

## ORIGINAL ARTICLE

**Genetic diversity of Chinese domestic goat based on the mitochondrial DNA sequence variation**Y.-P. Liu<sup>1,2,\*</sup>, S.-X. Cao<sup>1,\*</sup>, S.-Y. Chen<sup>2</sup>, Y.-G. Yao<sup>3,4</sup> & T.-Z. Liu<sup>1</sup>

1 Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, Nanjing, China

2 College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, China

3 State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

4 Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

**Keywords**

Domestic goats; genetic diversity; mtDNA; Yangtze River delta white goat.

**Correspondence**

Dr Tie-Zheng Liu, Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, 50# Zhongling Street, Nanjing, Jiangsu Province, China 210014. Tel &amp; Fax: 86 25 84390329; E-mail: tzliu@263.net

\*These authors contributed equally to this work.

Received: 23 October 2007;  
accepted: 9 March 2008**Summary**

The aim of this study was to characterize the genetic diversity of domestic goat in China. For this purpose, we determined the sequence of the mitochondrial DNA (mtDNA) control region in 72 individuals of the Yangtze River delta white goat, and reanalysed 723 published samples from 31 breeds/populations across China. All goat haplotypes were classified into four haplogroups (A–D) previously described. The phylogenetic pattern that emerged from the mtDNA control region sequence was confirmed by the analysis of the entire cytochrome *b* sequence of eight goats representative of the four haplogroups. It appeared that in Chinese domestic goat, haplogroups A and B were dominant and distributed in nearly all breeds/populations, while haplogroups C and D were only found in seven breeds/populations. Four breeds/populations contained all four haplogroups. When grouping the breeds/populations into five geographic groups based on their geographic distributions and ecological conditions, the southern pasturing area had the highest diversity whereas the northern farming area had the lowest diversity. 84.29% and 11.37% of the genetic variation were distributed within breeds and among breeds within the ecologically geographical areas, respectively; only 4% of genetic variation was observed among the five geographic areas. We speculate that the traditional seasonal pastoralism, the annual long-distance migrations that occurred in the past, and the commercial trade would account for the observed pattern by having favoured gene flows.

**Introduction**

Domestic goats have long been utilized for agricultural, economic, cultural, and even religious usages in human civilization (Tu *et al.* 1989; Zhao 1995; Joshi *et al.* 2004). There are numerous local goat breeds in China, which have a strong fitness and foraging capability under a wide range of habitats, from

the dry, cold, and harsh Qinghai-Tibet Plateau to the warm and humid lands of South China. Due to the large environmental difference across China, it is natural that there are phenotypic differences among different native breeds (Tu *et al.* 1989; Chen *et al.* 2005). Currently, 62 breeds of indigenous goats from China are listed in the Domestic Animal Diversity Information System (DAD-IS, 2007) from the Food

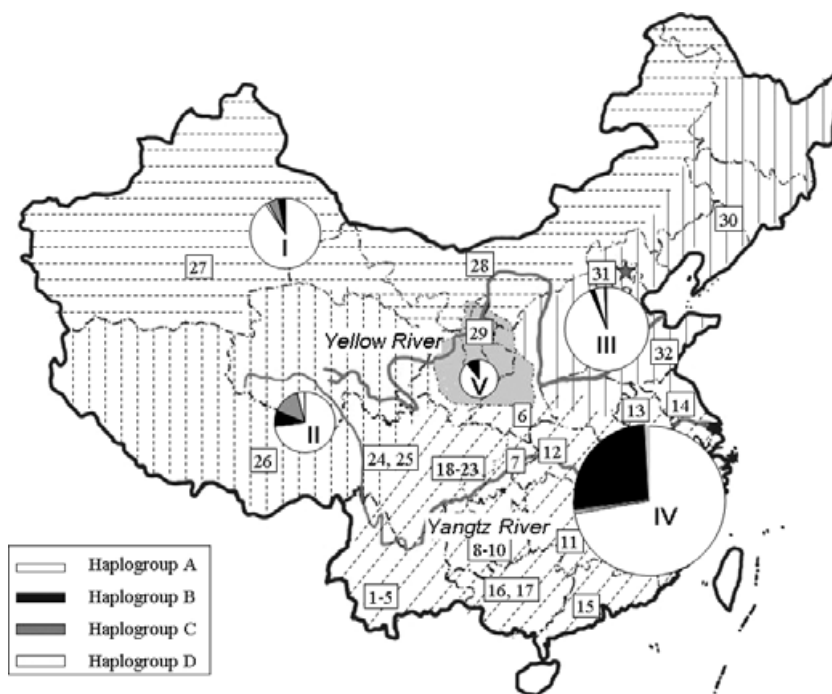
and Agriculture Organization of the United Nations (<http://www.dad.fao.org/>), which are classified based on the conformation traits, geographical distributions, ecological conditions, and historical literature or cultural relics. Twenty goat breeds/populations have been well described in a monograph (Tu *et al.* 1989). Here, the term 'population' refers to a cohort of individuals from the same location not necessarily related to a described breed. Based on the ecological distribution, Zhao (1995) classified all the Chinese goat breeds/populations into five geographic groups: northern pasturing area (I), Tibet region (II), northern farming area (III), southern pasturing area (IV), and farming and pasturing mixture area (V) (Figure 1).

Goat has long been used for trade and exchange in China and the earliest record could be dated back to 8000 years ago (Li 1993). With the development of modern Chinese economy, trade and exchange of goat became more and more common. Some local breeds became threatened with extinction or have already disappeared before necessary conservation efforts could be performed. For instance, the Zaobei large tail goat in Hubei Province went extinct more than 30 years ago; the Maguan Horn Down goat and the Guishan goat in Yunnan Province are also nearly extinct. Overall, more than 15% of goat breeds/populations in China are potentially threatened with extinction at present time. Fortunately, 14

breeds/populations (such as the Yangtze River delta white goat and the Zhongwei goat) have been approved as state-protected breeds in year 2000 (Ma *et al.* 2002).

In recent years, molecular studies offered new lights on the goat genetic diversity and origin. Based on the mtDNA RFLP data, Li *et al.* (1999) deduced that the origin and evolution of modern Chinese goat breeds were independent of those of exotic goats and that the indigenous goats could be grouped into two main types (the northern type and the southern type), which came from two distinct matrilineal ancestors. Chang *et al.* (2000) speculated that the Chinese goat might have been domesticated after its emergence in the Tibetan plateau, followed by the eastern and southern dispersal.

Hitherto, there are extensive studies on phylogeny and genetic structure of domestic goat based on mtDNA sequence variation. Luikart *et al.* (2001) assessed the phylogenetic history and matrilineal population structure of 406 domestic goats representing 88 breeds across the world and identified three highly divergent haplogroups A, B, and C. Later, Sultana *et al.* (2003) analysed the complete mtDNA D-loop and the cytochrome *b* gene sequences of 13 Pakistani domestic goat breeds and one wild goat, and found a new haplogroup D in addition to the three previously reported by Luikart *et al.* (2001). Chen *et al.* (2005) took advantage of



**Figure 1** Distribution of four known haplogroups A–D in 32 Chinese goat breeds/populations grouped according to five ecological areas (I–V). The area of circle is proportional to the sample size in that ecological area and each haplogroup was represented by different grey colour. The sample ID numbers were defined in Table 1 and were marked on the map according to provinces.

the available phylogeny of domestic goats and followed the same nomination system described by Luikart *et al.* (2001) and Sultana *et al.* (2003) to analyse the genetic diversity of Chinese goats. They analysed a 481-bp fragment of the first hypervariable region of the mtDNA control region from 368 individuals representing 18 indigenous breeds and detected all four haplogroups (A–D), in which haplogroup A was predominant, haplogroup B was moderate, whereas haplogroups C and D were present at low frequencies (Chen *et al.* 2005). Most recently, Naderi *et al.* (2007) analysed 2430 reported and new domestic goat samples from all over the old world and identified six haplogroups (A, B, C, D, F, and G). Among them, the newly described haplogroup G was presented around the Fertile Crescent. More goat breeds/populations from China were recently studied for mtDNA diversity (Liu *et al.* 2006a, 2007; Wang *et al.* 2008). All these studies only analysed limited samples (normally less than 20) for each Chinese goat breed/population and did not analyse mtDNA coding region information, which however is very useful to refine the phylogeny constructed by a short fragment of mtDNA control region (Yao *et al.* 2002; Wu *et al.* 2007).

In the present study, we determined the sequence of the mtDNA control region in 72 individuals of the Yangtze River delta white goat, and (re)analysed a total of 723 published samples from 31 breeds/populations across China. In addition, we sequenced the entire cytochrome *b* gene sequences in eight goat mtDNAs (representative of the four goat haplogroups) and analysed it together with 16 cytochrome *b* sequences from GenBank. The geographic structure of the genetic diversity (among regions of contrasted ecological traits) would be tested.

## Materials and methods

### Sampling

The Yangtze River delta white goat is a unique breed cultured in ancient China and distributed in the Yangtze River delta area. Due to the characteristics of appropriate rigidity and lustrousness, the Yangtze River delta white goat hair is widely used for making Chinese brush. In addition, the meat of the Yangtze River delta white goat has a specific flavour and is popular in the Yangtze River delta region. Fresh ear tissue or blood samples were collected from 72 individuals in three indigenous goat populations in Jiangsu Province and were stored at  $-20^{\circ}\text{C}$  until further processing.

### Polymerase chain reaction amplification and sequencing

Genomic DNA was extracted by a standard phenol/chloroform method. We first sequenced the first hypervariable segment (HVS-I) of the mtDNA control region in 72 individuals. Then, eight samples were randomly selected among them as representatives of the major haplogroups and used for sequencing the entire cytochrome *b*. We used the reported primer pairs (for HVS-I, 5'-CGTGTATGCA-AGTACATAC-3'/5'-CTGATTAGTCATTAGTCCATC-3'; for cytochrome *b*, 5'-AATGATATGAAAAACCATC-3'/5'-TAGATGTGGTTAATAGTGG-3') and amplification conditions to determine the sequence of the mtDNA control region (Luikart *et al.* 2001) and cytochrome *b* (Sultana *et al.* 2003). Polymerase chain reaction (PCR) were performed in a 50  $\mu\text{l}$  volume [500 mM Tris-HCl (pH 8.3), 0.1 % Triton X-100, 2.5 M KCl, 75 mM MgCl<sub>2</sub>, 5 mM of each dNTP, 10 pM of each primer, and 1 unit of Taq polymerase (S<sub>ABC</sub>)] following 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C. PCR products were purified on spin columns (Watson Biotechnologies Inc, Shanghai, China) and directly sequenced in both directions by using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM® 3100 DNA sequencer (Applied Biosystems) according to the manufacturer's manual. The mtDNA control region and cytochrome *b* sequences generated in this study have been deposited in GenBank under accession numbers EU130701–EU130772 and EU130773–EU130780.

### Data analyses

Altogether 723 Chinese domestic goat mtDNA control region sequences from 31 native breeds/populations were retrieved from GenBank and were compared to the new samples sequenced in this study (Table 1). Sequences were aligned by the Cluster W method included in the program MegAlign (DNAS Inc., Madison, WI, USA) and were cut to 481 bp fragment for accordance. Sequence variation was exported by using MEGA 3.1 (Kumar *et al.* 2004). Twenty-two goat mtDNA control region reference sequences belonging to the six known haplogroups that were summarized by Naderi *et al.* (2007) were also included in our analysis, to facilitate the recognition of haplogroup status of each individual. An unrooted neighbour-joining (NJ) tree was constructed for the mtDNA control region sequences

**Table 1** Distribution of mtDNA haplogroups in Chinese goat breeds/populations

Sample ID/regional area <sup>a</sup>	Breed/population	Location	GenBank accession number	Number	A	B	C	D	Number of haplotypes	Haplotype diversity ( $h \pm SD$ )	Nucleotide diversity ( $\pi \pm SD$ )
1	Heitou	Ninglang, Yunnan	DQ089106–33	28	23	5	0	0	16	0.942 ± 0.025	0.0279 ± 0.0046
2	Wujiào	Maguan, Yunnan	DQ089134–59	26	19	7	0	0	14	0.932 ± 0.028	0.0351 ± 0.0039
3	Gui dairy	Lulan, Yunnan	DQ089160–77	18	14	4	0	0	15	0.980 ± 0.024	0.0315 ± 0.0054
4	Longling yellow	longling, Yunnan	DQ089357–80	24	14	10	0	0	10	0.913 ± 0.028	0.0352 ± 0.0025
5	Yunling black	Yunling, Yunnan	DQ089381–433	53	42	11	0	0	25	0.940 ± 0.018	0.0329 ± 0.0032
6	Shaannan white	Ankang, Shaanxi	DQ121604–18; DQ089193–208; EF103519–27; EF368271–9	49	32	17	0	0	21	0.936 ± 0.026	0.0317 ± 0.0014
7	Chuangdong white	Kai, Chongqin	AY860880–84 <sup>b</sup> ; DQ089209–22	19	14	5	0	0	8	0.842 ± 0.066	0.0219 ± 0.0047
8	Guizhou black	Shuicheng, Guizhou	DQ121521–34; DQ089237–45	23	17	6	0	0	16	0.972 ± 0.018	0.0321 ± 0.004
9	Guizhou white	Yanhe, Guizhou	DQ121535–49; DQ089223–36	29	17	12	0	0	15	0.943 ± 0.022	0.0336 ± 0.0017
10	Qianbeima	Renhuai, Guizhou	DQ121591–603	13	9	4	0	0	11	0.962 ± 0.050	0.0351 ± 0.0046
11	Matou	Xinhuang, Hunan	DQ121577–90; DQ089269–80	26	15	11	0	0	13	0.914 ± 0.033	0.0340 ± 0.0024
12	Yichang white	Yichang, Hubei	DQ089246–58	13	8	5	0	0	7	0.897 ± 0.054	0.0329 ± 0.0037
13	Huanghuai	Fuyang, Anhui; Henan	DQ121550–63; EF103480–9; EF368254–8	29	26	3	0	0	23	0.985 ± 0.012	0.0239 ± 0.0039
14	Yangtze River delta white	Jiangsu	EU130701–72	72	69	1	1	1	36	0.947 ± 0.016	0.0205 ± 0.0022
15	Leizhou	Xuwen, Guangdong	DQ121564–76; DQ089295–309	28	12	16	0	0	15	0.876 ± 0.050	0.0312 ± 0.0029
16	Longlin	Longlin, Guangxi	DQ089259–68	10	5	5	0	0	7	0.911 ± 0.077	0.0391 ± 0.0047
17	Du'an	Du'an, Guangxi	DQ089281–94	14	12	2	0	0	11	0.967 ± 0.037	0.0292 ± 0.0061
18	Barjiao	Wanyuan, Sichuan	DQ121491–505; AY860875–79 <sup>b</sup>	20	18	2	0	0	10	0.900 ± 0.043	0.0136 ± 0.0052
19	Chengduma	Shuangliu, Sichuan	DQ121506–20; AY860885–93 <sup>b</sup> ; DQ089178–85	32	21	11	0	0	19	0.944 ± 0.024	0.0328 ± 0.0026
20	Jintang black	Jintang, Sichuan	AY860899–903 <sup>b</sup>	5	2	3	0	0	3	0.800 ± 0.164	0.0324 ± 0.0092
21	Jiayang daer	Jiayang, Sichuan	AY860904–8 <sup>b</sup>	5	3	2	0	0	4	0.900 ± 0.161	0.0399 ± 0.0073
22	Lezhi black	Lezhi, Sichuan	AY860914–19 <sup>b</sup>	6	3	3	0	0	3	0.733 ± 0.155	0.0344 ± 0.0073
23	Nangjiang yellow	Nangjiang, Sichuan	AY860920–33	14	11	3	0	0	10	0.956 ± 0.038	0.0280 ± 0.0059
Region IV	–	–	–	556	406	148	1	1	197	0.983 ± 0.002	0.0338 ± 0.0007
24	Baichuan white	Baichuan, Sichuan	AY860871–4 <sup>b</sup>	4	4	0	0	0	3	0.833 ± 0.222	0.0173 ± 0.0045
25	Jianchang black	Xichang, Sichuan	AY860894–98 <sup>b</sup> ; DQ188856–60	10	7	3	0	0	10	1.000 ± 0.045	0.0358 ± 0.0054
26	Tibet goat	Tibet	AY860934–42 <sup>b</sup> ; DQ188884–901; DQ089186–92	34	24	1	7	2	26	0.979 ± 0.014	0.0436 ± 0.0052
Region II	–	–	–	48	35	4	7	2	39	0.988 ± 0.008	0.0414 ± 0.0044
27	Xinjiang	Xinjiang	DQ089434–73	40	38	0	2	0	24	0.974 ± 0.010	0.0264 ± 0.0044
28	Inner Mongolia goat	Inner Mongolia	DQ188871–83; EF103509–18	24	20	2	1	1	22	0.993 ± 0.014	0.0315 ± 0.0054
Region I	–	–	–	64	58	2	3	1	46	0.989 ± 0.005	0.0296 ± 0.0036
29 / Region V	Zhongwei	Ningxia	EF103542–56; EF368280–83	19	17	2	0	0	17	0.988 ± 0.021	0.0291 ± 0.0046

Table 1 (Continued)

Sample ID/regional area <sup>a</sup>	Breed/population	Location	GenBank accession number	Number	A	B	C	D	Number of haplotypes	Haplotype diversity ( $h \pm SD$ )	Nucleotide diversity ( $\pi \pm SD$ )
30	Liaoning cashmere	Liaoning	AY860909–13 <sup>b</sup> ; DQ188861–70; DQ188902–03; DQ089310–56 EF103500–8; EF368264–9	79	78	0	0	1	36	0.944 ± 0.014	0.0161 ± 0.0010
31	Taihang	Hebei	EF103528–41	14	10	1	1	2	14	1.000 ± 0.027	0.0391 ± 0.0062
32	Jining grey	Shandong	EF103490–9; EF368259–63	15	13	1	0	1	12	0.943 ± 0.054	0.0279 ± 0.0054
Region III	–	–	–	108	101	2	1	4	60	0.969 ± 0.008	0.0214 ± 0.0017
Total	–	–	–	795	617	158	12	8	327	0.989 ± 0.001	0.0355 ± 0.0009

<sup>a</sup>We grouped the goat breeds/populations into five groups based on their ecological geographic distributions: samples 1–23 belonged to the southern pasturing area (IV), samples 24–26 belonged to the Tibet region (III), samples 27–28 belonged to the northern pasturing area (I), sample 29 belonged to the farming and pasturing mixture area (V) and samples 30–32 belonged to the northern farming area (III).

<sup>b</sup>Data only reported in GenBank by Zhang *et al.* (2006). Sequences DQ089106–DQ089480 were from Chen *et al.* (2005); EF103480–EF103556, EF368254–EF368283 were from Wang *et al.* (2008); DQ121491–DQ121618, and DQ18 8849–DQ188903 were from Liu *et al.* (2007).

based on the Kimura-2-parameters model and the alpha value of 0.28 for goat mtDNA gamma shape parameter (Luikart *et al.* 2001; Fan *et al.* 2007; Naderi *et al.* 2007) by using MEGA 3.1. We also constructed an unrooted NJ tree for goat mtDNA cytochrome *b* gene complete sequences based on 16 published sequences from GenBank (accession nos.: DQ089474–DQ089476, DQ089478, Chen *et al.* 2005; AB004070–AB004075, Takada *et al.* 1997; AB044307–AB044308, Mannen *et al.* 2001; AB110594–AB110597, Sultana *et al.* 2003) and eight new sequences from the current study. The robustness of internal branches was estimated based on 1000 bootstrap replications. Median-joining networks (Bandelt *et al.* 1999) of the four goat haplogroups were constructed by using Network 4.1 (<http://www.fluxus-engineering.com/sharenet.htm>), in which transitions, transversions, and insertions/deletions were equally weighted. In order to examine whether there are genetic differences among different geographical regions and breeds, we grouped the Chinese goat samples according to their ecological distributions and performed analyses of molecular variance (AMOVA; Excoffier *et al.* 1992), using the ARLEQUIN 3.0 (Excoffier *et al.* 2005). Haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) (Nei 1987) for each goat breed/population were also estimated by using DNASP 4.10 (Rozas *et al.* 2003).

## Results

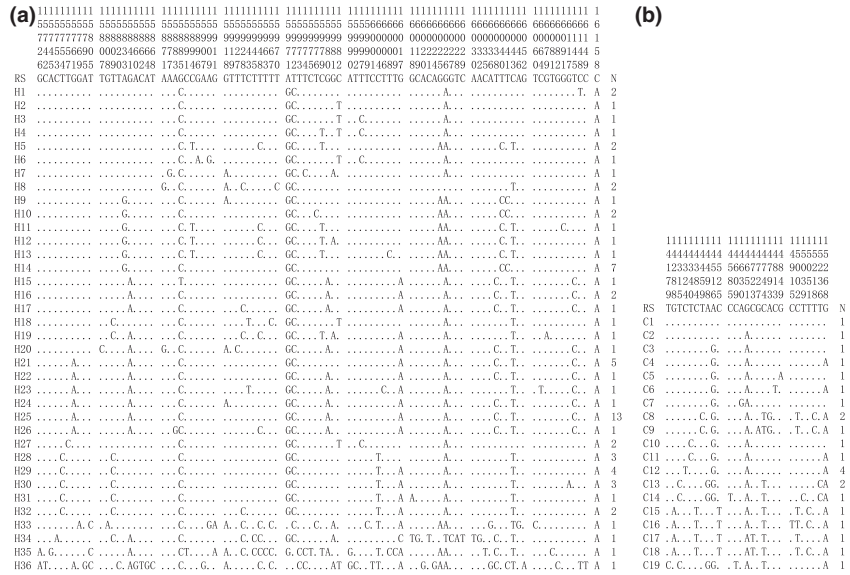
### Sequence variation

The HVS-I fragments of the mtDNA control region in all 795 goat samples were highly polymorphic, with 163 variable sites over the 481 bp of the alignment. Among them, 147 variants are transitions and 16 variants are transversions, and there is no insertion/deletion. Eighteen variable sites (involving three amino acids changes) were found in the eight complete cytochrome *b* sequences.

### Haplogroup classification and phylogenetic analysis

Thirty-six haplotypes were identified in 72 samples from the Yangtze River delta white goats analysed for the mtDNA control region sequence variation (Figure 2a). Using the available goat mtDNA haplogroup classification system (Luikart *et al.* 2001; Sultana *et al.* 2003; Naderi *et al.* 2007), 69 Yangtze River delta white goats could be classified into haplogroup A, whereas the remaining three samples were assigned to haplogroups B, C, and D, respectively. When the published Chinese goat mtDNAs were considered together with the newly obtained





**Figure 2** Sequence variations of (a) 72 Chinese Yangtze River delta white goat mtDNA HVS-I sequences (481 bp) and (b) 24 entire goat cytochrome *b* sequences. The haplotypes were scored relative to the reference sequence (accession no. NC\_005044; abbreviated as RS) on the basis of nucleotide substitutions. The number of individuals sharing the same haplotype were listed in the right column, under the capital letter N. Dots (·) denotes identical sites. The 481 bp fragment is located in region 15707–16187 in the goat mtDNA whole genome.

sequences in this study, we identified 327 haplotypes in 795 samples. Among them, 617 individuals (sharing 272 haplotypes) belonged to haplogroup A, 158 individuals (sharing 38 haplotypes) belonged to haplogroup B, 12 individuals (sharing nine haplotypes) belonged to haplogroup C, and eight samples (each with one unique haplotype) belonged to haplogroup D (Table 2). There were no novel haplogroup in our new data set and in the published 723 Chinese goat mtDNAs. In total, 19 haplotypes (which were defined by 27 variable sites) were identified in 24 goat complete cytochrome *b* sequences (Figure 2b).

Figure 3a presented the NJ tree of 795 mtDNA control region sequences together with the 22 reference sequences belonging to the known six haplogroups (Naderi *et al.* 2007). The six haplogroups were clearly separated from each other and received high bootstrap supports (not including haplogroup A with a low bootstrap value), as described by Naderi *et al.* (2007). All the Chinese goat samples were clustered into four haplogroups A, B, C and D. Within

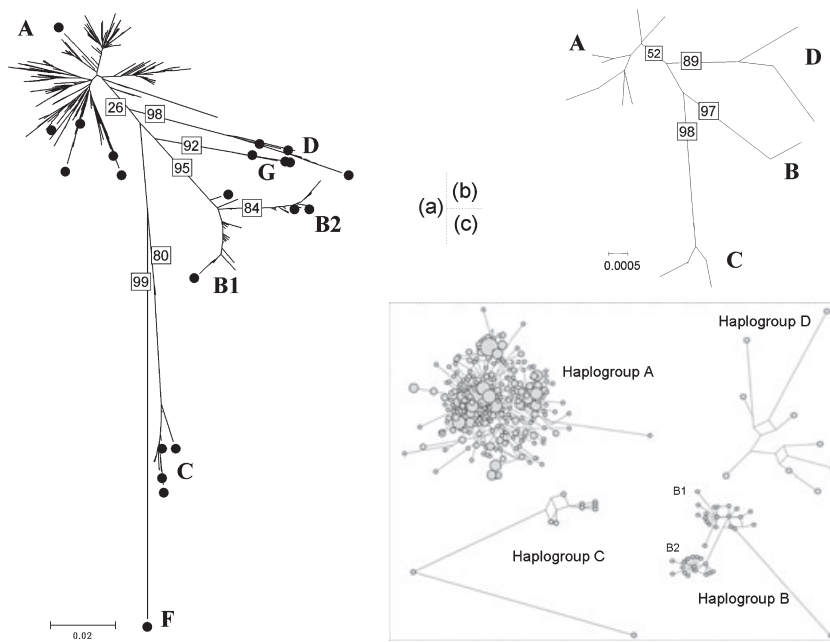
haplogroup B, two sub-haplogroups B1 and B2 were clearly discerned with a high bootstrap value, consistent with pattern described by Chen *et al.* (2005) and Naderi *et al.* (2007). The four haplogroups appeared in the mtDNA control region sequence tree were further verified by the cytochrome *b* sequence data (Figure 3b), thus validating their phylogenetic status. We further presented the genetic profile of goat samples in each of the four haplogroups by the network method (Bandelt *et al.* 1999). Haplogroups B1 and B2 showed a star-like profile (Figure 3c).

**Genetic diversity**

The genetic diversity estimated based on the mtDNA control region sequences varied substantially among the breeds: haplotype diversity values ranged from 0.733 in the Lezhi black goat to 1.000 in the Jianchang black goat and the Taihang goat (Table 1). The Tibetan breed displayed the highest nucleotide diversity value (0.0436 ± 0.0052), while the Banjiao

**Table 2** Genetic diversity of mtDNA haplogroups in Chinese domestic goat

Haplogroup	Number	Number of haplotypes	Haplotype diversity ( <i>h</i> ± SD)	Nucleotide diversity ( $\pi$ ± SD)
Haplogroup A	617	272	0.989 ± 0.001	0.0195 ± 0.0003
Haplogroup B	158	38	0.879 ± 0.019	0.0095 ± 0.0003
Sub-haplogroup B1	96	21	0.745 ± 0.046	0.0033 ± 0.0004
Sub-haplogroup B2	62	17	0.821 ± 0.042	0.0030 ± 0.0003
Haplogroup C	12	9	0.939 ± 0.058	0.0101 ± 0.0038
Haplogroup D	8	8	1.000 ± 0.063	0.0193 ± 0.0023
Total	795	327	0.989 ± 0.001	0.0355 ± 0.0008



**Figure 3** Phylogenetic trees of (A) 795 Chinese goat mtDNA control region sequences and 22 goat reference sequences and (B) 24 cytochrome *b* sequences. (C) Network profiles of the four haplogroups based on the mtDNA control region sequences. The phylogenetic positions of the 22 reference sequences, which were defined by Naderi *et al.* (2007), were marked by black dots in the neighbour-joining tree. The values on the branches were bootstrap support based on 1000 replications. In the networks, each circle represents a haplotype. The area of the circle is proportional to the sample size sharing that haplotype. The branch length between two circles is proportional to the number of mutations differed by the two haplotypes.

goat harbored the lowest one ( $0.0136 \pm 0.0052$ ), followed by the Liaoning cashmere breed ( $0.0161 \pm 0.001$ ). The haplotype and nucleotide diversities in the Yangtze River delta white goat were modest (Table 1). We also estimated genetic diversity for the four haplogroups. Among them, haplogroup A had the highest nucleotide diversity and haplogroup C had the lowest value. Within haplogroup B, subhaplogroup B1 had lower haplotype diversity and slightly higher nucleotide diversity than B2 (Table 2). When we grouped the goat samples according to the ecological distribution of Chinese goat, the southern pasturing area (IV) had the highest haplotype diversity ( $0.983 \pm 0.002$ ) and nucleotide diversity ( $0.0338 \pm 0.0007$ ), whereas the northern farming area (III) had the lowest diversity (haplotype diversity,  $0.969 \pm 0.008$ ; nucleotide diversity,  $0.0214 \pm 0.0017$ ). However, statistical test for the differences in genetic diversity were not significant ( $p > 0.05$ ).

### Haplogroup distribution

There was no haplogroup specific distribution pattern in breeds/populations or among the different ecological areas. Four breeds/populations (Tibetan goat, Taihang goat, Inner Mongolia goat, and Yangtze River delta white goat) contained all four haplogroups. The Baichuan white goat contained only haplogroup A while the Jining grey goat harboured three haplogroups (haplogroups A, B and D). The

**Table 3** Proportion of genetic variation among breeds and geographical regions

	Among regions	Among breeds within regions	Within breeds
d.f.	4	27	763
% of variation	4.35	11.37	84.29
p-value	<0.0001	<0.0001	0.0293

Note that the % of variation was calculated by using the amova (Excoffier *et al.* 1992).

other breeds/populations contained two haplogroups (A and B for most of them; A and C for Xinjiang; A and D for Liaoning cashmere goat). When we considered the ecological distribution, each ecological area contained all four haplogroups with the exception of the farming and pasturing mixture area V, which contained only one breed and had a small sample size (Table 1). Overall, 84.29% and 11.37% of the genetic variation were distributed within breeds and among breeds within the ecologically geographical areas, respectively. 4.35% of the genetic variation was attributed to the difference among the geographic areas ( $p < 0.001$ ) (Table 3).

### Discussion

Goat breeding has a long history, which approximately started at the end of Paleolithic and the beginning of Neolithic according to archaeological data (Tu *et al.* 1989; Zeder & Hesse 2000). Many indigenous breeds/populations were bred to meet

the diverse natural conditions across China. Due to the commercial trade, cultural communication, and the spread of agriculture, the original relationship among different goat breeds/populations were blurred to some extent, and this accounted for the weak phylogeographic structure in domestic goats (Luikart *et al.* 2001; Chen *et al.* 2005; Fan *et al.* 2007; Naderi *et al.* 2007). An analysis of amplified fragment length polymorphisms (AFLPs) markers in six autochthonous goat populations distributed in the middle and lower Yangtze River valley revealed no breed specific markers (Jiang *et al.* 2003). Recently, Chen *et al.* (2005) analysed the first hypervariable region of the mtDNA control region sequence variation in 18 Chinese goat populations and detected all four known haplogroups. Among them, haplogroups A and B were prevalent, haplogroup C (six samples) were only found in the Xinjiang and Tibetan breeds, and only one sample from the Liaoning cashmere breed belonged to haplogroup D (Chen *et al.* 2005). In another study, Liu *et al.* (2007) analysed the HVS-I sequence variation of 183 Chinese goats from 13 breeds and observed haplogroups C and D in the Tibetan breed. Most recently, Wang *et al.* (2008) analysed 107 individuals belonging to seven Chinese goat breeds from different geographic regions and detected haplogroup C in the Taihang and Inner Mongolia breeds and haplogroup D in the Taihang, Jining grey and Inner Mongolia breeds. Hitherto, almost all the major Chinese domestic goat breeds have been analysed except for the Yangtze River delta white goat in Jiangsu Province and the Fuqing goat in Fujian Province, both belong to the same ecological geography area – southern pasturing area (IV).

In this study, we analysed 72 Yangtze River delta white goats and identified all four known haplogroups in this breed. We speculate that there existed strong gene flow among goat populations, which was caused by extensive transportation of goats (as currency; *c.f.* Fan *et al.* 2007) in history. In all 795 Chinese goat samples, only four (A–D) out of the six known haplogroups were identified; there was no sample from haplogroup G or F, which were distributed in Middle East, Northern Africa, and Sicily (Naderi *et al.* 2007). We also sequenced cytochrome *b* gene complete sequences for representative samples from each haplogroup. The phylogenetic tree of the 24 new and published goat mtDNA cytochrome *b* sequences showed a consistent clustering pattern of the four haplogroups as observed in the mtDNA control region sequence tree. This result further validates the existing nomenclature of the four main

goat matrilineal haplogroups (Luikart *et al.* 2001; Sultana *et al.* 2003; Chen *et al.* 2005; Naderi *et al.* 2007). Two sub-haplogroups B1 and B2 of haplogroup B, which were first described by Chen *et al.* (2005) and further discussed by Naderi *et al.* (2007), were found in 96 and 62 Chinese goats, respectively. Moreover, these goat haplotypes from haplogroup B were mainly found in the southern pasturing area (region IV). This distribution pattern agreed with the results of Chen *et al.* (2005) and Naderi *et al.* (2007) that B2 individuals are restricted to China and Mongolia. The overall frequency distribution of the four haplogroups in all available Chinese goat samples was consistent with the reported pattern (Li *et al.* 1999; Joshi *et al.* 2004; Chen *et al.* 2005, 2006; Fan *et al.* 2007; Naderi *et al.* 2007; Wang *et al.* 2008), in which haplogroups A (77.61%, 617/795) and B (19.87%, 158/795) are the main components of Chinese goat. Note that haplogroups A and B at the worldwide scale, representing 91% and 6% of the goat haplotypes (Naderi *et al.* 2007).

Like in other domestic animals in China, such as chicken (Liu *et al.* 2006b,c), cattle (Lai *et al.* 2006), yak (Lai *et al.* 2007), and pig (Wu *et al.* 2007), we did not detect breed specific distribution of certain haplogroup. Moreover, the majority of genetic variation existed within breeds/populations and about 4% of genetic variation appeared among the five geographic regions, notwithstanding the great ecological difference. There was no clear geographic pattern in the network profile of haplogroup A, which was complicated by the abundance of sequence homoplasmy and was consistent with the low bootstrap support in the phylogenetic tree. The seemingly star-like network pattern of haplogroups B1 and B2 suggested for potential population expansion in the past, which was common in other domestic animals, such as chicken (Liu *et al.* 2006b) and pig (Wu *et al.* 2007).

In conclusion, we analysed both the mtDNA control region fragments and cytochrome *b* sequences to test Chinese goat phylogeny as well as to discern the genetic diversity of goat breeds/populations. Our results were in general agreement with the pattern described in previous studies (Chen *et al.* 2005; Fan *et al.* 2007; Liu *et al.* 2007; Naderi *et al.* 2007; Wang *et al.* 2008). We speculated that gene flow among goat populations facilitated by the traditional seasonal pastoralism and annual long-distance migrations in history as well as trade would account for the pattern discerned in regional goat pools. It is urgent to take measures that promote a sustainable management of these genetic resources (Taberlet *et al.* 2008).



## Acknowledgements

The authors acknowledged the two reviewers for constructive comments on the manuscript. This study was supported by the Chinese national postdoc trainee fund and a postdoc fund from Jiangsu Province, as well as, the natural science foundation of Jiangsu Province (BK2007121). YGY was supported by the 'Century Program' (or Hundreds-Talent Program) of the Chinese Academy of Sciences.

## References

- Bandelt H.-J., Forster P., Röhl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, **16**, 37–48.
- Chang H., Nozawa K., Liu X.L., Geng S.M., Ren Z.J., Qing G.Q. (2000) Study on phylogenetic relationship of native goat populations along the middle and lower Yellow River valley. *Sci. Agricult. Sinica*, **33**, 79–87.
- Chen S.-Y., Su Y.-H., Wu S.-F., Sha T., Zhang Y.-P. (2005) Mitochondrial diversity and phylogeographic structure of Chinese domestic goats. *Mol. Phylogenet. Evol.*, **37**, 804–814.
- Chen S., Fan B., Liu B., Yu M., Zhao S., Zhu M., Xiong T., Li K. (2006) Genetic variations of 13 indigenous Chinese goat breeds based on cytochrome B gene sequences. *Biochem. Genet.*, **44**, 87–97.
- Excoffier L., Smouse P.E., Quattro J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Excoffier L., Laval G., Schneider S. (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinform. Online*, **1**, 47–50.
- Fan B., Chen S.-L., Kijas J.H., Liu B., Yu M., Zhao S.-H., Zhu M.-J., Xiong T.-A., Li K. (2007) Phylogenetic relationships among Chinese indigenous goat breeds inferred from mitochondrial control region sequence. *Small Rumin. Res.*, **73**, 262–266.
- Jiang X.P., Liu G.Q., Ding J.T., Yang L.G., Cao S.X., Cheng S.O. (2003) Diversity in six Goat populations in the middle and lower Yangtze River valley. *Asian-australas. J. Anim. Sci.*, **16**, 277–281.
- Joshi M.B., Rout P.K., Mandal A.K., Tyler-Smith C., Singh L., Thangaraj K. (2004) Phylogeography and origin of Indian domestic goats. *Mol. Biol. Evol.*, **21**, 454–462.
- Kumar S., Tamura K., Jakobsen I.B., Nei M. (2004) MEGA3.1: Molecular Evolutionary Genetics Analysis Software. Arizona State University, Tempe, AZ, USA.
- Lai S.-J., Liu Y.-P., Liu Y.-X., Li X.-W., Yao Y.-G. (2006) Genetic diversity and origin of Chinese cattle revealed by mtDNA D-loop sequence variation. *Mol. Phylogenet. Evol.*, **38**, 146–154.
- Lai S.-J., Chen S.-Y., Liu Y.-P., Yao Y.-G. (2007) Mitochondrial DNA sequence diversity and origin of Chinese domestic yak. *Anim. Genet.*, **38**, 77–80.
- Li Z.L. (1993) Sheep and Goat Science in China. Chinese Agricultural Press, Beijing, pp. 1–3.
- Li X.L., Zhang Y.P., Chen S.G., Zeng F.T., Qiu X.P., Liu X.M. (1999) Study on the origin and differentiation of the goat based on the mtDNA diversity. *Acta Vet. Zootech. Sin.*, **30**, 313–319. (in Chinese).
- Liu R.Y., Yang G.S., Lei C.Z. (2006a) The genetic diversity of mtDNA D-loop and the origin of Chinese goats. *Acta Genet. Sin.*, **33**, 420–428. (in Chinese).
- Liu Y.-P., Wu G.-S., Yao Y.-G., Miao Y.-W., Luikart G., Baig M., Beja-Pereira A., Ding Z.-L., Palanichamy M.G., Zhang Y.-P. (2006b) Multiple maternal origins of chickens: out of the Asian jungles. *Mol. Phylogenet. Evol.*, **38**, 12–19.
- Liu Y.-P., Zhu Q., Yao Y.-G. (2006c) Genetic relationship of Chinese and Japanese gamecocks revealed by mtDNA sequence variation. *Biochem. Genet.*, **44**, 19–29.
- Liu R.Y., Lei C.Z., Liu S.H., Yang G.S. (2007) Genetic diversity and origin of Chinese domestic goats revealed by complete mtDNA D-loop sequence variation. *Asian-australas. J. Anim. Sci.*, **20**, 178–183.
- Luikart G., Gielly L., Excoffier L., Vigne J.D., Bouvet J., Taberlet P. (2001) Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc. Natl Acad. Sci. USA*, **98**, 5927–5932.
- Ma Y.H., Chen Y.C., Feng W.Q., Wang D.Y. (2002) Germplasm of Chinese domestic animals and their conservation. *Rev. China Agricult. Sci. Technol.*, **4**, 37–41. (in Chinese).
- Mannen H., Nagata Y., Tsuji S. (2001) Mitochondrial DNA reveal that domestic goat (*Capra hircus*) is genetically affected by two subspecies of bezoar (*Capra aegagurus*). *Biochem. Genet.*, **39**, 145–154.
- Naderi S., Rezaei H.R., Taberlet P., Zundel S., Rafat S.A., Naghsh H.R., Elbarody M.A.A., Ertugrul O., Pompanon F. (2007) Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. *PLoS ONE*, **2**, e1012 (doi:10.1371/journal.pone.0001012).
- Nei M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.
- Rozas J., Sachedz-Delbarrio J.C., Messeguer X., Rozas R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.
- Sultana S., Mannen H., Tsuji S. (2003) Mitochondrial DNA diversity of Pakistani goats. *Anim. Genet.*, **34**, 417–421.
- Taberlet P., Valentini A., Rezaei H.R., Naderi S., Pompanon F., Negrini R., Ajmone-Marsan P. (2008) Are cattle, sheep, and goats endangered species? *Mol. Ecol.*, **17**, 275–284.

- Takada T., Kikkawa Y., Yonekawa H., Kawakami S., Amano T. (1997) Bezoar (*Capra aegagrus*) is a matriarchal candidate for ancestor of domestic goat (*Capra hircus*): evidence from the mitochondrial DNA diversity. *Biochem. Genet.*, **35**, 315–326.
- Tu Y.R., Jiang Y., Han Z.Y., Feng W.Q. (1989) Sheep and Goat Breeds in China. Shanghai Scientific & Technical Publishers, Shanghai, pp. 1–25 (in Chinese).
- Wang J., Chen Y.L., Wang X.L., Yang Z.X. (2008) The genetic diversity of seven indigenous Chinese goat breeds. *Small Rumin. Res.*, **74**, 231–237.
- Wu G.-S., Yao Y.-G., Qu K.-X., Ding Z.-L., Li H., Palanichamy M.g., Duan Z.-Y., Li N., Chen Y.-S., Zhang Y.-P. (2007) Population phylogenomic analysis of mitochondrial DNA in wild boars and domestic pigs revealed multiple domestication events in East Asia. *Genome Biol.*, **8**, R245 (doi:10.1186/gb-2007-8-11-r245).
- Yao Y.-G., Kong Q.-P., Bandelt H.-J., Kivisild T., Zhang Y.-P. (2002) Phylogeographic differentiation of mitochondrial DNA in Han Chinese. *Am. J. Hum. Genet.*, **70**, 635–651.
- Zeder M.A., Hesse B. (2000) The initial domestication of goats (*Capra hircus*) in the Zagros Mountain 10,000 years ago. *Science*, **287**, 2254–2257.
- Zhao Y.Z. (1995) Goat Production. Chinese Agricultural Press, Beijing, pp. 24–26 (in Chinese).