CROSS-SPECIES AMPLIFICATION OF MICROSATELLITE LOCI IN JAPANESE QUAIL, CHICKEN AND GUINEA FOWL

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INTRODUCTION

Cross-species amplification of microsatellite loci among closely related species has been exploited in the construction of genetic maps for cattle (Kappes *et al.,* 1997), sheep (de Gortari *et al.,* 1997) and goat (Vaiman *et al.,* 1996) in the family Bovidae. Exchange of microsatellite markers has also been observed between related avian species (Primmer *et al.,* 1996). In the family Phasianidae, which includes several agriculturally important species of poultry, mapping efforts have so far concentrated on the chicken (*Gallus gallus*) and currently there is a consensus linkage map of over 2000 loci, more than 800 of which are microsatellite markers (Groenen *et al.,* 2000). Attempts have been made to utilize the large number of chicken-specific microsatellite markers to develop DNA markers for turkey, *Meleagris gallopavo* (Levin *et al.,* 1995 ; Liu *et al.,* 1996 ; Reed *et al.,* 2000) and Japanese quail, *Coturnix japonica* (Pang *et al.,* 1999 ; Inoue-Murayama *et al.,* 2001). However, comparative mapping in poultry species is currently not possible as genetic maps do not exist for other avian species. Since Japanese quail and guinea fowl (*Numida meleagris*) have the same diploid number of chromosomes as the chicken (2n = 78), exchange of marker information could be useful among them. As a preliminary effort towards the construction of a comparative genetic map in the family Phasianidae, we isolated microsatellite markers in Japanese quail and chicken and evaluated their usefulness as cross-reactive markers that could serve as anchor points for future comparative mapping purposes.

MATERIALS AND METHODS

Experimental animals. A wild-derived quail colony maintained at Gifu University was used in this study. A population of White Leghorn chickens was sampled from a stock at Gifu University Experimental Farm, while samples from guinea fowls were obtained from JAFRA TRADING CO., LTD., Ibaragi Prefecture, Japan. Blood was drawn from the jugular vein of quails and by wing venipuncture from chickens and guinea fowls, and DNA was extracted using the QIAamp Blood Kit (Qiagen Inc., CA).

Microsatellite-enriched libraries. Japanese quail and chicken genomic libraries enriched for the dinucleotide repeat array $(CA/GT)_n$ were constructed (Takahashi *et al.,* 1996) and screened

following standard procedures. Primers were designed and optimized for PCR as described previously (Kayang *et al.*, 2000).

Cross-species amplification. Japanese quail primer-pairs were tested on chicken and guinea fowl DNA. One male and one female of each species were used. Initially, the amplification conditions determined for Japanese quail were used for chicken and guinea fowl. Those markers that failed to amplify at the standard 1.5 mM $MgCl_2$ concentration were further tested at 2.0 mM and 2.5 mM concentrations of $MgCl_2$. Chicken-specific primers were also tested on Japanese quail and guinea fowl DNA using the annealing temperature optimized for chicken, but without adjusting the $MgCl_2$ concentration.

Microsatellite genotyping. Allele frequency, observed and expected heterozygosity, and polymorphism information content (*PIC*) were determined for each Japanese quail-specific marker by performing a PCR on DNA from 20 unrelated quails randomly sampled from a wild-derived quail colony. For cross-reactive markers, polymorphism and allele frequency at each locus were estimated in 20 chickens and 20 guinea fowls randomly sampled from their respective populations. PCR products were electrophoresed on an ABI Prism 377 DNA sequencer (Perkin-Elmer, Foster City, CA) and were sized using the GENESCAN system (Perkin Elmer). Allelic polymorphism of the chicken markers was also determined by genotyping 12 unrelated chickens, but the cross-reactive chicken markers were not typed.

Sequence homology. In order to confirm whether the products amplified by the cross-reactive markers were indeed the orthologous loci, 10 chicken loci and 10 guinea fowl loci that were amplified by Japanese quail-specific markers were randomly selected for DNA sequencing. PCR products were purified with the High Pure PCR Product Purification Kit (Boehringer Mannheim, IN) and cycle sequence was performed using the non-labeled primer of the same primer-pair used to amplify the locus. Sequences were determined by the dye termination method employing an ABI Prism 377 DNA sequencer (Perkin Elmer). Sequence comparisons were made with GENETYX-Homology v.2.2.2 (Software Development, Tokyo, Japan).

Table 1. Profile of Japanese quail and chicken microsatellite markers

	Japanese quail markers[A]	Chicken markers[A]
No. of markers	100	28
Observed number of alleles	1-6 (3.7)	1-6 (2.9)
Effective number of alleles	1.0-4.3 (2.45)	1.0-3.0 (1.63)
Observed heterozygosity	0.0-0.95 (0.42)	0.0-0.67 (0.19)
Expected heterozygosity	0.1-0.77 (0.53)	0.0-0.66 (0.32)
No. of polymorphic markers	98 (98.0%)	23 (82.1%)
Polymorphism information content	0.0-0.729 (0.477)	0.0-0.600 (0.290)
Amplification in chicken	42 (42.0%)	
Amplification in Japanese quail		11 (39.3%)
Amplification in guinea fowl	20 (20.0%)	2 (7.1%)

[A] Figures in parentheses are means unless when otherwise indicated

RESULTS AND DISCUSSION

The profile of the Japanese quail and chicken markers that were isolated and used in this study is summarized in table 1. A total of 100 markers were isolated in quail with an average of 3.7 alleles per locus, a mean observed heterozygosity of 0.42, and a mean *PIC* of 0.477. Fourty two loci in chicken and 20 in guinea fowl yielded analyzable PCR products that were mostly similar in size to that expected based on the fragment size of the orthologous quail loci. In chicken, however, 28 markers were isolated, with an average of 2.9 alleles per locus, a mean observed heterozygosity of 0.19, and a mean *PIC* of 0.290. 11 (39.3 %) chicken markers amplified loci in Japanese quail while 2 (7.1 %) amplified loci in guinea fowl.

The profile of the Japanese quail markers that produced positive results in chicken and guinea fowl is given in table 2. Nearly 60 % of the markers in chicken and 70 % of those in guinea fowl amplified loci at 1.5 mM $MgCl_2$ concentration, which is the same as that used in amplifying quail loci. In both chicken and guinea fowl, the observed number of alleles averaged 1.9. 57.1 % of the markers in chicken and 55.0 % of those in guinea fowl were polymorphic, while the *PIC* averaged 0.189 and 0.155, respectively. Hence, these markers would be useful for comparative mapping in Japanese quail, chicken and guinea fowl. Fifteen Japanese quail markers were found to cross-amplify both chicken and guinea fowl DNA. Of these, 5 markers were informative in all the three species of Phasianidae and would thus serve as the backbone of a future comparative map.

Table 2. Profile of Japanese quail markers cross-reacting in chicken and guinea fowl

		Japanese quail[A]	Chicken[A]	Guinea fowl[A]
No. of loci amplified		100	42	20
[$MgCl_2$]	1.5 mM	100	25 (59.5 %)	14 (70.0 %)
	2.0 mM	-	15 (35.7 %)	4 (20.0 %)
	2.5 mM	-	2 (4.8 %)	2 (10.0 %)
Observed number of alleles		1-6 (3.7)	1-4 (1.9)	1-5 (1.9)
Effective number of alleles		1.0- 4.3 (2.45)	1-3.6 (1.50)	1-4.1 (1.40)
Observed heterozygosity		0.0-0.95 (0.42)	0-0.95 (0.21)	0-0.45 (0.13)
Expected heterozygosity		0.1-0.77 (0.53)	0-0.72 (0.20)	0-0.75 (0.18)
No. of polymorphic markers		98 (98.0 %)	24 (57.1 %)	11 (55.0 %)
Polymorphism information content		0-0.729 (0.477)	0-0.671 (0.189)	0-0.710 (0.155)

[A] Figures in parentheses are means unless otherwise indicated.

Sequence information of a random sample of the cross-reactive markers revealed that all the markers shared sequence identity with quail (> 78.9 % in chicken and > 74.8 % in guinea fowl). Nine out of 10 sequences in chicken included $(CA/GT)_n$ microsatellite compared to 6 out of the 10 guinea fowl sequences. Although the data are limited, examination of the cross-species amplification results in chicken and guinea fowl show greater amplification in chicken than in guinea fowl, higher similarity of the quail-chicken flanking sequences compared to the quail-guinea fowl sequences, and a better conservation of microsatellite loci in orthologous

quail-chicken sequences than quail-guinea fowl sequences. These observations could be due to a closer phylogenetic relation between quail and chicken than between guinea fowl and chicken (Stock and Bunch, 1982). However, more data based on cross-reactive markers in quail, chicken and guinea fowl would be needed to complement our understanding of the phylogenetic relationships between these species.

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