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Araştırma Makalesi

Characterization of a Chicken x Peahen Intergeneric Hybrid Produced under Natural Mating

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Abstract

About 30 peafowls (Pavo cristatus) and one White Leghorn cock (Gallus gallus domesticus) were reared in the same cage for several years as pets. Out of the hatched eggs collected from the cage, one chick was phenotypically different and was presumed to be a hybrid between a peahen and the White Leghorn cock. The production of a chicken x peafowl hybrid under natural mating conditions is an unusual occurrence. This study therefore sought to confirm that the chick was indeed an inter-generic hybrid (F1) of the chicken and peafowl through phenotypic examination, analysis of blood protein, enzyme polymorphisms, and PCR-RFLP. It was concluded that this chick was, indeed, an intergeneric hybrid (F1) between the peahen and the White Leghorn cock.

Key words: inter-generic hybrid, peahen, White Leghorn cock

Doğal Çiftleştirme Koşullarında Üretilen Tavuk x Tavus Kuşu Melezinin Karekterizasyonu

Özet

Yaklaşık 30 tavus kuşu (Pavo cristatus) ve bir Beyaz Legorn horozu (Gallus gallus domesticus), pet hayvanı olarak birkaç yıl aynı kafeste yetiştirilmiştir. Bu kafesten toplanıp kuluçkaya konan yumurtalardan elde edilen bir civciv fenotipik olarak diğerlerinden farklı bulunmuş ve bunun bir tavus kuşu ve beyaz Legorn melezi olduğu kabul edilmiştir. Doğal çiftleşme koşullar altında bir tavuk x tavus kuşu melezi üretimi alışılmadık bir durum olduğundan, bu çalışma ile elde edilen civcivin fenotipik, kan proteinleri analizi, enzim polimorfizmleri ve PCR-RFLP yoluyla, tavuk ve tavus kuşunun bir inter-generik melezi (F1) olduğu teyit edilmeye çalışılmıştır. Sonuçta söz konusu civcivin gerçekten, bir tavus kuşu ile Beyaz Legorn horozu arasında bir inter-generik melezi (F1) olduğu sonucuna varılmıştır.

Anahtar kelimeler: Inter-generik melez, tavus kuşu, Beyaz Leghorn horoz

Introduction

Peafowls have been bred worldwide as pets from ancient times. The peacock belongs to the Pheasant Phasianidae family, which includes two species: Green peafowl (Pavo muticus) found in Southeast Asia, and the Common peafowl (Pavo cristatus) found in India and Sri Lanka. White peafowl is a domesticated sub-species of Pavo cristatus. Reports suggest that a hybrid can be produced between chicken (Gallus domesticus) and the following birds: Golden pheasant (Chrysolophus pictus), Japanese quail (Coturnix japonica), red jungle fowl (Gallus banliva), guinea fowl (Numida meleagris), and Ring-necked pheasant (Phasianus colchicus) (Bacharach et al., 1960; Ogasawara and Huang, 1963; Watanabe and Amano, 1967, 1971 a,b; Maru et al., 1967; Serebrovskii, 1929; Wilcox, 1961).

There are few reports on Gallus domesticus and Pavo cristatus hybrid birds. Annie (1958) reported two chicken-peafowl hybrid cases, while Gray (1958) reported of only two hybrid cocks being hatched from 73 hen and peacock eggs fertilized by artificial insemination. Tinhakov (1933) reported that the chicken-peafowl hybrid embryo lived for 6 days.

Materials and Methods

Thirty peafowls and one White Leghorn cock were kept under natural mating conditions for several years in the same cage in Mr. Wakiya's garden. One chick from the hatched eggs was phenotypically different from the rest. To confirm that the chick was a hybrid between a peahen and the White Leghorn cock, further investigations were conducted through analysis of blood protein, enzyme polymorphisms, and PCR-RFLP.

Phenotype

Body weight, head size, metacarpal bone, and tertii digiti pedis were measured on 5 peacocks and 5 peahens at the Animal Physiology Laboratory, Tokyo University of Agriculture. More phenotypic measurements were taken on 5 peacocks, 12 peahens and the F1 in reared in the same cage at Mr. Wakiya's residence in Shizuoka, Japan.

Table 1. Method of electrophoretic analysis of proteins and enzymes of blood from peafowl, cock and hybrid

Souces		Name of loci	Abridge	Reference for method
RBC	Protein	Hemoglobin A	HbA	Matuoka and Miyaji 1981
		Hemoglobin B	HbB	Matuoka and Miyaji 1981
	Enzyme	Non-specific esterase	EsRBC	Watanabe and Suzuki 1977
		Esterase-D	Es-D	Watanabe and Suzuki 1977
		Glucose-6-phosphate dehydrogenase	G6PDRBC	Nishimura et al. 1975
		Lactate dehydrogenase	LDHRBC	Nakayama 1981
		Catalase	CatRBC	Nishimura et al. 1975
Plasma	Protein	Transferrin	Tf	Nishimura and Watanabe 1973
		Albumin	Alb	Nishimura and Watanabe 1973
		Pre-albumin-1	Pr-1	Tanabe and Ogawa 1982
		Pre-albumin-2	Pr-2	Tanabe and Ogawa 1982
		Pre-albumin-3	Pr-3	Tanabe and Ogawa 1982
		Pre-albumin-4	Pr-4	Tanabe and Ogawa 1982
		Haptoglobin	Hp	Oshiro 1987
	Enzyme	Acid phosphatase	Acp	Nishimura et al. 1975
		Alkali phosphatase-1	Alp-1	Saeki and Takeuchi 1994
		Alkali phosphatase-2	Alp-2	Saeki and Takeuchi 1994
		Amylase	Amy	Watanabe et al. 1975
		Catalase	CatPlasma	Watanabe and Suzuki 1977
		Non-soecific esterase-1	Es-1Plasma	Watanabe and Suzuki 1977
		Non-soecific esterase-2	Es-2Plasma	Watanabe and Suzuki 1977
		γ-glutamyl transpeptidase-1	γGTP-1	Miyazaki and Okumura 1972
		γ-glutamyl transpeptidase-2	γGTP-2	Miyazaki and Okumura 1972
		Leucine aminopeptidase	LAP	Wajima and Takahashi 1963
		Lactate dehydrogenase	LDH	Nakayama 1981

Behaviors

The behavior of the hybrid in the breeding cage was observed for 1 hour.

Electrophoresis

The 23 elements of the 16 loci from blood were analyzed by polyacrylamide gel electrophoresis (Laemmli, 1970), starch gel electrophoresis, and agarose gel electrophoresis (Sakamuro et al., 1988; Sasaki et al., 1960, 1964; Watanabe et al., 1981). Heparinized blood was obtained from ten selected mature peahens, which were likely to be parents of the hybrid chick (F1). Heparinized blood was also obtained from one peacock, the White Leghorn cock, and the F1 hybrid. Following collection, all blood samples were kept on ice pending processing and analysis. The blood was centrifuged at 1,450 × g for 10 min, and the plasma was separated and collected. The packed cells were washed 3 times with 10 mL of ice-cold Dulbecco's phosphate buffered saline (pH 7.4, 279 mOsm/kg) and the buffy coat discarded. The plasma and washed red cells were stored at –50 ℃.

Proteins and enzymes from red blood cell lysates and plasma were typed using electrophoretic procedures to assay polymorphism at 23 loci: red cell protein: hemoglobin (HbA, HbB) (Matsuoka and Miyaji, 1981); red cell enzyme: non-specific esterase (Es/R), esterase-D (Es-D) (Watanabe and Suzuki, 1977), glucose-6-phosphate dehydrogenase (G6PD/R) (Nishimura et al., 1975a), and lactate dehydrogenase (LDH/R) (Nakayama, 1981); plasma protein: transferrin (Tf), albumin (Alb) (Nishimura and Watanabe, 1973), pre-

albumin (Pr-1, Pr-2, Pr-3, Pr-4) (Tanabe and Ogawa, 1982) and haptoglobin (Hp) (Oshiro, 1987, Malin et al., 1972); and plasma enzyme: acid phosphatase (Acp) (Nishimura et al., 1975b), alkali phosphatase (Alp-1, Alp-2) (Saeki et al., 1994), amylase (Amy) (Watanabe et al., 1975), catalase (Cat/P), non-specific esterase (Es-1/P, Es-2/P) (Watanabe and Suzuki, 1977), γ-glutamyl transpeptidase (γ GTP-1, γ GTP-2) (Miyazaki and Okumura, 1972) and leucine aminopeptidase (LAP) (Wajima and Takahashi, 1963; Law, 1967) (Table 1).

Figure 1. The phenotype of the hybrid bird

PCR-RFLP analysis

Genomic DNA was isolated from venous blood collected in heparin. A PCR was carried out with 500ng of genomic DNA from 19 peahens, 1 peacock, 1 cock, and F1 to investigate sequence polymorphisms of the B locus α1～α2 region of MHC class I in Japanese quail and PBR domain region in HSPA2 gene.

The PCR primers for the B locus α1～α2 region (QF63 primer: 5'-TAC AAC AGC ACC GCG CG-3'; QF2 primer: 5'-CTG TCC TCC CCA GCT CT-3') were designed to amplify a 428 bp fragment in quail. The PCR primers for PBR domain region in the HSPA2 gene (H1 primer: 5'-TTT TAT GAC TTT GAC AAC CG-3'; H2 primer: 5' GTT GTC TTA GTA GGT GGT GA-3') were designed to amplify a 615 bp fragment according to the chicken genomic sequence in the GenBank database (accession number J02679).

Figure 2. Leg phenotypes of chicken, peafowl and the hybrid bird

The reaction conditions were 96 ℃ for 3 min, followed by 30 cycles of 96 ℃ for 1 min, 62 ℃ for 30 s, and 72 ℃ for 1 min, and an extension at 72 ℃ for 3 min. The 25 µl reaction volume included 500 ng of template, 10 × reaction buffer, 6 pmol of each primer, 2.5 mM dNTP, 2.5 U of Ex Taq polymerase (TaKaRa).

The MHC PCR products were digested using EcoT14 I and Ban II at 37 ℃ for 3 hours. The HSPA PCR products were digested using Cfo I at 37 ℃ for 3 hours.

The restriction digests were electrophoresed for 1 h at 100V on a 1.0% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes for the gene were determined by visualizing the band pattern under ultraviolet light.

Results

Phenotype and Sex

Table 2 shows the body weight, head size, metacarpal bone length, and tertii digiti pedis length of the hybrid, male chicken parent, female chicken, peacock, and peahen. Hybrid body weight (3.9 kg) was heavier than that of White Leghorn cock (2.4 kg) and peahen, but lighter than peacock. The head and tertii digiti pedis sizes were smaller in the hybrid than peacock but larger than in peahens and the White Leghorn cock. The metacarpal bone of hybrid was longer than in White Leghorn cock and peahens but was shorter than in the peacock.

Figure 1 shows a photograph of the F1. The bill of hybrid was pink color. The plumage of the hybrid was white with brown spots. The hybrid had a black to dark brown scutum metatarsum in the front color while the rear had a variegation pattern of white, yellow, and dark brown colors (Figure 2). The comb of hybrid was different from both the comb of the chicken and the feather comb of the peafowl. The feathers on the head

of the hybrid seemed only to stand straight (crest) (Figure 3).

The hybrid's collum (neck) was heavier and shorter than in the peafowl, but thinner and longer than in the cock.

The hybrid had neither the super and extra main tail coverts nor the feather of the main tail coverts (Figure 4). The F1 was confirmed to be male due to the presence of a pair of immature testis in the peritoneum.

Behavior

The F1 was not able to fly, but could jump onto a perch placed at a height of 2 m. The movement of the neck of F1 was similar to that of the chicken.

Figure 3. Comb phenotypes of chicken, peafowl and the hybrid bird

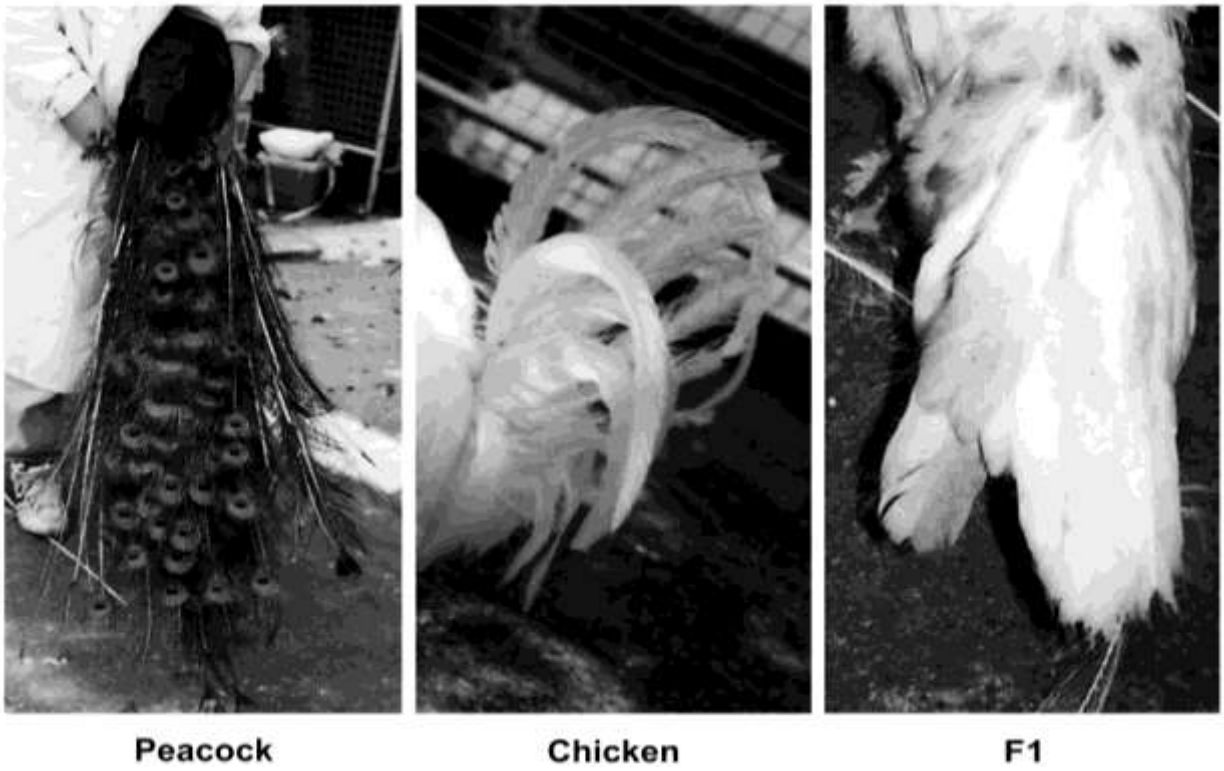

Figure 4. Tail phenotypes of chicken, peafowl and the hybrid bird

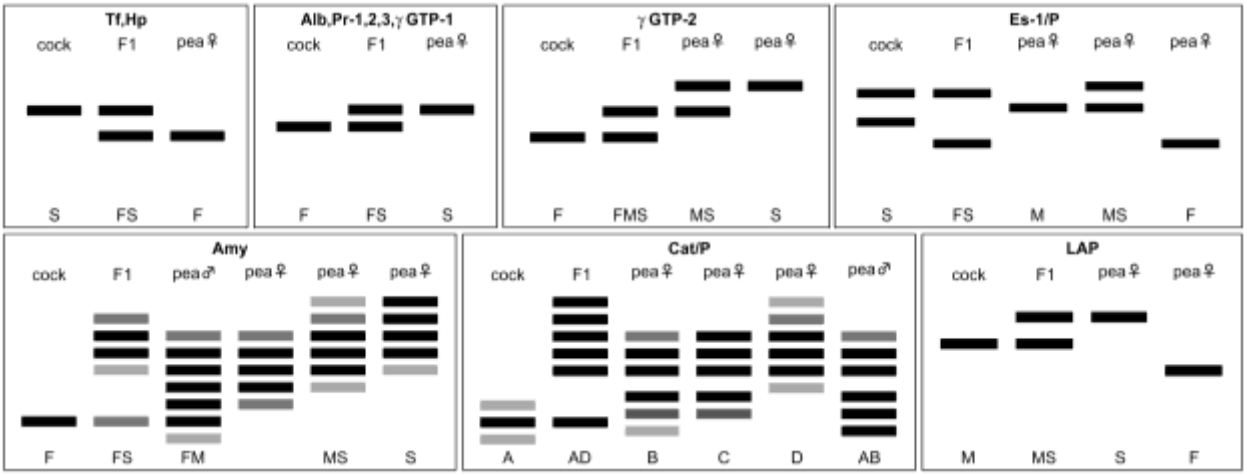

Figure 5. The diagrammatic patterns of blood proteins and isozymes of an intergeneric hybrid (Pea♀ : Peahen, Pea♂ : peacock, F1 : an intergeneric hybrid between the peahen and the cock. The top of the figure is a cathode, and the bottom is an anode. Black bars show meaty bands, and gray bars are light bands)

Electrophoresis

The 23 elements of 16 loci from blood were analyzed by polyacrylamide gel electrophoresis, starch gel electrophoresis, and agarose gel electrophoresis. Phenotypes of proteins and enzyme of red blood cell lysates and plasma from ten peahens, one peacock, F1 and the cock are shown in Table 3.

There was no difference between the type of Es/R from the red blood cells, Pr-4 and ALP-1 from the plasma of peahen, White Leghorn cock, and the F1.

Tf, Alb, Pr-1,-2,-3 Hp and γ GTP-1 zones from other electrophoretograms of peafowl and White Leghorn cock sera were divided into two patterns, namely faster migrating zone (F zone) and slower migrating zone (S zone). Amy, Es-1/P, LAP and γ GTP-2 zones from electrophoretograms of peafowl and White Leghorn cock sera were divided into three different patterns (migrated with three different mobilities), namely faster migrating zone (F zone), slower migrating zone (S zone) and intermediate migrating zone (M zone), as shown in Figure 5.

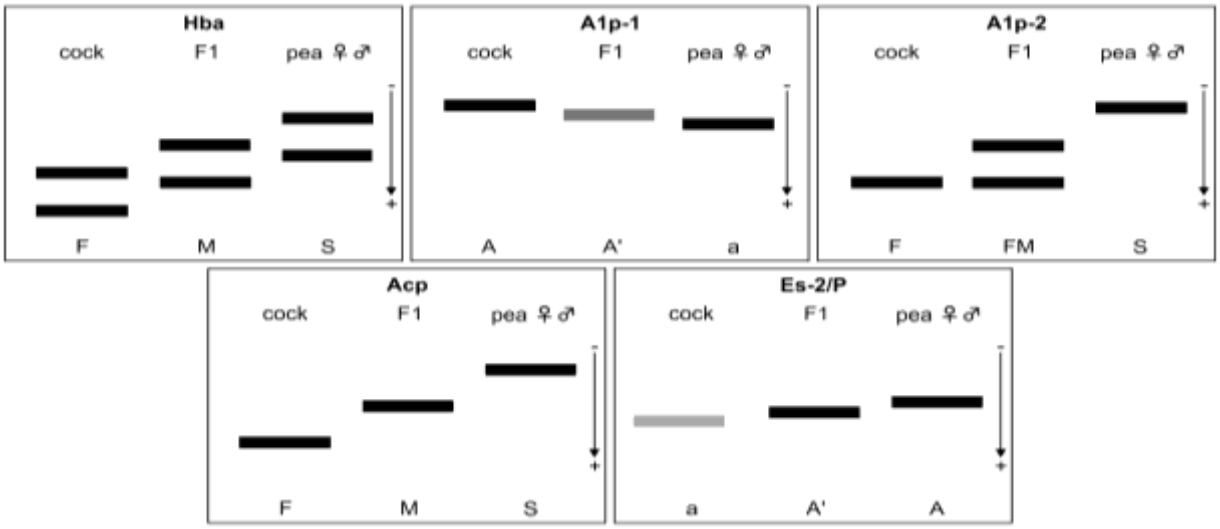

Figure 6. Diagrammatic patterns of blood proteins and isozymes of an intergeneric hybrid are specific type, that are not chicken and peahen (Pea♀ : peahen, Pea♂ : peacock, F1 : a intergeneric hybrid between the peahen and the White Leghorn cock. The top of the figure is a cathode, and bottom is an anode. Black bars show main bands, and gray bars are shown light bands)

Table 2. The body weight, head size, metacarpal bone length and Tertii digiti pedis length

	male chicken parent	male chicken	femal chicken	peacock	peahen	Hybrid
body weight(kg)	2.4	2.3±0.23	1.1±0.19	4.4±0.18	3.1±0.48	3.9
head size (cm)	2.9	2.7±0.17	1.8±0.20	4.9±0.09	4.5±0.33	4
metacarpal bone(cm)	12	11.4±0.27	9.7±1.78	13.9±0.27	11.9±1.37	13
tertii digiti pedis (cm)	6.5	7.18±1.2	3.1±0.22	7.6±3.5	7.2±0.51	7

Table 3. Comparison of phenotypes of protein and enzyme of blood from peafowls, cock and hybrid

Protein/ enzyme	Cock	Hybrid	Peafowl											Phenotype of hybrid
	R	F1	1	2	3	4	5	6	7	8	9	10	♂	
HbA	F	M	S	S	S	S	S	S	S	S	S	S	S	Specific
HbB	F	F	S	S	S	S	S	S	S	S	S	S	S	Cock
Es/R	S	S	S	FS	FS	FS	F	S	S	F	F	FS	F	Unclear
Es-D	F	FMS	S	S	S	S	S	S	S	S	O	O	O	Specotic+Hetero
G6PD/R	H	FS	FS	FS	FS	FS	FS	FS	FS	F	F	FS	FS	Peafowl
LDH/R	I	F	S	S	S	F	F	FS	F	S	S	S	F	Peafowl
Tf	S	FS	F	F	F	F	F	F	F	F	F	F	F	Hetero
Alb	F	FS	S	S	S	S	S	S	S	S	S	S	S	Hetero
Pr-1	F	FS	S	S	S	S	S	S	S	S	S	S	S	Hetero
Pr-2	F	FS	S	S	S	S	S	S	S	S	S	S	S	Hetero
Pr-3	F	FS	S	S	S	S	S	S	S	S	S	S	S	Hetero
Pr-4	N	N	N	N	N	N	N	N	N	N	N	N	N	Unclear
Hp	S	FS	F	F	F	F	F	F	F	F	F	F	F	Hetero
Acp	F	M	S	S	S	S	S	S	S	S	S	S	S	Specific
Alp-1	A	A'	a	a	a	a	a	a	a	a	a	a	a	Specific
Alp-2	S	FM	F	F	F	F	F	F	F	F	F	F	F	Specific+Peafowl
Amy	F	FS	M	M	M	M	M	S	MS	M	M	M	FM	Hetero
Cat/P	A	AD	B	B	C	B	C	D	D	B	B	C	AB	Hetero
Es-1/P	F	FS	F	M	F	M	MS	F	F	F	MS	S	F	Hetero
Es-2/P	a	A'	A	A	A	A	A	A	A	A	A	A	A	Specific
γGTP-1	F	FS	S	S	−	S	S	S	S	S	S	S	S	Hetero
γGTP-2	F	FMS	M	S	−	S	M	MS	MS	S	M	S	M	Hetero
LAP	M	MS	S	F	−	F	S	S	S	F	S	F	S	Hetero

Cat/P zones from electrophoretogram of peafowl and cock were divided into four patterns, namely cathode side migrating zones A, B, C, and D.

Tf, Alb, Pr-1, Pr-2, Pr-3, Hp, Amy, Cat/P, Es-1/P, LAP, γ GTP-1 and γ GTP-2 from plasma of F1 is a heterotype from cock and peahen (Figure 5).

Furthermore, HbA of the red blood cell lysates, plasma Alp-1 and the expression type of Alp-2, Acp, and Es-2/P in the F1 were intermediate (hybrid type) to those of the cock and the peahens (Figure 6). G6PD/R, Es/R, LDH/R zones from electrophoretograms of peafowl sera by polyacrylamide gel were observed to be divided into three different patterns (migrated with three different

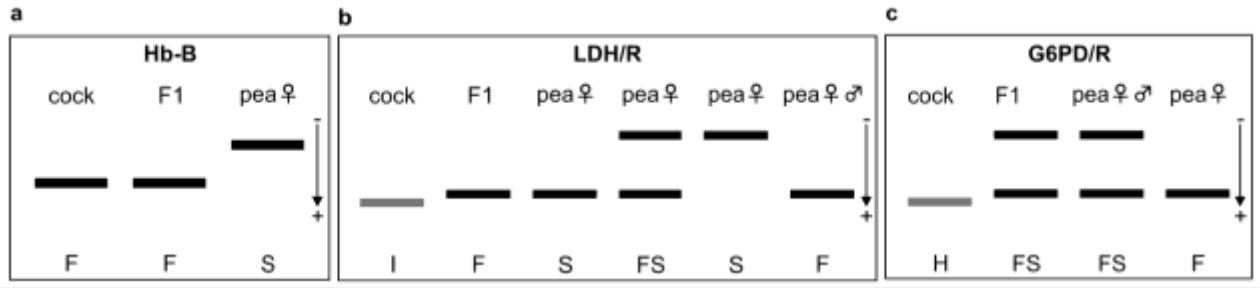

Figure 7. Diagrammatic patterns of blood proteins and isozymes of an intergeneric hybrid are peafowl or cock type (Pea♀ : peahen, Pea♂ : peacock, F1 : a intergeneric hybrid between the peahen and the White Leghorn cock. The top of the figure is a cathode, and bottom is an anode. Black bars show main bands, and gray bars are shown light bands. a: The diagrammatic patterns of an intergeneric hybrid are cock type. b: The diagrammatic patterns of an intergeneric hybrid are peafowl type.)

mobilities): faster migrating zone (F zone), slower migrating zone (S zone) and intermediate migrating zone (FS zone).

The phenotypes of HbB and LDH/R of the F1 were similar to those of the White Leghorn cock (Figure 7a and b). G6PG/R from the red blood cells of the F1 was similar to that of peafowl (Figure 7c).

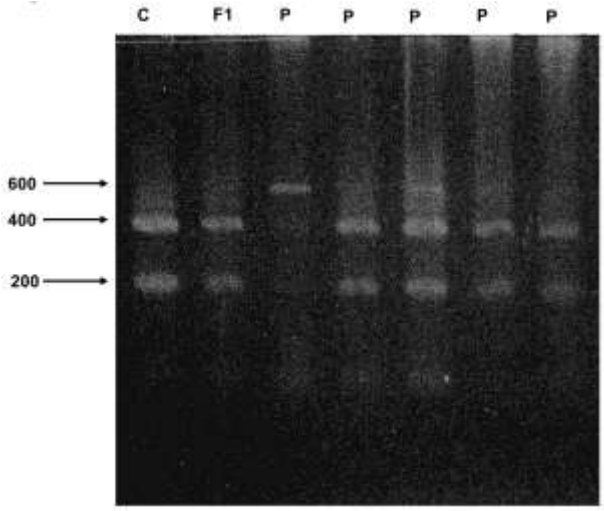

Figure 8. PCR-RFLP of products digested by EcoT14 restriction enzyme

PCR-RFLP

The amplification product of the MHC class I B locus α1~2 region was about 600bp in length. The restriction enzyme Eco T14 I-digested PCR products had fragment sizes of 600bp for peafowl (No.1), a plural fragment of sizes of 200bp and 400bp for cock, hybrid, peahen (individuals 2 and 5) and a plural fragment of sizes of 200bp, 400bp and 600bp for peahen (individuals 3 and 4), peacock (Figure 8). The restriction enzyme Bam II-digested PCR products had plural fragment of sizes of 200bp and 400bp for cock, hybrid, peahens (individuals 2, 4, and 5) plural fragment of sizes of 200bp, 300bp and 400bp for peahens (individuals 1 and 3) (Figure 9).

The amplification product of the HSPA2 gene PBR domain region had about 600bp in length. The restriction enzyme Cfo I-digested PCR products had a fragment of sizes of 600bp for cock, 510bp for peafowl, and plural fragment of sizes of 600bp and 510bp for the hybrid (Figure 10).

Discussion

The loss of flying ability of the F1 is probably due to the fact that its size is similar to the peafowl whereas the neck and wing structures are similar to those of the chicken.

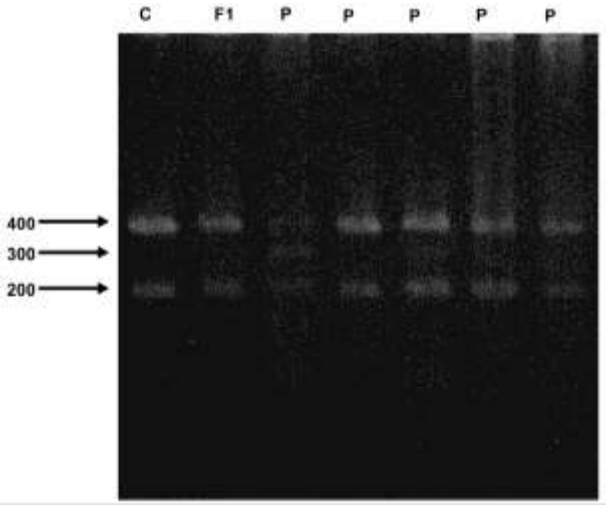

Figure 9. PCR-RFLP of products digested by Bam II restriction enzyme

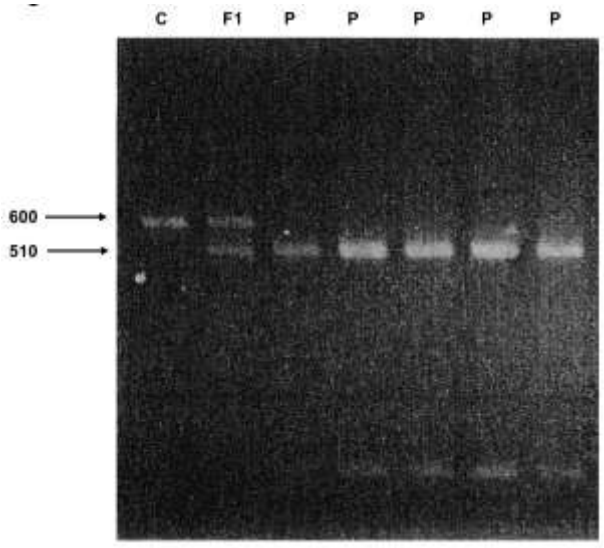

Figure 10. PCR-RFLP of products digested by Cfo I restriction enzyme

It has been demonstrated by Hashiguchi et al. (1970) that serum amylase in chicken was divided into five zones (A, B, C, D, and E) in agar gel electrophoretograms and that two isozymes were genetically controlled by a pair of autosomal codominant alleles Amy-1A and Amy-1B. Serum amylases of peafowl were divided into three zones: A, B, and C, in polyacrylamide gel electrophoretograms.

The transferrins of the White Leghorn cock were genetically controlled by three autosomal codominant alleles (TfA, TfB and TfC) on the same locus with six phenotypic variants (Watanabe and Suzuki, 1976). The primary data on the polymorphism of serum albumin in chickens were reported by McIndoe (1962). Stratil (1968) reported that the serum and yolk albumin in chickens is controlled by three autosomal codominant alleles (AlbA, AlbB and AlbC) on the same locus. Watanabe and Suzuki, (1977) also reported the existence of six serum albumin phenotypes controlled by three autosomal codominant alleles in Japanese native breeds of chickens. Stratil (1968) observed the AC and BC types of serum albumin in a few F1 hybrid birds between Gallus Lafayette and G. gallus.

A pair of alleles, a dominant and a recessive one, was first found by Law and Munro (1965) in serum alkaline phosphatase types. Afterwards, Kimura et al. (1971) classified genetic variations of serum alkaline phosphatase into two phenotypes controlled by the pair of alleles Akp-2a and Akp-2b.

Hb is divided into three patterns and is controlled by a pair of codominant alleles.

It was observed that the phenotypes of blood protein and isozymes in the hybrid are controlled by one or three alleles. These hybrid molecules were homo- or hetero-dimers due to transmutation in gene expression seen in the White Leghorn cock and peahen. The peafowl types of isozymes in F1 were observed in LDH, G6PD and ALP, enzymes that play an essential function in energy production.

The protein fractions of the hybrid contained all the serum protein fractions of both parents. Results of RFLP analysis show that HSPA gene type of the F1 is a hetero type from both chicken and peafowl. Heat shock protein (HSP) is one of the chaperone molecules that are required to fold proteins and to protect cells from heat stress. This suggests that a molecule specific type mutated to play these roles.

Collectively, phenotypic examination, analysis of blood protein, enzyme polymorphisms, and PCR-RFLP indicate clearly that this hybrid bird was an intergeneric hybrid (F1) produced by natural mating between the peahen and the White Leghorn cock.

References

Annie, P.G. 1958. Galliformes Order, Phasianidae Family. Bird hybrids, A check-list with bibliography. 85-118. Technical Communication No.13 of the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh, Farnham Royal: Commonwealth Agricultural Bureaux.

Bacharach, M.M., McGibbon, W.H., Irwin, M.R. 1960. An interaction product of the cellular antigens in species hybrids between Gallus gallus and Phasianus colchicus. J. Hered. 51: 122-126.

Gray, A.P. 1958. Bird Hybrids. A Check-List with Bibliography. Commonwealth Agricultural Bureaux (Ed), Buckinghamshire, pp. 98.

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

Kimura, M., Yokoyama, Y., Isogai, I. 1971. Plasma albumin polymorphisms in the chicken. Jpn. J. Poult. Sci. 8: 253-255.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Law, G.R.J. 1967. Alkaline phosphatase and leucine aminopeptidase association in plasma of the chicken. Science 156: 1106-1107.

Law, G.R., Munro, S.S. 1965. Inheritance of two alkaline phosphatase variants in fowl plasma. Science 149: 1518.

Malin, S,F., Baker, R.P.Jr., Edwards, J.R. 1972. Electrophoretic determination of haptoglobin as a hemoglobin complex. Biochem. Med. 6: 205-209.

Maru, N., Watanabe, S., Ichinoe, K., Suzuki, S. 1967. Serological studies on the intergeneric hybrid between Gallus gallus and Crysolophus pictus. Jpn. J. Poult. Sci. 4: 30-35.

Matsuoka, M., Miyaji, T. 1981. Region of Hemoglobin, in: All of electrophoresis, Ishiyaku Publishers Inc, Tokyo, pp. 218-224.

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

Miyazaki, S., Okumura, M. 1972. Change of serum γ-glutamyltranspeptidase level and isoenzyme pattern in hepatobiliary pancreatic disease. Intl. J. Clin. Chem. 40: 193-197.

Nakayama, N. 1981. LDH isozyme, in: All of electrophoresis. Ishiyaku Publishers Inc., Tokyo, pp. 174-181.

Nishimura, T., Watanabe, S. 1973. Studies on the polymorphisms of serum protein in pony. J. Agric. Sci. Tokyo 18: 6-13.

Nishimura, T., Watanabe, S., Matsushima, Y. 1975a. Studies on the polymorphism of erythrocyte catalase isozyme and hemoglobin in pony. J. Agric. Sci. Tokyo 20: 23-29.

Nishimura, T., Watanabe, S., Matsushima, Y. 1975b. Studies on the simultaneous electrophoresis for four enzyme systems and polymorphism of phosphohexose isomerase (PHI) and acid phosphatase (Acp) in erythrocytes of Pony. J. Agric. Sci. Tokyo 20: 72-80.

Ogasawara, F.X., Huang, R. 1963. A modified method of artificial insemination in the production of chicken-quail hybrids. Poultry Sci. 42: 1386-1392.

Oshiro, T. 1987. 2-I. Qualitative and Quantitative, Clinical Haptoglobin, Nagaishoten, Tokyo, Japan pp. 21-37.

Saeki, S., Ochi, M., Takeuchi, N. 1994. Analysis of ALP isozyme for electrophoresis. Jpn. J. Electrophoresis 38: 143-147.

Sakamuro, D., Yamazoe, M., Matsuda, Y., Kangawa, K., Taniguchi, N., Matsuo, H., Yoshikawa, H., Ogasawara, N. 1988. The primary structure of human gamma-glutamyl transpeptidase. Gene 73: 1-9.

Sasaki, K., Suzuki, S., Tanaka, K., Watanabe, S., Hosoda, T. 1960. Serobiological Studies on the Jungle Fowl. 1. Serological and Electrophoretic Examinations on the Relationship between Red Jungle Fowl and Domestic Fowl. Jpn. J. Zootechn. Sci. 31: 215-219.

Sasaki, K., Suzuki, S., Watanabe, S. 1964. Serological studies on the Jungle Fowl. Serological Relationship between White leghorn and Jungle Fowl or Japanese Native Fowl. Jpn. J. Zootechn. Sci. 35(Special Number): 61-66.

Serebrovskii, A.S. 1929. Observations on interspecific hybrids of the fowl. J. Genet. 21:327-340.

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

Tanabe, H., Ogawa, N. 1982. Comparative studies on physical and chemical property of avian eggs. 10. Electrophoretograms of prealbumins in plasma and yolk of quail. Jpn. Poult. Sci. 19: 15-19.

Tinhakov, G.G. 1933. A fowl x peacock hybrid. Animal Breeding Abstracts. Vol. 1, No: 118.

Wajima, T., Takahashi, H. 1963. Leucine aminopeptidase isoenzyme analyzed by means of agar-gel electrophoresis, application in serum and homogenized organ. Med. Biol. 68: 101-103.

Watanabe, S., Amano, T. 1967. Studies on the intergeneric hybrids between chicken and quail. I. Production of chicken-quail hybrids. Jpn. J. Zootechn. Sci. 38: 30-32.

Watanabe, S., Amano, T. 1971a. Studies on the chicken-quail hybrids. II. On the growth curve, morphological characters and testis of chicken-quail hybrids. J. Agric. Sci. Tokyo. Commemoration Number of the 80th Anniversary of Foundation. 91-96.

Watanabe, S., Amano, T. 1971b. Studies on the chicken-quail hybrids. III. On the antigenic constitution of serum and the polymorphism of serum protein in chicken-quail hybrids. J. Agric. Sci. Tokyo. Commemoration Number of the 80th Anniversary of Foundation. 97-104.

Watanabe, S., Yoshida, H., Kawahara, T. 1975. Serum amylase in the Japanese quail. Jpn. Poult. Sci. 12: 67-70.

Watanabe, S., Suzuki, S. 1976. Studies on the Polymorphisms of Protein and Isozyme of South East Asian Native Fowls and Jungle Fowls. Japanese International Society for Animal Blood Group Research Conference. 6(Suppl. 1): 93-94.

Watanabe, S., Suzuki, S. 1977. Studies on the polymorphism of serum proteins and isozymes in Japanese native breeds of chicken. J. Agric. Sci. Tokyo 22: 85-94.

Watanabe, S., Shibata, T., Kawahara, T. 1977. Estrase-D isozymes in Japanese quail. Jpn. Poult. Sci. 14: 66-70.

Watanabe, S., Munechika, I., Ichinohe, K., Miguel, P.P., Hilary, C. 1981. Studies on the Polymorphism of Serum Protein and Isozyme in the three Species of

Jungle Fowl. J. Agric. Sci. Tokyo. Commemoration Number of the 90th Anniversary of Foundation. 266-275.

Wilcox, F.H., Clark, C.E. 1961. Chicken-quail hybrids. J. Hered. 52: 167-170.