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Cross-species amplification and characterization of microsatellite DNA loci from *Gallus gallus* in *Bambusicola thoracica*

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Abstract The Chinese Bamboo Partridge (*Bambusicola thoracica*) is a gamebird endemic to China. Ten polymorphic microsatellite (simple sequence repeat) markers were obtained through cross-species amplification for this partridge from its relative species *Gallus gallus*. The number of alleles per locus varied from 4 to 13. The observed heterozygosity ranged from 0.1220 to 1.0000 and the expected heterozygosity from 0.1183 to 0.8898. Four microsatellite loci showed significant differences from the Hardy-Weinberg equilibrium. These polymorphic loci provide a valuable tool for the investigation of the phylogeography and conservation genetics of this partridge.

Keywords *Bambusicola thoracica*, microsatellite loci, polymorphism

Introduction

Microsatellites are also known as simple sequence repeats (SSRs), and are a few nucleotide sequence repeats distributed randomly in eukaryotic genomes. SSR markers are important tools to assess genetic diversity in avian because of their high level of polymorphism and codominant Mendelian inheritance (e.g. Randi et al., 2003). The Chinese Bamboo Partridge (*Bambusicola thoracica*) is an endemic gamebird that used to be widely distributed in temperate and subtropical forests of central and southeast China (Cheng, 1978; Johnsgard, 1999). At present, many studies have been conducted on the reproduction, habitat, anatomy and taxonomy of this bird (Chang et al., 1998; Lei and Lu, 2006; Huang et al., 2008; Yao et al., 2008). However, there are no molecular markers, such as SSRs isolated and applied in this species to date in spite of many molecular markers that have been developed and used broadly in other gamebirds (He et al., 2009; Wang et al., 2009; Zhou and Zhang, 2009). The lack of sufficient and polymorphic SSR markers limits research in genetic diversity for conservation purpose of this species. Thus, screening for polymorphic microsatellite markers in the Chinese Bamboo Partridge is very important and necessary for analyzing genome organization and evolution and developing marker-assisted breeding technology. In this study, we obtained ten polymorphic microsatellite loci for this partridge from its relative species, *Gallus gallus*, through cross-species amplification.

Materials and methods

In order to characterize isolated microsatellite markers, twenty samples of Chinese Bamboo Partridge were collected from Jinggangshan (26°22′N, 114°05′E), Jiangxi Province in China during two consecutive hunting seasons (2007 and 2008). Liver/muscle samples were dissected from birds and stored in 100% ethanol immediately after removal. Genomic DNA was extracted from livers/muscles using the standard phenol/chloroform method.

The sibling taxa of Chinese Bamboo Partridge is

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Table 1 Characteristics of ten polymorphic microsatellite loci in *Bambusicola thoracica*

Locus ID	Primer sequences	Repeat motif	T_a (°C)	Observed allele size range (bp)	N_a	H_O	H_E	p
ADL268	F: CTCCACCCCTCTCAGAACTA R: CAACTTCCCATCTACCTACT	$(GT)_{12}$	60	90–120	5	0.6098	0.4788	0.0887
ADL136	F: TGTCAAGCCCATCGTATCAC R: CCACCTCCTTCTCCTGTTCA	$(TG)_{20}$	56	98–134	13	0.7105	0.8898	0.0000*
MCW0016	F: ATGGCGCAGAAGGCAAAGCGATAT R: TGGCTTCTGAAGCAGTTGCTATGG	$(TG)_{16}$	62	104–126	4	0.6000	0.4459	0.0942
MCW067	F: GCACTACTGTGTGCTGCAGTTT R: GAGATGTAGTTGCCACATTCCGAC	$(TA)_6 + (TG)_{11}$	56	162–188	11	0.5000	0.8060	0.0000*
MCW069	F: GCACTCGAGAAAACTTCCTGCG R: TTGCTTCAGCAAGCATGGGAGGA	$(CA)_{11}$	58	152–166	8	0.7073	0.7148	0.1089
MCW0111	F: GCTCCATGTGAAGTGGTTTA R: ATGTCCACTTGTCAATGATG	$(CA)_7$	57	80–102	9	1.0000	0.7338	0.0000*
MCW0216	F: GGGTTTTACAGGATGGGACG R: AGTTTCACTCCCAGGGCTCG	$(GT)_9$	60	142–154	4	0.1220	0.1183	1.0000
MCW222	F: GCAGTTACATTGAAATGATTCC R: TTCTCAAAACACCTAGAAGAC	$(GT)_8$	62	190–216	5	0.2439	0.2671	0.1000
MCW0295	F: ATCACTACAGAACACCCTCTC R: TATGTATGCACGCAGATATCC	$(AC)_{10} + (AT)_4 + (ATAC)_3$	60	86–144	4	0.5854	0.5086	0.6300
LEI0192	F: TGCCAGAGCTTCAGTCTGT R: GTCATTACTGTTATGTTTATTGC	$(TTTC)_{12}$	56	250–376	12	0.4000	0.6671	0.0000*

T_a, annealing temperature; N_a, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.
* means $p < 0.05$.

Gallus gallus (Kimball et al., 1999). Therefore we selected a subset ($n = 50$) of *G. gallus* microsatellite primers for PCR amplification. PCR was carried out in a 30 μL mixture containing 100 ng DNA, 0.25 μM of each primer, 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 1U Taq polymerase (all reagents were from Dingguo Bio., Beijing, China). Amplification conditions were as follows: 94°C for 4 min, then 94°C for 30 s, annealing temperature for 15 s (Table 1), 72°C for 20 s for 30 cycles, then 72°C for 10 min in a PE9600 thermocycler. The PCR products were separated on an ABI377 PRISM™ DNA sequencer (ABI). Fragment lengths were assigned using Gene Scan software 3.1 (ABI). Of the 30 primer sets screened, 13 exhibited polymorphism (Table 1).

For each polymorphic locus, we calculated the observed heterozygosity (H_O) and expected heterozygosity (H_E) using GENEPOP version 3.4 (Raymond and Rousset, 2000). GENEPOP was also used to test for evidence of linkage disequilibrium and deviation from the Hardy-Weinberg equilibrium.

Results

The number of alleles per locus was 4–13. The observed heterozygosity ranged from 0.1220 to 1.0000 and the expected heterozygosity from 0.1183 to 0.8898 (Table 1). The observed heterozygosity of all loci was consistent with that expected under the Hardy-Weinberg equilibrium after a Bonferroni correction ($p < 0.05$), except for ADL136, MCW067, LEI0166 and LEI0192. Fifteen pairs of loci showed significant linkage disequilibrium values at $p < 0.05$ among the 78 pair-wise tests. These markers are potentially useful for studies on phylogeography and conservation genetics of the Chinese Bamboo Partridge.

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家鸡微卫星引物在灰胸竹鸡中的跨种扩增和特征分析

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摘要：灰胸竹鸡（*Bambusicola thoracica*）是中国特有鸟类。利用近缘种家鸡（*Gallus gallus*）中已发表的微卫星DNA标记引物，对灰胸竹鸡进行跨种扩增，得到10个多态位点。每个位点的等位基因数为4–13个，观察杂合度为0.1220–1.000，期望杂合度为0.1183–0.8898。4对引物显著偏离Hardy-Weinberg平衡。这些引物将为研究灰胸竹鸡的系统地理学和保护遗传学提供非常有用的工具。

关键词：灰胸竹鸡，微卫星，多态性