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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 F_2 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

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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 F_0 generation of the Gifu University resource population was composed of 24 males and 24 females single-pair mated to produce the F_1 generation. A total of 193 F_2 quail were produced by 25 single-pair mating of F_1 birds. Thus a total of 291 birds (48 P, 50 F_1 and 193 F_2) were used for the linkage analysis. These families included two plumage colour families: seven for *Y* (14 P, 14F_1 and 61 F_2) and eight for *Bh* (16 P, 16 F_1 and 51 F_2). Because homozygosity for *Bh* and *Y* is lethal, we designed the following cross: (*Bh* or *Y*/+) X (+/+) as P and (*Bh* or *Y*/+) X (+/+) as F_1. 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.

Genotyping

A total of 100 Japanese quail (Kayang *et al.* 2000, 2002) and three chicken-derived microsatellite markers (*ADL0037*, *ADL0142* and *ADL0255*; Inoue-Murayama *et al.* 2001) were genotyped in the above Japanese quail population. Among them, 80 microsatellite markers that showed polymorphism in the resource population were used for the linkage analysis.

DNA was extracted from the peripheral blood using the QIAamp DNA Blood Kit (Qiagen, Valencia, CA, USA). PCR amplifications were carried out on a PCR Thermal Cycler (TaKaRa Biomedicals, Shiga, Japan) in 10μl reaction mixtures containing 14 ng DNA template, 0.3 μM forward and reverse primers, 130 μM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.4 U AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA). After an initial incubation at 95 °C for 9 min, amplification reactions were performed for 30–42 cycles each with denaturing at 95 °C for 30 s, annealing for 1 min at 48–69 °C depending on the optimized annealing temperature of the primer used, and extension at 72 °C for 1 min. This was followed by a final cycle at 72 °C for 5 min. PCR products of three to five markers were combined and electrophoresed simultaneously on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer), and analysed using Genescan version 3.7 and the Genotyper version 3.7 softwares (Perkin-Elmer).

Quail from the Gifu University resource population were genotyped for three blood protein loci, specifically *haemoglobin* (*Hb-1*) (Maeda *et al.* 1975), *transferrin* (*Tf*) (Ito *et al.* 1981) and *prealbumin-1* (*Pa-1*) (Tanabe & Ogawa 1982).

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 (*GUJ0071*, *GUJ0065* 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 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

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

Locus	GenBank accession number	Japanese quail linkage group	Chicken chromosome number	Map position on the chicken chromosome (bp)	Nucleotide similarity between Japanese quail and chicken (%)[1]	
					5′ flank[2]	3′ flank[2]
GUJ0061	AB063129	QL13	GGA24	4 938 043–4 938 201	96.2 (104nt)	95.0 (37nt)
GUJ0065	AB063133	QL10	GGA20	7 845 119–7 845 404	96.8 (61nt)	90.0 (20nt)
GUJ0071	AB063139	QL8	GGA9	2 999 650–2 999 794	100 (12nt)	89.5 (111nt)

[1]Nucleotide 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/).

[2]5′ and 3′ flanking sequences of the microsatellite.

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).

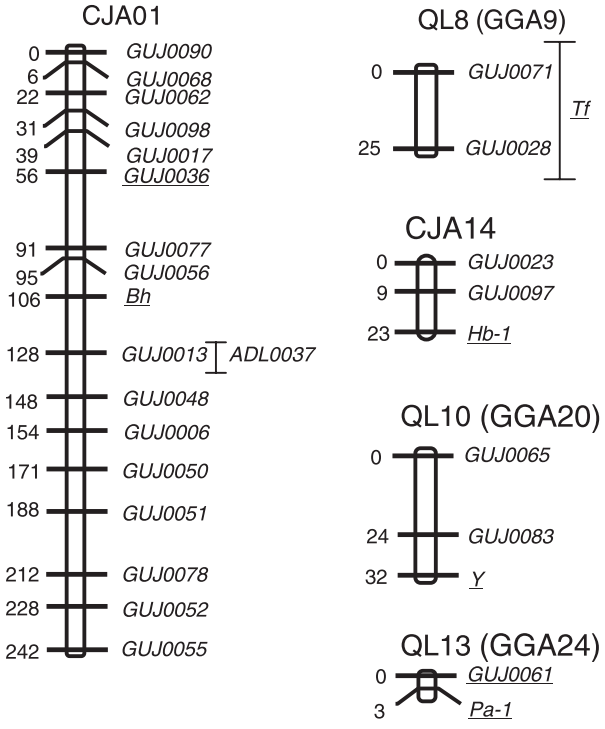

Figure 1 Sex-averaged genetic linkage map of CJA01, QL8, CJA14, QL10 and QL13 in Japanese quail (*Coturnix japonica*). The QL8, QL10 and QL13 linkage groups possibly correspond to chicken chromosome in the parenthesis based on BLAST homology search. The genetic linkage map based on a LOD score threshold of 3 is shown with the estimated Kosambi map distances in centimorgan (cM) on the left. The order of loci on the framework map is supported by odds >3 and the possible locations of the remaining loci are indicated by the error bar. Microsatellite markers named *GUJ* are original Japanese quail markers (Kayang *et al*. 2000, 2002) while *ADL0037* (Cheng *et al.* 1995) is a chicken-derived marker. Five classical markers (*Bh*, *Tf*, *Hb-1*, *Y* and *Pa-1*) and two microsatellite markers (*GUJ0036* and *GUJ0061*) newly mapped in this study are underlined.

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

dominant lethal genetic determinism and it induces a uniform yellow colour as does the *agouti* mutation A^y 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 α 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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