Polymorphic microsatellites for the lesser bulldog bat (*Noctilio albiventris*) cross-amplify with close and distant relatives

Dina K. N. Dechmann · Lena John · Tanja Noventa · Christian C. Voigt

Abstract We used the enriched genomic library to isolate 16 polymorphic microsatellite markers from the lesser bulldog bat (*Noctilio albiventris*). We analysed DNA from 226 individuals from two neighbouring populations to assess genetic polymorphism at these loci. Polymorphism varied from two to ten alleles per locus, with expected heterozygosity ranging from 0.309 to 0.766 (with the exception of one for which no *P*-value was calculated). Two loci, including the one mentioned above, departed from Hardy–Weinberg equilibrium and linkage disequilibrium was detected between one locus with two other loci after Bonferroni correction. All loci cross-amplified successfully with a member of at least one other bat family, several of them polymorphically. These loci will be used to study relatedness and parentage in a study on the social system of *Noctilio albiventris*.

Keywords Microsatellite loci · Population structure · Relatedness · Social system

The lesser bulldog bat, *Noctilio albiventris*, commonly occurs from Northern Honduras all the way down to Paraguay and the East coast of Brazil (Hood and Pitocchelli 1983). It naturally roosts in tree cavities, but also commonly inhabits buildings (Reid 1997). It forages in groups for swarming insects over open water bodies (Dechmann et al. published online; Hood and Pitocchelli 1983) As a basis for a project studying the reasons for sociality in bats, we developed and tested microsatellite primers for this species (primer development by Genetic Identification Services, Chatsworth, California USA).

Methods for DNA library construction, enrichment and screening were as described previously (Jones et al. 2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (Rsa I, Hae III, Bsr B1, Pvu II, Stu I, Sca I, Eco RV). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules.

Libraries were prepared in parallel using Biotin-AAC(12), Biotin-ATG(12), Biotin-CATC(8) and Biotin-TAGA(8) as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with HindIII to remove the adapters. The resulting fragments were ligated into the HindIII site of pUC19. Recombinant molecules were electroporated into *E. coli* DH5alpha. Recombinant clones were selected at random for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI 377, using ABI Prism Taq dye terminator cycle sequencing methodology.

The optimal amplification reaction mix for all primer pairs consisted of 1× BiolaseC Buffer (from 10× stock solution supplied by manufacturer), 2 mM $MgCl_2$, 0.2 mM each dNTP's, 6 M each primer (forward primer fluorescent-labeled), 0.025 U/l BiolaseC Taq polymerase, and 0.2 ng/l template DNA in 50 µl final reaction volume. Samples were amplified in a Perkin-Elmer-Cetus thermal cycler by an initial three minutes of denaturation at 94°C,

D. K. N. Dechmann · L. John · T. Noventa · C. C. Voigt
Leibniz Institute for Zoo and Wildlife Research,
Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

Present Address:
D. K. N. Dechmann (✉)
Department of Ornithology, University of Konstanz,
78457 Constance, Germany
e-mail: ddechmann@orn.mpg.de

Table 1 Primer pairs for 16 microsatellite loci derived from *Noctilio albiventris*. Locus size and repeat motif are derived from the original clone sequenced. Number of alleles are those detected in 226 samples from Barro Colorado Island and Gamboa, Panama. H_E Expected heterozygosity, H_O Observed heterozygosity

Locus	Primersequence (5′–3′)	Annealing temp. (°C)	Repeat motif	Locus size (bp)	Number of alleles detected	H_O	H_E	*P*-value	GenBank Accession No.
NA A103	GGC-ACT-ATC-AGA-GAT-GTC-CC GGA-AGG-AAG-CCA-GGA-TTC	56.7	$(AAC)_7$	177	3	0.450	0.432	NS	GQ183812
NA A108	CAG-GGA-ACA-TAG-CAG-CTC-TAC CTT-TCT-TTC-ATT-TGG-GAT-CTG	55.3	$(AAC)_7$	122	4	0.296	0.309	NS	GQ183813
NA B1	TGT-GGA-ATG-CCC-AGT-AAT-G GTC-ACG-GTT-GCC-AGA-AAG	58	$(CAT)_{12}$	292	4	0.542	0.543	NS	GQ183814
NA B4	GCC-CAC-TGT-GCT-ATC-TTT-C ACC-AGG-CTA-TTC-ACT-CAG-C	56.8	$(TGA)_{13}$	220	5	0.695	0.703	NS	GQ183815
NA B108	ATC-CCT-TTC-GCT-CTG-ATT-AG TGG-CTC-ATA-AGT-GGT-CAC-AT	57.4	$(CAT)_9$	269	4	0.609	0.662	NS	GQ183816
NA C2	AAA-ACC-TTT-CCA-CCT-TTG-AGT ATG-CGA-AGT-AAA-TGT-GTG-ATG	57.5	$(ATTC)_{11}$	281	4	0.486	0.565	NS	GQ183817
NA C8	GGT-GAG-GGC-AGG-TTA-GTC TGA-GCA-GAT-AGA-GAT-GGA-CAG	56.8	$(CATC)_7$	224	4	0.511	0.497	NS	GQ183818
NA C101	ATC-CAC-CCA-TCC-ATC-TGT-TC GCC-CAA-CCT-ACT-TGC-TCA-AC	56.6	$(TCCA)_{11}$	133	4	0.463	0.473	NS	GQ183819
NA C104	TTA-GGC-ATC-ACT-CTC-ACT-TGG CTC-CAG-CCA-CAG-GAC-TTT-AC	57.3	$(CATC)_6$	208	3	0.441	0.479	NS	GQ183820
NA C114	ACG-TGG-AAT-AAC-TGG-TTC-CC GAT-GGA-TGG-ATG-GAT-GAG-TG	58.1	$(CATC)_7$	268	2	0.013	0.013	–	GQ183821
NA D8	GGT-GAT-TCC-TGA-ATT-GAC-TG CCC-TTA-GGT-AAG-AAA-ACT-ACC-C	56.3	$(GATA)_2CATA(GATA)_6$	183	2	0.502	0.498	NS	GQ183822
NA D9	GCA-TAA-AAT-GAG-GCA-GTC-AG TCA-CCC-AGG-AAA-TAC-AAG-G	57.0	$(TCTA)_7TC(TCTA)_{13}$	225	6	0.811	0.736	NS	GQ183823
NA D103	GAA-AAG-AGC-GGA-GGA-AGA-G GGA-GAA-CAG-ACT-AAA-GGT-GGA-C	56.3	$(TATC)_{11}$	157	6	0.763	0.739	NS	GQ183824
NA D107	CGC-TTG-GGA-GAT-TAC-ATA-CAT-C TGC-AGC-AGT-AAC-CAC-ATT-TAT-G	58.2	$(GATA)_{12}$	283	10	0.647	0.763	**	GQ183825
NA D116	CCT-CAA-GGC-TAG-GTC-TCT-GAG CCA-AGG-GTC-CTT-ATG-ACT-TTG	56.5	$(GATA)_{12}$	146	6	0.765	0.766	NS	GQ183826
NA D118	TGT-GGT-GTA-GAA-ATG-CTA-CTT-G ATG-GGC-ATC-TGA-AGG-TAT-TT	56.9	$(ATCT)_{11}$	234	4	0.590	0.591	NS	GQ183827

Table 2 Cross-species amplification tests with eight species from five families, using the primers developed for *Noctilio albiventris minor* from Panama

Species (*n*)	Family	NA A103	NA A108	NA B1	NA B4	NA B108	NA C2	NA C8	NA C101	NA C104	NA C114	NA D8	NA D9	NA D103	NA D107	NA D116	NA D118
Saccopteryx bilineata (3)	Emballonuridae	1	2	1	2	0	0	0	3	2	0	1	1	3	0	0	2
Molossus molossus (3)	Molossidae	1	1	2	2	1	0	2	4	2	2	2	1	2	0	0	0
Pteronotus parnellii (5)	Mormoopidae	1	2	1	2	0	1	3	2	2	1	1	2	2	0	2	2
Noctilio albiventris affinis (1)	Noctilionidae	1	0	2	2	1	0	1	0	1	0	2	2	0	1	2	1
Noctilio leporinus (6)	Noctilionidae	2	1	2	2	2	2	1	3	1	1	2	5	6	1	1	4
Carollia perspicillata (8)	Phyllostomidae	1	2	1	1	1	1	1	4	2	4	3	2	5	1	0	0
Vampyressa pusilla (1)	Phyllostomidae	2	1	0	1	0	0	0	1	2	1	1	1	2	0	0	0

Number of alleles given (0, unsuccessful cross-species amplification; 1, successful cross-species amplification, but no polymorphism found among the analysed individuals; 2–6, polymorphic, number of alleles given)

followed by 35 cycles of denaturation (94°C, 40 s), annealing (55°C, 40 seconds), and extension (72°C, 30 s), with final extension time of 4 min at 72°C.

DNA was extracted using the PureGene DNA Extraction KitR kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Microsatellite loci were amplified in 10 µl reactions in the following reaction mix: $MgCl_2$, 2 mM; dNTPs (premixed), 0.2 mM each; primers, 0.3 µM each; BioTaq DNA PolymeraseR (Bioline USA, Canton, MA, USA), 0.025 U/µl; template DNA, 0.2 ng/µl. PCR was conducted in a RoboCycler Gradient 96R thermocycler (Stratagene, Inc., La Jolla, CA, USA) by an initial denaturation (94°C, 3 min), followed by 35 cycles of denaturation (94°C, 40 s), annealing (55°C, 40 s), and extension (72°C, 30 s), and a final extension at 72°C for 4 min. PCR products were labeled using one of the conventional sequencing dyes NED, HEX or FAM (Applied Biosystems, Inc.). Amplification products were separated on polyacrylamide gels in an ABI 377 DNA sequencer and sized using Genotyper 2.5 software and Rox 400 HD size markers (Applied Biosystems, Inc., Foster City, CA USA).

To test our microsatellite primers for applicability, 226 individual lesser bulldog bats, *Noctilio albiventris*, were captured in the village Gamboa (N 09.07°; W 079.41°) in Panama, and at surrounding water bodies. An additional capture site was Barro Colorado Island (BCI, N 09.10°, W 079.51°). Tissue samples were taken from the wing membrane with a sterile, 3 mm diameter biopsy punch (Stiefel; Germany; Worthington-Wilmer and Baratt 1996) and stored in 99% alcohol until extraction. Extracted samples from additional species (*Saccopteryx bilineata* (family Emballonuridae), *Molossus molossus* (Molossidae) *Pteronotus parnellii* (Mormoopidae), *Noctilio leporinus* (Noctilionidae) *Carollia perspicillata*, and *Vampyressa pusilla* (both Phyllostomidae), were already available from previous projects, and were used for crossamplification. Finally, one sample of the subspecies *Noctilio albiventris affinis* from Tiputini Biological Station Ecuador was also included in the analysis. DNA was extracted in the laboratory with the help of the QIAamp DNA Mini Kit™ and stored in purified water.

All amplifications were performed using one fluorescently labeled forward primer for each locus (6-Fam, locus NA A108, NA B1, NA C8, NA C104, NA D8, NA D107, NA D118; Hex, NA A103, NA B4, NA B108, NA C2, NA C101, C114, NA D9, NA D103, NA D116; Biotez, Berlin, Germany). PCRs were carried out in 10 µl reaction volumes containing 1 µl of undiluted DNA, 5 µl of Qiagen Multiplex mix (Qiagen Europe, Hilden, Germany), and 0.25 µl of each primer filling up the volume to 10 µl with RNase free water (Qiagen). Reactions were cycled using a Primus Gradient (MWG AG Biotech) or a 2720 Thermal Cycler (Applied Biosystems) and the following thermotreatment: 15 min at 94°C, followed by 30 cycles of 30 s at 94°C, 90 s at 58°C (adjusting the temperatures was not necessary when using the Multiplex mix), and 90 s at 72°C. All thermotreatments were concluded with a 30 min extension at 60°C. After amplification, 0.8 µl of 1:4 diluted PCR product were electrophoresed in 10 µl formamide (Hi-Di™ Formamide, Applied Biosystems) and 0.5 µl rox standard (GeneScan™-500ROX™ Size Standard, Applied Biosystems) using a ABI 3130xl Genetic Analyzer (Applied Biosystems) with a 36 cm 16-capillary Array (47 cm × 50 µm). Running conditions were as follows: Injection time, 5 s, injection voltage 1.2 KV, run time 880 min, run voltage 15 KV. Fragment analysis was completed using GeneMapper v. 3.7 (Applied Biosystems).

Expected and observed counts for heterozygotes, deviations from Hardy–Weinberg equilibrium, and linkage disequilibrium were calculated using Cervus 3.0.3 (Marshall et al. 1998) and Genepop 4.0, option 2 (Raymond and Rousset 1995).

We found between two and ten alleles per primer among our animal sample. All but NA D107 were in Hardy–Weinberg equilibrium (Table 1). For another primer, NA C114, which has only two alleles H_e was far below 0.5 and Hardy–Weinberg was not calculated. However as this primer was polymorphic in two other species (*C. perspicillata*, 4 alleles: *M. molossus*, 2 alleles; Table 2), it is nonetheless listed in the tables here, but was not used for analysis with *N. albiventris*. In fact, all of the microsatellites developed for *N. albiventris* cross-amplified with other species, some of them polymorphically (Table 2). We detected no linkage disequilibrium after Bonferroni except between NA C2 and NA C104 and between NA C2 and NA D116. Thus, the primer sets presented here do not offer a basis for the analysis of parentage and population structure in *N. albiventris*, but also potentially for the genetic study of other species and even families of bats.

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