Blood Protein Polymorphisms of Native Fowls in Laos[a]

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ABSTRACT : Blood protein polymorphism of fowls in Laos was analyzed by electrophoresis. Blood samples were collected in the area of Viangchan, Louangphrabang and Pakxe. Out of 17 loci, polymorphism was detected at the following seven loci: *Es-1*, *Amy-1*, *Akp-akp*, *Akp-2*, *Alb*, *Tf* and *Pas*. The other ten loci: *Amy-3*, *LDH*, *6-PGD*, *PGM*, *PHI*, *To*, *MDH*, *Es-D*, *Hb-1*, *Hb-2* were noted to be monomorphic. The proportion of polymorphic loci (P_{poly}), the expected average heterozygosity per individual ($\overline{H}$), and the subdivision index (G_{ST}) of the native fowl in Laos was 0.412±0.123, 0.106 and 0.026, respectively. Genetic distance between native fowls in Laos, Bangladesh, Nepal and Indonesia was estimated by a numerical taxonomy method. The population of Laos, Bangladesh and Nepal was clustered in one group. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1011-1014*)

Key Words : Protein Polymorphism, Native Fowl, Jungle Fowl, Genetic Variability, Genetic Distance

INTRODUCTION

Laos is a landlocked country which shares borders with Thailand, Cambodia, Vietnam, China and Myanmar, and its climate belongs to tropics, between latitudes 14° and 23° and longitudes 100° and 108°. The country's borders with Thailand as well as Myanmar is formed by the Mekong River whose source is far away in China's Quinghai Province. Over 70% of the country are mountains and plateaus.

There are many kinds of native fowls in Asia. Most of them have not been improved and have lower productive performance than the improved foreign breeds. Also in Laos, we can find many kinds of native fowl in all areas where farmers live. They rear the native chicken as the important protein source of themselves. So the farmer's flocks of fowl are very small in number. And now, many improved breeds have been introduced from foreign country, mainly Thailand and pure native fowl in Laos are gradually decreasing in number. In facts, the native chicken populations conserve genes which modern breeds have lost. In consequence, it is very important for practical use in future to survey the genetical characteristic of the native fowl and to evaluate it from a viewpoint of genetic resources. This study was conducted to clarify the gene constitution of protein polymorphism of native fowl in Laos.

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MATERIALS AND METHODS

Sampling and location

The surveyed areas and number of samples are shown in figure 1. The native fowls were sampled at Viangchan, Louangphrabang and Pakxe. Samples from a total of 136 Laos native fowls were collected in 1996~1997.

Figure 1. Surveyed areas in Laos
A: Viangchan; B: Louangphrabang;
C: Pakxe; ( ): Number of bird

Blood collection and electrophoresis

All blood samples collected were separated into plasma and erythrocyte and stored separately in a freezer (-40℃). Seventeen loci controlling 14 kinds of

Table 1. List of blood proteins examined

Symbol of locus	Name of blood protein	Cited from
Es-1	Plasma esterase	Okada et al. (1980)
Amy-1	Plasma amylase	Hashiguchi et al. (1970)
Amy-3	Plasma amylase	Hashiguchi et al. (1970)
Akp-akp	Plasma alkaline phosphatase	Okada et al. (19810)
Akp-2	Plasma alkaline phosphatase	Okada et al. (1980)
Alb	Plasma albumin	Mclndoe (1962)
Tf	Plasma transferin	Stratil (1968)
Pas	Plasma post albumin	Kuryl and Gasparska (1976)
LD	Erythrocyte lactate dehydrogenase	Manwell and Baker (1969)
6-PGD	Erythrocyte 6-phosphogluconate dehydrogenase	Bengtsson and Sandberg (1973)
PGM	Erythrocyte phosphoglucomutase	Bengtsson and Sandberg (1973)
PHI	Erythrocyte phosphoheose isomerase	Bengtsson and Sandberg (1973)
To	Erythrocyte tetrazolium oxidase	Baur and Schorr (1969)
MDH	Erythrocyte malate dehydrogenase	Davidson and Cortner (1967)
Es-D	Erythrocyte esterase	Watanabe et al. (1977)
Hb-1	Hemoglobin	Washburn (1968)
Hb-2	Hemoglobin	Washburn (1968)

blood protein were screened for genetic variation by starch gel or agar gel electrophoresis. The list of the genetic loci analyzed in this experiment is given in table 1.

Estimation of gene frequency, genetic variability and genetic distance

The genetic variability within population was quantified by measuring the proportion of polymorphic loci, P_{poly} (Lewontin and Hubby, 1966), and average heterozygosity, $\overline{H}$. The average heterozygosity was estimated using a formula $\overline{H}=\overline{1-q_i^2}$ (Lewontin and Hubby, 1966). Where q_I is the frequency of the Ith allele at a locus, and the bar means the average over all the loci estimated including monomorphic loci. The relative magnitude of gene differentiation among subpopulations was estimated by the coefficient of gene differentiation, *Gst* (Nei, 1975). The coefficient is given by $G_{st}=(\overline{H}_T-\overline{H}_S)\overline{H}_T)$, where $\overline{H}_T$ and $\overline{H}_S$ are the average heterozygosity of total population calculated from average gene frequencies of subpopulations, and a mean of average heterozygosity of subpopulations, respectively. The genetic distance was calculated by Nei's formula (Nei, 1972). From the matrix of the genetic distance values, dendrograms was drawn by the unweighted pair-group method of clustering in numerical taxonomy (Sokal and Sneath, 1963).

RESULTS

Protein polymorphism

Gene constitutions of the Laos native fowl were compared using gene frequencies at the 17 loci controlling blood protein type. The frequencies of allele at the polymorphic loci are given in table 2.

Table 2. Gene frequencies of polymorphic loci of native fowl in Laos

Locus	Area			Total (136)
	Viangchan (57)	Louangphra-bang (44)	Pakxe (35)	
Es-1A	0.254	0.205	0.257	0.239
Es-1B	0.746	0.795	0.743	0.761
Es-1C	0.000	0.000	0.000	0.000
Es-1D	0.000	0.000	0.000	0.000
Amy-1A	0.228	0.261	0.171	0.224
Amy-1B	0.772	0.739	0.829	0.776
Amy-1C	0.000	0.000	0.000	0.000
Amy-1D	0.000	0.000	0.000	0.000
Akp	0.054	0.108	0.044	0.069
akp	0.946	0.892	0.956	0.931
Akp-2a	0.516	0.542	0.455	0.509
Akp-20	0.484	0.458	0.545	0.491
AlbA	0.000	0.000	0.000	0.000
AlbB	0.851	0.852	0.914	0.868
AlbC	0.149	0.148	0.086	0.132
AlbD	0.000	0.000	0.000	0.000
TfA	0.000	0.000	0.000	0.000
TfB	0.939	0.977	0.986	0.963
TfC	0.061	0.023	0.014	0.037
PasA	0.121	0.121	0.014	0.093
Pasa	0.879	0.879	0.986	0.907

( ): Number of bird.

The three surveyed areas, Viangchan, Louangphrabang and Pakxe, showed almost the same gene frequencies in all loci. Of the 17 loci analyzed by electrophoresis, polymorphisms were detected at 7 loci: plasma

esterase (*Es-1*), plasma amylase (*Amy-1*), plasma alkaline phosphatase (*Akp-akp*, *Akp-2*), plasma albumin (*Alb*), plasma transferrin (*Tf*), and plasma post albumin (*Pas*). In the polymorphic loci, figure 2 shows three albumin phenotypes BB, BC and CC. The remaining 10 loci, *Amy-3*, *LDH*, *PGM*, *PHI*, *To*, *MDH*, *Es-D*, *Hb-1* and *Hb-2*, were monomorphic.

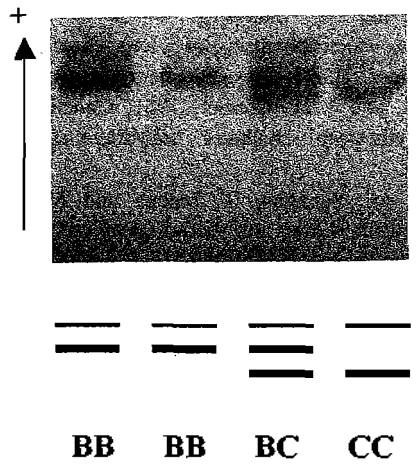

Figure 2. Phenotypic variations of plasma albumin of native fowls in Laos

Genetic variability

The proportion of polymorphic loci (P_{poly}), the expected average heterozygosity per individual ($\overline{H}$) and the effective number of alleles per locus (*Ne*) are shown in table 3. The P_{poly} calculated was 0.412± 0.123 for all local populations in Laos. The $\overline{H}$ varied from 0.137 to 0.162 and was lower in the southern part of Laos, Pakxe. The estimated $\overline{H}$ of the pooled data of Laos was 0.107. The coefficient of gene differentiation G_{ST} among the three populations was 0.026.

Table 3. Quantification of genetic variability in native fowl in Laos

Area	Number of samples	$P_{poly} \pm$ SE	$\overline{H}$	Ne
Viangchan	57	0.412±0.123	0.162	1.193
Louangphrabang	44	0.412±0.123	0.162	1.193
Pakxe	35	0.412±0.123	0.137	1.158
Total	136	0.412±0.123	0.107	1.119

G_{ST} = 0.026.

Genetic relationship

Genetic distance between every pair of the three native fowl populations in Laos are presented in table 4. The distance among the local populations of native fowl were very small and their average was 0.002.

Table 4. Matrix of genetic distance between the respective pair of 3 chicken populations used on 17 loci calculated by using Neis equation

Area	2	3
1. Viangchan	0.001	0.002
2. Louangphrabang		0.002
3. Pakxe		

By including the present data and the previous data of protein polymorphisms in Bangladesh (Okada et al., 1988), Nepal (Maeda et al., 1992) and Indonesia (Hashiguchi et al., 1993), the dendrogram among native fowls and jungle fowls in the South Asia was illustrated in figure 3. Laos native fowl was genetically close to Bangladesh native fowl, and the genetic distance between them was estimated as 0.010.

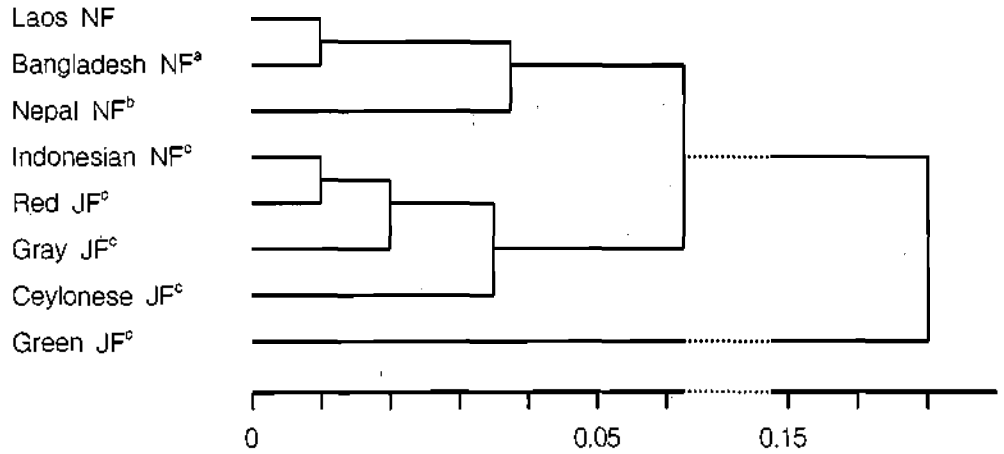

Figure 3. Dendtogram showing genetic similarities among native fowls and jungle fowls (NF: native fowl; JF: jungle fowl; [a] Okada et al. (1998); [b] Maeda et al. (1992); [c] Hashiguchi et al. (1993))

These populations and Nepal native fowl were clustered in one group. Indonesian native fowl formed another cluster with Red, Gray, and Ceylonese jungle fowl. The genetic distance between Indonesian native fowl and Red jungle fowl was estimated as 0.011. Green jungle fowl was genetically far from the both of native fowls and three jungle fowls, and the genetic distance was estimated as 0.171.

DISCUSSION

In the present study, we tried to clarify the blood protein polymorphisms of the native fowl populations in Laos and analyze the genetic relationship with other native fowls and four species of jungle fowls.

The new polymorphic locus was not found in Laos native fowl. Maeda et al. (1993) indicated that the gene frequency of *Amy-1*^{A} is gradually decreasing from south to north. It is well known that the frequency of *Amy-1*^{A} gene is higher in native fowl population in the South East Asia. In consequence, they suggested that the higher frequency in Bangladesh than in Nepal might reflect the influence of gene flow from South East Asia. In this study, it is also recognized that *Amy-1*^{A} gene is decreased in order of Indonesia (0.816), Laos (0.224) and Nepal (0.184).

The genetic variabilities (P_{poly}) of the native fowl in Viangchan, Louangphrabang and Pakxe were same and calculated as 0.412. The P_{poly} of the native fowl in Bangladesh, Nepal and Indonesia were calculated as $0.353 \pm 0.116 - 0.529 \pm 0.121$, 0.471 ± 0.121, 0.438 ± 0.124, respectively. The P_{poly} of the native fowl in Laos was the same as some population in Indonesia (Hashiguchi et al., 1993). The subdivision index (G_{st}) estimated from blood protein polymorphism among the local populations in Laos was calculated as 0.026. This value is about one third of Nepal native fowl (Maeda et al., 1992). It is suggested that the degree of genetic differentiation among the 3 native chicken populations in Laos is very small and migration of chicken might occur frequently.

The genetic distances among the 3 populations in Laos were very small. In the dendrogram by including the present data and the previous data, Laos, Bangladesh and Nepal native fowl belong to the same cluster. Especially, Laos native fowl is genetically close to Bangladesh native fowl. And they are separated from Indonesian native fowl and the 3 jungle fowls.

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