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

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 (Minvielle, 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 microsatellite 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
Microsatellite Analysis

imported from France, were also sampled from a commercial line, which was selected for increased body weight and was not available. Forty individuals of one meat-type quail underwent 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 are derived from the same breeding company. Breeding every 3 years. Farm 4 and Farm 8 have the same origin and these lines are renewed twice a year; in other words, 72 generations have passed in the 36 years since 1982. Farm 2 undergoes genetic exchange with three different farms every 5 years. Background information on the five introduction of male quails from other farms has been conducted every 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 μl reaction volume containing 1 μl of genomic DNA (20 ng/μl), 3 μl of RNase-free water, 5 μl of 2× Type-it Multiplex PCR Master Mix, and 1 μl of 10× primer mix (2 μM of each primer). Cycling conditions were as follows: 95°C for 5 min, 28 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 90 s and extension at 72°C for 30 s, followed by final extension at 60°C for 30 min.

Data Analysis

The number of alleles, observed heterozygosity (HO), unbiased expected heterozygosity (HE) (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), HO, and HE 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 (fij) (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 (GDt), contribution to total genetic diversity; GDW, contribution to within-line diversity; GDh, contribution to between-lines diversity (Caballero and Toro, 2002).

FST (Weir and Cockerham, 1984) between each pair of lines was obtained using FSTAT 2.9.3 (Goudet, 1995). Statistical significance of FST 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 (Dij) (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 (Dp) (Bowcock et al., 1994) calculated using MICROSATellite ANALYSE 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 1. Genetic diversity within 13 commercial Japanese quail lines based on 45 microsatellite genotypes

<table>
<thead>
<tr>
<th>Line</th>
<th>Location</th>
<th>Sample size</th>
<th>MNA</th>
<th>HO</th>
<th>HE</th>
<th>f0</th>
<th>Number of fixed loci (%)</th>
<th>Number of unique alleles</th>
<th>GDt</th>
<th>GDW</th>
<th>GDh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1−A</td>
<td>Hokkaido</td>
<td>40</td>
<td>5.38</td>
<td>0.581</td>
<td>0.602</td>
<td>0.405</td>
<td>1 (2.2%)</td>
<td>0</td>
<td>+0.095%</td>
<td>−0.030%</td>
<td>+0.125%</td>
</tr>
<tr>
<td>Farm 1−B</td>
<td>Hokkaido</td>
<td>40</td>
<td>5.60</td>
<td>0.566</td>
<td>0.613</td>
<td>0.394</td>
<td>1 (2.2%)</td>
<td>2</td>
<td>−0.105%</td>
<td>−0.178%</td>
<td>+0.073%</td>
</tr>
<tr>
<td>Farm 1−C</td>
<td>Hokkaido</td>
<td>40</td>
<td>5.47</td>
<td>0.568</td>
<td>0.614</td>
<td>0.394</td>
<td>1 (2.2%)</td>
<td>0</td>
<td>−0.034%</td>
<td>−0.177%</td>
<td>+0.143%</td>
</tr>
<tr>
<td>Farm 2</td>
<td>Saitama</td>
<td>40</td>
<td>5.40</td>
<td>0.584</td>
<td>0.601</td>
<td>0.407</td>
<td>1 (2.2%)</td>
<td>3</td>
<td>−0.002%</td>
<td>−0.005%</td>
<td>+0.003%</td>
</tr>
<tr>
<td>Farm 3</td>
<td>Shizuoka</td>
<td>40</td>
<td>5.64</td>
<td>0.580</td>
<td>0.605</td>
<td>0.402</td>
<td>1 (2.2%)</td>
<td>1</td>
<td>+0.099%</td>
<td>−0.068%</td>
<td>+0.167%</td>
</tr>
<tr>
<td>Farm 4</td>
<td>Shizuoka</td>
<td>40</td>
<td>5.44</td>
<td>0.599</td>
<td>0.612</td>
<td>0.396</td>
<td>1 (2.2%)</td>
<td>4</td>
<td>−0.018%</td>
<td>−0.155%</td>
<td>+0.136%</td>
</tr>
<tr>
<td>Farm 5−A</td>
<td>Aichi</td>
<td>39</td>
<td>5.69</td>
<td>0.602</td>
<td>0.614</td>
<td>0.394</td>
<td>1 (2.2%)</td>
<td>7</td>
<td>−0.050%</td>
<td>−0.177%</td>
<td>+0.128%</td>
</tr>
<tr>
<td>Farm 5−B</td>
<td>Aichi</td>
<td>40</td>
<td>5.67</td>
<td>0.583</td>
<td>0.618</td>
<td>0.390</td>
<td>1 (2.2%)</td>
<td>2</td>
<td>−0.106%</td>
<td>−0.238%</td>
<td>+0.132%</td>
</tr>
<tr>
<td>Farm 6</td>
<td>Aichi</td>
<td>40</td>
<td>5.51</td>
<td>0.582</td>
<td>0.603</td>
<td>0.405</td>
<td>1 (2.2%)</td>
<td>2</td>
<td>+1.135%</td>
<td>−0.025%</td>
<td>+0.160%</td>
</tr>
<tr>
<td>Farm 7</td>
<td>Aichi</td>
<td>40</td>
<td>5.69</td>
<td>0.560</td>
<td>0.604</td>
<td>0.404</td>
<td>0</td>
<td>1</td>
<td>+0.130%</td>
<td>−0.044%</td>
<td>+0.174%</td>
</tr>
<tr>
<td>Farm 8</td>
<td>Aichi</td>
<td>40</td>
<td>5.62</td>
<td>0.569</td>
<td>0.615</td>
<td>0.393</td>
<td>1 (2.2%)</td>
<td>0</td>
<td>−0.047%</td>
<td>−0.197%</td>
<td>+0.150%</td>
</tr>
<tr>
<td>Farm 9</td>
<td>Miyazaki</td>
<td>40</td>
<td>5.22</td>
<td>0.585</td>
<td>0.613</td>
<td>0.394</td>
<td>0</td>
<td>0</td>
<td>−0.242%</td>
<td>−0.177%</td>
<td>−0.065%</td>
</tr>
<tr>
<td>Meat-type</td>
<td></td>
<td>40</td>
<td>3.44</td>
<td>0.480</td>
<td>0.489</td>
<td>0.516</td>
<td>0</td>
<td>2</td>
<td>−0.174%</td>
<td>+1.473%</td>
<td>−1.647%</td>
</tr>
</tbody>
</table>

MNA, mean number of alleles per locus; HO, observed heterozygosity; HE, expected heterozygosity; f0, within-line molecular co-ancestry coefficient; GDt, contribution to total genetic diversity; GDW, contribution to within-line diversity; GDh, contribution to between-lines diversity. 1Seventeen of all 23 unique alleles (73.9%) were detected from only one individual within each line.
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 $\Delta K$ (Evanno et al., 2005) were computed to determine the optimum $K$ using STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt, 2012).
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 ≥ 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 ($f_0 = 0.390–0.407$) were lower than those within the experimental lines ($f_0 = 0.59–0.90$; 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
% 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 $\Delta K$ 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, $\Delta K$ 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
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.

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


1998.