Conservation genetics in Chinese sheep: diversity of fourteen indigenous sheep (Ovis aries) using microsatellite markers

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Introduction

The domestic sheep (Ovis aries) has been an economically and culturally important farm animal species worldwide, since domestication. However, commercial lines and industrialized livestock production systems have spread over all continents resulting in decreasing of large indigenous sheep breeds in comparison with some commercial breeds. Many studies have assessed the diversity of native local sheep in India (Dorji et al. 2010; Pandey et al. 2010; Arora et al. 2011), the Middle East and Europe (Alvarez et al. 2005; Lawson Handley et al. 2007), Asia (Dalvit et al. 2007), Africa (Gizaw et al. 2007; Gaouar et al. 2015), America (Crispim et al. 2013; Ferreira et al. 2014), and Eurasia (Blackburn et al. 2011a; Paiva et al. 2011a,b; Souza et al. 2012; Qwabe et al. 2013; Gaouar et al. 2015). In recent years, several microsatellite studies on diversity in Chinese sheep have been published (Jia et al. 2003; Gao and Wu 2005; Yuan et al. 2006; Sun et al. 2007; Zhong et al. 2011). However, these studies primarily considered a relatively small group of breeds. The Chinese mainland is a rich source of diverse ovine germplasm and contains 67 million sheep that belong to 42 described indigenous...
breeds (China National Commission of Animal Genetic Resources, 2011). This represents selection by man as well as the adaptation of sheep to different nutrient supplies and climates in China, which is a geographically complex continent and includes areas such as the Tibetan plateau regions. Currently, the number of breeds is rapidly decreasing because of increases in agriculture, industrialization, the no availability of proven rams, shifts in profession and the absence of any planned strategies for their conservation.

The objective of this study was to assess the genetic diversity and breed structure of fourteen Chinese local breeds, with the ultimate aim of maintaining and conserving those breeds. The results of this study allow us to have an idea about the genetic diversity and phylogenetic relationships between the studied breeds.

**Material and Methods**

**Animals and experimental methods**

We genotyped 611 individuals from 14 breeds from different geographic locations in the Chinese mainland (Table 1). Individuals were genotyped at the six microsatellite loci (Kappes et al. 1997; Maddox et al. 2001 and FAO 2011) that were suggested for biodiversity studies in sheep (Table 2). The methods of DNA extraction and the PCR protocols reference as Zhong et al. (2011). Approximately, 1–2 µL of PCR product was diluted with 10 µL of autoclaved distilled water for use in DNA genotyping. Two microliters of diluted products were added to 7.75 µL Hi Di™ formamide and 0.25 Gene Scan-500 LIZ™ (Applied Bio systems, USA). The mixtures were heated at 94°C for 5 min and then immediately chilled on ice for 2 min. Genotyping was performed on a Genetic Analyzer 3130 xl (Applied Bio systems, USA).

**Data analysis**

Genetic diversity expected ($H_S$), observed ($H_E$) heterozygosity, mean number of alleles ($N_A$), and polymorphism information content (PIC) were estimated from the allele frequencies using FSTAT 2.9.3.2 (Goudet 1995). For each locus-breeds combination of the global data set and breeds groupings, we used Fisher’s exact test with Bonferroni correction to test possible deviations from Hardy–Weinberg equilibrium (HWE) using GENEPOP 3.4 (Raymond and Rouset 1995). Pairwise differences in the populations ($F_{ST}$, Slatkin 1995) were displayed using the Arlequin software 3.5.1.3 (Excoffier and Lischer 2010). The Bayesian clustering algorithm was implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) to determine the population structure and to explore the assignment of individuals and populations to specific gene clusters using a burn-in of 50,000 followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations from $K=2$ to $K=14$, in 50 iterations. STRUCTURE_Harvester (Earl and vonHoldt 2012) was used to generate a graphical display of the simulated results and the most optimal $K$. To estimate the most optimal $K$, the number of clusters ($K$) was plotted against $\Delta K = |L(K)|/|\bar{L}(K)|$, and the optimal number of clusters was identified by the largest change in the log-likelihood ($L(K)$) values between the estimated number of clusters (Evanno et al. 2005).

**Results**

In total, 138 alleles were found in 14 Chinese native sheep breeds across six microsatellite loci. Across breeds, an average of 23 alleles per locus was observed, ranging from 12 in OarAE129 to 31 in OarFCB304. The two extreme

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**Table 1.** Sampling information of 14 native sheep in China.

| Name                  | Code | SZ | N    | E    | Location          |
|-----------------------|------|----|------|------|-------------------|
| Tibetan sheep         | TS   | 32 | 29±46±48.56* | 94±22±21.49* | Ling Zhi, Tibetan |
| ZhaoTong sheep        | ZT   | 48 | 27±20±17.65*  | 103±42±59.00* | Zhao Tong, YunNan |
| Anduo sheep           | AD   | 47 | 33±19±4.83*   | 90±33±41.33*  | AniDue, Tibetan   |
| Zazakh                | HZK  | 42 | 42±20±13.92*  | 93±31±16.51*  | Hami, Xinjiang    |
| Hu sheep              | HU   | 48 | 31±18±50.01*  | 120±36±33.48* | SuZhou, ZheJiang  |
| hulumber              | HBR  | 48 | 49±11±36.00*  | 119±44±49.59* | Hulunber, Inner Mongolian |
| Small-tailed Han      | STH  | 48 | 35±15±23.44*  | 115±27±3.60*  | HeZe, ShanDong    |
| Tan sheep             | TAN  | 48 | 37±37±6.05*   | 107±02±8.24*  | YanChi, NingXia   |
| Gangba sheep          | GB   | 44 | 28±18±51.22*  | 88±33±48.37*  | GangBa, Tibetan   |
| Ujumqin               | UQ   | 48 | 44±10±14.47*  | 116±07±24.96* | Xilihaoe, Inner Mongolian |
| Minxian black sheep fur | MXB | 40 | 34±25±30.71*  | 104±14±15.50* | Minxian, Gansu    |
| Mongolian sheep       | MGH  | 40 | 49±16±16.81*  | 120±01±44.86* | Hailaer, Inner Mongolian |
| Gansu alpine merino   | GSH  | 40 | 38±55±56.72*  | 100±27±6.38*  | Zhangye, Gansu    |
| Lanzhou fat-tailed sheep | LZD | 38 | 36±03±29.71*  | 103±48±51.92* | LanZhou, Gansu    |

SZ is Sample size, N is North latitude, E is East longitude, Code is short name of breed.
there were two breeds (ZT and TS, AD, and GB). There were two breeds (ZT and TS) carried the P-value of inbreeding coefficients are significantly different from zero.

In total, 18 private alleles were distributed across 14 breeds and 6 loci. The frequency of several private alleles within certain breeds was particularly high. For example, the frequency of a private allele (135 bp) at the locus MAF209 in TS was 20.31% (see Table S1).

In the pairwise difference analysis, the highest diversity within a breed was observed in TS, and the lowest was observed in GB. The group, including GSH, MXB, LED, and MGH, had the lowest difference between breeds compared with the others in the pairwise differences between populations (πXY) and consistency to that in corrected average pairwise difference (πXY−(πX + πY)/2) (Table 5 and Fig. 1).

The STRUCTURE software was used for clustering individuals into 2 ≤ K ≤ 14. At the lowest K-value (K = 2), the MXB, MGH, GSH, and LZD breeds split from the others to form their own cluster. At K = 3 to K = 14, the TS separated and formed an independent cluster base on the clustering diagrams of K2, the optimal K-value was thus 3 (Fig. 2).

### Discussion

The results obtained in a previous study for ĤE (ranging from 0.62 to 0.71), ĤO (ranging from 0.65 to 0.69), and NA (ranging from 5.22 ± 1.67 to 8.92 ± 3.20) in Mongolian sheep (Zhong et al. 2011) are consistent with those obtained in the current study. These six highly polymorphic microsatellite loci selected in this study allow us to present a general genetic pattern and the phylogenetic relationship of these breeds.

Deviations from HWE are expected if individual populations are substructured into flocks within populations that are isolated from each other or if inbreeding has

### Table 2. Primer information of six microsatellites in current study.

| Locus   | Chro. | Reference                   | TM(°C) | Sequences                                      |
|---------|-------|-----------------------------|--------|-----------------------------------------------|
| MCM527  | OAR 5 | Maddox et al. (2001)        | 56     | F:5’- GTCCATGGGCTAAATCGAATTGC3’               |
|         |       |                             |        | R:5’- AAACACTGACGCTAATCCCAAA3’               |
|         |       |                             |        | F:5’- GAGGCGAATGAAATCTATAGC3’                |
|         |       |                             |        | R:5’- GTGCTGTGATTGTTAAGC3’                   |
| ILSTS005| BTA 10| Kappes et al. (1997)        | 55     | F:5’- GATCACAAAAGTGGTACCAACGCGTG3’           |
|         |       |                             |        | R:5’- GTACACCTAATGTTAGGGTCTG3’               |
| MAF209  | OAR 17| Maddox et al. (2001)        | 65     | F:5’- GAAGTGAGAAAGGAGGAAAG’                  |
| OarJMP29| OAR 24| Maddox et al. (2001)        | 65     | F:5’- GCCTGGCGGCGTGG3’                       |
| OarAE129| OAR 5 | Kappes et al. (1997)        | 60     | F:5’- AACCCAGTGTTGAAAGACTAATCCAG’            |
| OarFCB304| OAR 19| Maddox et al. (2001)       | 60     | F:5’- GCCTAGGAGGCTTCAATAGAAGGCGGG3’          |

Chro is the Chromosomal location of microsatellite.

### Table 3. Genetics diversity of all populations by locus.

| Locus   | ĤO  | ĤE  | PIC  | Na  | dHWE |
|---------|------|------|------|-----|------|
| MCM527  | 0.7647| 0.8013| 0.7634| 22  | 4    |
| ILSTS005| 0.5107| 0.5275| 0.4824| 16  | 2    |
| MAF209  | 0.7279| 0.7484| 0.7134| 29  | 1    |
| OarJMP29| 0.7425| 0.7484| 0.7096| 27  | 1    |
| OarAE129| 0.3859| 0.5612| 0.4897| 12  | 7    |
| OarFCB304| 0.6972| 0.7287| 0.6976| 31  | 2    |
| Mean    | 0.6382| 0.6859| 0.6427| 23  | 2.83 |

dHWE is number of populations deviated from Hardy–Weinberg equilibrium.
that most of these indigenous breeds are close to the Hardy–Weinberg equilibrium state.

The pairwise difference, $F_{ST}$ value that was observed between some populations (LZD, MGH, GSH, and MXB), was generally lower than that observed between other breeds, thus indicating moderate-to-high genetic similarity in this subpopulation (Group 2). For the other subpopulation (Group 1), the high genetic differences indicated a more complex genetic background and different artificial selection direction during their domestica-

cution of these indigenous sheep and was consistent with
the pairwise \(F_{ST}\) value analysis described above (Fig. 1). For \(K = 3\) to \(K = 14\), the TS was independently clustered, and the Group 1 breeds (excluding TS) and Group 2 breeds were separated into their own clusters. In addition, the background of Group 1 was increasingly complex with increasing \(K\)-value, similar to the result of the pairwise \(F_{ST}\) value, which indicates that gene flow exists in exchange or during multi-complex ancient domestication. Gene flow between breeds can also be assessed by the abundance of a private allele (Slatkin and Barton 1989; and Granevitze et al. 2007). Therefore, the breed TS, which had the largest number of private alleles, with nine, was likely the first to split from the other breeds. Chinese indigenous sheep including three main pedigrees, such as Tibetan group, Mongolian group, and Kazak group. Their relative species are Urial (Ovis vignei) and Agarl (Ovis ammon). In addition, the ancestor of Tibetan sheep was demisted from Ovis vignei which living in Qinghai–Tibetan Plateau. However, Mongolian group sheep were derived from argali in central Asian mountains region (China National Commission of Animal Genetic Resources. 2011). Therefore, the different ancestor would create their different population structure and diversity level, too.

The optimal \(K\)-value was found to be 3 in STRUCTURE clustering. For \(K = 3\), three of the Group 2 breed (MXB, GSH, and LZD) were bred in Gansu Province, and one (MGH) was from Mongolian. This result suggests that the Gansu breeds and Mongolian sheep are indistinguishable, though they were separate for many hundreds of years at domestication sites and have different phenotypes. There may have been some gene flow between them in the past or shared ancestors. For a similar case, the Group 1 breeds, which represents an independent cluster, had a breed that was sampled over a large geographic region in the Chinese mainland and were not only separated into independent clusters but also carried a common large-complex genetic background, which indicated the general exchange of genetic material. The strong gene flow among regions induced by human migration, commercial trade, and the extensive transport of sheep was identified by the variability of mtDNA (Zhao et al. 2013) in China. Therefore, we could not conclude that there were two domestication sites or shared common ancestors in the China mainland according to the clustering diagrams. Thus, obtaining additional direct evidence from different regions is necessary and should include disciplines such as archeology. However, from the
Figure 2. Clustering diagrams of 14 Chinese sheep populations obtained from $K = 2$ to $K = 14$ with best similarities. *#label* is the most optimal $K$-value. Note: number of population: TS (1), ZT (2), AD (3), HZK (4), HU (5), HBR (6), STH (7), TAN (8), GB (9), UQ (10), MXB (11), MGH (12), GSH (13), LZD (14). Superscript letter (A) is $L(K)$, superscript letter (B) is $\Delta K = m_j \{ L(K) \} / \{ L(K) \}$.
clustering analysis and genetic diversity state, particularly the private alleles in the TS breed and other Tibetan breeds, it possible that there were more than two domestication sites of Tibetan region sheep in this study. However, this study only presents a general idea or retrieves a rough idea of genetic pattern and diversity status in those Chinese indigenous sheep. Therefore, in further study a more subtle population structure might be revealed using more genetic markers.

In short, six microsatellites were genotyped for 611 individuals from 14 breeds to investigate the breed structure of indigenous sheep in China. The results of the current study infer affluent genetic diversity within breeds and strong gene flow exchange between native sheep in the Chinese mainland.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Allelic frequency of six microsatellite in each population.

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