ) trees of 288 individuals based on *Dps* and *Dkf* distance matrixes. (a) NJ tree based on *Dps*; (b) NJ tree based on *Dkf*.

Table S1 Information of 26 microsatellite loci used in this study.

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