**INTRODUCTION**

Goats were the first animals to be domesticated (Porter, 1996; Pringle, 1998; Zeder et al., 2006). For the past several thousand years, domestic goats (*Capra hircus*) have been one of the most important livestock animals, providing people with milk, meat, and fiber (Joshi et al., 2004). China has one of the largest dairy goat populations in the world, and dairy goats are mainly located in the middle and lower reaches of the Yellow River. There are 9 dairy goat breeds in China: Wendeng (WDG), Laoshan (LSG), Xinong Saanen (XSG), and Guanzhong (GZG), Jianyang Big Ear (JYG), Lezhi Black (LZG), Laiwu Black (LWG), Saanen (SNG), and Nubian (NBG; Jingfa et al., 2004). However, until now, no comprehensive study of their genetic structure, origins, relevant relationships, or evolution has been performed based on mitochondrial DNA (mtDNA) analysis. There have been recent suggestions that the evolutionarily derived genetic diversity of livestock is an important resource through which domesticated species have maintained their breeding potential (FAO, 2007; Piras et al., 2012). Some important breeds exhibit signs of genetic erosion (Bruno-de-Sousa et al., 2011), and conservation measures must be taken to avoid an irremediable loss of their genetic resources (Taberlet et al., 2011).

Therefore, we investigated the genetic structure and origin of Chinese dairy goats by assessing intra- and interbreed genetic diversity present in the mtDNA to serve as a reference for defining conservation priorities and establishing breeding strategies.
MATERIALS AND METHODS

Collection of Blood Samples

Blood samples were obtained from 162 individual dairy goats from 4 provinces (Shaanxi, Shandong, Yunnan, and Sichuan) representing 4 cultivated dairy goat breeds (WDG, LSG, XSG, and GZG), 2 introduced dairy goat breeds (SNG and NBG), and 3 local breeds (JYG, LZG, and LWG) in China (Table 1). None of the sampled goats had common ancestors within 3 generations.

Mitochondrial DNA Displacement Loop Extraction and Sequence Analysis

Total DNA (genomic DNA and mtDNA) used in PCR amplification was extracted from whole blood using the Tianamp Blood DNA kit and 2x PCR MasterMix kit from China Tiangen Biotech Co., LTD (Beijing, China) according to the manufacturer’s instructions through the PCR instrument type FTC-200 (Fedbio; Shanghai, China). A 1,736-bp fragment corresponding to the complete mtDNA displacement loop (D-loop) region was amplified using the primers MT1R (forward primer) and MT2F (reverse primer) designed from GenBank accession number NC005044. Given the circular nature of mtDNA, these primers are positioned at bases 15,073 to 15,094 of the complete goat mtDNA sequence (GenBank accession number NC005044). Each 25-μL sequence amplification reaction was prepared with 0.8 μg of DNA, 0.5 μL of each primer, 2 μL of deoxyribonucleotide triphosphate mixture, 2.5 μL of 10x Taq buffer, and 0.5 μL of Taq DNA polymerase (from Tiangen Biotech-Beijing, Co.,Lto, Beijing, China) and performed in a GeneAmp PCR FTC-200 (FedBio) thermal cycler using the following conditions: an initial denaturing step at 94°C for 3 min; 34 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1.5 min; and a final extension at 72°C for 5 min. The PCR products were purified by Axygen (Shanghai, China) and directly sequenced with the MT1R, MT2F, and internal MT3 primers via an ABI 3730 automated DNA sequencer (Applied Bio systems, Carlsbad, California). A total of 162 complete mtDNA D-loop sequences were deposited in GenBank (accession numbers KP164647–KP164808). In total, 155 1,212- to 1,215-bp sequences exhibited good homology after they were aligned, and the remaining 7 sequences with low homology were not further considered.

Genetic Diversity Data Analysis

The D-loop sequence fragments were aligned using the ClustalW2 program (Larkin et al., 2007) to determine their degree of homology. Gene diversity parameters were obtained using the DnaSP program (Librado and Rozas, 2009) and included haplotype diversity and nucleotide diversity (an estimate of the population size). Tajima’s D was also calculated to summarize statistics for the entire frequency spectrum of variable sites. The neighbor-joining molecular phylogenetic trees and distance-based trees were generated and drawn with the MEGA 4.0 program (Kumar et al., 2008). Bayesian phylogenetic analyses were performed with MrBayes V3.1.2 as implemented at CIPRES (http://www.phylo.org/; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Median-joining networks were generated using the Network 4.6.1 program to investigate the possible relationships between haplotypes (Bandelt et al., 1999). Mismatch analysis, Fu’s Fs values (Fu, 1997), and analyses of molecular variance (AMOVA) were computed with Arlequin version 3.1 software (Excoffier et al., 2005).

Table 1. The breed names, geographic regions, sample sizes, haplotype, and nucleotide diversity for each breed used in this study

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sample</th>
<th>Geographic distribution</th>
<th>Number of haplotype</th>
<th>Haplotype diversity (±SE)</th>
<th>Nucleotide diversity (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDG</td>
<td>28</td>
<td>Wendeng, Shandong Province</td>
<td>8</td>
<td>0.69 ± 0.085</td>
<td>0.0053 ± 0.0027</td>
</tr>
<tr>
<td>LSG</td>
<td>21</td>
<td>Qingdao, Shandong Province</td>
<td>8</td>
<td>0.75 ± 0.086</td>
<td>0.0060 ± 0.0010</td>
</tr>
<tr>
<td>XSG</td>
<td>17</td>
<td>Yangling District, Shaanxi Province</td>
<td>11</td>
<td>0.94 ± 0.036</td>
<td>0.0098 ± 0.0027</td>
</tr>
<tr>
<td>GZG</td>
<td>20</td>
<td>Fuping County, Shaanxi Province</td>
<td>17</td>
<td>0.98 ± 0.024</td>
<td>0.012 ± 0.0027</td>
</tr>
<tr>
<td>SNG</td>
<td>19</td>
<td>Kunming, Yunnan Province</td>
<td>10</td>
<td>0.88 ± 0.056</td>
<td>0.0097 ± 0.0022</td>
</tr>
<tr>
<td>NBG</td>
<td>17</td>
<td>Kunming, Yunnan Province</td>
<td>12</td>
<td>0.95 ± 0.036</td>
<td>0.0157 ± 0.0010</td>
</tr>
<tr>
<td>JYG</td>
<td>11</td>
<td>Sichuan Province</td>
<td>8</td>
<td>0.95 ± 0.054</td>
<td>0.013 ± 0.0037</td>
</tr>
<tr>
<td>LZG</td>
<td>13</td>
<td>Sichuan Province</td>
<td>9</td>
<td>0.94 ± 0.051</td>
<td>0.014 ± 0.0028</td>
</tr>
<tr>
<td>LWG</td>
<td>9</td>
<td>Laiwu, Shandong Province</td>
<td>9</td>
<td>1.00 ± 0.052</td>
<td>0.0076 ± 0.0009</td>
</tr>
</tbody>
</table>

1WGG = Wendeng; LSG = Laoshan; XSG = Xinong Saanen; GZG = Guanzhong; SNG = Saanen; NBG = Nubian; JYG = Jianyang Big Ear; LZG = Lezhi Black; LWG = Laiwu Black.
RESULTS

Mitochondrial DNA Displacement Loop Sequence Polymorphism

Of the 155 sequences, we identified 97 polymorphic sites with only conversion substitutions and no transversion substitutions as well as 20 single polymorphic loci and 77 parsimony informative sites. Overall, we defined 62 different haplotypes, including 35 unique haplotypes, in the sequences from 155 individuals. The most common haplotype was observed 28 times. The haplotype diversity values in the dairy goats ranged from 0.69 ± 0.085 (WDG) to 0.98 ± 0.024 (GZG). The nucleotide diversity values ranged from 0.0053 ± 0.024 (WDG) to 0.0157 ± 0.001 (NBG) per site. The estimator theta(S) was 17.268 over the entire sequence or 0.014 per site. Tajima’s D was –0.688 and was not significant (P > 0.1). Detailed information regarding haplotype diversity and the nucleotide diversity of each population are presented in Table 2.

Phylogenetic Analysis

A neighbor-joining tree constructed with 62 haplotypes of Chinese dairy goat sequences indicated that the Chinese dairy goats were divided into 2 distinct mtDNA haplogroups, A and B. Haplogroup A was the predominant group, including 142 individuals and comprising 55 haplotypes. Haplogroup B included 13 individuals comprising 7 haplotypes (Fig. 1). Within the network, LSG and WDG exclusively appeared in haplogroup A, and all other dairy goat breeds were found in haplogroups A and B. Furthermore, the haplotypes of the breeds from each of the different geographic regions did not cluster together, and some haplotypes were shared by different breed individuals from different regions. To test this neighbor-joining tree, we reconstructed the tree using the 62 haplotypes of the Chinese dairy goat sequences indicated above and combined them with 8 sequences belonging to the Capra hircus mtDNA haplogroups that were published in GenBank (GenBank A, B, and C). The 8 sequences and their GenBank accession numbers are presented in Table 2, and the neighbor-joining tree is shown in Fig. 2. The Bayesian tree was constructed with 62 haplotypes using a model (Hasegawa, Kishino, and Yano [HKY] + γ) to accommodate the unusual GC content of the mtDNA D-loop of Chinese dairy goat sequences; this tree also exclusively exhibited a single central node. The credibility of the 4 clades was 61, 63, 66, and 100%. Using the same data set, the topology of the Bayesian tree was similar to that of the neighbor-joining tree, with the exception of minor incongruence among some internal branches.

Population Expansions

The mismatch distribution analysis of the complete dataset, including haplogroups A and B of the mtDNA D-loop, is presented in Fig. 3. The average number of nucleotide differences in all Chinese dairy goat breeds was 13.508, and haplogroups A and B exhibited differences of 9.305 and 3.740, respectively. Fu’s (1997) neutral Fs test was used to detect population expansion for all Chinese dairy goat breeds. Haplogroup A displayed

<table>
<thead>
<tr>
<th>Country</th>
<th>Haplogroup</th>
<th>Accession number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>A</td>
<td>DQ089106</td>
<td>Chen et al. (2005)</td>
</tr>
<tr>
<td>China</td>
<td>A</td>
<td>AJ317569</td>
<td>Luikart et al. (2001)</td>
</tr>
<tr>
<td>Germany</td>
<td>A</td>
<td>AJ317586</td>
<td>Luikart et al. (2001)</td>
</tr>
<tr>
<td>Azerbaijan</td>
<td>B</td>
<td>EF617706</td>
<td>Naderi et al. (2007)</td>
</tr>
<tr>
<td>Britain</td>
<td>A</td>
<td>AJ317655</td>
<td>Luikart et al. (2001)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>A</td>
<td>AJ317573</td>
<td>Luikart et al. (2001)</td>
</tr>
<tr>
<td>France</td>
<td>C</td>
<td>EF617786</td>
<td>Naderi et al. (2007)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>C</td>
<td>EF618486</td>
<td>Naderi et al. (2007)</td>
</tr>
</tbody>
</table>
Wang et al. exhibited a significant and smaller negative $Fs$ value ($-3.398; P < 0.01$). These results in-dicate a large and sudden expansion from the mismatch distribution (Table 3) of haplogroup A. The mismatch distribution revealed that haplogroup A exhibited a uni-modal peak at approximately 12 differences, whereas haplotype B showed bimodal peaks at approximately 1 and 10 differences. These results suggest that at least 1 expansion event occurred in the demographic history of haplogroup A Chinese dairy goats given the predominant departure and significant differences from neutrality. Haplogroup B exhibited no population expansion and relatively stable population sizes with no significant differences in neutrality.

Population Genetic Structuring

The hierarchical AMOVA revealed an increased percentage (89.34%; $P = 0.5572$) of total mtDNA variation within the populations; a smaller but significant percentage was attributed to geographical groups.
Diversity of Chinese dairy goats

(7.26%; P < 0.01) and populations (3.39%; P < 0.01), as presented in Table 4. For these groupings, the $F$-statistics were 0.0726, 0.0366, and 0.1066, respectively. The above results consistently demonstrated that no significant geographical structuring was observed in the Chinese dairy goat breeds and that the genetic variation mainly resulted from moderate differentiation.

**DISCUSSION**

**Chinese Dairy Goat Breed**

China has one of the largest dairy goat populations in the world, and dairy goats are mainly located in the middle and lower reaches of the Yellow River. Four dairy goat breeds, WDG, LSG, XSG, and GZG, are widely used in China. Each of these breeds was developed from mating imported SNG bucks with indigenous does. The NBG breed was also introduced to the Sichuan province and to some areas of southern China. Additionally, there are many local goat breeds, including the JYG, LZG, and LWG, that are used today to provide milk, meat, wool, and cashmere, but the yields of all of these products are low. These local goat breeds are also known as indigenous goats (Jingfa et al., 2004).

**Mitochondrial DNA Research Status**

Mitochondrial DNA has been commonly used for the study of genetic diversity. In particular, the mitochondrial control region has been used to describe genetic polymorphism in goats (Piras et al., 2012). Based on mtDNA studies, domestic goats are divided into 7 branches (A–G) based on their origins (Joshi et al., 2004; Sardina et al., 2006; Wang et al., 2013). A low frequency lineage was identified in Pakistan and India (Sultana et al., 2003; Joshi et al., 2004). In this study, Chinese dairy goats exhibited 2 maternal origins. Consistent with the previous studies on Chinese domestic goat breeds, genetic diversity occurred within the breeds, and the very weak geographic structure was interpreted to result from the extensive transportation of goats between continents (Sardina et al., 2006; Naderi et al., 2007). Chinese goats exhibit rich genetic diversity and are divided into 4 branches (A–D; Chen et al., 2006). Polymorphisms of the mtDNA control region are the most suitable metric for describing the diversity of extant breeds and for reconstructing the places and timing of domestication (Wu et al., 2009; Vacca et al., 2010; Doro et al., 2014) because this region is variable and sufficiently structured across the geographical range of the species and because it evolves at a constant rate (Bruford et al., 2003).

**Low Levels of Mitochondrial DNA Diversity**

Mitochondrial DNA haplotype and nucleotide diversity are 2 important indices for assessing population polymorphisms and genetic differentiation (Pereira et al., 2005; Benjelloun et al., 2011). High haplotype and nucleotide diversity values indicate an increased number of polymorphism in the population (Liu et al., 2006). In this study, the average haplotype diversity and nucleotide diversity of Chinese dairy goats were compared with values presented in other genetic diversity studies in Chinese domestic goat breeds (Liu et al., 2009; Kang et al., 2011; Zhao et al., 2014). Moreover, the level of diversity within Chinese dairy goats was also reduced compared with goat samples throughout the Eastern hemisphere (Luikart et al., 2001). This result may be due to differences in the study methods (Liu et al., 2006) and the limited number of breed samples (Zhao et al., 2014). In addition, it is likely that Chinese dairy goats have non-rich maternal origins (Chen et al., 2005, 2006).

**Origins of Chinese Dairy Goats**

In previous studies, goat mtDNA haplogroups were categorized into 6 highly divergent groups referred to as A, B, C, D, F, and G. The most common group is haplogroup A, which is highly frequent worldwide and in diverse geographical distributions (from 89% in Asia to 98% in Europe; Pereira et al., 2005). In this study, Chinese dairy goats exhibited 2 maternal origins. Consistent with the previous studies on Chinese domestic goat breeds,
haplogroup A was also the major haplogroup (Chen et al., 2006; Liu et al., 2006; Zhao et al., 2014). Some studies indicated that haplogroup B likely originated from Asia or China (Joshi et al., 2004; Chen et al., 2005; Liu et al., 2006). However, in our study, haplogroup B exhibited a lower frequency among breeds (7/62) than those reported by Zhao et al. (2011), Liu et al. (2006), and Chen et al. (2005; 30/148, 17/92, and 25/142, respectively). The LSG and WDG breeds did not appear in haplogroup B. Zhao et al. (2014) hypothesized that Southwestern Asia may serve as the origin area of haplogroup B. In this study, the results were largely consistent with the studies cited above. Given that 1) most of the dairy goat samples were collected from North China, 2) Chinese dairy goats originated from Europe, and 3) these breeds were subsequently subjected to natural selection by the local environment and to hybrid improvement, we hypothesized that the resulting breeds are currently considered naturalized, locally adapted, or native. It is also possible that numerous haplotypes resemble the original diversity before domestication.

No Significant Geographical Structure

Analyses of the genetic structure of the population revealed no significant geographical structure in mtDNA variation among Chinese dairy populations. This finding was revealed by the network analyses as well as the AMOVA results. Our results were consistent with the phylogeography patterns that were previously observed in Chinese goat populations (Fan et al., 2007; Wang et al., 2008; Liu et al., 2009). This result demonstrated that Chinese goats exhibit a weak phylogeographic structure as previously reported (Amills et al., 2004; Azor et al., 2005; Chen et al., 2005). The extent of genetic divergence in the study was similar to microsatellite DNA investigations using the same goat populations, in which approximately 89.5% of the total genetic variation was attributed to intrapopulation divergence (Li et al., 2002). Goats are easily transportable and manageable; therefore, these animals were commonly used as currency for equal exchange. Estimation of the demographic parameters via mismatch analyses indicated that haplogroup A experienced demographic expansion.

Expansion Events in Part of the Population

Population history has far-reaching implications on the patterns of genetic variation and genetic diversity in mtDNA. In domesticated animal groups, intense changes in the environment could lead to random drift and changes in allele frequencies. It is necessary to detect population expansion when the number of groups is dramatically altered. The existence of population expansion was tested by 2 different methods: Fu’s (1997) Fs statistic and mismatch distribution. In this study, haplogroup A yielded significant negative values with 1 major peak in mismatch distribution. This analysis indicated that haplogroup A experienced population expansion events. This result is consistent with a number of previous studies that suggested that domestic goats experienced population expansion events (Joshi et al., 2004; Hou et al., 2008; Zhao et al., 2011). Importantly, given the small sample size for the breeds in our sampling (<30 individuals; Zhao et al., 2014), the detection of population expansion was only performed at the individual breed level. We will improve these results with a more thorough test in the future.

In conclusion, mtDNA D-loop sequence analysis of 9 Chinese dairy goat breeds provided evidence for 2 maternal haplogroup origins, of which haplogroup A was most important. High mtDNA diversity was not observed, and the geographical structure was not significant. These results are consistent with the hypothesis that goats are easily transportable and were commonly used as currency for equal exchange. Estimation of the demographic parameters via mismatch analyses indicated that haplogroup A Chinese dairy goats experienced demographic expansion.

LITERATURE CITED


Diversity of Chinese dairy goats


