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Two Maternal Origins of the Chinese Domestic Grey Goose

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Abstract: The systematic study of genetic diversity and origin of Chinese indigenous geese will provide an important scientific basis for the conservation, utilization of the resource and human history. The 521 bp control region (D-loop) of mitochondrial DNA from 13 grey goose breeds collected from conservation farms and zones was sequenced. The results showed the average Haplotype Diversity (Hd) and nucleotide diversity (Pi) of domestic geese were 0.19245 and 0.00036, respectively.

Key words: Domestic goose, mtDNA, D-loop, origin, grey goose, analysis

INTRODUCTION

During thousands years of domestication, the goose has 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 an abundant variety of native goose resources. About 13 of 26 goose breeds are grey breeds which are mostly distributed in southern China and play a very important role in the agricultural and human history of China. These native goose breeds have better adaptability to extensive management, better immunity to diseases, a higher reproduction rate and better meat quality and they are also natural gene pool and the good original material of crossbreed predominance and high performance (Li *et al*., 2007). With the increasing demand for goose products including meat, down feather, fatty liver, the goose industry has flourished in China. In comparison to other domestic animals in China, the understanding of the domesticated goose is poor.

Microsatellite markers were used by Cathy (2001) described genetic diversity of Canadian geese and by Li *et al.* (2007) to evaluate genetic diversity of 26 Chinese native goose breeds. The genetic relationships among the populations had obvious association with their historical relations and geographical distribution.

Up to now, only a few researches of goose diversity and evolution have been performed at mitochondrial DNA (mtDNA) level. Liu (2003), Shi *et al.* (1998) and Wang *et al.* (2005) analysed mtDNA polymorphism of some goose breeds of different origins and genetic differentiations. Although, there were some mtDNA studies about partial native geese, it is essential to systemically study genetic diversity and origin of Chinese native grey goose breeds by using mtDNA markers. This will provide important scientific basis for the conservation and utilization of the resource in the future. In this study, we analyzed the genetic diversity and systemic evolution of 106 specimens from 13 Chinese domestic grey goose breeds which were collected from conserve farms or conserve zones.

MATERIALS AND METHODS

Blood was sampled from goose wing veins at conservation farms or zones and collected in test tube containing anticoagulant solution. The conservation farms or zones identified by the state were used for protecting purebred Chinese local goose breeds. About 13 native breeds were investigated: Yili goose (YL, N = 8 individuals), Shitou goose (ST, N = 8), Xingguo grey goose (XG, N = 8), Fengcheng grey goose (FC, N = 10), Youjiang goose (YOUJ, N = 8), Changle goose (CL, N = 8), Yongkang grey goose (YK, N = 8), Yan goose (Y, N = 8), Wuzong goose (WZ , N = 8), Yangjiang goose (YJ, N = 8), Magang goose (MG, N = 8), GANG goose (GANG, N = 8), Wugang goose (WG, N = 8). All the sample individuals represented their own breed, respectively. The geographic distributions of these breeds are showed in Fig. 1. Four sequences of goose included one Rhine goose (AY552169), one *Anser cygnoides* (AY552167) and two *Anser anser* (AF159961 and AF159963) two *Anser anser* (AF159961 and

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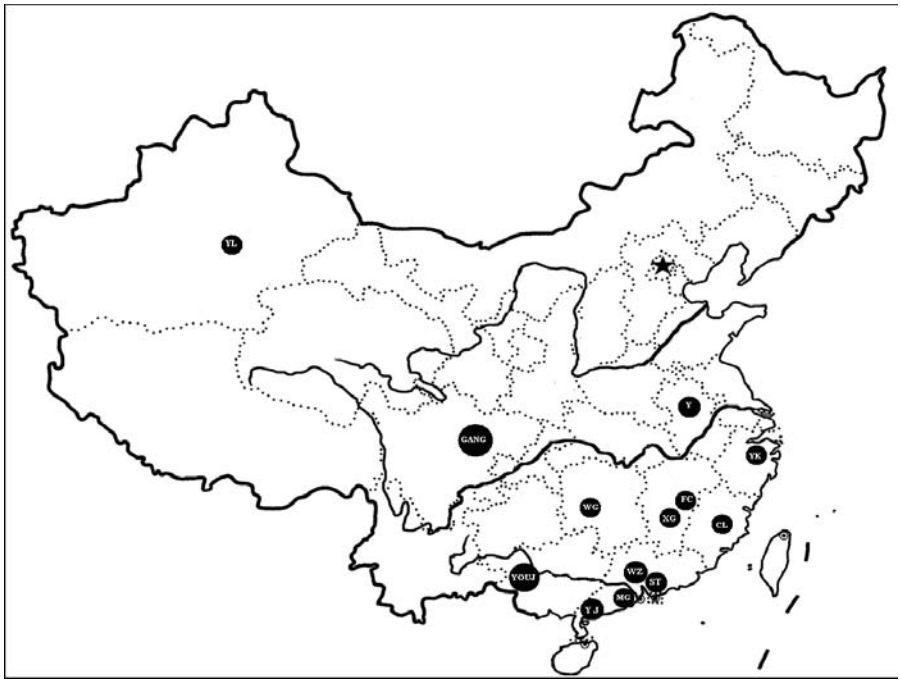

Fig. 1: The geographic distributions of 13 grey goose breeds. YL, ST, XG, FC, YOUJ, CL, YK, Y, WZ, YJ, MG, GANG, WG represented Yili goose, Shitou goose, Xingguo grey goose, Fengcheng grey goose, Youjiang goose, Changle goose, Yongkang grey goose, Yan goose, Wuzong goose, Yangjiang goose, Magang goose, GANG goose, Wugang goose respectively. Symbol plus a scale bar just represented the place of each goose breed, no matter what square was

AF159963) (Ruokonen *et al*., 2000) were collected from NCBI website. The primers reported by Wang *et al*. (2005) were used to amplify the target region. The corresponding sequences were L536 5'-CCTCTGGTTCCTCGGTCA-3' and H1248 5'-CAACTTCAGTGCCATGCTTT-3'. Polymerase Chain Reaction (PCR) was performed to amplify part of the mtDNA control region. 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^{-1}), 3.0 μL genomic DNA (50 ng μL^{-1}), 0.2 Taq polymerase (5 U μL^{-1}). The thermal cycling profile for mtDNA was 5 min preheat at 95°C followed by 35 cycles of 45 sec at 94°C, 45 sec at 56°C, 1 min at 72°C, a final extension of 10 min at 72°C and conservation at 4°C. 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).

Electropherograms were obtained using the program Chromas 1.45 reference downloaded from http://www.technelysium.com.au/chromas_lite.html website for free and manually checked insuring the veracity of the DNA sequences. Sequence alignments were performed using DNAman (version 6.0.40). Haplotype numbers, nucleotide variable sites, haplotype diversity, nucleotide diversity (Nei, 1982), mismatch distributions and Nm were calculated using DnaSP V.4.10.7 (Rozas *et al*., 2003). Analysis of molecular variance, Tajima (1989) 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. Another NJ tree was constructed based on the haplotypes identified in 106 Chinese domestic grey geese, 1 Rhine goose (AY552169), 1 swan goose (AY552167) and 2 greylag geese (AF159961 and AF159963) and the Kimura-2-parameters model using MEGA 4.0. 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 AND DISCUSSION

The average nucleotide composition was 23.8% T, 29.0% C, 32.3% A and 15.0% G in the 521-nucleotide mtDNA D-loop region of 106 domestic geese. The average

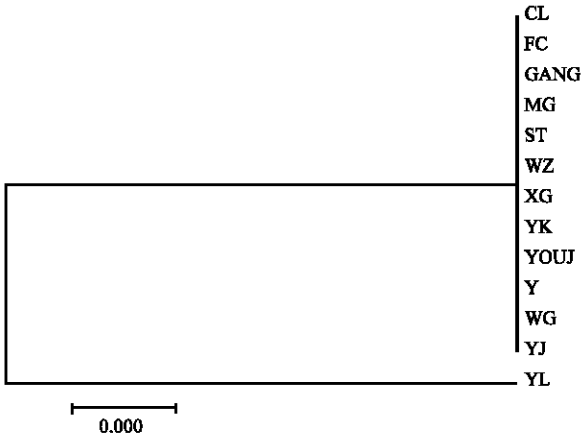

Fig. 2: Neighbor joining population tree of the domestic grey goose

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

Source of variation	df	Sum of squares	Variance components	Percentage variation	Fst fixation
Among populations	12	7.699	0.07522Va	72.53%	0.7253
Within populations	93	2.650	0.002849Vb	27.47%	
Total	105	10.349	0.103710		

percentage of A+T content (51.3%) was higher than G+C (48.7%). 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 domestic grey goose breeds (Fig. 2). The average Haplotype Diversity (Hd) and nucleotide diversity were 0.19245 and 0.00036, respectively.

AMOVA indicated that 72.53% of the genetic variation was present among breeds whereas 27.47% was within breeds (Table 1). Nm between YL goose breed and the other 12 goose breeds were ranged 0-0.05 but the Nm among the other 12 goose breeds was 1.02-10.68.

There was a peak in the mismatch distributions of the haplotypes of 13 domestic goose breeds (Fig. 3). Tajima test revealed that the 13 grey goose groups accorded with the standard neutral model ($p>0.10$). These indicated the 13 domestic grey groups had not existed before population expansion.

About 6 Haplotypes (H1-H6) were found in 110 sequences including 106 native grey goose and 4 sequences included one Rhine goose, one *A. cygnoides* and two *A. anser* from NCBI website. H1 haplotype was the biggest shared haplotype which was consisted of eight WG goose individuals, eight YAN goose individuals, nine FC goose individuals, seven XG goose

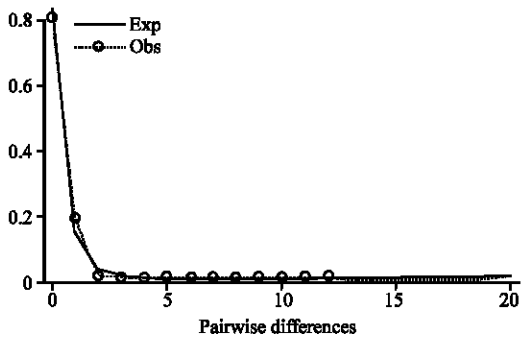

Fig. 3: The mismatch distributions of the haplotypes of 13 domestic grey goose breeds

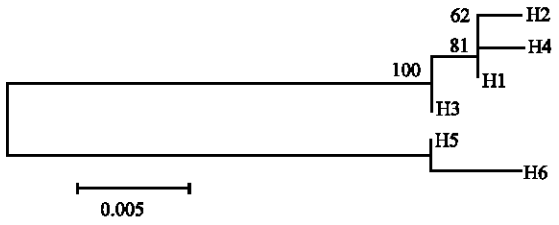

Fig. 4: A phylogenetic tree based on D-loop sequences constructed with NJ method using Kimura's two-parameter model. H: Haplotype, Six haplotypes (H1-H6) were explained in result part

individuals, eight ST goose individuals, eight WZ goose individuals, eight YJ goose individuals, eight MG goose individuals, eight GANG goose individuals, eight CL goose individuals, eight YOUJ goose individuals, seven YK goose individuals and one *A. cygnoides*. H2 haplotype consisted of one XG goose individual and one FC goose individual. H3 haplotype consisted of eight YL individuals and one Rhine goose. H3, H5 and H6 haplotypes only included one YK goose, one western greylag and one eastern greylag.

About 90.6% of the domestic grey geese shared the same haplotype H1 with *A. cygnoides* indicated that the maternal origin of these domestic grey geese was *A. cygnoides*. YL goose shared the same haplotype H3 with Rhine goose which originated from *A. anser* indicated that the maternal origin of YL goose was *A. anser*.

The NJ phylogenetic tree (Fig. 4) and reduced median-joining network chart (Fig. 5) were constructed by 6 haplotypes. The maternal lineage of H1, H2 and H4 was close to *A. cygnoides* and maternal lineage of H3 was close to *A. anser*.

Haplotype Diversity (Hd) and nucleotide diversity (Pi) of populations were main indexes for evaluating the mtDNA variation and genetic diversity of breed or

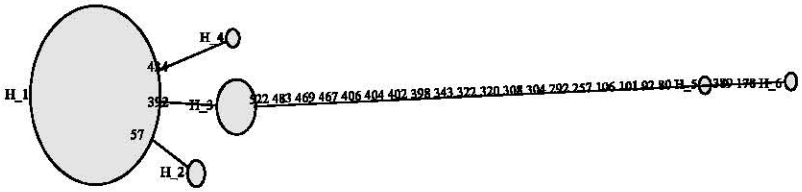

Fig. 5: Reduced median-joining networks of mtDNA D-loop haplotypes. H: Haplotype, Six haplotypes (H1-H6) were explained in result part. Red numbers indicated the mutation sites between two haplotypes

population. The greater Hd and Pi, the more rich the genetic diversity. Liu (2003) reported that three haplotypes were found in 1401 bp mtDNA sequence of 6 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 Pi of 15 Chinese domestic goose breeds was arranged from 0-0.00116. Li and Wangm (2007) analyzed nucleotide variables of partial sequence (621 base pairs) of the 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 on mtDNA level in the researches above. The same result was found in the research and we suggest that the conservation farms and zones should take scientific and useful measures to protect domestic goose resources.

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 size may decrease rapidly and genetic diversity may lose. Mismatch distributions of the haplotypes and Tajima test indicated that bottleneck event did not happen in the 13 grey goose populations 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 to affect the genetic differentiation of the YL goose breed (Nm = 0-0.05). On the other hand, gene flow is the main reason for the lack of a clear differentiation among the remaining 12 grey domestic goose breeds (Nm = 1.05-10.68).

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 angle, it indicated that there was no relationship between artificial selection focused on certain production performance and this D-loop region variance. It will need further research to study whether effects of cytoplasmic inheritance of production traits exist in domestic goose or not.

Liu (2003) analyzed nucleotide variation of 1,401 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 *A. anser*. The remaining 5 Chinese goose breeds originated from *A. cygnoides*. Shi *et al.* (1998) analyzed the polymorphisms of mtDNA in 11 Chinese goose breeds by RFLP method and stated the maternal lineage of YL goose was different from the other 10 goose breeds. In the study, haplotype analysis and systemic evolution analysis revealed that Chinese grey domestic goose were from two maternal origins. YL goose originated from *A. anser* and the other 12 grey goose breeds originated from *A. cygnoides*.

CONCLUSION

In this study, shared haplotype analysis and systemic evolution analysis revealed that Chinese grey domestic geese are derived from two maternal origins. Yili goose breed originated from the Greylag (*Anser anser*) and the other 12 grey goose breeds originated from the Chinese or Swan Goose (*Anser cygnoides*).

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