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Short communication

Genetic and phylogenetic studies of Chinese native sheep breeds (*Ovis aries*) based on mtDNA D-loop sequences

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Abstract

To determine the genetic diversity and the origin of Chinese sheep, we analyzed 83 complete sequences of mtDNA D-loop from nine Chinese sheep breeds and a foreign breed, together with nine sheep and cattle available sequences from GenBank. The length of the sequences was considerably variable between 1103 and 1225 bp. The hapolotype diversity was 92.7%. The nucleotide diversity was 3.058%. And the mean nucleotide composition of the 83 sequences was 32.9% A, 29.8% T, 22.9% C and 14.4% G, respectively. The NJ phylogenetic tree (the number of replications of bootstrap test is 1000) revealed that there were three distinct major domestic sheep lineages (termed as lineages A–C) in the 10 breeds. The result indicated that Chinese native sheep breeds derive from three different maternal sources. The mismatch distribution analysis showed that the Fs values were −25.15, −12.28, −8.60 for the lineages A–C, respectively ($P < 0.01$), which suggested that atleast one population expansion events occur in the demographic history of Chinese sheep breeds.

Keywords: Chinese native sheep; Maternal source; mtDNA; D-loop

1. Introduction

Mitochondrial DNA (mtDNA) is the genetic material that exists outside the nucleolus of eukaryotic cells. It has a simple molecular structure which is covalent and close. Including coding and non-coding regions, its length ranges from 15 to 20 kb in different species. The non-coding region is the control region. The rate of mtDNA evolution is about 5–10 times faster than that of nuclear DNA, and its genes do not recombine (Upholt and Dawid, 1977). So mtDNA analysis has often been used to investigate haplotype diversity within species. The mtDNA D-loop region is known to be more variable than other regions of it, and it is often used to analyze the phylogeny of closely related breeds within species (Wolf et al., 1999).

China has a centuries-old history of breeding sheep. It has abundant sheep breed resources, with more than 40 local sheep breeds, which serve as important genetic resources for the sustainable development of animal husbandry and the preservation of biological diversities. The purpose of the study was to investigate the genetic diversity and to discuss the origin of Chinese sheep breeds.

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2. Materials and methods

2.1. Sample collection

The edge tissues of ear samples from 10 different sheep breeds were collected at livestock farms throughout China according to the simple random sampling method. The breeds were chosen from Xinjiang province (Bashibai sheep, $n=48$; Tashikuergan sheep, $n=47$), Gansu province (Lanzhou big tail sheep, $n=42$; Ganjia sheep, $n=47$; Qiaoke sheep, $n=56$; Oula sheep, $n=48$ and Minshan black sheep, $n=48$), Shaanxi province (Hanzhong sheep, $n=50$), Henan province (Henan big tail sheep, $n=52$), Ningxia province (Zhongwei goat, $n=2$) and Beijing city (skudde, $n=30$). DNA was extracted from these specimens using phenol/chloroform as described by Sambrook et al. (2001). Eighty-three individuals among them were sequenced.

The primer sequences of mtDNA control regional were between $tRNA^{Phe}$ and $tRNA^{Pro}$ (forward: 5′ CTCACCATCAACCCCCAAAGC 3′; reverse: 5′ TCATCTAGGCATTTTCAGTG 3′) (Hiendleder et al., 2002). The volume of PCR amplification reaction system was 60 μL, consisted of genomic DNA 50 ng, dNTPs 200 μM, mixed primers 10 pmol, $MgCl_2$ 250 μM, Taq DNA polymerase 1 U. The reaction profiles included an initial denaturation at 95 °C for 5 min, followed by 30 cycles, each consisting of 30 s denaturation at 94 °C, 45 s primer annealing at 55 °C, 60 s extension at 72 °C, and then a final 8 min extension at 72 °C. The PCR products were electrophoresed through 2.0% (w/v) agarose gel which was stained with ethidium bromide solution.

The amplified products were purified with a DNA purification kit according to the manufacturer's instructions (TW-Biotech. Co. Ltd.). The purified fragments were cloned into pGEM-T easy vector and subsequently transformed into *Escherichia coli* Top10. After 16–20 h, single colonies were inoculated to obtain recombinant plasmid. The recombinant plasmid DNA was extracted and then sequenced using an ABI model 3730 automated sequencer. Unique polymorphisms in the results were confirmed by resequencing of independent clones.

2.2. Data analysis

Sequences were aligned using the BioEdit 7.0 software. Identical sequences were considered as the same haplotype. Generally, two different approaches were used to examine the traces of population expansion (Excoffier and Schneider, 1999). The first one approach is Fu's Fs statistic (Fu, 1997). The other approach to detect expansion is the distribution of the number of pairwise differences between sequences in a sample (mismatch distribution) (Rogers and Harpending, 1992). This model is based on an infinite-site model and assumes that a stepwise expansion occurred some time in the past from a stationary population to a large stationary population. The nucleotide mismatch analysis was used Arlequin 2.0 program. The NJ (neighbor-joining) molecular phylogenetic trees were

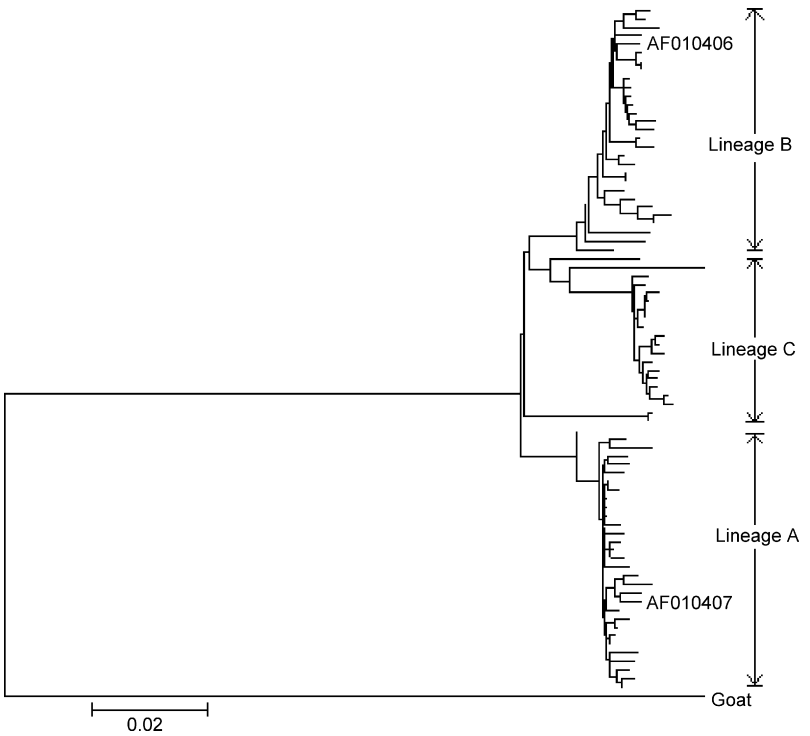

Fig. 1. The neighbor-joining phylogenetic tree was constructed by using the data from the study and GenBank (accession numbers AF010406 and AF010407). To achieve clarity, the taxon names and bootstrap values obtained from 1000 simulated replicates were not shown.

constructed to analyze sheep's evolution using the MEGA 2.0 program with a Kimura two-parameter model (Kumar et al., 2001), and the bootstrap value was 1000.

3. Results

3.1. Sequencing analysis of the sequences

With the reference (GenBank access number AF010406) as a criterion, all the sequences were aligned. The haplotype diversity of D-loop region was 92.7%. The nucleotide composition of all the haplotypes was 32.9% A, 29.8% T, 22.9% C, 14.4% G. The nucleotide diversity was 3.058%. There were a lot of transition sites but limited transversions. Under the Kimura two-parameter model, the mean pairwise genetic distance of all the haplotypes was 0.034. The percentage of sequence similarity between sheep and goat was 80%. The length of the sequences was considerably variable between 1103 and 1225 bp, and the majority was 1180 bp. Except minor insertions and deletions, the observed length variations were caused by different copy numbers of a 75 bp tandem repeat (5′ TACATAGTATTAATGTAATATAGACATTACATGTA-TAAAGTACATTAAATGATTTACCCCATGCA TAT-AAGCA 3′).

3.2. Phylogenetic analysis of Chinese domestic sheep breeds

A total of 78 mtDNA haplotypes (GenBank accession numbers DQ903228–DQ903304) could be distinguished from the 83 individuals analyzed. The D-loop sequences obtained in the study together with those from GenBank (AF010407, AF010406), were used to cluster (Fig. 1). The result showed that there existed three major lineages, which termed as lineages A–C. Lineages A and B were predominant in the figure, while lineage C was at lower frequency. In addition, only Chinese sheep breeds presented in lineage C. From the figure it could be seen that goat did not belong to any lineages, while it was as an outgroup.

3.3. Population expansion

The detection of population expansion was performed at lineage level because of the small size in our sampling. The Fs values were −25.15, −12.28, −8.60 for the lineages A–C, respectively ($P < 0.01$). The results were congruent with a demographic model showing a large and sudden expansion as inferred from the mismatch distribution (Fig. 2). These results suggested that atleast one

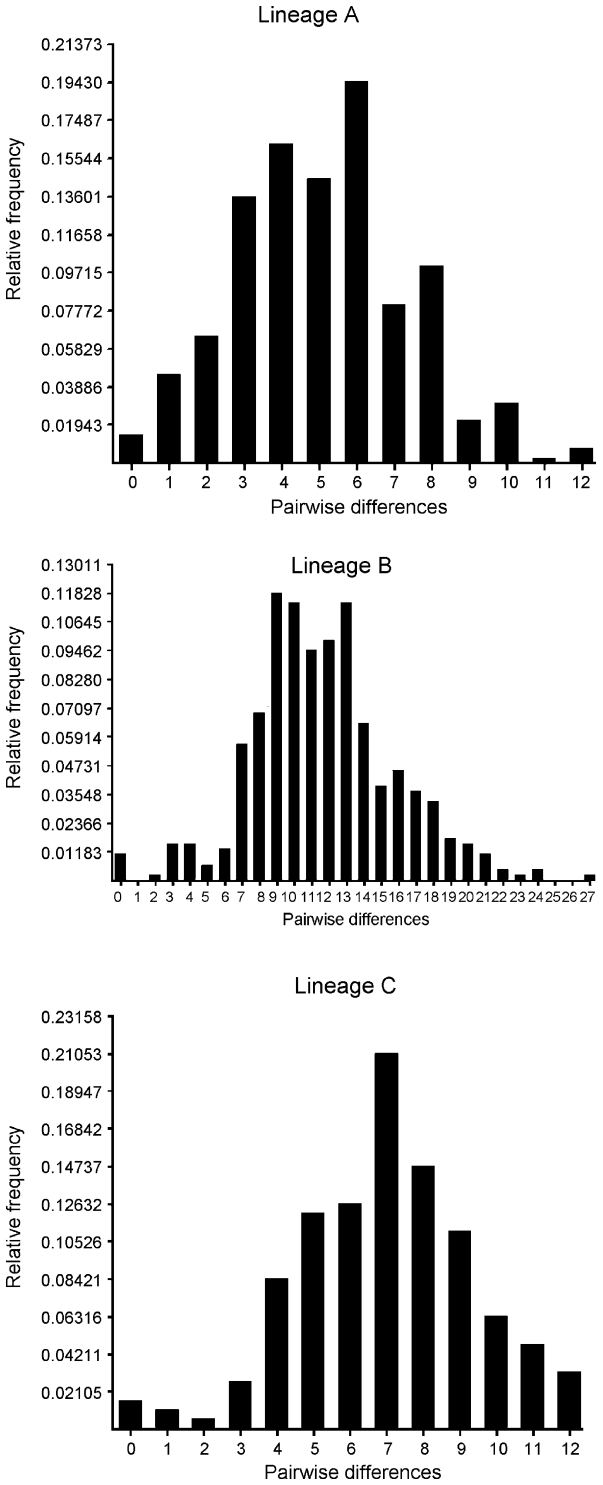

Fig. 2. Mismatch distributions of mtDNA types of the three lineages in Chinese sheep.

population expansion events occur in the demographic history of Chinese sheep breeds.

4. Discussion

Mitochondrial DNA had been very widely used by geneticists to analyze the phylogenetic relationships at or below the species level in cattle, swine, goats and water buffalo, and it had also been used to investigate the genetic variation of species (Giuffra et al., 2000; Sultana et al., 2003; Tanaka et al., 1996; Watanabe et al., 1989; Zhang et al., 2005). The nucleotides composition of the study was similar to that of Hiendleder (1998a). The shortest sequence length was 1103 bp, and the longest 1225 bp. The difference was caused by 3–5 copies of a 75 bp tandemly repeated sequence.

In this study, we conducted a mismatch analysis with 1000 simulations. The overall validity of the estimated demographic model was tested by comparing the distribution of a test statistic sum of squared differences (SSDs) between the observed and the estimated mismatch distribution by a bootstrap approach (Excoffier and Schneider, 1999; Schneider et al., 2000). Significant SSD values can be taken as an evidence for departure from the estimated demographic model, which can be either an expanding or a stationary population. The nucleotide mismatch analysis showed that the three lineages all underwent atleast one population expansion event in history ($P < 0.01$).

There has been a controversy over the issue of the origin of sheep. The previous opinion on the origin of domestic sheep was urial (*O. vignei bochariensis*) and argli (*O. ammon nigrimontana*) by using mtDNA PCR-RFLP and sequence analysis methods (Hiendleder et al., 1991, 1998b, 2002; Wood and Phua, 1996). They collected samples from Europe, Africa, New Zealand and central Asia, but not from China. The mtDNA D-loop sequencing by Guo et al. (2005) provided the evidence of a novel maternal lineage in six native Chinese sheep breeds. That was not consistent with the previous researches. It is well known that China is one of the most abundant countries of genetic resources. The samples used in the study were mostly distributed in remote areas, and hardly influenced by foreign sheep breeds, so they could represent the genetic situations of these domestic sheep breeds. The haplotypes of D-loop region we studied could be divided into three lineages, which indicated that the modern domestic sheep breeds derive from three maternal sources. It was consistent with the result of Guo et al's. The NJ phylogenetic result together with the sequences from GenBank further proved above.

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