《Research Note》

Genetic Differentiation and Phylogenetic Relationships among Species of *Gallus* (Jungle Fowl) and the Chicken

Noriko OHTA^{1,2}, Manabu KAJITA², Seiji KUSUHARA¹ and RyoZO KAKIZAWA²

¹ Graduate School of Science and Technology, Niigata University, Niigata 950-2181

² Yamashina Institute for Ornithology, Abiko, Chiba 270-1145

By means of protein electrophoresis on 32 loci (22 enzymes), genetic variation and differentiation among 20 individual birds of 4 species of *Gallus* (*Gallus gallus*, *G. lafayettei*, *G. sonneratii* and *G. varius*), and the chicken were analyzed. A genealogic dendrogram was constructed by the UPGMA method, using the genetic distances among species. In the present study, *G. gallus* and the chicken were shown to have genetic distance at the subspecies level, and genetic distance gradually increasing with the *G. sonneratii*, *G. lafayettei* and *G. varius*.

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Key words : *Gallus*, Jungle Fowl, Chicken, phylogeny, isozyme

Introduction

The genus *Gallus* (subfamily Phasianinae, the family Phasianidae, the order Galliformes) consists of 4 species ; Red Jungle Fowl (*Gallus gallus*), Ceylon Jungle Fowl (*G. lafayettei*), Grey Jungle Fowl (*G. sonneratii*) and Green Jungle Fowl (*G. varius*), (PETERS, 1976). *Gallus* members live on the ground and are distributed in India, southern China and Southeast Asia, and are characterized by sexual dimorphism. Some studies have investigated the relationships among the 4 species of *Gallus* by protein electrophoresis of blood samples (HASHIGUCHI *et al.* 1981, 1986, OKADA *et al.* 1984), but the results vary except for their demonstration that *G. varius* is distantly related to other *Gallus* members.

Chickens have long been domesticated, and are very important to human subsistence, and are sometimes kept for amusement. Since it is generally believed that the chicken originates from the Red Jungle Fowl (*G. gallus*), we studied its relationship to the *Gallus* species.

In the present study, the relationships among *Gallus* members and the chicken were examined by electrophoretic analysis on 32 loci (22 enzymes) with tissues (liver, muscle and heart) taken from the four *Gallus* species and the chicken.

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アイソザイムによるヤケイ属鳥類の類縁関係

太田紀子^{1,2}・梶田 学²・楠原征治¹・柿沢亮三²

1. 新潟大学大学院自然科学研究科, 新潟市 950-2181

2. (財)山階鳥類研究所, 我孫子市 270-1145

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Materials and Methods

G. gallus (n=7), *G. lafayettei* (n=3), *G. sonneratii* (n=1), *G. varius* (n=4) and the chicken (n=5) were collected between August 1993 and June 1996. Specimens were obtained from the Tokyo Tama Zoological Park, the Dusit Zoo in Thailand, bird markets in Bali, Indonesia and individual dealers in Japan and abroad. Birds were preserved at -80°C . Each bird was defrosted to remove samples of liver, muscle and heart, which were minced for experimental use and preserved at -80°C . Livers, muscles and hearts dissected from the birds were homogenized, and then centrifuged at $15,000 \times g$ for 20 minutes at 2°C . After centrifugation the supernatant was divided into aliquots and stored in capillary glass tubes at -80°C until electrophoresis. Extracts of liver, muscle and heart were subjected to starch gel electrophoresis to determine the allelic composition for each protein locus. The combinations of tissues and buffer systems for enzyme detection are shown in Table 1. The staining methods for enzymes were essentially the same as those described by BELL *et al.* (1982), SHAW and PRASAD (1970), SELANDER *et al.* (1971), HOPKINSON *et al.* (1973), Harris and HOPKINSON (1976), and NUMACHI *et al.* (1979). These enzymes and proteins are assumed to be coded by the 32 gene loci.

Genetic identities and genetic distances between species were calculated using

Table 1. Combinations of tissues and buffer systems for the detection of enzymes.

Protein	Abbr.	Tissues	Buffers*
Alcohol dehydrogenase	ADH	Liver	Tris-citrate
Creatine kinase	CK	Muscle, Heart	Tris-citrate
Esterase-D	ESD	Liver	Tris-citrate
Esterase	EST	Liver	Tris-HCl
Glutamate dehydrogenase	GDH	Liver	Tris-citrate
Glutamate oxaloacetate transaminase	GOT	Liver	Tris-citrate
Glutamate 6-phosphate dehydrogenase	G 6 PDH	Liver	Tris-citrate
α -Glycerophosphate dehydrogenase	α -GPDH	Muscle	Tris-citrate
Glucosephosphate isomerase	GPI	Muscle	Tris-citrate
Isocitrate dehydrogenase	IDH	Liver	Tris-citrate
Lactate dehydrogenase	LDH	Muscle, Heart	Tris-citrate
Malate dehydrogenase	MDH	Liver	Tris-citrate
Malic enzyme	ME	Liver	Tris-citrate
Mannosephosphate isomerase	MPI	Heart	Tris-citrate
Peptidase	PEP	Liver	Tris-citrate
6-Phosphogluconate dehydrogenase	6 PGD	Liver	Tris-citrate
Phosphoglucomutase	PGM	Liver	Tris-citrate
General protein	PT	Muscle	Tris-citrate
Sorbitol dehydrogenase	SDH	Liver	Tris-citrate
Superoxide dismutase	SOD	Liver	Tris-citrate
Xanthine dehydrogenase	XDH	Liver	Tris-citrate
Xylulose reductase	XR	Liver	Tris-citrate

* Tris-HCl : SELANDER *et al.* (1971), Tris-citrate : NUMACHI *et al.* (1983).

NEI's (1972) formula, and the values of genetic distances were clustered into a dendrogram using the unweighted pair-group method of analysis with arithmetic means (UPGMA) (SNEATH and SOKAL, 1973).

Results

(1) Genetic variation

Alleles detected at each locus which showed the variations, and the frequency of allele occurrence are shown in Table 2. The number of birds in each species ranged from one to seven, and genetic variation was found between the chicken and all species of *Gallus*, but *G. sonneratii*, in which only one specimen was examined. The species with the highest genetic variation was *G. varius* (7 loci), and the species showing the second-highest genetic variation was *G. lafayettei* (6 loci). The loci which showed the variation within a species were 11 loci : EST (two species of *Gallus* and the chicken), ME (three species of *Gallus*), ESD-1, PEP-1, PEP-2 (two species of *Gallus*), ADH-2 (one species of *Gallus* and the chicken), IDH-2, MPI, PT-3, SDH (one species of *Gallus*) and GOT-1 (the chicken). The largest number of alleles found at any one locus was three, found at ADH-2, EST and PEP-2.

Of the 32 loci tested, inter-specific variations were found in 13 loci (ADH-2, CK-2, ESD-1, EST, GOT-1, IDH-2, ME, MPI, PEP-1, PEP-2, PT-2, PT-3 and SDH). Nineteen loci (ADH-1, CK-1, ESD-2, GDH, GOT-2, G 6 PDH, α -GPDH, GPI, IDH-1, LDH-1, LDH-2, MDH-1, MDH-2, 6 PGD, PGM, PT-1, SOD, XDH and XR) were limited to single alleles and are therefore monotypic. CK-2 and PT-2 showed a single allele except for *G. varius*.

(2) Genetic differentiation

Genetic identity and distance were calculated from the data given in Table 3 according to NEI's (1972) formula. The results obtained from the analysis of the 32 loci showed that none of the species had the same genetic structure, therefore there is genetic variation among all *Gallus* members and the chicken. Within the *Gallus* group, the lowest genetic differentiation was shown between *G. gallus* and *G. sonneratii* (0.0619), and the highest genetic differentiation was shown between *G. sonneratii* and *G. varius* (0.1364). The mean genetic distance for the 4 species of *Gallus* was 0.092, which is almost the same as the currently documented mean genetic distance for Non-Passeriformes (KURODA *et al.* 1990, BARROWCLOUGH *et al.* 1981, NUMACHI *et al.* 1983 and WATADA *et al.* 1987). The extremely low genetic differentiation between the chicken and *G. gallus* was reflected by a genetic distance of only 0.0073. The highest value (0.1153) of genetic distance in the chicken was found between *G. varius*.

(3) Genetic relationships within *Gallus*

By means of the UPGMA procedure, a genealogic dendrogram (Fig. 1) was constructed, based on values for inter-specific distances within *Gallus*. The dendrogram shows that the chicken is most closely related to *G. gallus*. In descending order of distance, next comes *G. sonneratii*, *G. lafayettei* and finally, the most distantly related *G. varius*. However the placement of *G. sonneratii* was not confirmed since only one individual specimen was available for this study. Even if the *G. sonneratii* is dis-

Table 2. Alleles and their frequencies from the loci which

Species	ADH-2	CK-2	ESD-1	EST	GOT-1	IDH-2
1) <i>Gallus gallus</i>	b=0.857 c=0.143	a	a	b	b	a
2) <i>Gallus lafayettei</i>	b	a	a=0.333 b=0.667	b=0.667 c=0.333	b	a
3) <i>Gallus sonneratii</i>	a	a	a	b	b	a
4) <i>Gallus varius</i>	b	b	a=0.750 b=0.250	b=0.750 c=0.250	b	a=0.750 b=0.250
5) Chicken	a=0.200 b=0.800	a	a	a=0.400 b=0.600	a=0.100 b=0.900	a

Table 3. Matrix of genetic identity (above diagonal) and genetic distance (below diagonal) between species of *Gallus*.

	<i>G. gallus</i>	<i>G. lafayettei</i>	<i>G. sonneratii</i>	<i>G. varius</i>	Chicken
<i>Gallus gallus</i>	—	0.9363	0.9400	0.8964	0.9927
<i>G. lafayettei</i>	0.0659	—	0.9248	0.9056	0.9315
<i>G. sonneratii</i>	0.0619	0.0782	—	0.8725	0.9427
<i>G. varius</i>	0.1094	0.0992	0.1364	—	0.8911
Chicken	0.0073	0.0709	0.0590	0.1153	—

Fig. 1. Dendrogram of genus *Gallus* based on 32 loci, generated according to the UPGMA method.

counted, the order of the relation among the other four species is unchanged.

Discussions

(1) Genetic variation and differentiation of *Gallus*

Morphological studies have shown that the males of each species have strong phenotypic characteristics. *G. gallus*, *G. soneretti* and *G. lafayettei* all have a notched crown, two wattles and 14 tail feathers, but *G. varius* has a smooth crown, a large single

showed the variations. Alleles are shown as a, b and c.

ME	MPI	PEP-1	PEP-2	PT-2	PT-3	SDH
a=0.143 b=0.857	a	b	b	a	b	a=0.929 b=0.071
a=0.333 b=0.667	a	a=0.667 b=0.333	a=0.667 c=0.333	a	a=0.333 b=0.667	a
b	a	b	a	a	b	a
a=0.250 b=0.750	a=0.750 b=0.250	a=0.250 b=0.750	a=0.250 c=0.750	b	b	a
b	a	b	b	a	b	a

wattle and 16 tail feathers. As well, *G. varius* has a rounded fish-scale shaped neck feathers like the pheasant, but the other three species of *Gallus* have a long neck feathers.

Some studies have investigated the relationships among the 4 species of the *Gallus* by carrying out protein electrophoresis of blood samples (HASHIGUCHI *et al.* 1981, 1986, OKADA *et al.* 1984), but the results vary except for the finding that *G. varius* is distantly related to other *Gallus* members, the results of these experiments were not consistent. The results of the present study on the relationship among *Gallus* are similar to the findings of HASHIGUCHI *et al.* (1986). Both the morphological data and these experiments indicate that *G. varius* is distantly related to other *Gallus* members.

When considering the relationship between two species, it is important to determine if two species can be cross bred and if their hybrid (F1) is fertile. GRAY (1958) reported several studies on experimental crossing among *Gallus* members. It shows that only the hybrid between *G. gallus* and *G. lafayettei* has fertility, indicating that these two species are closely related. Hybrids between *G. sonneratii* or *G. varius* and other *Gallus* members occur infrequently, and are therefore considered to be distantly related. These crossing experiments show that *G. gallus* and *G. lafayettei* are closely related to each other, in contrast to the results of our study. In our study, only one specimen of *G. sonneratii* was analyzed, thus more data is needed to discuss the phylogenetic position of *G. sonneratii*.

(2) Relationship between the chicken and 4 species of *Gallus*

COLLIAS *et al.* (1967) studied the lives of *G. gallus*, *G. lafayettei* and *G. sonneratii*, and reported the similarities between *G. gallus* and the chicken regarding both breeding behavior and voice repertoires.

Morphological and biochemical reports on the native Jungle Fowl and the chicken in Southeast Asia (HAYASHI *et al.* 1983, NISHIDA *et al.* 1983), have shown that hybridization rarely occurs between the chicken and *G. varius* although they share the same territory. They also indicated that the chicken and *G. gallus* often hybridize successfully and frequently although they have distant home ranges. On the other hand, in the crossing experiments among the chicken and species of *Gallus*, only the chicken and *G. gallus* can be cross bred to create a hybrid with normal fertility. Cross

breeding between *G. lafayettei*, *G. sonneratii* or *G. varius* and the chicken result in F1 hybrids that produce fertilized eggs that do not hatch or that have a very low hatching rate (GRAY, 1958). Also, an analysis by AKISHINONOMIYA, F. *et al.* (1996), of the sequence of the D-loop region of the mitochondrial DNA of the chicken and *Gallus* in Thailand and Indonesia, produced data similar to ours, showing a close relationship between the chicken and *G. gallus*. These studies reinforce current research indicating that the original species of the chicken is *G. gallus*.

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