Conservation Genet Resour
DOI 10.1007/s12686-013-0125-0

MICROSATELLITE LETTERS

Spotted bat (*Euderma maculatum*) microsatellite discovery using illumina sequencing

Faith M. Walker · Jeffrey T. Foster · Kevin P. Drees · Carol L. Chambers

Received: 3 December 2013 / Accepted: 25 December 2013

Abstract The spotted bat (*Euderma maculatum*) is a rarely-encountered species for which behavior and population attributes are largely unknown. Using next-generation sequencing, we identified and characterized 17 microsatellite loci, which were screened for 31 individuals from northern Arizona. Allelic diversity, observed heterozygosity, and power of discrimination were high (N_A: 5–8 alleles per locus; H_O: 0.55–0.90; P_{ID}: 1.2×10^{-15}). All loci were in HWE, there was no evidence of null alleles or linkage disequilibrium, and five loci amplified and were variable in another Vespertillionid (*Eptesicus fuscus*). We will use these loci to evaluate gene flow and genetic diversity across the range of the spotted bat and determine population size in northern Arizona. The latter information is important to resource managers, who attempt to set mortality thresholds for bats at wind energy facilities in this region.

Keywords *Euderma maculatum* · Spotted bat · Next-generation sequencing · Microsatellite

The spotted bat (*Euderma maculatum*) is a charismatic species patchily distributed across western North America. Because spotted bats are cryptic (nocturnal, volant, solitary), much of their natural history and population biology is unknown; *E. maculatum* has been designated a species of concern in Canada and the United States, in part because of this lack of information. Using high-throughput sequencing, we generated a suite of microsatellite markers to elucidate aspects of *E. maculatum* biology.

Genomic DNA was extracted from an *E. maculatum* wing punch with a DNEasy Blood and Tissue Kit (Qiagen, Hilden, DEU). DNA was fragmented with a SonicMan sonicator (Brooks Life Science Systems, Spokane, WA). Whole genome sequencing libraries were prepared and quantified with qPCR using KAPA reagents (KAPA Biosystems, Woburn, MA). Fragments 500 bp long were selected with Agencourt AMPure magnetic beads (Beckman Coulter, Brea, CA). Sequencing was performed on an illumina MiSeq with v2 reagents, yielding 13 million paired 250 bp reads. We used ABySS 1.3.2 (Simpson et al. 2009) for de novo genome assembly. Microsatellites 4–6 bp in length with at least six repeats were discovered in this assembly and primers for the loci were designed with msatcommander-1.0.8-beta (Faircloth 2008).

We tested 56 primer pairs using the universal tail PCR labeling system of U'Ren et al. (2007). DNA from heart and kidney tissue samples of five individuals on loan from museums (Museum of Southwestern Biology, University of New Mexico: MSB 121373, 135536, and 22756; New Mexico Museum of Natural History and Science: catalog numbers 1901 and 4059) was used to assess optimal annealing temperature (Ta) and locus polymorphism. PCR amplifications were performed with MJ Research PTC-200 thermocyclers in 12 μL reactions containing 1× PCR Rxn Buffer (Invitrogen), 2 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTPs, 0.08 U/μL Platinum Taq DNA polymerase (Invitrogen), 0.02 μg/μL Ultrapure non-acetylated Bovine Serum Albumin (Ambion), 0.1 μM forward primer, 0.2 μM forward universal primer, 0.2 μM reverse primer, H_2O,

F. M. Walker (✉) · C. L. Chambers
School of Forestry, Northern Arizona University, 200 East Pine Knoll Dr., Flagstaff, AZ 86011, USA
e-mail: Faith.Walker@nau.edu

F. M. Walker · J. T. Foster · K. P. Drees
Center for Microbial Genetics and Genomics, Northern Arizona University, Bldg. 56, 3rd floor, 1298 S Knoles Dr., Flagstaff, AZ 86011-4073, USA

Published online: 04 January 2014

Table 1 Characterization of 17 polymorphic microsatellite loci for spotted bats

Locus	Genbank accession #	Primer sequence (5′–3′)	Repeat motif	Fluorescent tag	Ta (°C)	Size range (bp)[a]	N_A	H_O	H_E	*P* (HWE)[b]
EUMA2	KF922719	F: TGGGAGACAAAGGTGGAAGG R: GTTCACCCATTTGTCCGTCC	GGAT	6FAM	54	157–165	6	0.742	0.726	0.773
EUMA5	KF922720	F: CCCTGACTAATGCAATGCCC R: AAACCCAGGACCCTTGAGTC	CCTT	VIC	61	231–251	6	0.871	0.729	0.460
EUMA6	KF922721	F: CGAACTCAAGGCCAAACTCC R: ATTTGGCCTTCCCTTTGCAG	AGAT	6FAM	54	138–162	8	0.710	0.725	0.476
EUMA8	KF922722	F: CATGCATGGGTGGAAGGAAG R: AGCCTGGCCTTCTATGGATG	GGAT	PET	54	127–147	5	0.420	0.411	0.016
EUMA12	KF922723	F: AAGTGGTCAGAACTGGAGGG R: ACTGAGGCTTCTTCCGTGTC	AAGG	PET	54	159–191	8	0.710	0.631	0.193
EUMA18	KF922724	F: ACAAGTGTGAGTGCTGGGAC R: GGAGGTGAAGGGACAGATGG	AATG	6FAM	61	174–211	6	0.839	0.698	0.322
EUMA19	KF922725	F: TTGCAGAGCCTTGATGACAG R: GGTTGAACAGTTGGACGGTC	ATCT	NED	54	224–240	5	0.613	0.721	0.045
EUMA29	KF922726	F: GATTTCAGACTTGCCAGCCC R:CACACACACACCCTCTTATTGG	AGAT	VIC	54	216–236	6	0.742	0.777	0.707
EUMA32	KF922727	F: TGGGTTATGGTTTGCTGCTTC R: GCTGGATCCCACAATAGAGC	ATTT	PET	61	200–228	8	0.581	0.601	0.835
EUMA36	KF922728	F: ATGCTTCAGTGCCAGGTAGC R: GGGAGTATAGGAGGCAGCC	ATTT	PET	61	195–215	6	0.548	0.684	0.250
EUMA37	KF922729	F: TCATTCTGCTCCCTTCCCTG R: ACAGATGAGGCTAAATGACCC	AAAC	VIC	61	205–229	5	0.871	0.679	0.106
EUMA38	KF922730	F: GAAAGGCAGCACGTACAGG R: GAGGTCTATGGTGTGCAACTG	CTTT	6FAM	61	229–257	7	0.903	0.796	0.680
EUMA39	KF922731	F: GGCCTCTCCTTCATATTCAGG R: GTTGCTCCCTTGTTTCCTGC	AAGG	NED	61	202–238	8	0.800	0.754	0.887
EUMA40	KF922732	F: GCGGACTTCCCTTTATAGCTC R: TGTTCTCCCATGTCTTCCTCC	AGAT	PET	54	221–237	5	0.645	0.694	0.369
EUMA43	KF922733	F: TCTTCCCTGCTCTTGGATGC R:ACACAGATGGCAAACAATCAC	ATCT	NED	61	224–240	5	0.857	0.692	0.133
EUMA47	KF922734	F: TGAGAGTTGGATTCCTGGCC R:TCAGCTTAATCTTCACCTGAGG	ATCT	NED	61	197–213	5	0.742	0.735	0.596
EUMA55	KF922735	F:CCAGAGAAACAGAACCAACAAG R: TCCCAGTATAACAGCTGACCC	ATCT	NED	54	172–188	5	0.774	0.760	0.047

[a] Product size range includes the fluorescent dye tag (U'Ren et al. 2007)

[b] No loci were out of HWE after correction for multiple tests ($P < 0.003$)

and 2 μL DNA template (at 2 nm/μL). Thermal cycling conditions were as follows: 2 min denaturation at 94 °C; 35 cycles of 94 °C for 30 s, optimized Ta (47–63 °C) for 30 s, and 72 °C for 1 min; 72 °C extension for 2 min. Fragment analysis was performed on an Applied Biosystems 3130 Genetic Analyzer and results were visualized with GeneMapper 4.0 software.

Seventeen (36 %) loci amplified at either 54 or 61 °C annealing temperature, were polymorphic, and easily scored (Table 1). To assess genetic diversity and locus behavior, we genotyped DNA from 31 spotted bats captured in northern Arizona at all loci. We also genotyped 20 big brown bats (*Eptesicus fuscus*) from Flagstaff, Arizona, in order to test cross-species utility. GENEPOP V4.2 (http://genepop.curtin.edu.au/) was used to examine expected (H_E) and observed (H_O) heterozygosity, departures at each locus from Hardy–Weinberg equilibrium, and linkage disequilibrium between each locus pair. Micro-Checker (Van Oosterhout et al. 2004) was employed to evaluate presence of null alleles (1,000 randomizations). We calculated P_{ID} for an indication of the power of discrimination of the final panel of loci (Waits et al. 2001).

All loci adhered to Hardy–Weinberg expectations after correction for multiple tests, and exhibited no evidence of linkage disequilibrium or null alleles. Only 1.2×10^{-9} in a million pairs of spotted bats selected at random from the population would be expected to share a multilocus genotype, and 0.72 in a million pairs of full sibs would be expected to do so. Five loci (EUMA18, 29, 39, 43, and 55) were polymorphic and easily-scored in big brown bats.

The new loci will enable examination of genetic population structure, genetic diversity, and population size in a rarely-encountered species (e.g., 79 individuals in museum collections). The loci will be employed to address broad-scale questions (e.g., spotted bat movements between regions) and fine-scale questions (e.g., population size estimation and relatedness within a rarely-observed aggregation).

Acknowledgments Funding was provided by the State of Arizona Technology and Research Initiative Fund and the National Park Service. Research was facilitated by the Bureau of Land Management, Kaibab National Forest, and Navajo Nation.

References

Faircloth B (2008) Msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Res 8:92–94

Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I (2009) ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123

U'Ren J, Schupp J, Pearson T, Hornstra H, Clark Friedman C, Smith K, Leadem Daugherty R, Rhoton S, Leadem B, Georgia S, Cardon M, Huynh L, Deschazer D, Harvey SP, Robison R, Gal D, Mayo M, Wagner D, Currie B, Keim P (2007) Tandem repeat regions within the *Burkholderia pseudomallei* genome and their application for high resolution genotyping. BMC Microbiol 7:23–43

Van Oosterhout C, Hutchinson WF, Wills DMP, Shipley P (2004) Micro- checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249–256