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Mitochondrial DNA Diversity in Commercial Lines of Laying-type Japanese Quail

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The present study aims to investigate the maternal origin and genetic diversity of laying-type Japanese quail lines based on partial sequences (453 base pairs) of a mitochondrial DNA (mtDNA) control region. A total of 478 individuals from 12 lines were sequenced and six different haplotypes with eight variable sites were identified. All haplotypes, two of which were identical to previously reported sequences, were typical for the Japanese quail (*Coturnix japonica*) and were distinct from those of the common quail (*Coturnix coturnix*) in a phylogenetic analysis including other published haplotypes. One haplotype was distributed in the majority of individuals (84.9%, 406/478) across all lines. Within each line, 72.5−100% of individuals had this predominant haplotype. The second most common haplotype was detected in 12.8% (61/478) individuals. These two haplotypes accounted for 97.7% of all individuals. The remaining four haplotypes were distributed with a low frequency; these were observed in five, three, two, and one individuals across all lines, respectively. All lines showed a low degree of haplotype diversity ranging from 0.0000 to 0.4321. Genetic differentiation indexes (F_{ST}) were not significant in approximately 80% pairwise comparisons of lines. The results suggest limited maternal origin and low mtDNA diversity of laying-type quail lines and may reflect their breeding history where the present gene pool was rooted in a small number of founders.

Key words: genetic diversity, Japanese quail, maternal origin, mitochondrial DNA

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Introduction

In Japan, selective breeding of the Japanese quail (*Coturnix japonica*) for egg production started approximately in 1910 (Wakasugi, 1984). In the 1930s, the quail industry was first established in areas around Toyohashi City, Aichi Prefecture (Wakasugi, 1984). The number of commercial laying-type quails increased to two million in 1941 (Wakasugi, 1984). However, almost all laying-type quails were lost during World War II (Yamashina, 1961; Wakasugi, 1984). After World War II, the laying-type quails were restored from a small number of surviving individuals (Yamashina, 1961; Wakasugi, 1984). Thus, the existing gene pool of laying-type quails seems to have a few maternal origins, although approximately six million birds have been reared in recent years.

Analysis of maternally inherited mitochondrial DNA (mtDNA), particularly of a highly variable control region, is a useful approach to study the genetic diversity and maternal origin of domestic animal populations. Analysis of mtDNA control region has been mainly applied to detect maternal introgression of human-released domestic Japanese quails into the wild common quails (*Coturnix coturnix*) of Europe (Barilani *et al*., 2005; Chazara *et al*., 2010; Sanchez-Donoso *et al*., 2014). However, there are few mtDNA analyses of commercial Japanese quail lines (e. g., laying-type quail lines) raised in the quail industry. Thus, little is known about maternal lineages and mtDNA diversity of commercial Japanese quail lines. In the present study, the maternal origin and genetic diversity of commercial laying-type Japanese quail lines were estimated based on the analysis of partial sequences of the mtDNA control region.

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Materials and Methods

Samples and Sequencing

The samples used in the present study were the same samples used in a previously published microsatellite-based diversity study (Shimma and Tadano, 2019). One sample of Farm 5-A was excluded because reliable sequence data could not be obtained. As shown in Table 1, a total of 478 individuals from 12 laying-type lines and 40 individuals from one meat-type line were successfully sequenced using the

Table 1. **Distribution of haplotypes and genetic diversity estimates within 13 commercial Japanese quail lines based on analysis of mitochondrial DNA control regions**

Line	Location	Sample size	Number of haplotypes	Haplotype						Haplotype diversity	Nucleotide diversity
				Cj1	Cj2	Cj3	Cj4	Cj5	Cj6		
Farm 1-A	Hokkaido	40	2	32	8					0.3282	0.0022
Farm 1-B	Hokkaido	40	2	35	5					0.2244	0.0015
Farm 1-C	Hokkaido	40	2	39	1					0.0500	0.0003
Farm 2	Saitama	40	2	38	2					0.0974	0.0006
Farm 3	Shizuoka	40	3	30	8	2				0.4051	0.0024
Farm 4	Shizuoka	40	3	31	8				1	0.3679	0.0022
Farm 5-A	Aichi	38	2	33	5					0.2347	0.0016
Farm 5-B	Aichi	40	3	33	6		1			0.3038	0.0023
Farm 6	Aichi	40	3	29	9		2			0.4321	0.0034
Farm 7	Aichi	40	3	34	4	2				0.2718	0.0014
Farm 8	Aichi	40	4	32	5	1		2		0.3500	0.0018
Farm 9	Miyazaki	40	1	40						0.0000	0.0000
Meat-type	Saitama	40	2	39			1			0.0500	0.0007
Total		518	6	445	61	5	4	2	1		

following procedures. The laying-type lines were identified from nine commercial farms in five prefectures in Japan. The breeding histories of some lines are available in Shimma and Tadano (2019). The meat-type line selected for increased body weight was imported from France to Japan in September 2002.

PCR amplification of partial sequence of the mtDNA control region was performed using PHDL (5′-AGGACTACGGCTTGAAAAGC-3′) and PH-H521 (5′-TTATGTGCTTGACCGAGGAACCAG-3′) primers as described by Randi and Lucchini (1998). The 20 μL reaction volume contained 12 ng of total DNA, 1× GeneAmp PCR Buffer (Applied Biosystems, Foster City, CA, USA), 200 μM of deoxynucleoside triphosphate (Applied Biosystems), 0.25 μM of each primer, and 1.25 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). The cycling conditions were as follows: 95℃ for 10 min; followed by 35 cycles of 95℃ for 1 min, 55℃ for 1 min, 72℃ for 1 min; with a final extension of 72℃ for 10 min. PCR products were separated on 2% agarose gels and then stained with ethidium bromide and visualized under ultraviolet (UV) light. Purification of PCR products was performed using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA). The PHDL primer and BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) were used in sequencing reactions. Sequences were determined using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).

Data Analysis

The control region sequences were edited and aligned against a published sequence of the Japanese quail (GenBank accession number KF410830; Sanchez-Donoso *et al*., 2014) using BioEdit (Hall, 1999). To assess the genetic diversity within each line, the number of haplotypes, haplotype diversity, and nucleotide diversity were calculated using ARLEQUIN software version 3.5 (Excoffier and Lischer, 2010). Genetic differentiation indexes (F_{ST}) between each pair of lines and their statistical significance based on 10,000 permutations were computed using ARLEQUIN software version 3.5.

Phylogenetic relationships of haplotypes were inferred by constructing a neighbor-joining tree based on Tamura-Nei genetic distances (Tamura and Nei, 1993) with 1000 bootstrap replications using MEGA software version 7.0 (Kumar *et al*., 2016). Additionally, the haplotype network based on the TCS algorithm (Clement *et al*., 2002) was constructed using POPART version 1.7 (Leigh and Bryant, 2015). These two analyses included previously published haplotypes: four obtained from the Japanese quail (*Coturnix japonica*), which are indicated with the initial "F" (GenBank accession numbers KF410832, KF410833, KF410836, and KF410837; Sanchez-Donoso *et al*., 2014); five obtained from the common quail (*Coturnix coturnix*), which are indicated with the initial "W" (GenBank accession numbers from KF410844 to KF410848; Sanchez-Donoso *et al*., 2014); and one obtained from the blue-breasted quail (*Coturnix chinensis*) (GenBank accession number AB073301; Nishibori *et al*., 2002).

Results and Discussion

Haplotype Distribution

Partial sequences (453 base pairs) of the mtDNA control region were determined from 518 individuals from 12 laying-type lines and one meat-type line. These sequences were identified as six distinct haplotypes (Cj1−Cj6 in Table 1) defined by eight variable sites, all of which were transitions (Table 2). These six haplotypes were submitted to GenBank (accession numbers from LC492859 to LC492864). Cj1 and Cj2 found in the present study were identical to F1W1 (GenBank accession number KF410830; Sanchez-Donoso *et al*., 2014) and F5 (GenBank accession number KF410834; Sanchez-Donoso *et al*., 2014). These haplotypes were reported as the Japanese quail haplotypes, respectively, by using the Basic Local Alignment Search Tool (BLAST). The remaining four haplotypes (Cj3−Cj6) were thought to be new haplotypes because no identical sequences were found via BLAST. In a neighbor-joining tree (Fig. 1), all six hap-

Table 2. **Variable nucleotide sites of six haplotypes (453 base pairs) detected in the present study**

Haplotype	GenBank accession number	Variable nucleotide sites							
		66	211	219	222	228	231	251	272
Cj1	LC492859	C	A	G	C	G	A	A	G
Cj2	LC492860	T	·	·	T	·	G	·	·
Cj3	LC492861	·	·	·	·	·	·	·	A
Cj4	LC492862	T	G	·	T	A	G	G	·
Cj5	LC492863	·	·	A	·	·	·	·	·
Cj6	LC492864	T	·	·	·	·	·	·	·

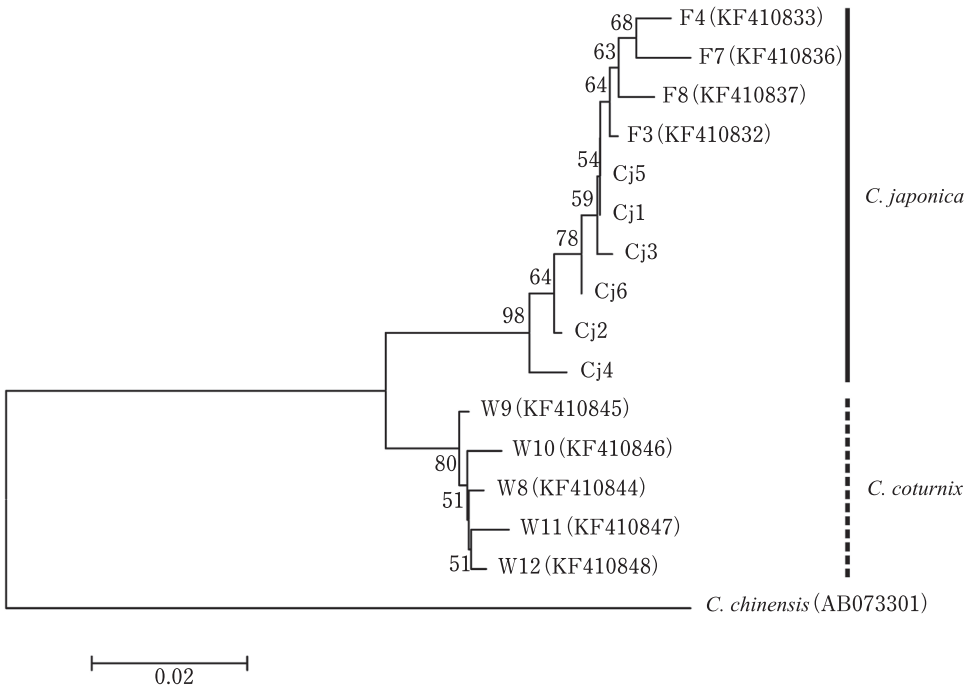

Fig. 1. **Neighbor-joining tree using Tamura-Nei genetic distances among mitochondrial DNA control region haplotypes of quails.** *Coturnix chinensis* was used as an outgroup. Six haplotypes identified in the present study are indicated by "Cj." "F" and "W" are published haplotypes of the Japanese quail (*Coturnix japonica*) and the common quail (*Coturnix coturnix*), respectively. GenBank accession numbers of haplotypes are shown in parentheses. Bootstrap values ＞50% are shown at each node.

lotypes were located in the Japanese quail (*C. japonica*) clade and were clearly different from the common quail (*C. coturnix*) haplotypes. Similarly, in the TCS network (Fig. 2), the six haplotypes were clustered with other Japanese quail haplotypes and sharply diverged from the common quail haplotypes with a large number of nucleotide substitutions.

Remarkably, a single haplotype was distributed with extremely high frequency in the gene pool of laying-type lines: 84.9% (406/478) of the laying-type quails had Cj1 (Table 1). When the data were stratified by farm, the Cj1 had the highest prevalence: 72.5% (29/40) in Farm 6 to 100% (40/40) in Farm9. The second most frequent haplotype was Cj2, which was found in 12.8% (61/478) of the laying-type quails. The Cj1 and Cj2 jointly made up 97.7% (467/478) of the total. On the other hand, Cj3, Cj4, Cj5, and Cj6 were found in only five, three, two, and one individuals, respectively. The haplotype distribution indicated that the maternal origin of laying-type lines was limited. Cj1 is the main haplotype in the present gene pool, although this was obtained from partial sequences of the control region (less than 500 base pairs). This finding was also in agreement with the fact that laying-type quails were threatened with extinction during World War II, and the present gene pool was derived from a

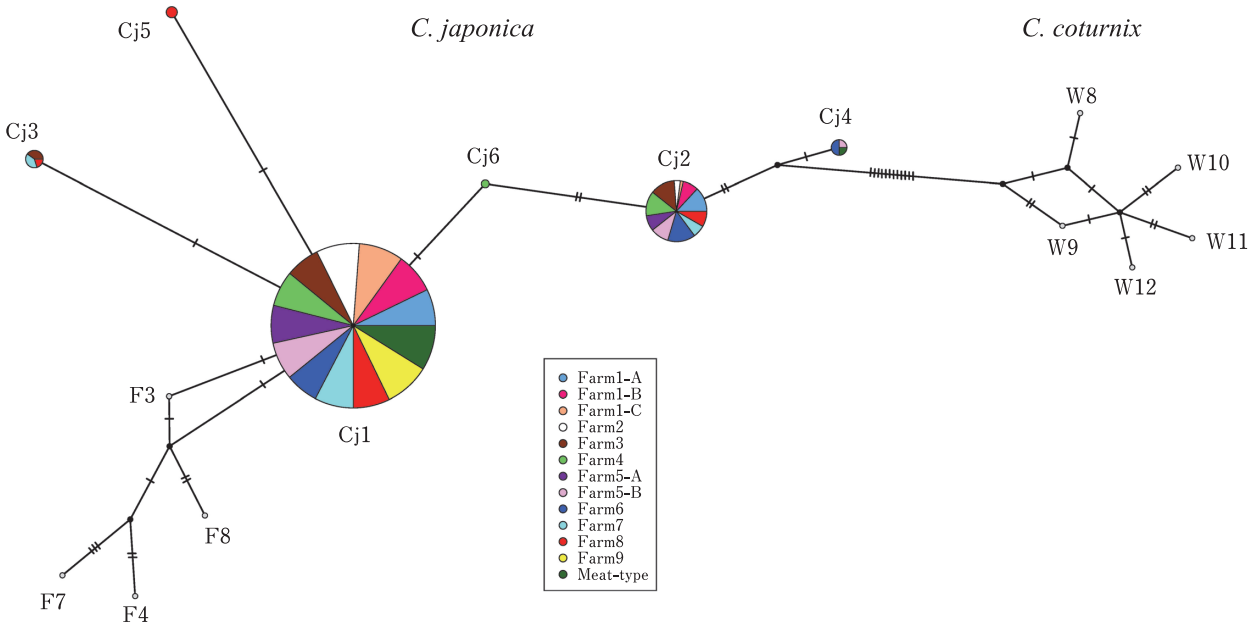

Fig. 2. **TCS network of mitochondrial DNA control region haplotypes of quails.** "Cj" represents haplotypes identified in the present study. Published haplotypes of the Japanese quail (*Coturnix japonica*) and the common quail (*Coturnix coturnix*) are indicated by "F" and "W," respectively. Circle corresponds to one haplotype. Small black circles represent hypothetically intermediate haplotypes that were not identified in the present study. Circle size and different colors of a "Cj" haplotype represent relative frequency and distinct quail lines, respectively. Tick marks on each branch correspond to numbers of nucleotide substitutions.

small number of individuals after World War II (Yamashina, 1961; Wakasugi, 1984). The uniformity of the haplotype distribution in domestic Japanese quail lines has been observed in other studies. For example, Barilani *et al*. (2005) reported that 12 individuals of a Japanese quail line maintained by the University of Renne in France were analyzed, and all of them showed a single haplotype of the mtDNA control region. This haplotype (374 base pairs) (GenBank accession number DQ087957) showed 100% similarity to the prevalent Cj1 haplotype (473 base pairs) in the present study. Therefore, these two haplotypes are possibly the same. Sanchez-Donoso *et al*. (2014) also reported that 22 of 29 (75.9%) individuals of farm quail in Spain, which have Japanese quail maternal origin, had a single haplotype "F1W1" identical to the Cj1 haplotype in the present study. Furthermore, Nunome *et al*. (2017) reported a predominant mtDNA haplotype of the control region across various experimental lines of domestic Japanese quail. The Cj2 haplotype had a relatively high frequency (12.8%) in the present study. However, Sanchez-Donoso *et al*. (2014) reported that a haplotype "F5" identical to Cj2 was distributed with low frequency (one of 29 individuals of farm quail in Spain). This may result from the differences in the number of samples analyzed among the two studies. In the present study, meat-type line, which was bred in France, showed the same haplotype distribution as that shown by laying-type lines, and no haplotypes unique to the meat-type line were detected. This observation may reflect that meat-type quails in Europe have their roots in domestic Japanese quails (i.e., laying-type quails) introduced from Japan after World War II.

Genetic Diversity and Differentiation

Within each laying-type line, the number of haplotypes varied from one (Farm 9) to four (Farm 8) (Table 1). As mentioned above, all lines had Cj1 with high frequency. In contrast, Cj5 and Cj6 were unique to Farm 8 and Farm 4, respectively, although these occurred with low frequency. Haplotype diversity and nucleotide diversity ranged from 0.000 (Farm 9) to 0.4321 (Farm 6), and from 0.000 (Farm 9) to 0.0034 (Farm 6), respectively. The results indicate low mtDNA diversity of laying-type lines. As mentioned in a previous paper (Shimma and Tadano, 2019), each farm generally incorporates quails from other farms every three or five years in order to avoid inbreeding depression. For example, Farm 2 in the present study introduces quails from three different farms into its breeding stocks every three years. However, the number of distinct haplotypes is low: only two haplotypes (Cj1 and Cj2) were found in Farm 2, despite the existence of gene flows from other farms. This may suggest that there are only a small number of maternal lineages throughout the entire gene pool of laying-type lines.

Table 3. **Genetic differentiation index (F_{ST}) between pairs of commercial Japanese quail lines based on analysis of mitochondrial DNA control regions**

Line	Farm 1-A	Farm 1-B	Farm 1-C	Farm 2	Farm 3	Farm 4	Farm 5-A	Farm 5-B	Farm 6	Farm 7	Farm 8	Farm 9	Meat-type
Farm 1-A													
Farm 1-B	−0.0047^{NS}												
Farm 1-C	0.1205*	0.0457^{NS}											
Farm 2	0.0747^{NS}	0.0099^{NS}	−0.0168^{NS}										
Farm 3	−0.0231^{NS}	−0.0029^{NS}	0.1136*	0.0714^{NS}									
Farm 4	−0.0250^{NS}	0.0002^{NS}	0.1296*	0.0832*	−0.0225^{NS}								
Farm 5-A	−0.0092^{NS}	−0.0261^{NS}	0.0526^{NS}	0.0146^{NS}	−0.0073^{NS}	−0.0047^{NS}							
Farm 5-B	−0.0218^{NS}	−0.0143^{NS}	0.0840^{NS}	0.0460^{NS}	−0.0195^{NS}	−0.0199^{NS}	−0.0174^{NS}						
Farm 6	−0.0056^{NS}	0.0416^{NS}	0.1726**	0.1312*	−0.0044^{NS}	−0.0083^{NS}	0.0348^{NS}	−0.0001^{NS}					
Farm 7	0.0145^{NS}	−0.0187^{NS}	0.0228^{NS}	−0.0038^{NS}	0.0095^{NS}	0.0204^{NS}	−0.0179^{NS}	0.0002^{NS}	0.0644*				
Farm 8	−0.0029^{NS}	−0.0213^{NS}	0.0415^{NS}	0.0108^{NS}	−0.0040^{NS}	0.0016^{NS}	−0.0219^{NS}	−0.0117^{NS}	0.0401^{NS}	−0.0185^{NS}			
Farm 9	0.1795**	0.1026^{NS}	0.0000^{NS}	0.0256^{NS}	0.1677**	0.1887**	0.1118*	0.1346*	0.2209***	0.0696^{NS}	0.0883*		
Meat-type	0.1079*	0.0389^{NS}	−0.0170^{NS}	−0.0125^{NS}	0.1026*	0.1166*	0.0446^{NS}	0.0705^{NS}	0.1548**	0.0193^{NS}	0.0361^{NS}	0.0000^{NS}	

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS Not significant.

No significant F_{ST} values were obtained in 78.8% (52/66) pairwise comparisons of lines (Table 3). This result indicates that laying-type lines are genetically close to each other in terms of mtDNA variations. This lack of differentiation may result from a small number of common founders of these lines. A similar tendency was observed in a previous study with the same samples that were used in the present study, which was based on nuclear microsatellite variations (Shimma and Tadano, 2019) in which the 42.4% (28/66) F_{ST} values between pairs of lines were not significant.

In the present study, most individuals of different laying-type quail lines shared a single mtDNA haplotype. In contrast to relatively high nuclear microsatellite diversity reported in a previous study (Shimma and Tadano, 2019), mtDNA diversity of the laying-type lines is low. Genetic differentiation was not estimated in most pairwise comparisons of the laying-type quail lines. These observations are thought to be associated with the history that the laying-type lines were restored from a small number of individuals after World War II. In the future, it may be necessary to implement strategies for preventing loss of genetic diversity and/or introducing novel genetic diversity in the gene pool of laying-type lines.

Conflicts of Interest

The authors declare no conflict of interest.

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