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PRIMER NOTE

Characterization of microsatellite loci in the Jamaican fruit-eating bat *Artibeus jamaicensis* and cross-species amplification

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Abstract

***Artibeus jamaicensis* is one of the most common bat species in the neotropics, with a well-defined polygynous social structure in caves. In order to study behaviour and to examine patterns of paternity and relatedness between different harem groups, we developed 14 microsatellite loci from two different enriched genomic libraries. We screened 125 individuals from two different bat colonies and found that polymorphism ranged from five to 13 alleles. Heterozygosity ranged from 63 to 95%. The primers amplified across 14 bat species, indicating their potential utility for population-level studies in several closely related bat species.**

Keywords: *Artibeus jamaicensis*, cross-species amplification, heterozygosity, microsatellite, Phyllostomidae

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The Jamaican fruit-eating bat (*Artibeus jamaicensis*) is an abundant and widely spread species in the neotropics, living in a wide variety of roosting sites, such as caves, hollow trees, buildings, etc. A complex social organization based on a polygynous mating system was described for the species in the caves of Yucatán (Ortega & Arita 1999). Harem group size varies from four to 18 females with one adult male in the smaller and medium sized groups and two males in the largest groups (> 14 females). Associated males defend females from foreign males and potentially sire the broods in the groups (Ortega & Arita 2000). In large groups, the second associated male invests little energy in defending the harems and obtains no obvious immediate benefit. We developed microsatellite loci to examine paternity in the harem groups and to assess reproductive output of both associated males. Harems are highly cohesive and potentially comprised of females of the same family. Microsatellites will be used to determine relatedness among members of any particular group, including relationships between females and associated males.

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Wing tissue samples were collected from *A. jamaicensis* and immediately stored in 70% ethanol. Genomic DNA was isolated from the samples using the Dneasy®tissue kit (QIAGEN®). DNA was digested with *Nhe*I, *Rsa*I, *Hae*III and *Xmn*I (New England Biolabs). Following the standard protocol of Hamilton *et al*. (1999), microsatellite loci were isolated from two genomic libraries enriched for di- and trinucleotide repeat motifs. Enriched genomic libraries were cloned into *Xba*I-digested P-bluescript SK+ (Stratagene), and the resultant plasmids were transformed into *Escherichia coli* Supercompetent Cells (Stratagene). Colonies were lifted onto 3 MM nitrocellulose paper (Whatman) following the protocol of Sambrook *et al*. (1989). After probing, filters were washed to exclude unbound DNA as follows: 15 min at room temperature in 2× SSC [0.1% sodium dodecyl sulphate (SDS)]; 15 min at 45 °C in 2× SSC (0.1% SDS); 15 min at 65 °C in 1× SSC (0.1% SDS); and 15 min at 65 °C in 1× SSC (0.1% SDS); and later exposed to X-ray film (Kodak BioMax). To screen and detect positive colonies, the Photo Star Detection protocol (New England Biolabs) was used in paper filters. Positive colonies were picked and heated for 10 min at 100 °C in 200 μL TE [10 mM Tris–HCl, 0.1 mM ethylenediaminetetraacetic acid (pH 8.0)].

Table 1 Primer sequences and characteristics of the 14 microsatellite loci developed for *Artibeus jamaicensis*

Locus	Repeat motif	Primer sequence 5′–3′	No. of alleles	Allele size range (bp)	H_E	H_O	GenBank accession number
AjB464	$(CT)_{27}(CA)_{14}$	F:**TET**-CACCAGCCAGGGCTTATTTA R:AGCCCCAAGAATTTCTTCG	13	230–272	0.83	0.63	AY099072
AjA123	$(CA)_1A(CA)_{17}CT(CA)_2$	F:**TET**-GACCACTTTTCCTCCCATGAC R:CCTGAGCTAATACTCCAGAGGAAG	10	242–272	0.90	0.73	AY099073
AjA40	$(GT)_{13}N_6(CT)_3$	F:**TET**-GATGTGAATGGTGTTTTTAGAGCTT R:CTCTACAGTGGACCCACATCATT	9	190–220	0.92	0.87	AY099074
AjA47	$(CA)_2CT(CA)_{18}$	F:**TET**-CATTGTGTAGCACAAAGTAAGTGTG R:CATGGTGGAAAAAGAATGGACT	8	168–194	0.90	0.91	AY099075
AjA151	$(CA)_3N_9(GT)_{17}$	F:**TET**-GGGTGGAAAGGGAGAGAAAA R:GAAGCTCTTCCCTGACCACTTA	10	158–192	0.92	0.95	AY099076
AjA74	$(GT)_7(GA)_2TT(GT)_2N_9(GT)_4N_4(GT)_4N_5(GT)_2N_6(GT)_4N_8(GT)_4$	F:**HEX**-GGCAAAGGCTTTTACAAGTATG R:GCAGTGGAGGAGAAAGCTAGAC	7	150–166	0.85	0.89	AY099077
AjA185	$(CA)_{14}$	F:**HEX**-CAATAAGAAATGGTGCAGGA R:CTCAGTGTCTAGCACAGTGGTT	10	102–136	0.73	0.72	AY099078
AjA180	$(GT)_{21}N_2(GT)_4AT(GT)_8$	F:**HEX**-CACTGGCTGCGCACATATCAT R:CTGCGAGGCGGTTATCCATT	7	128–152	0.78	0.75	AY099079
AjA2	$(CA)_6N_{14}(CA)_5GT(CA)_4N_{10}(CA)_3N_4(CA)_7$	F:**HEX**-CTAGACCTCCAGGACTGTAGCAC R:TGGGCAAAGGCTTTTACAG	5	122–134	0.77	0.87	AY099080
AjA84	$(CA)_3TA(CA)_6N_3(CA)_5N_9(CA)_9$	F:**HEX**-ACTGTGACTGGGAGTAAACTTCTGT R:TCCTGCACTCAGGACACTTC	11	112–150	0.92	0.88	AY099081
AjA80	$(CA)_4A(CA)_{10}$	F:**FAM**-ATGTGCTCAATCCACTGAACTAGA R:ATCCACTGACAGATGAATGGATAAA	5	120–136	0.83	0.79	AY099082
AjA199	$(CA)_4GA(CA)_1N_6(CA)_4TA(CA)_{12}$	F:**FAM**-CCGTGGTGTGCGAGGGCA R:TGTTTTTCTGAATGCCTCTG	6	90–108	0.78	0.79	AY099083
AjA107	$(CA)_{12}$	F:**FAM**-CGGTTATCCATTGGAGTTGG R:CGCACAAACATTCTGCGTAA	6	120–136	0.84	0.82	AY099084
AjA110	$(CA)_{13}TA(CA)_5$	F:**FAM**-CTCCCCCTACTCCTCACACA R:CATGGTGGAAAATGAATGGA	7	94–114	0.89	0.86	AY099085

H_E, expected heterozygosity; H_O, observed heterozygosity.

Polymerase chain reactions (PCR) were performed using a PTC-100 Programmable Thermal Cycler (MJ Research Inc.). Amplifications contained the following in a total volume of 25 μL: 50–100 ng of DNA, double-distilled H_2O, 0.1 U of Ampli*Taq*®DNA polymerase (Applied Biosystems), 10 μM of both primers, 25 mM of $MgCl_2$, 10× PCR Buffer II (Applied Biosystems), and 2 mM of dNTPs. Products were run in 2% ethidium bromide agarose gels. DNA from agarose gels was purified and extracted using the QIAquick Gel kit protocol (QIAGEN®). A total of 400 clones in the size range of 70–300 bp were sequenced using ABI BigDye ready reaction kit (Applied Biosystems), and compared using SEQUENCHER® (Gene Codes Corp.).

All clone sequences that contained one or more microsatellite loci were selected. Flanking primer pairs were designed using the program PRIMER3® (Rozen & Skaletsky 1996). PCR amplifications, 25 μL volume, contained the above components, but some dNTPs were substituted by 2 mM FdUTPs (R110; Applied Biosystems). Tests for primer pair amplification and polymorphism were performed in 10 individuals from two different localities of Yucatán, México (Ortega & Arita 1999). Fourteen primer pairs that showed positive results were selected, and their forward primer was labelled with fluorescents phosphoramidites (6-FAM, TET, or HEX; Operon Technologies Inc.). Primer pair optimization cycling program consisted of: 5 min at 96 °C, 30 cycles of 96 °C for 45 s, 56 °C for 45 s (annealing temperature), 72 °C for 45 s, followed by a final step of 72 °C for 5 min. We added 5 M Betaine (Sigma) and 10 μg/L bovine serum albumin to enhance the PCR reaction.

A total of 125 individuals were genotyped at all 14 loci (Table 1). Alleles were separated on a 5% polyacrylamide gel using an ABI 377 DNA Sequencer and evaluated using the GENESCAN™ 3.1 software. Expected heterozygosity (H_E) and deviations from Hardy–Weinberg equilibrium were calculated using GENEPOP® (Raymond & Rousset 2000). The mean observed heterozygosity (H_O = 0.818) was not significantly different from the mean expected heterozygosity (H_E = 0.847). Most loci were in Hardy–Weinberg equilibrium in both of the study areas with the exception of two loci, AjB464 and AjA123, which showed low observed

Table 2 Cross-species amplification test in 17 bat species using the primers developed for *Artibeus jamaicensis*

Species	AjB464	AjA123	AjA40	AjA47	AjA151	AjA74	AjA185	AjA180	AjA2	AjA84	AjA80	AjA199	AjA107	AjA110
Epomophorus gambianus (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Saccopteryx bilineata (2)	0	M	0	0	0	0	0	0	0	M	0	1	0	0
Nycteris thebaica (2)	1	0	0	0	0	1	0	0	0	0	1	0	0	M
Rhinolopus darlingi (2)	0	0	1	0	0	0	M	0	0	1	0	M	0	0
Mormoops megalophylla (2)	1	M	0	M	0	0	0	0	1	0	0	0	0	0
Pteronotus parnellii (3)	0	P (2)	M	0	0	1	0	0	1	1	0	0	0	0
Phyllostomus hastatus (4)	M	0	M	P (2)	0	P (4)	P (2)	P (3)	0	P	M (3)	0	P	0 (2)
Glossophaga soricina (1)	0	0	0	0	1	1	1	1	1	0	0	1	1	1
Leptonycteris nivalis (2)	0	P (4)	M	0	P (4)	0	P (2)	M	M	M	M	0	0	M
Carollia perspicilliata (1)	M	M	0	0	M	P (2)	0	0	M	M	M	M	0	M
Uroderma bilobatum (1)	M	P (2)	M	P (2)	P (2)	M	M	M	M	M	0	P (2)	P (2)	M
Lasiurus borealis (1)	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Myotis adversus (2)	0	0	0	0	0	P (2)	0	0	0	0	0	P (2)	0	0
Myotis sodalis (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nycticeinops schlieffeni (2)	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Nycticeius humeralis (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaerephon pumilus (2)	0	0	1	0	0	0	M	0	0	0	0	0	0	0

P, polymorphic (no. of alleles); M, monomorphic; 0, unsuccessful cross-species amplification; and 1, successful cross-species amplification but polymorphism not demonstrated.

heterozygosities relative to expected ones. Finally, all 14 loci showed moderate to high polymorphism, and ranged from five to 13 alleles per locus.

All polymorphic loci were tested on 17 other bat species from North and South America, and Africa (Table 2). Similar PCR conditions were used to test cross-species amplification. These primers had null utility in three species but were more useful in the rest. Members of the family Phyllostomidae showed higher cross-species amplification than any other taxonomic group. These microsatellites can therefore become an important molecular tool for future behavioural and ecological studies within the genus *Artibeus*, and potentially in population studies in other bat species.

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