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Mitochondrial DNA Diversity of Korean Ogol Chicken

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ABSTRACT : Korean Ogol chicken has been registered as a natural monument in Korea and regarded as a valuable genetic resource for the world. As an initial step to investigate the genetic structures of this breed, phylogenetic analysis and calculation of genetic diversities have been performed using mitochondrial DNA (mtDNA) sequence variations. A total of 31 Korean Ogol chicken was grouped into four haplotypes and the large haplotype was represented in 12 individuals. The unrooted neighbor-joining tree indicates that the Korean Ogol chicken shared three (A to C) major chicken lineages representing the high genetic variability of this breed. These results can be used for making the breeding and conservation strategies for the Korean Ogol chicken. (**Key Words :** Phylogenetic Analysis, Korean Ogol Chicken, Mitochondrial DNA, D-loop)

INTRODUCTION

There have been two hypotheses for the origins of the current domestic chicken. One is monophyletic origin, mainly contributed by red jungle fowl (*Gallus gallus*). The other is the multiple origins that the current chicken is formed from several *Gallus* subspecies (Crawford, 1990). However this is still controversial and uncertainty has been remained how many subspecies have contributed to the origin of chicken. Based on the written evidence, 120 chicken breeds have been lived in the world (http://www.poultrypages.com/chicken-breeds.html) and the number of breeds is now decreasing because of their low productivities as well as pressure from the commercial farms with high productive strains. Recently, the native livestock genetic resources become more important and large efforts have been concentrated for maintaining minimum number of animals for each native species (http://www.fao.org/dad-is/).

The mitochondrial DNA (mtDNA) polymorphism, especially the displacement loop (D-loop) region, has been largely applied to understand phylogenetic relationships in many animal species, including cattle (Loftus et al., 1994; Bradley et al., 1996; Mannen et al., 1998; Troy et al., 2001; Mannen et al., 2004), pig (Giuffra et al., 2000), sheep (Hiendleder et al., 1998, 2002), horse (Vila et al., 2001), goat (Luikart et al., 2001; Mannen et al., 2001; Sultana et al., 2003; Joshi et al., 2004; Sultana et al., 2004; Chen et al., 2005; Odahara et al., 2006), and chicken (Niu et al., 2002; Liu et al., 2004; Liu et al., 2006). Along with mtDNA polymorphism, microsatellite markers were also investigated in chicken for delineating the breed structures and phylogenetic relationships with other breeds for the conservation perspectives (Hillel et al., 2003).

The Korean Ogol chicken has been registered as a natural monument (registration number 265) and the conservation program has been being carried out for this breed. The Korea Ogol chicken typically has black feathers, beak, comb, legs, bone, skin, and meat. The meat is often eaten as a folk remedy to improve people's health. In this study, the current phylogenetic status and genetic diversities of Korean Ogol chicken have been investigated in order to understand the genetic basis of this breed and ultimately contribute to make better breeding and conservation strategies.

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Table 1. Mitochondrial D-loop sequence variations among Korean Ogol chicken

Haplotypes	Nucleotide position[1]																	
	6	190	203	208	213	233	236	237	247	252	272	301	306	321	333	354	358	437
Type 1(12)[2]	T	C	G	C	A	G	C	C	C	T	A	T	C	C	A	C	T	T
Type 2 (9)	A	T	-	T	-	A	-	-	-	C	G	-	-	-	G	T	C	C
Type 3 (5)	-	T	-	-	G	-	-	-	-	-	-	-	-	T	-	-	-	-
Type 4 (5)	-	T	A	T	-	-	T	T	T	C	A	C	T	-	-	-	-	-

[1] Numbers indicate nucleotide base position in mitochondrial D-loop region and hyphen represents the identical nucleotide with the type 1 sequence.
[2] Numbers in parentheses indicate the observed number of Korean Ogol chicken.

MATERIALS AND METHODS

Sampling

Blood samples of 31 Korean Ogol chicken (*Gallus gallus domesticus*) were collected from National Livestock Research Institute (NLRI) in Korea. Approximately 500 birds of this breed have been maintained per year at NLRI for conserving this valuable genetic resource. Genomic DNAs were extracted according to the manufacturer's standard protocol using Magextractor (Toyobo Ltd, Japan) for investigation of sequence analysis in mitochondrial D-loop region. We included published mtDNA sequence data from domestic chicken populations of Japan, China, India and Thailand in our analyses (GenBank accession numbers AB098692 - AB098697, AB086102, AB114089, AB009441 - AB009443, AY465989, AY465988, AY644966 - AY644973, AY704710 - AY704719, AF512273-AF512282, AF512060 - AF512075, AF512189- AF512209, AF512221-AF512236).

PCR amplification, purification and DNA sequencing

The D-loop region of Korean Ogol chicken was amplified directly from the genomic DNA by polymerase chain reaction (PCR). The primer pair, L16750 (5'-AGGACTACGGCTTGAAAAGC–3') and H547 (5'-ATGTGCCTGACCGAGGAACCAG -3'), described by Niu et al. (2002), was used to amplify first 510 bp segment of the D-loop hypervariable region. In the primer names, L and H refer to the light and heavy chains, respectively, and the number designates the position of the 3'-end of the primer on the complete chicken mtDNA sequence (Desjardins and Morais, 1990). PCR reactions were carried out in 25 μl volumes using 1× buffer, 1.5 mM $MgCl_2$, 2.5 mM each of dNTP, 10 pM each primers and 1 unit TAKARA EX Taq polymerase (TAKARA, Japan). The PCR cycle profile included the initial denaturation at 94°C for 10 min following 35 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 40 sec with a final extension at 72°C for 10 min using GenAmp 9700 (Applied Biosystems, CA, USA). The PCR products were purified with Accuprep® PCR purification kit (Bioneer, Korea) according to the manufacturer's instructions. Sequencing reaction was performed by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (v3.0, Applied Biosystems, CA, USA) and electrophoresis was done by a 3100 DNA sequencer (Applied Biosystems, CA, USA). The obtained nucleotide sequences of Korean Ogol chicken have been submitted to the GenBank database (accession numbers from DQ629864 to DQ629894).

Data analyses

The mtDNA nucleotide sequences obtained in this study were aligned by using the ClustalW program (Thompson et al., 1994) and identical sequences were considered as the same haplotypes. Phylogenetic and molecular evolutionary analyses were performed using MEGA software version 3.1 (Kumar et al., 2004). Bootstrap confidence levels of the unrooted neighbor-joining (NJ) phylogenetic tree were estimated by 1,000 random bootstrap resampling of the data (Felsenstein, 1985). Genetic distances between chicken populations were estimated by Kimura-2 parameter distance matrix using MEGA software version 3.1 (Kumar et al., 2004).

RESULTS AND DISCUSSION

Sequence and mtDNA D-loop variation in Korean Ogol chicken

Analysis of 31 mitochondrial sequences from Korean Ogol chicken identified a total of 18 nucleotide changes grouped into four haplotypes. The large majority of haplotype group consisted of 12 individuals and one haplotype group comprised of 9 individuals, whereas the remaining 2 haplotypes were represented by 5 individuals each (Table 1). No deletion or insertion was detected in our sequences. The average percentage of polymorphic sites was 3.53 for 510 bp of 31 DNA sequences. But Liu et al. (2004) and Fu et al. (2001) reported the average percentage of polymorphism in D-loop region to be 6.4 and 4.45, respectively, for Chinese native chicken breeds. This lower polymorphic value might be due to sampling from one location (NLRI) where selection pressure continued for a long time. Moreover, It might be interpreted that chicken mtDNA had gone through an evolutionary bottleneck during the course of domestication (Niu et al., 2002).

Table 2. Sequence divergence of Korean Ogol chicken with Asian native chicken populations[1]

	1	2	3	4	5
1 Japan	0.018	*0.019*	*0.018*	*0.017*	*0.054*
2 China	0.018	0.009	*0.018*	*0.018*	*0.056*
3 Korea	0.017	0.018	0.014	*0.014*	*0.051*
4 India	0.016	0.017	0.014	0.010	*0.047*
5 Thailand	0.052	0.054	0.049	0.045	0.009

[1] Below the diagonal and on the diagonal are the average sequence divergences between and within populations respectively. Above the diagonal *(italic)* are genetic distances between populations.

Genetic distances by comparing with other Asian chicken populations

Using previously published Asian domestic chicken mtDNA sequence information, mean sequence divergence values among five Asian chicken populations (China, Japan, Korea, India and Thailand) and within each population were calculated (Table 2). The highest sequence divergence value 0.054 was observed between China and Thailand chicken populations, whereas the lowest value (0.014) displayed in between Korea and India. Mean sequence divergence values between populations ranged from 0.014 to 0.018 among the countries of Japan, China, Korea and India. This supported that Korean Ogol chicken population was more closely related with above mentioned four Asian countries than Thailand.

The highest mean sequence divergence value within population was found in Japanese chicken (0.018) and relatively low values (0.009-0.010) were found in the chicken populations of China, India and Thailand. However, a higher sequence divergence value (0.016) in Chinese domestic chicken was reported by Liu et al. (2004). The Korean Ogol chicken showed high level of mean divergence (0.014) than China, India and Thai populations, which supports the existence of more genetic variability in Korean Ogol chicken than the three Asian countries. The present findings are supported by the results of Fumihito et al. (1996) that the sequence divergence among D-loop segments of domestic chicken breeds and *G. g. gallus* in Thailand was 0.5 to 3.0%.

The estimated genetic distances between populations also indicated that Thailand and Chinese chicken populations are most far away (0.056) and interestingly, Korean Ogol chicken population is closely related with Indian chicken population (0.014) than Japanese and Chinese (0.018) chicken. These findings further reveal that genetic differentiation, in general, is low among the Asian domestic chicken populations except chicken from Thailand.

Phylogenetic analysis

Phylogenetic tree was constructed with mtDNA D-loop sequences from Korean Ogol chicken and published sequences from Japanese, Chinese, Thai and Indian domestic chicken populations (Figure 1). The unrooted neighbor-joining tree indicates that Japanese, Korean and Indian chicken belong to the three major chicken lineages (A to C), representing these populations have the highest mtDNA sequence diversity in the Asian chicken populations investigated. Here, it is noted that the present Korean Ogol chicken breed shared 3 common maternal lineages. On the other hand, lineage B and C contained Chinese native chicken population only, which indicates the genetic diversity is relatively low in that chicken group. Similar result was found by Niu et al. (2002) in Chinese native chicken breeds.

The phylogenetic tree also indicates that the lineage B (11 haplotypes) and C (5 haplotypes) include Japanese, Chinese, Indian and Korean populations. The lineage A (10 haplotypes) includes three chicken populations (Japan,

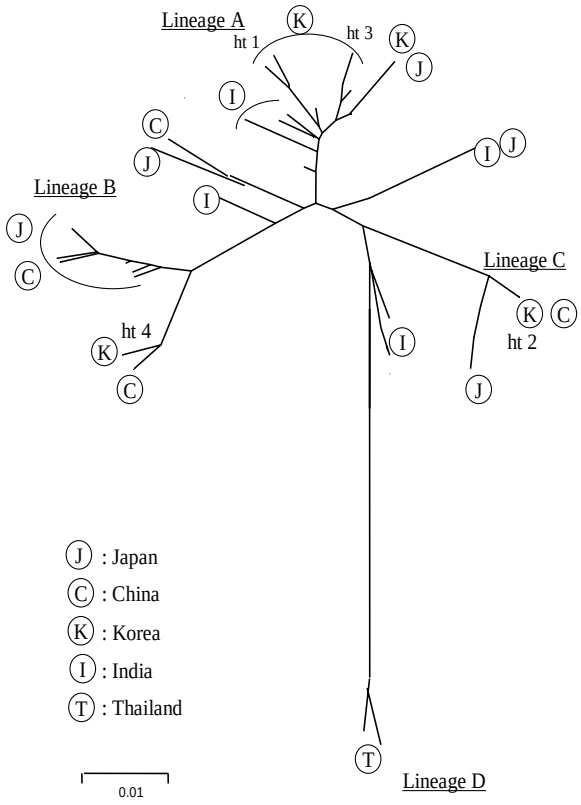

Figure 1. Unrooted neighbor-joining tree constructed from Japan, China, Korea, Thailand and Indian native chicken populations. This tree contains four lineages indicating diversity of the Asian domestic chicken populations. The largest lineages B and C include four chicken populations, and lineage A includes three populations. Also lineage D includes only Thailand native chicken population. Note that Korean Ogol chicken appeared in lineage A, B and C. The Korean Ogol chicken haplotypes in Table 1 also indicated in this figure (ht 1, 2, 3 and 4 represents haplotype 1, 2, 3 and 4 respectively).

India and Korea) except the Chinese chicken, whereas lineage D (2 haplotypes) shares only Thai chicken. Korean Ogol chicken, represented as four haplotypes, were distributed into mt lineage A, B and C (Figure 1), and are concentrated mainly in a specific region of mt lineage A indicates they were inbred within Korean peninsula for a long time.

In this study, we found that the Korean Ogol chicken still maintains genetic variability to some extent within the small population in Korea. In order to maintain this genetic variation for this valuable breed, appropriate conservation breeding program is ultimately needed. However more detailed molecular studies are required in near future.

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