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Analysis of Genetic Characteristics of Korean Native Chicken Using DNA Marker

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DNA Marker를 이용한 한국 재래닭의 유전특성 분석

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ABSTRACT

This study was conducted to analyze genetic characteristics of Korean Native Chicken three lines classified on the basis of the feather color and appearance (Red, Yellow, and Black) using DNA fingerprinting method. To estimate the genetic relatedness among breeds and similarities within breeds, we collected blood samples from Korean Native Chicken (KNC), Rhode Island Red (RIR), White Leghorn (WL), and Cornish(CN) and obtained genomic DNA from the blood of 10 individuals randomly selected within the breeds and lines. The genomic DNA samples were digested with restriction enzymes (*Hinf* Ⅰ, *Hae* Ⅲ) and hybridized with various probes (Jeffreys' probes 33.15, 33.6 and M13) after Southern transfer. Genetic similarities within breeds were characterized by band sharing (BS) value, estimated by the DFP band pattern between the pair of lanes. BS values within WL, RIR, and KNC were 0.82, 0.70 and 0.56, respectively. Relative genetic diversity (BS value) of KNC was higher than those two breeds (WL, RIR). Estimation of genetic similarity between KNC lines and control breed (RIR) was 0.32, whereas similarity within KNC lines (6 groups) was 0.50. In this analysis, KNC was showed to have a highly genetic diversity at the DNA level, and to be closer in genetic distance to RIR (0.67) than any other breeds.

(Key words : Korean native chicken, DNA fingerprinting, band sharing, genetic similarity)

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INTRODUCTION

Most of the early efforts to apply Mendelian genetics to the breeding of domestic animals were concentrated on the aspects of phenotype selection(Hartman, 1988). The selection on phenotype was most powerful approach for quantitative traits. Nevertheless, the possibility of linkages between easily detectable markers and genes responsible for variation in quantitative performance traits continued to provoke scientific interest. Molecular biology offers breeders the technologies to address certain questions on domestication and breeding, because it allows genetic evaluation of breeding animals at genotypic level rather than phenotypic traits. In addition, molecular biology techniques can be applied to classical breeding processes and increase their efficiency. With the advent of molecular biological techniques, DNA-based approaches for genetic analysis have been proposed in numerous organisms. Many hypervariable regions have been discovered in human DNA (Jeffreys et al, 1985; Nakamura et al., 1987). The hypervariable region consists of tandem repeats of short DNA sequence, so-called "minisatellites", and the polymorphism are generated from allelic difference in the number of tandem repeat (VNTR). DNA fingerprints (DFP) are the result of genetic polymorphism of fragments hybridized with multiple tandem repetitive sequences or hypervariable minisatellites (Jeffreys et al, 1985). The use of hypervariable minisatellites in production of DNA fingerprints (DFP) has also provided a powerful tool for identification of individuals and for detecting such polymorphisms in animal species(Jeffreys et al., 1987). When Georges et al. (1988) applied DNA fingerprinting to various species of domestic animals with different minisatellite probe, they found numerous polymorphic loci (VNTR loci) and considerable variation at the level of DNA. Recent work has shown that DNA fingerprints can be used to estimate the levels of inbreeding and genetic distance. Examples include assessment of genetic distance between strains of poultry (Kuhnlein et al., 1989), estimation of relative genetic variability in natural populations of birds and mammals (Wetton et al., 1987 ; Gilbert et al., 1990), and correlations between DFP patterns and inbreeding levels in domestic poultry (Kuhnlein et al., 1990). In addition, genetic information derived from DFP has been employed to identify the bands linked to quantitative trait loci (QTL) (Plotsky et al., 1990) and to prove availability of DFP in identifying the degree of gene introgression in genomes (Hillel et al., 1990). Although there may be some clustering and cosegregation of DFP markers, most DFP bands represent independent loci, and therefore the DFP pattern reflects a broad screening of the genome (Hillel et al., 1989).

The objectives of this study was to evaluate the genetic variation within chicken breeds and to estimate the genetic relationship among poultry breeds (Korean Native Chicken, White Leghorn, Rhode Island Red, Cornish) by DNA fingerprints.

MATERIALS AND METHODS

1. Genetic stocks

We used four breeds of chickens including Korean Native Chicken (KC), White Leghorn (WL), Rhode Island Red (RIR), and Cornish (CN). KNC has been classified into three lines according to their feather color and shape. The first line is Korean Native Yellow Chicken

(KNYC) with yellow feather color, the second one is Korean Native Red Chicken (KNRC) with red feather color, and the third one is Korean Native Black Chicken (KNBC) with black feather color. KNC classified into three lines are phenotypically very different from other foreign breeds. Ten individuals of each breed were randomly selected and studied.

2. Preparation of DNA

Venous blood was collected in vacuum blood collection tubes containing heparin from wing vein of chickens. Five ml of blood samples were diluted into 1 X SSC (0.15M NaCl, 15mM trisodium citrate, pH 7.0) and washed twice with 1X SSC . One hundred microliter of the pellet was resuspended with high TE (100mM Tris-Cl pH8.0, 40mM EDTA pH 8.0) and lysed with lysis buffer (100mM Tris-Cl pH 8.0, 40mM EDTA pH 8.0, 0.2% SDS). The lysates were extracted twice with phenol : chloroform : isoamyl alcohol (25:24:1) and once with 24:1 (v/v) mixture of chloroform : isoamyl alcohol as described by Sambrook et al. (1989) and DNA was precipitated by the addition of 2.5 volumes of ethanol, rinsed with 70% ethanol and dissolved in TE, pH 8.0.

3. DNA fingerprinting

1) Probe preparation

The human minisatellite probe, 33.6, was kindly provided by Dr. Alec J. Jeffreys, University of Leicester, UK. Probe 33.6 consisted of a 0.7kb *Bam*H I plus *Eco*R I insert in pBluescript II vector. The plasmid was transformed into *E. coli* (DH5α) by $CaCl_2$ method. The transformed *E. coli* was incubated in LB media with ampicillin (60μg/ml). Prepared plasmid was digested with restriction enzymes. The probes were purified in low-melting temperature agarose gel. Also, the protein Ⅲ gene of M13 was isolated for probe as by Vassart et al. (1987).

2) Southern hybridization

Ten μg of DNA samples were digested with 30 unit of restriction enzymes *Hinf* I or *Hae*Ⅲ at 37℃ for 24hr. The samples were electrophoresed (1V/cm) in a 0.8~1% agarose gel in TAE running buffer. The DNA was transferred onto nylon membrane (Hybond-Nfp, Amersham) and prehybridized in 0.263 M sodium phosphate pH 7.2, 7% SDS, 1mM EDTA, and 1% BSA for 1-2h at 65℃. Hybridization was performed in the same buffer with the addition of ^{32}P- labeled probes (33.6, 33.15, or M13 mp18 single strand DNA) for 12-16h at 65℃. Probe labeling was processed according to the procedures of random primed DNA labeling Kit (Boehringer Mannheim Biochemica) or nick translation kit (Amersham). Washes were carried out at low stringency - twice with 2 X SSC, 0.5% SDS, 10 min each at room temperature, and then twice with 0.1 X SSC, 0.5% SDS for 30 min at 50℃. Membranes were autoradiographed for 2~7 days at −70℃, using AGFA CURIX X-ray film, in the presence of two intensifying screens.

4. Statistical analysis

Band sharing (BS) levels were calculated to estimate the degree of genetic distance and similarity between two populations (Jeffreys and Morton, 1987; Wetton et al., 1987). Band sharing(BS)=2 Nab/(Na+Nb). Where BS is the level of band sharing between lane a and b, Nab is number of bands shared by lane a and b, Na is total number of bands for lane a, Nb is total number of bands for lane b. Genetic distance (D) between two populations was calculated as D=1−BS. Genetic distance based on

the band sharing level of DFP between breeds was used to construct dendrogram using the cluster analysis programmed in SAS package (SAS, 1985).

RESULTS AND DISCUSSION

Figure 1 shows DFP band patterns in four chicken breeds with probe 33.6. Pooled DNA were prepared from equal amounts of blood from 10 individuals per breed. Probe 33.6 detected the more scorable bands with homogeneous intensity than other two probes(figures not shown), which was in agreement with the results reported by Hillel et al. (1989) and Vassart et al. (1987). Pooling DNA from individuals within a lines or breeds was an effective tool for comparing DFP band patterns among various groups. Two mixed DNA samples per breed were made to estimate genetic similarities within breed using pooled DNA of randomly chosen individuals. The degree of genetic variation within breed could be represented by the value of band sharing between the chickens of each breed (Kuhnlein et al., 1989).

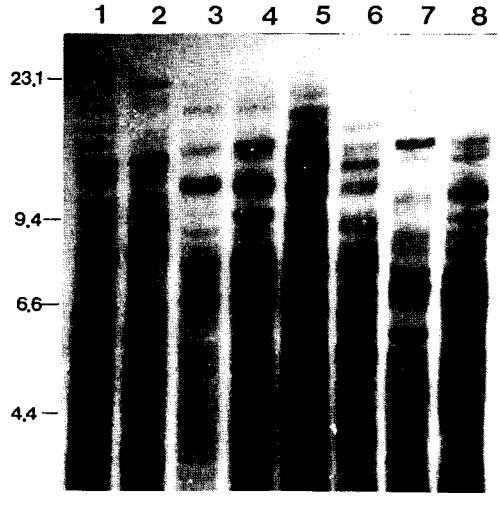

Figure 1. DNA fingerprints of KNBC, WL, RIR, and CN using Jeffreys' probe 33.6 and *Hae* Ⅲ (lanel, 2 : Korean Native Black Chicken ; lane 3, 4 : Cornish ; lane 5, 6 : White Leghorn ; lane 7, 8 : Rhode Island Red).

Table 1. Band sharing level and band number for various breeds using the Jeffreys' probe 33.6 and Hinf I

Breed	KNBC		WL		RIR		CN	
Replication group	1	2	1	2	1	2	1	2
No. of bands	12	13	10	12	11	12	10	11
Band sharing level	0.56		0.82		0.70		0.68	
Common bands	7		9		8		7	

KNBC : Korean Native Black Chicken, WL : White Leghorn, RIR : Rhode Island Red, CN : Cornish.

Table 1 shows band sharing levels within each chicken breed and band number per lane of DNA fingerprints using probe 33.6 and Hinf Ⅰ. Those estimations of band sharing value were reflected as parameters of genetic diversity within breed based on pooled DFP patterns. According to table 1, band sharing levels based on the DFP patterns of two groups within breed showed more identical patterns in WL and RIR. On the basis of band sharing level, WL(0.82) and RIR (0.70) seemed to be pure breeds as like the report by Dunnington et al. (1991). In other hands, BS value between two groups of DFP band pattern within KNC was much low comparing with those of previous two breeds. Therefore, KNC was thought to have high genetic diversity from the evaluation of BS value. These results reflected the breeding background of KNC that it had began to be bred systematically in early 1980.

To estimate genetic variation and diversity

in detail within the KNC, DFPs included 6 KNC population and RIR as a control breed. Table 2 showed the estimation of BS values among the 6 population of KNC and RIR using Jeffreys' 33.6 and M13. Average BS value for the 6 population of KNC was 0.50 and that of between KNC and RIR was 0.32. Plotsky et al. (1995) reported that BS values of DFP band pattern within breeds were higher than those among breeds.

Table 2. Band sharing coefficients between strains of Korean Native Chicken and RIR

	Lines						RIR
	K_1	K_2	K_3	K_4	K_5	K_6	
K_1		0.58	0.48	0.44	0.38	0.53	0.37
K_2			0.63	0.50	0.51	0.56	0.32
K_3				0.50	0.34	0.38	0.32
K_4					0.65	0.44	0.33
K_5						0.59	0.27
K_6							0.32

Average BS within KNC = 0.50 ± 0.09, RIR vs. KNC = 0.32

K_1~K_2: Korean Native Black Chicken,
K_3~K_4: Korean Native Red Chicken,
K_5~K_6: Korean Native Yellow Chicken,
RIR:Rhod Island Red

Table 3. Matrix for genetic similarity(BS) and genetic distance(D) between pair of breeds by DFP generated with M13 and Hae Ⅲ

	KNBC	RIR	WL	CN
KNBC		0.33	0.30	0.21
RIR	0.67		0.26	0.14
WL	0.70	0.74		0.23
CN	0.79	0.86	0.77	

Note : BS is above the diagonal, D is below the diagonal
KNBC : Korean Native Black Chicken
WL : White Leghorn, RIR : Rhode Island Red
CN : Cornish

To differentiate genetic identity of each breed of chicken, strategy for mixing the individual DNA was used. We obtained DNA fingerprinting of KNC and other foreign control breeds with Jeffreys' 33.6 and M13 probe with various restriction enzymes for those purposes (Hillel et al., 1990). Genetic similarity and distance were calculated from the BS values of DFP band pattern among breeds and those result were shown in Table 3. This matrix was used to generate a dendrogram by UPGMA method (Figure 2). The level of genetic similarity between breeds appeared to be the highest in the pair of KNC and RIR (0.33) than any other pairwise comparison. These results were compatible with those relationship among chicken breeds in previous study of DNA fingerprinting study including Korean Native Ogol Chicken (Lee et al., 1995). Also it means that genetic characteristics of KNC are more close to RIR than other breeds. Additionally, genetic diversity within each strain of KNC was much higher than any other

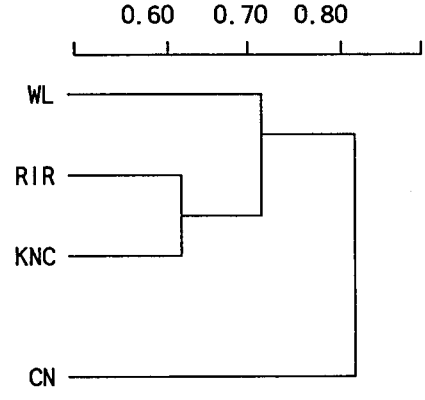

Figure 2. Genetic relationship among four breeds of chickens. A dendrogram was based on genetic distance(D) calculated using DFP(KNC: Korean Native Chicken, WL: White Leghorn, RIR: Rhode Island Red, CN: Cornish).

purebred and also, as expected, genetic diversity within strains of KNC was lower than the comparison between KNC and other breeds.

In conclusion, KNC was not a highly inbred line yet(BS<0.80), therefore, the efforts to establish the KNC line should be maintained more several generations. Genetic similarity between KNC and RIR was the greatest (0.33) in the pairwise comparison among chicken breeds in this study. The greatest genetic distance was observed in the comparison between KNC and CN breed, which were consistent with the results of Yeo et al. (1994) for KNC with gray feather color. However, for the correct conclusion about genetic relationships of KNC with other breed, there will be some needs in near future to perform the study on genetic characteristics of KNC with more large samples and various DNA probes.

적 요

본 연구는 DNA fingerprinting (DFP) 기법을 이용하여 한국 재래닭 집단의 유전특성을 파악하기 위하여 실시되었다. 사용된 공시축은 국내에서 외모 및 우모색으로 구분하여 분리 육성중에 있는 3종류의 재래닭 집단을 대상으로 하였으며 (황갈색계통, 적갈색계통, 흑색 계통), 외래종과의 유전적 거리, 집단내 상대적 유전변이를 추정하기 위해 White Leghorn (WL), Rhode Island Red (RIR) 및 Cornish (CN) 등 각각의 외래종 집단에서 10수씩 혈액을 채취한 후 DNA를 추출하였다. 추출한 DNA를 제한효소 (*Hae* Ⅲ, 또는 *Hinf* Ⅰ)로 자른 후 0.8~1.0% agarose gel에서 전기영동을 실시하였고 Southern blotting 방법에 의해 nylon membrane에 전이한 후 Jeffreys' probe 33.15와 33.6 및 M13을 이용하여 hybridization 시켰다. White Leghorn과 Rhode Island Red의 품종내 유사도 (BS : band sharing)는 0.82 및 0.70으로 추정되었고 동일 계통내 재래닭 (흑색계통)의 2 group 간에는 0.56으로 나타난 것으로 보아 외래종 순종계통내 유전적 변이성보다 재래닭 집단내 유전적 변이성이 높은 것으로 나타났다. 재래닭 집단내 계통간 유전적 유사성을 추정하기 위해 각 계통별 2 group씩 각각 5수의 혈액을 혼합하여 DFP를 실시하였으며 이때 재래닭 집단내 각 계통간 평균 BS수준은 0.50이었으며 이들 재래계 집단과 대조집단인 Rhode Island Red와는 평균 0.32로 추정되었다. 품종간 유전적 거리 (D)에 있어서 재래계는 Rhode Island Red (0.67)와 가장 가까운 것으로 나타났으며 다음으로 White Leghorn (0.70), 육용계인 Cornish (0.79) 순으로 나타났다.

(색인: 한국재래닭, DNA 표지, 유전특성)

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