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Characterization and cross-species amplification of microsatellite markers in African Silverbill (*Lonchura cantans*)

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ABSTRACT. We tested the cross-amplification of eight microsatellites developed for Bengalese finch in African Silverbill (*Lonchura cantans*). In order to develop resources for conservation genetic studies in the species *L. cantans*, we tested the amplification success and polymorphism in eight previously developed microsatellite loci, in *L. cantans*. All eight microsatellite markers were successfully amplified, of which all were polymorphic, with 3 to 9 alleles and an expected heterozygosity (H_E) ranging from 0.606 to 0.718. On average, there were 5.25 alleles/locus and a mean H_E of 0.6456. These eight polymorphic markers could be of potential use in studies of genetic variability, population structure, and reproductive strategy of African Silverbills. The markers tested should be useful for population and conservation genetic studies in this genus, and, in particular, for species closely related to the source species, *L. cantans*.

Key words: Cross-species amplification; Microsatellite markers; *Lonchura cantans*; African Silverbil

INTRODUCTION

Lonchura cantans, a small passerine bird (Family Estrildidae), is endemic to Africa and the Middle East (Clements et al., 2009). Along with this introduced finch, *Lonchura malabarica* (Indian Silverbill) is also found in the Middle East and Saudi Arabia (Clements et al., 2009). The African Silverbill presently has a conservation status of "least concerned" (IUCN, 2012). However, the species is continuously declining as a result of habitat loss, illegal trading, and other anthropogenic interferences (BirdLife International, 2013).These factors have led to the loss of genetic diversity in the extant population of African Silverbill. Therefore, studies on the genetic diversity in surviving populations are warranted. So far, no studies have been conducted to reveal the population genetic diversity of these munias.

Microsatellite DNA markers are simple sequence repeats (Tautz, 1989) distributed along the genome (Litt and Luty, 1989). By virtue of this feature, they have become powerful tools, used in forensic studies, kinship investigation, gene mapping, conservation biology, and population genetics (Jarne and Lagoda, 1996; Zane et al., 2002; Gibson et al., 2005). Despite their usefulness, the isolation and development of microsatellite markers are most time-consuming, labor-intensive, and expensive processes, and require a skilled molecular biologist. Thus, using loci already developed in a related species will provide a cost-effective alternative to microsatellite isolation, development, and characterization in the species of interest (Moore et al., 1991; Peakall et al., 1998).

Cross-species amplification is only effective if the primer sequences are conserved between species (Wilson et al., 2004). Generally, the number of amplifying loci tends to decrease with increasing divergence between species (Moore et al., 1991; Peakall et al., 1998). Besides this, the relationship between amplification success and the evolutionary divergence of source-target species has also been widely studied in birds (Primmer et al., 1996; Dawson et al., 2000; Galbusera et al., 2000). Several early studies have shown the applicability of microsatellite loci among closely related species by means of cross-species amplification (Moore et al., 1991; Primmer et al., 1996; Baratti et al., 2001; Huang et al., 2005; Zhou et al., 2009). In this paper, we report the cross-species amplification of eight polymorphic microsatellites characterized and developed from Bengalese finch in African Silverbill.

MATERIAL AND METHODS

Sample collection and DNA extraction

Feather samples of African Silverbill were collected from the Riyadh region of Saudi Arabia. Dead birds were collected from their natural habitat and samples were collected after proper identification. A total of 22 individuals were sampled. Genomic DNA was extracted from the feather sample using conventional DNA extraction method (Sambrook et al., 1989). DNA was then dissolved in TE buffer and stored at -20°C until further use.

PCR amplification and microsatellite genotyping

A total of eight microsatellite loci, developed for Bengalese finch by Yodogava et

al. (2003), were selected for the present study. Polymerase chain reactions (PCRs) were performed using an Eppendorf ep gradient S master cycler (Hamburg, Germany) in a 15-µL reaction mixture containing 2 µL 1 X PCR buffer (50 mM KCl, 10 mM Tris-HCl), 2 µL 25 mM $MgCl_2$, 1.6 µL 2.5 mM dNTPs (Applied Biosystems, Weiterstadt, Germany), 2 µL 10 X BSA, 5 pmol of each primer, 0.2 µL 5 U *Taq* DNA polymerase (AmpliTaq Gold, ABI), and 4 µL 10 ng/µL genomic DNA. PCRs for all primers pairs were performed using an initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 50 s, annealing at the temperature given in Table 1 for 50 s, and extension at 72°C for 55 s, and a final extension at 72°C for 10 min. The annealing temperature for each microsatellite locus was first tested as defined in the target species and then modified accordingly to get the optimum amplification.

Approximately 5µL PCR products was mixed with 1µL loading buffer and then loaded onto a 2.5% agarose gel along with the size standards, and visualized over UV light to detect amplification. Primers producing visible and expected bands were labeled with fluorescent dyes (FAM, HEX) at the 5 end. The PCR products were pooled and denatured at 95°C for 5 min and electrophoresed using the ABI 3730 Genetic Analyzer (Applied Biosystems) with LIZ500 as the internal lane size standard. Data collected were analyzed using the GeneMapper version 3.7 software (Applied Biosystems).

In order to confirm whether the product amplified by the cross-reactive markers was indeed the orthologous locus, the PCR products from two individuals for each locus were randomly selected for DNA sequencing. The PCR products were purified with the High Pure PCR Product Purification Kit (Roche Applied Science Mannheim, Germany) and the cycle sequence was performed using the non-labeled primer of the same primer pair used to amplify the locus. The purified products were sequenced in both directions on an Applied Biosystems 3730xl DNA analyzer. The sequences were compared with entries in the GenBank database using the BLASTN program (Altschul et al., 1990). The obtained sequences were deposited in GenBank.

Data analysis

The Micro-Checker version 2.2.3 program (Van Oosterhout et al., 2004) was used to test for null alleles, large allele dropout, and scoring errors due to stutter peaks. The observed and effective number of alleles, percentage of polymorphic loci observed, and expected heterozygosity estimates were computed according to Nei (1973), as executed in the Popgene software (Yeh et al., 1999). Using allelic frequencies, the polymorphic information content (PIC), a measure of a marker's informativeness, was calculated with Cervus3.0 (Kalinowski et al., 2007). Deviations from Hardy-Weinberg equilibrium (HWE) expectations and linkage disequilibrium were calculated using Arlequin version 3.5.1 (Schneider et al., 2000).

RESULTS AND DISCUSSION

A total of eight polymorphic microsatellite loci were amplified in African Silverbill by means of cross-species amplification, and all of them showed significant polymorphism and potential for evaluating genetic variation. All the 22 samples of African Silverbill were amplified, and a total number of 52 distinct alleles were scored over the eight microsatellite loci. However, most loci investigated here showed more number of alleles when compared with previous studies carried out on Bengalese finch (Yodogava et al., 2003).

The number of alleles detected at each locus ranged between 3 and 9, with an overall mean number of 5.25 alleles per locus. The expected heterozygosity ranged from 0.606 to 0.718. The observed heterozygosity ranged between 0.591and 0.975. All the microsatellite loci showed PIC values higher than 0.5, which is normally considered as informative in population genetic analyses (Botstein et al., 1980). The mean PIC in our samples was 0.5685. The mean observed heterozygosity over the eight loci was 0.752, which was more than the expected heterozygosity of 0.6456.

Out of eight loci, three (viz., BF03, BF05, and BF10) deviated significantly from HWE (Table 1). Deviations of these three loci from HWE could be due to several reasons, such as Wahlund effect, inbreeding due to consanguineous mating, and assortative mating (Hedrick, 2005). All the data were evaluated using Micro-Checker 2.2 for the presence of null alleles, large allele dropout, and scoring errors associated with peak stuttering. We found that none of these factors was a cause for the existing Hardy-Weinberg disequilibrium.

Table 1. Characterization of nine polymorphic microsatellite loci in African Silver bill.

Locus name (GenBank accession No.)	Primer sequence	T_A(°C)	Size	Dye (forward primer)	Repeat motif	N_A	H_O	H_E	PIC	HWE (P)
BF02 JX069753	F: 5'-GCCTAAAGAGTATCCCATGA-3' R: 5'-AAATCTCCCACAACCCCCT-3'	58	336	FAM	$(CAAA)_8$	5	0.773	0.613	0.518	0.1965^{ns}
BF03 JX069752	F: 5'-GGCTTAGCAGACAGCTTTGG-3' R: 5'-GGAACAAGCAGCCAGCAC-3'	60	191	HEX	$(CA)_{10}AA(CA)_{20}$	3	0.773	0.606	0.505	<0.05^{ns}
BF04 JX069751	F: 5'-CAAGGTAAATAACTCTCATGTAC-3' R: 5'-TCCCATTATATTTGCACCTCTTG-3'	54	169	FAM	$(CA)_9AA(CA)_3$ $AA(CA)_5$	6	0.682	0.655	0.591	0.8041^{ns}
BF05 JX069750	F: 5'-CTCTTCCCTAGTGCTTTGTC-3' R: 5'-GCAATAAACAACCCCTCTCC-3'	54	158	FAM	$(GT)_5A_5(GT)_6$	4	0.818	0.610	0.610	<0.05^{ns}
BF08 JX069749	F: 5'-CGATTTGGATGGTTATTTGC-3' R: 5'-TTTTTTTACTGACCACTGTTC-3'	58	133	HEX	$(CA)_3AA(CA)_2$ $GA(CA)_4$	5	0.591	0.661	0.598	0.6562^{ns}
BF10 JX069748	F: 5'-CCTGTGAAGTTGTACCTG-3' R: 5'-AGAAGTGACATGGGCTAC-3'	52	133	FAM	$(GTTT)_6$	3	0.975	0.601	0.503	<0.005***
BF17 JX069747	F: 5'-TGGGCAGGACTCTCAAGCCA-3' R: 5'-CCTCTGCCTGCCTGTCTTGT-3'	59	139	FAM	$(CA)_{10}$	9	0.682	0.701	0.643	0.6562^{ns}
BF18 JX069746	F: 5'-GGTGGTGCGTGGTGAGAGTA-3' R: 5'-TCACCCCGGATTCTAGCACG-3'	59	98	HEX	$(GT)_2GA(GT)_6$ $GC(GT)_9$	7	0.727	0.718	0.675	0.9150^{ns}

T_A = annealing temperature, size range of alleles (base pairs), N_A = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphism information content and probability of deviation from Hardy-Weinberg proportions [HWE (P)] are reported. Each locus was genotyped at a minimum of 22 African Silverbills (range 20-22), NS = not significant with Bonferroni correction. ***Significant with Bonferroni correction.

Genotype biases were evaluated with Micro-Checker (Van Oosterhout et al., 2004), and null alleles were not found with any of the eight loci used. The genotype data revealed a reasonable amount of polymorphism (Table 1). Similarly, no linkage disequilibrium was observed between the loci when corrected for multiple tests (Bonferroni correction $\alpha = 0.05/8$).

The present study is the first exhaustive attempt to test and to ensure the applicability of eight Bengalese finch microsatellite loci in African Silverbill. These loci could be employed for further studies on ecological traits such as kin selection and mating systems in African Silverbill, and the recommendations could be used for better conservation of this species. Increased sampling may also provide insight into the existing genetic diversity in the population and for inbreeding level estimates as well. The applicability of these microsatellites would allow the evaluation of genetic characteristics of other *Lonchura* species in order to understand different aspects of their conservation in relation to dispersal and colonization, genetic aspects of mating, and breeding performance (Aparicio et al., 2007).

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