PROTEIN POLYMORPHISMS IN NATIVE AND RED JUNGLE FOWLS IN NEPAL

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Summary

Protein polymorphism of native and red jungle fowls in Nepal was analyzed by electrophoresis. Blood samples were collected in the areas of Solu, Jomson road, Kathmandou, Pokhara and Low land. Out of 17 loci, polymorphism were found at nine loci in native fowls and at three loci in red jungle fowls. The proportion of polymorphic loci (P_{poly}) of native and red jungle fowls were 0.529 + 0.121 and 0.176 ± 0.095, respectively. The five fowl populations in Nepal formed a different cluster from Sri Lankan and Bangladeshi fowl populations. When the gene frequencies of polymorphic loci were compared between the native fowl populations of Sri Lanka, Bangladesh and Nepal, *Amy* 1^A, *Es-*1^A *and Akp-*2^A genes showed inclination of south to north.

(Key Words: Protein Polymorphism, Nepalese Fowls, Red Jungle Fowl)

Introduction

Nepal is located on the south side of the Himalayas, and people inhabit on low land as Tarai plain to mountain side as Namche Bazal located on 3500 m above of the sea level. In all areas where people inhabits, the native chickens are reared as the important protein source of inhabitants. Although the native fowls in Nepal have lower productive performance than the improved foreign breeds, their character of broodiness is very important for farmers to breed the chicks. Usually, flocks of the fowl of farmer in Nepal are small in number (4-5 birds) because of lack of feed for fowl. The native fowl of Nepal are medium birds with 1.2-1.5 kg and their annual egg production is 40-60 in number with weight of about 45 g.

Since 1960's, improved breeds like New Hampshire, Australorp, White Leghorn and their crosses and also the synthetic breeds have been introduced from foreign countries to improve the economical performances of the native fowl. At present, these foreign improved breeds are being distributed to the farmers by the government and private hatchery stations. In consequence, crosses of the native breed and the foreign breeds are advancing and pure native fowl are gradually decreasing in number. 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. Recently, Yamamoto et al. (1991) surveyed the blood group polymorphism in the native fowl population in Nepal.

This study was conducted to clarify the gene constitution of protein polymorphism of native fowl and red jungle fowl in the Nepal.

Materials and Methods

Sampling and location

The surveyed areas and the number of samples are shown in figure 1. The native fowls were sampled at Solu and Jomson Road areas (about 2500 m of above sea level), Kathmandu and Pokhara areas (1500-1700 m of above sea level) and low land area (0-200 m of above sea level). The red jungle fowl was caught at Tarai plain in low land. Forty-eight fowls and 6 red jungle fowl were collected in 1987, and 224 fowls were collected in 1988.

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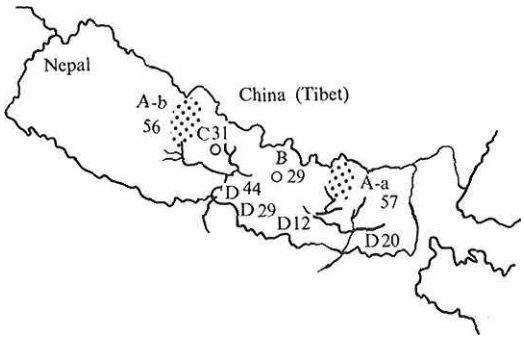

Figure 1. Surveyed areas and number of native fowl examined A-a: High land (Solu), A-b: High land (Jomson road), B: Kathmandu, C: Pokhara, D: Low land (No. of birds in low land include 6 birds of red jungle fowl).

Blood collection and electrophoresis

All blood samples collected were separated into plasma and erythrocyte and stored separately in a freezer (−40℃). These were transported with dry ice air to Japan. Electrophoretic examination were carried out in the Laboratory of Animal Breeding, Hiroshima University, and in Laboratory of Animal Breeding, Kagoshima University.

Seventeen loci controlling 14 kinds of 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, $\bar{H}$. The average heterozygosity was estimated using a formula $\bar{H} = \overline{1 - q_i^2}$ (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 examined including monomorphic loci. The relative magnitude of gene differentiation among subpopulations was estimated by the coefficient of gene differentiation, G_{ST} (Nei, 1975). The coefficient is given by $G_{ST} = (\bar{H}_T - \bar{H}_S)/\bar{H}_T$, where $\bar{H}_T$ and $\bar{H}_S$ 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, dendrogram was drawn by the unweighted pair group method of clustering in numerical taxonomy (Sokal and Sneath, 1963).

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. (1980)
Akp-2	Plasma alkaline phosphatase	Okada et al. (1980)
Alb	Plasma albumin	McIndoe (1962)
Tf	Plasma transferin	Stratil (1968)
Pas	Plasma post albumin	Kuryl and Gasparska (1976)
LDH	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 phosphohexose 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)

Results

Protein polymorphism

Out of 17 loci examined in the present experiment, nine loci (*Es-1, Amy-1, Akp, Akp-2, Tf, Pas, Alb, 6-PGD* and *Hb-1*) in native fowl showed polymorphism. Eight loci in native fowl were monomorphic. The frequencies of allele at the polymorphic loci are given in table 2.

The *Pas* locus was polymorphic in the populations of Kathmandu, Pokhara and low land, but monomorphic in those of Solu and Jomson Road areas in high land (fixed in Pas^A). The frequencies of both genes (Pas^A and Pas^B) in pooled data from five areas were calculated as 0.72 in Pas^A and 0.28 in Pas^B. In the *Akp* locus, the gene frequency (0.243) of *Akp* at low land areas was slightly higher than those (0.147-0.178) of other areas. In the *6-PGD* locus, the frequency of $6\text{-}PGD^A$ gene in low land was higher (0.81) than those (0.48-0.67) of the other areas. In the *Hb-1* locus, phenotype *AB* was observed in the populations of Jomson Road and Pokhara, and frequencies of $Hb\text{-}1^A$ gene in both populations were calculated as 0.02 and 0.03, respectively.

The red jungle fowl caught at Tarai area in low land showed polymorphism at three loci

TABLE 2. GENE FREQUENCIES OF POLYMORPHIC LOCI OF NATIVE CHICKEN AND RED JUNGLE FOWL IN NEPAL

Locus	Area						
	Jomson road (56)	Solu (57)	Kathmandu (29)	Pokhara (31)	Low land (99)	Total (272)	R.J.F[1] (6)
$Es\text{-}1^A$	0.300	0.353	0.428	0.516	0.192	0.315	1.000
$Es\text{-}1^B$	0.700	0.588	0.554	0.452	0.786	0.658	
$Es\text{-}1^C$		0.059	0.018	0.032	0.022	0.027	
$Amy\text{-}1^A$	0.145	0.169	0.161	0.218	0.194	0.184	1.000
$Amy\text{-}1^B$	0.855	0.824	0.839	0.772	0.742	0.804	
$Amy\text{-}1^C$		0.007		0.010	0.064	0.012	
Pas^A	1.000	1.000	0.537	0.618	0.746	0.716	0.184
Pas^B			0.463	0.382	0.254	0.284	0.816
Akp	0.147	0.178	0.176	0.158	0.243	0.192	0.423
akp	0.853	0.822	0.824	0.842	0.757	0.808	0.577
$Akp\text{-}2^a$	0.330	0.470	0.598		0.406	0.410	
$Akp\text{-}2^b$	0.670	0.530	0.402	1.000	0.594	0.590	1.000
Alb^A	0.009		0.018		0.034	0.016	
Alb^B	0.964	0.926	0.911	0.952	0.951	0.944	1.000
Alb^C	0.027	0.074	0.071	0.048	0.015	0.040	
Tf^A	0.009	0.015	0.018	0.048	0.034	0.024	
Tf^B	0.836	0.904	0.893	0.774	0.908	0.874	1.000
Tf^C	0.155	0.081	0.089	0.178	0.058	0.102	
$6\text{-}PGD^A$	0.683	0.478	0.589	0.629	0.809	0.662	0.883
$6\ PGD^B$	0.327	0.552	0.411	0.371	0.191	0.338	0.167
$Hb\text{-}1^A$	0.018			0.032		0.007	
$Hb\text{-}1^B$	0.982	1.000	1.000	0.968	1.000	0.993	1.000

[1] Red jungle fowl.
( ): Number of bird.

(*Pas*, *Akp* and *6-PGP*) and no-polymorphism at fourteen loci. Out of three polymorphic loci, the gene frequencies of *Pas* and *Akp* loci were different from those of the native chicken populations. The gene frequencies of *Pas* locus showed 0.184 in Pas^A and 0.816 in Pas^B.

Genetic variability of the native fowls in Nepal

Genetic variability of each local population was quantified from frequencies at 17 loci controlling blood protein, and the results are shown in table 3. P_{poly} was calculated as 0.471 in all local populations. The P_{poly} of the pooled data was estimated as 0.529. The $\bar{H}$ were varied from 0.12 to 0.17, and were lower in low land population. The coefficient of gene differentiation G_{ST}, among the populations was 0.09. The P_{poly} of the red jungle fowl was estimated as 0.176.

Genetic relationship among five native chicken populations in Nepal, native fowl populations in South Asia and red jungle fowl in Nepal

Genetic distance between every pair of the five native fowl populations in Nepal are presented in table 4. The distance among the local populations of native fowl were generally very small, and their average was 0.018.

By including the present data and the previous data of protein polymorphisms in Sri Lanka (Hashiguchi et al., 1986) and Bangladesh (Okada et al., 1988), the dendrogram among the native and red jungle fowls in the South Asia was illustrated in figure 2. The five fowl populations in Nepal were clustered in one group. Although people can not migrate directly between Solu and Jomson Road, genetic relationship between both populations is relatively close. The population of Pokhara area is separated from other populations.

The Populations of Sri Lanka and Bangladesh formed another cluster. Genetic distance between Nepal population and Sri Lanka-Bangladesh populations was estimated as 0.055. Genetic distance between native fowl and red jungle fowl estimated as 0.130.

TABLE 3. QUANTIFICATION OF GENETIC VARIABILITY IN CHICKENS AND RED JUNGLE FOWL IN NEPAL

Area	Number of sumples	*Ppoly* ± S.E.		$\bar{H}$	*Ne*
Solu khumbu	68	0.471	0.121	0.1424	1.1661
Jomson	55	0.471	0.121	0.1284	1.1474
Kathmandu	28	0.471	0.121	0.1701	1.2049
Pokhara	31	0.471	0.121	0.1511	1.1780
Low land	103	0.471	0.121	0.1528	1.1804
	285	$\bar{H}_T = 0.1636$	$\bar{H}_S = 0.1490$	$G_{ST} = 0.0925$	
Red jungle fowl	6	0.176	0.095	0.1216	1.1384

$\bar{H}_T$: Average heterozygosity of total population.
$\bar{H}_S$: Average heterozygosity of subpopulations.
$\bar{G}_{ST}$: Coefficient of gene differentiation.

TABLE 4. MATRIX OF GENETIC DISTANCE BETWEEN THE RESPECTIVE PAIR OF 5 CHICKENS POPULATION USED ON 17 LOCI CALCULATED BY USING NEI'S EQUATION

Area	2.	3.	4.	5.
1. Solu khumbu	0.005	0.017	0.025	0.021
2. Jomson		0.022	0.017	0.014
3. Kathmandu			0.031	0.011
4. Pokhara				0.025
5. Low land				

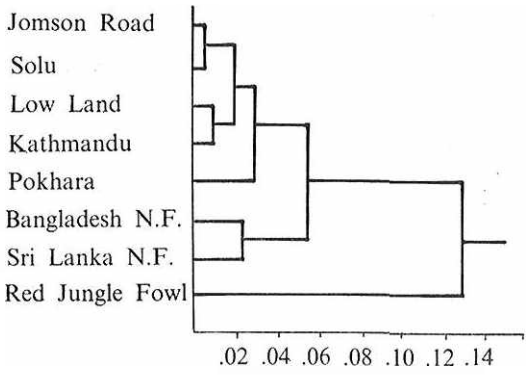

Figure 2. Dendrogram drawn from the Nei's genetic distance matrix among native fowl populations in Nepal, Sri Lanka and Bangladesh.

Discussion

In the present study, (1) the protein polymorphisms of the native fowl population in five areas and red jungle fowl in Nepal, and (2) genetic relationship among native fowl and red jungle fowl in the South Asia, were analyzed. The genetical studies of the native fowls in the South Asia have been studied for those of Sri Lanka and Bangladesh. The genetic variabilities (P_{poly}) of the native fowl in Sri Lanka and Bangladesh were calculated as 0.353 ± 0.116 and $0.353 \pm 0.116 \sim 0.529 \pm 0.121$, respectively. The P_{poly} of the native fowl in Nepal was the same as some populations in Bangladesh. The P_{poly} (0.176) of the red jungle fowl in Nepal is lower than that of the South East Asia (Hashiguchi et al., 1986).

Five regional fowl populations in the central part of Nepal were clustered into three groups. The populations of Solu and Jomson road were belonged to the same cluster. Two areas of Solu and Jomson Road are separated from each other by mountains and valleis, therefore people of both areas cannot migrate between both areas without pass through Kathmandou and Popkhara regions. In spite of no direct migrate between Solu Kuhmbu and Jomson Road areas, genetical relationship is close. Both locations are fixed in Pas^A gene. From the result of principal component of analysis, it is likely that *Pas* locus contribute to the formation of the cluster. Yamamoto et al. (1991) reported that a difference in frequency was found in that the frequency of A^E allele at the blood group *A* locus was higher in the high altitude areas.

Genetical studies on the native fowl in the South Asia had been conducted for those of Sri Lanka (Hashiguchi et al., 1986) and Bangladesh (Okada et al., 1988). When the gene frequencies of 6 polymorphic loci are compared among the native fowl populations of Sri Lanka (Hashiguchi et al., 1986), Bangladesh (Okada et al., 1988) and Nepal, the gene frequencies of $Amy\text{-}1^A$, $Es\text{-}1^A$, and $Akp\text{-}2^A$ genes showed the inclination from south to north. $Amy\text{-}1^A$ gene are gradually decreased in order of Sri Lanka, Bangladesh and Nepal. It is well known that the frequency of $Amy\ 1^A$ gene are higher in native fowl populations of the South East Asia. As Okada et al. (1988) indicated, the higher frequency in Bangladesh than in Nepal might reflect the influence of gene flow from South East Asia. The frequency of $Es\text{-}1^A$ gene are gradually increase in order of Sri Lanka, Bangladesh and Nepal. The frequency of $Akp\text{-}2^B$ gene are gradually decreased in order of Sri Lanka, Bangladesh and Nepal.

$Hb\text{-}1^A$ gene was found in the Athens-Canadian population, Chabo (Japanese Bantam) and native fowl of Bangladesh. Okada et al. (1988) suggested that $Hb\ 1^A$ gene might be flowing out from the Rangamati District because of two homozygote *AA* birds were found in the Rangamati District and 3 heterozygotes in the surrounding districts in Bangladesh. Four heterozygotes were found in Pokhara and Jomson Road in Nepal. The gene frequency in Nepal is lower than in Bangladesh. This result also suggests that $Hb\text{-}1^A$ gene originates from Bangladesh.

In the dendrogram, native fowl population in Nepal formed another cluster from the populations of native fowl in Bangladesh and Sri Lanka and red jungle fowl in South Asia. It seems that the native fowls of Nepal are not influenced by the native fowl population of the South East Asia in comparison to those of Sri Lanka and Bangladesh.

Literature Cited

Baur, E. W. and R. T. Schorr 1969. Genetic poly-

morphism of tetrazolium oxidase in dogs. Science 166:1524-1525.

Bengtsson, S. and K. Sandberg. 1973. A method for simultaneous electrophoresis of four horse red cell enzyme systems. Anim. Blood Grps. Biochem. Genet. 4:83-87.

Davidson, R. G. and J. A. Cortner. 1967. Genetic variant of human erythrocyte malate dehydrogenase. Nature 215:761-762.

Hashiguchi, T., S. Okamoto, T. Nishida, Y. Hayashi, H. Goto and H. W. Cyril. 1986. Blood protein variations of the Ceylon Jungle Fowl and native fowl in Sri Lanka (Japanese with English summary). Rep. Soc. Res. Native Livestock 11:193-207.

Hashiguchi, T., M. Yanagida, Y. Maeda, and M. Taketomi. 1970. Genetical studies on serum amylase isozyme in fowls. Jpn. J. Genet. 45:341-349.

Kuryl, J. and J. Gasparska. 1976. Observations on blood plasma postalbumin and hatchability of chickens. Anim. Blood Grps. Biochem. Genet. 7: 241-246.

Lewontin, R. C. and J. L. Hubby. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetcs 54:595-609.

Manwell, C. and C. M. Baker. 1969. Hybrid proteins, heterosis and the origine of spacies. 1. Unusual variation of polychaete Hyalinoecia "nothing dehydrogenases" and of quail Coturnix erythrocyte enzymes. Comp. Biochem. Physiol. 28:1007-1028.

McIndoe, W. M. 1962. Occurrence of two plasma albumins in the domestic fowl. Nature 195:353-354.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106:283-292.

Nei, M. 1975. Molecular population genetics and evolution. North-Holland, Amsterdam & Oxford.

Okada, I., Y. Maeda, T. Hashiguchi, M. A. Hasnath, M. O. Frauque, and M. A. Majid. 1988. Gene constitution of indigenous chickens in Bangladesh. Jpn. Poult. Sci. 25:15-26.

Okada, I., K. Toyokawa, and I. Takayasu. 1980. Genetic relationships of some native chicken breeds in the northern Tohoku district of Japan (Japanese with English summary). Jpn. Poult. Sci. 17:334-343.

Sokal, R. R. and P. H. A. Sneath. 1963. Principles of numerical taxonomy. Freeman, San Francisco and London.

Stratil, A. 1968. Transferrin and albumin loci in chickens, *Gallus gallus* L. Comp. Biochem. Physiol. 24:113-121.

Watanabe, S., T. Shibata, and T. Kawahara. 1977. Esterase-D isozyme in Japanese quail (Japanese with English summary). Jpn. Poult. Sci. 14:66-70.

Washburn, K. W. 1968. Inheritance of an abnormal hemoglobin in a randombred population of domestic fowl. Poult. Sci. 47:567-574.

Yamamoto, Y., I. Okada, Y. Maeda, K. Tsunoda, T. Namikawa, T. Amano, T. Shotake, T. Nishida and H. B. Rajubhandore. 1991. Blood groups in the Nepalese native chicken. Jap. J. Zootechnical Sci. 62:1-5.