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Chinese Geese Would Have Contributed to the Commercial Fatty Liver Line of Landes Geese

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Abstract: According to the public declaration by commercial companies, the commercial fatty liver line of Landes geese is grey feather. However, about 0.3% varieties of pure white or white with grey spots feather phenotype were observed in our purebred stock. So, we tentatively put forward a doubt whether some alien genetic components coexist in this commercial line. In order to prove this proposition, the mtDNA D-loop sequence variation were analyzed among 115 domestic goose individuals from this commercial fatty liver line and Chinese indigenous breeds. Total 11 haplotypes were determined and all, which could be classified into Asian haplogroup and European haplogroup. Seven landes geese residing in the E1-E3 haplotypes were clustered into the European haplogroup together with the wild greylag geese, while the remaining 45 Landes geese were grouped into the Asian haplogroup, which was predominantly occupied by the Chinese geese haplotypes. Although, the frequency (45/52) would be overestimated, we also reasonably believed that the matrilineal components of Chinese geese coexist in this commercial fatty liver line of Landes geese. In addition, the extremely low genetic diversity has been retained in the 2 sampled populations of Chinese geese, especially, in Wanxi white geese population.

Key words: Chinese geese, landes geese, commercial fatty liver line, D-loop, genetic diversity

INTRODUCTION

Commercial lines of geese had been successfully developed for fatty liver production from Landes geese (*Anser anser domestica*), which is an indigenous breed in France (Buckland and Guy, 2002; Chen *et al*., 2004). As the increase of fatty liver consumption, these commercial fatty liver lines have been wildly introduced into a number of other countries and regions to produce fatty liver either utilized as a pure breed or crossbred. The initial importation to China of these breeds took place almost 30 years ago and have been distributed over the country.

Due to the severe implementation of animal welfare criteria in many European countries and the fast development of Chinese agricultural economy, China will become a new fatty liver production center. The statistics showed that the fatty liver production volume in China remarkably increased during the last 30 years and reached to about 700 tons in 2006, which contributed 23% to global fatty liver production (http://www.poultryinfo.org).

China abounds in genetic resources of domestic geese, consisting of 20 indigenous breeds and a few introduced and cultivated breeds (Chen *et al*., 2004). The genetic improvement appears to have been relatively under-utilized. To improve the fatty liver production of Chinese indigenous geese, many attempts have been made by adopting the commercial fatty liver line of Landes geese as a male or female line in breeding programs and have achieved significant progress (Geng *et al*., 1993; Yin *et al*., 1996; Zhang *et al*., 2006). However, the lack of understanding for the genetic structure of this commercial fatty liver line has prevented such crossbreeding. The commercial breeding companies suggested that the commercial fatty liver line of Landes geese was developed from three indigenous goose breeds in France (Landes geese, Toulouse geese and Gers geese), which are a pure grey feather phenotype (Buckland and Guy, 2002). We observed about 0.3% varieties of pure white or white with grey spots feather phenotype in our purebred stock of this commercial fatty liver line (Fig. 1). Then, we speculated whether, some alien genetic components coexist in this commercial line.

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(a)

(b)

Fig. 1: The standard grey feather appearance, a): The pure white or white with grey spots feather varieties and b): The commercial fatty liver line of Landes geese observed in our purebred reproduction stock

Owing to the different origins and breeding histories, the worldwide domestic geese have resulted in a wide range of colors, sizes and shapes. However, a rough classification based on their appearance and production characteristics could be determined. Most of Chinese domestic geese are white feather with a few brown varieties and could be readily identified by the knob at the base of its beak. The Chinese geese are relatively small in body size and known for high egg production averaging 60 eggs per laying period. In contrast, European goose breeds show predominant grey feather phenotype, relatively larger body size and better meat production. As to the phylogenetic relationship, it is widely accepted that there are 2 matrilineal origins for the modern domestic geese (Qiu *et al*., 1998; Buckland and Guy, 2002; Wang *et al*., 2005a, b; Shi *et al*., 2006). Both European geese and the Yili geese (distributed in the Xinjiang Uigur Autonomous Region in China) derived from greylag goose (*Anser anser*) and that of Asian geese were derived from swan goose (*Anser cygnoides*). Shi *et al*. (2006) estimated the divergence between the *Anser anser* and *Anser cygnoides* is about 360,000 years ago.

Mitochondrial DNA (mtDNA) sequence variation has been successfully applied to deduce the matrilineal origin and genetic structure in many farm animals (Lai *et al*., 2006, 2007; Liu *et al*., 2006a, b). However, the same tactics has not been applied to commercial lines. In this study, we traced the matrilineal components for a commercial fatty liver line of Landes geese maintained in China based on mtDNA control region (D-loop) sequence variation. To clearly understand this issue is important for its utilization in crossbreeding.

MATERIALS AND METHODS

Sampling and sequencing: A purebred stock with the number of 5000 birds for the commercial fatty liver line of Landes geese were raised in the farm of Shanghai Academy of Agricultural Sciences. All these birds have derived from 2 breeding stocks. The 1st breeding stock (500 parent generation birds) was introduced from France about 15 years ago and the second descended from the initially introduced population containing 500 birds of grandparent generation and 2000 birds of parent generation from France in 2004. Because we have been trying to genetically improve the egg production using pure breeding tactics, strict mating system has been applied to avoid the influence by Chinese goose in this population. We randomly collected 45 samples (41 vein blood and 4 muscle tissues). In addition, the vein blood samples of 18 Sichuan White geese and 43 Wanxi White geese were also sampled from other 2 farms (Shanghai and Sichuan) and the latter one also consisted of the high production strain and low production strain (Table 1).

Total Genomic DNA was extracted by standard phenol/choloroform method. The reported primers of H1248: 5'-CAGCTTCAGT GCCATGCTTT-3' (Ruokonen *et al*., 2000) and L16643: 5'-CCATAATACGG CGAAGGATTC-3' (Wang *et al*., 2005b) were used to amplify the mtDNA D-loop sequences. The numbers in the primer name refer to the homologous positions of 3' base of the primer on the chicken mtDNA complete sequence (Desjardins and Morais, 1990). The H1248 was also used as sequencing primer. PCR amplification was performed in a 50 μL reaction mixture containing 100 ng of DNA, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 50 mM KCl, 10 pM of each primer and 1 unit of *Taq* polymerase (S_{ABC}) following 35 cycles of 50 sec at 94°C, 50 sec at 53°C and 90 sec at 72°C. PCR products were purified on spin columns and were directly sequenced for light strand using Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI PRISM® 3100 DNA sequencer according to the manufacturer's manual.

Authenticity of the sequences: It is well-known that the nuclear copies of mtDNA (NUMTs) have widely distributed in bird species (Sorenson and Quinn, 1998).

Table 1: The sample sources and genetic diversity for the domestic geese in this study

Breeds/strains (Abbreviation)[a]	No. of samples	Sampling locations	Genetic origins	Sources[b]	Haplotype diversity (H)	Nucleotide diversity (π)
Sichuan white goose (SC)	18	Sichuan	*Anser cygnoides* (China)	Our study	0.667±0.103	0.00142±0.00033
Wanxi white goose strain H (WH)	21	Shanghai	*Anser cygnoides* (China)	Our study	0.000±0.000	0.00000±0.00000
Wanxi white goose strain L (WL)	22	Shanghai	*Anser cygnoides* (China)	Our study	0.000±0.000	0.00000±0.00000
Zi goose (ZI)	01	-	*Anser cygnoides* (China)	AY552167	-	-
Huoyan goose (HY)	01	-	*Anser cygnoides* (China)	AY552166	-	-
The commercial fatty liver line	-	Shanghai	-	Our study; Wang (2003)	-	-
of Landes goose (LD)	52	Sichuan	*Anser anser* (France)	AY552168	0.345±0.083	0.01021±0.00293
Total	115	-	-	-	0.632±0.030	0.00514±0.00145
Asian haplogroup	108	-	-	-	0.583±0.028	0.00089±0.00012
European haplogroup	07	-	-	-	0.667±0.160	0.00085±0.00026

[a]The Wanxi white goose strain H and Wanxi white goose strain L refer to the high production strain and low production strain of Wanxi White geese, respectively; The European haplogroup did not contain the 3 wild greylag geese when calculated the genetic diversity; [b]The 52 Landes geese have 3 sources. A 45 individuals were sequenced in this study; 6 indiviudals were artificially revivified according to variations reported in Wang's doctor thesis (2003); the rest 1 individual was retrieved from GenBank

The PCR amplification for targeted mtDNA sequence from total genomic DNA would heighten the risk for nuclear sequences to be amplified and further lead to mistaken conclusion, especially in blood sample (Ruokonen *et al*., 2000; Pereira and Baker, 2004). To verify the mtDNA sequences were authentically amplified in this study, 12 Landes geese blood samples representing different haplogroups were selected for pure mtDNA isolation. Considering the fact that the hemolysis always occurs in frozen blood sample, the leucocytes were isolated after breaking erythrocytes into pieces by chemical method, containing 0.25 M sucrose, 1 mM EDTA, 2.5 mM $MgCl_2$, 30 mM Tris-HCl and 0.015% Triton-X100 (pH = 7.5) (Zhai *et al*., 2007). MtDNA was further extracted from purified leucocytes using regular alkaline lysis method. The amplified sequences when the pure mtDNA was used as template were compared with the sequences amplified from total genomic DNA from the same individuals.

Data analysis: The sequencing chromatogram files were qualitatively clipped and edited using the DNAstar program. Nine published domestic goose mtDNA D-loop sequences were retrieved from GenBank or artificially revivified based on the reported variable nucleotide sites (Wang, 2003) and compared with our new data (Table 1). A total of 115 samples were scored relative to the white fronted goose (*Anser albifrons*) mtDNA complete sequence (accession No. AF363031). The variations were subsequently exported using MEGA 3.1 (Kumar *et al*., 2004) and gaps in the aligned sequences were excluded in the following analyses. A Neighbor Joining (NJ) phylogenetic tree was constructed together with 3 wild greylag geese (*Anser anser*) from GenBank (accession No. AF159961-AF159963), rooted by *Branta canadensis* (Acc. No. AY112974) using the Kimura 2-parameters model in MEGA 3.1. The sequences Haplotype diversity (H) and nucleotide diversity (π) of the different breeds/strains were calculated using DnaSP 4.10 (Rozas *et al*., 2003) to provide more information on the genetic variability. The haplotype sequences generated in this study have been deposited in GenBank under Acc. No. EU571949-EU571959.

RESULTS

Variations and haplotypes distribution: We obtained the reliable mtDNA D-loop fragments ranging from 899-901 bp length, which referred to the 15840-16742 homologous fragments on the white-fronted goose (*Anser albifrons*) mtDNA complete sequence. The identity among sequences amplified from the total genomic DNA and pure mtDNA for the same individual were identified and confirmed that the potential NUMTs amplification was absent from this study. Although, the ratio of nuclear to mitochondrial DNA is much higher in blood than in muscle tissue, we did not find the tissue-specific haplotype. After excluding the gaps, we detected 48 variations, which defined 11 haplotypes out of the 115 samples. The 11 haplotypes were further classified into 2 haplogroups, Asian haplogroup and European haplogroup. The Asian haplogroup consisted of eight haplotypes (A1-A8) that identified 108 individuals (93.9%), while the remaining seven individuals (6.1%) resided in the European haplogroup (Fig. 2).

In the Asian haplogroup, A3 was the predominant haplotype, which was shared by 56 individuals from 5 breeds/strains and followed by A1 containing exclusively 42 Landes geese. There was only 1 or 2 nucleotide difference among A1-A3 haplotypes. The other 6 haplotypes were shared by 1 or 3 individuals. In contrast, there was no obvious bias for the haplotypes distribution in European haplogroup. The number of samples occurred in E1-E3 haplotypes were 2, 4 and 1, respectively (Fig. 2 and Table 2). Among the 6 haplotypes detected in Landes geese, 3 of them were distributed in the Asian haplogroup and the other

Table 2: The haplotypes distribution in the different breeds or strains

	Asian haplogroups								European haplogroups			
Samples	A1	A2	A3	A4	A5	A6	A7	A8	E1	E2	E3	Total
SC	-	-	10	3	3	1	1	-	-	-	-	18
WH	-	-	21	-	-	-	-	-	-	-	-	21
WL	-	-	22	-	-	-	-	-	-	-	-	22
ZI	-	-	1	-	-	-	-	-	-	-	-	1
HY	-	1	-	-	-	-	-	-	-	-	-	1
LD	42	-	2	-	-	-	-	1	2	4	1	52
Total	42	1	56	3	3	1	1	1	2	4	1	115

The abbreviations for breeds/strains are consistent with the definition in Table 1

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                1111111111 1111111111 1111111111 1111111111 11111111
                5555555555 5555555566 6666666666 6666666666 66666666
                8888888889 9999999901 1222333334 4455555566 66666677
                4556677790 0011445921 9069011992 7801233500 00188903
                5130303700 3738689571 5463549050 0757135623 45102694
RS              CCCGCAACCA TTCTGTGCTT CCCTCCGCAG CCATTCGCAT GTTAATCC N
A1              TT..T.CT.G CC.C.CA..C ...CT..... ..GGCT.TG. A.CG.C.. 42
A2              TT..T.CT.G CC.C.CA..C ...CT..... ..GGCT.TG. A.CG.CT. 1
A3              TT..T.CT.G CC.C.CA..C ...CT..... ..GGCT.TG. ACCG.C.. 56
A4              TT..T.CT.G CC.C.CA..C ..TCT..... ..GGCT.TG. ACCG.C.. 3
A5              TT..T.CT.G CC.C.CA..C T.TCT..... ..GGCT.TG. ACCG.C.. 3
A6              TT..T.CT.G CC.C.CA..C T.TCT...C. ..GGCT.TG. ACCG.C.. 1
A7              TT..T.CT.G CC.C.CA..C TTTCT..... .AGGCT.TG. ACCG.C.. 1
A8              TT..T.CT.G CC.C.CA.CC ...CT..... ..GGCT.TG. ACCG.C.. 1
E1              .TTA.GCTT. CCT....T.. .T...TAT.A T..C..A.G. A...G..T 2
E2              .TTA.GCTT. CCT....T.. .T...TAT.A T..C..A.GG A...G..T 4
E3              .TTA.GCTT. CCT.A..T.. .T...TAT.A T..C..A.GG A...G..T 1
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Asian group

European group

Fig. 2: MtDNA sequence variations among the 115 domestic geese. Variable sites were scored relative to the standard sequences of white-fronted goose (*Anser albifrons*) mtDNA complete sequence (GenBank accession No. AF363031). The number of individuals sharing a haplotype is listed under the capital N. Gaps were excluded and dots (·) denoted identity with the reference sequence. The Asian haplogroup and European haplogroup were further marked

haplotypes occurred in the European haplogroup. Five haplotypes were detected in Sichuan white geese, whereas only one haplotype was observed in Wanxi white geese (Table 2).

Phylogenetic relationship analysis: Combined with 3 wild greylag goose (*Anser anser*) mtDNA D-loop sequences, 2 clades were clearly separated in the NJ tree, which referred to the Asian haplogroup and European haplogroup, respectively (Fig. 3). Thirty three mutations distance existed between the 2 clades. Both the 7 Landes geese residing in the E1-E3 haplotypes and the 3 wild greylag geese were clustered closely together, while the remaining 45 Landes geese were grouped into the Asian haplogroup, which was predominantly occupied by the Chinese geese haplotypes.

Genetic diversity: The mean haplotype diversity and nucleotide diversity in the 115 domestic geese were 0.632±0.030 and 0.00514±0.00145, respectively. The highest haplotype diversity was 0.667±0.103 in Sichuan

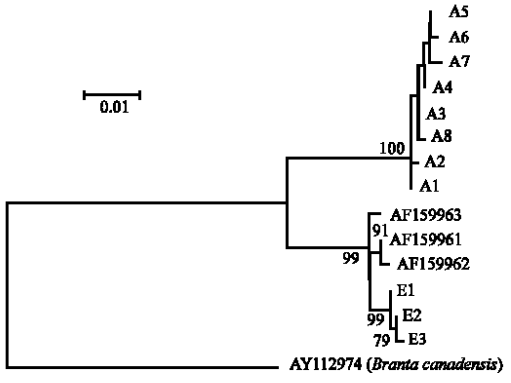

Fig. 3: The Neighbor-Joining (NJ) tree of 11 domestic geese haplotypes and 3 wild greylag geese (*Anser anser*) retrieved from GenBank (GenBank accession No. AF159961-AF159963). The NJ tree was rooted by Branta canadensis (GenBank accession No. AY112974). The values on the branch are bootstrap support based on 1000 replications and these under 70% were excluded

white geese, while the highest nucleotide diversity was in Landes geese (0.01021±0.00293). There was no genetic variability detected in Wanxi white geese. Compared with Asian haplogroup, the European haplogroup had higher haplotype diversity (0.667±0.160 vs. 0.583±0.028) and slight lower nucleotide diversity (0.00085±0.00026 vs. 0.00089±0.00012).

DISCUSSION

The commercial fatty liver line of Landes geese was grey feather appearance according to the statements by commercial breeding companies (Fig. 1). In contrast, the Chinese geese were absolutely charactered by white feather phenotype. The occurrence of white feather varieties of Landes geese made us put forward a doubt whether the commercial fatty liver line contains the genetic components introduced from Chinese geese. It is commonly known that the same tactics has been successfully applied to develop European modern pig breeds by introducing the Taihu pig from China. Although, the earliest recorded exportation of Chinese white geese was to America in 1788 (Zhang and Zhu, 1986), we think the diffusion into European countries is also possible.

Based on the mtDNA sequence variation, the *Anser anser* and *Anser cygnoides* have been thought to be the wild ancestors for European geese and Chinese geese with independent domestication (Qiu *et al*., 1998; Buckland and Guy, 2002; Wang *et al*., 2005a; Shi *et al*., 2006). According to this fact, we would expect to discern the genetic components of Chinese geese from European geese in one commercial line. At least, it is acceptable on the point of the matrilineal component. In this study, 52 mtDNA D-loop sequences of the commercial fatty liver line were classified into 2 haplogroups representing the Asian and European geese, respectively. Up to 45 Landes geese were clustered closely together with Chinese geese in the Asian haplogroup, while the European haplogroup contained exclusively 7 Landes geese. The predominant haplotype A3 in Asian haplogroup was shared by only 2 Landes geese. However, there was no Chinese goose found in haplotype A1, which was occurred exclusively by 42 Landes geese. The results seem to be illogical to a certain extent. Despite this, one fact has to be taken into account that the 3 studied Chinese indigenous goose populations in this study were sampled from different farms and thus, the biased haplotype distribution would have been enlarged by genetic drift because of the small population and/or artificial selection. Although, it was impossible to focus mtDNA types, such artificial selection would inevitably narrow genetic variation in commercial population and then influenced indirectly mtDNA diversity. Only one haplotype detected in 43 Wanxi white geese further confirmed this proposition. In addition, we detected only 1 or 2 nucleotide variations among A1-A3 haplotypes, which suggested a very close relationship. Based on this, the bias of haplotype distribution would be insignificant. Based on the same consideration, we suggest that such high frequency (45/52) of Chinese geese haplotypes in this commercial line would be also overestimated. At least, it is likely acceptable that the matrilineal component of Chinese geese coexist in this commercial fatty liver line of Landes geese. The favorable explanation is that Chinese geese had been adopted in the breeding program to intentionally improve the reproduction performance of this commercial line. The occurrence for Asian haplogroup in Landes geese could also result from the gene introgression after the commercial lines were introduced into China. However, the pure white or white with grey spots feather varieties immediately appeared after to be introduced into China according to our records. On the other hand, those varieties were simultaneously observed in the 2 introduced populations, which having completely distinct introduction history. Combining with the strict mating system applied in this purebred stock, we think the introgression of Chinese geese into this commercial line after introduction would be out of the range of possibility. Ideally, to further clarify the matrilineal components in those commercial lines of Landes geese kept in France or Europe could perfectly resolve this issue. In addition, whether, there existed gene introgression between the *Anser anser* and the *Anser cygnoides* before the domestication, more wild and domestic samples should be further studied.

In contrast, Wang *et al*. (2005a) compared the mtDNA cytochrome b gene sequence variation among fifteen Chinese indigenous goose breeds (n = 33) and 2 European goose breeds (Landes and Rhin, n = 11). They found that the European and Asian haplogroups were essentially distinguishable and the Asian haplogroup was observed exclusively in Chinese geese. The same conclusion was proposed by another report (Shi *et al*., 2006), which was conducted by mitochondrial DNA cleavage pattern. However, if the frequency of Chinese geese haplotypes in this commercial line is not as high as we discussed above, only 6 Landes geese sampled in Wang's study might not be enough to sample the Chinese geese haplotype. The genetic marker adopted in the Shi's study was Restriction Fragment Length Polymorphism (RFLP), which has relatively low resolution compared with nucleotide variation. To shade more light on this subject, the cleavage pattern for our sequences was also, checked by using the restriction endonucleases adopted in Shi *et al*. (2006). Only one nucleotide site could be cut

by HaeII, out of the 4 enzymes and it was uninformative for discerning the European from Asian haplogroups.

Hitherto, there is no excellent specialized line developed for production of fatty liver in China and this fact has strongly blocked the development for Chinese fatty liver production industry. The pure white feather varieties would be an excellent material available for developing an independent commercial line. In addition to select for fatty liver production characteristic, to intentatively improve the reproduction performance is also charming by adopting the Chinese indigenous geese as a female line in the breeding program. Considering, the close genetic relationship between this commercial fatty liver line and Chinese geese as our results suggested, this scheme will become better applicable. The Sichuan white geese population showed the higher genetic diversity compared with Wanxi white geese. In addition to the true narrow genetic origin, the relatively small population size of Wanxi white geese and intense artificial selection would partially account for the low genetic diversity. No genetic differentiation between the high production strain and low production strain of Wanxi white geese was detected.

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