家禽誌 23(3) : 135~144(1996) Korean J. Poult. Sci. 23(3) : 135~144(1996)

Analysis of Genetic Characteristics by Biochemical Genetic Markers in Korean Native Chicken

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생화학적 유전표지인자에 의한 한국재래닭의 유전특성 분석

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ABSTRACT

This study was carried out to clarify the genetic constitution of biochemical polymorphic loci controlling blood protein and enzymes as genetic markers in Korean native chicken(KNC) population. Blood samples were collected from 230 KNC representing three colored-lines(reddish-, yellowish- and blackish- brown) raised in Daejeon branch of National Livestock Research Institute. Eight blood marker loci, transferrin(Tf), post-albumin(Pas), albumin(Alb), amylase-1(Amy-1), esterase-1(Es-1), alkaline phosphatase(Akp), catalase(Cat) and hemoglobin(Hb) were analyzed by using starch, agarose and polyacrylamide gel electrophoresis. Based on the gene frequencies of polymorphic marker loci, the genetic characteristics of KNF population was analyzed, and the genetic variability within population was quantified. The genetic relationships between KNC and other native fowls or improved breeds were also estimated. The gene frequencies of Tf, Pas and Alb loci were similar to those of improved breeds among the seven biochemical polymorphic loci, while gene frequencies of Cat and Es-1 loci were remarkably different between KNC and improved breeds. Gene frequencies of amy-1 and Akp loci were similar to those of New Hampshire and Rhode Island Red and White Leghorn, respectively. However in comparison with other improved breeds, great differences were observed in gene frequencies of these loci. The average heterozygosity, effective number of alleles and homogeneity index for the seven loci combined were estimated to be .334, 1.639 and .373, respectively. Based on the dendrogram and genetic distances, the KNC was genetically closer to New Hampshire, Plymouth Rock and Rhode Island Red breeds than to the White Leghorn breed.

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(Key words: biochemical polymorphism, genetic markers, genetic analysis, Korean native chicken)

INTRODUCTION

Korean native chickens(KNC) have been reared in the country as purebred chicken for a long period of time and their breeding rate reached about 85.6% in 1922. However with the introduction of improved breeds and the increased crossbreeding, the number of KNC has drastically decreased. Today, the pure KNC are in serious danger of extinction. Every native breed has its own specific history and is adapted to the local climate, even though its productivity may be low. Native chickens seem to have a particular genetic background, different from improved breeds of chicken, and will be important as genetic resources for future use in poultry breeding. Therefore, it is urgent to conserve the endangered breeds such as pure native chickens in the viewpoint of preservation of animal genetic resources. Nowadays, due to changing circumstances in livestock production and animal products, genetic variations which exist in native breeds have become highly valuable.

Although morphological and archaeological evidence based on classical literature has still plays an important role, current trends on the studies of the native breeds are the utilization of biochemical and molecular data. In genetic analysis of breed populations, genetically determined variants of protein and enzyme are widely used as genetic markers of corresponding structural genes. The biochemical markers used do not depend on environmental factors and are stable throughout ontogenesis and have a simple mode of inheritance. A great deal of data concerning gene frequencies at biochemical polymorphic loci are now available in literature. These data can provide more reliable evaluation of the genetic constitution, variability and divergence in various native breeds. During the last decade, information on gene frequency of biochemical polymorphic marker loci such as blood protein and enzymes has been used for genetic characterization and differentiation among Asian native chicken breeds (Okada et al., 1980, 1984, 1988, 1989; Hashiguchi et al., 1981, 1986, 1993 ; Cheng et al., 1991a,b; Yamamoto et al., 1991, Maeda et al., 1992). Recently, Chung et al.(1994) reported genetic characteristics of the KNC population raised in the Kang Won Do province using gene frequencies at seven blood marker loci.

This study was conducted to clarify the genetic constitution of the KNC population using marker genes controlling the blood protein polymorphisms.

MATERIALS AND METHODS

1. Blood samples

The blood samples of KNC were collected from population maintained at the Daejeon branch of National Livestock Research Institute. A total of 230 blood samples consisted of three lines designated yellowish-brown line (YBL; 100 birds), reddish-brown line(RBL; 80 birds) and blackish-brown line(BBL; 50 birds) according to the their feather colors.

All blood samples were separated into plasma and red cells and stored at −20℃ until used in the analysis.

2. Blood typing

Eight biochemical polymorphic loci controlling blood protein and enzymes were screened for genetic variation by means of starch, agarose and polyacrylamide gel electrophoresis. The list of the genetic loci examined in the present study is given in Table 1.

3. Statistical analysis

Gene frequencies were calculated by the direct gene counting method for the loci with codominant alleles, while the square-root method was used for the Akp locus with a recessive null allele. Chi-square tests for the goodness of fit by the Hardy-Weinberg equilibrium were carried out according to the method of Falconer(1989). The genetic variability within populations was quantified by measuring the expected heterozygosity(H), the effective number of alleles(Ne) and genetic homogeneity(H. I)(Nei, 1975,1978; Chung et al., 1994). Genetic distances were estimated from gene frequencies of every pair of the populations(Nei, 1972). The genetic similarity between breed population was presented in form of a dendrogram. The dendrogram was constructed from the genetic distance matrix by the unweighted pair-group arithmetic average clustering method(UPGMA) of Sneath and Sokal(1973). BIOSYS program (Swofford and Selander, 1981) was used for statistical analyses.

RESULTS AND DISCUSSION

Gene frequencies for the eight blood protein and enzyme loci are given in Table 2, and gene frequencies of KNC population were compared with those of the east Asian native and improved breeds in Table 3. Of the eight loci examined, seven loci(Tf, Pas, Alb, Cat, Amy-1, Akp and Es-1) showed polymorphism. However, Hb locus was monomorphic in all populations. In the Tf locus, the frequency of Tf^B allele was considerably higher than that of Tf^C allele in all three lines. This result was very similar to that of a previous study of Chung et al.(1994). Similarly higher frequency of Tf^B allele was observed in other native chickens and improved breeds(Tanabe et al., 1977; Hashiguchi et al., 1981; Kimura et al., 1981; Okada et al., 1984). Although most chicken breeds have a high frequency of Tf^B allele, Tf^C allele was observed at a high frequency(o.82) in green jungle fowl of Indonesia(Hashiguchi et al., 1993). Stratil(1968) reported that the Tf locus was controlled by three codominant alleles, Tf^A, Tf^B and Tf^C in domestic fowl. The Tf^A allele, which is present

Table 1. List of blood protein and enzymes examined

Locus	Name of blood protein & enzyme	Reference
Tf	Plasma transferrin	Juneja (1981)
Pas	Plasma post albumin	Kuryl and Gasparska (1976)
Alb	Plasma albumin	Stratil (1968)
Amy-1	Plasma amylase-1	Hashiguchi et al. (1970)
Es-1	Plasma esterase-1	Grunder (1968)
Akp	Plasma alkaline phosphatase	Tanabe et al. (1977)
Cat	Red cell catalase	Ueda and Hachinohe (1984)
Hb	Red cell hemoglobin	Juneja (1981)

Table 2. Gene frequencies of blood protein and enzyme loci in Korean native chicken

Locus	Allele	YBL[1]	BBL[1]	RBL[1]	Total		SE
Tf	A	–	–	–	–		
	B	.9156	.9609	.8387	.9019	±	.035
	C	.0844	.0391	.1613	.0981	±	.035
Pas	A	.3539	.3587	.3666	.3574	±	.005
	B	.6461	.6413	.6334	.6426	±	.005
Alb	A	.0939	.0325	.1563	.0826	±	.037
	B	.9061	.9675	.8437	.9174	±	.037
	C	–	–	–	–		
Cat	A	.0603	–	–	.0335	±	.020
	B	.9397	1	1	.9665	±	.020
Amy-1	A	.4900	.5195	.7100	.5048	±	.011
	B	.5100	.4805	.2900	.4952	±	.011
	C	–	–	–	–		
Akp	Akp	.3775	.0800	.2308	.2593	±	.083
	akp	.6225	.9200	.7692	.7407	±	.083
Es-1	A	.1402	.1266	.1563	.1380	±	.008
	B	.4100	.5696	.4531	.4660	±	.048
	C	.4497	.3038	.3906	.3960	±	.043
Hb	A	0	0	0	0		
	B	1	1	1	1		

[1] YBL: yellowish-brown line, BBL: blackish-brown line, RBL: reddish-brown line

at low frequencies in Indonesian and Nepalese native fowl populations, was not found in the KNC. At the Pas locus, the predominant gene was Pas^B with a frequency of 0.643 in total line, whereas Pas^A was in low frequency(0.357). However, these values were somewhat different from those of other KNC population(Chung et al., 1994). Chung et al.(1994) reported that the frequencies of Pas^B and Pas^A alleles were 0.833 and 0.167 in the KNF population of the Kang Won Do province, respectively. This difference may be due to limited sampling in a particular region. Based on the allele frequencies, it was assumed that Pas locus is probably controlled by two codominant alleles, Pas^A and Pas^B. For the Alb locus, frequency of the Alb^B allele overwhelmingly predominates all three lines. This gene has a frequency of 92% in total line. Contrary to this, the Alb^A allele has a frequency of only 8%. These results appeared to be similar to gene frequencies reported for those of other breeds. In the previous study, Chung et al. (1994) reported the existence of Alb^C allele with a frequency of 12.7% in the KNC population. However, this allele was not observed in the present study. Stratil(1968) suggested that Alb polymorphisms were controlled by three codominant alleles, Alb^A, Alb^B and Alb^C, at a single autosomal locus. Concerning the Cat locus, the frequency of Cat^B allele(0.966) was much higher than that of Cat^A allele(0.034) in the total line. The Cat^A alleles, which are present at extremely low frequencies, were found only in KNCY line. These allelic frequencies were very similar to those observed in the previous study of Chung et al.(1994). When these results were compared

Table 3. Comparison of gene frequencies between Korean native chicken and foreign breeds

Locus	Allele	KNC (230)[1]	GIFU[2] (47)	IND (138)	NEP. (272)	PHI (64)	WL (2133)	NH (637)	PR (824)	RIR (462)	CN (533)
Tf	A	–	–	.004	.024	–	–	–	–	–	–
	B	.902	1	.992	.874	1	.990	.990	.980	.980	.990
	C	.098	–	.004	.102	–	.010	.010	.020	.020	.010
Pas	A	.357	–	–	–	–	.249	–	.290	–	–
	B	.643	–	–	–	–	.751	1	.710	1	1
Alb	A	.083	–	.000	.016	–	.048	.072	.010	.010	.010
	B	.917	1	.982	.994	1	.952	.906	.960	.990	.950
	C	.000	–	.018	.040	–	.000	.022	.030	–	.040
Cat	A	.034	–	–	–	–	.740	.560	.390	.460	.470
	B	.967	–	–	–	–	.260	.440	.610	.540	.530
Amy-1	A	.505	1	.862	.184	.675	.050	.530	.730	.500	.680
	B	.495	–	.138	.804	.325	.950	.470	.270	.500	.320
	C	.000	–	–	.012	–	–	–	–	–	–
Akp	F	.259	.039	.119	.192	.311	.250	.120	.430	.180	.390
	S	.741	.961	.881	.808	.689	.750	.880	.570	.820	.610
Es-1	A	.138	.375	.406	.315	.273	.780	.380	.190	.560	.300
	B	.466	.514	.545	.658	.696	.170	.600	.800	.430	.690
	C	.396	.111	.049	.027	.031	.050	.020	.010	.010	.010
Hb	A	–	–	–	–	–	–	–	–	–	–
	B	1	1	1	1	1	1	1	1	1	1

[1] ( )=Number of birds examined

[2] GIFU = Gifugidori as Japanese native breed(Hashiguchi et al., 1981)
IND = Indonesian native breed(Hashiguchi et al., 1981)
NEP = Nepalese native breed (Maeda et al., 1992)
PHI = Philippine native breed(Hashiguchi et al., 1981)
W L = White Leghorn(Kimura, 1981)
N H = New Hampshire(Kimura, 1981)
P R = Plymouth Rock(Kimura, 1981)
RIR = Rhod Island Red(Kimura, 1981)
C N = Cornish(Kimura, 1981)

with those of other improved breeds, as shown in table 3, it was obviously different from the data of improved breeds. The Cat polymorphism was shown to be controlled by two alleles, Cat^A and Cat^B, at an autosomal locus(Ueda and Hachinohe, 1984). In the case of Amy-1 locus, the frequencies of two alleles, Amy-1^A and Amy-1^B, were nearly equal in YBL and BBL, whereas frequency of Amy-1^A allele was higher than that of Amy-1^B in RBL line. The gene frequencies of Amy-1^A and Am-1^B was 0.505 and 0.495, respectively in total population. These values were similar to the values obtained previously (Chung et al., 1994). The Amy-1^C allele was found previously from Japanese Onagadori and Nepalese native breeds(Hashiguchi et al., 1981), but this allele was not observed in KNC. Generally, the gene frequencies of Amy-1 locus

tend to be considerably different between the breeds. Tanabe et al.(1977) reported that Amy-1 isozymes are controlled by three autosomal codominant alleles, Amy-1^A, Amy-1^B and Amy-1^C. In the Akp locus, the frequency of the akp allele was higher than that of the Akp allele in all three lines. The BBL has relatively high frequencies of akp allele and a lower frequency of Akp allele compared to the other two lines. The frequencies of Akp and akp alleles in total line were 0.259 and 0.741, respectively. The Akp isozyme types were shown to be controlled by a dominant(Akp) and a recessive(akp) allele(Wilcox, 1966). Among the three alleles of Es-1 locus, Es-1^B and Es-1^C alleles were most frequently observed, whereas the frequencies of the Es-1^A allele were relatively low. The frequencies of Es-1^B allele were somewhat higher than those of Es-1^C allele in BBL and RBL, but the frequencies between Es-1^B and Es-1^C alleles were observed to be similar to those of YBL. In total line, gene frequencies were 0.14 for Es-1A, 0.47 for Es-1^B and 0.39 for Es-1^C alleles. However, Chung et al.(1994) reported that gene frequencies of Es-1^A, Es-1^B and Es-1^C in the KNC population of the Kang Won Do province were 0.04, 0.87 and 0.08, respectively. Therefore, there appeared to exist some differences between gene frequencies of two populations. Especially, the Es-1^C allele of the KNC population appeared with much higher frequency than those from improved breeds as shown in Table 3. Grunder(1968, 1971) reported that the Es-1 variants were controlled by three codominant alleles, Es-1^C, Es-1^A and Es-1^B.

The results of the chi-square tests for the goodness of fit by the Hardy-Weinberg equilibrium are presented for seven blood protein and enzyme loci in Table 4. In all loci except for Tf and Amy-1, the χ^2 values were statistically significant in total population. Therefore, this population was not in genetic equilibrium for Pas, Alb, Cat, Akp and Es-1 loci as a whole. The small population sizes, genetic drift, isolation and an obvious inbreeding in the KNC population may be the major cause of a genetic disequilibrium.

For the evaluation of genetic variability within KNC population, the average heterozygosity, the effective number of alleles and the genetic homogeneity per locus were calculated as shown in Table 5. The genetic variability of BBL population was lower than those of YBL and RBL populations. This result may be due to inbreeding in a small population size. Among the seven loci, the Es-1 locus showed the highest

Table 4. Hardy-Weinberg equilibrium test for the blood protein and enzyme loci in the Korean native chicken population

Locus	YBL			BBL			RBL			Total		
	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
TF	.489	2	<.750	1.680	2	<.250	.268	2	<.750	2.120	2	<.250
Pas	12.101	1	<.001	19.643	1	<.001	4.133	1	<.025	37.599	1	<.001
Alb	5.823	2	<.050	2.187	2	<.250	1.129	2	<.500	10.857	2	<.001
Cat	108.796	1	<.001	.003	1	<.900	.007	1	<.900	196.823	1	<.001
Amy-1	.024	2	<.975	.008	2	<.995	2.720	2	<.250	.003	2	<.995
Akp	94.775	1	<.001	62.960	1	<.001	21.591	1	<.001	184.818	1	<.001
Es-1	31.508	2	<.001	73.824	2	<.001	35.851	2	<.001	118.196	2	<.001

Table 5. Genetic variabilities in the population of Korean native chicken

Loci	YBL			BBL			RBL			Total		
	He[1]	Ne[2]	Ho[3]	He	Ne	Ho	He	Ne	Ho	He	Ne	Ho
TF	.155	1.183	.691	.075	1.081	.849	.271	1.371	.459	.177	1.215	.646
Pas	.457	1.843	.085	.460	1.852	.079	.464	1.867	.071	.459	1.850	.081
Alb	.170	1.205	.659	.063	1.067	.874	.263	1.358	.473	.152	1.179	.697
Cat	.113	1.128	.773	.000	1.000	1	.000	1.000	1	.065	1.069	.871
Amy-1	1.500	1.999	.001	.499	1.997	.002	.412	1.700	.176	.500	1.998	.001
Akp	.470	1.887	.059	.147	1.173	.706	.355	1.550	.289	.384	1.624	.232
Es-1	1.610	2.564	.085	.567	2.311	.149	.618	2.616	.074	.607	2.544	.089
Means	.353	1.687	.336	.258	1.497	.522	.340	1.637	.363	.334	1.639	.373

[1] Heterozygosity

[2] Number of effective alleles

[3] Gene homogeneity

expected heterozygosity value(.567~.618) in all three lines. The average heterozygosity(H) for three lines expected from the estimated gene frequencies was in a range .258~.353. The average degree of heterozygosity in total population was estimated as 33.4%. This value was higher than that estimated by Chung et al.(1994), who reported that average heterozygosity in the KNC population was 18.3%. Okada et al.(1984) showed that H values from the data of seven blood protein loci in Japanese native breeds were in the range of 30~40%. These results are similar to those observed in the present study. However, Hashiguchi et al.(1981) reported that H values based on gene frequencies of 18 loci were 4.6~8.6% for Japanese native fowls, 10.5% for Indonesian native fowls, 6.7~13.5% for jungle fowls and 3.4~10.9% for commercial chickens. These differences among the H values may be probably due to the different number of loci and population size examined. In effective number of alleles as the reciprocal of the degree of homozygosity, the highest value among seven loci was 2.544 of the Es-1 locus, whereas the lowest value was the Cat locus(1.069) in total population. The average effective number of alleles calculated for all loci was 1.687 for YBL, 1.497 for BBL, 1.637 for RBL and 1.639 for all three lines. These values were slightly higher than those reported for the KNC population (Chung et al., 1944), for Indonesian native fowls(Hashiguchi et al., 1981) and for Nepalese native fowls(Maeda et al., 1992). When the genetic homogeneity of the population was compared, BBL was the highest(52.2%). The average homogeneity value for all loci was estimated as 37.3% in total population. From the above results, this population had a genetic variability relatively higher than those of other native and improved breeds. This may be due to small number of protein loci excluding monomorphic loci. Therefore, as more loci are assayed, these values of genetic variability are likely to decrease, since loci known to be polymorphic have been included here. In general, protein polymorphisms include many monomorphic loci, so their H value estimated from the data of protein loci will be small(Hashiguchi et al., 1986; Maeda et al., 1988).

The matrix of genetic similarity and genetic distance coefficients among each pair of ten breed populations is given in Table 6 and the

Table 6. Matrix for normalized genetic identity (I) and the standard genetic distance(D) between every pair of breeds at six blood loci

Population	KNC	GIF	IND	NEP	PHI	W L	N H	P R	RIR	C N
KNC	✶✶✶✶	.076	.055	.048	.033	.116	.031	.050	.041	.040
GIF	.927	✶✶✶✶	.005	.151	.040	.235	.047	.057	.059	.049
IND	.947	.995	✶✶✶✶	.105	.019	.178	.024	.036	.032	.026
NEP	.953	.860	.901	✶✶✶✶	.058	.055	.031	.084	.036	.066
PHI	.968	.961	.981	.944	✶✶✶✶	.145	.016	.006	.027	.002
W L	.890	.790	.837	.946	.865	✶✶✶✶	.090	.196	.056	.149
N H	.970	.954	.976	.970	.984	.914	✶✶✶✶	.039	.009	.023
P R	.951	.944	.965	.919	.994	.822	.962	✶✶✶✶	.056	.003
RIR	.960	.943	.968	.965	.973	.946	.991	.946	✶✶✶✶	.032
C N	.961	.953	.975	.937	.998	.862	.977	.997	.968	✶✶✶✶

Below diagonal: genetic identity
Above diagonal: genetic distance

dendrogram derived from genetic distances in Fig. 1. The genetic distances between KNC and other fowl breeds ranged from 0.031~0.116. The closest genetic distance was obtained between KNC and New Hampshire, whereas the highest distance was found between KNC and White Leghorn. The dendrogram diagramatically expressing the genetic similarities between populations may be considered as reflecting their phylogenetic relationships. The results showed

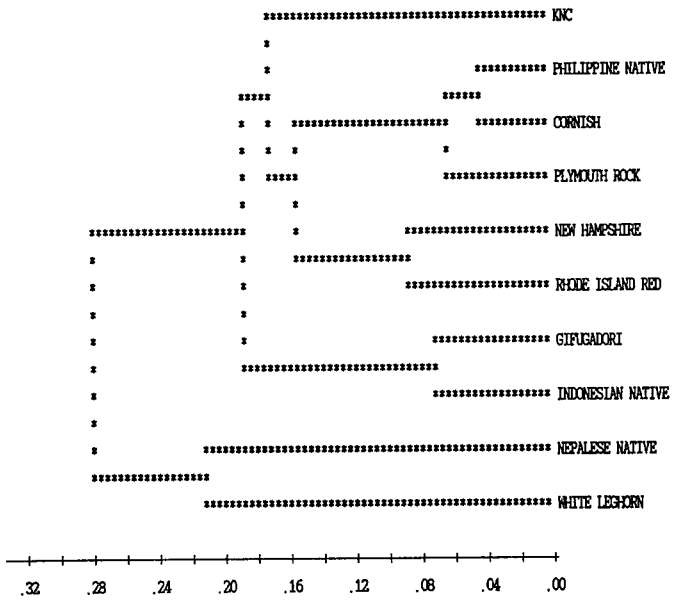

Figure 1. Dendrogram showing phylogenetic relationships among ten fowl breeds, constructed by UPGMA clustering methods.

showed that the native fowl constituted one group with Philliphine native fowl, Cornish, Plymouth Rock, New Hampshire and Rhode Island Red breeds. However, KNC population showed the greatest differentiation from White Leghorn. The results of the present study indicate that among the improved breeds compared the KNC was genetically closer to New Hampshire and Plymouth Rock than to White Leghorn.

적 요

본 연구는 축산기술연구소 대전 지소에서 보존하고 있는 한국재래닭 3계통(적갈색, 황갈색 및 흑갈색) 230수를 대상으로 생화학적 유전 표지인자로서 혈액 단백질(transferrin, albumin, post-albumin 및 hemoglobin) 및 효소(amylase-I, esterase, catalase 및 alkaline phosphatase) 9개 좌위의 생화학적 다형현상을 starch, agarose 및 polyacrylamide gel 전기영동법을 이용하여 분석하고 각 다형좌위별 대립유전자 빈도를 추정하여 집단의 유전적 구조 및 특성을 파악하고 유전적 변이성을 정량화했으며 동시에 개량종 및 동아시아 재래계와의 유전적 근연관계를 분석하였다. 생화학적 다형좌위가운데 Tf, Pas 및 Alb 좌위의 대립유전자빈도는 개량종과 유사했으나 Cat와 ES-좌위는 유전자빈도에 현저한 차이가 인정되었다. 그리고 Amy-1좌위는 New Hampshire와 Rhode Island Red종과 각각 매우 유사한 대립유전자빈도를 나타 냈으나 나머지 개량품종들과는 출현빈도에 많은 차이가 존재하였다. 품종집단의 생화학적 다형좌위별 평균 이형접합성은 .334, 평균 대립유전자 유효수는 1.639 그리고 유전적 균질도는 .373으로 추정되었으며 품종간 유전거리 및 계통도를 비교했을 때 한국재래닭은 White Leghorn 종 보다는Plymouth Rock, New Hampshire 및 Rhode Island Red 종과 더 가까운 유전적 근연관계를 보였다.

(색인: 생화학적 다형현상, 유전적 표지인자, 유전분석, 한국재래닭)

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