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Original article

Microsatellite loci in Japanese quail and cross-species amplification in chicken and guinea fowl

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Abstract – In line with the Gifu University's initiative to map the Japanese quail genome, a total of 100 Japanese quail microsatellite markers isolated in our laboratory were evaluated in a population of 20 unrelated quails randomly sampled from a colony of wild quail origin. Ninety-eight markers were polymorphic with an average of 3.7 alleles per locus and a mean heterozygosity of 0.423. To determine the utility of these markers for comparative genome mapping in Phasianidae, cross-species amplification of all the markers was tested with chicken and guinea fowl DNA. Amplification products similar in size to the orthologous loci in quail were observed in 42 loci in chicken and 20 loci in guinea fowl. Of the cross-reactive markers, 57.1% in chicken and 55.0% in guinea fowl were polymorphic when tested in 20 birds from their respective populations. Five of 15 markers that could cross-amplify Japanese quail, chicken, and guinea fowl DNA were polymorphic in all three species. Amplification of orthologous loci was confirmed by sequencing 10 loci each from chicken and guinea fowl and comparing with them the corresponding quail sequence. The microsatellite markers reported would serve as a useful resource base for genetic mapping in quail and comparative mapping in Phasianidae.

Japanese quail / microsatellite loci / chicken / guinea fowl / comparative genetic map

1. INTRODUCTION

Microsatellite loci have gained widespread use in genome mapping, phylogenetics, and conservation genetics due to their abundance in eukaryotic

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genomes, high polymorphism, codominant nature, high reproducibility, and relative ease of scoring by the polymerase chain reaction (PCR). In recent years, genetic linkage maps based on microsatellite markers have been constructed for a number of livestock species including cattle (*Bos taurus*) [17], sheep (*Ovis aries*) [9], goats (*Capra hircus*) [42], and pigs (*Sus scrofa*) [35]. In the poultry species however, mapping efforts have been slowed by the fewer number of microsatellites present in the avian genome compared to that of mammals [31], and by the large number of cytogenetically similar microchromosomes. In spite of the problems inherent in mapping avian genomes, significant progress has been made for chickens (*Gallus gallus*) and recently a consensus linkage map of the chicken genome based on Compton [2], East Lansing [4], and Wageningen [11] linkage maps has been published [12]. At present, genetic maps do not exist for other economically important poultry species, including the Japanese quail (*Coturnix japonica*).

The Japanese quail is valued for its egg and meat, which are enjoyed for their unique flavor [23]. Advantages of small body size, rapid generation turnover, and high egg production [43] make it particularly suited for laboratory research [26], and it has been recommended as a pilot animal for poultry [45]. In the light of this, genetic mapping of this species would be especially desirable if the Japanese quail is to be promoted as a model for poultry. Until now, only two autosomal linkage groups based on plumage color and blood protein markers [15,16,36] and one sex-linked plumage color linkage group [24] have been reported, while DNA markers have not been developed for the Japanese quail. Thus, the quail genome mapping effort was initiated in our laboratory based on the isolation and characterization of microsatellite markers [14,19]. As the number of quail microsatellite markers increases, comparative genome analysis of the quail with other closely related species, especially with the more extensively studied chicken, could facilitate the construction of a comparative genetic map in the Phasianidae family, which is our ultimate objective. A step towards achieving this goal would be to uncover cross-reactive markers that could serve as anchor points for future comparative mapping purposes.

Cross-species amplification of microsatellite loci has been reported within closely related livestock species [3,28,37] and has been exploited in the construction of genetic maps for cattle [17], sheep [9], and goats [42] in the Bovidae family. Exchanges of microsatellite markers have also been observed between related avian species [8,29,30,34]. In the Phasianidae family, attempts have been made to use the large number of chicken-specific microsatellites available to develop DNA markers for turkeys (*Meleagris gallopavo*) [21,22,32,33] and Japanese quail [14,27]. However, for comparative mapping purposes, it is also necessary to determine the utility of markers isolated from other Phasianidae species in the chicken. In a preliminary effort, we isolated 50 original quail microsatellite markers and found 46.0% of them to be polymorphic in two

unrelated quails [19]. Furthermore, we observed positive amplification for 28.0% of the loci in the chicken. In this article, we report 50 new quail microsatellite markers and provide a more extensive characterization of all the 100 loci including an evaluation of their usefulness as cross-reactive markers for comparative mapping in chicken and guinea fowl (*Numida meleagris*), all of which belong to the Phasianidae family.

2. MATERIALS AND METHODS

A quail colony maintained at Gifu University was used in this study [14, 19]. A population of White Leghorns was sampled from a stock at the 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 White Leghorns and guinea fowls, and DNA was extracted using the QIAamp Blood Kit (Qiagen Inc., CA).

A quail genomic library enriched for the dinucleotide repeat array $(CA/GT)_n$ was constructed [40] and screened following standard procedures, and primers were designed and optimized for PCR as outlined previously [19], with the exception that 1.5 mM $MgCl_2$ concentration was used as the standard to test all markers.

Using the annealing temperature optimized for quail, primer-pairs were tested on chicken and guinea fowl DNA to determine cross-reactive markers. One male and one female of each species were used. Initially, the amplification conditions determined for quail were used for chicken and guinea fowl. Those markers that failed to amplify were further tested at 2.0 mM and 2.5 mM concentrations of $MgCl_2$.

Allelic polymorphism was determined for each marker by performing a PCR on DNA from 20 unrelated quails (10 males and 10 females) randomly sampled from a colony of wild quail origin. For cross-reactive markers, polymorphism and allele frequency at each locus were estimated in 20 chickens and 20 guinea fowls made up of 10 males and 10 females 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).

In order to confirm whether the product amplified by the cross-reactive markers was indeed the orthologous loci, 10 chicken loci and 10 guinea fowl loci 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).

3. RESULTS

3.1. Fifty new Japanese quail microsatellite loci

A total of 100 microsatellite markers were isolated and characterized. The first 50 (*GUJ0001–GUJ0050*) of these markers have been published elsewhere [19] while the remaining 50 markers (*GUJ0051–GUJ0100*) are being reported for the first time. The locus name, GenBank accession number, microsatellite repeat array, as well as primer pairs designed for these markers are shown in Table I. The number of $(CA/GT)_n$ repeats in the newly sequenced clones varied between 7 and 19. According to the criteria used by Weber [44], most of the new microsatellites were perfect repeats (82.0%) and the remaining arrays were either interrupted (imperfect 6.0%) or a compound of two perfect repeats (12.0%). The optimized annealing temperature was from 50 to 64 °C.

3.2. Profile of Japanese quail microsatellite markers

The characteristics of all 100 microsatellite markers based on genotyping data from 20 unrelated quails are shown in Table I. All loci (98.0%) except *GUJ0038* and *GUJ0096* were polymorphic, and the average number of alleles per locus was 3.7 (range 1 to 6 alleles). The allele sizes were between 87 and 298 bp (mean range 12.6 bp) and the effective number of alleles was from 1.0 to 4.3 (mean 2.45). The observed and expected heterozygosities ranged from 0.00 to 0.95 (mean 0.423) and 0.00 to 0.77 (mean 0.527), respectively. Values for the polymorphism information content (*PIC*) varied between 0.000 and 0.729 (mean 0.4769). Based on the classification of Botstein *et al.* [1], 59.2% (58/98) of the polymorphic markers were highly informative ($PIC > 0.50$), 28.6% (28/98) were reasonably informative ($0.50 > PIC > 0.25$), and 12.2% (12/98) were slightly informative ($PIC < 0.25$).

3.3. Cross-species amplification of Japanese quail markers in chicken and guinea fowl

Table I also shows the results of cross-species amplification of all 100 quail markers in chicken and guinea fowl. In all, 42 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.

The profile of the Japanese quail markers that produced positive results in the chicken is given in Table II. An average of 1.9 alleles per locus (range 1 to 4 alleles) was observed. 57.1% (24/42) of the markers were polymorphic with

Table I. Profile of one hundred Japanese quail microsatellite markers[#]. *(continued on next pages)*

Locus name	GenBank accession number	Repeat array	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size range (bp)	T_A (°C)	N_O	N_E	H_O	H_E	*PIC*	Amplification in chicken	Amplification in guinea fowl
GUJ0001	**AB035652**	**(CA)7TG(CA)13**	**GAAGCGAAAGCCGAGCCA**	**CAGCACTTCGGAGCACAGGA**	231-239	**56**	4	3.3	0.70	0.70	0.645	+	+
GUJ0002	**AB035813**	**(CA)13**	**AGGTTGTGCTTTGCTTGTAT**	**GAGCATGTTGCACATTTCTT**	141-157	**50**	3	2.0	0.00	0.51	0.442	**0**	0
GUJ0003	**AB035814**	**(CA)9**	**AGGGAAGAAGCAACTGTTC**	**ATTCCAGAATCTGGACTGG**	144-148	**48**	2	1.9	0.50	0.48	0.365	+	0
GUJ0004	**AB037157**	**(CA)10**	**AGCTCTCCTATGGGGCAAC**	**CTGAGCACGAGGACTGGGAA**	183-233	**59**	3	2.5	0.20	0.60	0.515	**0**	0
GUJ0005	**AB035815**	**(CT)11CG(CA)13**	**GCTCTGCTCTCACAGCAGT**	**TGGATCTGGAGCTGCAACGC**	127-149	**59**	4	3.0	0.30	0.67	0.620	**0**	0
GUJ0006	**AB035816**	**(CA)14**	**TGGGATGATAATGAGGTACGG**	**AGGATAGCATTTCAGTCACGG**	117-121	**55**	4	2.7	0.30	0.63	0.562	**0**	0
GUJ0007	**AB035817**	**(CA)15**	**TGACTGCTTTCCACACACA**	**CAGAAGGTAAAAGGACGGA**	87-89	**51**	2	1.5	0.25	0.35	0.288	**0**	0
GUJ0008	**AB035818**	**(CA)10**	**CATGGTTATCAACCTGCAGA**	**ACATGCCAGTCCTTCACAAT**	170-174	**58**	3	2.8	0.85	0.64	0.562	+	0
GUJ0009	**AB035819**	**(CA)14**	**CACGCTTGCTTCTTGCTTCA**	**TATGTTTGGTGCCCTGCTAG**	199-203	**60**	2	1.2	0.20	0.18	0.164	**0**	0
GUJ0010	**AB035820**	**(CA)15**	**TTCCTTCTGGGTGCTGCTCA**	**CATAGACACATCCCTCCCTC**	154-158	**62**	2	1.5	0.35	0.35	0.288	+	0
GUJ0011	**AB035821**	**(CA)13**	**TACTTGATACACCAGCTGTC**	**CACCCTATACCAATGAAAGG**	159-167	**58**	4	2.3	0.24	0.56	0.469	**0**	0
GUJ0012	**AB035822**	**(CA)6TA(CA)6**	**TTTATGTACTGTTTGGGCGC**	**CTTGGACATAGAGTAAGCCA**	140-146	**58**	3	2.7	0.35	0.63	0.555	**0**	0
GUJ0013	**AB035823**	**(CA)10**	**ACCAAACCCGAGATCCGACA**	**AGCGTTCGCGTTCCTCTTTC**	127-139	**55**	4	3.0	0.75	0.67	0.611	+	+
GUJ0014	**AB035824**	**(CA)9**	**TGCTGGGGTTGCTTTCTCCA**	**TCTCGGTGGTTTGCTCTGAC**	143-147	**60**	3	1.7	0.45	0.41	0.345	+	0
GUJ0015	**AB035825**	**(CA)9**	**AGGTGGTCCCCAATGCCCTT**	**GGAAGCAGAGCATCGTTCCC**	135-139	**60**	2	1.2	0.05	0.14	0.130	**0**	0
GUJ0016	**AB035826**	**(CA)9**	**AATGAATGTCTGGGTGGTGC**	**CATGGAGTGTTGGGTATTGC**	235-249	**55**	2	1.1	0.00	0.10	0.090	**0**	0
GUJ0017	**AB035827**	**(CA)14**	**AGAGAGATTAGAGGAGCTGC**	**GGCACTAAAACCATCGAGAG**	153-165	**60**	2	1.9	0.30	0.48	0.365	+	+
GUJ0018	**AB035828**	**(CA)10**	**ATCCCGCGCCGTCCTTTGTT**	**CGGCACCACGAAGTACTCCA**	237-243	**55**	2	1.8	0.30	0.46	0.351	+	0
GUJ0019	**AB035829**	**(CA)21**	**GGGGGCTGTAGGTCTGGATC**	**ATCGGGCACGCGAGGACCAT**	183-191	**50**	4	2.4	0.40	0.58	0.495	**0**	0
GUJ0020	**AB035830**	**(CA)8**	**AATGTCCTTGTGCAGCTCCA**	**CAGCATTGTGCAAAGCAGTG**	205-207	**64**	2	1.2	0.00	0.18	0.164	**0**	0
GUJ0021	**AB035831**	**(CA)11**	**GAGCATTTCTAGTCTGTCTC**	**GATCAATACACAGGCTAAGG**	143-157	**62**	4	3.9	0.65	0.74	0.696	+	+
GUJ0022	**AB035832**	**(CA)15**	**AAACTTATTCTCGCGCTCCC**	**TAAGCAAGGAAGAGGTGGCA**	126-132	**69**	3	2.1	0.95	0.52	0.409	**0**	0
GUJ0023	**AB035833**	**(CA)7TA(CA)11**	**GAGAGGTACAGCAACACTTT**	**CGTTTCTTTCTGGAGTGTCT**	219-237	**55**	4	2.6	0.40	0.61	0.545	+	+
GUJ0024	**AB035834**	**(CA)13AA(CA)3**	**TCACACCTTCGGGCTGATCT**	**ATGCGACGGGGTGCCTTAAA**	162-174	**55**	6	4.3	0.80	0.77	0.725	**0**	0
GUJ0025	**AB035835**	**(CA)9**	**CCTGAGCGAATACACAACTG**	**AGTGTTAGGTGAGGACTGCT**	243-247	**60**	2	2.0	0.35	0.50	0.374	**0**	0
GUJ0026	**AB035836**	**(CA)16**	**CATGAACATCTCTCTTCATG**	**GTGTTCTGCATCACAAACAT**	112-118	**60**	2	1.1	0.00	0.10	0.090	**0**	0
GUJ0027	**AB035837**	**(CA)15**	**TTCACAGATGACAATCTAGC**	**CTGCAAGTAACAGAAGGTAA**	163-177	**55**	4	1.6	0.40	0.38	0.359	+	0
GUJ0028	**AB035838**	**(CA)9**	**TGAACAAAGCAGAAAGGAGC**	**CCTTACCTACATGAAACGTC**	150-178	**55**	5	2.7	0.55	0.63	0.579	**0**	0
GUJ0029	**AB035839**	**(CA)11CT(CA)2**	**GAGCATTTCTAGTCTGTCTC**	**ATACACAGGCTAAGGAAACC**	140-152	**55**	5	2.9	0.80	0.66	0.598	+	+
GUJ0030	**AB035840**	**(CA)31**	**TGCACCAATCCCAGCTGTTT**	**AACGCACAATGGAAAGTGGG**	167-179	**64**	5	4.2	0.35	0.76	0.727	**0**	0
GUJ0031	**AB035841**	**(CA)9**	**AAGGGCAGGGGCTGGGAACA**	**CGCCTCTGCGGTGTGCAACT**	160-166	**55**	4	3.1	0.45	0.68	0.612	+	0
GUJ0032	**AB035842**	**(CA)5CTG(CA)9**	**GAGGCTGCGAACAACACACA**	**GCTAAGACGAGGTGAAGGCT**	161-197	**55**	3	1.6	0.25	0.36	0.310	**0**	0

Table I. Continued.

Locus name	GenBank accession number	Repeat array	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size range (bp)	T_A (°C)	N_O	N_E	H_O	H_E	*PIC*	Amplification in chicken	Amplification in guinea fowl
GUJ0033	**AB035843**	**(CA)13**	**TCTGCTCTCACAGCAGTGCA**	**GCATAGAGCCCAGCAGTGTT**	193-203	**55**	5	2.1	0.45	0.51	0.483	**0**	0
GUJ0034	**AB035844**	**(CA)9CG(CA)2**	**CGTAACGGTCCAATATGGAT**	**TCCACGATGCAGAGGTATTT**	219-241	**55**	5	4.2	0.60	0.76	0.727	+	0
GUJ0035	**AB035845**	**(CA)14**	**AATACTGGTTTTGTGATGGC**	**GGGCAATAAAAGAAAGACTG**	144-150	**55**	3	2.6	0.75	0.61	0.539	**0**	0
GUJ0036	**AB035846**	**(CA)9TA(CA)4**	**CTTTCACATTGCTTTTGCCT**	**CACTAAAGATTGGCTAACAG**	147-155	**55**	4	1.4	0.10	0.27	0.250	**0**	0
GUJ0037	**AB035847**	**(CA)10C(CA)2**	**CCATTCCTCCATCGTTCTGA**	**GGGAAGGAGTGTAGGAAAGA**	178-194	**55**	4	1.9	0.30	0.48	0.448	**0**	0
GUJ0038	**AB035848**	**(CA)19**	**TACATCCAGCAATCGCCCAC**	**CACGGGTGAGTCCATTAGTG**	262	**60**	1	1.0	0.00	0.00	0.000	**0**	0
GUJ0039	**AB035849**	**(CA)19**	**CAAAGAGCAGAGGGAATGGA**	**CCGAGAGATGGGTTTTTTCC**	164-188	**60**	4	3.4	0.70	0.71	0.659	**0**	+
GUJ0040	**AB035850**	**(CA)12**	**GTTGAAGCTCCCATCCCTCC**	**ACACCCCCACGGTCTTTGCA**	176-192	**55**	4	2.3	0.20	0.56	0.494	**0**	+
GUJ0041	**AB035851**	**(CA)11**	**AAAATGTCTGCAAAATGGGC**	**TGAAACATACCTGAGTGCTA**	114-126	**55**	4	3.9	0.45	0.75	0.697	**0**	0
GUJ0042	**AB035852**	**(CA)8**	**TCAGTGCCTTTGTGTTGTCC**	**ACAGCCTTCCCCAAATTCCT**	189-191	**55**	2	1.3	0.00	0.26	0.222	+	0
GUJ0043	**AB035853**	**(CA)9TGTG(CA)2**	**GAGACCAGGTGGTCCCCAAT**	**GGAAGCAGAGCATCGTTCCC**	141-145	**55**	2	1.2	0.00	0.18	0.164	**0**	0
GUJ0044	**AB035854**	**(CA)16**	**GCCTTGAAACCTGAGTGATC**	**TGCATTTCAGCAGCTCTCAG**	180-220	**55**	5	3.5	0.75	0.72	0.666	+	0
GUJ0045	**AB035855**	**(CA)18**	**ACATGCACCACCATTCTTGC**	**CATGCACAAATGAGCGTGCA**	241-251	**60**	2	1.1	0.05	0.05	0.048	**0**	0
GUJ0046	**AB035856**	**(CA)9**	**GCCATGTTTGTCACCTTGCA**	**ACTGGTTGGGACTGAAGGAT**	206-210	**55**	3	2.2	0.35	0.54	0.481	+	0
GUJ0047	**AB035857**	**(CA)23**	**GAGATAAGACTGGCTGGGGC**	**TCACCGTGGCTGGCCAACTT**	262-292	**55**	5	2.4	0.55	0.59	0.555	+	0
GUJ0048	**AB035858**	**(CA)14**	**AACGCATACAACTGACTGGG**	**GGATAGCATTTCAGTCACGG**	130-138	**55**	4	3.8	0.85	0.74	0.688	**0**	0
GUJ0049	**AB035859**	**(CA)11**	**GAAGCAGTGACAGCAGAATG**	**CGGTAGCATTTCTGACTCCA**	229-241	**55**	5	4.2	0.75	0.76	0.725	+	0
GUJ0050	**AB035860**	**(CA)8**	**CTGCCATGTTACTAATCTAG**	**TGGTTTCTTTACACTTGACA**	143-153	**55**	3	1.1	0.10	0.10	0.094	+	0
GUJ0051	AB063119	(CA)10	CCTTAACCACTCCTACTGAC	TTTTGTAAGTGGCCCCGTAC	184-188	55	2	1.1	0.00	0.10	0.090	0	0
GUJ0052	AB063120	(CA)12	AAACTACCGATGTAAGTAAG	ATGAGATATATAAGGAACCC	96-108	55	5	3.7	0.55	0.73	0.681	0	0
GUJ0053	AB063121	(CA)19	GCTGGAGTTTTACATGCACG	TGGATTATGATGCTGACATAAG	151-159	64	4	3.0	0.60	0.67	0.608	0	0
GUJ0054	AB063122	(CA)7	GTGTTCTCTCACTCCCCAAT	ATGTGAGCAATTGGGACTG	120-146	55	4	2.7	0.55	0.63	0.569	+	0
GUJ0055	AB063123	(CT)12(CA)11	GCATACTGCAATATACCTGA	TTGACATACTTGGATTAGAGA	159-183	55	5	2.5	0.20	0.59	0.540	0	0
GUJ0056	AB063124	(CA)7	GTTACATCCATCCTGCCTCA	CTCTTGAGCCTACCAGTCTG	181-185	55	3	2.7	0.15	0.63	0.532	+	0
GUJ0057	AB063125	(CA)12	GGAATGGAAAATATGAGAGC	CAGGTGTTAAAGTCCAATGT	132-154	62	5	2.4	0.65	0.59	0.544	+	0
GUJ0058	AB063126	(CA)10	CCCTTCCAAGTTCCTGG	ATGACAGGTCCAGCCTG	103-109	55	4	3.1	0.35	0.67	0.598	+	0
GUJ0059	AB063127	(CA)10	GACAAAGTTACAGCTAGGAG	TAGGTGCGAAAATCTCTGAC	207-219	50	5	3.4	0.85	0.71	0.670	+	+
GUJ0060	AB063128	(CA)9	ATGCTATGGGAACCTCACTC	TATAAAGCAGGGGGACATGG	132-168	60	5	1.6	0.40	0.38	0.357	0	0
GUJ0061	AB063129	(CA)15	CCACGCTCCCCAATTTCCTG	CCTTGGAGTGCTTCCAAGCG	157-171	55	5	3.2	0.60	0.69	0.620	+	+
GUJ0062	AB063130	(CA)13	TTATGTTTGATGGGCAGAGG	CATGGCAAAAACTGAAGAGC	171-201	60	4	1.5	0.40	0.35	0.329	0	0
GUJ0063	AB063131	(CA)7CT(CA)2CT(CA)7	GCTCAGGTTCTCAGCTGATG	GGGAGAGATCAAGGGAACAG	242-250	55	4	2.5	0.60	0.61	0.538	+	+
GUJ0064	AB063132	(CA)8	AAGCCTGATTCCCTGCCTTG	TTAAAGCTGGGAGGTGGAGG	214-220	55	4	1.6	0.20	0.38	0.351	0	+

Table I. Continued.

Locus name	GenBank accession number	Repeat array	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size range (bp)	T_A (°C)	N_O	N_E	H_O	H_E	*PIC*	Amplification in chicken	Amplification in guinea fowl
GUJ0065	AB063133	(CA)13	GCGTGCCATTTACTTCCCGG	AGCCAGGATGACCAGGAAGG	109-131	55	5	2.3	0.55	0.57	0.536	+	0
GUJ0066	AB063134	(CA)12TA(CA)2	GGGAAAACAATCACTGCCTC	TCTGCAAATCCCCCTTAGAG	167-175	55	3	1.1	0.10	0.10	0.094	+	+
GUJ0067	AB063135	(CA)14	ACGTACGAGCTCAACATTTG	GCGTGCATAAAGGCAACTTA	121-131	55	5	2.8	0.85	0.65	0.594	0	0
GUJ0068	AB063136	(CA)13	TAGGAGAGGTCACGATTTGC	ATCTTAACTCGCCCAGCCTT	204-216	54	5	3.6	0.60	0.72	0.668	0	0
GUJ0069	AB063137	(CA)11	TTCAGGGTAGCAGTCATCTC	CACCAACCACCTTCATCTTC	201-211	54	2	1.7	0.40	0.42	0.332	0	0
GUJ0070	AB063138	(CA)9	AAACCCCAAAGAAGCTGTCC	ACGTTGTCACCATCAGCTTG	196-206	54	6	4.3	0.62	0.77	0.729	+	0
GUJ0071	AB063139	(CA)8	AGATCCTGCTCCTGGAATTG	CAGCTGCACTTAATACAGGC	160-178	54	6	2.0	0.30	0.49	0.468	0	0
GUJ0072	AB063140	(CA)13	CTTTCTTTCTGGCATTGTAC	ATGGGAAGTTGTAGTAGTAG	114-120	50	3	1.6	0.50	0.39	0.618	0	0
GUJ0073	AB063141	(CA)13	GCTGCTATTCTGTTGATGTG	CAACTGCAAAGACAACATCC	144-160	52	4	3.1	0.55	0.68	0.618	0	+
GUJ0074	AB063142	(CA)10	GTTGTCCTGGCTGAGATGGC	GGGTTTGAGGGCTTGGGGTT	290-298	59	3	2.2	0.60	0.54	0.455	0	0
GUJ0075	AB063143	(CA)8	CTCCAATCACACTAGCTCTG	CCTGCTTTTTTTGGGAGAGG	122-126	54	2	1.2	0.15	0.14	0.121	0	0
GUJ0076	AB063144	(CA)4AA(CA)9	GTATCAGTGCATGCTCGTCC	TCGAGGACTGGCTGGAAAAT	208-230	57	5	2.3	0.80	0.57	0.494	0	0
GUJ0077	AB063145	(CA)8	TATAAGATGGGGAGTGGCAG	ATTTTGCTGACCCCCTTCTG	228-232	54	4	2.1	0.60	0.52	0.443	+	0
GUJ0078	AB063146	(CA)14	TCTTTGATTGATGGCTTGCG	GTTATCCTCTGAAGTGTAGC	141-149	55	4	2.2	0.30	0.55	0.495	0	0
GUJ0079	AB063147	(CA)12	GAAAGATAAGCATGAGTGAC	GTTTTGGCATTCACTTCAGA	121-135	55	6	3.0	0.65	0.67	0.626	0	0
GUJ0080	AB063148	(CA)9	TTGAAGGGACATAGGGAAGC	GAAAACGGTGAAGTCTGGTG	151-167	54	6	4.2	0.35	0.76	0.728	0	0
GUJ0081	AB063149	(CA)14	AGGAACGAGTGGAAGTGAAG	TTGGAAAGACACGTTGGGCT	134-144	54	3	2.4	0.65	0.59	0.506	0	0
GUJ0082	AB063150	(CA)9	CTTGGAACACACGGGATGGC	TTACCCCTCTTTTCCCCCCG	142-156	59	5	2.7	0.30	0.63	0.558	+	0
GUJ0083	AB063151	(CA)11	CCATCTCTGTGCCTTTCCAA	GCTGAAAACATTGGGCGTAG	118-128	55	3	2.8	0.45	0.64	0.567	0	0
GUJ0084	AB063152	(CA)10	ACTCCTCCTCTTTCTCCCTC	TCCCGTCTCCCGATGTGTTT	159-165	55	3	2.6	0.55	0.61	0.531	+	+
GUJ0085	AB063153	(GT)14	ACAACCACTTCTCCAGCTAC	GCTTGTGCTGCTGTTGCTAA	245-265	55	5	2.4	0.65	0.59	0.548	+	0
GUJ0086	AB063154	(CA)19	AGCTGCCATATCTACTGCTC	TGGCTTAGTGCTTTCAGAGG	197-207	55	4	3.8	0.40	0.73	0.684	+	+
GUJ0087	AB063155	(CT)12AA(CA)11	CATGCCGGCTGCTATGACAG	AAGTGCAGGGAGCGAGGAAG	151-155	55	3	2.8	0.65	0.65	0.572	+	+
GUJ0088	AB063156	(CA)21	TCTTCACCCTCACTGTATGC	ATCCACGTACAAAGCGTTGC	165-189	55	3	2.6	0.11	0.61	0.542	0	0
GUJ0089	AB063157	(CA)12	CCAGTTTAAGCACCAGCATC	TGGCAAGTAGTCGTGGAAGA	131-145	55	5	2.5	0.79	0.60	0.524	0	+
GUJ0090	AB063158	(CA)11(TA)4	GCCTTCAGAGTGGGAAAT	TCTCACAGAAACAGCTCC	96-106	55	4	2.9	0.20	0.66	0.588	0	0

Table I. Continued.

Locus name	GenBank accession number	Repeat array	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size range (bp)	T_A (°C)	N_O	N_E	H_O	H_E	*PIC*	Amplification in chicken	Amplification in guinea fowl
GUJ0091	AB063159	(CA)9	AAACCGCCATCCCCATTCC	AGCACGTGGGCAAAGGAAC	172-188	55	3	2.7	0.70	0.63	0.645	+	+
GUJ0092	AB063160	(TA)7(CA)12	GTACATTGCTTGCCAGTA	TCCAAGTATGTTGCTTGC	117-123	55	4	3.0	0.55	0.66	0.599	0	0
GUJ0093	AB063161	(CA)16	CTCTTGTATTGTAACTGGGC	AGCCATAGAGGGCTATTAAG	213-231	60	4	3.1	0.45	0.68	0.612	+	0
GUJ0094	AB063162	(CA)16	ATTTTCCCCTCCTTGTCATG	CACTGTTCACTGTTATTCCC	237-249	55	4	2.3	0.15	0.56	0.522	+	+
GUJ0095	AB063163	(CA)12	GCAACATTTTCAGTCAGATC	AATTCTCATCAGTCTCCAAC	120-126	55	2	1.4	0.37	0.30	0.255	0	0
GUJ0096	AB063164	(A)10(CA)14(A)20	GTACCAAAAGTGAATAGTGG	CAGATCACAGACTTAGAAAG	157	55	1	1.0	0.00	0.00	0.000	0	0
GUJ0097	AB063165	(CA)14	GGATGCTCAGTGTGGAAAAG	GAGCAAGAGGTGAGTGTTTC	131-157	55	5	3.6	0.40	0.72	0.672	+	0
GUJ0098	AB063166	(CA)12	GCATAACTGAACTACCACGC	GCATCAGTTCCATCAGCTAG	197-205	55	4	2.5	0.73	0.60	0.539	+	0
GUJ0099	AB063167	(CA)16GA(CA)5(TA)7	CTCTTATCCATCCTTCCTTC	TTTTAAGTTTCCCCAGGCAG	246-284	55	3	3.0	0.30	0.66	0.590	+	0
GUJ0100	AB063168	(CA)12	GCATTTCCATCAGTACAACC	CAGAATATAAGGTCACAGCC	278-290	55	5	2.8	0.45	0.65	0.602	0	0

[#] The locus code *GUJ* stands for Gifu University Japanese quail and is in accordance with the standardized nomenclature rules adopted for poultry [5]. T_A, annealing temperature; N_O, observed number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *PIC*, polymorphism information content; +, amplification products were obtained using the annealing temperature optimized for quails; 0, amplification products were not obtained using the annealing temperature optmized for quails.
The information provided in bold type for the first 50 markers, *GUJ0001–GUJ0050*, has been originally published in The Journal of Heredity [19].

2 to 4 alleles per locus and 42.9% (18/42) were monomorphic. The observed heterozygosity and *PIC* were on average 0.205 and 0.1888, respectively. Based on the *PIC*, 12.5% (3/24) of the polymorphic markers were highly informative, 58.3% (14/24) reasonably informative, and 29.2% (7/24) slightly informative. Nearly 60.0% (25/42) of the markers amplified chicken loci at 1.5 mM $MgCl_2$ concentration, which is the same as that used in amplifying quail loci. However, the $MgCl_2$ concentration had to be adjusted to 2.0 mM for 15 markers and 2.5 mM for the *GUJ0018* and *GUJ0098* markers.

The characteristics of the Japanese quail microsatellite loci that were amplified in guinea fowl are shown in Table III. The observed number of alleles per locus averaged 1.9 (range 1 to 5 alleles). A polymorphism was observed in 55.0% (11/20) of the markers having 2 to 5 alleles per locus, while the rest were monomorphic. The mean observed heterozygosity was 0.127 and that of *PIC* was 0.1553. Of the polymorphic markers, 18.2% (2/11) were highly informative, 36.4% (4/11) were reasonably informative, and 45.5% (5/11) were slightly informative. Similar to chicken, 70.0% (14/20) of the markers amplified guinea fowl loci at 1.5 mM $MgCl_2$ concentration, with four markers requiring 2.0 mM $MgCl_2$ and two markers (*GUJ0089* and *GUJ0091*) requiring 2.5 mM of $MgCl_2$.

3.4. Japanese quail, chicken and guinea fowl loci amplified by the same quail markers

Fifteen Japanese quail markers were found to cross-amplify both chicken and guinea fowl DNA. To illustrate how informative these markers would be for comparative mapping, their observed heterozygosities were plotted in Figure 1. Generally, nearly all the 15 loci had high heterozygosities in Japanese quail, which is not unexpected since they are quail-specific markers. Five loci in chicken (*GUJ0059*, *GUJ0061*, *GUJ0066*, *GUJ0087*, and *GUJ0094*) and 7 loci in guinea fowl (*GUJ0001*, *GUJ0013*, *GUJ0021*, *GUJ0029*, *GUJ0061*, *GUJ0087*, and *GUJ0091*) were not heterozygous and therefore uninformative in our test populations. However, 5 loci (*GUJ0017*, *GUJ0023*, *GUJ0063*, *GUJ0084*, and *GUJ0086*) were informative in all three species of Phasianidae and would thus be useful for comparative mapping. The average observed heterozygosities for these 15 loci in the Japanese quail, chicken and guinea fowl were 0.547, 0.297, and 0.145, respectively.

3.5. Sequence analysis of chicken and guinea fowl loci amplified by Japanese quail markers

The sequence information of 10 chicken loci amplified by cross-species PCR is summarized in Table IV. Nine chicken loci contained $(CA/GT)_n$ repeats, 5 (*GUC0002*, *GUC0003*, *GUC0006*, *GUC0007*, and *GUC0009*) of

Table II. Characteristics of 42 Japanese quail microsatellite loci amplified in chicken[#].

Locus name	Size range (bp) in quail	T_A (°C)	[$MgCl_2$] (mM)	Size range (bp) in chicken	N_O	N_E	H_O	H_E	*PIC*
*GUJ0001**	231-239	56	1.5	225-247	4	2.3	0.40	0.56	0.516
GUJ0003	144-148	48	1.5	134	1	1.0	0.00	0.00	0.000
GUJ0008	170-174	58	1.5	168	1	1.0	0.00	0.00	0.000
GUJ0010	154-158	62	1.5	160	1	1.0	0.00	0.00	0.000
*GUJ0013**	127-139	55	1.5	140-144	3	2.4	0.35	0.58	0.494
GUJ0014	143-147	60	2.0	159-163	2	1.1	0.05	0.05	0.048
*GUJ0017**	153-165	60	1.5	149-151	2	1.2	0.20	0.18	0.164
GUJ0018	237-243	55	2.5	231	1	1.0	0.00	0.00	0.000
GUJ0021	143-157	62	1.5	137-141	2	1.1	0.10	0.10	0.090
*GUJ0023**	219-237	55	1.5	208-222	3	1.7	0.40	0.41	0.368
GUJ0027	163-177	55	1.5	167-169	2	1.8	0.65	0.44	0.343
*GUJ0029**	140-152	55	1.5	132-136	2	1.1	0.10	0.10	0.090
GUJ0031	160-166	55	2.0	212	1	1.0	0.00	0.00	0.000
GUJ0034	219-241	55	2.0	163	1	1.0	0.00	0.00	0.000
*GUJ0042**	189-191	55	1.5	199	1	1.0	0.00	0.00	0.000
*GUJ0044**	180-220	55	1.5	187	1	1.0	0.00	0.00	0.000
GUJ0046	206-210	55	1.5	227-229	2	1.1	0.05	0.50	0.048
GUJ0047	262-292	55	2.0	225-233	2	2.0	0.25	0.50	0.374
*GUJ0049**	229-241	55	1.5	239-241	3	1.8	0.35	0.43	0.390
GUJ0050	143-153	55	2.0	147	1	1.0	0.00	0.00	0.000
GUJ0054	120-146	55	2.0	127	1	1.0	0.00	0.00	0.000
GUJ0056	181-185	55	2.0	180	1	1.0	0.00	0.00	0.000
GUJ0057	132-154	62	1.5	120-126	4	1.9	0.15	0.47	0.433
GUJ0058	103-109	55	2.0	97-99	2	2.0	0.67	0.49	0.369
*GUJ0059**	207-219	50	1.5	196-216	2	1.8	0.00	0.45	0.351
GUJ0061	157-171	55	1.5	158	1	1.0	0.00	0.00	0.000
*GUJ0063**	242-250	55	1.5	231-235	2	1.8	0.65	0.44	0.343
GUJ0065	109-131	55	1.5	112-126	3	1.6	0.15	0.39	0.329
GUJ0066	167-175	55	2.0	176	1	1.0	0.00	0.00	0.000
GUJ0070	196-206	54	2.0	200-204	2	1.7	0.55	0.40	0.319
GUJ0077	228-232	54	2.0	214	1	1.0	0.00	0.00	0.000
GUJ0082	142-156	59	2.0	140	1	1.0	0.00	0.00	0.000
GUJ0084	159-165	55	1.5	164-176	4	3.6	0.95	0.72	0.671
GUJ0085	245-265	55	2.0	225	1	1.0	0.00	0.00	0.000
GUJ0086	197-207	55	1.5	209-215	3	2.7	1.00	0.63	0.555
GUJ0087	151-155	55	1.5	145	1	1.0	0.00	0.00	0.000
GUJ0091	172-188	55	2.0	162-164	2	1.3	0.30	0.26	0.222
GUJ0093	213-231	60	2.0	218-224	2	1.2	0.15	0.14	0.129
GUJ0094	237-249	55	1.5	291	1	1.0	0.00	0.00	0.000

Table II. Continued.

Locus name	Size range (bp) in quail	T_A (°C)	[$MgCl_2$] (mM)	Size range (bp) in chicken	N_O	N_E	H_O	H_E	*PIC*
GUJ0097	131-157	55	1.5	123-129	3	2.1	0.30	0.53	0.468
GUJ0098	197-205	55	2.5	196-210	4	2.2	0.75	0.54	0.483
GUJ0099	246-284	55	1.5	237-253	2	1.7	0.10	0.42	0.332

[#] Amplification products were obtained in 20 randomly sampled chicken using the annealing temperature optimized for quails.
T_A, annealing temperature; N_O, observed number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *PIC*, polymorphism information content.
[*] Loci for which sequences were determined.

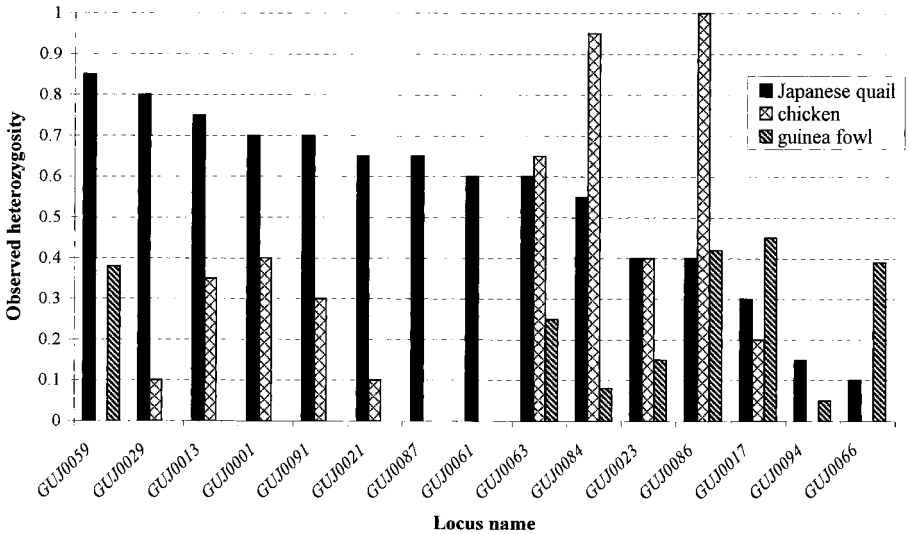

Figure 1. Observed heterozygosity in Japanese quail, chickens, and guinea fowl for the 15 quail markers found to cross-amplify DNA from the two other species. Observed heterozygosities of the 15 cross-reactive quail markers were estimated in random samples of 20 Japanese quail, 20 chickens, and 20 guinea fowls, each sample made up of 10 males and 10 females. The markers were ordered, from left to right, by decreasing heterozygosity in Japanese quail.

which were perfect repeats and 2 (*GUC0001* and *GUC0010*) were imperfect repeats as found in their corresponding quail loci. For the remaining 2 loci, the repeat array was either perfect in the chicken, as opposed to imperfect (*GUC0004*), or *vice versa* (*GUC0008*) in the quail. The *GUC0005* locus only had a poly A. Sequence alignment of the 5′ flanks of the corresponding quail

Table III. Characteristics of 20 Japanese quail microsatellite loci amplified in guinea fowl[#].

Locus name	Size range (bp) in quail	T_A (°C)	[$MgCl_2$] (mM)	Size range (bp) in guinea fowl	N_O	N_E	H_O	H_E	*PIC*
*GUJ0001**	231-239	56	1.5	226	1	1.0	0.00	0.00	0.000
*GUJ0013**	127-139	55	1.5	139	1	1.0	0.00	0.00	0.000
*GUJ0017**	153-165	60	1.5	153-161	3	2.7	0.45	0.63	0.550
*GUJ0021**	143-157	62	1.5	135	1	1.0	0.00	0.00	0.000
GUJ0023	219-237	55	2.0	233-245	3	1.2	0.15	0.14	0.140
*GUJ0029**	140-152	55	1.5	130	1	1.0	0.00	0.00	0.000
GUJ0039	164-188	60	2.0	159-163	2	1.1	0.13	0.12	0.110
GUJ0040	176-192	55	1.5	171	1	1.0	0.00	0.00	0.000
*GUJ0059**	207-219	50	1.5	204-226	4	1.5	0.38	0.33	0.311
*GUJ0061**	157-171	55	1.5	158	1	1.0	0.00	0.00	0.000
GUJ0063	242-250	55	2.0	220-224	2	2.0	0.25	0.50	0.374
GUJ0064	214-220	55	2.0	220-224	2	2.0	0.20	0.50	0.372
*GUJ0066**	167-175	55	1.5	186-194	5	4.1	0.39	0.75	0.710
*GUJ0073**	144-160	52	1.5	147-149	2	1.0	0.04	0.04	0.040
*GUJ0084**	159-165	55	1.5	168-170	2	1.1	0.08	0.08	0.077
GUJ0086	197-207	55	1.5	211-215	2	2.0	0.42	0.50	0.373
GUJ0087	151-155	55	1.5	137	1	1.0	0.00	0.00	0.000
GUJ0089	131-145	55	2.5	123	1	1.0	0.00	0.00	0.000
GUJ0091	172-188	55	2.5	165	1	1.0	0.00	0.00	0.000
GUJ0094	237-249	55	1.5	313-317	2	1.1	0.05	0.05	0.048

[#] Amplification products were obtained in 20 randomly sampled guinea fowls using the annealing temperature optimized for quails.
T_A, annealing temperature; N_O, observed number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *PIC*, polymorphism information content.
* Loci for which sequences were determined.

and chicken loci revealed significant homologies ranging from 78.9% to 93.9%. A BLAST search with sequences in GenBank showed no significant homology except for similarity with orthologous quail sequences that we had registered previously [19].

Table V shows the sequence results of 10 guinea fowl loci amplified by cross-reactive quail markers. The sequence of 6 loci included $(CA/GT)_n$ repeats. Two loci (*GUG0006* and *GUG0010*) had perfect repeats and 2 (*GUG0001* and *GUG0008*) had imperfect repeats similar to their orthologous loci in the quail, while 2 loci (*GUG0002* and *GUG0003*) had imperfect repeats as opposed to the perfect repeats found in their corresponding quail loci. The remaining 4 guinea

Table IV. Sequence results of 10 Japanese quail and chicken loci amplified by the same quail markers.

Japanese quail			Chicken			
Locus name	GenBank accession number	Repeat array	Locus name*	GenBank accession number	Repeat array	% similarity between Japanese quail and chicken 5′ flank
GUJ0001	AB035652	(CA)7TG(CA)13	*GUC0001*	AB063261	(CA)2CG(CA)3TG(CA)5GA(CA)11	84.1 (176 nt)
GUJ0013	AB035823	(CA)10	*GUC0002*	AB063262	(CA)5	85.7 (91 nt)
GUJ0017	AB035827	(CA)14	*GUC0003*	AB063263	(CA)8	93.9 (98 nt)
GUJ0023	AB035833	(CA)7TA(CA)11	*GUC0004*	AB063264	(CA)17	78.9 (152 nt)
GUJ0029	AB035839	(CA)11CT(CA)2	*GUC0005*	AB063265	(A)14	92.7 (124 nt)
GUJ0042	AB035852	(CA)8	*GUC0006*	AB063266	(CA)7	81.0 (147 nt)
GUJ0044	AB035854	(CA)16	*GUC0007*	AB063267	(CA)3	85.4 (123 nt)
GUJ0049	AB035859	(CA)11	*GUC0008*	AB063268	(CA)2A(CA)5	80.0 (200 nt)
GUJ0059	AB063127	(CA)10	*GUC0009*	AB063269	(CA)11	82.7 (110 nt)
GUJ0063	AB063131	(CA)7CT(CA)2CT(CA)7	*GUC0010*	AB063270	(CA)6CC(CA)8	85.5 (138 nt)

* The locus code *GUC* stands for Gifu University chicken and is in accordance with the standardized nomenclature rules adopted for poultry [5].

fowl loci had no repeat arrays. However, for all 10 loci, the sequences of the 5′ flanking regions were very similar to the corresponding quail sequences (74.8% to 95.1%). When searched against the database in GenBank, no matches were found for these sequences except our registered quail sequences.

4. DISCUSSION

The isolation of 50 new microsatellite markers in Japanese quail is a follow up on our earlier success in targeting simple sequence repeat (SSR) loci from an enriched genomic library [19] aimed at generating sufficient original quail markers for constructing a genetic map for this economically important poultry species. Previous attempts to localize quail SSR using chicken-specific primers have not been very successful. In one report [27], 22.9% specific amplification was obtained from 48 chicken markers tested in quail but eventually only 6 markers were developed. In a related study [14], we could only amplify 31 (25.8%) of 120 chicken microsatellite markers in Japanese quail, 22 of which were non-specific amplifications. This led us to the conclusion that chicken microsatellite primers are not efficient markers for Japanese quail, thereby underscoring the need to develop original markers for quail.

In our earlier report [19], 46.0% (23/50) of the markers showed polymorphism in two unrelated quails. However, in this expanded study 98.0% (98/100) were polymorphic in 20 unrelated quails, thus clearly indicating that the larger sample size is more informative. Values of 75.8% (25/33) [6] and 93.2% (259/278) [7] polymorphisms have been reported for chicken-specific markers tested in the chicken. The very high level of polymorphism seen in the quail markers could, in part, be a reflection of the genetic constitution of the test population, which was derived from a colony of wild quail origin and is thus considered to be genetically diverse as a result of its shorter history of domestication [18]. The average number of alleles observed in the Japanese quail was 3.7, ranging from 1 to 6. This is similar to a mean of 4 and a range of 2 to 9 [7] or a mean of 5.6 and a range of 2 to 10 [41] reported for the chicken. Based on the *PIC* values, nearly 60.0% of the polymorphic markers were highly informative and only a few (12.2%) were slightly informative. Therefore, we conclude that these markers have a high utility for mapping the quail genome.

As a step towards constructing a comparative genetic map in the Phasianidae family, which includes a number of agriculturally important species of poultry, cross-species amplification was carried out to determine the usefulness of Japanese quail markers in chicken and guinea fowl. The level of amplification observed in the chicken in the present study (42.0%) is consistent with the results of other studies of cross-species amplification involving chicken markers applied to turkeys (91.7% [21], 51.1% [22], 55.6% [13], 55.3% [32], and 53.8% [33] specific amplifications), or chicken markers tested in the Japanese

Table V. Sequence results of 10 Japanese quail and guinea fowl loci amplified by the same quail markers.

Japanese quail			Guinea fowl			
Locus name	GenBank accession number	Repeat array	Locus name*	GenBank accession number	Repeat array	% similarity between Japanese quail and guinea fowl 5′ flank
GUJ0001	AB035652	(CA)7TG(CA)13	*GUG0001*	AB063271	(CA)2CG(CA)12	83.1 (148 nt)
GUJ0013	AB035823	(CA)10	*GUG0002*	AB063272	(CA)7CC(A)19	81.9 (83 nt)
GUJ0017	AB035827	(CA)14	*GUG0003*	AB063273	(CA)2(A)20	87.3 (134 nt)
GUJ0021	AB035831	(CA)11	*GUG0004*	AB063274	X	83.7 (135 nt)
GUJ0029	AB035839	(CA)11CT(CA)2	*GUG0005*	AB063275	X	85.5 (124 nt)
GUJ0059	AB063127	(CA)10	*GUG0006*	AB063276	(CA)11	84.7 (196 nt)
GUJ0061	AB063129	(CA)15	*GUG0007*	AB063277	X	87.8 (90 nt)
GUJ0066	AB063134	(CA)12TA(CA)2	*GUG0008*	AB063278	(CA)27CG(CA)2CG(CA)5	74.8 (135 nt)
GUJ0073	AB063141	(CA)13	*GUG0009*	AB063279	X	79.6 (142 nt)
GUJ0084	AB063152	(CA)10	*GUG0010*	AB063280	(CA)12	95.1 (143 nt)

* The locus code GUG stands for Gifu University guinea fowl and is in accordance with the standardized nomenclature rules adopted for poultry [5].

X, No repeats detected.

quail (22.9% [27] and 25.8% [14] specific PCR products). Although we adjusted the $MgCl_2$ concentration, we did not attempt to optimize the amplification condition for any locus. Hence, it is likely that such an effort would yield more positive amplifications. In our earlier study using chicken primers on quail, no adjustment was made in the $MgCl_2$ concentration, and this could partly account for the lower amplification success of 25.8% [14]. The average observed number of alleles for quail markers tested in the chicken was 1.9. This value is lower than the 3.7 number of alleles observed for quail in this study, but is, however, close to the value of 1.4 reported for chicken markers tested in turkeys [33]. The lower value of the number of alleles observed in chickens as compared to quail could, in part, be due to the characteristics of the test populations, since wild-derived quail were used on the one hand and White Leghorn chickens on the other. However, studies on cross-reactive markers have shown that microsatellite repeats tend to be generally longer, and thus more polymorphic, in the species of origin than in the comparison species, thus suggesting an ascertainment bias [10,33]. This could have also contributed to the differences observed. From the *PIC* data, the polymorphic cross-reactive markers were reasonably informative and would be useful for comparative mapping in chickens and Japanese quail.

In guinea fowl, 20 of the quail markers amplified loci, with the observed number of alleles per locus averaging 1.9, and 11 of them were polymorphic. Although the mean observed number of alleles per locus was similar to that in chickens, the mean observed heterozygosity and *PIC* were lower in guinea fowl. This is particularly evident in Figure 1 for the 15 markers that cross-amplified Japanese quail, chicken and guinea fowl DNA. Apart from the possible ascertainment bias mentioned earlier, one reason for this might be due to the low heterogeneity suspected in the guinea fowl population that was sampled, since it is probable that only a very small number of founders were introduced into Japan as is evidenced by the few guinea fowl farms that exist. In spite of this, a considerable number of the cross-reactive markers in guinea fowl are reasonably informative and would be useful for comparative mapping.

Out of the 15 markers cross-reacting in Japanese quail, chickens and guinea fowl, five markers (*GUJ0017*, *GUJ0023*, *GUJ0063*, *GUJ0084*, and *GUJ0086*) were informative in our test populations and would thus serve as the backbone of a comparative map in these Phasianidae species. Although the remaining 10 markers were not polymorphic in all three species, it is likely that they would be polymorphic when tested in a larger population, or they could be useful in the future as markers for radiation hybrid mapping [20].

By sequencing PCR products of a random sample of the cross-reactive markers, we observed that all the markers shared sequence identity with the quail ($>$ 78.9% in chicken and $>$ 74.8% in guinea fowl). Nine out of 10 sequences in chickens included $(CA/GT)_n$ microsatellites compared to 6 out

of the 10 guinea fowl sequences. Similar observations have been made in other studies on cross-species amplification involving chicken markers in quail in which 2 out of 10 loci [27] and three out of 9 loci [14] sequenced had no microsatellites. In this study, three of the guinea fowl sequences lacking microsatellites were not polymorphic. The greater number of quail markers that amplified chicken DNA as opposed to guinea fowl DNA, and the higher similarity of the quail-chicken flanking sequences compared to the quail-guinea fowl sequences, coupled with a better conservation of microsatellite loci in orthologous quail-chicken sequences than quail-guinea fowl sequences, are useful observations pointing to a closer relation between quail and chickens and could thus contribute to the discussion on the phylogenetic relationship of the three species. However, our data was limited and therefore inconclusive in this regard. Studies on phyletic relationships based on homologies of chromosome banding patterns have placed *Gallus*, *Coturnix* and *Numida* in the same subfamily, with *Coturnix* and *Gallus* being more closely related than *Numida* and *Gallus* [39]. It has been recently confirmed that chromosome homology between Japanese quail and chickens is highly conserved, with very few chromosome rearrangements after divergence of the two species (Matsuda Y., personal communication). Sequencing and microsatellite genotyping data based on cross-reactive markers in quail, chickens, and guinea fowl could, therefore complement our understanding of the phylogenetic relationships between these species.

From this study, we report 9 $(CA/GT)_n$ microsatellite-containing quail markers as new markers for chickens. Similarly, six quail markers are being reported as the first novel microsatellite markers registered for guinea fowl. The guinea fowl has been reputed to be a species with great potential, able to adapt easily to all kinds of climate in spite of its African origin [25]. In view of this, DNA markers for this species would help promote their genetic improvement. Based on our results, we recommend the isolation of original microsatellite markers for mapping in guinea fowl rather than attempting to adapt markers isolated from other species for studies in guinea fowl.

In conclusion, we have described informative Japanese quail microsatellite markers that would form a useful resource base of DNA markers as part of our initiative to develop a genetic map for Japanese quail. Since cross-species amplification indicated that several of the cross-reactive markers are informative in chickens (57.1%) and guinea fowl (55.0%), these markers may be useful for comparative genome analysis in Phasianidae. Furthermore, the cross-reactive markers could be used as a tool in future phylogenetic studies aimed at improving our understanding of the relatedness of Japanese quail to chickens and guinea fowl. The trend in comparative mapping in poultry is taking several directions including the analysis of cDNA clones [38] and radiation hybrid mapping [20], and our results would contribute to this collective effort.

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