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Isolation and Characterization of Microsatellite Markers in Tsaiya Duck

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ABSTRACT : An enrichment library of GATA-repeats from genomic DNA was constructed in this study to isolate and characterize microsatellite loci in Tsaiya duck (*Anas platyrhynchos*). Thirty-three microsatellite markers were developed and used to detect polymorphisms in 30 Tsaiya ducks. A total of 177 alleles were observed and all loci except APT022 were polymorphic. The number of alleles ranged from 2 to 9 with an average of 5.5 per microsatellite locus. The observed and expected heterozygosity of these polymorphic markers ranged from 0.07 to 0.93 with an average number of 0.60 and 0.10 to 0.86 with an average number of 0.61, respectively. Among the polymorphic markers, the observed heterozygosities of 23 loci were higher than 0.50 (69.70%). The polymorphism information content (PIC) in the 32 loci ranged from 0.09 to 0.83 with an average of 0.57. Seven of the 33 duck microsatellite loci had orthologs in the chicken genome, but only APT004 had a similar core repeat to chickens. These microsatellite markers will be useful in constructing a genetic linkage map for the duck and a comparative mapping with the chicken can also provide a valuable tool for studies related to biodiversity and population genetics in this duck species. (**Key Words :** Microsatellite Marker, Polymorphism, Tsaiya Duck)

INTRODUCTION

Many important agricultural traits are quantitative traits controlled by multiple genes. The recent development of molecular genetic mapping tools has enabled the identification of quantitative trait loci (QTL) in the genome. The application of marker assisted selection for QTL has the potential to enhance the accuracy of animal breeding programs, particularly for traits that are difficult to improve through traditional selection methods (Meuwissen and Goddard, 1996). Microsatellites, also known as short tandem repeats (STR), are tandem repeated motifs of 1-6 bases. They are found abundantly and at random throughout most eukaryotic genomes (Stallings et al., 1991). Microsatellites are highly polymorphic and have become one of the most useful tools for population genetic studies, linkage mapping, parentage determination and QTL analysis. In chicken, swine and cattle populations a large number of microsatellites have been isolated and widely used for these purposes (Kong et al., 2006; Liu et al., 2006). In contrast, fewer genetic markers have been established in the duck and only a few articles have been published for some species including the Peking duck, eider duck, muscovy duck, mallard, white-headed duck, ruddy duck and musk duck (Maak et al., 2000; Maak et al., 2003; Paulus and Tiedemann, 2003; Stai and Hughes, 2003; Denk et al., 2004; Munoz-Fuentes et al., 2005; Guay and Mulder, 2005; Huang et al., 2005; Huang et al., 2006). Although the first duck genetic linkage map has been developed (Huang et al., 2006), it only spans 1,353.3 cM with an average interval distance of 15.04 cM. More microsatellites are needed to establish a complete duck genetic map. We attempted to isolate microsatellite markers for the Tsaiya duck (*Anas platyrhynchos*) and investigated its polymorphisms.

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MATERIALS AND METHODS

Collection and extraction of Tsaiya duck genomic DNA

Thirty individuals (15 males and 15 females) were selected from a germplasm preservation population of Tsaiya ducks kept at the Ilan Branch since 1984. In the beginning, the population was reproduced via natural mating. At the 8th generation, we randomly divided this germplasm preservation duckling population into 15-sire families. The rotational crossbreeding system was then used

to avoid inbreeding. We randomly chose one male and one female as samples from each sire family from the 9th generation. Genomic DNA was extracted from fresh blood using the GenoMaker kit (Watson BioTech, Taiwan) following the manufacturer's instructions. Briefly, 40 μl blood was lysed in 1 ml GenoMaker reagent and extracted with chloroform. DNA was precipitated with isopropanol and quantified using a spectrophotometer.

Construction of genomic DNA libraries enriched for microsatellites

The library was enriched for GATA repeats following a combination of modified procedures according to Hamilton et al. (1999) and Hsu et al. (2003). A pooled genomic DNA of 3 Tsaiya ducks was digested with *Alu*I, *Hae*III and *Rsa*I. The fragments were then ligated with SNX linkers (Hamilton et al., 1999). The ligated products were amplified using PCR at 94°C for 5 min, 30 cycles of 94°C for 1 min,

Table 1. Characteristics of 33 novel microsatellite loci in the Tsaiya duck

Locus	Repeat motif in clone	Primer sequences (5'-3')	Size from clone (bp)	T_a (°C)	$MgCl_2$ (mM)	Fragment (bp)	No. of alleles	H_O	H_E	PIC	GenBank accession no.
APT001	$(GATA)_{12}$	F: GTC CCA CTG GTT TGC TGT CC R: ACT ACG CAT GGC AGT GAG GTT	206	55	2.5	178-206	3	0.23	0.52	0.46	DQ884881
APT002	$(GATA)_7GACA(GATA)_3$	F: ACC CTC CCA CAG ATT AAA GAG AAG T R: GGA AGG ATG CCC TGA TTT ACA C	133	55	2.5	129-145	5	0.70	0.66	0.61	DQ884882
APT003	$(GATA)_{11}$	F: GAT CAT TGC ACT TGA AAT TAT TGT TAT TT R: TGT GCA TTA CTG TGG CAG ATC TG	228	55	2.5	220-236	4	0.57	0.54	0.49	DQ884883
APT004	$GATAGAT(GATA)_{15}$	F: GGG CAG GAA AAT CTC CTG AAT R: TCT CAG TGG CTG AGC GGT C	306	55	2.5	294-322	9	0.80	0.86	0.83	DQ884884
APT005	$(GATA)_{17}$	F: TCC GTA CAG ACC AAC ATC GG R: AGG TCT TTA CAG CCC ACT CCC	311	55	2.5	283-319	9	0.83	0.80	0.77	DQ884885
APT006	$(GATA)_{12}$	F: CTT CCC ATT GCA GTG TTG GTC R: TTG GCA TCT TTG TTC TGC AGA	326	50	2.5	318-342	6	0.57	0.64	0.59	DQ884886
APT007	$(GATA)_{14}$	F: TCT TAA ATG TTA GTG ATA CCA GCA TCT TT R: TTC GGG ATG AGA AGG AAG GA	218	50	2.5	194-230	6	0.80	0.72	0.67	DQ884887
APT008	$(GATA)_{12}$	F: CAA AGA AAT CCT AGA ACA TCA TTC AAA T R: TCT TCT GGC TTT TCA CCT TAG TTT AGT A	188	50	2.5	184-208	7	0.87	0.79	0.75	DQ884888
APT009	$(GATA)_2GAT(GATA)_{15}$	F: CCA GGC AGT TGC TGT GTA ACA R: GGC GCT TTC TTC TAT GAT CGA	334	55	2.5	330-354	7	0.50	0.81	0.77	DQ884889
APT010	$(GATA)_9GAT(GATA)_3$	F: CAC TCA GGC TTT TAG GTC CAT TAA TA R: CAT CTG AGA ATG CAC TTA CTG TCA AA	204	55	2.5	192-215	6	0.70	0.63	0.56	DQ884890
APT011	$(GATA)_8$	F: CAT ACA GGG AGT CTG AGA TGA TCA A R: TTA TGT TCC ATT CAG GGC TTT CTT	160	50	2.5	152-164	3	0.07	0.13	0.12	DQ884891
APT012	$(GATA)_{16}$	F: TTG AGC CTC AGG TTC TAA ACT CCT A R: TCA TAA CAT TTC AGA CCA GTT TTC AGA	205	50	2.5	185-205	6	0.87	0.73	0.67	DQ884892
APT013	$(GATA)_{10}$	F: CCA ACC ACC AGG AAG TAC TGT AAA TA R: AGG AAA GTT CAG ACA CAT GGA TTG	131	50	2.5	127-171	9	0.77	0.81	0.78	DQ884893
APT014	$(GATA)_{11}$	F: GCA CCA GGT AAT TTA TGT CAG AAA TAA T R: GAA GTG CAA AAC ATG GTT CAG G	321	50	2.5	317-325	3	0.10	0.16	0.15	DQ884894
APT015	$(GATA)_{13}$	F: CTG TTA TGA CAC CAT GTT TGG ATT TA R: CGT GCT CTG CAA CAA CTG AAA	138	55	2.5	126-150	7	0.77	0.75	0.71	DQ884895
APT016	$(GATA)_{10}$	F: TCT TAA ATG GGA CTG ATG GAG AGA G R: ACC TAT TTT ATC TCA GGA TGC AAT TAT G	112	50	2.5	112-120	3	0.53	0.54	0.45	DQ884896
APT017	$(GGAT)_6(GATA)_{12}$	F: TGG ATG GAC AGA CGG GTG A R: TGG AAG TTT TGA TTT CTA GTG CTT ACA	185	55	2.5	161-189	5	0.77	0.74	0.69	DQ884897
APT018	$(GATA)_9(GAAA)_{14}(GA)_2$ $(GAAA)_2$ $(GA)_6(GAAA)_2$	F: GTG GCA GTT TAA TGA AAG CGA AA R: TGG AGG TAC CCA AAG GAG AAT TC	267	50	2.5	267-295	8	0.20	0.86	0.83	DQ884898
APT019	$(GATA)_{11}$	F: CCA AGA TTA GGG CTA TGT GGT GAT R: AAG GAT TGA GAC AGG AGA TGG G	218	50	2.5	206-218	2	0.37	0.35	0.28	DQ884899
APT020	$(GATA)_{14}$	F: TTC CAA GTT TGT CAT GCC AAT AGA R: CTG ACC ATG TTA GGG CGT TTT AG	201	50	2.5	177-205	8	0.93	0.83	0.79	DQ884900
APT021	$(GATA)_{10}$	F: GCA CTC CCT AAC TAG TAG CGC TCT R: GAA GCA TTG TCA TAC TTG CCT GA	133	50	2.5	133-169	6	0.80	0.79	0.75	DQ884901
APT022	$(GATA)_{12}$	F: TCA GTG AAA GCC ACA GTC AGA TC R: TTT AGG CAC TGA AGC CCA ACA	120	50	2.5	120	1	0.00	0.00	0.00	DQ884902
APT023	$(GATA)_4AATA(GATA)_7$	F: CCA AAC AAG AGA AGA TGA TAG AGA GAC A R: GAA TCA ATA AAC TGC TTT GAT CCT GAC	117	50	2.5	113-121	3	0.57	0.51	0.43	DQ884903
APT024	$GATAGACA(GATA)_7$	F: TGT GGG CAG TTC CTC AAC AA R: GCC CAC CCT CTC TTT CTG AAG	102	55	2.5	102-118	4	0.50	0.51	0.44	DQ884904
APT025	$(GATA)_{13}$	F: TCC TAA GAA ACG TTG CTT CAT AGA CC R: GAG TTA AGC TTC ATC ACT CTG TGA CTG	121	50	3	105-133	7	0.70	0.67	0.63	DQ884905
APT026	$(GATA)_{10}$	F: CCC TGA AAG GCT GTT TTA TAT ATC CA R: ATG TAA ATA AAG TAG CCT TGC ACG GT	138	55	2.5	138-142	2	0.60	0.51	0.38	DQ884906
APT027	$(GATA)_{10}$	F: ATT TCC AAA ATC TTG TGC TTT AAG C R: TTT TTG TTC TTT CTC TCT CTC CCT CT	151	50	3	151-155	2	0.10	0.10	0.09	DQ884907
APT028	$(GATA)_{10}$	F: CAT TCA TGT TTA TTT CTT CTG GTA TGT G R: GTT AAA ATG GGA AGG CTT CAC TAG A	167	55	2.5	131-187	7	0.83	0.82	0.78	DQ884908
APT029	$(GATA)_{14}$	F: TCT GCA AGG TAT TCT CAT TCT TAT TCT T R: GAT ACG TAG AGT GGA TGC TGG AGA T	171	50	3	143-179	6	0.73	0.66	0.60	DQ884909
APT030	$(GATA)_{13}$	F: TGG ATA TAC CAT GCC AGT GCA R: TGG CTT GTG GGA GAG ATG ATG	206	50	2.5	190-226	8	0.67	0.54	0.52	DQ884910
APT031	$(GATA)_{12}$	F: GCT GGA AGA AAG GAG AAG GAG G R: AGA AAA ACA GTA TGA GCG AAC AGG T	210	50	2.5	194-234	8	0.90	0.84	0.81	DQ884911
APT032	$GATA(GACA)_2GACT$ $(GATA)_4$	F: TCA CTT TCT TGA CTC TCC TTG GTT T R: TGA CTT GAA TTC TGT TCA GGA TAA ATG	259	55	2.5	207-259	4	0.70	0.67	0.60	DQ884912
APT033	$(GATA)_{13}$	F: CTT CAC CCT ACC TCA TAA GGA ACT G R: ATT CCA AAT CTG CAA GGT GAG TAT TA	266	55	2.5	266-274	3	0.13	0.19	0.17	DQ884913

T_a = Annealing temperature, H_O = Observed heterozygosity, H_E = Expected heterozygosity, PIC = Polymorphism information content.

Table 2. The orthologous microsatellite duck DNA in the chicken genome

Locus	Score (bits)	E value	Chromosome*	Physical position[1]	Chicken repeat	Duck repeat
APT004	154	2e-35	3	6514982-6515130	GATAAATA$(GATA)_{10}(CATA)_6$	GATAGAT$(GATA)_{15}$
APT005	119	5e-25	1	8485714-8485875	T_{21}	$(TATC)_{17}$
APT006	117	1e-24	4	27975792-27975899	No	$(GATA)_{12}$
APT012	196	3e-48	2	185809-185999	A_{13}	$(GATA)_{16}$
APT014	106	4e-21	un	5986-6145	No	$(GATA)_{11}$
APT016	167	1e-39	4	8793108-8793307	No	$(GATA)_{10}$
APT031	204	1e-50	4	77150-77368	No	$(GATA)_{12}$

* Chromosome is the pair of chromosome in the chicken. 'un' means that the position in the chicken genome is uncertain.
[1] The physical position in the chicken genome.

55°C for 1 min and 72°C for 2 min, followed by a final cycle at 72°C for 7 min. The amplified products were used for subtractive hybridization with 3'-biotinylated $(GATA)_{10}$ oligonucleotides to select the microsatellite-containing DNA fragments. The biotin-labeled oligonucleotides were eluted using Dynabeads MyOne Streptavidin (Dynal, Norway) according to the manufacturer's protocol. Repeat-enriched DNA was made double-stranded and amplified under the above described PCR conditions and then cloned in the pGEM-T Easy Vector (Promega, USA). After transformation into JM109 competent cells, 800 colonies containing inserts were lifted to Nylon membranes (Roche, Germany) and hybridized with 3'-DIG-labeled $(GATA)_8$ oligonucleotides. The positive colonies were cultured to extract their plasmids and then sequenced using the BigDye Terminator Kit on a 3730xl DNA Analyzer (Applied Biosystems).

Genotyping

Sequences were aligned with SeqWeb Version 2.1 (Wisconsin Package). The Primer Express software (Applied Biosystems) was used to design PCR primers. The primer pairs showing the expected PCR products were selected for polymorphism screening. The forward primers of these primer pairs were labeled with FAM or HEX fluorescent dye. PCR reaction mixtures (10 μl) containing 10 ng genomic DNA, 2.5 mM or 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 200 μM of each dNTP, 0.4 μM of forward and reverse primer and 0.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems/Roche) were prepared. After initial incubation at 95°C for 10 min, each PCR amplification was performed for 30 cycles of denaturing at 95°C for 20 s, annealing for 30 s at an appropriate annealing temperature (50 or 55°C) for each primer pair and extension at 72°C for 30 s. This was followed by a final cycle at 72°C for 1 h. The PCR products were analyzed in a MegaBACE 1000 auto sequencer (Amersham Biosciences). The sizes of the DNA fragments were investigated using the Genetic Profiler Version 2.2 software (Amersham Biosciences).

Statistics and similarity searching

The observed and expected heterozygosities and polymorphism information content (PIC) were calculated using the CERVUS 2.0 program (Marshall et al., 1998). Hardy-Weinberg expectation and linkage disequilibrium tests were performed with the FSTAT 2.9.3.2 software (http://www2.unil.ch/popgen/softwares/fstat.htm). The sequences were analyzed using the BLAST program (NCBI) to identify the orthologous microsatellite duck DNA in the chicken genome. The unique match sequences with E-values smaller than e-20 from the chicken were used as orthologs to the duck microsatellite DNA.

RESULTS AND DISCUSSION

Eighty positive clones out of 800 colonies screened from the GATA-enriched genomic library were sequenced. There were 47 different loci from 75 sequences containing GATA repeats and 33 of these loci were chosen for further polymorphism tests in 30 Tsaiya ducks. After searching further with the BLAST program, we found that these 33 microsatellite Tsaiya duck loci were novel. The characteristics of the 33 microsatellite loci are summarized in Table 1. A total of 177 alleles were observed and all loci except APT022 were polymorphic. The number of alleles ranged from 2 to 9 with an average of 5.5 per microsatellite locus. The observed and expected heterozygosity of these polymorphic markers ranged from 0.07 to 0.93, with an average number of 0.60, and 0.10 to 0.86, with an average number of 0.61, respectively. Among the polymorphic markers, the observed heterozygosities of 23 loci were higher than 0.50 (69.70%). The polymorphism information content (PIC) of the 32 loci ranged from 0.09 to 0.83, with an average number of 0.57. Based on the classification of Botstein et al. (1980), twenty-one (65.63%) polymorphic markers were highly informative (PIC>0.50), seven (21.88%) were reasonably informative (0.50>PIC>0.25), and four (12.50%) were slightly informative (PIC<0.25). No locus showed significant deviation from Hardy-Weinberg equilibrium and there was no linkage disequilibrium among the loci (p>0.05). The orthologous microsatellite duck DNA in the chicken genome search results are presented in Table 2. Only APT004 out of the seven duck microsatellite loci with orthologs in the chicken genome had a similar core repeat. The others had different

core repeats or were absent from the orthologous loci in the chicken genome.

Values of 94.44% (Maak et al., 2003), 80.00% (Huang et al., 2005) and 77.89% (Huang et al., 2006) polymorphisms have been reported for duck-specific microsatellite markers tested in the duck genome. The higher (96.97%) polymorphism seen in this study could be a reflection of the test population genetic constitution, which was derived from a germplasm preservation population without artificial selection. Based on the PIC values, most polymorphic markers were highly or reasonably informative and only a few were slightly informative. Therefore, these markers will be very useful for mapping the duck genome. As the Chicken Genome Project moves toward functional genomics studies, the availability of the chicken genome sequence has proven to be an invaluable tool in studying the genomes of other avian species, including the duck. A good comparative genetic map based on the orthologous microsatellite markers will provide the substrates for major gene identification (Reed et al., 2005). After a similar BLAST search, 21.21% of the microsatellite loci were conserved between the duck and the chicken. This result was similar to a previous report (20.42%, Huang et al., 2006). In conclusion, these microsatellite markers will be useful in construction of a duck genetic linkage map. Comparative mapping with the chicken can provide a valuable tool for studies related to duck biodiversity and population genetics.

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