

Genetic Differentiation among Commercial Lines of Laying-type Japanese Quail

Kiyohito Shimma and Ryo Tadano

Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan

Recently in Japan, approximately six million quails were primarily being reared for commercial egg production. It is believed that almost all commercial quails in the country became extinct during World War II, and that the present commercial gene pool was restored from the limited number of surviving birds. The present study evaluates the genetic diversity within and differentiation between 12 laying-type Japanese quail lines on the basis of 45 microsatellite genotypes. The mean number of alleles per locus and the expected heterozygosity within a quail line were 5.22–5.69 and 0.601–0.618, respectively. These results showed that laying-type quail lines in the present study exhibited a higher degree of genetic diversity than experimental quail lines in a previous study. Pairwise genetic differentiations (F_{ST}) between lines were significant but weak ($F_{ST}=0.0028-0.0254$; 57.6%), and no significant differentiations were found between the remainder. This was also confirmed by genetic clustering analyses, in which individuals did not form independent clusters consistent with their line origins. The results of the present study indicate relatively high genetic diversity within and no clear genetic differentiation between laying-type quail lines. Absence of genetic differentiation may reflect the breeding history of laying-type quails.

Key words: genetic differentiation, genetic diversity, Japanese quail, microsatellites

J. Poult. Sci., 56: 12–19, 2019

Introduction

The Japanese quail (*Coturnix japonica*) is utilized globally for egg and meat production. For instance, quails are reared commercially for eggs in Japan and meat in Spain and France (Minville, 2004). In Japan, both the utilization of quails for egg production and their improvement were initiated around 1910 (Wakasugi, 1984). The quail industry was initially developed in Aichi Prefecture in central Japan in the 1930s, and the number of commercial quails in the country reached two million by 1941 (Wakasugi, 1984). However, quails were almost extinct during World War II (Yamashina, 1961; Wakasugi, 1984). It is believed that the present gene pool of commercial quail was mainly restored from the few surviving individuals after World War II in Toyohashi City, Aichi Prefecture (Yamashina, 1961; Wakasugi, 1984). At present, it is estimated that there are approximately six million commercial quails in Japan.

Microsatellite markers are widely used for assessing the genetic diversity and population structure of farm animals, although single nucleotide polymorphism markers are becoming increasingly common. The high degree of microsa-

tellite polymorphism is believed to enable the detection of genetic variation among closely related breeds or lines of farm animals (FAO, 1998). Multilocus microsatellite analysis has mainly been applied to quail lines selected for experimental use (Kim *et al.*, 2007; Tadano *et al.*, 2014). These studies revealed that experimental quails had considerably low genetic diversity and that there was high genetic differentiation between lines. However, genetic diversity and differentiation of commercial quails, such as laying-type lines, are poorly documented.

In the present study, we examined genetic diversity and differentiation of laying-type quail lines on the basis of microsatellite analysis and compared these estimates with those of experimental quail lines obtained from a previous study (Tadano *et al.*, 2014).

Materials and Methods

Quail Lines

In total, 479 individuals were sampled from 12 laying-type quail lines reared for egg production at nine commercial farms in five prefectures in Japan (Hokkaido, Saitama, Shizuoka, Aichi, and Miyazaki) (Table 1). These lines are thought to be descended from the restored quail population at Toyohashi City, Aichi Prefecture after World War II. Three lines from Farm 1 (Farm 1-A, B, and C) have been managed as independent stock. More specifically, Farm 1-A, B, and

Received: December 25, 2017, Accepted: April 11, 2018

Released Online Advance Publication: May 25, 2018

Correspondence: Dr. Ryo Tadano, Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan. (E-mail: tadano@gifu-u.ac.jp)

The Journal of Poultry Science is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. To view the details of this license, please visit (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

Table 1. Genetic diversity within 13 commercial Japanese quail lines based on 45 microsatellite genotypes

Line	Location	Sample size	MNA	H_O	H_E	f_{ij}	Number of fixed loci (%)	Number of unique alleles ¹	GD_T	GD_W	GD_B
Farm 1-A	Hokkaido	40	5.38	0.581	0.602	0.405	1 (2.2%)	0	+0.095%	-0.030%	+0.125%
Farm 1-B	Hokkaido	40	5.60	0.566	0.613	0.394	1 (2.2%)	2	-0.105%	-0.178%	+0.073%
Farm 1-C	Hokkaido	40	5.47	0.568	0.614	0.394	1 (2.2%)	0	-0.034%	-0.177%	+0.143%
Farm 2	Saitama	40	5.40	0.584	0.601	0.407	1 (2.2%)	3	-0.002%	-0.005%	+0.003%
Farm 3	Shizuoka	40	5.64	0.580	0.605	0.402	1 (2.2%)	1	+0.099%	-0.068%	+0.167%
Farm 4	Shizuoka	40	5.44	0.599	0.612	0.396	1 (2.2%)	4	-0.018%	-0.155%	+0.136%
Farm 5-A	Aichi	39	5.69	0.602	0.614	0.394	1 (2.2%)	7	-0.050%	-0.177%	+0.128%
Farm 5-B	Aichi	40	5.67	0.583	0.618	0.390	1 (2.2%)	2	-0.106%	-0.238%	+0.132%
Farm 6	Aichi	40	5.51	0.582	0.603	0.405	1 (2.2%)	1	+0.135%	-0.025%	+0.160%
Farm 7	Aichi	40	5.69	0.560	0.604	0.404	0	1	+0.130%	-0.044%	+0.174%
Farm 8	Aichi	40	5.62	0.569	0.615	0.393	1 (2.2%)	0	-0.047%	-0.197%	+0.150%
Farm 9	Miyazaki	40	5.22	0.585	0.613	0.394	0	0	-0.242%	-0.177%	-0.065%
Meat-type		40	3.44	0.480	0.489	0.516	0	2	-0.174%	+1.473%	-1.647%

MNA, mean number of alleles per locus; H_O , observed heterozygosity; H_E , expected heterozygosity; f_{ij} , within-line molecular co-ancestry coefficient; GD_T , contribution to total genetic diversity; GD_W , contribution to within-line diversity; GD_B , contribution to between-lines diversity.

¹Seventeen of all 23 unique alleles (73.9%) were detected from only one individual within each line.

C were initiated in 1982, and since then, these lines have been maintained as closed flocks. In each line of Farm 1, 600 males and 2,000 females are reserved as breeding stock and these lines are renewed twice a year; in other words, 72 generations have passed in the 36 years since 1982. Farm 2 was initiated in 1956 and was established from 200 individuals derived from a population at Toyohashi City. Farm 2 undergoes genetic exchange with three different farms every 3 years. Farm 4 and Farm 8 have the same origin and are derived from the same breeding company. Breeding stock of this company was initiated in 1960 and male quails from other farms have been introduced into this stock every 5 years. Farm 7 was established from individuals of a population at Toyohashi City approximately 20 years ago and the introduction of male quails from other farms has been conducted every 5 years. Background information on the five other lines (Farm 3, Farm 5-A, Farm 5-B, Farm 6, Farm 9) was not available. Forty individuals of one meat-type quail line, which was selected for increased body weight and was imported from France, were also sampled from a commercial farm for comparison with laying-type lines.

Microsatellite Analysis

Genomic DNA was extracted from liver tissue using the phenol-chloroform method (Sambrook and Russell, 2001). Forty-five microsatellite markers (Table 2) were chosen from a previous study (Tadano *et al.*, 2014). Simplex PCR and genotyping were performed, as described in Tadano *et al.* (2014). In addition, multiplex PCR was performed using Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany) in a 10 μ l reaction volume containing 1 μ l of genomic DNA (20 ng/ μ l), 3 μ l of RNase-free water, 5 μ l of 2 \times Type-it Multiplex PCR Master Mix, and 1 μ l of 10 \times primer mix (2 μ M of each primer). Cycling conditions were as follows: 95 $^{\circ}$ C for 5 min, 28 cycles of denaturation at 95 $^{\circ}$ C for 30 s, annealing at 55 $^{\circ}$ C for 90 s and extension at 72 $^{\circ}$ C for 30 s,

followed by final extension at 60 $^{\circ}$ C for 30 min.

Data Analysis

The number of alleles, observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) (Nei, 1987), and polymorphic information content (PIC) (Botstein *et al.*, 1980) for each locus were calculated using CERVUS 3.0.3 (Kalinowski *et al.*, 2007). The mean number of alleles per locus (MNA), H_O , and H_E within each line were calculated using MICROSATELLITE TOOLKIT 3.1 (Park, 2001). The level of inbreeding within each line was estimated by computing the molecular co-ancestry coefficient (f_{ij}) (Caballero and Toro, 2002) using MOLKIN 3.0 (Gutiérrez *et al.*, 2005). MOLKIN 3.0 was also used to calculate the contribution of each line to the genetic diversity (GD_T , contribution to total genetic diversity; GD_W , contribution to within-line diversity; and GD_B , contribution to between-lines diversity) (Caballero and Toro, 2002).

F_{ST} (Weir and Cockerham, 1984) between each pair of lines was obtained using FSTAT 2.9.3 (Goudet, 1995). Statistical significance of F_{ST} was evaluated using the permutation test implemented in FSTAT. In addition, genetic differentiation between lines was also estimated by calculating the modified Cavalli-Sforza chord distances (D_A) (Nei *et al.*, 1983) and by constructing a neighbor-joining tree with 1,000 bootstrap replications using POPTREE2 (Takezaki *et al.*, 2010).

To reveal the genetic structure, a neighbor-joining tree of individuals was constructed using NEIGHBOR in PHYLIP 3.6 (Felsenstein, 2005) and TREEEXPLORER in MEGA 3.0 (Kumar *et al.*, 2004) from the genetic distance based on the proportion of shared alleles (D_{ps}) (Bowcock *et al.*, 1994) calculated using MICROSATELLITE ANALYSER 4.00 (Dieringer and Schlötterer, 2003). Bayesian model-based clustering was also performed using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). Under the admixture models with

Table 2. Characterization of 45 microsatellite markers analyzed in 13 commercial Japanese quail lines ($n=519$).

Locus name	GeneBank accession number	T _a (°C)	Number of alleles	Allele size range (bp)	H _O	H _E	PIC	Missing (%)
NGJ0001	AB973827	55	7	225-247	0.613	0.640	0.604	0.0
NGJ0002	AB973828	55	3	307-315	0.439	0.424	0.376	0.0
NGJ0003	AB973829	55	10	196-213	0.830	0.815	0.789	0.0
NGJ0004	AB973830	55	7	157-168	0.674	0.703	0.653	0.0
NGJ0005	AB973831	55	9	203-216	0.753	0.742	0.700	0.0
NGJ0006	AB973832	55	6	244-250	0.663	0.708	0.663	0.0
NGJ0007	AB973833	50	3	118-122	0.033	0.601	0.533	0.6
NGJ0008	AB973834	55	4	188-193	0.657	0.654	0.586	0.0
NGJ0009	AB973835	55	7	200-216	0.667	0.655	0.609	0.0
NGJ0010	AB973836	55	6	249-258	0.705	0.702	0.648	0.0
NGJ0011	AB973837	55	7	138-166	0.580	0.572	0.542	0.0
NGJ0012	AB973838	55	4	257-263	0.418	0.424	0.383	0.0
NGJ0013	AB973839	55	10	236-277	0.705	0.738	0.710	0.0
NGJ0014	AB973840	55	3	126-130	0.212	0.538	0.450	1.0
NGJ0015	AB973841	55	5	93-103	0.304	0.544	0.511	0.0
NGJ0017	AB973843	55	5	108-139	0.374	0.504	0.453	1.5
NGJ0018	AB973844	55	5	240-245	0.620	0.636	0.577	0.0
NGJ0019	AB973845	55	8	248-268	0.631	0.715	0.679	0.2
NGJ0020	AB973846	55	10	161-183	0.705	0.731	0.695	0.0
NGJ0021	AB973847	55	4	247-260	0.634	0.641	0.588	0.0
NGJ0022	AB973848	55	4	163-167	0.368	0.376	0.316	0.0
NGJ0023	AB973849	55	2	238-240	0.040	0.047	0.046	0.0
NGJ0024	AB973850	55	12	294-335	0.752	0.791	0.761	0.4
NGJ0025	AB973851	50	9	187-206	0.761	0.765	0.727	0.0
NGJ0026	AB973852	55	8	118-138	0.618	0.637	0.598	0.0
NGJ0027	AB973853	55	7	261-282	0.703	0.702	0.661	0.0
NGJ0029	AB973855	55	7	152-166	0.601	0.582	0.545	0.0
NGJ0030	AB973856	55	11	169-194	0.647	0.654	0.606	0.0
NGJ0031	AB973857	55	3	209-216	0.222	0.301	0.280	0.0
NGJ0032	AB973858	55	10	149-168	0.784	0.789	0.765	0.0
NGJ0033	AB973859	55	10	116-137	0.757	0.774	0.738	0.8
NGJ0034	AB973860	55	10	204-231	0.734	0.755	0.718	0.0
NGJ0035	AB973861	55	7	143-160	0.326	0.328	0.313	0.0
NGJ0036	AB973862	55	6	278-285	0.773	0.765	0.726	0.0
NGJ0037	AB973863	55	4	313-319	0.380	0.401	0.349	0.0
NGJ0038	AB973864	55	12	249-284	0.800	0.822	0.798	0.0
NGJ0040	AB973866	55	5	232-241	0.582	0.572	0.523	0.0
NGJ0041	AB973867	55	8	185-196	0.676	0.687	0.646	0.0
NGJ0043	AB973869	55	9	253-273	0.678	0.678	0.627	0.0
NGJ0044	AB973870	55	3	209-213	0.320	0.336	0.288	0.0
NGJ0046	AB973872	55	10	189-216	0.685	0.691	0.661	0.2
NGJ0047	AB973873	55	3	271-292	0.489	0.512	0.458	0.0
NGJ0048	AB973874	55	8	283-295	0.684	0.710	0.665	0.0
NGJ0049	AB973875	55	5	193-199	0.459	0.487	0.455	0.0
NGJ0050	AB973876	55	12	268-298	0.688	0.831	0.810	1.2

T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; Missing, proportion of missing data.

correlated allele frequencies, 20 runs were performed for each K (the number of clusters) ranging from 1 to 20, with a burn-in period of 100,000 and 100,000 iterations. CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) was used to average individual's membership coefficients for the 20 runs based on the LargeKGreedy algorithm. DISTRUCT 1.1 (Rosenberg, 2004) was used to visualize the results. The mean likelihood

[$L(K)$] (Pritchard *et al.*, 2000) and ΔK (Evanno *et al.*, 2005) were computed to determine the optimum K using STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt, 2012).

Table 3. F_{ST} (top diagonal) and D_A (lower diagonal) between pairs of commercial Japanese quail lines based on 45 microsatellite genotypes

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		0.0058 ^{NS}	0.0007 ^{NS}	0.0156 **	0.0054 *	0.0053 *	0.0078 **	0.0062 **	0.0057 **	0.0043 ^{NS}	0.0046 **	0.0221 **	0.1154 **
Farm 1-B	0.027		0.0036 ^{NS}	0.0202 **	0.0083 ^{NS}	0.0094 **	0.0087 **	0.0102 **	0.0083 **	0.0072 **	0.0089 **	0.0207 **	0.1189 **
Farm 1-C	0.023	0.024		0.0159 **	0.0013 ^{NS}	0.0028 *	0.0070 **	0.0041 *	0.0035 ^{NS}	0.0022 ^{NS}	0.0047 ^{NS}	0.0200 **	0.1153 **
Farm 2	0.041	0.046	0.045		0.0094 **	0.0173 **	0.0152 **	0.0096 **	0.0112 **	0.0079 **	0.0128 **	0.0254 **	0.1263 **
Farm 3	0.027	0.026	0.023	0.033		0.0024 ^{NS}	0.0015 ^{NS}	0.0013 ^{NS}	-0.0033 ^{NS}	-0.0010 ^{NS}	0.0010 ^{NS}	0.0130 **	0.1276 **
Farm 4	0.033	0.031	0.029	0.042	0.025		0.0067 **	0.0020 NS	0.0030 ^{NS}	0.0030 ^{NS}	0.0002 ^{NS}	0.0182 **	0.1191 **
Farm 5-A	0.034	0.033	0.033	0.039	0.025	0.031		0.0058 *	0.0014 ^{NS}	0.0021 ^{NS}	0.0009 ^{NS}	0.0117 **	0.1217 **
Farm 5-B	0.029	0.030	0.028	0.034	0.022	0.023	0.028		0.0012 ^{NS}	0.0030 ^{NS}	0.0007 ^{NS}	0.0180 **	0.1258 **
Farm 6	0.029	0.029	0.027	0.033	0.019	0.028	0.028	0.022		0.0036 ^{NS}	0.0028 ^{NS}	0.0143 **	0.1296 **
Farm 7	0.027	0.030	0.027	0.033	0.020	0.024	0.028	0.023	0.019		0.0036 ^{NS}	0.0155 **	0.1169 **
Farm 8	0.028	0.032	0.027	0.037	0.022	0.024	0.026	0.021	0.025	0.025	0.0036 ^{NS}	0.0187 **	0.1167 **
Farm 9	0.050	0.047	0.051	0.052	0.039	0.047	0.045	0.042	0.038	0.041	0.047	0.0187 **	0.1296 **
Meat-type	0.147	0.153	0.145	0.152	0.152	0.152	0.155	0.152	0.152	0.141	0.151	0.164	

Significant F_{ST} values are in bold. * $P < 0.05$, ** $P < 0.01$, NS Not significant. The highest and lowest D_A values are in bold.

Results and Discussion

Microsatellite Polymorphism and Genetic Diversity

Table 2 shows the degrees of polymorphism for 45 microsatellite markers calculated from the genotype data of 13 quail lines (519 individuals). The highest proportion of missing data (8/519 genotypes, 1.5%) was observed at *NGJ0017*. In total, 308 distinct alleles were detected at the 45 microsatellite loci, with the number of alleles per locus ranging from 2 (*NGJ0023*) to 12 (*NGJ0024*, *NGJ0038*, and *NGJ0050*). H_O and H_E per locus ranged from 0.033 (*NGJ0007*) to 0.830 (*NGJ0003*) and 0.047 (*NGJ0023*) to 0.831 (*NGJ0050*), respectively. Rosenberg *et al.* (2001) suggested that H_E is a useful criterion for selecting effective markers for genetic clustering and assignment. Based on H_E , 36 of 45 markers (80.0%) showed a high degree of polymorphism ($H_E > 0.500$). PIC per locus ranged from 0.046 (*NGJ0023*) to 0.810 (*NGJ0050*). According to the criteria of Botstein *et al.* (1980), 73.3% (33/45) were highly informative ($PIC \geq 0.500$).

Table 1 summarizes the genetic diversity within the 12 laying- and one meat-type quail lines. All laying-type lines exhibited greater diversity than meat-type line. In laying-type lines, MNA ranged from 5.22 (Farm 9) to 5.69 (Farm 5-A and Farm 7). H_O and H_E varied from 0.560 (Farm 7) to 0.602 (Farm 5-A) and from 0.601 (Farm 2) to 0.618 (Farm 5-B), respectively. No large differences were observed among diversity estimates of laying-type lines. However, these were much higher than those previously reported for experimental quail lines ($MNA = 1.3-2.7$, $H_O = 0.11-0.42$ and $H_E = 0.11-0.43$; Tadano *et al.*, 2014). In addition, the degrees of inbreeding within laying-type lines ($f_{ij} = 0.390-0.407$) were lower than those within the experimental lines ($f_{ij} = 0.59-0.90$; Tadano *et al.*, 2014). In the present study, a maximum of 2.2% of genotyped loci were fixed within a laying-type line. The proportion was much smaller than those within an experimental line (14.9%-72.3% of genotyped loci; Tadano *et al.*, 2014). These results indicate that laying-type lines have a higher level of genetic diversity than experimental lines. In fact, farmers periodically introduce quails from other farms into their own breeding stocks to prevent inbreeding depressions. In general, exchange of male quails among farms is conducted every 3 or 5 years. This breeding procedure may result in high genetic diversity within laying-type lines. In the present study, closed lines (Farm 1-A, B, and C), which have been maintained without gene flow for 36 years, showed high genetic diversity similar to other lines. This may be attributable to the large population size of these closed lines; that is, 600 males and 2,000 females contribute to the production of the next generation. In contrast, experimental lines have undergone intense selection on the basis of specific traits in their small closed flocks. This may result in low levels of genetic diversity within experimental lines.

In all the lines analyzed, the highest contribution to total genetic diversity ($GD_T = -0.242\%$) was found in Farm 9, indicating that its loss would lead to the greatest loss, 0.242

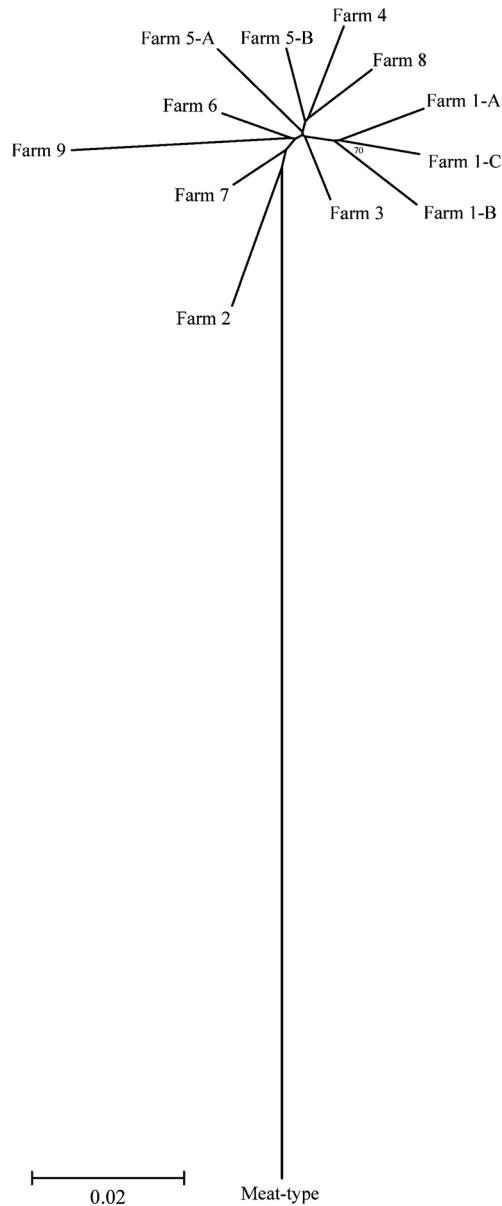


Fig. 1. Neighbor-joining tree for 13 commercial Japanese quail lines, using D_A (Nei *et al.*, 1983) calculated from 45 microsatellite genotypes. Bootstrap values $> 50\%$ are shown.

% of total genetic diversity, from the whole population. This result indicates that Farm 9 is relatively distinct from other laying-type lines. This is further supported by the finding that Farm 9 showed significant genetic differentiation (F_{ST} in Table 2) between all other laying-type lines. All laying-type lines contributed negatively to within-line diversity (GD_W ranging from -0.238% to -0.005%). In contrast, the meat-type line made a high positive contribution ($GD_W = +1.473\%$). This suggests that the degree of inbreeding within laying-type lines was much lower than that within the meat-type line.

Genetic Differentiation

Of all 66 F_{ST} values between each pair of laying-type lines, 28 (42.4%) were not significant (i.e., no genetic differentiation) (Table 3). In particular, although three lines (Farm 1-A, B, and C) have been closed for 36 years, significant F_{ST} values were not estimated between each pair of these lines. The remaining 38 (57.6%) F_{ST} values were significant but low ($F_{ST} = 0.0028-0.0254$) and were much lower than those of experimental quail lines ($F_{ST} = 0.13-0.83$; Tadano *et al.*, 2014). This result indicates that there is no clear genetic differentiation between laying-type lines. Similarly, small Nei's D_A (range: 0.019-0.052) values were estimated between laying-type lines (Table 3). These were considerably smaller than those of experimental lines ($D_A = 0.10-0.60$; Tadano *et al.*, 2014). In a neighbor-joining tree based on Nei's D_A (Fig. 1), low genetic differentiation was found in laying-type lines with short branch lengths.

The absence of genetic differentiation between lines was further supported by genetic clustering analyses. In a neighbor-joining tree based on D_{ps} (Fig. 2), individuals of laying-type lines did not form defined clusters corresponding to their line origins. This suggests that the individuals are genetically similar to each other and a number of laying-type lines can be, to a large extent, regarded as a single population. In Bayesian model-based clustering, two independent analyses were conducted using different data sets (i.e., laying-type and meat-type lines or laying-type lines only). In the analysis including the meat-type line, both $L(K)$ and ΔK indicated that the most likely number of clusters (K) was two (data not shown). Laying-type and meat-type lines were separated into two distinct clusters at $K=2$ (Fig. 3a). No independent cluster was detected in the gene pool of laying-type quails at $K=3-5$, although Farm 9 exhibited a genetic component different from others (Fig. 3a). In the analysis of laying-type lines only, the highest $L(K)$ was observed at $K=1$ (data not shown), indicating no genetic differentiation between laying-type lines. Meanwhile, ΔK had a maximum at $K=2$ (data not shown), indicating the presence of two genetically distinct groups. However, the result of $K=2$ showed no independent cluster and a pattern with a high degree of admixture (Fig. 3b). The same pattern was also found at $K=3$. Ultimately, these results suggest that there was no obvious genetic differentiation in laying-type lines, although Farm 9 showed a slight difference at $K=4$ and $K=5$ (Fig. 3b). This weak structuring between laying-type lines (i.e., high genetic similarity between individuals of different lines) may be attributed to the reconstruction of the present gene pool from a limited number of individuals after World War II. In addition, sufficient selection to generate genetic differentiation between the lines has not occurred. The clustering patterns of laying-type lines were considerably different from those of the experimental lines. In a previous study (Tadano *et al.*, 2014), the experimental lines formed well-defined clusters corresponding to the line origin, reflecting high levels of genetic differentiation between lines.

In conclusion, this study revealed that commercial laying-type quail lines have high genetic diversity and show no

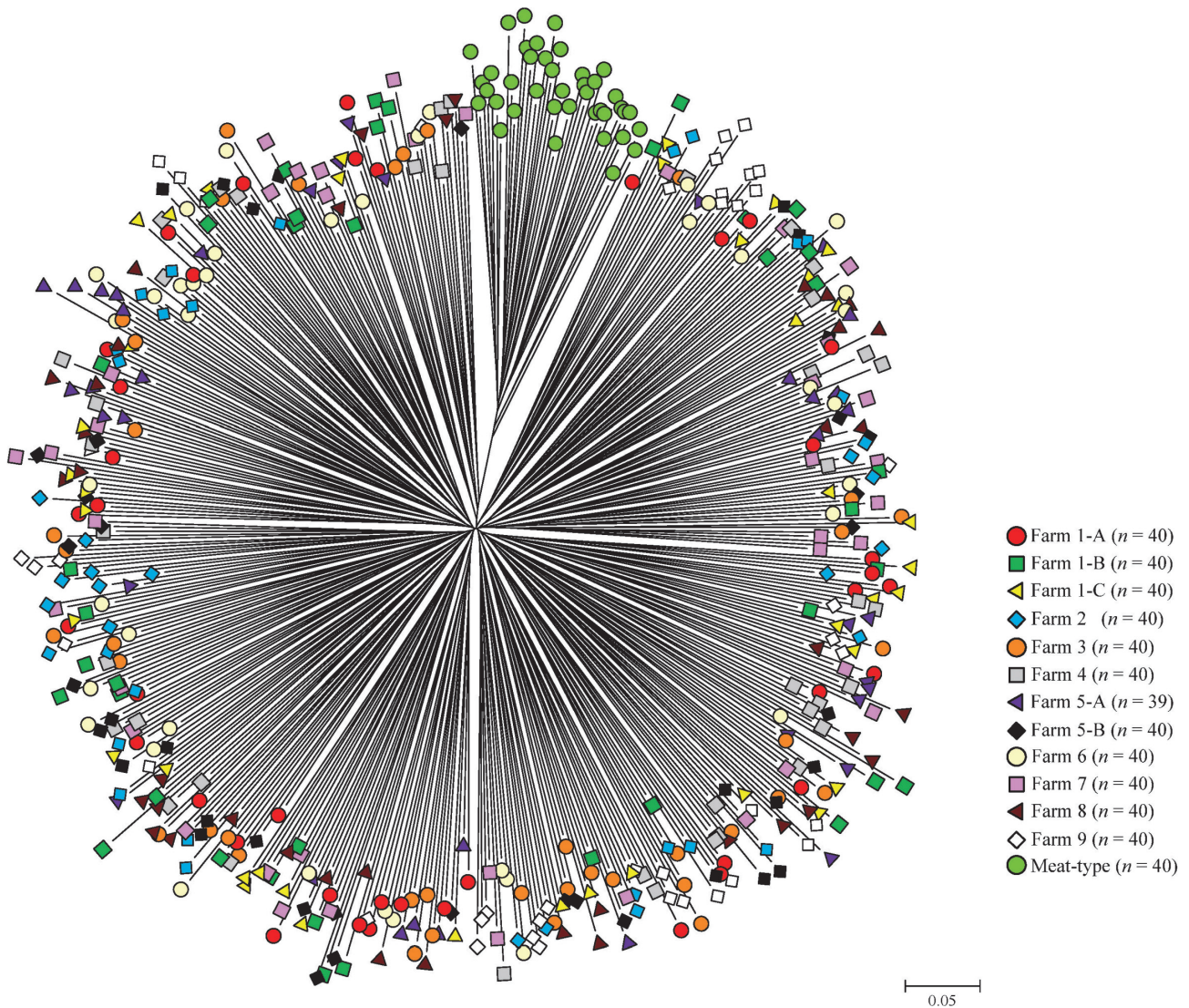


Fig. 2. Neighbor-joining tree for 519 individuals from 13 commercial Japanese quail lines, using D_{ps} (Bowcock *et al.*, 1994) calculated from 45 microsatellite genotypes.

inbreeding signatures as compared with experimental quail lines. In addition, the lack of clear genetic differentiation between lines was observed, which may be associated with the breeding history of laying-type quail lines.

Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research (Grant No. 26850166 and 16K18781) from the Japan Society for the Promotion of Science to R. Tadano.

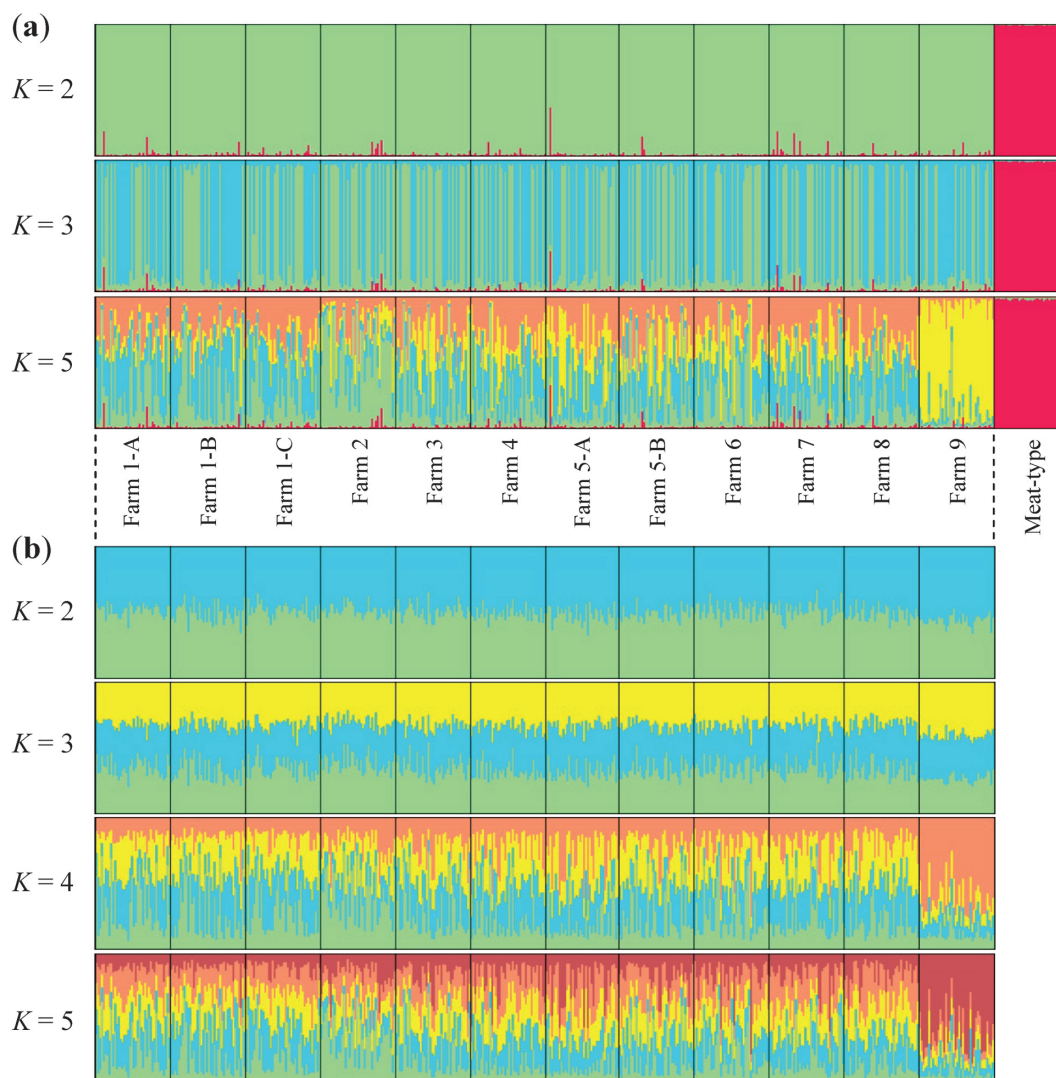


Fig. 3. **Bayesian model-based clustering in STRUCTURE based on 45 microsatellite genotypes.** (a) 12 laying-type lines and one meat-type line ($K=2, 3,$ and 5); (b) 12 laying-type lines ($K=2, 3, 4,$ and 5). Each individual is represented by a vertical bar. Each color corresponds to one cluster, and the length of the colored segment represents the individual's membership coefficient in this cluster.

References

- Botstein D, White RL, Skolnick M and Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32: 314-331. 1980.
- Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR and Cavalli-Sforza LL. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, 368: 455-457. 1994.
- Caballero A and Toro MA. Analysis of genetic diversity for the management of conserved subdivided populations. *Conservation Genetics*, 3: 289-299. 2002.
- Dieringer D and Schlötterer C. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, 3: 167-169. 2003.
- Earl DA and vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359-361. 2012.
- Evanno G, Regnaut S and Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14: 2611-2620. 2005.
- FAO. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Original Working Group Report. Food and Agriculture Organization, Roma.

- 1998.
- Felsenstein J. Phylip (Phylogeny Inference Package) version 3.69. Department of Genome Sciences, University of Washington, Seattle. 2009.
- Goudet J. FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, 86: 485–486. 1995.
- Gutiérrez JP, Royo LJ, Álvarez I, and Goyache F. MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. *Journal of Heredity*, 96: 718–721. 2005.
- Jakobsson M and Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23: 1801–1806. 2007.
- Kalinowski ST, Taper ML and Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16: 1099–1106. 2007.
- Kim SH, Cheng KM, Ritland C, Ritland K and Silversides FG. Inbreeding in Japanese quail estimated by pedigree and microsatellite analyses. *Journal of Heredity*, 98: 378–381. 2007.
- Kumar S, Tamura K and Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, 5: 150–163. 2004.
- Minvielle F. The future of Japanese quail for research and production. *World's Poultry Science Journal*, 60: 500–507. 2004.
- Nei M. *Molecular Evolutionary Genetics*. Columbia University Press, New York. 1987.
- Nei M, Tajima F and Tateno Y. Accuracy of estimated phylogenetic trees from molecular Data. II. Gene frequency data. *Journal of Molecular Evolution*, 19: 153–170. 1983.
- Park SDE. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. PhD Thesis, University of Dublin, Ireland. 2001.
- Pritchard JK, Stephens M, and Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959. 2000.
- Rosenberg NA. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4: 137–138. 2004.
- Rosenberg NA, Burke T, Elo K, Feldman MW, Freidlin PJ, Groenen MAM, Hillel J, Mäki-Tanila A, Tixier-Boichard M, Vignal A, Wimmers K and Weigend S. Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. *Genetics*, 159: 699–713. 2001.
- Sambrook J and Russell DW. *Molecular Cloning: A Laboratory Manual*, Third Edition. Cold Spring Harbor Laboratory Press, New York. 2001.
- Tadano R, Nunome M, Mizutani M, Kawahara-Miki R, Fujiwara A, Takahashi S, Kawashima T, Nirasawa K, Ono T, Kono T, and Matsuda Y. Cost-effective development of highly polymorphic microsatellite in Japanese quail facilitated by next-generation sequencing. *Animal Genetics*, 45: 881–884. 2014.
- Takezaki N, Nei M and Tamura K. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Molecular Biology and Evolution*, 27: 747–752. 2010.
- Wakasugi N. Japanese quail. In: *Evolution of Domesticated Animals* (Mason IL ed.), pp. 319–321. Longman Inc., New York. 1984.
- Weir BS and Cockerham CC. Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38: 1358–1370. 1984.
- Yamashina Y. Quail breeding in Japan. *Journal of the Bombay Natural History Society*, 58: 216–222. 1961.