

## PRIMER NOTE

# Development and characterization of microsatellite markers in the Point Arena mountain beaver *Aplodontia rufa nigra*

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## Abstract

The Point Arena mountain beaver (*Aplodontia rufa nigra*) is an endangered subspecies. Efforts to recover this sub-species will be aided by advances in molecular genetics, specifically the ability to estimate population size using noninvasive genetic sampling. Here we report on the development of nine polymorphic loci for the Point Arena mountain beaver. These markers provide us sufficient power to distinguish individuals, and thus can be used for calculating population size and delineating population substructure.

*Keywords:* *Aplodontia rufa nigra*, microsatellite, mountain beaver, noninvasive genetic sampling

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The mountain beaver (*Aplodontia rufa*) is the sole genus and species within the family Aplodontidae, and represents the oldest known group of living rodents (Nowak 1991). One of seven recognized subspecies, the Point Arena mountain beaver (*Aplodontia rufa nigra*) was listed as endangered under the US Endangered Species Act in 1991 (50 FR 64716). *A. r. nigra* is isolated from other subspecies of mountain beaver, and occupies a small range (approximately 60 km<sup>2</sup>) in Mendocino County in Northern California (Steele & Litman 1998). Its endangered status is due to its small population size, limited distribution and effects of livestock grazing, urban development, and human activities (Steele & Litman 1998).

Currently, information is lacking for this subspecies with respect to breeding season, population density, activity patterns, population structure, recruitment rates, dispersal distances, barriers to dispersal, and movements. Our attempts to determine home range size were unsuccessful using traditional radio-telemetric methods despite considerable effort. Marked individuals were rarely located outside their den locations, either because they spend very little time elsewhere or because they returned to their dens quickly when they detected the presence of the observer. More problematic, however, was that few of the 16 radio-marked individuals survived for more than 3

months after radio collars were attached. Therefore, rather than risking additional mortality, collars were removed and other means for collecting information such as non-invasive DNA methods were considered.

Noninvasive genetic sampling to monitor populations of elusive or rare species has become commonplace (Taberlet *et al.* 1997; Flagstad *et al.* 2004; Schwartz *et al.* 2004; Zielinski *et al.* in press). However, there are currently no microsatellites available for mountain beaver and the monotypic nature of the species reduces the likelihood of success using markers developed for other rodents. For use with noninvasive samples, we also wanted markers with enough variability to distinguish individuals. Here we report on the first microsatellite markers developed for mountain beaver. These markers were developed in the Point Arena mountain beaver, and are a key first step in the ability to address the research needs for this subspecies.

Genomic DNA was extracted using the DNeasy tissue kit (QIAGEN) and partially restricted with a cocktail of seven blunt-end cutting enzymes (*RsaI*, *HaeIII*, *BsrB1*, *PvuII*, *StuI*, *ScaI*, *EcoRV*). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG Inc.), using biotinylated capture molecules.

Libraries were prepared in parallel using biotin-AAC(12), biotin-CAG(10), 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

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**Table 1** Primer sequences and features of nine microsatellite loci developed for *Aplodontia rufa nigra*. All loci conform to Hardy–Weinberg proportions

Locus	Accession no.	Repeat motif	Primer (5'–3') F: forward; R: reverse	Size (bp)	$T_a$ (°C)	$H_O$	$H_E$	No. of alleles
AruA1	DQ288272	(CA) <sub>15</sub>	F: CATGATTCATTGGTCCATTCT R: GGGAAACTGTTAATCCTTGCT	126–142	53	0.61	0.72	5
AruA12	DQ288273	(CA) <sub>21</sub>	F: TGCATCACAGTGAATTCA R: CCTTTCTTCCACTCATTTTAG	84–102	53	0.48	0.63	4
AruA104	DQ288274	(CA) <sub>12</sub>	F: GGACCTAAGTCCAAAGACTGGT R: CTGCCTCCATATTCAACATAGC	86–92	53	0.34	0.42	2
AruA114	DQ288275	(CA) <sub>5</sub> CT(CA) <sub>19</sub>	F: AGAAGAGCAACACCTGTCTC R: GTGAGCAGAAGTTCAGAGAATC	148–154	56	0.46	0.46	3
AruB8	DQ288276	(GA) <sub>16</sub>	F: ACTGGAGAAAGTGGGTGCTAT R: ATGTGGCAGAGGAAGTTACTC	133–141	60	0.54	0.63	3
AruB12	DQ288277	(GA) <sub>16</sub>	F: CAGGGGAAGAGAGAGAGG R: CCCTTTTCACCTTGGTACAA	89–97	56	0.40	0.51	2
AruC3	DQ288278	(AAAG) <sub>9</sub>	F: CTCTCTGACTGACCCCTCTCTC R: GGAAGGGAGACCATTGTGAC	111–127	58	0.66	0.70	4
AruC6	DQ288279	(AAAG) <sub>6</sub>	F: TGGGGATTTAACCTCAGTG R: CAGGTGTGGCCTAAAGTGT	77–85	56	0.53	0.66	3
AruD101	DQ288280	(TAGA) <sub>10</sub>	F: GACTCCCTCCCTCTCTCTC R: TCCTCCCTCTCACTCTGTC	91–95	60	0.09	0.08	2

$T_a$ , annealing temperature.

were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *Escherichia coli* DH5 $\alpha$ . 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. Forty-five of 81 positive clones contained microsatellite sequences, and 24 sets of primers were designed using Designer PCR version 1.03 (Research Genetics).

Primer optimization reaction mix for all primer pairs consisted of 1 $\times$  reaction buffer (Applied Biosystems), 2 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP's, 1  $\mu$ M reverse primer, 1  $\mu$ M dye-labelled forward primer, 1 U *Taq* polymerase (Applied Biosystems), and ~20 ng genomic DNA in 10  $\mu$ L final reaction volume. Samples were amplified in a PTC200 DNA Engine (MJ Research) thermal cycler by an initial 5 min of denaturation at 94 °C, followed by 35 cycles of denaturation (94 °C, 40 s), annealing (X °C, 40 s; see Table 1 for annealing temperatures), and extension (72 °C, 30 s), with final extension time of 4 min at 72 °C. Amplification products were separated on 6.5% polyacrylamide gels and visualized using a Li-Cor DNA Analyser 4300 (Li-Cor Biotechnology).

Of the 24 sets of primers designed, nine loci have currently been optimized that provide easily scorable and repeatable patterns. The characteristics of each of the microsatellite markers are shown in Table 1. Microsatellites included two dinucleotide repeats (CA and GA), and two

tetranucleotide repeats (AAAG and TAGA). An estimate of the variability at each locus was determined by scoring 35 individual tissue samples from unique individuals. All nine loci were polymorphic with 28 alleles discovered across the nine loci; observed heterozygosity ( $H_O$ ) ranged from 0.09 to 0.66 (Table 1). All loci conform to Hardy–Weinberg proportions as tested in program GENEPOP (Raymond & Rousset 1995). Because of their variable nature, these markers will be useful for population genetic analyses of the Point Arena mountain beaver as the probability of identity is  $9.16 \times 10^{-6}$  and the probability of identity given siblings is  $4.86 \times 10^{-3}$  (Evet & Weir 1998). All primers were specifically designed to produce small amplification products (< 160 bp) to be more likely to successfully amplify noninvasively collected hair samples in the future.

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