PCR-RFLP Analysis of Cytochrome b (cyt b) Inheritance in the Wild-Type Strain and Laboratory Population of Japanese Quail

PCR-RFLP Analysis of Cytochrome *b (cyt b)* Inheritance in the Wild-Type Strain and Laboratory Population of Japanese Quail

Xiang-Jun SHEN, Masao KIMURA[a], Atsushi IWASAWA[b] and Takao NAKAMURA[b]

The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
[a]National Institute for Environmental Studies, Ibaragi 305-0053, Japan
[b]Department of Animal Science and Technology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

SUMMARY

PCR-RFLP analysis was used to examine the 1,075 bp cytochrome *b* (*cyt b*) region in 26 birds from two families of laboratory quail and 26 birds of wild-type Japanese quail (*Coturnix japonica*). The total *cyt b* amplicon was digested with ten restriction endonucleases, and its electrophoretic pattern was investigated. A variation was observed only in the *Alu* I digestion. Two kinds of haplotypes, A and B, were found in the laboratory population, and their frequencies were 92.3% and 7.7%, respectively. Only the unique haplotype A was found in the wild population. The estimated nucleotide sequence divergence between the two haplotypes observed in the laboratory population of Japanese quail was 0.770 %, and the nucleotide diversity (π) within the laboratory population was 0.159 %. No variation was found in the wild quail population. These results indicated a higher genetic diversity in the laboratory strain than in the wild-type strain of quail. The results of progeny tests for cytochrome *b* inheritance in two families of the laboratory population revealed that cytochrome *b* was inherited maternally. *Alu* I is suitable for detecting cytochrome *b* variations in Japanese quail.

INTRODUCTION

The Japanese quail (*Coturnix japonica*) belongs to the family Phasianidae[7]. In Japan, there are three kinds of Japanese quail: wild, commercial and laboratory quail[21]. Research on the wild quail has revealed that their body weight and shank length are smaller by about 20 per cent than those of domestic quail. Other characteristics of wild quail in captivity are late sexual maturity, a high incidence of non-layers and a low egg-production rate[12, 13, 14]. There are no differences in karyotype between wild and domestic quail. The colony of wild quail maintained in laboratory conditions became tame after the sixth generation[22].

Maternal transmission of mitochondrial (mtDNA) has been reported in humans, amphibians, insects, and birds[17]. Maternally transmitted point mutations and duplications in the human mitochondrial genome have been reported in Leber disease, cardiomyopathy[5], retinitis pigmentosa, merrgf disease, diabetes mellitus, and cerebellar ataxia[19]. The avian mitochondrial genome has been shown to be inherited uniparently and maternally. However, there has been no detailed report on the maternal inheritance conducted with the *cyt b* gene of mtDNA in birds.

Avian mtDNA is a circular genome approximately 16,770 bp in length, encoding the same set of genes, 13 proteins, 2 rRNAs, and 22 tRNAs, as does mammalian mtDNA, and it consists of coding and non-coding regions[8]. Cytochrome *b* is one of 13 protein genes encoded by mtDNA, and it is one of the best known of the nine proteins that make up Complex III of the mitochondrial oxidative phosphorylation system[11].

Cytochrome *b* is the only one of the Complex III proteins that is encoded by the mitochondrial genome. The entire *cyt b* gene of Japanese quail is 1,143 bp nucleotides in length, and it appears to encode a 380-amino-acid protein [16] (GenBank accession number L08377).

Mitochondrial *cyt b* genes have been used for the identification of meat species [6], and for phylogenetic analysis in perching bird species [9] and in the *Francolinus* [4]. A dedicated series of studies on the nucleotide sequence, PCR-RFLP, and a phylogenetic analysis of the D-loop region of avian mtDNA have been carried out on domestic fowl and their related species [1, 2, 3]. No reports are available, however, on the PCR-RFLP of *cyt b* in the Japanese quail. The purposes of this study were to investigate the RFLPs for the *cyt b* region of the Japanese quail mtDNA ,to examine its *cyt b* inheritance, and to compare the genetic diversity between wild and laboratory birds of this species.

MATERIALS AND METHODS

Samples. Twenty-six birds from two families (I and II) of the RM (random mating) laboratory population of Japanese quail maintained in Gifu University, and 26 wild-type quail captured in Japan and maintained as a closed population in the National Institute of Animal Industry of Japan for some 30 years, were used in the present study. Family I included parents and their nine offspring, and family II included parents and their thirteen offspring. The 26 wild-type quail had no blood relationships with each other.

MtDNA preparation. All the quail were killed by decapitation after food had been withheld from them overnight. MtDNAs were prepared from the quail livers with the alkaline lysis method as described by Tamura and Aotsuka [20], with a slight modification. The extracted mtDNA was used as template DNA for use with PCR.

Survey of mtDNA concentration. The mtDNA concentrations of all the samples were measured with a Pharmacia Biotech GeneQuant spectrophotometer.

PCR primer. To design PCR primers for the *cyt b* region, we compared the published sequences for mammals, chickens [8], and quail [9], and designed the following two primers:

L 14963 5'-CTCCCCCAAACATCTCCGCCTGATGAA-3'

H 16092 5'-GTCTTGTAAACCAAAAACTGAAGACTC-3'

The letters L and H refer to the light and heavy strands of DNA, and the numbers refers to the positions for the 3' bases of the primer in the complete mtDNA sequence of the chicken.

PCR. Amplification was performed in a 100 μl solution containing 6.7 mM Tris, 6.7 mM $MgSO_4$, 16 mM $(NH_4)_2SO_4$, 10 mM mercaptoethanol, 1 mM dNTP, each of 1 μM primers, 50 ng of mtDNA, and 2.5 units of TaqI polymerase. PCR was performed at 94℃ for 40 sec, and then for 34 cycles of denaturation at 94℃ for 40 sec, annealing at 55℃ for 45 sec, with extension at 70℃ for 1 min.

Purification and restriction analysis. PCR products were purified with Centricon (Pharmacia) to remove primers and any impurities. Eight restriction enzymes (*AluI*, *Bsh*1236I, *Bsu*RI, *CfoI*, *Hin*6I, *Msp*I, *Rsa*I, and *TaqI*) that recognize 4 base pairs, and two more (*Cfr*13I and *Hinf*I) that recognize 5 base pairs were used in this experiment to cleave the *cyt b* region that had been amplified by PCR. The samples treated with restriction endonucleases were purified with phenol-chloroform purification and ethanol extraction.

Electrophoresis. The samples were analyzed by gel electrophoresis with 13% acrylamide.

Data analysis. The length of restriction fragments was compared with the DNA standards-50-2,000 bp Ladder of BIO-RAD on the acrylamide gel, and the band sizes were determined by using a computer program ImageMaster 1D of Pharmacia Biotech.

Nucleotide sequence divergence. Nucleotide sequence divergence between haplotypes were estimated according to the formula of Nei [18] in the laboratory and the wild population of quail from the different haplotypes regarding RFLPs of the *cyt b* region. Nucleotide sequence divergence within a

population (Nucleotide diversity: π) was calculated by the Ewens method [10] from RFLPs of *cyt b*.

RESULTS

MtDNA contents. The average mtDNA contents of liver tissues measured in two families (I and II) of Japanese quail were 367.6 μ g/g and 365.8 μ g/g, respectively. The average mtDNA content in the liver tissue of the laboratory population was 366.6 μ g/g.

PCR and purification. A DNA band of 1,045 bp obtained using PCR was identified by 1% agarose gel electrophoresis. The amplified PCR products of mtDNA were verified by the PCR method using the original two primers and dot-blot hybridization. The products were purified in a MicroSpin column (Pharmacia Biotech).

Restriction analysis. The sizes of bands produced by electrophoresis were determined by comparison with standard size markers using graphical methods. The cleavage patterns, including band numbers and sizes, are shown in Table 1.

Electrophoretic pattern. Each of 5, 2, 2, 4, 2 and 4 cleavage fragments was observed from *Bsu*RI, *Cfo*I, H*in*6I, *Rsa*I, *Taq*I, and *Hinf*I digests, respectively. No cleavage sites were found in the *Bsh*1236I, *Msp*I, and *Cfr*13I digestions. In the laboratory population, only the *Alu*I digestion contained two morphologies, A and B (Fig. 1). Type A consisted of three fragments of 430, 374, and 271 bp, while type B consisted of four fragments of 430, 315, 271, and 59 bp. No morphs were found in the wild-type quail population.

Haplotypes. Haplotypes were determined on the basis of the electrophoretic patterns as shown in Table 2. In the two families of the laboratory population, two haplotypes, A and B, were observed. Only haplotype A was found in the wild-type strain, and that was the same as the one (haplotype A) observed in the laboratory population.

Nucleotide sequence divergence. Nucleotide sequence divergence estimated between the haplotypes from the RFLP data of the cytochrome *b* gene was 0.770% in the laboratory strain, and the nucleotide diversity within the laboratory population was 0.159%. However, neither sequence divergence nor nucleotide diversity was investigated in the wild-type strain.

Progeny test. The sires of both families yielded type B morphology with 4 bands in response to *Alu* I digestion, while both dams yielded the type A morphology with 3 bands (Fig. 2). Regardless of their sex, all chicks, 9 chicks in family I and 13 chicks in family II, yielded three bands like their dam.

Pedigree analysis. Pedigree analysis was carried out in the two families of the laboratory population according to the pedigree registration. As shown in the pedigree registration file in Fig. 2, No. 9317 and No. 9343 are full siblings.

DISCUSSION

In the laboratory population, of the 10 kinds of restriction endonucleases used, polymorphism was only found in the *Alu* I digestion. No variations were observed in the wild population. Three enzymes, *Bsh*1236I, *Msp*I, and *Cfr*13I, were not suitable for the detection of the RFLPs of *cyt b* in Japanese quail, because these enzymes had no cleavage site at the *cyt b* region in either of the two populations. The PCR-RFLP analysis of the *cyt b* region revealed two haplotypes in the laboratory population, but only one unique haplotype was observed in the wild-type quail population. The nucleotide sequence divergence (0.770%) and nucleotide diversity (π = 0.159%) estimated in the laboratory population were apparently higher than those in the wild-type quail population. This result indicates that the genetic diversity existed in the cytochrome *b* region in the laboratory population. The lack of variation in the wild quail population implies that the *cyt b* gene region had remained in the original wild-gene state as a unique gene conformation.

Our results obtained using mtDNA *cyt b* were in accordance with those reported by Kimura and Fujii [15], who reported on the genetic variability between wild and domestic Japanese quail populations using the 34 enzyme and protein loci. The heterozygosity ($\overline{H}$) estimated for the wild and domestic quail were 0.078 and 0.109, respectively [15]. The $\overline{H}$ value estimated for the wild quail was significantly lower than that for the domestic quail [15].

The distribution of wild Japanese quail covers Japan, Korea, east China, Mongolia, Siberia, and Sakhalin. They usually breed in northern Japan in spring and summer, and then migrate south in autumn. However, some quail breed in the mountains of their winter habitat without migrating north [21].

The domestic quail was bred from the song quail by an enthusiastic breeder around 1910, thus inithiating the genetic differentiation of wild and domestic quail. During the following 89-year period, the body weight and reproductive performance of Japanese quail improved markedly under artificial selection. In 1984, the number of domestic quail was around 6 million [21].

Even within such a short differentiation period, artificial selection may have played an important role in the evolution of a number of gene mutations and variations in the domestic population. We consider that artificial selection may be the main cause of genetic variation. In contrast, the wild population underwent only mild natural selection, the results of which are not as serious as those of artificial selection. Therefore, the *cyt b* gene conformation of the wild type population is unique and without diversity, contrary to that observed in the laboratory population.

Cyt b-RFLP reflects the sequence polymorphism that occurred in the mtDNA *cyt b* exon region, and thus represents the *cyt b* gene sequence conformation and characteristics of the quail population. The RFLPs of the mitochondrial *cyt b* region were determined for representatives of both laboratory and wild-type quail populations.

From the pedigree analysis using *cyt b*-RFLP data, the sequences were compared for parent-child pairs and, as expected, typical maternal transmission of *cyt b* was observed. The sires, No. 9317 and No. 9343, of both families in the laboratory population possess the same B type of *cyt b* that may have been transmitted from their mother (No. 7176), but which they can not pass on to the next generation. The cytochorme *b* pattern in offspring is determined only by their mothers, but has no relevant to their fathers. This result is in accordance with the formal theory of maternal inheritance of human mtDNA. The sequence polymorphism that occurred in the mtDNA *cyt b* region is, therefore, useful as a cytoplasmic DNA marker.

Alu I RFLP was effective in detecting *cyt b* variations in Japanese quail. *Alu* I digestion of PCR-RFLP of the *cyt b* in the Japanese quail can, therefore, be used as an ideal cytoplasmic marker for tracing maternal genealogies and establishing special maternal lines, and as the gene marker for discrimination between specific lines or individuals in making various maternal models.

PCR-RFLP analysis of mtDNA is a powerful and comprehensive tool for the characterization of maternal lines, and is useful for detecting polymorphism of the *cyt b* region in Japanese quail. Most differences in mtDNA within and among different species can be detected directly by the PCR-RFLP method. This method saves time and money compared to the sequencing method, especially when dealing with a large number of samples. These techniques can also be used to study relationships within lines, breeds, and species of Japanese quail, Chinese painted quail, and chickens.

Acknowledgement

The authors are grateful to Dr. S. ITO for his critical comment and reading of the manuscript.

References

1) Akishinonomiya, F. Miyake, T. Sumi, S. Takada, M. Ohno, S. & Kondo, N. : One subspecies of the red

junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. Proc. Natl. Acad. Sci. USA. 91 : 12505-12509, 1994.

2) Akishinonomiya, F. Miyake, T. Takada, M. Ohno, S. & Kondo, N. : The genetic link between the Chinese bamboo partridge (*Bambusicola thoracica*) and the chicken and junglefowls of the genus Gallus. Proc. Natl. Acad. Sci. USA. 92 : 11503-11056, 1995.

3) Akishinonomiya, F. Miyake, T. Takada, M. Shingu, R. Endo, T. Gojobori, T. Kondo, N. & Ohno, S. : Monophyletic origin and unique dispersal patterns of domestic fowls. Proc. Natl. Acad. Sci. USA. 93 : 6792- 6795, 1996.

4) Bloomer, P. & Crowe T. M. Francolin phylogenetics: molecular, morphobehavioral, and combined evidence. Mol. Phylogen. Evol. 9 : 236-254, 1998.

5) Casali, C. Santorelli, F. M. D'Amati, G. Bernucci, P. DeBiase, L. & DiMauro, S. : A novel mtDNA point mutation in maternally inherited cardiomyopathy. Biochem. Bioph. Res. Co. 213 : 588-593, 1995.

6) Chikuni, K., Tabata, T., Saito, M. & Monma, M. : Sequencing of mitochondrial cytochrome b genes for the identification of meat species. Anim. Sci. J. 6 : 571-579, 1993.

7) Crawford, R. D. Japanese quail. In: Poultry breeding and genetics, (R. D. Crawford ed.) 23-26.Elsevier, Amsterdam, Oxford, New York, Tokyo. 1990.

8) Desjardins, P. & Morais, R. : Sequence and gene organization of the chicken mitochondrial genome. J. Mol. Biol. 212 : 599-634, 1990.

9) Edwards, S. V. & Arctander, P. Congruence and phylogenetic reanalysis of perching bird cytochrome b sequences. Mol. Phylogen. Evol. 7 : 266-271, 1997.

10) Ewens, W. J. Speilman, R. S. & Harris, H. : Estimation of genetic variation at the DNA level from restriction endonuclease data. Proc. Natl. Acad. Sci. USA. 78: 3748-3750, 1981.

11) Hatefi, Y. : The mitochondrial electron transport and oxidative phosphorylation system. Ann. Revie. Biochem. 54 : 1015-1069, 1985.

12) Kawahara, T. : Comparative study of quantitative traits between wild and domestic Japanese quail (*Coturnix coturnix japonica*). Exp. Anim. 22 : 138-150, 1973.

13) Kawahara, T. : History and useful characters of experimental strains of Japanese quail (*Coturnix coturnix japonica*). Exp. Anim. 25 : 351-354, 1976.

14) Kawahara, T. : Body traits and behaviour in wild Japaese quail *Coturnix coturnix japonica*. Tori (Bulletin of the Ornithological Society of Japan), 27 : 105-112, 1978.

15) Kimura, M. & Fujii S. : Genetic variability within and between wild and domestic Japanese quail populations. Jpn. Poult. Sci. 26 : 245-256, 1989.

16) Kornegay, J. R. Kocher, T. D. Williams, L. A. & Wilson, A. C. : Pathway of lysozyme evolution inferred from the sequence of cytochrome b in birds. J. Mol. Evol. 37 : 367-379, 1993.

17) Lin, L. Y. Cheng, I. P. Tzeng, C. S, & Huang, P.C. : Maternal inheritance of mitochondrial DNA in ducks. Biochem. Biophy. Re. Com. 168 : 188-193, 1990.

18) Nei, M. T. Molecular Evolutionary Genetics. In Columbia University Press. Newyork. 1987.

19) Rotig, A, Bessis, J. L. Romero, N. Cormier, V. Saudubray, J. M. Narcy, P. Lenoir, G. Rustin, P. & Munnich, A. Maternally inherited duplication of the mitochondrial genome in a syndrome of proximal tubulopathy, diabetes mellitus, and cerebellar ataxia. Am. J. Hum. Genet. 50 : 364-370, 1992.

20) Tamura, K. & Aotsuka, T. : Rapid isolation method of animal mitochondrial DNA by the alkaline lysis procedure. Biochem. Genet. 26 : 815-819, 1988.

21) Wakasugi, N. Japanese quail. In : Evolution of domesticated animals. (Mason I. L. ed.) 319-321. Longman, London and New York. 1984.

22) Wakasugi, N. and Kondo, K. Breeding methods for maintenance of mutant genes and establishment of strains in the Japanese quail. Exp. Anim. 22 (Suplement) : 151-159. 1973.

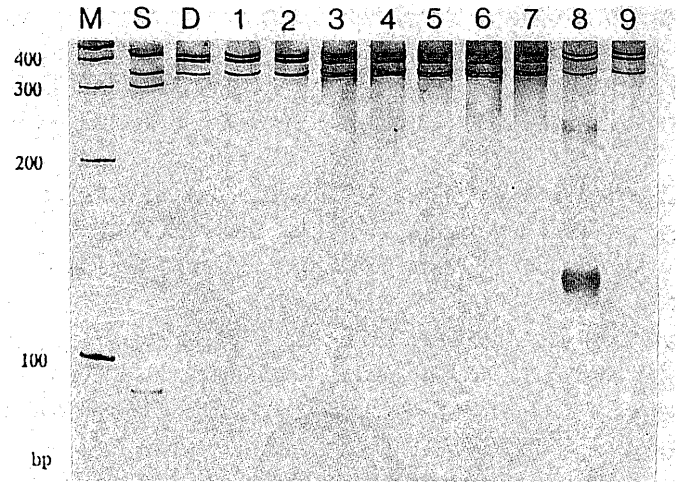

Fig.1. Acrylamide gel electrophoretic pattern of cytochrome b region that digested with *Alu*I in the laboratory quail populaton. S and D is the sire and dam of the family I, and 1 to 9 are their nine offspring, respectively. The individual number is the same as the sequences shown in the Table I. M is the DNA standard size marker, 100 bp ladder.

6812 (♂- ? type) × 7176 (♀- B type ?)

↓

9317 (♂- B type) 9343 (♂- B type)

Family I

9317 (Sire) × 9210 (Dam)
(B type) ↓ (A type)

8953	♂	(A)
9098	♂	(A)
8954	♀	(A)
8955	♀	(A)
8956	♀	(A)
9016	♀	(A)
9017	♀	(A)
9019	♀	(A)
9020	♀	(A)

Family II

9343 (Sire) × 9225 (Dam)
(B type) ↓ (A type)

9024	♂	(A)
9030	♂	(A)
9120	♂	(A)
9124	♂	(A)
9125	♂	(A)
9128	♂	(A)
9129	♂	(A)
8959	♀	(A)
9022	♀	(A)
9028	♀	(A)
9029	♀	(A)
9031	♀	(A)
9123	♀	(A)

Fig.2. Pedigree analysis in two families of Japanese quail according to the electrophoretic patterns of *Alu*I digestion of the cytochrome b region.

Table 1. The numbers and the sizes of electrophoretic bands of cytochrome b after treatment with ten different restriction endonucleases

Restriction endonuclease	Number of fragments	The size of each fragment (bp)
*Alu*I (A) *	3	430, 374, 271
*Alu*I (B) *	4	430, 315, 271, 59
*Bsh*1236I	1	1075
*Bsu*RI	5	595, 176, 147, 84, 73
*Cfo*I	2	824, 251
*Hin*6I	2	822, 253
*Msp*I	1	1075
*Rsa*I	4	389, 293, 219, 174
*Taq*I	2	887, 188
*Cfr*13I	1	1075
*Hin*fI	4	399, 308, 193, 175

**Alu*I has two cleavage patterns A and B.

Table 2. Restriction endonuclease cleavage patterns of cytochrome b haplotypes distributed in the wild-type and laboratory quail populations

	Restriction endonuclease cleavage pattern *											
Population	*Alu*	*Bsh*	*Bsu*	*Cfo*	*Hin*6	*Msp*	*Rsa*	*Taq*	*Cfr*	*Hin*f	n	Ratio
▲ Wild-type	A	A	A	A	A	A	A	A	A	A	26	(100%)
▲ Haplotype A	A	A	A	A	A	A	A	A	A	A	24	(92.3%)
❋ Haplotype B	B	A	A	A	A	A	A	A	A	A	2	(7.7%)

* Abbreviations of enzymes: *Alu*, *Alu*I; *Bsh*, *Bsh*1236I; *Bsu*, *Bsu*RI; *Cfo*, *Cfo*I; *Hin*6, *Hin*6I; *Msp*, *Msp*I; *Rsa*, *Rsa*I; *Taq*, *Taq*I; *Cfr*, *Cfr*13I and *Hin*f, *Hin*fI.

▲ indicates haplotype A, ❋ indicates haplotype B.

研究用ウズラと野生系ウズラ集団におけるシトクローム*b*領域のPCR-RFLP解析

沈相　俊・木村正雄[1)]・岩澤　淳[2)]・中村孝雄[2)]

岐阜大学大学院連合農学研究科

[1)] 環境庁国立環境研究所，茨城県305-0053

[2)] 岐阜大学農学部動物生産学講座，岐阜市501-1193

要　　約

2家系の研究用日本ウズラ26羽、野生系日本ウズラ26羽を供試し、PCR-RFLPにより、1,075bpのシトクローム*b*（*cytb*）領域の制限酸素断片長多型解析を行った。PCRにより増幅した全てのシトクローム*b*領域を10種類の制限酸素で処理し、アクリルアミド電気泳動法により、泳動パターンを観察した。

その結果、変異は研究用集団で*Alu*Iの処理のみで認められ、2種類のハプロタイプAとBが観察され、それぞれ92.3%と7.7%を示したが、それに対して野生系集団では、10種類の制限酸素においてはそれぞれ同じ泳動パターンを示した。研究用集団における2種類のハプロタイプ間で求められた塩基置換率は0.77%であったが、野生系集団では塩基置換が見出されなかった。本実験において、研究用集団における遺伝的変異性は、野生系集団より高いことが示唆された。シトクローム*b*-RFLPの遺伝による研究用2家系におけるの後代検定結果、シトクローム*b*が母性遺伝をすることを示した。これらの結果より*Alu*Iは日本ウズラのシトクローム*b*遺伝子領域の変異を観察するのに適切な制限酸素であることが明かになった。

岐阜大農研報（64）：13－20，1999