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Molecular genetic diversity and origin of Chinese domestic duck breeds

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Abstract

The 667 bp control region of mitochondrial DNA from 8 representative domestic duck breeds in China, which were all native preserved duck breeds, and a mallard (6 *Anas zonorhyncha* individuals) were sequenced. The genetic polymorphism and the origin of the 8 domestic ducks were analysed. The result showed that the haplotype diversity (Hd) and average nucleotide diversity (Pi) were 0.67136 and 0.19 %, respectively. Hd and Pi of Youxian Sheldrake were the highest in the 8 domestic duck breeds. Kimura-2 parameter genetic distance between the breeds ranged from 0.00056 to 0.00414. The NJ phylogenetic tree and reduced median-joining network chart were constructed by the total 38 haplotypes and 96 sequences, which included 12 sequences of *Anas platyrhynchos* from GenBank, 6 sequences of *Anas zonorhyncha* from GenBank and 78 sequences of this study (72 domestic ducks and 6 *Anas zonorhyncha*). The maternal origin of the 8 domestic ducks all originated from *Anas platyrhynchos*.

Keywords: domestic duck, D-loop region, genetic diversity, origin

Zusammenfassung

Molekulargenetische Vielfalt und die Herkunft der chinesischen Hausentenrassen

Die 667 bp-Kontrollregion der mitochondrialen DNS von 8 Hausentenrassen aus China, die Maßnahmen zur Lebenderhaltung erfahren, und einer Stockente (6 *Anas zonorhyncha* Individuen) wurde sequenziert. Der genetische Polymorphismus und die Herkunft der 8 Hausentenrassen wurden analysiert. Die Haplotyp-(Hd) und die Nukleotid-Diversität (Pi) betrugen 0.67136 und 0.19 %. Der höchste Hd- und Pi-Wert der 8 Hausentenrassen fand sich bei der Youxian-Sheldrake-Ente. Die genetische Distanz (Kimura-2-Parameter) zwischen den Rassen reichte von 0.00056 bis 0.00414. Der »Neighborhood joining« (NJ) phylogenetische Baum und das reduzierte »Median-Joining«-Netzwerkdiagramm wurde aus 38 Haplotypen und 96 Sequenzen erstellt, einschließlich 12 Sequenzen von *Anas platyrhynchos* und 6 Sequenzen von *Anas zonorhyncha* aus GenBank sowie 78 Sequenzen dieser Untersuchung (72 Hausenten und 6 *Anas zonorhyncha*). Alle 8 Hausentenrassen stammen mütterlichseits von *Anas platyrhynchos* ab.

Schlüsselwörter: Hausente, D-loop-Region, genetische Vielfalt, Herkunft

Introduction

Taxonomically, the duck species (domestic duck) belongs to the Anseriformes, Anatidae, *Anas platyrhynchos* domestic. There were two views about the origin and evolution of Chinese domestic ducks in academia. The monadism view affirmed by overseas scholars was that Chinese domestic ducks were originated from wide *Anas platyrhynchos*. The dualism view was that Chinese domestic ducks were originated from archaic *Anas platyrhynchos* and *Anas zonorhyncha*, domesticated in different areas respectively, or from the generations crossed by *Anas platyrhynchos* and *Anas zonorhyncha* (QIU 1998, CHANG 1995).

The mitochondrial DNA (mtDNA) was considered to be the effect genetic marker for the genetic structure among populations and species and the phylogenetic evolution, because of the characters of classical maternal inheritance, less recombinant, rapid evolution, less selection pressure, great genetic variance and so on, and the displacement region (D-loop) especially. Recently, this molecule marker was extensively used on the animal phylogenetic evolution and genetic diversity (RANDI *et al.* 1998, SACCONE *et al.* 2000, NIU *et al.* 2002, LI *et al.* 2008).

The Chinese domestic duck in particular is one of the earliest domesticated fowls in the world (CASS 1979). China is particularly rich in Waterfowl genetic resources; in 2002, the state identified 27 domestic duck breeds. But, there were only a few studies on the origin of these domestic ducks. The 8 representative breeds including various breed types in China were selected in our study. In this survey, combined with the sequences of *Anas platyrhynchos* and *Anas zonorhyncha*, the mtDNA D-loop sequences from 8 representative domestic duck breeds were amplified, sequenced, and analysed to reveal the diversity, origin and evolution of these breeds. The conclusion could provide theoretic basis for the reasonable taxon, protection, development and utilization of the local ducks and offered basic material for the origin and genetic differentiations of the domestic ducks.

Material and methods

Specimen collection and DNA extraction

The blood samples of the 8 representative duck breeds were collected from the preservation farms respectively, as follows (Table 1): Beijing (BJ, n=8), Jianchang (JC, n=8), Youxian Sheldrake (YX, n=12), Shaoxing (SX, n=8), Putian Black (PT, n=8), Jinding (JD, n=8), Liancheng White (LC, n=8), Gaoyou (GY, n=12). The sample of *Anas zonorhyncha* (BZ, n=6) was collected from the Qianjiang fowl breeding farm in Hangzhou City, Zhejiang Province, P.R. China. All the sample individuals represented their own breed respectively. The sex ratio of each breed was 1 male to 1 female. The genetic relationship between ducks should be avoided as much as possible.

PCR amplification and DNA sequencing

Polymerase chain reaction (PCR) was performed to amplify part of the mtDNA control region. The primers reported by SORENSON *et al.* (1999) were used to amplify the target region. The corresponding sequences were L78 5′-GTT ATT TGG TTA TG CAT ATC GTG-3′, and H774 5′-CCA TAT ACG CCA ACC GTC TC-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), 2.5 µl Mg^{2+} (25 mM), 1µl each primer (25 pmol/µl), 3.0 µl genomic DNA (50 ng/µl), 0.2 Taq polymerase (5U/µl). The thermal cycling profile for mtDNA was 5 min preheat at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 58 °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 3 730 DNA Analyzer in both directions by primer walking using a BigDye Terminator V. 3.1 Cycle Sequencing Kit (ABI, Foster City, CA).

Table 1
Conservation status and characteristics of the 8 domestic duck breeds
Erhaltung und Eigenschaften der 8 Hausentenrassen

Breed	Amount	Conservation type	Longitude and latitude	Type	Feather color
BJ	200 000	Conservation farm	39° 15′ N, 116° 10′ E	Meat	White
JC	2 000	Conservation farm	26° 54′ N, 102° 12′ E	Meat and Egg	Spotty
YX	500 000	Conservation farm/zone	26° 72′ N, 113° 27′ E	Egg	Spotty
SX	20 000000	Conservation farm/zone	30° 12′ N, 120° 12′ E	Egg	Spotty
PT	1 000000	Conservation farm/zone	25° 24′ N, 119° 08′ E	Egg	Spotty
JD	3 500000	Conservation farm/zone	24° 18′ N, 117° 48′ E	Egg	Spotty
LC	2 000000	Conservation farm/zone	25° 42′ N, 116° 42′ E	Egg and medicine	Spotty
GY	100 000	Conservation farm/zone	32° 47′ N, 119° 25′ E	Egg and meat	Spotty

Data analysis

Electropherograms were obtained using the program Chromas and manually checked insuring the veracity of the DNA sequences. Sequence alignments were performed using DNAman (6.0.40). Haplotype numbers, nucleotide variable sites, haplotype diversity, nucleotide diversity (NEI 1982) were calculated using DnaSP V.4.10.7 (ROZAS 2003). Ignoring the insertion/deletion mutations, the same sequences were considered to be one haplotype. The same mtDNA D-loop control region sequences of *Anas platyrhynchos* and *Anas zonorhyncha* were acquired from GenBank. Kimura 2-parameter distances between breeds were estimated in Mega v. 3.1 (KUMAR *et al.* 2004) and a neighbour-joining tree was then constructed. The haplotype network was constructed using the software NETWORK 4.5.0.1.

Table 2
Haplotype diversity (Hd), average number of differences (K) and nucleotide diversity (Pi) of D-loop in 8 domestic duck breeds
Haplotypdiversität (Hd), durchschnittliche Anzahl der Unterschiede und Nukleotiddiversität (Pi) der D-loop bei 8 Hausentenrassen

Breeds	Size	No. of haplotypes	Haplotypes, %	Hd	K	Pi
BJ	8	2	25.0 %	0.42857	0.42857	0.00064
JC	8	3	37.5 %	0.46429	0.50000	0.00075
YX	12	7	58.3 %	0.83333	2.13636	0.00320
SX	8	2	25.0 %	0.25000	0.25000	0.00037
PT	8	3	37.5 %	0.46429	0.75000	0.00112
JD	8	3	37.5 %	0.7500	0.96429	0.00145
LC	8	3	37.5 %	0.60714	0.78571	0.00118
GY	12	4	33.3 %	0.56061	0.63636	0.00095

```
[        111111112 2223344455 5]
[       9134577882 3594928824 6]
[       6632702567 9518272519 5]
 A7     CTCAAAACCG CTGCGTTATT T
 H1     ..T....... .......... .
 H2     .......... .......... .
 H3     .......... .C........ .
 H4     .......... ........G.. .
 H5     .....G.... .......... .
 H6     .........A .......... .
 H7     .........A .......... .
 H8     ....G.G... .......... .
 H9     ....G..... .......... .
 H10    .......... ..A.C..... .
 H11    .......... ..A....... .
 H12    ...G...... .......... .
 H13    .C........ .......... .
 H14    .......... T......... .
 H15    ........T.. .......... .
 H16    .........T. .......... .
 H17    .......... ...A....C. .
 H18    .......... ...A...... A
 H19    T......... ...A.A.... .
 H20    T......... ...A..G..C .
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Figure 1
H1-H20 indicates the haplotypes of 72 domestic ducks
Variable sites in mtDNA D-loop of haplotpyes of domestic ducks
H1 – H20 zeigt die Haplotypen von 72 Hausenten an
Unterschiedliche Standorte in der mtDNA D-loop der Haplotypen von Hausenten

Table 3
Kimura 2-parameter distances (above diagonal) and standard error (below diagonal) of D-loop in 8 domestic duck breeds
Kimura 2-Parameter-Distanzen (über der Diagonalen) und Standardfehler (unter der Diagonalen) in der D-loop von 8 Hausentenrassen

Breed	BJ	JC	YX	SX	PT	JD	LC	GY
BJ		0.00044	0.00088	0.00039	0.00046	0.00120	0.00149	0.00047
JC	0.00075		0.00080	0.00031	0.00040	0.00119	0.00149	0.00040
YX	0.00226	0.00226		0.00076	0.00082	0.00143	0.00163	0.00079
SX	0.00056	0.00056	0.00207		0.00036	0.00119	0.00149	0.00036
PT	0.00094	0.00094	0.00241	0.00075		0.00123	0.00148	0.00043
JD	0.00207	0.00207	0.00348	0.00188	0.00211		0.00191	0.00121
LC	0.00263	0.00263	0.00414	0.00244	0.00282	0.00395		0.00151
GY	0.00088	0.00088	0.00238	0.00069	0.00106	0.00219	0.00276	

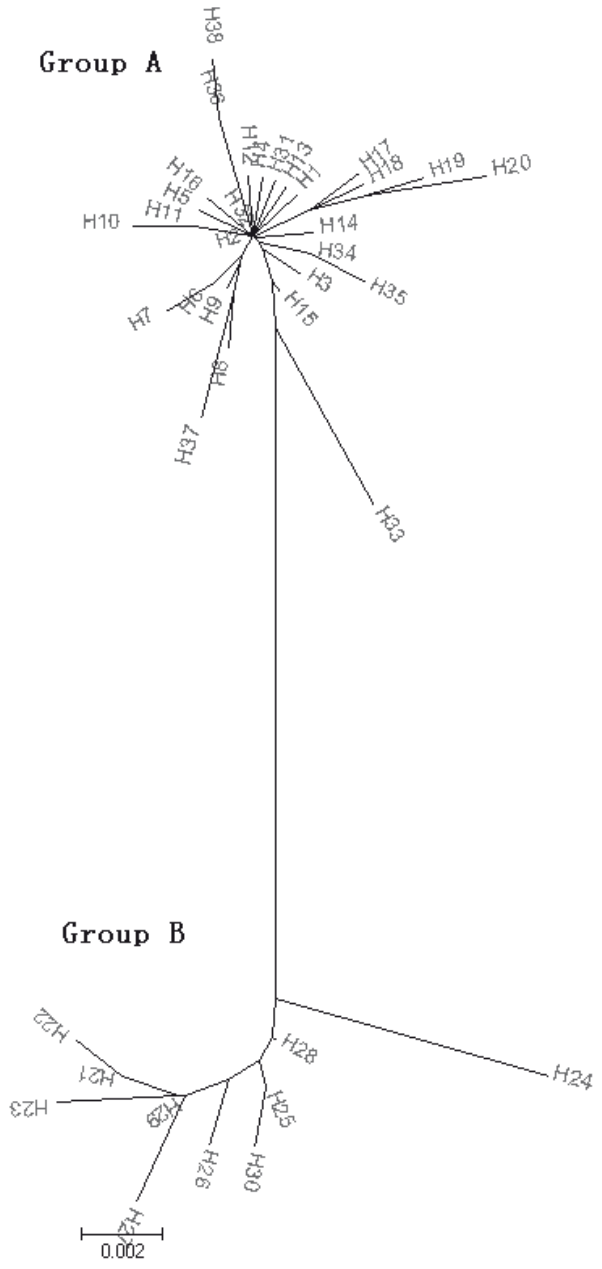

Figure 2
H1-H20 represented the haplotypes in the 8 domestic duck breeds
H21~H30 represented haplotypes of *Anas zonorhyncha*
H31~H38 represented haplotypes of *Anas platyrhynchos*
H1-H20 stellt die Haplotypen bei 8 Hausentenrassen dar
H21~H30 stellt die Haplotypen bei Anas zonorhyncha dar
H31~H3 stellt die Haplotypen bei Anas platyrhynchos dar

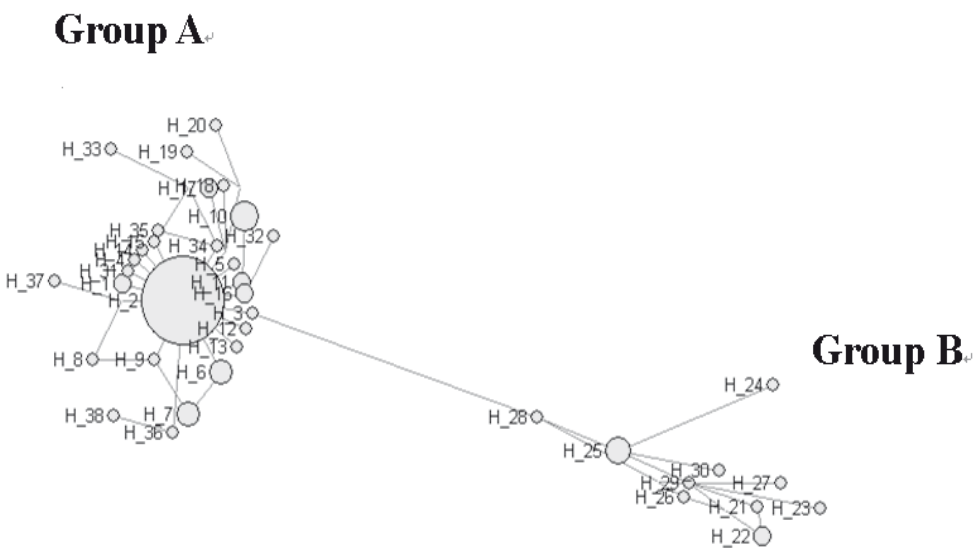

Figure 3
H1-H20 represented the haplotypes in the 8 domestic duck breeds
H21~H30 represented haplotypes of *Anas zonorhyncha*
H31~H38 represented haplotypes of *Anas platyrhynchos*
H1-H20 stellt die Haplotypen bei 8 Hausentenrassen dar
H21~H30 stellt die Haplotypen bei Anas zonorhyncha dar
H31~H3 stellt die Haplotypen bei Anas platyrhynchos dar

Results and discussion

Genetic diversity and distance among 8 domestic duck breeds

Table 2 showed that the largest number of haplotype was found in YX (7) with the haplotype proportion of 58.3 %, the second in GY (4), 33.3 %, and then in the JC, PT, JD and LC (3), 37.5 %, respectively, the lowest in BJ and SX (2), 25.0 %, respectively. The average haplotype diversity (Hd), nucleotide diversity (Pi) and nucleotide difference (K) of the 8 domestic duck populations was 0.67136, 0.00192 and 1.27856, respectively, of which YX was the highest (Hd=0.83333, Pi=0.00322, K=13 636).

Kimura 2-parameter distances of mtDNA D-loop in 8 duck breeds were showed as table 3. The genetic distances between the 8 duck breeds were ranged from 0.00056 to 0.00414, the largest was between LC and YX, followed by LC and JD, and the lowest was between SX and JC.

Hd and Pi of 8 domestic duck breeds (0.67136, 0.19 %) were lower than *Anas platyrhynchos* of that region (0.987, 0.83 %) (KULIKOVA *et al.* 2005). Compared to other animals, this result was similar to swine (0.122 %) (LAN *et al.* 1995), significantly less than yak (1.231 %) (LAI *et al.* 2005) and scalper (2.16 %) (LIU *et al.* 2006) and significantly higher than chicken (<0.001 %) (WAKANA *et al.* 1986). Haplotype diversity (Hd) and nucleotide diversity (Pi) of populations were main indexes for evaluating the mtDNA variation and genetic diversity of breed or population. The greater Hd and Pi, the more rich the genetic diversity. Hd and Pi were lower in BJ and SX, and highest in YX. The genetic diversity was mainly affected by the selection period and pressure. The selection period and pressure of BJ and SX were earlier and bigger

than YX's. In general, we considered that the nucleotide diversity of Chinese domestic ducks was low, and average nucleotide difference (K) was greater compared to nucleotide diversity. It indicated that the genetic diversity of Chinese domestic ducks was low, the interspecies of which was less than within-species.

In recent years, genetic distance and genetic differentiation of Chinese domestic ducks were analysed by SSR marker (LI *et al.* 2006 a,b and 2007), and some valuable conclusions were obtained. In our investigation, the genetic distances were 0.00056-0.00414. The greatest genetic distance in our test was between LC and other breeds, which was consistent with the report of YAN *et al.* (2005). LC with a dual purpose for medicine and egg, which was bred for the medicinal trait and laying performance for a long time, differed from other domestic ducks in phenotype and genome.

Origin and evolution of 8 domestic duck breeds

The mtDNA phylogeny of the ducks comprises two divergent haplotype groups, group A and group B (AVISE *et al.* 1990, JOHNSON *et al.* 1999, KULIKOVA *et al.* 2004 & 2005). The NJ phylogenetic tree (Figure 2) and reduced median-joining network chart (Figure 3) were constructed by the total 38 haplotypes and 96 sequences which included 12 *Anas platyrhynchos* (AY506877, AY506906, AY506925, AY506930, AY506941, AY506974, AY506977, AY506982, AY506883, AY506873, AY506874, AY506904), 6 *Anas zonorhyncha* (AY506946, AY506958, AY506960, AY506964, AY506949, AY506945) and 78 sequences of this study (72 domestic ducks and 6 *Anas zonorhyncha*). As the figure 2 and figure 3 showed, Chinese domestic ducks were clustered into the haplotype cluster A, and the maternal origin of the 8 domestic ducks was *Anas platyrhynchos*.

Most of Chinese duck breeds were formed in Ming and Qing dynasty, such as Pekin duck, Shaoxing duck, Gaoyou duck, the domesticated history was less than one thousand years. At present, there are lots of reports about the origin of Chinese domestic duck, but the origin dispute still existed. Based on mtDNA molecular marker, 8 representative domestic duck breeds were analysed to reveal the diversity, origin and evolution of Chinese domestic duck breeds.

Parts of domestic ducks genetic diversity and the genetic relationship among domestic ducks, *Anas platyrhynchos* and *Anas zonorhyncha* were analysed by CHEN *et al.* (2001) and the results showed that both of *Anas platyrhynchos* and *Anas zonorhyncha* contributed to domestic duck evolution. Genomic DNA polymorphism among eight domestic ducks and two wild ducks by using AFLP technique was estimated. The eight domestic duck breeds were originated from both *Anas platyrhynchos* and *Anas zonorhyncha* by genetic distance and variance analysis (YAN *et al.* 2005). The analysis of Genomic DNA polymorphism among three famous duck breeds of domestic ducks (Jinding duck, Liancheng white duck, Shanma duck) and two wild ducks (*Anas platyrhynchos* and *Anas zonorhyncha*) in Fujian province by SSR indicated that *Anas platyrhynchos* contributed more to domestic duck evolution than *Anas zonorhyncha* (CHEN *et al.* 2006). In general, the three reports showed that the Chinese domestic ducks were originated from *Anas platyrhynchos* and *Anas zonorhyncha* which supported the dualism of duck origin.

The mtDNA phylogeny of the duck comprises two divergent haplotype groups, group A and group B (AVISE *et al.* 1990). Group A haplotypes are most common and are found worldwide.

B haplotypes occur at high frequencies throughout North America and in frequently in North Asia. Mallards from Asia are attributed to the Group A haplotype clade; spot-billed ducks are attributed to group A or B haplotype clades. The B haplotype from North Asia, however, differs from all other group B haplotypes, forming a separate subclade (SB) nested within the group B clade (accession nos. AY506873, AY506874, and AY506904). Actually, the spot-billed duck was not closely related to Asian Mallards but instead is more closely related to the Mottled Duck (JOHNSON and SORENSON 1999). DNA sequencing revealed clades that correspond to AVISE *et al.* (1990) group A and group B mtDNA. In this study, 20 haplotypes of domestic ducks were only clustered to group A and also no evidence of contribution of *Anas zonorhyncha* to the maternal origin of domestic duck breeds. The study conclusion is that Chinese domestic ducks were only originated from *Anas platyrhynchos* based on mitochondrial DNA analysis.

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