Genetic Variability of mtDNA Sequences in Chinese Native Chicken Breeds

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ABSTRACT : The variability of mtDNA hypervariable segment I (HVS I) sequences was investigated in a total of 48 birds belonging to 12 Chinese native chicken breeds. Sixteen haplotypes were identified from 35 polymorphic nucleotide sites which accounted for 6.4% of a sequenced 544 bp fragment. Diversity analysis of the haplotypes showed that Tibetan, Langshan and Henan cockfight chicken had only one haplotype, while ancient haplotypes existed in Taihe silky and Chahua chicken. Phylogenetic analysis of the haplotypes suggested that Chinese native chicken breeds shared 5 maternal lineages and some breeds would share the same maternal lineage, regardless of their external features and ecological types. Both divergent and phylogenetic analysis of the haplotypes indicated the close genetic relationships between the Chinese native chicken breeds and *G. g. gallus* and *G. g. spadiceus* from different areas, which implied that *G. g. gallus* and *G. g. spadiceus* were the original ancestors of the Chinese native chicken breeds. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 903-909)***

Key Words : Chinese, Native Chicken, Jungle Fowl, mtDNA, Haplotype, Original Ancestor

INTRODUCTION

Domestic chicken taxonomically belongs to *Galliformes, Pharsianidae, Gallus*. Chicken breeds are considered to have originated either from *G. gallus* or from *G. sonnerati* (grey jungle fowl), *G. lafayettei* (Ceylonese jungle fowl) or *G. varius* (green jungle fowl), respectively (Mason, 1987). Domestic chicken is the earliest domesticated fowl and there are 60 native chicken breeds in China (Chang, 1995). At present, researchers have analyzed the genetic characteristics of Chinese native chicken breeds using methods such as cytogenetics, biochemical genetics and DNA fingerprinting (Zeng, 1987; Cheng et al., 1991; Wang et al., 2003).

Mitochondria, one of the important organelles in eukaryotic cells, are presumed to represent bacteria-like organisms incorporated into eukaryotic cells over 700 million years ago (perhaps even as far as 1.5 billion years ago) and function as the sites of energy metabolism. In higher vertebrates, mitochondria strictly follow the path of maternal transmission, i.e., the descendents of the same maternal ancestor have almost identical mitochondria. Mitochondrial DNA (mtDNA) is the genetic material in mitochondrion. Generally, evolution of mtDNA occurred primarily as single base pair substitutions, with only infrequent major sequence rearrangements. Moreover, the rate of mtDNA evolution was about 5 to 10 times faster than nuclear DNA, and its genes did not recombine. So mtDNA analysis has been used to investigate the genetic backgrounds of both closely related species and individuals within species. The D-loop region of mtDNA is known to be more variable in sequence than in other regions and thus has been frequently used by geneticists for phylogenetic analysis of closely related breeds within species. Phylogenetic relationships among Chinese native chicken breeds had been determined before using RFLP of mtDNA (Wang et al., 1994; Zhou et al., 1997). However, results of these studies provided only limited information. Sequencing of mtDNA allowed for more powerful phylogenetic inference because it can detect all the polymorphic sites present. Fu et al. (2001) were the first to use D-loop sequence polymorphism to determine phylogenetic relationships among 5 native chicken breeds in Zhejiang province. In our study, we used the D-loop hypervariable segment Ⅰ (HVS Ⅰ) of mtDNA to determine the genetic differentiation backgrounds and to probe into the origin of Chinese native chicken breeds.

MATERIALS AND METHODS

Specimen collection

Based on related documents (Chang, 1995; Qiu et al., 1988; Compilation Committee of Annals of Domestic Animal and Poultry Breeds in Yunnan Province; Guizhou Provincial Development station of Animal Breeds, 1997; Lai et al., 2001), information on the native chicken breeds we examined are listed in Table 1. We typically collected 12 relatively ancient native chicken breeds from different animal husbandry culture areas in China. Each breed was represented by 4 specimens from four different lines.

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Table 1. List of native breeds examined

Breed	Breed code	Ecological type	Collection site	Primary location	Animal husbandry culture area	Time of breed origin
Tibetan chicken	ZJ	Other	[a]	Tibet	Qing Zang area [b]	1,000 years ago
Qingyuan blotted chicken	QY	Meat	[a]	Qingyuan county, Guangdong	Min Yue area [c]	900 years ago
Henan cockfight	DJ	Recreation	[a]	Kaifeng city, Henan	Southeast of China	2,000 years ago
Chahua chicken	CH	other	[a]	Dehong county, Yunnan	Southwest of China	Many years ago
Big bone chicken	DG	Meat and egg concurrent	[a]	Zhuanghe county, Liaoning	Northeast of China	200 years ago
Beijing youkei	BY	Meat and egg concurrent	[a]	An'ding area, Beijing	North of China	250 years ago
Langshan chicken	RS	Meat and egg concurrent	[a]	Rudong county, Jiangsu	Southeast of China	Very ancient breed
Yugan Wugu chicken	YG	Medicine	Yugan breed farm	Yugan county, Jiangxi	Xiang'er'Gan area [d]	Han dynasty (2,000 years ago)
Souguang chicken	SG	Meat and egg concurrent	Cilun breed farm	Souguang city, Shandong	North of China	1,500 years ago
Taihe Silky chicken	SY	Medicine	Taihe breed farm	Taihe county, Jiangxi	Xiang'er'Gan area [d]	1,000 years ago
Wumeng Wugu chicken	WM	Medicine	Bijie city, Guizhou	Bijie city, Guizhou	Southwest of China	Many years ago
Yanjing Wugu chicken	YJ	Medicine	Yanjing county, Yunnan	Yanjing county, Yunnan	Southwest of China	Han dynasty (2,000 years ago)

[a] represents Poultry Institute, Chinese Academy of Agri. Sci., [b] contains Qinghai, Tibet and adjacent areas.
[c] contains Fujian, Guangdong and adjacent areas, [d] contains Hunan, Hubei, Jiangxi and adjacent areas.

DNA extraction

The blood specimens were preserved by 75% ethanol at the volume rate of 1:4 (blood:ethanol) and stored at room temperature. DNA was extracted from these specimens using phenol/chloroform.

PCR amplification

The primers used were those of Dejardins et al. (1990) and Randi et al. (1998) which amplified a 544 bp fragment between sites 16,750 and 543 (GenBank NC-001323), the forward primer was L_{16750}: 5'-AGGACACGGCTTGAAA AGC-3', and the reverse primer was H_{543}: 5'-ATGTGCC TGACCGAGGAACCAG-3'. The reagent kits used for PCR were from Shanghai Biologic Engineering Company. The PCR reaction was carried out in a total volume of 50 μl, and the final concentration or content of each component was as follows, 1×PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.7 U DNA polymerase, 0.3 μM of each primer and 150 ng DNA template. PCR was performed in a Progene thermal cycler (PE 9600). The reaction profiles included an initial denaturation at 95°C for 3 min, followed by 33 cycles, each consisting of 30 sec denaturation at 94°C, 36 sec primer annealing at 63°C, 56 sec extension at 72°C, and then a final 5 min extension at 72°C. The amplified products were all electrophoresed by 2.0% (wt/vol) agarose gel in 1×TBE buffer, with 5 v/cm voltage for 1 h. After the run, the gel was stained with ethidium bromide.

PCR products sequencing

The amplified products were purified by using the Wizard™ PCR Preps DNA purification kit (Promega) according to the manufacturer's instructions. Sequencing was performed by using an ABI model 377 automated sequencer (PE). The sequencing primers were the forward primer L16750 and the reverse primer H543. A consensus sequence of approximately 544 bp for each bird resulted from assembling of sequence reads in both strands.

Data analysis

All mtDNA nucleotide sequences obtained in this work were aligned by using the Clustal X software (Thompson et al., 1997), and identical sequences were considered as the same haplotype. Referring to the D-loop sequences of several chicken breeds in GenBank, haplotypes dissimilar to those found in the present study were selected to enrich our data. Using MEGA software (Kumar et al., 1993), Kimura 2-parameter distance matrix of all haplotypes were calculated to construct the unrooted NJ phylogenetic tree with *G. lafayettei* and *G. varius* as outgroups. Bootstrap confidence levels (BCL) of the phylogenetic tree were estimated by 1,000 random bootstrap resampling of the data. The haplotypes of the D-loop sequences for all of the species of *Gallus* in GenBank were used to draw the unrooted NJ cladogram of haplotypes for both Chinese native chicken breeds and *Gallus*. Bootstrap confidence levels were evaluated by 1,000 random bootstrap resampling of the data.

RESULTS

Sequence and genetic variation of mtDNA D-loop

The sequences we studied have submitted to GenBank (from AY465960 to AY466007). The nucleotide

Table 2. Haplotypes of studied breeds obtained from GenBank

Breed	Code of haplotype	Accession No. in GenBank	Author	Collection site
Chahua chicken	CH5-6	AF512085, AF512089	Y. P. Liu, et al.	Yunnan, China
Yanjing Wugu chicken	YJ5-7	AF512324, AF512326, AF512327	Y. P. Liu, et al.	Yunnan, China
Qinyuan blotted chicken	QY5	AF512260	Y. P. Liu, et al.	Guangdong, China
Gushi chicken	GuS5-8	AF512144, AF512145, AF512146, AF512150	Y. P. Liu, et al.	Henan, China
Bai'er yellow chicken	BeH5	AF128322	Y. Fu et al.	Jiangxi, China
G. g. spediceus	spadiceus1-5	AF512182, AF512185, AF512186, AF512187, AF512188	Y. P. Liu et al.	Myanmar
	spadiceus6	AF512174	Y. P. Liu et al.	Yunnan, China
	spadiceus7	AB009442	Miyake, T.	Laos
	spadiceus8-9	AB009443, D82907	Miyake, T.	Thailand
G. g. gallus	gallus1-5	AB007720, AB007725, AB007752, AB007756, AB007757	Miyake, T.	Unknown
	gallus6-7	AB009440, AB009439	Miyake, T.	Sumatra
	gallus8-9	AB009438, AB009437	Miyake, T.	Lombok
	gallus10-11	AB009435, AB009434	Miyake, T.	Vietnam
	gallus12	AB009433	Miyake, T.	Philippines
	gallus13	AB009432	Miyake, T.	Thailand
G. g. bankiva	bankiva1-2	AB009430, AB009431	Miyake, T.	Indonesia
	bankiva3	AB007718	Miyake, T.	Unknown
G. sonnerati	sonnerati	D82911	A. Fumihito, et al.	India
G. lafayettei	lafayette1-2	D66893, D82910	A. Fumihito. et al.	Sri Lanka
G. varius	varius1-2	D82913, D82915	A. Fumihito. et al.	Indonesia

Table 3. Nucleotide substitutions in D-loop HVS I of Chinese native fowl

Substitution	Polymorphic sites
A/T	268 th, 381 st.
C/A	289 th, 316 th, 454 th, 479 th.
G/A	232nd, 259th, 262nd, 301st, 362nd, 456th.
T/C	153rd, 187th, 219 th, 237 th, 245 th, 263 rd, 266 th, 276 th, 281 th, 289 th, 316 th, 322nd, 330 th, 333 rd, 335 th, 342 nd, 375 th, 383 rd, 387 th, 414 th, 416 th, 467 th, 501st, 505 th, 534 th.

Table 4. Haplotypes shared among Chinese native chicken breeds

Code of haplotype	Breeds	Code of haplotype	Breeds
ZJ1	ZJ, CH.	YG2	YG, CH, GuS.
SG2	SG, SY.	BY1	BY, CH, SY, YJ.
YJ4	YJ, SY.	QY1	QY, CH, DJ, DG, BY, SG, SY,
SG4	SG, CH, GuS.		YG, WM, RS, BeH.

substitutions found in the mtDNA D-loop region of Chinese domestic fowl are shown in Table 3. Analysis showed that the lengths of all the sequences we studied were 5 bases (TACCT at 3' end, not including the primers) longer than those sequences amplified before by the same primers (Fu et al., 2001; Liu et al., see Table 2). When we aligned our sequences with some D-loop sequences of *G. g. bankiva* (AB009430 and AB009431) and *G. g. gallus* (AB007720 and AB007725), the lengths of which were all more than 600 bp, the results showed that the 5 bases (TACCT) should be at the 3' end. Therefore, it is believed that the sequences in Chinese native chicken breeds amplified by the primers in this study should be 544 bp rather than 539 bp (not including the primers).

The 48 samples represented 16 haplotypes of the D-loop hypervariable region. These samples altogether showed 35 variable sites of base substitution. No deletion or insertion was observed. The average percentage of polymorphic sites was 6.4% for the 544 bp region of D-loop HVS I, but the average percentage of polymorphic sites of native chicken breeds in Zhejiang province was just about 4.45% (Fu et al., 2001). This might be ascribed to the differences of sampling areas.

11 haplotypes of some related breeds were identified and chosen from GenBank (Table 2). Among these breeds, Bai'er Yellow chicken (BeH) was an egg type breed while Gushi chicken (GuS) was a relatively ancient breed from Henan province that was part of the original domestication area of Chinese native chicken. There are 27 different haplotypes from 14 breeds in the study.

The results showed that most of these breeds had more than one haplotype, but Tibetan chicken (ZJ), Henan cockfighting chicken (DJ) and Langshan chicken (LS) had only one haplotype each. On the other hand, some of these 27 haplotypes were found in more than one breed (Table 4).

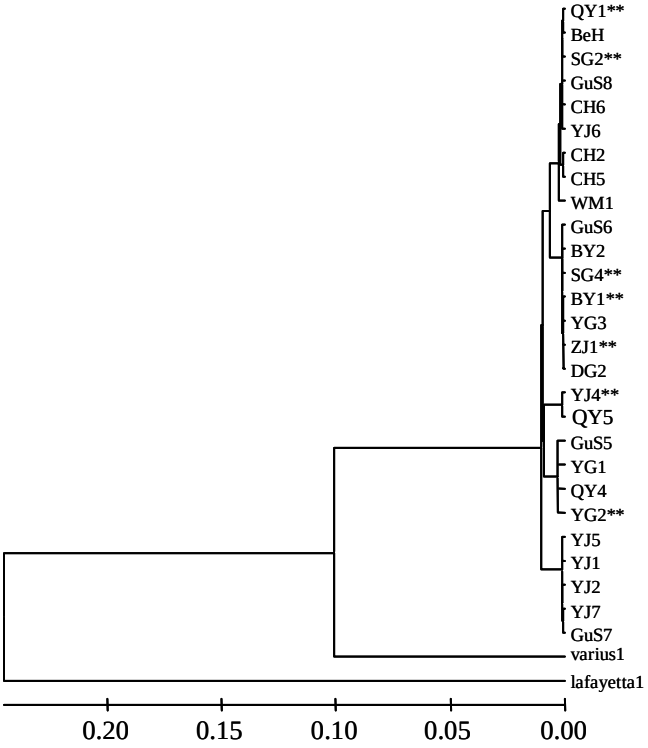

Figure 1. Unrooted NJ phylogenetic tree in Chinese native chicken breeds. Haplotypes in the figure with ** meant they were found in more than one breed.

Phylogenetic analysis in Chinese native chicken breeds

An unrooted NJ phylogenetic tree of haplotypes in Chinese native chicken breeds was constructed (Figure 1). As a whole, these 27 haplotypes were placed into 5 clusters. However the haplotypes of native fowls in Zhejiang province were grouped into only 2 clusters (Fu et al., 2001). Such clustering disparity might be caused by differences in sampling areas. Moreover, Chinese native chicken breeds fell into 4 clusters based on blood-groups and blood protein polymorphisms (Cheng et al., 1991) and microsatellite analysis (Wang et al., 2003). The differences in the genetic markers used in the studies might affect the clustering results as well.

Phylogenetic relationship of Chinese native fowl and other species of *Gallus*

Some haplotypes of other species of *Gallus* were selected from GenBank (Table 2). The average divergence of haplotypes found in Chinese native fowl and other species of *Gallus* (Table 5) showed that the mean divergence indices between Chinese native chicken and *G. g. gallus* or *G. g. spediceus* were much lower than those between Chinese native fowl and *G. g. bankiva* or other species of *Gallus*, which indicated that Chinese native chicken was more genetically close to *G. g. gallus* and *G. g. spadiceus*. Table 5. Also showed that, as far as *G. lafayettei*, *G. sonnerati* and *G. varius*, the genetic divergence between *G. lafayettei* and *G. sonnerati* was relatively lower, which was similar to the result of Hashiguchi et al. (1993). The phylogenetic tree of haplotypes in Chinese native chicken breeds and other *Gallus* (Figure 2) also showed that, except that *G. g. gallus* from Sumatra Island (gallus6 and gallus7) was slightly remote from Chinese native fowl, all of the haplotypes in *G. g. gallus* and *G. g. spadiceus* from different areas fell into the primary 5 clusters of Chinese native chicken, which indicated the very close genetic relationships between these two subspecies and Chinese native chicken. But the genetic distances between Chinese native chicken and *G. g. bankiva*, *G. lafayettei*, *G. sonnerati* and *G. varius* were relatively further. In addition, Both Table 5 and Figure 2. showed that the genetic distance between *G. varius* and native chicken was not as the largest as had reported (Hashiguchi et al., 1993; Cheng et al., 1996; Fumihito et al., 1996). It maybe necessary to further clarify this issue by increasing the sample size of the chicken populations studied.

DISCUSSION

Diversity analysis of mtDNA in Chinese native chicken breeds

Generally, the more ancient the population was, the longer they had to mutate and accumulate the mutations. So ancient populations would be more diversified genetically and the haplotypes present in them would have more opportunities to be shared by other populations (Torroni et al., 1993; Ward et al., 1993). The results of the present study showed that Tibetan chicken (ZJ), Henan cockfighting chicken (DJ) and Langshan chicken (LS) had only one haplotype each, but they were all relatively ancient breeds (Qiu et al., 1988). This could be attributed to the following reasons. (i) These breeds, as germplasm, were introduced

Table 5. The average divergence of haplotypes found in Chinese native fowl and other *Gallus*

	1	2	3	4	5	6	7
1 Native fowl	0.016726						
2 *G.g.pediceus*	0.020569	0.021462					
3 *G.g.allus*	0.022627	0.024819	0.020141				
4 *G.g.bankiva*	0.326823	0.327016	0.32779	0.293508			
5 *G.sonnerati*	0.557238	0.559667	0.571293	0.419746	0.000000 [a]		
6 *G.afayettei*	0.554068	0.558349	0.562595	0.375933	0.353075	0.010166	
7 *G.varius*	0.423851	0.423586	0.429228	0.42855	0.451837	0.402868	0.549169

[a] *G.sonnerati* had only one haplotype studied.

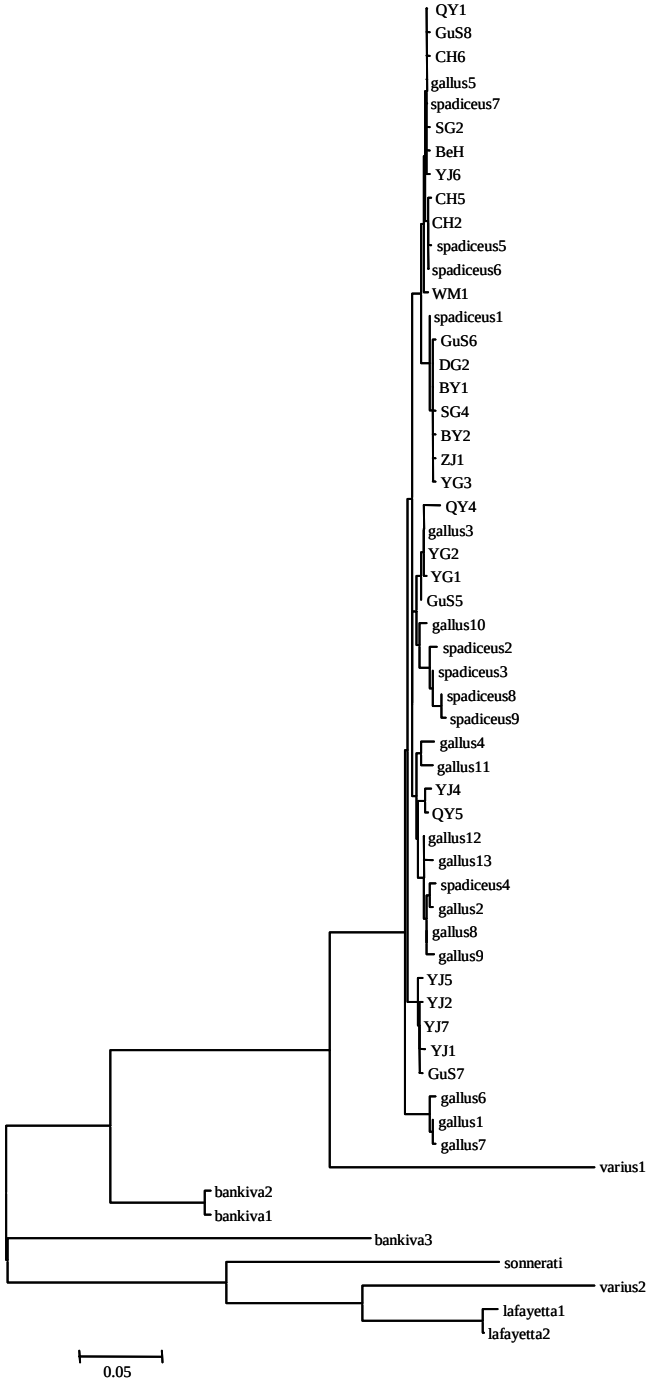

Figure 2. Unrooted NJ phylogenetic tree of haplotypes found in Chinese native fowls and four species of *Gallus*.

on a small scale from their primary distribution regions to a new location, the Poultry Institute of the Chinese Academy of Agricultural Sciences. This process would likely result in genetic drift (founder effect). (ii) The long history of selection and breeding or organized production might have imposed high selection pressure on these ancient breeds (Pandey et al., 2002). For example, the cockfighting breed had been bred for fighting for more than 2,000 years ago. Thus, the selection pressure on this breed would be high because of the needs of cockfighting. (iii) These breeds were likely to have encountered serious genetic drift (bottleneck effect) during their evolutionary process. For example, Henan cockfighting chicken was almost extinct during the Cultural Revolution of the 1970s' (Wu, 2003). (iiii) It might be caused by the small sample sizes of the present study.

Lai et al. (2001) noted that Taihe silky chicken (SY) had been bred for more than 1,000 years, and the Annals of Native Livestock and Poultry Breeds in Yunnan Province (Compilation Committee) reported that Chahua chicken (CH) often mated with *G. gallus* inhabiting the nearby areas during harvest time. *G. gallus* is considered to be the original ancestor of domestic chicken. Our results (Table 4) showed that Chahua chicken had the most haplotypes shared with other breeds, and the number of haplotypes in Taihe silky chicken shared with other breeds ranked second. This suggested that Taihe silky and Chahua chicken were likely to be ancient breeds or they shared ancient lineages. This was consistent with the descriptions given in both the Annals refered to above.

Genetic differentiation of chinese native chicken breeds

Ohno (1997) deduced that a set of full or maternal half-sisters should have inherited identical mitochondrial genome from their mother, but each sister's female descendants invariably establish an independent lineage which in time would accumulate its own characteristic mutations to become a distinct sublineage. As a whole, Figure 1 showed that the phylogenetic tree of haplotypes in the Chinese native chicken breeds had five clusters which represented 5 lineages, so we concluded that the present Chinese native chicken breeds likely shared 5 common maternal lineages.

As illustrated in both Figure 1 and Table 4, some chicken breeds had more than one haplotype, and some of these haplotypes belonged to different lineages. For example, some haplotypes in Gushi chicken (GuS) belonged to lineages I, II, IV and V. Some haplotypes in Yanjin Wugu chicken (YJ) belonged to lineages I, II, III and IV. Some haplotypes in Taihe silky chicken (SY) belonged to lineages I, II and III. Some haplotypes in Qingyuan blotted chicken (QY) belonged to lineages I, III and V, and some haplotypes in Chahua chicken (CH) belonged to lineages I, II and V. All of the above suggested that these breeds shared lineages, that their genetic backgrounds were complex, and that the 5 lineages might not have evolved independently. Mating might have occurred between lineages or some of them might have differentiated during the process of evolution.

Figure 1 also showed that each lineage contained more than one breed. Lineages I and II contained most of breeds we examined in the study, they might be the primary

evolutionary lineages. Table 4 also illustrated that several breeds shared the same haplotype. Both results suggested that these breeds belonged to the same lineage or that they shared the common maternal ancestor regardless of external features and ecological types of these breeds. How could some breeds with different features share a common lineage? Zhu (1958) suggested that mutation was the main reason and that the more than 30 subspecies or variants of domestic chicken were formed by gradual mutations. For example, in 1921, Joes discovered a Leghorn with fuzz which was similar to silky chicken. Then Joes mated the strange Leghorn with normal Leghorn and silky chicken, respectively, and analyzed the inheritance and differentiation of the feather in their offspring. It was found that both the Leghorn with fuzz and silky chicken had essentially the same mutation. But how could different breeds share the same maternal lineage? Chinese researchers presumed that the primary reason for domestication of chicken was to make available a source of meat and for religious purposes. Later on the cockfighting breed was bred for recreation and lastly, egg type breeds were developed (Cheng et al., 2000). But foreign researchers speculated that domesticated chicken was first used as recreational breeds, such as gamecock, then for various religious purposes, and eventually as the source of meat and egg (Mason, 1987). The differences between these two viewpoints were attributed to different citations. The former mainly referred to Chinese archaeological and other related documents, while the latter mainly referred to Indian archaeological and other related documents. Although the viewpoints were not consistent with each other, both of them implied that various types of chicken breeds might have originated from common ancestors.

Original ancestor of Chinese native chicken breeds and original domestication site of native chicken

In so far as original ancestor of the native fowl, some studies before have probed into it using methods such as biochemical genetics and nuclear DNA (Hashiguchi et al., 1993; Cheng et al., 1996; Mohd-Azmi et al., 2000). All of them concluded that the domestic chicken from different areas or countries was genetically very close to their indigenous red jungle fowl (*G. gallus*). Our results (both Table 5 and Figure 2) showed that Chinese native chicken breeds showed a low genetic relationship with *G. varius*, *G. lafayettei* and *G. sonnerati*. For *G. gallus*, however, the Chinese native fowl was just genetically close to two subspecies of *G. gallus* (i.e., *G. g. gallus* and *G. g. spadiceus*), but another subspecies, *G. g. bankiva* was remote from them. This is in accordance with the results before (Fumihito et al., 1996). Our results (Figure 2) further showed that all of the haplotypes in *G. g. spadiceus* from Thailand, Laos, Myanmar and Yunnan province of China, and in *G. g. gallus* from Vietnam, Thailand, Philippines, Lombok Island and an unknown sampling area can be found in the primary 5 haplotype clusters of Chinese native chicken. For each chicken lineage, the mean rates of base substitutions were equivalent (Zheng, 1995). Therefore, the current native chicken and jungle fowls can respectively be assimilated to domestic chicken and jungle fowls at the beginning of domestication. It is concluded that Chinese native chicken breeds originated from two subspecies of *G. gallus*, namely *G. g. gallus* and *G. g. spadiceus*. While another subspecies, i.e., *G. g. jabouillei* was not included in this study.

Fumihito et al. (1996) reported that the sequence divergence among D-loop segments of domestic chicken breeds and *G. g. gallus* in Thailand was only 0.5 to 3.0%, and haplotypes of domestic chicken and *G. gallus* from Asian populations including *G. g. gallus* and *G. g. spadiceus* from Thailand and its adjacent regions were clustered in the same group. Thus suggested that the all domestic chicken breeds were likely to have originated from a single domestication event in Thailand and its adjacent regions, and these domesticated chicken then dispersed northwards to China in accordance with the findings of West and Zhou (1988). However, a different viewpoint was proposed due to the following reasons: (i) The present distribution area of *G. gallus*, including all kinds of subspecies, stretched from the northwest of India eastwards to the north of China, including Hainan province, and southwards to Indonesia. Moreover, *G. gallus* had emigrated to the Pacific islands (Mason, 1991). According to Fumihito et al. (1996), the distribution of *G. g. gallus* and *G. g. spadiceus* which included Thailand and South Sumatra was just part of the distribution area of *G. gallus*. In the present study, except that *G. g. gallus* from Sumatra was slightly remote from native fowl as the result before (Fumihito et al., 1996), the genetic relationship between Chinese native chicken breeds and *G. g. gallus* and *G. g. spadiceus* from different areas, such as Myanmar, China, Laos, Thailand, Vietnam, Philippines and Lombok Island was very close. (ii) Some pictures, statues and skeletons of chicken were excavated in Harapa site and Mohenjo-Doro site along the Indus Valley. These remains were considered the most ancient and evident proof of chicken domestication. The skeletons were larger than those of *G. gallus*, which meant chicken had been domesticated at that time. Based on these remains, Zeuner speculated that the domestication event had already been finished about 2000 B.C., but Wood-Gush presumed that the domestication event had been dated to about 3200 B.C. (Mason, 1987). Since the founding of P. R. China, early Neolithic sites were discovered in Wannian county of Jiangxi province and Banpo in Xi'an, Shaanxi province. Bones of ancient jungle fowls were found in both sites, which implied that ancient jungle fowls had inhabited regions along both the Huanghe and Yangtse Valleys.

Besides those sites, chicken bones were unearthed in other Neolithic sites in Xinzheng county of Henan province, Tengxian county of Shandong province and Wu'an county of Hebei province as well. Archaeological analysis of these remains suggested that these bones were the most ancient chicken bones dating about 6000 to 5500 B.C. (Cheng et al., 2000). Although it was not able to be determined whether the bones were from domestic chicken or jungle fowls, researchers agreed that the bones were at least from some species of *Gallus*. Thus, there should have been similar or more ancient remains of some species of *Gallus* in Thailand and its adjacent regions if the original domestication event had occurred there. However, before the finding of such convincing archaeological evidence, it is reasonable to assume that because of the suitable ecological environment, the present *G. gallus* which share common lineages with domestic chicken, inhabit their own distribution regions which are not necessarily the original domestication sites of the domestic chicken.

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