Studies of Genetic Differentiation within Breeds of Japanese Indigenous Chickens

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Genetic differentiation within breeds was analyzed using 30 populations belonging to six Japanese and one Bangladesh indigenous breeds and to two improved breeds of chickens. Gene frequencies at four blood group (*A*, *B*, *D* and *E*) and 7 blood protein (*Akp*, *Akp-2*, *Es-1*, *Amy-1*, *Alb*, *Tf* and *Pas*) loci were examined. Although some specific patterns of gene frequencies were observed between breeds, no such patterns were found between local populations within each breed. Genetic distances between the populations within breeds were small. Cluster analysis showed that almost all populations within each breed belonged to one cluster. The estimates of genetic differentiation between local populations within indigenous breeds were smaller than those between strains within improved breeds. These results suggest that in most of Japanese indigenous breeds genetic differentiation between local populations is small.

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Key words: gene frequency, genetic distance, coefficient of gene differentiation, indigenous breed

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Introduction

During the last decade several studies of phylogenetic relationships among Japanese indigenous breeds of chickens have been reported¹⁻⁴⁾. Although some breeds were common to all of these studies, the results of the studies were inconsistent. Since these past studies examined relatively small samples of the birds collected from different areas, the inconsistencies may have been caused either by random fluctuation or by genetic differentiation within breeds.

The object of the present study was to compare local populations within Japanese indigenous breeds of chickens in order to get a clearer insight into local differentiation within the breeds.

Materials and Methods

Breeds of chickens examined

Six Japanese indigenous breeds, Hinai-dori, Koeyoshi, Tomaru, Satsuma-dori, Chabo and Utai-chahn, were examined. Two of them, Hinai-dori and Satsuma-dori, developed in Akita and Kagoshima Prefectures, respectively, are nondescript native chickens. Another three breeds, Koeyoshi from Akita area, Tomaru from Niigata Prefecture and Utai-chahn from Ryukyu Islands, are breeds bred for long lasting crowing patterns in males. The sixth breed, Chabo, is the Japanese Bantam, believed to be descendants of chickens imported from Indo-China in the 16th or 17th century.

The samples of birds of these breeds, except the Chabo, were classified by the area where they were collected (Table 1). Most of the samples were collected from areas typical for the breed. However, some samples were collected from the outside of such area and were designated as "others". Samples of the Chabo were collected within the same area, and were classified by plumage color varieties.

For comparison, three foreign breeds, Bangladesh Deshi, White Leghorn and Rhode Island Red, were also tested. The detailed description of Bangladesh Deshi was given by OKADA *et al.*⁵⁾. The samples of White Leghorn and Rhode Island Red chickens were classified by strains. All samples of the strains were obtained from the Shirakawa Livestock Breeding Station, Shirakawa, except for the Hiroshima strain of Rhode Island Red which has been kept at the Hiroshima Prefectural Animal Husbandry Experiment Station, Miyoshi.

Number of birds and populations examined are listed in Table 1.

Blood collection, blood typing and electrophoreses

Heparinized blood samples were collected from a wing vein. Four blood group systems, A, B, D and E, were examined by hemagglutination. The number of reagents used for blood typing were four for the A, nine for the B and two each for the D and E systems. Seven loci controlling blood plasma proteins were examined by starch or agar gel electrophoresis. The list of loci examined is given in Table 2.

Estimation of gene frequencies and genetic distances

Gene frequencies were estimated by the direct counting method for the loci known to have only codominant alleles. For the loci including recessive alleles gene frequencies were estimated by the method presented by NEIMANN-SØRENSEN¹¹⁾.

Genetic variability within populations was quantified by measuring the average heterozygosity per individual, $\bar{H}$. The heterozygosity was estimated using the following formula¹²⁾:

$$\bar{H} = \overline{2n(1 - \sum q_i^2)} / (2n - 1),$$

where q_i is the frequency of the i th allele at a locus, n is the number of birds of the population concerned, and the bar indicates averaging over all the loci examined.

The relative magnitude of genetic differentiation among populations within breeds was estimated by the coefficient of gene differentiation, G_{ST} ¹³⁾. The coefficient is given by

$$G_{ST} = D_{ST} / H_T,$$

Table 1. List of populations examined in each breed

Breed	Abbreviation	No. of populations	No. of birds ¹	Description of populations
Hinai-dori	HIN	4	191	A : Akita ² (private farms), B : Akita (Experiment Station), C : Aomori ² , D : Others ³
Koeyoshi	KOE	3	128	A : Akita, B : Aomori, C : Others
Tomaru	TOM	3	127	A : Niigata ² (private farms), B : Niigata (Experiment Station), C : Others
Satsuma-dori	SAT	2	83	A : Kagoshima ² , B : Others
Chabo	CHA	3	74	A : Katsura ⁴ , B : Goishi ⁴ , C : Ginzasa ⁴
Utai-chahn	UTA	2	128	A : Central area of Okinawa ² , B : Southern area of Okinawa
Bangladesh Deshi	BAN	2	170	A : Dhaka Division ² , B : Chittagong Division
White Leghorn	LEG	6	180	A: 11 ⁵ , B: 62, C: 68, D: 74, E: 76, F: R1 ⁵
Rhode Island Red	RIR	5	150	A: 06, B: R4, C: 48, D: 47, E: Hiroshima ⁵

¹ At least, more than 20 birds were examined for each population.

² Location of sampling.

³ Others indicate the samples which were collected from the outside of areas typical for the breed.

⁴ Name of variety.

⁵ Name of strain.

Table 2. List of polymorphic characteristics examined

Characteristics	Locus	Method of analysis	References
Blood group	<i>A</i>	Hemagglutination	MATSUMOTO and OKADA ⁶⁾
"	<i>B</i>	"	"
"	<i>D</i>	"	"
"	<i>E</i>	"	"
Alkaline phosphatase	<i>Akp</i>	Starch gel electrophoresis	OKADA <i>et al.</i> ²⁾
"	<i>Akp-2</i>	"	"
Esterase	<i>Es-1</i>	"	"
Amylase	<i>Amy-1</i>	Agar gel electrophoresis	HASHIGUCHI <i>et al.</i> ⁷⁾
Albumin	<i>Alb</i>	Starch gel electrophoresis	McINDOE ⁸⁾
Transferrin	<i>Tf</i>	"	STRATIL ⁹⁾
Postalbumin	<i>Pas</i>	"	KURL and GASParska ¹⁰⁾

where H_T is the average heterozygosity of the breed, calculated from average gene frequencies of local populations or strains, and D_{ST} is the difference between the H_T and H_S , the mean of average heterozygosity of local populations or strains.

The genetic distance was measured by a jackknife method using the following formula¹⁴⁾:

$$\overline{D_r} = \frac{1}{r} \sum [rD_r - (r-1)D_{r,i}],$$

where D_r is the Nei's genetic distance¹⁵⁾ and $D_{r,i}$ is the same as D_r except that i th locus has been omitted. The Nei's genetic distance was calculated by the method

modified for small populations¹²⁾.

The dendrogram was constructed from the genetic distance matrix by the unweighted pair-group method of SNEATH and SOKAL¹⁶⁾.

Results

Out of 11 loci examined, gene frequencies are given in Table 3 for two loci, blood group *A* and plasma esterase *Es-1*, as examples. The frequencies of blood group alleles, especially at the *A* and *B* loci, showed considerable variation among the breeds. There were ten alleles at the *A* locus and 23 alleles at the *B*. The number of alleles within the individual breeds varied from 3 to 8 at the *A* locus and from 3 to 12 at the *B*. The variation between local populations within breeds, however, was not as large

Table 3. Gene frequencies at the blood group *A* and plasma esterase *Es-1* loci in each population

Popu- lation ¹	No. of birds	<i>A</i>						<i>Es-1</i>			
		<i>F</i>	<i>R</i>	<i>Z</i>	<i>C'</i>	<i>a</i>	Others	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
HIN-A	63	0	0.960	0.040	0	0	0	0	1	0	0
HIN-B	62	0	0.815	0.186	0	0	0	0.444	0.556	0	0
HIN-C	37	0	0.762	0.014	0	0.224	0	0.149	0.851	0	0
HIN-D	29	0	0.707	0.190	0.103	0	0	0.259	0.724	0.017	0
KOE-A	42	0.071	0.556	0	0	0	0.372	0.238	0.762	0	0
KOE-B	57	0	0.635	0	0	0	0.365	0.579	0.421	0	0
KOE-C	29	0.035	0.218	0.367	0.035	0	0.346	0.190	0.810	0	0
TOM-A	61	0	0.758	0.016	0.033	0.193	0	0.131	0.656	0.213	0
TOM-B	43	0	0.784	0	0	0.216	0	0.233	0.767	0	0
TOM-C	23	0.044	0	0.398	0	0.343	0.215	0.565	0.413	0.022	0
SAT-A	45	0	0.067	0.392	0.097	0.340	0.105	0.689	0.311	0	0
SAT-B	38	0.025	0.197	0.448	0.039	0.177	0.112	0.487	0.500	0.013	0
CHA-A	30	0.166	0	0.253	0.071	0.510	0	0.345	0.293	0.362	0
CHA-B	23	0.109	0	0.428	0.185	0.039	0.239	0	0.870	0.130	0
CHA-C	21	0.106	0	0.136	0.337	0.422	0	0.476	0.524	0	0
UTA-A	46	0.420	0	0.185	0.079	0.247	0.069	0.087	0.891	0.022	0
UTA-B	82	0.246	0.043	0.211	0.090	0.266	0.144	0.171	0.829	0	0
BAN-A	83	0.421	0.073	0.189	0.106	0.165	0.046	0.247	0.753	0	0
BAN-B	87	0.418	0.088	0.130	0.130	0.212	0.023	0.207	0.776	0.012	0.006
LEG-A	30	0	0	0.182	0.034	0.784	0	0.633	0.367	0	0
LEG-B	30	0	0	0	0.051	0.949	0	1	0	0	0
LEG-C	30	0	0	0	0	1	0	0.783	0.217	0	0
LEG-D	30	0	0	0.017	0.245	0.738	0	0.867	0.133	0	0
LEG-E	30	0	0	0	0.270	0.730	0	0.967	0.033	0	0
LEG-F	30	0	0	0	0.204	0.796	0	0.183	0.817	0	0
RIR-A	30	0.151	0.203	0.147	0.045	0.069	0.385	0.183	0.817	0	0
RIR-B	30	0.035	0.484	0	0.092	0.389	0	0.050	0.950	0	0
RIR-C	30	0	0.166	0.184	0.034	0.616	0	0.017	0.983	0	0
RIR-D	30	0.102	0.039	0	0.250	0.422	0.188	0.067	0.933	0	0
RIR-E	30	0.017	0.678	0	0	0.039	0.267	0.367	0.633	0	0

¹See Table 1 for the population symbols.

Table 4. Mean genetic distances within breeds (on the diagonal) and between breeds (off diagonal)

Breed ¹	HIN	KOE	TOM	SAT	CHA	UTA	BAN	LEG	RIR
HIN	.0849	.1564	.1459	.2373*	.2276	.2105	.1930	.3766*	.2144*
KOE		.1014	.1170	.1553	.1964	.1650	.1402	.2471	.1747
TOM			.0937	.1867	.2209	.1875*	.1210*	.1851	.1679
SAT				.0597	.1377	.1312	.1810	.3086	.1998
CHA					.1289	.1416	.1890	.3781*	.2133*
UTA						.0131	.1361*	.3419	.1382
BAN							.0069	.2202*	.1794
LEG								.0867	.2573
RIR									.1414

* Significant at 5% level, compared with mean distances within relevant breeds.

¹ See Table 1 for the breed abbreviation.

Fig. 1. Dendrogram showing genetic relationships among 30 populations belonging to seven indigenous and two improved breeds. For the population symbols, see Table 1.

as that among breeds. Furthermore, some features typical for specific breeds were observed. For example, the A^F allele was found with high frequencies in all populations of Chabo, Utai-chahn and Bangladesh Deshi, but not in the populations of Hinai-dori and White Leghorn (Table 3). Similar phenomena were also observed at the B locus.

As for blood protein loci, typical features for specific breeds were somewhat less pronounced than those observed at the blood group loci, except for the esterase alleles. At the $Es-1$ locus, the breeds could be divided into three groups: high frequency of $Es-1^A$ (White Leghorn), medium frequency (indigenous breeds) and low frequency (Rhode Island Red) (Table 3).

Genetic distances between every pair of the 30 populations studied were calculated and averaged for every pair of breeds (Table 4). As expected, mean genetic distances between populations from different breeds were considerably larger than those within breeds. Especially, the distances between the populations of White Leghorn and the other breeds were large.

The dendrogram drawn from the genetic distances among the 30 populations is given in Fig. 1 and that drawn from the mean distances within and between breeds in Fig. 2. The cluster analysis assigned populations within a breed generally to the same cluster, except for the Chabo populations that were not classified by the sampled area but by plumage color varieties.

To evaluate genetic differentiation among local populations within breeds, the coefficients of gene differentiation (G_{ST}) were estimated as shown in Table 5. The

Fig. 2. Dendrogram drawn from the mean genetic distances among 30 populations within and between breeds. Shaded parts show mean distances within each breed. For the breed abbreviations, see Table 1.

Table 5. Gene differentiation among populations within each breed

Breed ¹	No. of populations	No. of loci	H_T ²	H_S	G_{ST}
HIN	4	11	.3552	.3283	.0755
KOE	3	11	.3714	.3290	.1141
TOM	3	11	.3469	.3195	.0791
SAT	2	11	.4284	.4091	.0451
CHA	3	11	.4151	.3570	.1401
UTA	2	11	.4455	.4382	.0163
BAN	2	11	.4638	.4620	.0039
LEG	6	11	.2459	.1878	.2363
RIR	5	11	.3699	.2931	.2077

¹See Table 1 for the breed abbreviation.

² H_T =the average heterozygosity of the breed; H_S =the mean of average heterozygosity of populations within a breed; G_{ST} =the coefficient of gene differentiation. For details, see text.

estimates of G_{ST} of the indigenous breeds were similar in magnitude to the mean genetic distances within breeds (Table 4), while those of improved breeds, namely White Leghorn and Rhode Island Red, were higher than their mean distances.

Discussion

Genetic differentiation between local populations or strains within breeds was found in several farm animal species¹⁷⁻²⁰⁾. In chickens, similar differentiation was observed empirically in some indigenous breeds such as Shamos, distributed widely throughout Japan²¹⁾. However, to the authors' knowledge, there has been no scientific survey of genetic differentiation of local populations within Japanese indigenous breeds of chickens.

As was shown by cluster analysis in the present study, local populations within each breed formed generally a group in the same cluster (Fig. 1). Thus, the genetic differentiation within breeds was not pronounced. One exception was the Chabo breed. The genetic distances observed among the populations within the Chabos were comparable with those among breeds. In Chabos, the populations sampled in this study were plumage varieties rather than local populations as in the remaining indigenous breeds. It is known that Chabos are usually mated within plumage varieties, and such mating may have caused the genetic differentiation observed. In the other indigenous breeds, Koeyoshi-C and Tomaru-C deviated from the other populations of the respective breed. Both were comparatively small populations kept in locations distant from the area where the original breed was developed. Such deviation might be caused by random genetic drift.

The G_{ST} values of the Japanese indigenous breeds were generally less than half of those of improved breeds (Table 5). Low estimates of G_{ST} for local populations were also reported in cattle²²⁾ and cats²³⁾. The present results suggest that local genetic differentiation within breeds did not yet occur to any significant degree in most of Japanese indigenous chickens. The degree of such differentiation was considerably

smaller than that between strains within the improved breeds of White Leghorns or Rhode Island Reds tested.

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日本鶏の品種内分化に関する研究

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日本鶏 6 品種 (比内鶏, 声鶏, 蜀鶏, 薩摩鶏, 矮鶏及びウタイチャー), バングラデシュ在来種 1 品種及び改良種 2 品種 (白色レグホン及びロードアイランドレッド) に属する 30 集団を用いて, 鶏の品種内における遺伝的分化を分析した。調査した遺伝形質は血液型 4 座位 (*A*, *B*, *D* 及び *E*) 並びに血液蛋白型 7 座位 (*Akp*, *Akp-2*, *Es-1*, *Amy-1*, *Alb*, *Tf* 及び *Pas*) の計 11 座位である。これらの座位における遺伝子頻度には品種差が観察されたが, 各品種内の地域集団間にはあまり差

はみられなかった。品種内の各集団間の遺伝距離は小さく, デンドログラムではそれぞれの品種内の各集団はほぼ同一のクラスターに属していた。日本鶏各品種の地域集団間の遺伝的分化の程度は, 改良種における系統差より小さいと推定された。このように, 日本鶏では有意な品種内分化はまだ生じていないと推測される。

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キーワード: 遺伝子頻度, 遺伝距離, 遺伝的分化係数, 在来種