Inbreeding in Japanese Quail Estimated by Pedigree and Microsatellite Analyses

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Accurately estimating inbreeding is important because inbreeding reduces fitness and production traits in populations. We analyzed information from pedigrees and from microsatellite markers to estimate inbreeding in a line of Japanese quail derived from a randombred line (QO) and maintained for 17 generations by pedigreed matings of brothers to groups of sisters. Pedigree data were used to calculate the inbreeding coefficient \( F_{IT} \), which is the level of inbreeding based on a reference ancestor. Data from analysis of 14 microsatellite markers in the inbred and QO lines were used to calculate the population differentiation \( F_{ST} \) of the lines caused by inbreeding. The \( F_{IT} \) was then calculated as \( F_{IT} = F_{ST} + (1 - F_{ST}) \times F_{IS} \), where \( F_{IS} \) is the level of inbreeding in the inbred line. Observed heterozygosity from analysis of the microsatellite markers of the QO and inbred lines was 0.43 and 0.21, respectively, and the number of alleles was 3.29 and 1.93, demonstrating a reduction of genetic diversity in the inbred line. The \( F_{IT} \) of the inbred line calculated from the pedigree and microsatellite marker analyses was 0.69 ± 0.07 and 0.57 ± 0.33, respectively. These data suggest that pedigree analysis was more accurate than microsatellite marker analyses for estimating inbreeding in this line of Japanese quail.

Inbreeding increases homozygosity and exposes deleterious recessive mutations carried by a population causing inbreeding depression that reduces the fitness of the population (Frankham et al. 2002; Keller and Waller 2002). Frankel and Soule (1981) suggest that an increase in inbreeding of 10% accounts for a reduction of up to 5–10% in fitness components. Keller (1998) reported a 17.5% reduction in annual survival rate of offspring of a full-sib mating of wild song sparrows.

Japanese quail \( (Coturnix japonica) \) are very susceptible to inbreeding depression (Sittmann et al. 1966), probably because of the heavy genetic load that they carry. Sittmann et al. (1966) estimated that 3 generations of full-sib matings reduced the probability of a zygote reaching sexual maturity and leaving offspring to practically zero, and only 6 of the 17 full-sib inbred lines started by Kulenkamp et al. (1973) survived to the fifth generation. No one has reported an inbred Japanese quail line surviving more than 8 consecutive generations of full-sib matings (Okimoto R, personal communication). This is unfortunate because highly inbred lines are very useful in genome analysis and gene mapping (e.g., Hoti and Sillanpää 2006). At the University of British Columbia, a less intensive inbreeding scheme, adapted from that used for the development of the I-420 inbred White Leghorn chicken line (Shoffner et al. 1953), was applied to a line of Japanese quail. This inbred line has undergone 17 generations of inbreeding and still appears to be vigorous. It is therefore of interest to estimate the inbreeding coefficient of this population and compare it to the inbreeding level that would have been attained with full-sib mating.

The inbreeding coefficient \( (F) \) is often calculated from pedigree information, but it is also possible to estimate inbreeding using information from genetic markers (Keller and Waller 2002). In the absence of pedigree information, genetic markers have been used extensively to estimate relatedness between the individuals and the level of inbreeding in populations (e.g., Vargo et al. 2003). Using a simulation study, Baumung and Sölkner (2003) found that inbreeding calculated from pedigrees was more closely related to the proportion of alleles that were identical by descent than that obtained from microsatellite marker information.

The aims of this study were to 1) compare genetic diversity between the inbred quail line and the randombred line from which the inbred line was derived and 2) compare the level of inbreeding calculated from the pedigree with that from analysis of microsatellite markers.

Materials and Methods

A line of Japanese quail (QO) that had previously been selected for 45-day body weight (Caron et al. 1990) was
acquired from the Deschambault Research Station, Quebec, Canada, in 1990. Subsequently, the line was maintained at the University of British Columbia as a randomized population with 48 males and 96 females per generation. In 1992, an inbred line was started from this population (Aggrey 1994) using the breeding scheme described in Figure 1, although some generations had fewer than 4 females per cage. At the time of this study, the inbred line had gone through its 17th generation. We sampled genomic DNA from whole blood of 40 individuals from the QO line and 31 from the inbred strain using a protocol provided by J. Fulton (Hy-Line International, personal communication).

Twenty-four highly polymorphic markers were selected from the Japanese quail microsatellite markers developed by Kayang et al. (2002). These markers were screened based on polymorphism, their distribution across the genome, and the clarity of the bands that they produced. We chose to proceed on polymorphism, their distribution across the genome, and by Kayang et al. (2002). These markers were screened based on polymorphism, their distribution across the genome, and the clarity of the bands that they produced. We chose to proceed on polymorphism, their distribution across the genome, and the clarity of the bands that they produced.

From 0.5 to 2 μl of 20 ng/μl of genomic DNA were added to a mixture of 1 μl Taq enzyme buffer (Roche, Mannheim, Germany), 1 μl of 2.0 mM dNTPs (Roche), 0.5 μl of each of 1.0 pmol/μl forward and reverse primers (Operon Biotechnologies, Huntsville, AL), 0.5 μl of 1 pmol/μl M13 primer (LiCor, Lincoln, NE), and 0.5 unit of Taq polymerase (0.2 μl, Roche), with dH2O to make up 10 μl. After the polymerase chain reactions (PCR), we analyzed the products on a LiCor 4200 DNA sequencer. We scored the bands using RFLPscan (LiCor) and optimized the PCR conditions necessary for adjusting the amount of genomic DNA or the annealing temperature.

We calculated the inbreeding coefficient of an individual relative to the total population, \( F_{IT} \), from microsatellite marker analyses. We considered the QO line to be the total population because the inbred line was derived from it, and we calculated \( F_{IT} \) of an individual in the inbred line in relation to the QO line. We estimated \( F_{IT} \) as \( (1 - F_{IS}) (1 - F_{ST}) = 1 - F_{IT} \) (Keller and Waller 2002), where \( F_{IS} \) is the level of inbreeding (heterozygosity observed relative to that expected from random mating) in the inbred population, calculated as \( F_{IS} = 1 - (H_{EINB}/H_{ERAND}) \), and \( F_{ST} \) is the accumulated effect of inbreeding over time. We used Genetic Data Analysis, v1.0 (Lewis and Zaykin 2001) after Frankham et al. (2004) to calculate \( F_{ST} \) as \( F_{ST} = 1 - (H_{EINB}/H_{ERAND}) \), where \( H_{EINB} \) and \( H_{ERAND} \) are the expected heterozygosity of the inbred and randombred strains, respectively.

We calculated \( F_{IT} \) from pedigrees of 2 individuals from the last generation of the inbred line that were as unrelated as possible. Two pedigrees for each individual representing the maximum and the minimum possible coancestry matings were used, and the \( F_{IT} \) was calculated using the 4 pedigrees by ENDOG (v3.0: Gutiérrez and Goyache 2005). We calculated the standard deviations of the \( F_{IT} \) from analysis of microsatellite data and pedigree information as

\[
s = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2},
\]

where \( N \) is the sample size (14 microsatellite loci and 4 pedigrees), \( x_i \) is the \( F_{IT} \) of each pedigree or locus, and \( \bar{x} \) is the mean of 14 and 4 values for \( F_{IT} \) calculated from pedigree and microsatellite data, respectively.

**Results and Discussion**

In the QO line, all of the markers were polymorphic (Table 1), with up to 6 alleles at a locus (GU0030). Four loci had 2 alleles, 4 had 3 alleles, and 5 had 4 alleles. The mean number of alleles observed from the analysis of microsatellite markers in the inbred line (3.29) was higher than that reported by Pang et al. (1999, 2.45 per locus) and Inoue-Murayama et al. (2001, 2.3 per locus) for the populations of Japanese quail that they studied. Our results are closer to those reported by Kayang et al. (2002, 3.7 per locus) and lower than those reported by Chang et al. (2005, 4.67 per locus) in wild Japanese quail in China. From these reports, it appears that the number of microsatellite alleles that we found is typical of Japanese quail populations.

The mean observed heterozygosity (\( H_0 \)) for the randombred line we studied (0.43) was higher than that (0.31) of the populations studied by Inoue-Murayama et al. (2001) and similar to that described by Kayang et al. (2002, 0.42). In all 3 studies, the heterozygosity observed for domestic quail populations was lower than that reported for wild Japanese quail (0.66, Chang et al. 2005). Cheng et al. (1992) and Kimura and Fuji (1989) used isozyme polymorphism to assess genetic variability in domestic and wild Japanese quail and also reported a higher proportion of polymorphic loci in wild populations than in domestic populations. Variation between studies could represent real population differences or could be the result of using different marker sets.

For the inbred line, the loci were less polymorphic than for the randombred line and 5 loci were monomorphic, 6 had 2 alleles, 2 had 3 alleles, and 1 had 4 alleles. The mean number of alleles observed in the inbred line was 1.93 and the mean observed heterozygosity was 0.21. The difference between these values for the randombred and inbred lines indicate that the inbreeding scheme that we applied was effective in reducing heterozygosity (Maeda and Hashiguchi 1981; Slate et al. 2004).
Table 1. Profiles of 14 Japanese quail microsatellite markers amplified in the randombred (QO) and inbred lines

<table>
<thead>
<tr>
<th>Locus</th>
<th>TA</th>
<th>Allele size (base pairs)</th>
<th>N</th>
<th>HD</th>
<th>HE</th>
<th>FIS</th>
<th>Allele size (base pairs)</th>
<th>N</th>
<th>HD</th>
<th>HE</th>
<th>FIS</th>
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<tr>
<td>GUJ0001</td>
<td>58</td>
<td>230, 234</td>
<td>39</td>
<td>0.03</td>
<td>0.03</td>
<td>0.00</td>
<td>230</td>
<td>13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>GUJ0024</td>
<td>58</td>
<td>162, 164</td>
<td>39</td>
<td>0.00</td>
<td>0.14</td>
<td>1.00</td>
<td>162</td>
<td>31</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>GUJ0030</td>
<td>64</td>
<td>171, 173, 175, 177, 181</td>
<td>40</td>
<td>0.43</td>
<td>0.69</td>
<td>0.39</td>
<td>171, 173, 175, 177</td>
<td>31</td>
<td>0.48</td>
<td>0.52</td>
<td>0.06</td>
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<tr>
<td>GUJ0034</td>
<td>55</td>
<td>217, 233, 237, 239</td>
<td>40</td>
<td>0.45</td>
<td>0.68</td>
<td>0.34</td>
<td>239</td>
<td>31</td>
<td>0.00</td>
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<td>0.00</td>
</tr>
<tr>
<td>GUJ0040</td>
<td>55</td>
<td>178, 182</td>
<td>31</td>
<td>0.42</td>
<td>0.46</td>
<td>0.08</td>
<td>178, 182</td>
<td>28</td>
<td>0.25</td>
<td>0.36</td>
<td>0.32</td>
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<tr>
<td>GUJ0044</td>
<td>57</td>
<td>188, 198, 216</td>
<td>28</td>
<td>0.54</td>
<td>0.65</td>
<td>0.18</td>
<td>188, 198, 216</td>
<td>25</td>
<td>0.32</td>
<td>0.52</td>
<td>0.39</td>
</tr>
<tr>
<td>GUJ0057</td>
<td>64</td>
<td>138, 144, 146, 152</td>
<td>24</td>
<td>0.67</td>
<td>0.70</td>
<td>0.04</td>
<td>146, 152</td>
<td>14</td>
<td>0.36</td>
<td>0.52</td>
<td>0.32</td>
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<tr>
<td>GUJ0059</td>
<td>50</td>
<td>211, 215, 217, 235</td>
<td>39</td>
<td>0.46</td>
<td>0.55</td>
<td>0.16</td>
<td>211</td>
<td>31</td>
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<td>0.00</td>
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<td>GUJ0060</td>
<td>60</td>
<td>246, 252, 258, 258</td>
<td>38</td>
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<td>0.59</td>
<td>−0.25</td>
<td>246, 252, 258, 258</td>
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<tr>
<td>GUJ0065</td>
<td>53</td>
<td>118, 122, 134, 32</td>
<td>32</td>
<td>0.69</td>
<td>0.59</td>
<td>−0.17</td>
<td>118, 122</td>
<td>27</td>
<td>0.37</td>
<td>0.35</td>
<td>−0.05</td>
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<td>25</td>
<td>0.44</td>
<td>0.49</td>
<td>0.10</td>
<td>212, 242</td>
<td>9</td>
<td>0.11</td>
<td>0.11</td>
<td>0.00</td>
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<tr>
<td>GUJ0070</td>
<td>56</td>
<td>196, 202, 204, 206</td>
<td>36</td>
<td>0.64</td>
<td>0.54</td>
<td>−0.18</td>
<td>202, 204</td>
<td>9</td>
<td>0.11</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>GUJ0071</td>
<td>54</td>
<td>165, 167, 175, 181</td>
<td>37</td>
<td>0.49</td>
<td>0.49</td>
<td>0.01</td>
<td>165, 181</td>
<td>26</td>
<td>0.31</td>
<td>0.32</td>
<td>0.03</td>
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<tr>
<td>GUJ0085</td>
<td>58</td>
<td>257, 263</td>
<td>40</td>
<td>0.05</td>
<td>0.05</td>
<td>−0.01</td>
<td>257</td>
<td>31</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>3.29</td>
<td>34.9</td>
<td>0.43</td>
<td>0.48</td>
<td>0.10</td>
<td>1.93 alleles</td>
<td>23.9</td>
<td>0.21</td>
<td>0.24</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

TA is the annealing temperature. N is the number of individuals amplified. HD is the observed heterozygosity. HE is the expected heterozygosity calculated after Lewis and Zaykin (2001). FIS is the inbreeding coefficient.

The mean expected heterozygosity (HE) for the randombred line was 0.48 compared with 0.24 for the inbred line, resulting in FIS values for the randombred and inbred lines of 0.10 and 0.13, respectively. We estimated the population differentiation (FST) from microsatellite marker analysis as 0.50 and the inbreeding coefficient (FIT) as 0.57 ± 0.33. From the pedigree, we calculated FIT of the inbred line as 0.69 ± 0.07 (the mean FIT of 4 individual values: 0.75, 0.75, 0.64, 0.61), which would be comparable to the inbreeding achieved by 5 generations of consecutive full-sib matings (FIT = 0.67). The less intensive inbreeding scheme should allow purging of deleterious genes by natural selection to play a larger role in shaping the population (Kulenkamp et al. 1973) and may allow a higher degree of inbreeding without inbreeding depression (Ballou 1997; Boakes and Wang 2005).

The FIT of the inbred line estimated from microsatellite marker analyses is close to that calculated from the pedigree, but the standard deviation was much larger, showing that the FIT estimate calculated from microsatellite markers is less precise than that calculated from the pedigree. Use of a larger number of microsatellite markers may have reduced the standard deviation. However, Baumung and Sölkner (2003) also concluded from their simulation study that good pedigree information, traceable to the base population, is a good measure of autozygosity. They believed that microsatellite marker information without multigeneration genotyping tends to overestimate heterozygosity, providing a lower than true estimate of inbreeding coefficient. Variation in the length of sequences flanking the microsatellites (Grimaldi and Crouau-Roy 1997) could cause individuals with identical microsatellite repeats to show different length alleles. Variation in the flanking regions can also generate homoplasy, where individuals with different microsatellite repeats show the same alleles. Therefore, heterozygosity of microsatellite markers can underestimate inbreeding. Our slightly lower estimate of inbreeding from microsatellite data than pedigree analysis appears to support the conclusions from the simulation study of Baumung and Sölkner (2003).

In many situations, especially when working with wild populations, good pedigree information is not available. Using poor pedigree information that includes gaps and false parentages may not be a good measure of autozygosity (Roughsedge et al. 2001; Baumung and Sölkner 2003). However, Baumung and Sölkner (2003) argued that low-quality pedigrees are still better indicators of autozygosity than microsatellite analysis. Our data suggest that in situations where no pedigree information is available, microsatellite analysis could provide a comparable measure of autozygosity.

The purpose of this research was to investigate the level of inbreeding in an inbred line of Japanese quail and to compare 2 methods (analysis of microsatellite markers and pedigrees) for estimating the level of inbreeding in this line. We demonstrated that the less intensive inbreeding scheme used was effective in developing an inbred line of Japanese quail and provided empirical data to support the notion that whenever pedigree information is available, it provides a better estimate of inbreeding than microsatellite marker information from a single generation.

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**References**


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