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Matrilineal Genetic Structure of Domestic Geese

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In contrast to other farm animals, the matrilineal structure of domestic goose has remained an open question. In this study, we analyzed mitochondrial DNA control region sequence variation of 245 domestic geese to discern the main matrilineal components and their phylogenetic relationships. The result of phylogenetic analysis indicated the distinct lineage of clade II and the result of network analysis further suggested the major group of Chinese native goose breeds (subclade S) and introgressive goose breeds (subclade G). Except Yili geese, all Chinese domestic goose breeds were clustered in S, whereas clade II were widely distributed in 2 European breeds, Yili geese as well as 3 domestic Graylag geese. The results support that Chinese domestic goose breeds (except the Yili breed) originated from Swan goose (*Anser cygnoide*s) while European goose breeds originated from Graylag goose (*Anser anser*). In total, 17 Landoise and 16 White Roman geese were clustered in subclade G together with 1 domestic goose (*A. anser*, AY112966), which supported the previous finding that European geese originated from Graylag goose (*A. anser*). But 9 unanticipated samples of two Chinese domestic goose breeds including 7 Lion-head geese and 1 Zhedong White goose in haplotype H9 were also clustered in subclade G as well as 3 Lion-head geese in haplotypes H13, H15 and H16 and 2 Zhedong White geese in haplotypes H18 and H19, which may be caused by the potential gene introgressions between swan goose breeds and graylag breeds with a directional contribution towards graylag geese.

Key words: domestic geese, matrilineal pattern, mitochondrial DNA D-loop region

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Introduction

Dissecting the genetic structure of domestic animals has important implications for understanding the history of human civilization. Phylogenetic analyses of mitochondrial DNA (mtDNA) sequence variation of main domestic animals, such as pig (Wu *et al*., 2007), cattle (Lai *et al*., 2006), goat (Liu *et al*., 2009), and chicken (Liu *et al*., 2006), have shown that these animals contained many divergent matrilineal components and underwent multiple domestication. In contrast, a homogenous nature of native Chinese duck matrilineal pool was observed (He *et al*., 2008). There are insufficient studies for genetic structure of domestic goose by far. Whether this domestic bird has a divergent or a homogenous matrilineal genetic pool is an intriguing question.

China has a very long history of raising geese (Qiu *et al*., 1989; Chen, 1990) and owns enormous genetic resources, with 29 breeds being registered in the Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organization of the United Nations (http://www.fao.org./dad-is). Based on morphological features, geese in China were grouped into "Chinese goose" breeds and the Yili goose. Shi *et al*. (1998) claimed that all Chinese native goose breeds were domesticated from swan goose (*Anser cygnoides*) but only the Yili breed originated from the graylag goose (*Anser anser*), which has been subsequently supported by several recent studies (Wang *et al*., 2005; Zhu *et al*., 2010; Li *et al*., 2011). Gene flow was suggested to be the main reason for the lack of geographic differentiations among Chinese geese (Li *et al*., 2010). In this study, we enlarged sample size of domestic geese for analysis of the mtDNA sequence variation and origin of 245 domestic geese including 16 Chinese domestic goose breeds, 3 European goose breeds that were collected from conserve farms in

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various geographical regions, 3 Wild Swan geese (*A. cygnoides*) under ex-situ conservation as well as 4 known Graylag geese (*A. anser*), to clarify the phylogenetic relationships and matrilineal genetic structure of domestic geese.

Materials and Methods

Collection Site of Goose Samples

In total, 146 newly collected domestic geese and 99 goose specimens that were reported in the Ph D thesis of Wang (2003) were added together to analyze in the present study (Tables 1 and 2). Fig. 1. showed the detail distributions of sample collection sites in China, and permission for sampling was given by the owners of all birds used in this study.

Method of Sampling Collection

Blood samples were collected from 146 individuals from eight goose breeds and one Wild Swan population in China and were stored at −80℃ until further processing (Table 1). Each breed was collected from the native habitat or reservation farms, which followed the 'family method of breeding' (Bao, 1993). The introduced European goose breeds Landoise and Rhine goose that were from the study of Wang (2003) (Table 2) had the same breeding strategy. Samples

Table 1. **Sample information for domestic geese newly investigated in this study**

Breed / Population	No.	Plumage color	Location	Haplotype diversity (Hd)	Nucleotide diversity (Pi)
Lion-head	19	gray	Guangdong	0.754±0.071	0.01170±0.00077
Zhedong White	13	white	Shanghai	0.718±0.128	0.00759±0.00217
Yangjiang[a]	21	gray	Guangdong	ND	ND
Magang[a]	17	gray	Guangdong	ND	ND
Sichuan White	18	white	Sichuan	0.111±0.096	0.00045±0.00039
Hybrid (Sichuan White ♀× Yangjiang ♂)[a]	19	white	Guangdong	ND	ND
Landoise[a]	18	gray	Shanghai	0.399±0.138	0.00353±0.00140
White Roman	18	white	Shanghai	0.216±0.124	0.00526±0.00302
Wild Swan	3	brownish gray	Chengdu Zoo	1.000±0.272	0.00122±0.00430
Total	146	—	—	0.563±0.037	0.01016±0.00063

[a] Only one haplotype was observed in these breeds / populations. We did not determine (ND) haplotype diversity and nucleotide diversity for these samples.

Table 2. **Samples information of the reported data analyzed in this study**

Breed	No.	Location	Haplotype (no.)[a]	Reference/GenBank accession number
Taihu	5	Jiangsu	H23 (5)	(Wang, 2003)
Zhedong	5	Zhejiang	H23 (5)	(Wang, 2003)
Sichuan White	5	Sichuan	H23 (5)	(Wang, 2003)
Wanxi	5	Anhui	H23 (5)	(Wang, 2003)
Yan	9	Anhui	H22 (1); H23 (8)	(Wang, 2003)
Gushi	4	Henan	H22 (1); H23 (3)	(Wang, 2003)
Xupu	6	Hunan	H23 (6)	(Wang, 2003)
Wugang	5	Hunan	H23 (5)	(Wang, 2003)
Lion-head	9	Guangdong	H23 (8); H25 (1)	(Wang, 2003)
Wuzong	5	Guangdong	H23 (5)	(Wang, 2003)
Yangjiang	4	Guangdong	H23 (4)	(Wang, 2003)
Magang	6	Guangdong	H22 (1); H23 (5)	(Wang, 2003)
Yunnan	4	Yunnan	H23 (4)	(Wang, 2003)
Huoyan	7	Xinjiang	H23 (7)	(Wang, 2003)
Yili	5	Xinjiang	H24 (5)	(Wang, 2003)
Landoise	6	Zhejiang	H12 (5); H24 (1)	(Wang, 2003)
Rhine	5	Zhejiang	H12 (5)	(Wang, 2003)
Western Graylag	2		H1 (1); H2 (1)	AF159961; AF159962
Eastern Graylag	1		H20 (1)	AF159963
Domestic goose	1		H21 (1)	AY112966
Total	99			

[a] The number in parentheses refer to the number of individuals shared certain haplotype.

Fig. 1. **Map of collection sites in this study.** It contains the provinces for sample collection, and different capital letter/number combinations in the parentheses represent different chicken populations: S - Wild Swan goose; L - Lion-head; Z - Zhedong White; Y1 - Yangjiang; M - Magang; S1- Sichuan; G - Gushi; H - Huoyan; T - Taihu; W1 - Wanxi; W2 - Wugang; W3 - Wuzong; X - Xupu; Y2 - Yan; Y3 - Yunnan; Y4 - Yili; H1 - Hybrid; R1 - White Roman; R2 - Rhine; L1 - Landoise.

were collected from different family lines or unimproved village where geese were raised under free-range scavenging system, with two birds per line. In this study, the protocol was approved by the Committee on the Care and Use of Laboratory Animals of the State-level Animal Experimental Teaching Demonstration Center of Sichuan Agricultural University (Approval ID: Decree No. 20 [2003]).

DNA Amplification and Sequencing

We extracted mtDNA by an improved method of alkaline lysis (Wang and Shi, 1993), and the mtDNA control region were amplified and sequenced using primers L536 (5′-CCTCTGGTTCCTCGGTCA-3′) (Wang *et al*., 2005) and H16557 (5′- GGGGTAGTTTGCTGGGATTG-3′) (newly designed in this study). The numbers in the primer names refer to the homologous positions in the White-fronted goose (*Anser albifrons*) (GenBank: AF363031). PCR was carried out in a volume of 50 μL solution containing 500 mM Tris-HCl (pH 8.3), 0.1% Trition X-100, 2.5 M KCl, 75 mM $MgCl_2$, 5 mM of each dNTP, 10 pM of each primer and 1 unit of Taq polymerase and was amplified by 35 cycles of 40 s at 94℃, 40 s at 56℃ and 1 min at 72℃. PCR products were detected by 2% agarose gel electrophoresis and purified by TIANgel Midi Purification Kit (product ID: DP209-02). And the final purified PCR products were directly sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM® 3100 DNA sequencer according to manufacturer protocol.

Data Analyses

In total, 245 sequences including the newly generated 146 mtDNA control region sequences, 99 sequences of domestic geese including the 4 known Graylag geese used in Wang (2003) (the dissertation was freely available, www.cnki.net) (listed in Table 2). Sequences were aligned by using the DNAStar package (DNASTAR) and were truncated to 491 bp fragments for uniformity. Sequence variation and Tajima's D-value (Tajima, 1989) was exported using MEGA 4.0 (Tamura *et al*., 2007). Phylogenetic relationship among 237 domestic geese and 21 published sequences was evaluated by neighbor-joining method. A rooted neighbor-joining (NJ) tree of all haplotypes was reconstructed under the Kimura 2-parameter model with 1,000 replications, using Canada goose (*Branta canadensis*) (GenBank: AY112974) as the outgroup. Relationship among the haplotypes was further demonstrated by a median-joining network recon-

	1111111111	1111111111	111111111	
	6666666666	6666666666	666666666	
	1222222333	3333444445	555555555	
	1000129001	1899226770	011233445	
	1479673154	9801013085	607135016	
AF363031.1	TCATGATACC	GTCCGGACCA	CCTTCGATC	No.
H1	.T.....T	A.....T..	..C..A...	1
H2	.T.....T	A.....GT..	..C..A...	1
H3	.TCC.....	.C.....	.TC.....	89
H4	.TCCTC....	.C.....	.TC.....	1
H5	.TCC.....	.C.....	.TG.....	4
H6	.....C.T.	.....	..GCT....	1
H7	.T.C..C...	.C.....	.TG.T....	1
H8	.....C.T.	.....	.....	2
H9	.....C.T.	.....G	..GCT....	38
H10	.T.....T.	.C.....	.....	1
H11	.....C.T.	.....G	..GCT.TC.	1
H12	.T.....T	A.T.A..T..	..C..A...	11
H13	.T...C.T.	.....	..CCT....	1
H14	.TCC.....	.C.....	.TC.T....	1
H15	.T...C.T.	.....G	..GCT....	1
H16	.T...C.T.	.....	..GCT....	1
H17	.T.C....T.	.C.....	.TG.T....	1
H18	.T...C.T.	.C.....	..G.T....	1
H19	.T.....T.	.C.....	..GCT....	1
H20	.T.....T	A..T...T..	..C..A...	1
H21	C.....CGT.	.....AG	A.GCT...T	1
H22	CTCC.....	.C.....T..	.TC.....	3
H23	CTCC.....	.C.....	.TC.....	75
H24	.T.....T	A.T.A..T..	.TC..A...	6
H25	CTCC.....	.C...A....	.TC.....	1

Fig. 2. **Variation sites in the sequences of mtDNA control region of domestic geese.** The sequences of mtDNA control region were truncated into 490 bp, which is located in region between 16068 and 16558 (first position corresponds to first position in homologous mtDNA genome of *Anser albifrons* (GenBank: AF363031)).

structed by using Network 4.611 (http://www.fluxus-engineering.com) (Bandelt *et al*., 1999). Haplotype diversity and nucleotide diversity for each goose breed were computed using DnaSP 5.0 (Librado and Rozas, 2009).

Results

Mitochondrial DNA Haplotypes of Domestic Geese

A total of 29 sequence variations were detected among the 491 bp fragments of 245 domestic goose samples (Fig. 2), including 11 singleton polymorphic sites and 18 parsimony informative polymorphic sites. They finally determined 25 haplotypes (H1 to H25). Among them, haplotypes H1 and H2 were only observed in one Western Graylag goose that used in Wang's study, respectively. Haplotype H3, the largest shared haplotype, consisted of 1 White Roman goose and 88 Chinese domestic geese from 5 Chinese domestic goose breeds and a hybrid goose strain (Sichuan White ♀ × Yangjiang ♂) (Table 3). Haplotype H4 consisted of only 1 Sichuan White goose that newly sequenced in the present study while H5 consisted of 1 Lion-head goose, 2 Zhedong White geese and 1 wild Swan goose. Two wild Swan geese were respectively present in haplotypes H6 and H7 whereas haplotype H8 contained two Landoise geese. Haplotype H9 consisted of 38 individuals from 4 domestic goose breeds. Haplotypes H10 and H11 contained 1 Landoise goose, respectively. Haplotype H12 consisted of 1 White Roman goose and 11 European domestic geese that recovered from the Wang's study (including 10 Rhine geese and 1 Landoise goose). Haplotypes H13 to H16 were only found in 1 Lion-head goose, respectively; while haplotypes H17 to H19 were only found in 1 Zhedong White goose, respectively. Haplotype H20 was only observed in 1 Eastern Graylag used in Wang (2003) while 1 domestic goose (AY112966) was only present in haplotype H21. Haplotype H22 consisted of 1 Gushi goose, 1 Yan goose and 1 Magang goose. Haplotype H23, the second largest shared haplotype, consisted of 75 Chinese domestic geese that were recovered from the study of Wang (2003), covering 14 goose breeds (detailed in Table 3). Haplotype H24 was found in 5 Yili geese and 1 Landoise goose whereas haplotype H25 consisted of 1 Lion-head goose. The GenBank accession numbers of the newly generated 146 mtDNA control region sequences in this study

Table 3. **Distribution of 12 mtDNA haplotypes in geese**

Haplo-type	No.[1]	Frequency (%)	S	L	Z	Y1	M	S1	G	H	T	W1	W2	W3	X	Y2	Y3	H1	Y4	R1	L1	R2	G1	G2	G3	G4
H1	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
H2	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
H3	89	36.33	0	7	7	21	17	17	0	0	0	0	0	0	0	0	0	19	0	1	0	0	0	0	0	0
H4	1	0.41	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H5	4	1.63	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H6	1	0.41	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H7	1	0.41	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H8	2	0.82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
H9	38	15.51	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	14	0	0	0	0	0
H10	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
H11	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
H12	11	4.49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	5	0	0	0	0
H13	1	0.41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H14	1	0.41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H15	1	0.41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H16	1	0.41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H17	1	0.41	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H18	1	0.41	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H19	1	0.41	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H20	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
H21	1	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
H22	3	1.22	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H23	75	30.61	0	8	5	4	5	5	3	7	5	5	5	5	6	8	4	0	0	0	0	0	0	0	0	0
H24	6	2.45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1	0	0	0	0	0
H25	1	0.41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	245	100.00	3	28	18	25	23	23	4	7	5	5	5	5	6	9	4	19	5	18	24	5	1	1	1	1

[1] The total numbers of geese that used for haplotype analysis consist of 146 birds collected in this study and 99 sequences that used in Wang (2003).

S - Wild Swan goose; L - Lion-head; Z - Zhedong White; Y1 - Yangjiang; M - Magang; S1- Sichuan; G - Gushi; H - Huoyan; T - Taihu; W1 - Wanxi; W2 - Wugang; W3 - Wuzong; X - Xupu; Y2 - Yan; Y3 - Yunnan; Y4 - Yili; H1 - Hybrid; R1 - White Roman; R2 - Rhine; L1 - Landoise; G1 - Western Graylag 1 (AF159961); G2 - Western Graylag 2 (AF159962); G3 - domestic goose (AY112966); G4 - Eastern Graylag (AF 159963).

were JN382329-JN382470, and KF057078-KF057081.

Genetic Diversity of Domestic Goose Breeds

The average haplotype diversity (Hd) and nucleotide diversity (Pi) of 146 sequences newly collected in this study were 0.563±0.037 and 0.01016±0.00063, respectively. The estimated level of haplotype diversity and nucleotide diversity varied in different domestic goose breeds: the nucleotide diversity and haplotype diversity of Chinese domestic geese (including 18 Sichuan White, 13 Zhedong White, 19 Lion-head geese and 3 wild Swan geese) were 0.356±0.058 and 0.00502±0.00097, respectively; while that of two European domestic goose populations (18 White Roman and 18 Landoise geese) were 0.308±0.099 and 0.00436±0.00172, respectively. The genetic diversity (Hd and Pi) of each goose breed newly collected in this study was detailed in Table 1. The insignificant results for Tajima's D test were showed in Zhedong White geese (0.19755, $p>0.10$) and Landoise geese (−1.19798, $p>0.10$) whereas the statistical significant results for Tajima's D test were showed in Lion-head goose breed (2.95207, $p<0.001$) and White Roman goose breed (−1.83994, $p<0.05$).

Phylogenetic Analysis of Haplotypes and Network Profile

We investigated phylogenetic relationship of 245 goose samples based on the neighbor-joining (NJ) method of mtDNA D-loop control fragments (491 bp) using Canada goose (*B. canadensis*, AY112974) as the outgroup (Fig. 3). In total, two clades (I and II) were observed. Clade II contained haplotypes H1, H2, H12, H20 and H24 that were found in 6 Landoise geese, 6 Rhine geese, 5 Yili geese as well as 4 known Graylag geese that extracted from NCBI (1 Eastern Graylag, 2 Western Graylag, and 1 domestic Graylag, Table 2). Conversely sample in clade I were widely distributed in 15 Chinese domestic goose breeds. Of interest, all Chinese goose samples were clustered into two subclades (S and G) in the tree with the low bootstrap values (Fig. 3). Among them, haplotypes H3, H4, H5, H7, H14, H17, H22, H23 and H25 were located in subclade S, which harbored samples from 15 Chinese domestic goose breeds. Interestingly, haplotypes H6, H8 to H11, H13, H15, H16, H18, H19 and H21 were located in subclade G, which harbored samples from 2 European goose breeds (17 Landoise geese and 16 Roman geese), 2 Chinese domestic goose

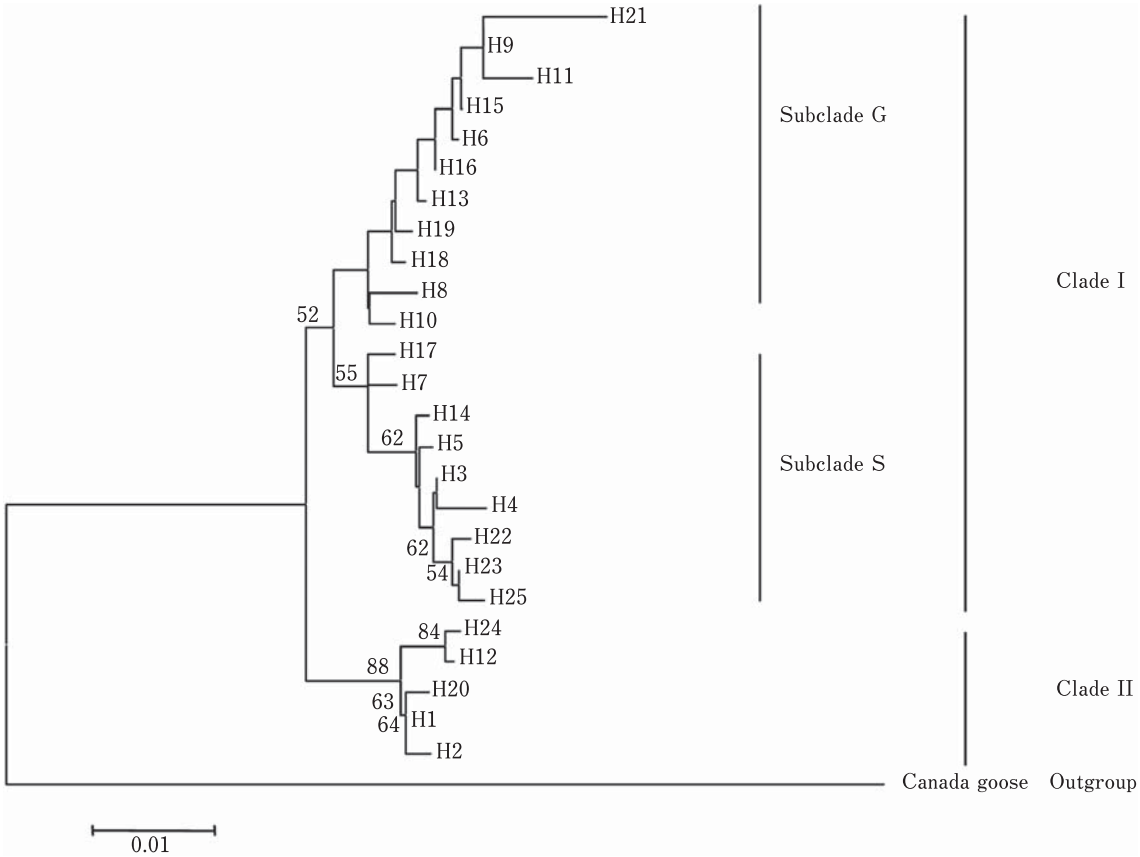

Fig. 3. **Rooted neighbor-joining tree of 25 hyplotypes identified in 245 geese.** The numbers above each branch denote the percentage bootstrap that supports the monophyly of the node. The bootstrap values below 50% were not showed on the phylogenetic tree. The tree was rooted with a Canada goose (*Branta canadensis*, AY112974). The scale length 0.01 means the number of substitutions per site.

breeds (3 Zhedong White geese and 10 Lion-head geese) and 1 Wild Swan goose. Specially, one Zhedong White goose and 7 Lion-head geese shared the same haplotype H9 with the European goose breeds (14 Landoise geese and 16 Roman white geese). Due to the little bit low bootstrap values for supporting the clade I (52%) and subclade S (55%) in the phylogenetic tree, the result of median-joining (MJ) network profile goose distribution further suggested that the existence of major group of Chinese native goose breeds (subclade S) and introgressive breeds (subclade G) (Fig. 4).

Discussion

No mtDNA sequence variation for three Chinese domestic goose populations (including 21 Yangjiang, 17 Magang and 19 Hybrid goose specimens) was observed in the present study, which would suggest a serious founder effect during the selection and breeding of these breeds (Hedrick *et al*., 2001). The statistical significant results for Tajima's D test in Lion-head goose breed (2.95207, $p<0.001$) and White Roman goose breed (-1.83994, $p<0.05$) suggest that the two goose populations might have undergone different demographic histories in the past (Tajima, 1989). Up to now, a lot of studies have indicated that genetic diversity of Chinese domestic geese is generally not rich (Li *et al*., 2010, 2011; Liu, 2003), and the similar level of genetic diversity of domestic geese is also showed in the present study (Hd$=$ 0.563$\pm$0.037, Pi$=$0.01016$\pm$0.00063 in average). Wang (2003) indicated that Graylag goose (*A. anser*, Pi$=$1.179) had the higher nucleotide diversity than Swan goose (*A. cygnoides*, Pi$=$0.088), but when we expanded the number of samples within goose breeds, an opposite result is showed in this study that Swan geese have a higher level of genetic diversity (Hd$=$0.356 and Pi$=$0.00502) than Graylag geese (Hd$=$0.308 and Pi$=$0.00436). But anyway, these above results indicate the fact that domestic geese in China seem to have the low level of genetic diversity. Besides, Poyarkov *et al*. (2010) focused on the maternal investigation on the swan goose breeds in Russia, and they found a similar low value of nucleotide diversity (Pi$=$0.0074) in 48 geese from two groups nesting. Among them, in those from Khabarovsk

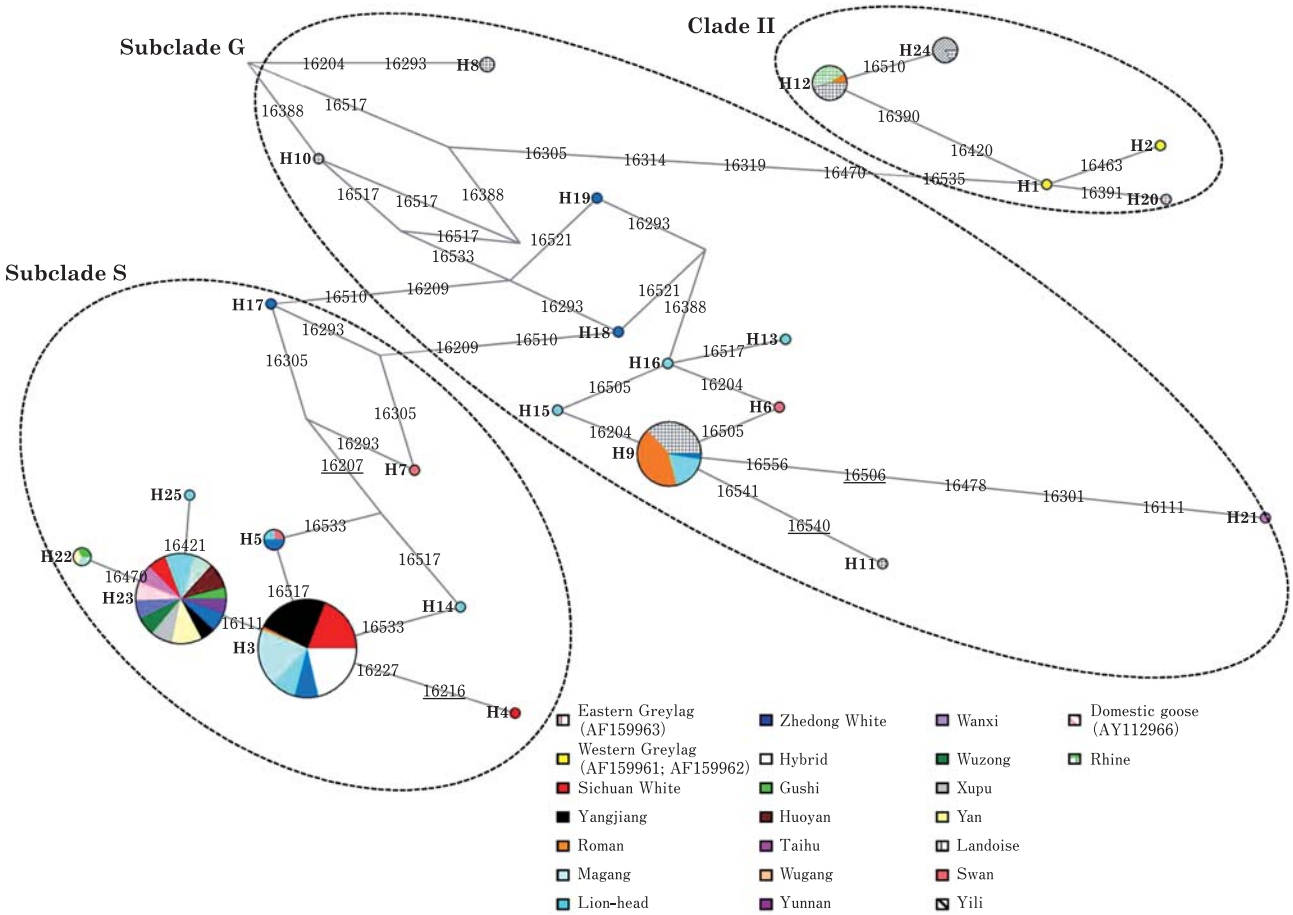

Fig. 4. **Network profile of 25 mtDNA haplotypes identified in 245 geese.** Twenty-five haplotypes (H1 to H25) were found in 245 geese. Circled areas are proportional to haplotype frequencies. Each breed of domestic geese is marked by one color. The links are labeled by nucleotide positions to designate transitions; transversions are underlined.

krai region, nucleotide diversity of swan geese was only 0.0031. Therefore, it suggests that, geographically different regions of swan goose breeds may have comparatively genetic differentiation.

So far, all studies about the maternal origin of domestic geese have pointed to the conclusion that Chinese domestic goose breeds originated from Swan goose (*A. cygnoides*) except the Yili goose breed, which originated from Graylag goose (*A. anser*) (Wang *et al*., 2005; Zhu *et al*., 2010; Li *et al*., 2011). In this study, we analyzed 490 bp sequences of the mtDNA control regions of 245 domestic geese (including 16 Chinese domestic goose breeds, 2 European goose breeds, 3 Wild Swan geese and 4 known Graylag geese, Tables 1 and 2), and phylogenetic result detected the distinct lineage of clade II (88% bootstrap value), in which samples of the European goose breeds (Landoise, White Roman and Rhine geese) shared the same haplotype H12, 5 Yili geese shared the same haplotype H24 with 1 Landoise goose, and 3 Graylag geese that separately in haplotypes H1, H2 and H20 (Table 3 and Fig. 3). It indicated that the maternal origin of these European geese and Yili geese was Graylag goose (*A. anser*). Considering insufficient supports for the clade I (52%) and subclade S (55%) in the phylogenetic analysis, the result of network analysis (Fig. 4) also suggested that the existence of graylag goose breeds (clade II), swan goose breeds (subclade S) and introgressive breeds (subclade G). Subclade S has the largest goose specimens that were used in this study, which harbored all Chinese domestic goose breeds except Yili breed (about 71.84%) that used in this study. The above results did support the previous finding that Chinese domestic geese (except the Yili goose breed) originated from the swan goose (*A. cygnoides*) and European geese originated from the Graylag goose (*A. anser*).

Specially, a finding by Li *et al*. (2011) catches our attention, that 1 Linxian white goose and 1 Wanxi white goose shared the same haplotype with the European goose breeds. Interestingly, 7 Lion-head geese and 1 Zhedong White goose shared the same haplotype H9 with two European goose

breeds (14 Landoise geese and 16 White Roman geese) that originated from the Graylag goose (*A. anser*) in the previous studies, and finally clustered into the subclade G in the results of phylogenetic and network analysis (Figs. 3 and 4). A reason to suspect this result is that samples of the two Chinese domestic goose breeds (Zhedong White and Lion-head goose breeds) used in the present study might have some lineage of Graylag goose (*A. anser*) besides swan goose (*A. cygnoides*) contribution, specially noticing these samples of Zhedong White goose, Landoise and White Roman goose breeds were collected from conservation farms in Shanghai. In other word, gene introgressions may occur between swan goose breeds and graylag breeds in the corresponding geographical region, with a directional contribution towards graylag geese.

Another possible guess of this result was that the Chinese domestic goose breeds may have the multiple maternal origins after all 1 Wild Swan goose in haplotype H6 was also clustered into subclade G (Fig. 4). But lacking of powerful and believable bootstrap values in the subclade G of the phylogenetic tree using the NJ methods, it seems to be more reasonable that the occurrence of hybridization caused gene introgression between swan goose breeds and graylag goose breeds with a directional contribution towards graylag geese. In other words, we preferred suggesting that 1 Zhedong White geese and 7 Lion-head geese may contain graylag goose genetic components besides swan goose (*A. cygnoides*) contribution. But coupled with the finding that the haplotypes including H6, H13, H15, H18 and H19 in the network spread across in the subclade G, it may indicate more complicated domestication and breeding history of Chinese domestic goose. Further studies will help to clarify these issues.

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