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Microsatellite analysis revealed genetic diversity and population structure among Chinese cashmere goats

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Summary

Most cashmere goats are found in northern China and Mongolia. They are regarded as precious resources for their production of high quality natural fibre for the textile industry. It was the first time that the genetic diversity and population structure of nine Chinese cashmere populations has been assessed using 14 ISAG/FAO microsatellite markers. In addition, two Iranian populations and one West African goat population were genotyped for comparison. Results indicated that the genetic diversity of Chinese cashmere goats was rich, but less than those of the Iranian goat populations. All pairwise F_{ST} values between the Chinese cashmere goat populations reached a highly significant level ($P < 0.001$), suggesting that they should all be considered as separate breeds. Finally, clustering analysis divided Chinese cashmere goats into at least two clusters, with the Tibetan Hegu goats alone in one cluster. An extensive admixture was detected among the Chinese goat breeds (except the Hegu), which have important implications for breeding management.

Keywords Chinese cashmere goat, genetic diversity, microsatellite, population structure.

Cashmere goats are only found in specific areas of Asia. However, this resource is rich in China with more than ten different breeds developed after long-term natural and human selection (Qi 2004). Among them, the Liaoning and Inner Mongolian (including three types of Alashan, Erlangshan and Wuzhumuqin) cashmere goats are well known worldwide for their high cashmere yield and cashmere fineness respectively. In the past three decades, cashmere yield has been the primary breeding objective. Genetic admixture among some breeds and inbreeding within the breeds/types is possible. This may have led to the loss of genetic structure and diversity in some of these breeds/types. It is the first time that 14 microsatellite markers were applied to detect the genetic structure and variation of these breeds. Meantime, this study was expected to provide genetic evidence for the previous classification of these breeds/types, which has been based primarily on their morphology and geographical distribution.

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A total of 376 goats from nine breeds/types spanning the entire distribution range of Chinese cashmere goats were analysed. Additionally, we collected 76 Iranian goats (two populations) and 41 Guinea Bissau goats (one population) in West Africa as reference samples (Fig. S1 and Table 1). Genomic DNA was recovered from ear tissues or blood samples of Chinese or Iranian goats (Sambrook *et al.* 1989) and from FTA paper-preserved blood samples of the West African goats (Qi *et al.* 2005). Information about the 14 ISAG/FAO microsatellite loci is shown in Table S1. We conducted genotyping using an ABI 3130XL Genetic Analyzer.

The absence of linkage between loci was verified using GENEPOP 3.4 (Raymond & Rousset 1995). The polymorphism parameters were obtained using POPGENE 1.31 (Yeh & Boyle 1997), FSTAT 2.9.3 (Goudet 2001) and Excel Microsatellite Toolkit Version 3.1 (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/index.php). Results showed that Chinese cashmere goat breeds/types exhibited a rich genetic diversity (Table 1). Expected heterozygosity (He) and allelic richness (AR) ranged from 0.54 to 0.65 and from 3.90 to 5.96 respectively, with the lowest value found in HG and the highest in XJ and ELS. He of Chinese goats was similar to that of West African goats, but lower than that of Iranian goats. In this study, TUR in Southern Zagros in Iran had

Table 1 Sampling information and basic parameters of genetic diversity for 12 goat populations.

				Allelic diversity					Genetic diversity					
Area	Population	Code	N	TNA	NEA (SD)	MNA (SD)	AR (SD)	NPA	He (SD)	Ho (SD)	HWE	NHD	NHE	F^{IS}
China	Alashan	ALS	38	81	2.84 (1.01)	5.79 (2.75)	4.99 (2.05)	2	0.60 (0.18)	0.59 (0.20)	3	7	1	0.02
	Erlangshan	ELS	43	99	3.71 (1.88)	7.07 (2.81)	5.96 (2.31)	3	0.64 (0.24)	0.62 (0.26)	1	1	0	0.03
	Wuzhumuqin	WZ	35	91	3.69 (1.85)	6.50 (2.68)	5.67 (2.20)		0.64 (0.24)	0.61 (0.26)	2	2	0	0.04*
	Liaoning	LN	44	79	3.27 (1.75)	5.64 (2.59)	5.07 (2.18)	1	0.60 (0.24)	0.60 (0.27)	4	4	1	0.02
	Chaidamu	CDM	45	93	3.56 (1.75)	6.64 (2.56)	5.80 (2.22)	1	0.63 (0.24)	0.59 (0.25)	1	1	0	0.05**
	Shanbei	SB	38	84	3.64 (1.97)	6.00 (2.35)	5.17 (2.04)	1	0.59 (0.24)	0.55 (0.26)	2	2	0	0.07**
	Xinjiang	XJ	40	88	3.05 (1.38)	6.29 (2.76)	5.57 (2.37)	1	0.65 (0.19)	0.64 (0.25)	2	1	1	0.02
	Hexi	HX	47	75	3.06 (1.45)	5.36 (2.37)	4.82 (2.00)	1	0.58 (0.25)	0.63 (0.30)	6	1	3	–0.10***
	Hegu	HG	46	59	2.63 (1.20)	4.21 (1.97)	3.90 (1.59)		0.54 (0.23)	0.58 (0.26)	1	0	1	–0.07**
	Mean			83	3.27 (1.58)	5.94 (2.94)	5.21 (2.10)	1.1	0.61 (0.06)	0.60 (0.02)	2.4	2.1		
Iran	Naini	NAI	35	90	3.57 (2.02)	6.43 (2.65)	6.34 (2.58)	3	0.65 (0.19)	0.66 (0.22)	2	0	0	–0.01
	Turki	TUR	41	101	3.75 (1.85)	7.21 (3.04)	6.56 (2.63)	6	0.67 (0.18)	0.64 (0.20)	1	2	0	0.04
	Mean			96	3.66 (1.96)	6.82 (2.85)	6.45 (2.61)	4.5	0.66 (0.05)	0.65 (0.03)				
Africa	Guinea Bissau	GUI	41	76	2.84 (1.29)	5.43 (2.28)	4.90 (1.94)	2	0.60 (0.18)	0.61 (0.19)	1	2	2	–0.02

N, sample size; TNA, total number of alleles; NEA, number of effective alleles; SD, standard deviation; MNA, mean number of alleles; AR, allelic richness; NPA, number of private alleles; He, expected heterozygosity; Ho, observed heterozygosity; HWE, number of loci deviated from Hardy Weinberg equilibrium; NDH, number of loci whose heterozygote deficit are significant; NHE, number of loci whose heterozygote excess are significant; F_{IS}, population inbreeding coefficient.

Significant values are as indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

higher He and AR than NAI sampled from Central Zagros, supporting the hypothesis that domestication of goats in the Southern Zagros took place earlier than in Central Zagros (Naderi *et al.* 2008).

The *F*-statistic values were estimated using FSTAT 2.9.3. The inbreeding coefficients (F_{IS}) for seven Chinese and one Iranian goat population were positive, and three of them (WZ, CDM and SB) reached significant levels ($P < 0.01$, Table 1). Although LN, HX, SB, CDM and ALS were each sampled from two separate flocks, we did not find obvious sub-structure within these breeds/types (except SB) using the STRUCTURE 2.1 program (Pritchard *et al.* 2000). The global F_{ST} value of 0.063 for Chinese cashmere goats in this study was similar to that of the Chinese goats (0.054, Li & Valentini 2004) and the European and Middle Eastern goats (0.069, Canon *et al.* 2006), but lower than the value of 0.143 of the Southeast Asian goats (Barker *et al.* 2001).

Usually, Inner Mongolian cashmere goats are considered as one breed. However, all pairwise F_{ST} values reached highly significant levels ($P < 0.001$, Table S2) except that between NAI and TUR, indicating that ALS, ELS and WZ from Inner Mongolia could be considered as independent populations for breeding. Of the Chinese cashmere goats, the Tibetan HG was the most distinct according to the F_{ST} values and structure plot (Fig. 1). The mtDNA data showed that haplogroup A was predominant (>60%) in most populations of this study and that haplogroup D was at quite a high frequency (50.0%) only in the Tibetan HG, whereas it was lower than 50% in other goat breeds (Chen *et al.* 2005; Ji *et al.* 2008; Naderi *et al.* 2008; Wu *et al.* 2009). The haplogroup D identified by Sultana *et al.* (2003) was as old as A, and may even be the oldest haplogroup. Combined with archaeological records, we hypothesized that the Tibetan goats are of an ancient breed and might carry unique adaptation alleles. Therefore this breed should be considered as a priority group for conservation and further research.

Genetic phylogeny was analysed with three methods following that of Peter *et al.* (2007). Using STRUCTURE 2.1, we determined an optimum K value at 4 following Evanno *et al.* (2005), who found that an ad hoc quantity based on the second order rate of change of the likelihood function with respect to *K* (ΔK) did show a clear peak at the true value of *K*. Figure 1 shows a clear clustering ($K = 4$), which was in accordance with breed types. HG, which produces very little cashmere, was genetically distinct and was the first to separate. The other Chinese goats were then divided into two sub-clusters: LN, SB and XJ in one cluster belonging to the high-yield-cashmere type; and CDM, HX, ALS, ELS and WZ in the other cluster belonging to the fine-cashmere type. Surprisingly, HG was farther from other Chinese goats than Iranian populations. An early study proposed the Tibetan Plateau to be a place of origin for Chinese goats (Chen *et al.* 2006). Our previous mtDNA data indicated that the maternal ancestors of HG were different from those of other Chinese cashmere goats (Ji *et al.* 2008). Whether other Chinese cashmere goats and Iranian goats share the same ancestors needs further research. A dendrogram (Fig. S2) and principal component analysis (Fig. S3) also obviously separated HG from other groups, but these methods could not sub-divide the other Chinese goats. According to our investigation, the individual exchanges among cashmere goat breeds for improving cashmere quantity (You & Li 2004; Wang *et al.* 2008) might have led to a close relationship among cashmere goat breeds. Thus, an extensive admixture among populations was also detected (Fig. 1). To maintain the present genetic diversity and structure of these breeds, genetic exchanges between breeds must be controlled on every conservation farm.

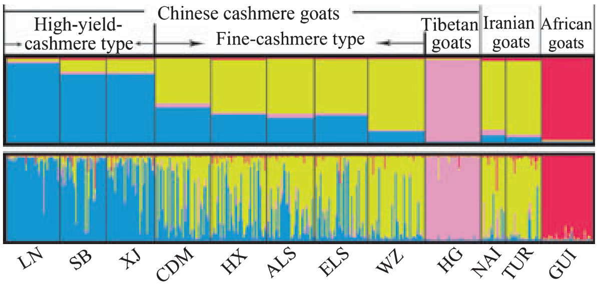

Figure 1 Twelve population structures displayed with population (top half of figure) and individual (bottom half of figure) Q-matrices at $K = 4$ using STRUCTURE 2.1 following ten independent runs for $2\leq K \leq 6$ with 10^6 Markov Chain Monte Carlo repetitions.

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Supporting information

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

Figure S1 Geographical location of the 12 goat populations sampled.

Figure S2 UPGMA tree based on Nei's D_A distances for 12 goat populations. See Table 1 for definitions of population abbreviations.

Figure S3 Principal component analysis of 11 Asian goat populations. (a) projection of populations on axis 1 and axis 2; (b) projection of populations on axis 2 and axis 3. See Table 1 for definitions of population abbreviations.

Table S1 Primer, loci data and polymorphisms for 14 microsatellite loci.

Table S2 Pairwise F_{ST} (above the diagonal) and N_m (below the diagonal) among 12 goat populations.

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