Mapping of plumage colour and blood protein loci on the microsatellite linkage map of the Japanese quail

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Summary

The objective of this work was to map classical markers (plumage colours and blood proteins) on the microsatellite linkage map of the Japanese quail (Coturnix japonica). The segregation data on two plumage colours and three blood proteins were obtained from 25 three-generation families (193 F2 birds). Linkage analysis was carried out for these five classical markers and 80 microsatellite markers. A total of 15 linkage groups that included the five classical loci and 69 of the 80 microsatellite markers were constructed. Using the BLAST homology search against the chicken genome sequence, three quail linkage groups, QL8, QL10 and QL13, were suggested to be homologous to chicken chromosomes GGA9, GGA20 and GGA24, respectively. Two plumage colour loci, black at hatch (Bh) and yellow (Y), and the three blood protein loci, transferrin (Tf), haemoglobin (Hb-1) and prealbumin-1 (Pa-1), were assigned to CJA01, QL10, QL8, CJA14 and QL13, respectively.

Keywords blood protein, Japanese quail, linkage map, microsatellite, plumage colour.

Introduction

The Japanese quail (Coturnix japonica) was originally domesticated in Japan around the 11th century as a pet song bird (Crawford 1990). Nowadays, this poultry is commercially raised for egg production in Japan and East Asian countries, and for meat production in Western European countries such as Spain and France (Minvielle 2004). The domestic Japanese quail is also used as a laboratory animal for research in biomedical sciences and as a pilot animal for poultry production because of its small body size, short generation interval and high egg production (Padgett & Ivey 1959; Wilson et al. 1961). Twenty-seven plumage colours and over 70 biochemical markers have been reported so far (Cheng & Kimura 1990). While these are easily identifiable classical markers, only three linkage groups based on them are known (Ito et al. 1988a,b; Shibata & Abe 1996; Minvielle et al. 2000).

Recently, 100 microsatellite markers were developed for Japanese quail (Kayang et al. 2000, 2002) and used to build the first microsatellite linkage map, which spans 576 cM and contains 58 loci assigned to 12 linkage groups (Kayang et al. 2004). As was the recently published AFLP map for Japanese quail (Roussot et al. 2003), this map is composed solely of type II markers. Morphological traits or type I markers have not been mapped in the Japanese quail yet.

The chicken (Gallus gallus) linkage map includes loci for plumage and skin colour, such as dermal melanin inhibitor (Levin et al. 1993), dominant white (Ruyter-Spira et al. 1997) and extension (Kerje et al. 2003), which were mapped on linkage groups GGAZ, E22C19W28 and GGA11, respectively. However, a relatively small number of classical markers have been mapped.

The objective of the present work was to map two quail plumage colour loci, yellow (Y) (Homma et al. 1967) and black at hatch (Bh) (Minezawa & Wakasugi 1977), and three blood protein loci, specifically haemoglobin (Hb-1), transferrin (Tf) and prealbumin-1 (Pa-1) (Cheng & Kimura 1990). These plumage colour traits are controlled...
by autosomal dominant alleles with homozygous lethality. The heterozygote Y/+ shows a golden wheat-straw colour while the heterozygote Bh/+ shows an overall black colour that obscures the pattern of black and yellow stripes. Neither of these two loci has been reported in other Phasianidae species.

**Materials and methods**

**Japanese quail population**

The F0 generation of the Gifu University resource population was composed of 24 males and 24 females single-pair mated to produce the F1 generation. A total of 193 F2 quails were produced by 25 single-pair matings of F1 birds. Thus a total of 291 birds (48 P, 50 F1 and 193 F2) were used for the linkage analysis. These families included two plumage colour families: seven for Y (14 P, 14 F1 and 61 F2) and eight for Bh (16 P, 16 F1 and 51 F2). Because homozygosity for Bh and Y is lethal, we designed the following cross: \( (\text{Bh} \, \text{or} \, Y)X^\text{+/+} \) as P and \( (\text{Bh} \, \text{or} \, Y)X^{+/+} \) as F1. These two plumage colour families did not overlap. In addition to the former 15 families, 10 families were used for linkage analysis of microsatellite markers and blood protein markers.

**Data analysis**

To perform the comparative mapping with chicken, we checked the orthologous positions of quail microsatellite flanking sequences that were linked with classical markers using BLAST homology search against the chicken draft genome sequence (http://www.ncbi.nlm.nih.gov/genome//guide/chicken/).

Linkage analysis was performed using CriMap version 2.4 software (Green et al. 1990). Our genotyping data were merged with available microsatellite genotyping data from the INRA resource population \( (n = 497) \) (Kayang et al. 2004) to construct more informative microsatellite linkage map of the Japanese quail. A two-point linkage analysis of all markers was then made, based on a LOD score threshold of 3.0. Subsequently, the markers belonging to the same linkage group were analysed using the BUILD option and the order of different loci was examined with the FLIPS option. Map distances were derived using the Kosambi mapping function.

**Results**

Polymorphism was found in 80 of the 103 microsatellite markers tested in the two resource populations. Among them, 75 were polymorphic in the Gifu University resource population. The other five markers were polymorphic only in the INRA resource population. Linkage analysis was thus performed using a total of 85 loci composed of 80 microsatellites, two plumage colours (Bh and Y) and three blood proteins (Tf, Hb-1 and Pa-1). A total of 14 autosomal linkage groups and a Z chromosome-specific linkage group were obtained with the five classical markers and 69 microsatellite markers. These linkage groups covered a total map distance of 921 cM with an average spacing of 11.8 cM between loci. Informative meiosis of classical markers, Bh, Y, Tf, Hb-1 and Pa-1, were 51, 61, 28, 58 and 237, respectively. The average informative meiosis of each microsatellite marker was 650 (7–1037).

Using BLAST homology search, orthologous sequences for quail microsatellite flanking sequences \( (\text{GUJ0071}, \text{GUJ0065} \text{and GUJ0061}) \) were detected on the chicken chromosomes GGA9, GGA20 and GGA24, respectively (Table 1). Thus, three linkage groups, QL8 (with GUJ0071), QL10 (with GUJ0065) and QL13 (with GUJ0061) (Kayang et al. 2004), were homologous to the chicken chromosomes GGA9, GGA20 and GGA24, respectively based on the high level of karyotype conservation between chicken and Japanese quail (Schmid et al. 2000; Shibusawa et al. 2001; Kayang et al. 2004).

The plumage colour loci Bh and Y were mapped on CJA01 and the QL10 linkage group (homologous to GGA20), respectively (Fig. 1). The Bh locus was linked to GUJ0077, GUJ0056 and ADL0037 (LOD = 4.30, 7.99 and 3.34, respectively) and the marker order was GUJ0077-GUJ0056-Bh-ADL0037. The Y locus was linked to GUJ0083 (LOD = 9.26), but it was not significantly linked.
with GUJ0065 because GUJ0065 was polymorphic only in two families (number of available $F_2 = 16$, $\theta = 0.25$, LOD = 0.91). Because GUJ0065-GUJ0083 linkage was also supported by a high LOD score ($= 35.3$) and double recombination rarely occurs in a short chromosome region, marker order was calculated GUJ0065-GUJ0083-Y using CriMap version 2.4 software.

The blood protein loci Tf, Hb-1 and Pa-1 were linked to GUJ0071, GUJ0097 and GUJ0061 (LOD = 3.80, 5.44 and 28.9), respectively, and were mapped on QL8 (homologous to GGA9), CJA14 and QL13 (homologous to GGA24), respectively (Fig. 1).

### Discussion

Three linkage groups were suggested to be homologous to chicken chromosomes by the BLAST homology search. Cytogenetic studies based on banding patterns or chromosome painting using fluorescent in situ hybridization (FISH) revealed highly conserved chromosome homology and orthologous chromosome number between Japanese quail and chicken (Schmid et al. 2000; Shibusawa et al. 2001). In the previous study, six linkage groups including CJA01 and CJA14 have provisionally been assigned to quail chromosomes through comparative mapping with chicken using the cross-species markers (Kayang et al. 2004). Because results in this study were not enough to assign linkage groups to quail chromosomes, we have used linkage group numbers from the previous study (Kayang et al. 2004).

The location of Bh around the middle of the CJA01 linkage group supports the observation from FISH studies that this locus was mapped on the long arm of chromosome 1 using the flanking sequence of Bh as a probe (Niwa et al. 2003). The analysis of the expression pattern of genes relating to melanocyte development and melanins pigment production in Bh and wild-type quail embryos throughout development revealed an abnormal expression pattern of the MelEM antigen in homozygous and heterozygous embryos (Niwa et al. 2002). Identification of the Bh gene will be possible in the near future by combining information of chromosome location, chicken genome sequence (International Chicken Genome Sequencing Consortium 2004), and gene expression pattern. In contrast, there is no direct evidence for the function of Y, which was mapped on the QL10 linkage group (homologous to GGA20) in Japanese quail. This mutation might be agouti-like; it has the same

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### Table 1 BLAST search of Japanese quail microsatellite flanking sequences with the chicken draft genome sequence.

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank accession number</th>
<th>Japanese quail linkage group</th>
<th>Chicken chromosome number</th>
<th>Map position on the chicken chromosome (bp)</th>
<th>Nucleotide similarity between Japanese quail and chicken (%)</th>
<th>5' flank</th>
<th>3' flank</th>
</tr>
</thead>
<tbody>
<tr>
<td>GUJ0061</td>
<td>AB063129</td>
<td>QL13</td>
<td>GGA24</td>
<td>4 938 043–4 938 201</td>
<td>96.2 (104nt)</td>
<td>95.0 (37nt)</td>
<td></td>
</tr>
<tr>
<td>GUJ0065</td>
<td>AB063133</td>
<td>QL10</td>
<td>GGA20</td>
<td>7 845 119–7 845 404</td>
<td>96.8 (61nt)</td>
<td>90.0 (20nt)</td>
<td></td>
</tr>
<tr>
<td>GUJ0071</td>
<td>AB063139</td>
<td>QL8</td>
<td>GGA9</td>
<td>2 999 650–2 999 794</td>
<td>100 (12nt)</td>
<td>89.5 (111nt)</td>
<td></td>
</tr>
</tbody>
</table>

1Nucleotide similarities of original Japanese quail markers were calculated by the BLAST homology search against the chicken draft genome sequence (http://www.ncbi.nlm.nih.gov/genome//guide/chicken/).

25' and 3' flanking sequences of the microsatellite.
dominant lethal genetic determinism and it induces a uniform yellow colour as does the agouti mutation A\(^o\) in the mouse (Michaud et al. 1993). The chicken expressed sequence tag (EST) homologous to agouti signalling protein (ASIP) has already been sequenced (BBSRC ChickEST Database: http://chick.umist.ac.uk/) and was mapped on GGA20 by BLAST search. Because of these points, ASIP is suggested to be the candidate gene for the Y locus.

Blood protein loci have been located in many species such as humans, mouse and chicken (NCBI Genomic Biology web page: http://www.ncbi.nih.gov/Genomes/). Transferrin, haemoglobin and prealbumin-1 are mapped onto the genetic linkage map of the Japanese quail for the first time in this study. In the present study, genetic information in the chicken suggests that the Hb-1 polymorphism mapped on CJA14 is based on the polymorphism of the haemoglobin \(x\) chain locus (HBA), because the HBA locus in chicken is located on homologous GGA14 (ARKdb: http://www.thearkdb.org/). The Tf locus was mapped on the QL8 linkage group (homologous to GGA9) in Japanese quail, and the ovotransferrin locus (Jeltsch & Chambon 1982) is also located on homologous GGA9, which suggests that both ovotransferrin and serum transferrin polymorphisms may be controlled by the same locus in the Japanese quail (Kimura et al. 1978).

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