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Genetic Diversity Measures of 8 Local Sheep Breeds in Northwest of China for Genetic Resource Conservation

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ABSTRACT : The aim of this study was to evaluate, through the use of microsatellite markers, the current genetic diversity and the relationships of 375 individuals from 8 local sheep breeds reared in typical breeding farms in the northwest of China, and moreover, to offer a contribution towards genetic conservation decisions for the studied breeds. The expected heterozygosities and allelic richness for the 8 breeds varied from 0.474 to 0.623 and from 3.8 to 5.4, respectively. All the populations showed a significant deficit in heterozygosity and a relatively low level of genetic diversity. Furthermore, the high positive F_{IS} value (ranging from 0.255 to 0.556) indicated inbreeding to be one of the main causes for high genetic homogeneity and lack of heterozygosity in all breeds. The clustering analysis performed with the DISPAN package showed that Aletai, Kazak, Bashibai and Bayinbuluke were grouped together, and Hetian, Qira black and Duolang were grouped together, which indicated that the relationship among breeds displayed some degree of consistency with their geographical distribution, production and origin. These findings indicate that improved conservation measures must be undertaken to avoid further losses of genetic diversity and minimize inbreeding represented by these breeds. (**Key Words :** Sheep, Microsatellite, Genetic Diversity, Phylogenetic Relationship, Conservation)

INTRODUCTION

China has a long history of sheep raising and breeding, and there are a total of 15 indigenous and 7 improved sheep breeds (Ma et al., 2006). The northwest of China is one of the main areas of sheep breeding in the country, and has abundant breed resources such as Kazak, Aletai, Hetian, and Bayinbuluke sheep. Most local sheep are not only adapted to the local environment, but are regarded also as important genetic resources and one of the major components of agro-pastoral societies. Recently, in China, in order to compete for high market returns, a large number of indigenous sheep breeds were replaced by other breeds and their crosses. According to a survey carried out by the Xinjiang province Government in 2007, there were approximately 34,000,000 sheep in northwest China, and the population of Hetian, Bayinbuluke, Duolang and Qira black sheep was 1,580,000, 1,000,000, 720,000, and 30,000, respectively (Xiao et al., 2009). In fact, the indigenous sheep breeds have relatively small numbers, which have declined steadily over the past two decades (Chen et al., 2002; Jiang, 2006; Gao et al., 2008). There has been concern that such a great reduction may have led to losses of genetic diversity in the sheep population.

Considering that local sheep breeds are important for biological diversity, concerns about the conservation of genetic diversity have arisen since the late 1990s (Chen, 1995; Chen et al., 2002; Gao et al., 2008). Subsequently, several breeding farms were established to avoid further losses of genetic diversity and to maximize gene purity. However, few reports have been published on assessing the contribution of the local breeding farms in northwest China in terms of genetic conservation schemes. Moreover, a deeper knowledge of the genetic diversity is essential for conservation decisions on maintenance and/or improvement. Therefore, it was meaningful to perform a study on investigating the genetic diversity within and among the local breeds from breeding farms in northwest China.

Microsatellite markers are the most useful markers for genetic diversity and variability and have been successfully used in many studies (Glowatzki-Mullis et al., 2008; Kusza et al., 2008; Santos-Silva et al., 2008; Takahashi et al., 2008; Dixit et al., 2009; Ligda et al., 2009; Qi et al., 2009). In the case of sheep populations in northwest China, only a few reports on these breeds have been published to date;

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Table 1. The geographic locations and the numbers of samples recruited for each sheep breed

Breed	Location	Breeding farm	No. of samples
Kazak (KAZ)	North of Xinjiang province	Jimunai	45
Aletai (ALT)	Aletai	Fuhai	50
Bashibai (BSB)	Taicheng	Yumin	50
Duolang (DUL)	Along Yeerqiang River	Maigaiti	45
Qira black (QIB)	Qira	Qira	50
Bayinbuluke (BYB)	Bayinbuluke prairie	Hejing	45
Hetian (HET)	South of Xinjiang province	Hetian	45
Chinese Merino sheep (CMC)	North of Xinjiang province	Kuitun 129 mission	45

among these are the work of Qi et al. (2007) who estimated the genetic distances in Qira black, Hetian and Karaku sheep breeds, Chen et al. (2006) who estimated the genetic diversity and distances in Altai, Hetian and Karaku sheep breeds, and Hao et al. (2009) who studied the genetic diversity between Bashibai and Qira black sheep breeds. These past trends indicated that the study of the genetic diversity and variability of these sheep populations is not adequate.

In this study, efforts were made to evaluate genetic diversity and relationships of 8 local sheep breeds from breeding farms in northwest China based on standard microsatellite markers. The goal was to have a better understanding of current diversity and the level of inbreeding in these breeds, and offer a contribution towards further improvement of conservation schemes.

MATERIALS AND METHODS

Animals and sampling

Blood samples were obtained from a total of 375 sheep in 8 populations at breeding farms (Table 1). To avoid closely related individuals, samples were collected from different flocks on the breeding farm. The breeds included in this study were local sheep breeds raised in Xinjiang province, located in northwest China (Table 1). They were Chinese Merino sheep (CMC), Kazak (KAZ), Aletai (ALT), Bashibai (BSB), Duolang (DUL), Qira black (QIB), Bayinbuluke (BYB) and Hetian (HET).

The CMC, KAZ, ALT, BSB and BYB breeds are raised in northern Xinjiang province; and DUL, QIB and HET are reared in southern Xinjiang province. Except for CMC, all breeds had coarse wool character, resistance to crude feed and strong adaptability. The ALT and KAZ are fat-tail breeds which are well known for high meat-grease yield, and ALT sheep originated from KAZ sheep. The QIB and DUL are breeds with high prolificacy and year-round estrus, and QIB is also well known for its lamb skin. Unlike other breeds, CMC is the famous fine wool breed in China, which was bred in Xinjiang province by crossing the Australian Merino sheep with local sheep. DNA was extracted from these specimens using phenol/chloroform as described by Liu et al. (1997).

Microsatellite analysis

A total of 10 microsatellite markers (BM1824, MAF109, MAF64, OarAE57, OarDB6, OarFCB20, BMS2508, OarAE101, MAF65 and OarFCB48) were selected according to the level of polymorphism detected in previous studies (Zeng et al., 2007; Xu et al., 2008). The PCR amplifications were carried out in a 25 μl volume including 2.5 μl 10×polymerase buffer, 1.2 μl of 25 mM $MgCl_2$, 0.25 μl of 10 mmol/L dNTPs, 0.5 μl of 10 μmol/L of each primer, 0.6 units Taq polymerase (Sangon) and approximately 100 ng of genomic DNA. The annealing temperatures are shown in Table 2. PCR products were analyzed by vertical electrophoresis in 10% non-denaturing polyacrylamide gel.

Data analysis

Gene frequencies, the number of alleles, Hardy-Weinberg equilibrium (HWE), and heterozygosis (He) were estimated from the genotyping data using GENEPOP version 3.4 (Raymond and Rousset, 1995). The polymorphic information content (PIC) was calculated using the EXCEL software package. The coefficient of inbreeding (F_{IS}) was calculated using the FSTAT software (Goudet, 2002). The total genetic heterozygosity (H_T), average heterozygosity within a population (H_S), coefficient of gene differentiation (G_{ST}) at each of the 10 microsatellite markers, Nei's standard genetic distances among breeds (Nei, 1978) and UPGMA diagram were obtained using the DISPAN package (Ota, 1993). Bootstrap (n = 1,000) re-sampling was performed to test the robustness of the dendrogram topology.

RESULTS

A total of 55 alleles were detected across the 10 microsatellite markers, and all markers were found to be polymorphic in each of the 8 analyzed populations. The number of alleles per locus ranged from 3 (BM1824 and MAF65) to 8 (MAF64 and OarDB6), with a mean of 5.5 in all (Table 2). The majority of the markers were found to have high polymorphism, with PIC ranging from 0.192 (BM1824) to 0.724 (MAF64). Overall, the mean He across locus was 0.536, while the mean PIC was 0.492. He was

Table 2. Chromosome (Cr), annealing temperature (TA°C), number of alleles (Na), heterozygosity (He), polymorphic information content (PIC), inbreeding coefficient within populations (F_{IS}), average heterozygosity within each population (H_S), total gene diversity (HT) and coefficient of gene differentiation (Gst) for each locus

Locus	Cr	TA (°C)	Na	He	PIC	F_{IS}	H_T	H_S	G_{ST}
BM1824	1	60	3	0.206	0.192	0.323	0.210	0.206	0.017
BMS2508	6	55	6	0.581	0.525	0.345	0.656	0.581	0.114
MAF109	1	58	4	0.536	0.467	0.487	0.655	0.536	0.182
MAF64	1	59	8	0.758	0.724	0.371	0.803	0.758	0.055
MAF65	15	60	3	0.429	0.383	0.660	0.451	0.429	0.047
OarAE101	6	63	4	0.379	0.319	0.371	0.415	0.379	0.088
OarAE57	1	62	5	0.669	0.613	0.166	0.717	0.669	0.067
OarDB6	1	61	8	0.590	0.559	0.395	0.632	0.590	0.066
OarFCB20	2	55	7	0.702	0.665	0.552	0.753	0.702	0.067
OarFCB48	17	55	7	0.511	0.469	0.366	0.563	0.511	0.092
Mean	-	-	5.5	0.536	0.492	0.404	0.586	0.536	0.080

highest for MAF64 (0.758) and lowest for BM1824 (0.206), and the same loci also had the highest and lowest PIC, respectively (Table 2).

The unbiased coefficients of gene differentiation (G_{ST}) of the loci due to breed differences ranged from 0.017 to 0.182, with an overall average of 0.080. Moreover, the F_{IS} statistic differed widely between loci, with values ranging between 0.323 and 0.660 for the majority of the loci, except for the OarAE57 loci with an F_{IS} estimate of 0.166 (Table 2).

As shown in Table 3, He was lowest in QIB (0.474) and HET (0.476) and highest in ALT (0.623), while PIC displayed its minimum value in QIB and HET (0.425) and its maximum in ALT (0.562). All breeds had all the loci not complying with the HWE. In general, this non-compliance with HWE was due to a deficit in heterozygosity, with positive F_{IS} estimates which were significant in all breeds. The estimated F_{IS} were especially high among these breeds, with values ranging from 0.255 to 0.556, and probably reflect accumulated inbreeding in these breeds.

The matrix of Nei's standard genetic distances (Ds) among breeds is presented in Table 4 and the corresponding UPGMA phylogenetic tree is presented in Figure 1. The largest genetic distances were observed between CMC and QIB, HET, BYB and DUL, with estimated values for Ds ranging from 0.180 to 0.212. The closest relationships were those of KAZ with ALT (0.002) and KAZ with BSB (0.023), which can be appreciated in the phylogenetic tree (Figure 1). ALT and KAZ were grouped with a high bootstrap value (82%), and then the group clustered with BSB with a bootstrap value of 56%. In addition, HET and QIB clustered together with a bootstrap value of 53% and presented a relatively close relationship with the Ds value of 0.040.

Table 3. Mean number of alleles (MNA), mean heterozygosity (He), mean no. of effective alleles (Ne), mean polymorphic information content (PIC) and inbreeding coefficient within populations (F_{IS}) for each of the 8 breeds of sheep

Population	MNA	Ne	He	PIC	F_{IS}
DUL	3.8	2.2	0.512	0.446	0.462
BYB	4.5	2.6	0.574	0.508	0.386
HET	4.5	2.1	0.476	0.425	0.527
QIB	4.3	2.1	0.474	0.425	0.411
ALT	4.9	2.9	0.623	0.562	0.556
KAZ	5.1	2.7	0.581	0.524	0.356
BSB	4.9	2.9	0.545	0.490	0.255
CMC	5.4	3.0	0.610	0.552	0.381

DISCUSSION

Currently, much attention is focused on the issue of preserving biological diversity in livestock. In China, in order to conserve the genetic resources in domestic animals, many breeding farms have been established in the past decades. Moreover, the genetic diversity can be considered as an initial guide for conservation decisions. In this study, the first attempt was made to quantify the genetic diversity through microsatellite markers of the 8 local sheep breeds reared on typical breeding farms in northwest China. This will provide genetic information to be used for conservation and improvement of this population.

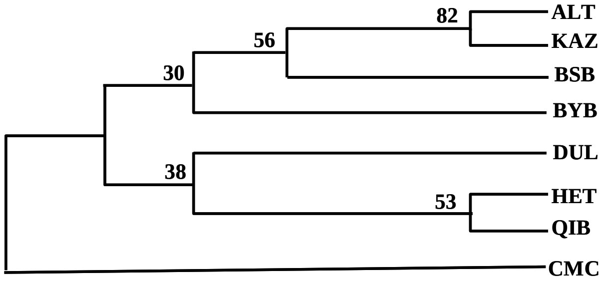

Figure 1. UPGMA phylogenetic of 8 sheep breeds by Ds genetic distance.

Table 4. Matrix of Nei's standard genetic distances among sheep breeds

Breed	DUL	BYB	HET	QIB	ALT	KAZ	BSB
BYB	0.175	-	-	-	-	-	-
HET	0.046	0.099	-	-	-	-	-
QIB	0.143	0.082	0.040	-	-	-	-
ALT	0.043	0.082	0.078	0.137	-	-	-
KAZ	0.088	0.049	0.071	0.109	0.002	-	-
BSB	0.094	0.066	0.096	0.125	0.050	0.023	-
CMC	0.185	0.180	0.200	0.212	0.058	0.068	0.111

The allele diversity (mean number of alleles) values can be considered as a reasonable parameter of genetic variation within a population (Bhatia and Arora, 2007). Allelic richness ranged from 3.8 (DUL) to 5.4 (CMC), which was similar to that observed by Ma et al. (2006) with a study on 13 other Northern Chinese sheep breeds, but lower than reported by Sun et al. (2007), Lv et al. (2006), Luo et al. (2009) and Qu et al. (2009) studying the Mongolia sheep, 7 native sheep breeds in Western China, two plateau Merino populations and 6 sheep populations in the Ningxia province in China, respectively.

On the other hand, estimates of heterozygosity confirmed the low level of diversity in the studied populations. Compared with the study reported by Zhong et al. (2008) and Hao et al. (2009), this study showed much lower heterozygosity of KAZ, BSB and QIB. This difference is possibly due to the different markers and the different geographic location of the samples. Our results suggest that the studied populations show much lower genetic diversity compared to others, as observed by Ma et al. (2006), Lv et al. (2006) and Hao et al. (2009) in studies on sheep breeds in middle and northern China. In addition, some other local sheep breeds were studied and results showed that the levels of genetic diversity in those breeds were remarkably higher than those reported here (Sun et al., 2007; Zhong et al., 2008; Cheng et al., 2009; Qu et al., 2009). These results may be due to the fact that the animals of the present 8 breeds were sampled on the breeding farms where artificial selection or inbreeding might be the major concern, and selection of no related samples in the populations is not easy and can also directly influence the results in relation to the HWE. Also, the results reflected that these populations have controlled management in order to maximize gene purity and homozygosis.

In our work, all the studied breeds did not comply with HWE and presented high positive values of the F_{IS} index, ranging between 0.255 (BSB) and 0.556 (ALT). In spite of higher genetic diversity (0.623), inbreeding was high in ALT sheep, and higher estimates of inbreeding coefficient were also reported in HET (0.527) and DUL (0.462). In general, these results probably reflect accumulated inbreeding in these breeds. It is well known that a high level of inbreeding might result in a decline of genetic diversity and animal adaptability, moreover, it probably affects some biophysical and biochemical phenotype (Arora and Bhatia, 2009). The present study suggested the need to set up an improvement scheme for the frequent exchange of rams among farms or flocks rearing the same breed, aimed to increase genetic diversity and maintenance of breed purity. The reason was that animals of the same breed but belonging to different farms showed a different genetic pool (Dalvit et al., 2008). In addition, either high variability rams or decreasing directional selection could also help maximize genetic diversity.

Phylogenetic and phylogeographic analyses based on the standard genetic distance between breeds indicated that the relationship among breeds displayed some degree of consistency with their geographical distribution, production and origin. These results indicated that CMC (fine wool) was the more distanced breed, which was formed in the 1980s by crossing with Australian Merino rams, while all the other breeds were native and coarse-wool sheep breeds. The observation that ALT, KAZ and BSB were clustered together on the UPGMA phylogenetic tree may be attributed to the fact that all three breeds were raised in northern Xinjiang province. Although BYB was reared in southern Xinjiang province, they were classified into a group with ALT, KAZ and BSB instead. Further, ALT and KAZ were clustered together with the highest bootstrap value (82%), which was consistent with the report of Chen et al. (2006). In the other main lineage of the UPGMA phylogenetic tree, we observed that DUL, HET and QIB were clustered together, which may be explained by all three breeds being raised in southern Xinjiang province.

In conclusion, the results from this study indicated that low genetic diversity and a high level of inbreeding existed in 8 populations from typical breeding farms located in northwest China. Such information provides an overview of the effect of conservation activities on breeding farms, suggesting that we should take some measures to avoid further losses of genetic diversity and minimize inbreeding represented by these breeds as soon as possible.

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