

PERMANENT GENETIC RESOURCES NOTE

Isolation and characterization of polymorphic microsatellite loci in muskrat, *Ondatra zibethicus*

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Abstract

We describe the isolation and characterization of 12 highly polymorphic microsatellite loci for the muskrat, *Ondatra zibethicus*. Microsatellite markers from three other rodent species were cross-amplified in muskrat and one of them was polymorphic. We observed moderate to high levels of genetic variability in these 13 polymorphic loci (five to 22 alleles per locus) with observed heterozygosity ranging from 0.48 to 0.96. These markers will be useful for further studies on population genetic structure in muskrat and potentially in other rodent species.

Keywords: cross-species amplification, microsatellite, muskrat, *Ondatra zibethicus*, rodent

Received 25 August 2008; revision accepted 4 November 2008

Anthropogenic disturbances and natural events can result in habitat fragmentation leading to heterogeneous landscapes, and therefore, to the creation of barriers to dispersal. The study of population genetic structure in heterogeneous landscapes is necessary in order to understand how such landscape characteristics affect dispersal and gene flow (Manel *et al.* 2003). Microsatellite markers have been shown to be useful genetic markers for studying population structure because they are relatively abundant, codominant and they have high mutation rates (Lowe *et al.* 2004). In order to assess the effect of heterogeneous landscapes on gene flow, we are using the muskrat, *Ondatra zibethicus*, as a model. The muskrat is a widespread mammal in North America that uses the hydrogeographical network as well as terrestrial corridors for dispersal. The study of a semi-aquatic species allows consideration of both terrestrial and aquatic features of complex landscapes in the context of population structure and gene flow. Furthermore, the muskrats' wide geographical range will allow us to investigate the effects of fragmentation on gene flow at macrogeographical and microgeographical scales. Despite its wide geographical range, the muskrat has not been extensively studied and very few population studies based on molecular analyses have been conducted (Marinelli *et al.* 1997; Zachos *et al.* 2007). Moreover, microsatellite markers

have not been reported as of yet for the muskrat. To address these questions, we have developed polymorphic microsatellite markers for *O. zibethicus*. Here we characterize 12 polymorphic microsatellite markers and test their potential for amplification in five other rodent species (*Microtus pennsylvanicus*, *Clethrionomys gapperi*, *Peromyscus maniculatus*, *Napaeozapus insignis* and *Tamiasciurus hudsonicus*) in order to evaluate their usefulness for further studies on population structure. We also cross-amplified microsatellite markers developed for *M. pennsylvanicus* (Spritzer *et al.* 2005), *Clethrionomys rufocanus bedfordiae* (Ishibashi *et al.* 1995) and *P. maniculatus* (Mullen *et al.* 2006) for their use in muskrat and report the successful amplification of one polymorphic microsatellite locus.

Microsatellites developed in this study were cloned from an enriched genomic library created using the protocol described in Hamilton *et al.* (1999). Of 48 clones sequenced, seven resulted in poor or unreadable sequences and eight revealed the repeat region was too close to one end of the clone to allow for the placement of a polymerase chain reaction (PCR) primer. Thirty-five primer pairs were designed from the remaining 33 clones using Primer 3 (version 0.3.0; Rozen & Skaletsky 2000).

Muskrats were collected from trappers across Ontario, Canada, during the 2005–2006 trapping season. Genomic DNA was extracted from muscle tissue using QIAGEN DNeasy procedure. PCR was performed in a 10- μ L reaction volume containing 2 μ L (> 10 ng) genomic DNA, 10 \times PCR

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Table 1 Characterization of 12 microsatellite loci for the muskrat (*Ondatra zibethicus*) and successful amplification of one microsatellite locus from the grey red-backed vole (*Clethrionomys rufocanus bedfordiae*)

Locus	GenBank Accession no.	Primer sequence (5'-3')	Repeat motif	T_a (°C)	Size range (bp)	A	H_E	H_O
Oz06	EU999728	F: GGACAACAGAGAGGGAAGGA R: CTCATATTGTAAGAAGCCTGCTG*	(AC) ₂₀	65	135–181	15	0.93	0.93
Oz08	EU487259	F: CCTATGGGACTGACGGCTAA* R: AGTTTGGGACTCTGCCTTGA	(CA) ₅ AACACA(TC) ₅ TG(TC) ₇ (AC) ₈	63	231–251	8	0.82	0.79
Oz16	EU999729	F: TGACTGCACTGTCCACACA R: AAGCATCTCTGCTGGGTCAT†	(CA) ₂₂	67	289–335	18	0.93	0.96
Oz17	EU487260	F: GCAAGGCACCTAAGTGTGTG R: TTGGGCTTCACTGGGTAGC†	(GT) ₁₈ ... (AG) ₂₂	63	174–228	22	0.96	0.89*
Oz22	EU487262	F: GTCTGTCTCGCTCTCTCT† R: CGTCCCCAACCTGTACTA	(CT) ₂₃ (CA) ₁₂	63	207–241	15	0.90	0.48*
Oz27	EU999730	F: GCTGAAATGAACTGGCTAA R: TCTGAACTGGTGTGGGATTG*	(AC) ₁₈	64	197–217	10	0.89	0.82
Oz30	EU487263	F: GCTTCGGTGACAATGGAAAT R: TTCGTGGCTGAATACCCAGT*	(GA) ₃₁ AACA(GA) ₁₂	63	212–254	19	0.95	0.74
Oz32	EU999731	F: TTGACTTTTTCCAACATTCAGAG† R: TTGCAATTCTGTGGCTAGGA	(GA) ₁₈ TA(GA) ₁₂	65	172–206	13	0.91	0.96
Oz34	EU999732	F: ACCTTCCATTCTTAAATAGC* R: GATTCCTCTCTTTCATCTCAT	(GT) ₅ AT(GT) ₅ ... (GA) ₇ ... (GT) ₁₃	60	216–232	9	0.83	0.79
Oz41	EU487264	F: ATGACATTGACCCAGGGAAG* R: GACGGTGCAGATTTGGTTCT	(CA) ₄₀	63	180–248	21	0.95	0.83
Oz43	EU487265	F: AGAAGGGAGTGACACCTGAT R: CTAGCCCCCATAGGCATGTA	(TG) ₁₆	64	230–278	15	0.91	0.83
Oz44	EU999733	F: TCCAGAGAGGTTACCGAAATG* R: CCCTTCAGGACCAGTGTCTAT	(CA) ₁₉	63	215–257	11	0.68	0.50
MSCRB5§	—	F: GGTGGTGTTTGCATTTAGG† R: CTCCTGGTAATTTTCATCTTACC	CA-, ATAC-, ATGT-	48	186–202	5	0.68	0.50

Number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O) based on 30 individuals. Significant deviation from Hardy–Weinberg equilibrium (*) are indicated. Primers labelled with: *6-FAM; †HEX. §Ishibashi *et al.* (1995).

buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM of each forward and reverse primers (labelled with HEX or 6-FAM; Table 1), and 0.5 U *Taq* DNA polymerase. PCR amplification was performed using a Mastercycler gradient (Eppendorf). PCR profiles consisted of 2-min initial denaturation at 94 °C, followed by 30 cycles of 1-min denaturation at 94 °C, 30 s at primer-specific annealing temperature (Table 1), 30-s extension at 72 °C, and a final extension at 72 °C for 2 min except for locus Oz17 and Oz32 for which the final extension was 45 min. Amplification products were verified on a 1% agarose gel.

Nineteen of the 33 microsatellite loci amplified successfully and polymorphism was assessed by genotyping 10 individuals per locus on a 3730 DNA Analyser (Applied Biosystems) using a GeneScan 600 Liz Size Standard (Applied Biosystems) at MOBIXLab. Results were analysed using GeneMapper software version 4.0 (Applied Biosystems). Among these 19 loci, 12 loci were polymorphic and genetic variability at each locus was determined by genotyping 30 individuals. Moderate to high levels of

genetic variability were observed with a number of alleles per locus ranging from eight to 22 and observed heterozygosity (H_O) ranging from 0.48 to 0.96 (Table 1).

The 12 polymorphic loci developed for *O. zibethicus* showed some level of consistent cross-amplification in five other rodent species (Table 2). The PCR protocols followed those used for muskrat with the exception of the annealing temperature, which is indicated in Table 2. The success of these cross-specific amplifications was verified using gel electrophoresis; however, the amplified products were not genotyped on a sequencer. These loci may have some potential for further studies on population structure in these species with the exclusion of *T. hudsonicus*.

Eight markers from other rodent species were also tested on *O. zibethicus* including four loci developed for *M. pennsylvanicus* (AV13, AV14, AV15 and MSMM2; Spritzer *et al.* 2005), two loci developed for *C. rufocanus* (MSCRB2 and MSCRB5; Ishibashi *et al.* 1995) that have been successfully used for *C. gapperi* in other studies (Mech & Hallett 2001; Reese *et al.* 2001), and two loci developed for *P. maniculatus*

Table 2 Cross-species amplification of 12 microsatellite loci developed for the muskrat, based on five individuals per species. Annealing temperature (°C) is shown in parentheses

	<i>Microtus pennsylvanicus</i>	<i>Clethrionomys gapperi</i>	<i>Peromyscus maniculatus</i>	<i>Napaeozapus insignis</i>	<i>Tamiasciurus hudsonicus</i>
Oz06	–	–	+ (61)	–	–
Oz08	–	–	–	–	–
Oz16	–	+ (59)	+ (60)	+ (58)	–
Oz17	–	+ (64)	–	–	–
Oz22	–	–	+ (56.5)	+ (59)	–
Oz27	+ (60)	+ (64)	+ (55)	–	–
Oz30	+ (62.5)	–	–	–	–
Oz32	–	+ (64)	–	–	–
Oz34	–	–	–	–	–
Oz41	+ (63)	+ (63)	+ (60)	–	–
Oz43	+ (62)	+ (61)	–	–	–
Oz44	+ (61)	–	–	–	–

–, no amplification or inconsistent product; +, amplification product.

(Bw4-28 and Bw4-249, Mullen *et al.* 2006). Three of these eight loci showed amplification in *O. zibethicus*; one was polymorphic (MSCRB5; Table 1), one was monomorphic (MSCRB2; $T_a = 54$ °C) and one exhibited inconsistent amplification and was therefore removed from further analysis (AV13; $T_a = 59$ °C).

For the 13 polymorphic loci amplifying in *O. zibethicus*, we tested for departure from Hardy–Weinberg equilibrium (HWE) using the software GenePop version 4.0.7 (Rousset 2008). Two loci exhibited significant departure from HWE (Oz17 and Oz22) even after sequential Bonferroni correction ($P = 0.0035$ and $P < 0.0001$ respectively). By using only 10 individuals from one region (Sudbury), only one locus (Oz22) deviated from HWE after sequential Bonferroni correction ($P < 0.0001$). The deviation from HWE may be explained by the presence of null alleles. The presence of null alleles was tested using Micro-Checker version 2.2.3 (van Oosterhout *et al.* 2004) with a confidence interval of 99% and 5000 randomizations. One of the loci showing deviation from HWE (Oz22) showed significant excess of homozygotes ($P < 0.001$) which could indicate the presence of null alleles (frequency of null alleles $r = 0.296$). Linkage disequilibrium was tested on all loci using FSTAT version 2.9.3.2 (Goudet 2002) and results suggested that loci Oz08 and Oz16 as well as loci Oz27 and Oz32 displayed significant linkage disequilibrium after Bonferroni correction ($P = 0.00064$ for both pairs). These new microsatellite loci will be useful to estimate population genetic structure in *O. zibethicus* and may potentially be useful for population studies in other rodent species.

Acknowledgements

We thank the trappers from the trapping councils of Sudbury, North Bay and Midhurst that donated carcasses. We also thank L. DeSousa and G. Kataeva for their technical help and comments, C.

Andrews for her help in the laboratory, and E. Gauthier for the use of equipment. Funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant and Canadian Foundation for Innovation grant (CFI) to AIS-H, and an Alberta Ingenuity and NSERC Discovery Grant to DWC.

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