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Original article

Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds

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Abstract – In order to study duck microsatellites, we constructed a library enriched for (CA)n, (CAG)n, (GCC)n and (TTTC)n. A total of 35 pairs of primers from these microsatellites were developed and used to detect polymorphisms in 31 unrelated Peking ducks. Twenty-eight loci were polymorphic and seven loci were monomorphic. A total of 117 alleles were observed from these polymorphic microsatellite markers, which ranged from 2 to 14 with an average of 4.18 per locus. The frequencies of the 117 alleles ranged from 0.02 to 0.98. The highest heterozygosity (0.97) was observed at the CAUD019 microsatellite locus and the lowest heterozygosity (0.04) at the CAUD008 locus, and 11 loci had heterozygosities greater than 0.50 (46.43%). The polymorphism information content (PIC) of 28 loci ranged from 0.04 to 0.88 with an average of 0.42. All the above markers were used to screen the polymorphism in other bird species. Two markers produced specific monomorphic products with the chicken DNA. Fourteen markers generated specific fragments with the goose DNA: 5 were polymorphic and 9 were monomorphic. But no specific product was detected with the peacock DNA. Based on sequence comparisons of the flanking sequence and repeat, we conclude that 2 chicken loci and 14 goose loci were true homologous loci of the duck loci. The microsatellite markers identified and characterized in the present study will contribute to the genetic map, quantitative traits mapping, and phylogenetic analysis in the duck and goose.

duck / microsatellite marker / enriched library / polymorphism / cross-species amplification

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1. INTRODUCTION

Molecular genetic maps will provide insight into the genome organization and chromosomal localization of cloned genes, and also provide a framework for the identification and location of major genes associated with economically important traits [7]. In recent years, rapid advances have been made in the development of molecular genetic maps. High-density linkage maps are now available for many farm animals, such as cattle, pigs, and goats. In contrast, mapping studies in avian species are much less advanced except in the chicken. In order to construct saturated genetic maps for more bird species, the isolation of many polymorphic genetic markers, particularly microsatellite markers is an eventual pre-requisite.

Microsatellites or simple sequence repeats (SSR) are tandem repeated motifs of 1–6 bases found in all prokaryotic and eukaryotic genomes, and are present both in coding and noncoding regions. Because of their high polymorphism, they have been extensively used in forensics, genetic mapping, population genetics, evolutionary studies and investigation of social systems [3–5,13].

Although microsatellites are very informative genetic markers, the need for prior sequence information to produce locus-specific primer sets is a major limitation. Traditionally, microsatellite loci have been isolated from partial genomic libraries (selected for small insert size) from the species of interest, and several thousands of clones screened through colony hybridization with repeat-containing probes [30]. Although relatively simple, especially for microsatellite rich genomes, this approach can be extremely tedious and inefficient for species with low microsatellite frequencies, such as avian species. Several procedures for microsatellite library enrichment have been developed to improve the efficiency of microsatellite isolation. They can be classified according to the capture technique: (1) streptavidin-coated magnetic beads; (2) microsatellite probes attached to small nylon membranes; (3) other not frequently used procedures, such as the use of the *dut ung* strain of *E.coli* or magnetic capture of phage DNA [32].

The Peking duck, the most common type of duck bred for meat, was exported to the United States and Britain from China in the last century. Many meat type ducks have originated from this breed. Ducks, which are different from the chicken (order Galliformes), belong to the order Anseriformes. According to paleontological data, the main radiation of modern ducks took place during the Miocene, 5–23 million years ago [26]. Ducks together with the ostrich, emu, peacock, turkey, quail, and other birds play a major role in studies on bird evolution. Up to now, most available data concerning ducks have come from heritabilities and genetic correlations of some

traits [2, 12, 33, 39] and epidemiology [11, 20, 21]. Most available molecular data have come from evolution studies based on the analysis of mitochondrial DNA sequence [6, 8, 14, 15, 22, 34]. However, information about genetic markers in the duck is limited [3, 23, 24, 29, 35], and therefore we isolated and characterized 35 novel microsatellite markers for this species. In the hopes of developing universal microsatellite primers for birds, 35 duck microsatellite markers were used to identify the homologous loci in the chicken, peacock and goose.

2. MATERIALS AND METHODS

2.1. Construction of the microsatellite-enriched library

A microsatellite-enriched partial genomic library was constructed using total genomic DNA extracted from blood collected from the wing vein of one female Peking duck (*Anas platyrhynchos)*. The library was enriched for (AC)n, (ACG)n, (GCC)n and (TTTC)n by following a combination of modified procedures according to Kanpdal *et al.* [16], Fischer *et al.* [10], Takashi *et al.* [36] and Tang [37]. Genomic DNA fragments ranging in size from 300–1000 bp were recovered using the Geneclean kit (Q BIO GENE) after digestion with *Hae*III and *Rsa*I (Promega). The recovered fragments (1 μg) were ligated to an adaptor (5 μg, consisting of a 21-mer: 5'CTCTTGCTTGAATTCGGACTA3' and a phosphorylated 25-mer: 5'pTAGTCCGAATTCAAGCAAGAGCACA3') with T4 DNA ligase. The fragments with adaptors were denatured at 98 °C for 10 min, then, quick chilled in an ice bath. At the same time, 450 pmol (CA)n, 250 pmol(CAG)n, 250 pmol (GCC)n, 250 pmol (TTTC)n biotinylated oligonucleotide were attached to 900 ng of streptavidin magnetic beads (Biolabs) in 200 μL 5×SSC at room temperature for 1 hour. The excess unbound probe was removed twice with 200 μL 5×SSC. Then, the streptavidin magnetic beads were re-suspended in 20×SSC (final concentration 6×SSC) and hybridized with 100 μL predenatured fragments at 65 °C overnight. The beads were then washed three times at low stringency conditions (room temperature in 2×SSC, 1% SDS, 5 min each), six times at high stringency conditions (3 times in 1×SSC at room temperature, and 3 times in 1×SSC at 65 °C, 5 min each) and once in cold low salt buffer (0.15M NaCl, 20mM Tris-HCl (pH 7.5), 1mM EDTA) for 2 min. Finally, the beads were eluted as single-stranded fragments using an elution buffer (10 mM Tris-HCl (pH 7.5), 1mM EDTA). The final elution served as a template for PCR (the 21-mer oligonucleotide as the

unique primer, 20–25 cycles of 94 °C for 1min, 60 °C for 1 min, and 72 °C for 1 min) in order to obtain double-stranded fragments. The PCR products were purified, ligated to pMD 18-T vector (Takara) and transformed into *E.coli DH5α* competent cells to produce a microsatellite-enriched library.

2.2. Sequencing of positive clones and designing of primers

Plasmids, extracted from clones of the microsatellite-enriched library, were sequenced with the BigDye Terminator Kit on ABI PRISM 377 DNA sequencers (Perkin-Elmer, USA). Oligo6.0 and the Primer3 web site [28] were used to design PCR primers. The length of the primers ranged from 18 to 25 bp, and was designed to give PCR products ranging from 100 to 400 bp. One primer in each pair was labeled with either 6-FAM or HEX fluorescent dye (Augct Biotechnology Co. Ltd).

2.3. Birds

Thirty-one unrelated individuals from a breeding population (15 males and 16 females) were sampled from Gold Star Duck Production Ltd (Peking). A total of 30 individuals were used for cross-species amplification with 35 duck-specific microsatellite primers: 10 chickens (3 Silkies, 3 Beijing Fatty chickens and 4 Brown Shell Layers) from the Chicken Breeding Farm of China Agricultural University; 10 Peacocks (3 Blue-Peacocks, 3 White-Peacocks and 4 Green-Peacocks) from Yingjieli Co., Ltd, Guangdong province; 10 geese (2 Rhin, 2 Landaise, 3 Zi and 3 Xiayan) from Fangzheng Agro-Industry Co., Ltd, Jilin province. A routine phenol/chloroform extraction method was used to extract and purify the duck, chicken, peacock and goose genomic DNA. The DNA was qualified using agarose gel electrophoresis. The DNA concentration was estimated by comparison with molecular markers.

2.4. Optimization of mutiplex PCR and muti-run

The annealing temperature of the microsatellite primers was determined using an Authorized Thermal Cycler (Eppendorf). DNA amplification was performed in a total volume of 10 μL, with 40 ng duck DNA, 50mM KCl, 1.5mM $MgCl_2$, 10mM Tris.HCl (pH 8.3), 1mM Tetramethylammoniumchloride (TMAC), 0.1% Triton X-100, 0.01% gelatin, 200mM dNTP, 0.2 to 2 pmol of each primer and 2.5 U Taq polymerase. The PCR reaction conditions

were denaturing for 5 min at 94 °C, followed by 94 °C for 40 s, 58 ± 10 °C for 30 s or 1 min, and 72 °C for 30 s or 1 min, with a final 30 min elongation step at 72 °C. PCR primer pairs with similar annealing temperatures and different amplification product sizes were combined in mutiplex PCR reactions. Primer pairs unsuitable for mutiplex PCR were used in independent reactions, however, the products could be run in the same lane (muti-run) of the gel if their sizes were sufficiently different (> 60 bp).

2.5. Cross-species amplification and sequence

PCR parameters used for cross-species amplification were the same as in the duck except for annealing temperature. Initially, PCR was tested at the annealing temperature suggested for the duck. If the PCR product was not found on the 2% agarose gel, PCR was optimized using a 12-degree annealing temperature (± 10 °C) in an Authorized Thermal Cycler. Markers that generated specific products similar to the size fragments of the duck were selected for genotyping. These specific amplified products were sequenced with a reverse primer, or ligated to pMD 18-T vector and transformed into *E.coli DH5α* competent cells, then sequenced with the universal primer (M13).

2.6. Genotyping

Mutiplex PCR products or independent PCR products were diluted by 10–70 times. A mixture of 1 μL diluted PCR product, 12 μL deoinized formadide (Amresco), and 0.2 μL Genescan-350 ROX^{TM} or Genescan-500 ROX^{TM} (ABI) internal standard was run on a 3100 pop-4^{TM} (ABI) using a 3100 genetic analyzer (ABI). The fragment sizes of PCR products were analyzed using the Genescan 3.7 and Genemapper 1.1 software (ABI).

2.7. Statistics

Statistical evaluations of the microsatellite markers, including the allele frequency, observed heterozygosity, expected heterozygosity and polymorphism information content (PIC), were performed using the Popgene analysis software (Version 1.31) developed by Francis Yeh (ftp://ftp.microsoft.com/Softlib/MSLFILES/HPGL.EXE). DNAman Version 5.2.2 was used to search for sequence homology among the duck, chicken and goose.

3. RESULTS

3.1. Characteristics of the microsatellite markers

The characteristics of 35 novel microsatellite markers, including GenBank accession number, microsatellite repeat sequence, sequence of the PCR primers, the optimal annealing temperature for PCR, as well as the length of PCR products are shown in Table I.

The number of repeats in the microsatellites ranged from 6 to 52 with an average of 18.29/sequence (see Tab. I). Polymorphisms were detected in 31 unrelated individuals according to the conditions of the optimized mutiplex PCR and muti-run. Twenty-eight (80%) of the 35 primer pairs exhibited sequence length polymorphisms. A total of 117 alleles were observed from these polymorphic microsatellite markers, and the number of alleles ranged from 2 to 14 with an average of 4.18 per microsatellite locus. The frequencies of the 117 alleles ranged from 0.04 to 0.98 (Tab. II). Twenty-three frequencies of the 117 alleles were higher than 0.5 (Tab. II). Among the polymorphic markers, the highest heterozygosity (0.97) was observed at CAUD019 and the lowest heterozygosity (0.04) at CAUD008 (Tab. II). A total of 11 loci had heterozygosities greater than 0.50 (39.29%). The polymorphism information content (PIC) of 28 loci ranged from 0.04 to 0.88. The percentage of the loci with a PIC greater than 0.50 was 32.14% (9), with a PIC between 0.25 and 0.5 was 50% (14), and with a PIC lower than 0.25 was 17.86% (5). Sequences of 35 duck microsatellites were submitted to GenBank (AY493246–AY493280). Seven markers that were monomorphic in the genotyped individuals, are also listed in Table I.

3.2. Cross-species amplification

Thirty-five duck-specific primers were employed to screen the homologous loci in the chicken, peacock and goose. Amplification products were obtained with 5 (14.29%) markers in the chicken, with 4 (11.43%) markers in the peacock, with 22 (62.86%) markers in the goose. Amplified products with the sizes of the duck in each species were to investigate the polymorphism in 10 individuals. The characteristics of 16 homologous microsatellite markers, including GenBank accession number, repeat unit, sequence similarity between species, annealing temperature for PCR, as well as the length of the PCR products are shown in Table III. Homologous markers of CAUD016 and CAUD027 were detected in the chicken (CAUD-C016 and CAUD-C027) and goose (CAUD-G016 and CAUD-G027) respectively. All of them were

Table I. GenBank accession numbers, Tm, primer sequences and repeat type of the microsatellite markers in the duck.

GenBank accession	Locus[1]	Tm[2] (°C)	Primer	Dye[3]	Sequence	Repeat
AY493246	CAUD001	55.5	LEFT	HEX	ACAGCTTCAGCAGACTTAGA	(AAAAC)3A6G(CA)9A33
			RIGHT		GCAGAAAGTGTATTAAGGAAG	
AY493247	CAUD002	60.8	LEFT	6-HEX	CTTCGGTGCCTGTCTTAGC	(CAG)6
			RIGHT		AGCTGCCTGGAGAAGGTCT	
AY493248	CAUD003	51.4	LEFT	6-FAM	CCTGGCATTCTGCTAAGTTC	(CA)8
			RIGHT		TGGGTTTGAACAGTGTAGCC	
AY493249	CAUD004	60.8	LEFT	6-FAM	TCCACTTGGTAGACCTTGAG	(AC)20
			RIGHT		TGGGATTCAGTGAGAAGCCT	
AY493250	CAUD005	60.8	LEFT	6-FAM	CTGGGTTTGGTGGAGCATAA	(TC)18
			RIGHT		TACTGGCTGCTTCATTGCTG	
AY493251	CAUD006	63.5	LEFT	6-FAM	ATGGTTCTCTGTAGGCAATC	(CAAA)4A3(AC)9
			RIGHT		TTCTGCTTGGGCTCTTGGA	
AY493252	CAUD007	60.8	LEFT	6-FAM	ACTTCTCTTGTAGGCATGTCA	(CAG)7
			RIGHT		CACCTGTTGCTCCTGCTGT	
AY493253	CAUD008	63.5	LEFT	6-FAM	GTTAAGAAAATCAGAAGAGCG	(TA)9
			RIGHT		CTTGAGTTAGGCTAAGTGTG	
AY493254	CAUD009	60.8	LEFT	6-HEX	AGGGATTTTGGAGCGGAGC	(CAG)7
			RIGHT		TGTGCGGCGTTTTCCTCTG	
AY493255	CAUD010	50.3	LEFT	HEX	GGATGTGTTTTTCATTATTGAT	(CA)3(TA)2(CA)9
			RIGHT		AGAGGCATAAATACTCAGTG	
AY493256	CAUD011	50.3	LEFT	6-FAM	TGCTATCCACCCAATAAGTG	(CA)13
			RIGHT		CAAAGTTAGCTGGTATCTGC	
AY493257	CAUD012	63.5	LEFT	HEX	ATTGCCTTTCAGTGGAGTTTC	(CA)2CG(CA)7
			RIGHT		CGGCTCTAAACACATGAATG	

Table I. Continued.

GenBank accession	Locus[1]	Tm[2] (°C)	Primer	Dye[3]	Sequence	Repeat
AY493258	CAUD013	58.1	LEFT	HEX	ACAATAGATTCCAGATGCTGAA	(AC)25
			RIGHT		ATGTCTGAGTCCTCGGAGC	
AY493259	CAUD014	58.1	LEFT	HEX	CACAACTGACGGCACAAAGT	(AC)7GC(AC)6
			RIGHT		CTGAGTTTTTCCCGCCTCTA	
AY493260	CAUD015	68.1	LEFT	6-FAM	ACAACCCACTTCCAGAACTG	(CA)3(CG)2(CA)25…A9
			RIGHT		GCATGTCAGAGATGCGTGC	
AY493261	CAUD016	51.4	LEFT	6-FAM	TTTAGGTAAAACTGTGAATCAA	T8(TTTC)8T13
			RIGHT		ATCAAAGCAGGGAGCTAAG	
AY493262	CAUD017	58.1	LEFT	6-FAM	AGAAATACACTTACAGCACT	(TC)4(TTTC)2CTTC(TTTC)2CTTC(TTTC)9(TC)20
			RIGHT		TGTCATAAAATGGTTAATTGC	
AY493263	CAUD018	50.3	LEFT	6-FAM	TTAGACAAATGAGGAAATAGTA	(CA)9
			RIGHT		GTCCAAACTAAATGCAGGC	
AY493264	CAUD019	58.1	LEFT	6-FAM	CTTAGCCCAGTGAAGCATG	(TTTC)23
			RIGHT		GCAGACTTTTACTTATGACTC	
AY493265	CAUD020	53.2	LEFT	HEX	TAGGGTCAATAGTAAGAAACA	(CA)n
			RIGHT		TAACTGTGTGATAAGGGAGA	
AY493266	CAUD021	60.8	LEFT	6-FAM	TGCAGGTTCCATGTGTTAGA	(CA)9
			RIGHT		TAGCAACAAATGAGAAATGAGT	
AY493267	CAUD022	55.5	LEFT	6-FAM	CATGCTGAGTGTCCTATCCT	(GCA)8
			RIGHT		CCAGGTCAGGCGTGTGCT	
AY493268	CAUD023	51.4	LEFT	HEX	CACATTAACTACATTTCGGTCT	(AC)17
			RIGHT		CAGCCAAAGAGTTCAACAGG	
AY493269	CAUD024	55.5	LEFT	HEX	TCGCATTAAGCTCTGATCT	(TTTC)33…T19
			RIGHT		ATCAACAGAATCCAAAATATG	

Table I. Continued.

GenBank accession	Locus[1]	Tm[2] (°C)	Primer	Dye[3]	Sequence	Repeat
AY493270	CAUD025	63.5	LEFT	6-FAM	AGTTCATCCCGATTTGTAGC	(CA)4A3(CA)10
			RIGHT		AAATGCAGTGAGGTAAACCC	
AY493271	CAUD026	60.8	LEFT	6-FAM	ACGTCACATCACCCCACAG	(AC)17
			RIGHT		CTTTGCCTCTGGTGAGGTTC	
AY493272	CAUD027	66	LEFT	6-FAM	AGAAGGCAGGCAAATCAGAG	(CA)11
			RIGHT		TCCACTCATAAAAACACCCACA	
AY493273	CAUD028	55.5	LEFT	6-FAM	TACACCCAAGTTTATTCTGAG	(AC)8
			RIGHT		ACTCTCCAGGGCACTAGG	
AY493274	CAUD029	55.5	LEFT	6-FAM	GACCTCAAGAATTTACCAC	(CA)14A11
			RIGHT		ATTATTTTCTTCTGGCAGCA	
AY493275	CAUD030	50.3	LEFT	6-FAM	ATTATTCCTGATGGCGTGGT	(CA)9...(AT)6T10
			RIGHT		TCATGCTGAATTTGGCTGTT	
AY493276	CAUD031	51.4	LEFT	HEX	AGCATCTGGACTTTTTCTGGA	(TTTC)9(TC)25
			RIGHT		CACCCCAGGCTCTGAGATAA	
AY493277	CAUD032	58.1	LEFT	HEX	GAAACCAACTGAAAACGGGC	(CA)n
			RIGHT		CCTCCTGCGTCCCAATAAG	
AY493278	CAUD033	58.1	LEFT	6-FAM	ACCCAGAAGAGTCAAGAATAG	(AC)10...T9
			RIGHT		GAGTATTCCTGGTCTGTGCT	
AY493279	CAUD034	55.5	LEFT	HEX	TACTGCATATCACTAGAGGA	(CA)9
			RIGHT		TAGGCATACTCGGGTTTAG	
AY493280	CAUD035	63.5	LEFT	HEX	GTGCCTAACCCTGATGGATG	(CA)n
			RIGHT		CTTATCAGATGGGGCTCGGA	

[1] The markers code CAU stands for China Agricultural University, D stands for duck.

[2] The optimal annealing temperature in the PCR.

[3] Fluorescent amidite dye labeled on the 5‘ end of the left primer.

Table II. The characteristics of the novel microsatellite markers in the duck.

Locus	Fragment	Allele No.	Obs_Ht[1]	PIC[2]	Allele	size	Frequency
CAUD001	315-331	5	0.68	0.51	A	315	0.02
					B	317	0.39
					C	321	0.02
					D	323	0.50
					E	331	0.08
CAUD002	188-188	1	0.00	0.00	A	188	1.00
CAUD003	115-115	1	0.00	0.00	A	115	1.00
CAUD004	199-221	5	0.59	0.56	A	199	0.02
					B	201	0.19
					C	209	0.55
					D	211	0.03
					E	221	0.21
CAUD005	250-284	5	0.32	0.30	A	250	0.02
					B	259	0.10
					C	267	0.82
					D	282	0.03
					E	284	0.03
CAUD006	210-210	1	0.00	0.00	A	210	1.00
CAUD007	108-108	1	0.00	0.00	A	108	1.00
CAUD008	102-104	2	0.04	0.10	A	102	0.06
					B	104	0.94
CAUD009	127-127	1	0.00	0.00	A	127	1.00
CAUD010	112-116	3	0.16	0.20	A	112	0.05
					B	114	0.89
					C	116	0.06
CAUD011	121-140	4	0.58	0.57	A	121	0.02
					B	127	0.23
					C	131	0.27
					D	140	0.48
CAUD012	202-204	2	0.05	0.04	A	202	0.02
					B	204	0.98
CAUD013	85-113	7	0.68	0.63	A	85	0.10
					B	91	0.24
					C	95	0.10
					D	105	0.02
					E	107	0.50
					F	111	0.03
					G	113	0.02
CAUD014	113-117	2	0.42	0.32	A	113	0.73
					B	117	0.27
CAUD015	118-122	2	0.19	0.27	A	118	0.20
					B	122	0.80

Table II. Continued.

Locus	Fragment	Allele No.	Obs_Ht[1]	PIC[2]	Allele	size	Frequency
CAUD016	189-217	5	0.37	0.46	A	189	0.67
					B	193	0.22
					C	209	0.03
					D	213	0.02
					E	217	0.07
CAUD017	216-262	3	0.25	0.16	A	216	0.17
					B	218	0.88
					C	262	0.02
CAUD018	98-100	2	0.37	0.30	A	98	0.75
					B	100	0.25
CAUD019	132-213	13	0.97	0.87	A	132	0.08
					B	136	0.10
					C	140	0.02
					D	145	0.06
					E	149	0.19
					F	153	0.10
					G	162	0.05
					H	188	0.05
					I	192	0.15
					J	196	0.02
					K	200	0.15
					L	209	0.02
					M	213	0.03
CAUD020	111-115	2	0.37	0.32	A	111	0.28
					B	115	0.72
CAUD021	184-184	1	0.00	0.00	A	184	1.00
CAUD022	128-140	4	0.57	0.62	A	128	0.40
					B	133	0.05
					C	137	0.23
					D	140	0.32
CAUD023	163-183	4	0.39	0.40	A	163	0.16
					B	165	0.73
					C	171	0.10
					D	183	0.02
CAUD024	270-340	13	0.93	0.88	A	270	0.16
					B	278	0.07
					C	281	0.02
					D	284	0.02
					E	288	0.05
					F	292	0.14
					G	296	0.16
					H	300	0.09

Table II. Continued.

Locus	Fragment	Allele No.	Obs_Ht[1]	PIC[2]	Allele	size	Frequency
CAUD024	270-340	13	0.93	0.88	I	304	0.05
					J	308	0.12
					K	324	0.03
					L	328	0.02
					M	340	0.09
CAUD025	289-291	3	0.63	0.49	A	289	0.37
					B	290	0.10
					C	291	0.53
CAUD026	140-150	4	0.70	0.67	A	140	0.18
					B	146	0.28
					C	148	0.37
					D	150	0.17
CAUD027	111-119	3	0.60	0.48	A	111	0.55
					B	115	0.35
					C	119	0.10
CAUD028	143-143	1	0.00	0.00	A	143	1.00
CAUD029	112-114	2	0.23	0.22	A	112	0.85
					B	114	0.15
CAUD030	257-261	2	0.48	0.37	A	257	0.53
					B	261	0.47
CAUD031	112-126	4	0.48	0.40	A	112	0.62
					B	114	0.02
					C	118	0.02
					D	126	0.34
CAUD032	115-121	3	0.27	0.31	A	115	0.18
					B	117	0.78
					C	121	0.03
CAUD033	200-206	4	0.35	0.39	A	200	0.74
					B	202	0.10
					C	204	0.13
					D	206	0.03
CAUD034	215-217	2	0.27	0.27	A	215	0.80
					B	217	0.20
CAUD035	223-237	4	0.60	0.52	A	223	0.13
					B	231	0.23
					C	233	0.60
					D	237	0.05

[1] Obs_Ht is the observed heterozygosity.

[2] PIC stands for the polymorphism information content.

monomorphic in three chicken breeds, and only one was polymorphic in three goose breeds (CAUD-G016). Sequencing results showed that the simple tandem repeat was present in both loci. For CAUD-C027, the flanking sequences and core repeats had high similarity (94%) for the chicken and the duck, and for the chicken and the goose, but the number of repeats in the microsatellite

in the duck was higher than that in the chicken. However, the similarity was moderate in the flanking sequences of CAUD-C016 and CAUD-G016, but a repeat unit found in the duck was absent in the chicken and the goose.

A total of 14 alleles were observed from 5 polymorphic microsatellite markers, which ranged from 2 to 3 with an average of 2.8 alleles per locus in the goose. In the other 9 loci only one allele was observed (see Tab. III). Sequencing results showed that the simple tandem repeat was contained in all 14 goose homologous loci (CAUD-G002, CAUD-G005, CAUD-G006, CAUD-G007, CAUD-G011, CAUD-G012, CAUD-G013, CAUD-G016, CAUD-G018, CAUD-G021, CAUD-G023, CAUD-G026, CAUD-G027, CAUD-G028) (see Tab. III). All of the flanking sequences were highly similar to the corresponding ones in the duck (90%–98%), but a bit different for CAUD-G005 (83%). The variance in the core sequence was higher than that in the flanking sequence. For CAUD-G002, CAUD-G005, CAUD-G011, CAUD-G013, CAUD-G018, CAUD-G021, CAUD-G023, CAUD-G026, CAUD-G027, and CAUD-G028, the core sequences in the goose were shorter than those in the duck. Conversely, the core sequence in CAUD-G007 and CAUD-G012 in the goose was longer than that in the duck. Moreover, part of the repeat unit found in the duck was absent in the goose at CAUD-G016 and CAUD006.

Sequences of 2 chicken and 14 goose microsatellite loci were submitted to GenBank (AY866384–AY866399).

4. DISCUSSION

4.1. Evaluation of the microsatellite markers

Although the repeat number in the 35 microsatellite markers was high (18.28/sequence), 20% of the markers had only one allele in the genotyped population. The main reason was that the population we examined had been selected for several generations. These markers may show polymorphism if more individuals or more populations or breeds are analyzed. Five out of 28 polymorphic microsatellite markers had a PIC lower than 0.25, and only 9 loci were informative.

4.2. Evaluation of cross-species amplification

This study was the first attempt to detect a duck-specific primer in pheasant birds. The results indicate only 5.7% of the primers designed for the duck could be useful in the analysis of the chicken genome, and none of them could be

Table III. GenBank accession numbers, repeat types, Tm, sequence similarity, number of alleles and size of the duck-specific markers in the chicken and goose.

GenBank accession No.	Locus[a]	Species	Repeat1[b]	Repeat2[c]	Similarity[d]	Tm(°C)	Allele No.1[4]	Allele No.2[4]	Size1[e]	Size2[e]
AY866384	CAUD-C016	Chicken	A15	T8(TTTC)8T13	76%	51.4	1	5	189-189	189-217
AY866385	CAUD-C027	Chicken	(CA)9	(CA)11	94%	66	1	3	116-116	111-119
AY866386	CAUD-G002	Goose	(GCA)2CAGGCA	(CAG)6	96%	60.8	1	1	131	188-188
AY866387	CAUD-G005	Goose	(TAAA)3AATTA9 TA5(GA)3	(TC)18ORA23	77%	60.8	1	5	233	250-284
AY866388	CAUD-G006	Goose	(AC)2(CA)6	(CAAA)4(AC)9	88%	51.2	1	1	178	210-210
AY866389	CAUD-G007	Goose	(CAG)5(GCA)5	(CAG)7OR(CAG)5	97%	60.8	3	1	113-119	108-108
AY866390	CAUD-G011	Goose	(CA)2A6C3A	(CA)13	90%	50.3	3	4	113-127	121-140
AY866391	CAUD-G012	Goose	(AC)10	(CA)2CG(CA)7	91%	51.2	3	2	201-207	202-204
AY866392	CAUD-G013	Goose	(AC)9	(AC)25	96%	58.1	2	7	85-89	85-113
AY866393	CAUD-G016	Goose	(A)14	T8(TTTC)8T13	83%	51.4	3	5	174-184	189-217
AY866394	CAUD-G018	Goose	(CA)4	(CA)9	90%	50.3	1	2	84	98-100
AY866395	CAUD-G021	Goose	(CA)6	(CA)9	92%	60.8	1	1	181	184-184
AY866396	CAUD-G023	Goose	(AC)11	(AC)17	89%	51.4	1	4	133	163-183
AY866397	CAUD-G026	Goose	(AC)5	(AC)17	92%	60.8	1	4	135	140-150
AY866398	CAUD-G027	Goose	(CA)9	(CA)11	94%	66	1	3	120	111-119
AY866399	CAUD-G028	Goose	(AC)3T(CA)3	(AC)8	94%	55.5	1	1	139	143-143

[a] CAUD-C016–CAUD-C022 are chicken loci developed from the duck, CAUD-G002–CAUD-G028 are goose loci developed from the duck.

[b] Repeat1 is the repeat in the chicken or goose, Repeat2 is the repeat in the duck.

[c] Similarity is the sequence similarity between the chicken or goose and duck.

[d] Allele No.1 is the number of alleles in the chicken or goose, Allele No.2 is the number of alleles in the duck.

[e] Size1 represents the size of product in the chicken or goose, Size2 represents the size of product in the duck.

useful in the analysis of the peacock genome. In contrast to the pheasant bird, 40% of the 35 duck primers yielded specific PCR products. The difference in the success rates could be attributed to the relationship among the four birds. Both the chicken and peacock belong to the order Galliformes, but the duck and goose belong to the order Anseriformes. There should be high variance in the flanking sequence of the microsatellite between the two orders. The results showed that it was difficult to screen the microsatellite marker for the duck by cross-species amplification from chicken loci. But it was possible to isolate a marker from the same order. The success rates of the heterologous amplification with microsatellite primers in closely related species ranged from 16.23% to 62.89% [1, 9, 17–19, 25, 31]. Besides the above factor, difference in stringency used for amplification could also be attributed to this result [27].

Although the numbers of alleles ranged from 3 to 5 with an average of 4 at CAUD016 and CAUD027 in the Peking duck, there was only one allele in three chicken breeds. Five of 14 markers were polymorphic in the genotyped goose, with an average of 2.8 per locus. The number of alleles at all common loci in the chicken or goose was less or equal to that of the duck except CAUD-G007 and CAUD-G012. There are two possible reasons for this result. One is that the mutational rate in the core sequence at the loci in the chicken or goose is lower than in the original species (duck). The other is that the population used in the study was small, and therefore the effect of random variation is less acute.

With regards to the product size in cross-species amplification, the results were inconsistent in the different taxa [1, 9, 38]. In this study, only one locus (CAUD-G007) in the goose was larger than that in the duck, seven loci in the chicken or goose were equivalent to that in the duck, and nine loci in the goose were smaller than that in the duck. In addition, the size of one homologous locus in the chicken (CAUD-C016) was larger than that in the goose (CAUD-G016). The size of the other homologous loci was the same in the two birds.

Based on sequence comparisons of the flanking sequence and repeat, we conclude that 2 chicken loci and 14 (see Tab. III) goose loci are true homologous loci of the duck loci from which the markers were developed. These loci should be useful for comparative mapping among the birds.

Although microsatellites are informative and powerful genetic markers, only 102 microsatellite markers specific for waterfowl were preciously reported [3, 23, 24, 29, 35]. The goal of the present study was to isolate novel microsatellite markers in the duck by an enriched library. The majority of loci developed in this study are polymorphic in our population. These loci will be

useful for linkage and QTL mapping, population genetics and phylogenetic studies. In addition, the novel goose microsatellite loci developed by cross-species are useful for population genetic study and linkage mapping.

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