ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS IN TSAIYA DUCK

M. C. Hsiao[1], Y. C. Hsu[2], Y. H. Hu[1], S. H. Li[3], S. R. Lee[1], and S. C. Liu[1*]

[1] Ilan Branch, Livestock Research Institute, Council of Agriculture, Executive Yuan, Yilan 268 Taiwan, R.O.C.
[2] Institute of Natural Resources, National Dong Hwa University, Hualien 974, Taiwan, R.O.C.
[3] Department of Life Sciences, National Taiwan Normal University, Taipei 116, Taiwan, R.O.C.
***** Corresponding author, e-mail: scliu@mail.tlri.gov.tw

ABSTRACT

In this study, an enrichment library with GATA-repeats from genomic DNA was constructed to isolate and characterize microsatellite loci in Tsaiya duck (*Anas platyrhynchos*). Thirty-three microsatellites were used to detect polymorphisms in 30 Tsaiya ducks. A total of 177 alleles were observed and all loci except *DRC022* 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 makers 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 32 loci ranged from 0.09 to 0.83 with an average of 0.57. Seven of 33 duck microsatellite loci had the orthologs in the chicken genome, only *DRC004* had the similar core repeats with chicken. These microsatellite markers will be useful for constructing the genetic linkage map of the duck and a comparative mapping with the chicken, also can provide a valuable tool for studies related to biodiversity and parentage determination of the duck.

KEY WORDS: Microsatellite marker, Polymorphism, Tsaiya duck.

INTRODUCTION

Many important agricultural traits are quantitative traits controlled by polygene. The recent development of molecular genetics mapping tools enables the identification of quantitative trait loci (QTL) in the genome. Application of marker assisted selection for QTL has the potential to enhance the accuracy in animal breeding program, particularly for the 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 were 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 the populations of chicken, swine and cattle, a

large number of microsatellites have been isolated and widely used for these purposes described above. In contrast, less genetic markers have been established in the duck. Although the first genetic linkage map of the duck has been developed (Huang *et al*., 2006), only spans 1353.3 cM and with an average interval distance of 15.04 cM, more microsatellites are needed for constructing the completed genetic map of the duck. Thus, we attempted to isolate microsatellite markers for Tsaiya duck (*Anas platyrhynchos*) and to investigate their polymorphism.

MATERIALS AND METHODS

Collection and extraction of Tsaiya duck genomic DNA

Thirty individuals (15 males and 15 females) were selected from the germplasm preservation population of Tsaiya duck kept in I-Lan Branch since 1984. Genomic DNA was extracted from fresh blood by using the GenoMaker kit (Watson BioTech, Taiwan) following the manufacturer's instruction.

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, and then the fragments were ligated with SNX linkers (Hamilton *et al*.1999). The ligated products were amplified using PCR at 94℃ for 5 min, 30 cycles of 94℃ for 1 min, 55℃ for 1 min and 72℃ for 2 min, and followed by a final cycle at 72℃ 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 Strepta-vidin (Dynal, Norway) according to the manufacturer's protocol. Repeat-enriched DNA was made double-stranded and amplified with the same PCR conditions described above then cloned in the pGEM-T Easy Vector (Promega, USA). After transformed to 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 with the BigDye Terminator Kit on 3730xl DNA Analyzer (Applied Biosystems).

Genotyping

Sequences were aligned with SeqWeb Version 2.1 (Wisconsin Package). Primer Express software (Applied Biosystems) was used to design PCR primers. The primer pairs showen a expected PCR product were selected to screen for polymorphism. The forward primers of these primer pairs were labelled with FAM or HEX fluorescent dye. After initial incubation at 95℃ for 10 min, PCR amplification was performed for 30 cycles of denaturing at 95℃ for 20 sec, annealing at 50℃ or 55℃ for 30 sec and extension at 72℃ for 30 sec. This was followed by a final cycle at 72℃ for 1 hr. The PCR products were analyzed in MegaBACE 1000 autosequencer (Amersham Biosciences). The size of DNA fragments were investigated with the software Genetic Profiler Version 2.2 (Amersham Biosciences).

Statistics and similarity searching

The observed, expected heterozygosities and polymorphism information content (PIC) were calculated using the program CERVUS 2.0 (Marshall *et al*. 1998). The sequences were analyzed by using the BLAST program (NCBI) to identify the orthologous microsatellite DNA of the duck in the chicken genome. The unique match sequences with an E-value smaller than e-20 from the chicken were regarded 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 had 47 different loci from 75 sequences containing GATA repeats and 33 of these loci were chosen for further polymorphism test in 30 Tsaiya ducks. The characteristics of the 33 microsatellite loci summarized as table 1. A total of 177 alleles were observed and all loci except *DRC022* 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 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).

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

Locus	Repeat motif in clone	Fragment (bp)	No. of alleles	H_O[1]	H_E[2]	PIC[3]
DRC001	$(GATA)_{12}$	178-206	3	0.23	0.52	0.46
DRC002	$(GATA)_7GACA(GATA)_3$	129-145	5	0.7	0.66	0.61
DRC003	$(GATA)_{11}$	220-236	4	0.57	0.54	0.49
DRC004	$GATAGAT(GATA)_{15}$	294-322	9	0.8	0.86	0.83
DRC005	$(GATA)_{17}$	283-319	9	0.83	0.8	0.77
DRC006	$(GATA)_{12}$	318-342	6	0.57	0.64	0.59
DRC007	$(GATA)_{14}$	194-230	6	0.8	0.72	0.67
DRC008	$(GATA)_{12}$	184-208	7	0.87	0.79	0.75
DRC009	$(GATA)_2GAT(GATA)_{15}$	330-354	7	0.5	0.81	0.77
DRC010	$(GATA)_9GAT(GATA)_3$	192-215	6	0.7	0.63	0.56
DRC011	$(GATA)_8$	152-164	3	0.07	0.13	0.12
DRC012	$(GATA)_{16}$	185-205	6	0.87	0.73	0.67
DRC013	$(GATA)_{10}$	127-171	9	0.77	0.81	0.78
DRC014	$(GATA)_{11}$	317-325	3	0.1	0.16	0.15
DRC015	$(GATA)_{13}$	126-150	7	0.77	0.75	0.71
DRC016	$(GATA)_{10}$	112-120	3	0.53	0.54	0.45
DRC017	$(GGAT)_6(GATA)_{12}$	161-189	5	0.77	0.74	0.69
DRC018	$(GATA)_9(GAAA)_{14}(GA)_2(GAAA)_3(GA)_6(GAAA)_3$	267-295	8	0.2	0.86	0.83
DRC019	$(GATA)_{11}$	206-218	2	0.37	0.35	0.28
DRC020	$(GATA)_{14}$	177-205	8	0.93	0.83	0.79
DRC021	$(GATA)_{10}$	133-169	6	0.8	0.79	0.75
DRC022	$(GATA)_{12}$	120	1	0	0	0
DRC023	$(GATA)_4AATA(GATA)_7$	113-121	3	0.57	0.51	0.43
DRC024	$GATAGACA(GATA)_7$	102-118	4	0.5	0.51	0.44
DRC025	$(GATA)_{13}$	105-133	7	0.7	0.67	0.63
DRC026	$(GATA)_{10}$	138-142	2	0.6	0.51	0.38
DRC027	$(GATA)_{10}$	151-155	2	0.1	0.1	0.09
DRC028	$(GATA)_{10}$	131-187	7	0.83	0.82	0.78
DRC029	$(GATA)_{14}$	143-179	6	0.73	0.66	0.6
DRC030	$(GATA)_{13}$	190-226	8	0.67	0.54	0.52
DRC031	$(GATA)_{12}$	194-234	8	0.9	0.84	0.81
DRC032	$(GATA)(GACA)_2GACT(GAT$	207-259	4	0.7	0.67	0.6
DRC033	$(GATA)_{13}$	266-274	3	0.13	0.19	0.17

[1]Observed heterozygosity [2]Expected heterozygosity [3] Polymorphism information content

DRC004, *DRC005*, *DRC006*, *DRC012*, *DRC014*, *DRC016* and *DRC031* loci of the duck found the corresponding orthologs in the chicken genome after a similarity searching of BLAST. Only *DRC004* contained the similar core repeats, while the others had different core repeats or absent form 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. Higher (96.97%) polymorphism seen in this study could be a reflection of the genetic constitution of the test population, 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 can provide a high utility for mapping the duck genome. As the Chicken Genome Project moves toward to the functional genomics study, availability of the chicken genome sequence has already proved 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 similarity searching of BLAST, 21.21% microsatellite loci were conserved between the duck and the chicken, this result was similar to the previous report (20.42%, Huang *et al*., 2006). Overall, these microsatellite markers will be useful for constructing the genetic linkage map of the duck and a comparative mapping with the chicken, can also provide a valuable tool for studies related to biodiversity and parentage determination of the duck.

REFERENCE

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314–331.

Hamilton, M. B., E. L. Pincus, A. Di Fiore, and R. C. Fleischer. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques, 27:500-507.

Hsu, Y. C., L. L. Severinghaus, Y. S. Lin, and S. H. Li. 2003. Isolation and characterization of microsatellite DNA markers from the Lanyu scops owl (*Otus elegans botelensis*). Mol. Ecol. Notes 3:595-597.

Huang, Y. H., J. F. Tu, X. B. Cheng, B. Tang, X. X. Hu, Z. L. Liu, J. D. Feng, Y. K. Lou, L. Lin, K. Xu, Y. L. Zhao, and N. Li. 2005. Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds. Genet. Sel. Evol. 37:455–472

Huang, Y. H., Y. H. Zhao, C. S. Haley, S. P. Hu, J. P. Hao, C. X. Wu, and N. Li. 2006. A Genetic and Cytogenetic Map for the Duck (*Anas platyrhynchos*). Genetics 173:287-296.

Maak, S., K. Wimmers, S. Weigend, and K. Neumann. 2003. Isolation and characterization of 18 microsatellites in the Peking duck (*Anas platyrhynchos*) and their application in other waterfowl species. Mol. Ecol. Notes 3:224–227.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7:639-655.

Meuwissen, T. H. E. and M. E. Goddard. 1996. The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol. 28:161-176.

Reed, K. M., J. Holm, M. Morrisson, S. Leroux, and A. Vignal. 2005. Assignment of non-informative turkey genetic markers through comparative approaches. Cytogenet. Genome Res. 109:527–532.

Stallings, R. L., A. F. Ford, D. Nelson, D. C. Torney, C. E. Hildebrand, and R. K. Moyzis. 1991. Evolution and distribution of (GT)n repetitive sequences in mammalian genomes. Genomics 10:807-15.