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Mitochondrial D-Loop Variations for Discrimination of Commercial Korean Native Chicken Populations

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ABSTRACT The increasing demand for Korean native chicken meat indicates that the discovery of haplotypes is very important from both economic and conservation points of view. In this study, mtDNA D-loop sequences from two crossbred Korean native chicken populations of 138 individuals were investigated. Twenty six nucleotide substitutions were identified from sequence analysis and were classified into 12 haplotypes. The haplotype H_8 represents 73.47% of Woorimatdag (chicken population) sequences, which were identified in all five Woorimatdag chicken populations investigated. The H_7 haplotype (Dhap1) for D population covers 45% sequences, which indicate maternal inheritance from black Korean native chicken. On the other hand, Chap3 and Chap4 for C population are specific haplotypes, as H_5 and H_2, respectively. Based on the network profiles, six SNPs (C199T, A239G, G242A, A291G, T330C and C391A) of the D-loop region are effective markers for discrimination between Woorimatdag and Hanhyup chicken populations. Also, the phylogenetic analyses of Woorimatdag and Hanhyup chicken populations were used to identify the genetic relationships among the haplotypes. The results presented here can be used for developing molecular markers to discriminate between two commercial Korean native chickens.

(Key words : D-loop variation, haplotype, Korean native chicken)

INTRODUCTION

In Korea, there has been a strong increasing demand for meat of the Korean native chicken. Two commercial Korean native chicken populations (Woorimatdag and Hanhyup) have recently been developed by crossing Korean native chickens with other commercial chicken breeds in order to increase the meat-related traits. Thus, 'Woorimatdag' has been developed by a government institution, the National Institute for Animal Science (NIAS), and the Hanhyup chicken population was developed by a private company in Korea. Based on the crossing experiments, five distinct Woorimatdag chicken populations have been developed in Korea. For the comparison of Woorimatdag and Hanhyup chicken populations, the D-loop variations in the mitochondrial genome have been initially investigated (Hoque et al., 2011).

In order to understand the evolutionary relationship of domestic and wild populations, DNA technology is routinely applied. This mtDNA has been considered as a powerful source of molecular information to identify the ancestors of many species (Harpending et al., 1998). Polymorphisms in the mtDNA are successfully used for the analysis of phylogenetic and genetic diversity. MtDNA is inherited maternally and has a high mutation rate as well as an absence of recombination (Coble et al., 2004; Vallone et al., 2004). It is not only an ideal marker for the reconstruction of evolutionary relationships among species, but also enables the tracing of hybridization between species and subspecies of domesticated animals (Nijman et al., 2003). The control region or D-loop of the mitochondrial genome is considered more suitable for inter-specific population studies (Baker and Marshall, 1997) than the use of coding genes for distinction of species or breeds by phylogenetic analysis (Moore and Defilippis, 1997). The analysis of the D-loop region in mtDNA sequences from Gallus species and domestic chickens suggests the presence of monophyletic origin in domestic chicken, the sole ancestor of all domestic chickens being the red jungle fowl subspecies (*Gallaus gallus gallus* in Southeast Asia) of Thailand and adjacent areas (Fumihito et al., 1994, 1996). Niu et al. (2002) examined the mtDNA of Chinese native chicken populations

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and confirmed that their most likely origin was Thailand and adjacent geographic areas. Also, the phylogenetic relationships between Korean native chicken and other breeds have recently been investigated using D-loop sequence variations in mtDNA, and attempts to discriminate between Korean native chickens have been made using mtDNA and the LEI0258 marker (Cho et al., 2011; Hoque et al., 2011; Hoque et al., 2009; Lee et al., 2007).

In this study, the chicken mtDNA D-loop region was used for the demarcation of haplotypes in two commercial Korean native chicken populations (Woorimatdag and Hanhyup) in order to construct an appropriate breeding plan as well as to develop molecular markers for these breeds.

MATERIALS AND METHODS

1. Sampling and DNA Extraction

A total of 98 individuals of five different versions of Woorimatdag chicken populations were used in this study. The number of animals used in this study are version 1 (K, n=20), version 2 (A, n=20), version 3 (C, n=20), version 4 (B, n=18), version × (D, n=20) and these were collected from National Institute of Animal Science (NIAS). The blood and muscle samples were used for genomic DNA extraction using modified manual methods (Miller et al., 1988). For the comparison of haplotypes, a previously determined D-loop sequence data from the Hanhyup commercial chicken population (40 samples) was included in the analysis (Hoque et al., 2011).

2. PCR Amplification and Sequencing

The D-loop hyper-variable region in mtDNA was used to PCR amplify a 591 bp fragment by the following primer pair (Forward: 5'-AGGACTACGGCTTGAAAAGC-3' and Reverse: 5'-ATGTGCCTGACCGAGGAACCAG-3'). The PCR reactions included approximately 50 ng of genomic DNA and 2X *Prime Taq* Premix (GeNet Bio, Korea) in a 25 uL reaction volume. PCR was performed in a My-Genie96 Thermal Block (Bioneer, Korea) with an initial denaturation step at 94℃ for 10 min, followed by 35 cycles of 30 sec at 94℃, 30 sec at 61℃, 40 sec at 72℃, and a final extension step at 72℃ for 10 min. The PCR products for mtDNA were electrophoresed on 1.5% agarose gels with ethidium bromide, and DNA bands were visualized under ultraviolet light. Purification of PCR products was performed using an Accuprep®PCR purification kit (Bioneer, Korea) according to the manufacturer's instructions. Purified PCR products were also confirmed using agarose gels for sequencing. All the purified PCR products were sequenced by Genotech (www.genotech.co.kr).

3. Sequence Analysis

The chicken mtDNA D-loop nucleotide sequence data were aligned using the ClustalW program (Thompson et al., 1994). Nucleotide replacement export data from mtDNA were carried out in haplotype sequences by using MEGA5 (Tamura et al., 2011). A rectangular Kimura 2-parameter model neighbor-joining (NJ) phylogenetic tree with 1000 bootstrap replications was constructed using MEGA5. Also, the median-joining network profile with positional SNPs for the differentiation of haplotypes was constructed using NETWORK4610 software (Bandelt et al., 1999).

RESULTS AND DISCUSSION

1. Haplotype Analysis

Based on the analysis of 138 D-loop sequences from Woorimatdag and Hanhyup chicken populations, 26 nucleotide substitutions were identified, representing 12 haplotypes (Table 1). Among these haplotypes, seven haplotypes were identified in Woorimatdag chicken populations. All of the A, B and K populations had haplotype H_8, which may due to their having a common origin in the Rhode Island Red (RIR) breed. However, C and D populations are the offspring of red and black Korean native chicken breeds, which were used as maternal sources. While, the Chap1 haplotype in the C population contains 50% of sequences which were found in H_8 haplotype. Based on their haplotype sharing, RIR and red Korean native chicken are possibly related. Also, H_8 haplotype (Dhap3) in the D population accounts for 20% of sequences. Thus, the concern of specific haplotype, 45% of the H_7 haplotype (Dhap1) of the D population was indicated to have been maternally inherited from black Korean native chicken. Additionally, H_2 (Chap4) and H_5 (Chap3) haplotypes are specific haplotypes in C population. Whereas, C and D chic-

Table 1. Haplotypes identified using the mitochondrial D-loop sequence polymorphisms between Woorimatdag and Hanhyup chicken populations

Haplotype	D-loop variation																									Haplotype within population (No.)[1]
	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3		
	6	7	9	1	1	2	2	3	4	4	4	5	6	6	8	9	0	1	1	1	3	4	6	6	7	9	
	7	7	9	2	7	0	5	9	2	3	6	6	1	5	1	1	6	0	3	5	0	2	3	7	0	1	
H_1	T	A	T	G	C	T	C	A	G	C	C	C	T	C	A	A	T	T	C	C	C	A	C	T	T	A	Hfhap2 (10)
H_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	Chap4 (2)
H_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	Hfhap3 (8)
H_4	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	Hfhap1 (13)
H_5	C	.	.	.	T	.	T	.	.	T	.	T	C	.	.	G	.	C	T	.	.	.	.	C	C	C	Chap3 (3)
H_6	C	.	C	.	T	.	T	.	.	T	.	T	C	.	.	G	.	C	T	.	.	.	.	C	C	C	Chap2 (3), Dhap4 (1)
H_7	C	.	.	.	T	C	T	.	.	T	.	T	C	.	.	.	.	C	.	.	.	.	.	.	.	C	Dhap1 (9)
H_8	C	.	.	.	T	.	T	.	.	T	.	T	C	.	.	.	.	C	.	.	.	.	.	.	.	C	Ahap1 (20), Bhap1 (18), Chap1 (10), Dhap3 (4), Khap1 (20)
H_9	C	.	.	.	T	.	T	G	.	T	.	T	C	.	.	.	.	C	.	.	.	.	.	.	.	C	Hfhap4 (7)
H_10	.	.	.	A	T	.	.	.	.	T	T	T	C	.	.	.	.	C	.	T	.	.	.	.	.	C	Dhap5 (1)
H_11	.	T	.	.	T	.	.	.	.	.	.	.	.	T	G	.	C	.	.	.	.	G	T	.	.	C	Chap5 (2), Dhap2 (2)
H_12	.	.	.	.	T	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	T	C	.	C	Hfhap5 (2)
																											138

[1] The mtDNA haplotypes for the Hanhyup chicken population has been previously published (Hoque et al., 2011)

ken populations are more diverse among Woorimatdag chicken populations, indicating multiple maternal origins for these populations. On the other hand, Hanhyup chicken populations had five haplotypes, H_1, H_3, H_4, H_9 and H_12, as detailed in Table 1. Interestingly, D-loop variations in mtDNA have been clearly differentiated between Woorimatdag and Hanhyup chicken populations. In our analysis, six SNPs of D-loop region can be used as effective markers for discrimination between Woorimatdag and Hanhyup chicken populations by using positional SNPs at C199T, A239G, G242A, A291G, T330C and C391A. Our previous studies also suggested that C225T, A239G and G243A SNPs are effective for chicken population discrimination (Hoque et al., 2011). This study conveys the vital importance of differential markers between commercial Korean native chicken populations.

2. Network Profiling and Phylogenetic Analysis

Based on the network profiles, the relationship of 12 haplotypes from D-loop sequences was identified between Woorimatdag and Hanhyup chicken populations (Fig. 1). This result can also be used to select nucleotide positions for the discrimination of Korean native chicken populations. As has been previously mentioned, Woorimatdag and Hanhyup haplotypes are completely separated by the D-loop nucleotide positions. Three haplotypes, H_1, H_3 and H_4, are representative of Hanhyup haplotypes, which can be differentiated from Woorimatdag haplotypes by observation of the C391A, T330C and C199T nucleotide positions. Among these, the C199T nucleotide position is also used for distinguishing H_5 and H_6 haplotypes. In this case, H_4 is differentiated from H_6 by using A291G or T370C nucleotide positions. Two further Hanhyup haplotypes, H_9 and H_12, are also different to Woorimatdag haplotypes by using A239G and G242A nucleotide substitutions. This network profile is effective for the selection of markers between and within populations. Other study also reported that allele-specific SNP typing in mtDNA D-loop region at the five SNP sites (C310T, A342G, C446T, A686G and C1213T) were SNP haplotypes that can be used for identification of the origins of chicken meat (Harumi et al., 2011).

The phylogenetic analysis between Woorimatdag and Hanhyup chicken populations was conducted in order to identify

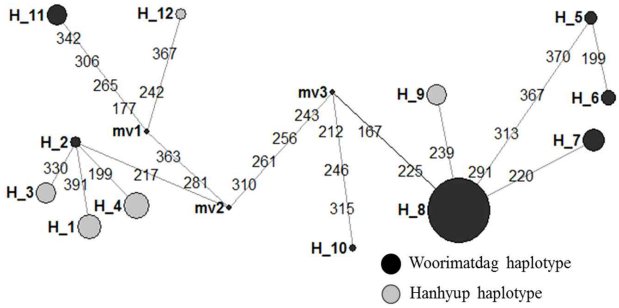

Fig. 1. Network profiles of the 12 haplotypes, which indicate the linked nucleotide positions between and within Woorimatdag and Hanhyup chicken populations. The order of the mutations on the branch is arbitrary. The median vectors (mv) are nucleotide junctions.

the genetic relationships among the haplotypes. Based on the Kimura's 2-parameter model, a neighbor-joining phylogenetic tree was constructed based on the frequencies of transitional and transversional substitution rates (Fig. 2). Besides, five haplotypes were distributed among the Hanhyup chicken population which is distinguishable to Woorimatdag chickens. Based on phylogenetic analysis, three haplotypes in Hanhyup chickens (H_1, H_3 and H_4) are closely related to the Woorimatdag C population. Only one haplotype (H_12) is closely located to Woorimatdag C and D populations. However, H_9 haplotype is closely related with H_7 (Dhap1) and H_8 (Ahap1, Bhap1, Chap1, Dhap3 and Khap1). This result may indicate that Hanhyup chickens were also developed by using red and black Korean native chickens as maternal sources. Therefore, our phylogenetic analyses also provide some clues

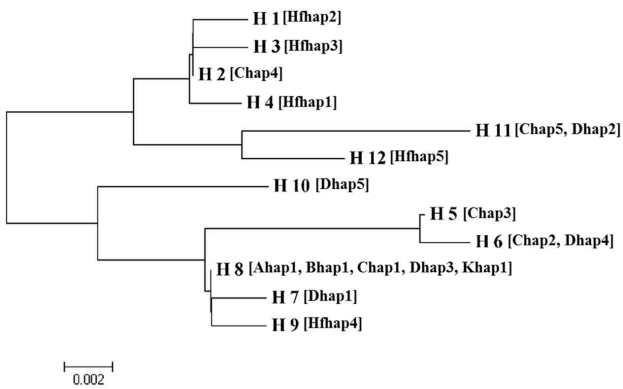

Fig. 2. The constructed neighbor-joining phylogenetic tree using the Kimura 2-parameter model between Woorimatdag and Hanhyup chicken populations.

as to the relationship between Woorimatdag and Hanhyup chicken populations in Korea.

In this study, we investigated the D-loop variations to identify haplotypes, and to compare Woorimatdag and Hanhyup chicken populations. Also, the network profiles and phylogenetic relationships may be helpful for the selection of genetic markers for discrimination among chicken populations.

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