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Full Length Research Paper

Two maternal origins of Chinese domestic light-body type goose

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China is particularly rich in goose genetic resources. The systemic study of genetic diversity and origin of Chinese indigenous geese will provide important scientific basis for the conservation, utilization of resources and human history. The 521 bp control region (D-loop) of mitochondrial DNA from 13 light-body type breeds was sequenced. The results showed that in the D-loop region of the 13 gray goose breeds, the content of T, C, A and G nucleotides was 23.8, 29.0, 32.2 and 15.1%, respectively. The average haplotype diversity (Hd) and nucleotide diversity (Pi) of domestic geese were 0.2153 and 0.00046, respectively. The 13 light-body type breeds had bigger nucleotide variance value among populations than the value within populations and all the breeds did not exist population expansion. Shared haplotype analysis and systemic systematic evolution analysis revealed that Chinese light-body type domestic goose owned two maternal origins. YL goose breed originated from greylag goose (anser anser), and the other 12 light- body type goose breeds originated from swan goose (anser cygnoides).

Key words: Domestic goose, mtDNA D-loop, systematic evolution.

INTRODUCTION

During thousands years of domestication, geese have been considerably differentiated by natural and artificial selections (Romanov and Weigend, 2001). With its long history of animal husbandry and diversified geographical conditions, China has abundant variety of native goose resources. 26 goose breeds distributed in China, play a very important role in the agricultural and human history of China. According to body weight, the 26 goose breeds were divided into three types such as heavy-body type goose breeds, median-body type goose breeds and light-body type goose breeds. The light-body type goose breeds include Changle (CL) goose, Youjiang (YouJ) goose, Yangjiang (YJ) goose, Wuzong (WZ) goose, Taihu (TH) goose, Lianhua (LH) goose, Yili (YL) goose, Huoyan (HY) goose, Minbei white (MB) goose, Yongkang gray (YK) goose, Linxian (LX) goose, Baizi (BZ) goose and Zi goose. These 13 native goose breeds have better adaptability to extensive management, better immunity to diseases, higher reproduction rate and good meat quality, which are natural gene pool and good original material of crossbreed predominance and high performance. With the increasing demand for goose products including meat, down feather, and fatty liver, the goose industry has flourished in China. But in comparison to other domestic animals in China, there are fewer biological researches in geese than in chickens' and ducks' thus far reported.

Li et al. (2007) evaluated the genetic diversity of 26 Chinese native goose breeds by microsatellite markers. The Chinese geese had strong genetic potential and strong adaptability. The genetic relationships among the populations had significant association with their historical

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Abbreviations: T, Thymine; **G,** guanine; **A,** adenine; **C,** cytosine; **Hd,** haplotype diversity; **Pi,** nucleotide diversity; **RFLP,** restriction fragment length polymorphism; **mtDNA,** mitochondrial deoxyribonucleic acid; **PCR,** polymerase chain reaction; **Nm,** product of migrants and deme size; **CL,** Changle goose; **YouJ,** Youjiang goose; **YJ,** Yangjiang goose; **WZ,** Wuzong goose; **TH,** Taihu goose; **LH,** Lianhua goose; **YL,** Yili goose; **HY,** Huoyan goose; **MB,** Minbei white goose; **YK,** Yongkang gray goose; **LX,** Linxian goose; **BZ,** Baizi goose; **Zi,** Zi goose.

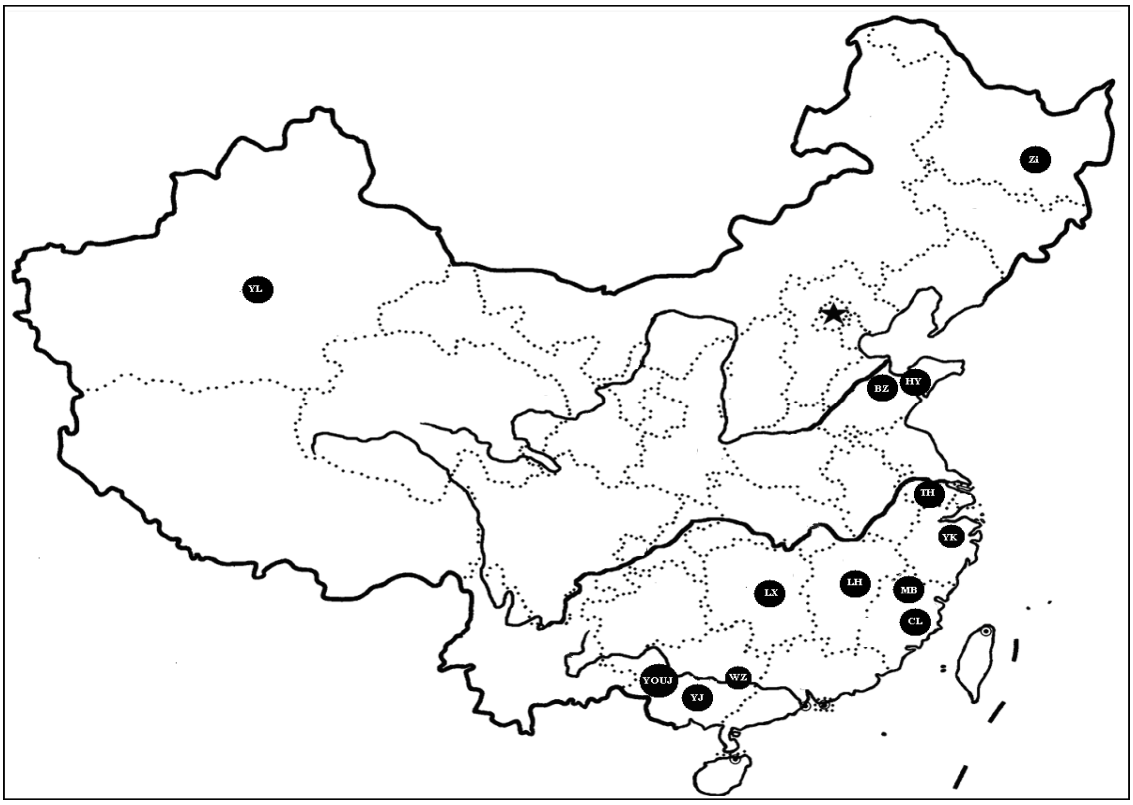

Figure 1. The geographical distributions of 13 light- body type goose breeds in China.

relations and geographical distribution. Up to now, only a few researches of goose diversity and origin have been performed at mitochondrial DNA level using two methods of mtDNA RFLP and mtDNA sequencing. Shi et al. (1998), Liu (2003) and Wang et al. (2005) studied mitochondrial DNA polymorphisms of some goose breeds, analyzing their different origins and their genetic differentiations. Although, there are some mtDNA studies on partial native geese, it is still essential to study genetic diversity and origin of Chinese native light- body type goose breeds using mtDNA marker in order to provide important scientific basis for the conservation and utilization of the resource in the future. In the present study, the genetic diversity and systematic evolution of 102 specimens from 13 Chinese light-type goose breeds collected from conserve farm or conserve zone were analyzed, and the results may help to understand the origin of these goose breeds.

MATERIALS AND METHODS

Specimen collection

The blood samples of the 13 native light- body type goose breeds were collected from the conservation farms or zones respectively, which were as follows: Changle goose (CL, N = 8), Youjiang goose (YouJ, N = 8), Yangjiang goose (YJ, N = 8), Wuzong goose (WZ, N = 8), Taihu goose (TH, N = 8), Lianhua goose (LH, N = 6), Yili goose (YL, N = 8), Huoyan goose (HY, N = 8), Minbei white goose (MB, N = 8), Yongkang gray goose (YK, N = 8), Linxian goose (LX, N = 8), Baizi goose (BZ, N = 8) and Zi goose (Zi, N = 8). All sampled individuals represented their own breed. The geographic distributions of 13 light- body type goose breeds were shown in Figure 1. Four sequences included one Rhine goose (AY552169), one swan goose (AY552167) and two greylag goose (AF159961 and AF159963) were collected from NCBI website.

PCR amplification and DNA sequencing

Polymerase chain reaction (PCR) was performed to amplify part of the mtDNA control region. The primers reported by Wang et al. (2005) were used to amplify the target region. The corresponding sequences were L536 5'-CCTCTGGTTCCTCGGTCA-3', H1248 5'-CAACTTCAGTGCCATGCTTT-3'.

The PCR reaction was carried out on an Eppendorf Mastercycle. The reaction recipe contained 2.5 µl 10×Buffer, 2.5 µl dNTPs (2.5 mM), 2.0 µl Mg^{2+} (25 mM), 1 µl each primer (25 pmol/µl), 3.0 µl genomic DNA (50 ng/µl), 0.2 Taq polymerase (5 U/µl). The thermal cycling profile for mtDNA was 5 min preheat at 95℃, followed by 35 cycles of 45 s at 94℃, 45 s at 56℃, 1 min at 72 ℃, a final extension of 10 min at 72℃, and conservation at 4℃. PCR products were agarose gel-purified and sequenced on an ABI Prism 3730 DNA analyzer in both directions by primer walking using a BigDye Terminator V. 3.1 cycle sequencing kit (ABI, Foster City, CA).

Data analysis

Electropherograms were obtained using the program Chromas and

Table 1. The hierarchical composition of mtDNA variation analysis of molecular variance of 13 goose breeds.

Source of variation	d.f.	Sum of squares	Variance components	Percentge variation	Fst Fixation Index
Among populations	12	7.596	0.07444 Va	60.23%	0.6023
Within populations	89	4.375	0.04916Vb	39.77%	
Total	101	11.971	0.12359		

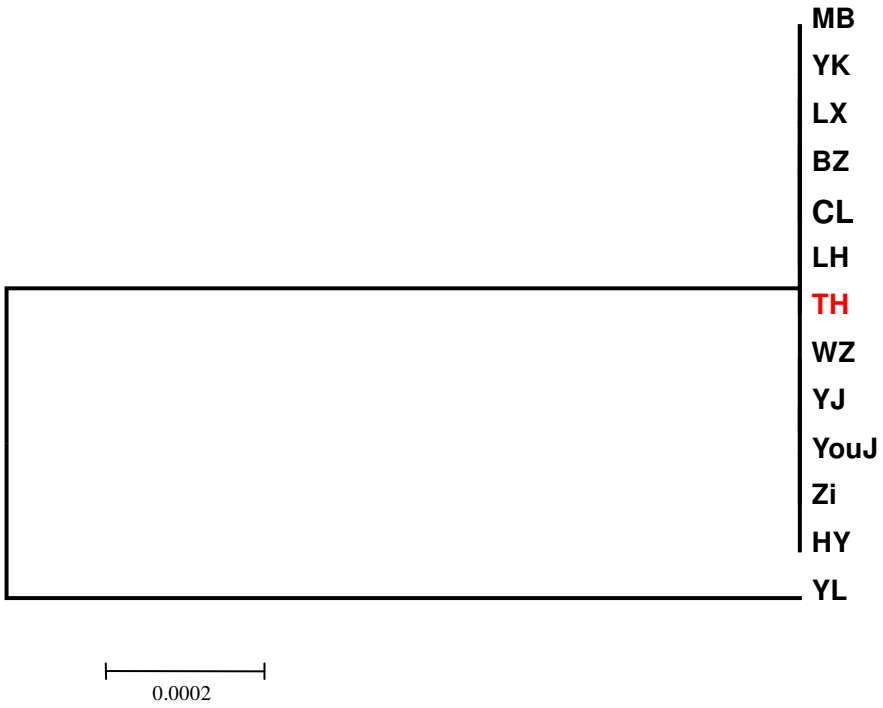

Figure 2. Neighbor joining population tree of the domestic light- body type goose.

manually checked insuring the veracity of the DNA sequences. Sequence alignments were performed using DNAman (6.0.40). The average nucleotide composition was calculated by Mega v.4.0 (Kumar et al., 2004). Haplotype numbers, nucleotide variable sites, haplotype diversity, nucleotide diversity (Nei, 1982), mismatch distributions and Nm were calculated using DNASP V.4.10.7 (Rozas, 2003). Analysis of molecular variance, Fst and Tajima's D value were implemented using Arlequin 3.0 (Excoffier et al., 2005). Kimura 2-parameter distances between breeds were estimated in Mega v.4.0 (Kumar et al., 2004) and a neighbor-joining tree was then constructed. Median-joining network of the mtDNA control region sequence haplotypes was constructed according to Bandelt et al. (1999) using program Network 4.5.0.1 http://www.fluxus-engineering.com/sharenet.htm.

RESULTS

Genetic variation and polymorphism of mtDNA D-loop

The average nucleotide composition was 23.8 T, 29.0 C, 32.2 A and 15.0% G in the 521-nucleotide mtDNA D-loop region of 102 domestic geese. The average percentage of A+T content (56%) was higher than G+C (44%). There were three polymorphic sites with two singleton polymorphic sites and one parsimony informative polymorphic sites. The variable types were transitions and transversions. Four haplotypes were identified in 13 light- body type domestic goose breeds. The average haplotype diversity (Hd) and nucleotide diversity were 0.2153 and 0.00046, respectively.

Light-body type goose population differentiation and expansion

AMOVA analysis indicated that 60.23% of the genetic variation was present among breeds, whereas 39.77% was within breeds (Table 1). Nm between YL goose breed and the other 12 goose were ranged from 0 to 0.14, while the Nm among the other 12 goose breeds was 1.22-11.36.

It can be inferred from Figure 2 that the 12 goose breeds including CL, YouJ, YJ, WZ, TH, LH, HY, MB, YK, LX and BZ as well as Zi goose were clustered into one

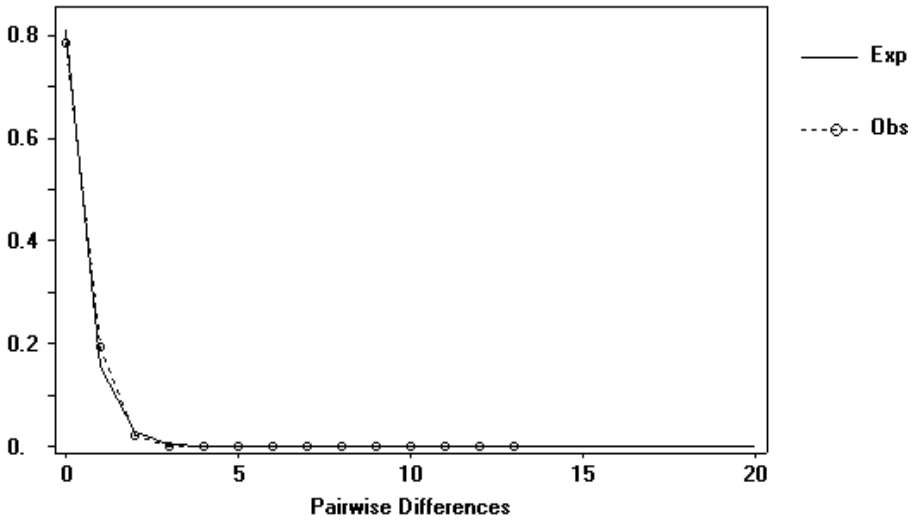

Figure 3. The mismatch distributions of the haplotypes of 13 domestic goose groups.

group while YL goose formed another group alone (Figure 2). There was a peak in the mismatch distributions of the haplotypes of 13 domestic goose breeds (Figure 3). Tajima's test revealed that the 13 light- body type goose groups were accorded with the standard neutral model ($P > 0.10$). These indicated that the 13 domestic light-body type goose groups did not exist population expansion.

Composition and distribution of haplotype in 13 light-body type goose breeds

Six haplotypes (H1-H6) were found in 106 sequences including 102 native geese and 4 goose) sequences including one rhine goose, one swan goose and two greylag geese from NCBI website. H1 haplotype was the biggest shared haplotype, which consisted of eight goose individuals for CL, YouJ, YJ, WZ, TH, BZ, Zi, LH; seven goose individuals for HY, MB, YK, LX and one swan goose. H2 haplotype consisted of one HY goose individual. H3 haplotype consisted of eight YL individuals, one LX goose individual and one Rhine goose individual. H4 consisted of one MB goose individual and one YK goose individual. H5 and H6 haplotype contained one greylag goose individual independently. 89.22% of domestic light- body type geese shared the same haplotype H1 with swan goose. This indicates that the maternal origin of these domestic geese might be swan goose (*anser cygnoides)*. YL goose and one LX goose individual shared the same haplotype, H3, with Rhine goose which originated from greylag goose indicating that the maternal origin of YL goose might be greylag (*anser anser)*.

Systematic evolution of Chinese light-body type goose

The NJ phylogenetic tree (Figure 4) and reduced median-joining network chart (Figure 5) were constructed by 6 haplotypes. The maternal lineage of H1, H2 and H4 was close to swan goose, and maternal lineage of H3 was close to greylag goose.

DISCUSSION

Genetic diversity of Chinese native goose breeds

Haplotype diversity (Hd) and nucleotide diversity (Pi) of populations were the main indexes for evaluating mtDNA variation and genetic diversity of breed or population. The greater the Hd and Pi, the richer the genetic diversity. Liu (2003) reported that three haplotypes were found in 1401 bp mtDNA sequence of six breeds of Chinese goose and two breeds of domestic Europe goose. Hd and Pi were 0.547 and 0.00775, respectively. Wang et al. (2005) reported that the Pi of 15 Chinese domestic goose breeds was arranged from 0 to 0.00116. Li and Wang (2007) analyzed nucleotide variables of partial sequence (621 bp) of ND4 gene in 6 native goose breeds, and found the Hd was 0.582, and Pi was arranged from 0-0.01417. Hd and Pi were all low at mtDNA level in the researches above. The same result was found in the present research. From these results, we suggest that the conservation farm and zone should take scientific and useful measures to protect domestic goose resources.

Genetic differentiation of 13 light- body type goose populations

A population bottleneck (or genetic bottleneck) is an evolutionary event in which a significant percentage of a population or species is killed or otherwise prevented from reproducing. As for the bottleneck effect, population

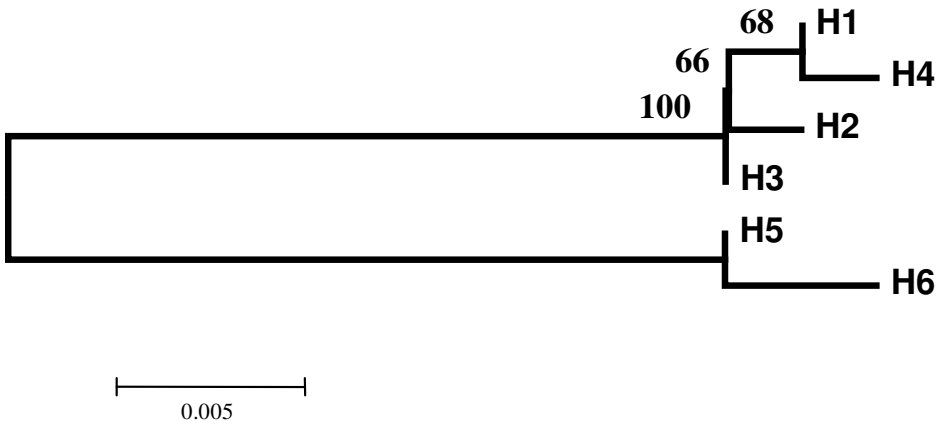

Figure 4. A phylogenetic tree based on D-loop sequences constructed with NJ method using Kimura's two-parameter model Key: H: Haplotype, Six haplotypes (H1-H6) were explained in Results.

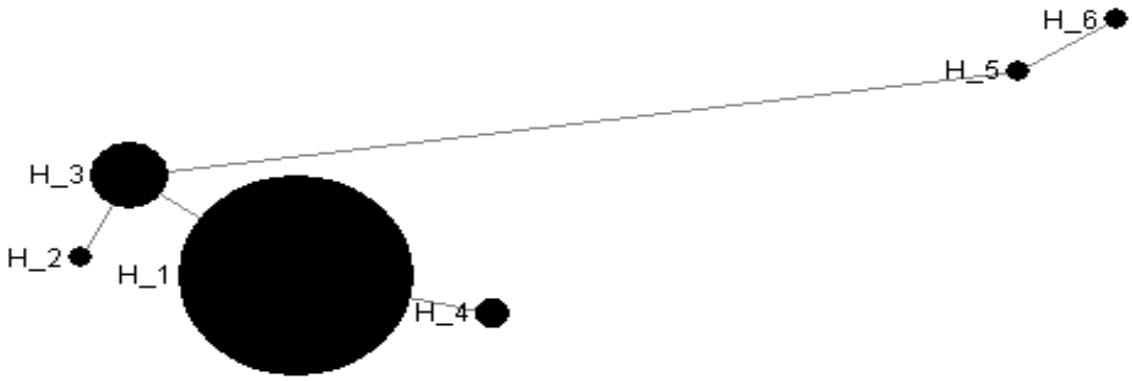

Figure 5. Reduced median-joining networks of mtDNA D-loop haplotypes. H: Haplotype. The six haplotypes(H1-H6) were explained in Results.

size may decrease rapidly and genetic diversity may be lost. Mismatch distributions of the haplotypes and Tajima's test indicated that the 13 light- body type goose populations did not exist bottleneck event in its evolution progress. Nm value below 0.5 indicated that genetic drift played a main role in population genetic differentiation. Nm value above 0.5 indicated that gene flow played a main role in population genetic differentiation. Genetic drift may have been the main factor that affected the genetic differentiation of the YL goose breed (Nm = 0-0.14). On the other hand, gene flow is the main reason for the lack of a clear differentiation among the remaining 12 light- body type domestic goose breeds (Nm = 1.22 - 11.36).

Neutral test revealed that nucleotide variable in the 521 bp D-loop region accorded with neutral theory. Variations in this D-loop region were mainly affected by neutral mutation, and this region sequence had not been changed by artificial selection. From another point of view, it indicated that there was no relationship between artificial selection focused on certain production performance and this D-loop region variance. However, Bell (1985), Mannen et al. (2003) and Oh et al. (2003) reported that effects of sequence variation in bovine mitochondrial DNA on milk production, meat quality and rate of back fat were found, respectively. It will need further research to study whether effects of cytoplasmic inheritance on production traits exist in domestic goose or not.

Systemic evolution of Chinese light- body type goose

Liu (2003) analyzed nucleotide variation of 1401 bp mtDNA sequence in 6 breeds of Chinese goose and 2 breeds of domestic Europe goose. YL goose and the 2 Europe goose breeds originated from greylag goose. The remaining 5 Chinese goose breeds originated from swan goose. Shi et al. (1998) analyzed the polymorphisms of mtDNA in 11 Chinese goose breeds by RFLP method and stated that the maternal lineage of YL goose was different from the other 10 goose breeds. In the present study, haplotype analysis and systematic evolution analysis

revealed that Chinese light- body type domestic goose owned two maternal origins. YL goose originated from greylag goose (*anser anser)*, and the other 12 light- body type goose breeds originated from swan goose (*anser cygnoides)*.

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