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Development of 10 Polymorphic Microsatellite Loci Isolated From The Mountain Beaver, *Aplodontia rufa rufa* (Rafinesque)

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PERMANENT GENETIC RESOURCES

Development of 10 polymorphic microsatellite loci isolated from the mountain beaver, *Aplodontia rufa rufa* (Rafinesque)

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Abstract

We developed 10 microsatellite markers for the mountain beaver, *Aplodontia rufa rufa*. In three populations of *A. r. rufa*, the number of alleles for these loci ranged from monomorphic to nine. Average observed heterozygosities in these populations ranged from 0.29 to 0.60. We also tested previously published markers from the endangered subspecies *A. r. nigra* in *A. r. rufa* populations.

Keywords: *Aplodontia rufa*, microsatellite, mountain beaver

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Aplodontia rufa rufa is a species from a monotypic genus, which is endemic to the Pacific Coast of North America. *A. r. rufa* populations are found in California, Nevada, Oregon, Washington, and in British Columbia, Canada. There are seven subspecies (Hall 1981); one, *A. r. nigra*, is considered endangered under the US Endangered Species Act. There are significant concerns over the status of other populations as well. However, in some portions of *A. rufa*'s range, it is managed as a pest species where it causes significant economic damage to forestry interests (Campbell & Evans 1988). Studies of *A. r. rufa* populations are critical for understanding their status and for informing management practices, whether it is a region where there are conservation concerns or intensive management of this species. Molecular genetics techniques are particularly useful for elucidating population demographics. For such studies, we have developed and characterized 10 microsatellite markers from the *A. r. rufa* genome.

Tissue samples of *A. r. rufa* were obtained from 30 Washington individuals and from 18 individuals from British Columbia. A single population was sampled in Washington and two populations were sampled in British Columbia: Chilliwack ($n = 8$) and Sumas ($n = 10$). One individual from Washington was used for enrichment and development of a microsatellite library with an adapted method from Glenn & Schable (2005). Genomic DNA was digested with *Rsa*I. Fragments were ligated using double-stranded SNX-24 linkers and the resulting library was hybridized to 12 biotinylated microsatellite oligonucleotide probes with dinucleotide and trinucleotide repeats (e.g. CT, CA, TC, AAG, and GGA). Hybridized fragments were captured on streptavidin-coated Dynabeads (Dynal Biotech). These fragments were then amplified and cloned with the TOPO TA cloning kit (Invitrogen). Insert sequences from 96 colonies were obtained with M13 forward and reverse primers and run on an ABI 3730xl genetic analyser (Applied Biosystems). Fifty-eight clone sequences had recognizable microsatellite sequences, of which 64% (37) had adequate flanking regions to design primers. Staden Package (Staden *et al*. 1998), TROLL (Castelo *et al*. 2002; Martins *et al*. 2006), and Primer3 (Rozen & Skaletsky 2000) software packages were employed to detect repeat regions and design primers within flanking regions. Parameters for the Staden Package were set according to step-by-step instructions provided on the website for TROLL software. Thirty-seven primer pairs were designed and tested; 10 pairs amplified and were variable. To save time and reagent costs, these markers have been optimized to run in three multiplex panels. Nine microsatellite markers have previously been generated for the endangered subspecies *A. r. nigra* (Pilgrim *et al*. 2006). We multiplexed and amplified these markers in the Washington population to characterize them in this more widely distributed subspecies.

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Polymerase chain reactions (PCRs) for all markers, including those from Pilgrim *et al*. (2006) were performed with 5′-end fluorescent label primers for visualization on an ABI 3130. PCRs were carried out using 0.25–0.90 µL of 1 µm primer, 1.0 µL of ABI Ampli*Taq* 10× Buffer, and 1.0 µL of 10 mm dNTP (Table S1, Supporting Information). Markers from panels A, B, and C were run individually on a LI-COR 4200S with M13-tailed primers for visualization. These PCRs were performed in 10 µL reaction volumes consisting of 1× *Taq* buffer (10 mm Tris, 1.5 mm $MgCl_2$, 50 mm KCl, pH 8.3; Roche Mannheim), 0.8 mm dNTP, 0.50–0.60 µL of 1 µm primer, 0.2–0.6 µm M13 IRD-labelled primer (LI-COR Inc.), 1 U *Taq* DNA polymerase (Roche), and 1 µL of 25 ng DNA template (Table S1, Supporting Information). Details for thermal profiles for all loci are provided (Table S1, Supporting Information), but generally, each consisted of an initial denaturation at 94 °C for 5–10 min, followed by 40–45 cycles of 94 °C for 30 or 45 s, 52–64 °C annealing for 45 s, and a 72 °C extension for 45 s or 1 min. Cycling was followed with a 30- or 45-min extension at 60 or 65 °C for ABI genotyping. For LI-COR, the PCR cycles were followed by a final extension step of 4 min at 72 °C or 30 min at 60 °C. Visualized products were analysed with GeneMapper software (Applied Biosystems) or RFLPscan (LI-COR Inc.).

Each locus was tested for evidence of null alleles with Micro-Checker (Van Oosterhout *et al*. 2004). Tests for genotypic disequilibrium between pairs of loci were performed using FSTAT 2.9.3 (Goudet 2001). Hardy–Weinberg equilibrium (HWE) tests and number of alleles were obtained from Arlequin (Excoffier *et al*. 2005). There was evidence of null alleles in only two loci in the Washington population at moderate rates (Table 1). There was no linkage disequilibrium between loci. Locus AruB8 (Pilgrim *et al*.

Table 1 Characteristics of the 10 microsatellite loci that were developed and optimized for *Aplodontia rufa rufa*

Multiplex: loci	Primer sequence (5′–3′) F, forward; R, reverse	Repeat motif	Size range (bp)	PCR primer quantity using 1 µm primer (µL)	N_A Total	H_O	H_E
Panel A:							
ArE04F_H05R	F: **NED**-CTGAGTGCTGGACTGAAGGT	$(AC)_{19}$	WA:267–279	0.65	WA:7	WA:0.77	WA:0.79
	R: AGCCCTCCTTGTTCAATTTT		C:254–260	0.50	C:4	C:0.38	C:0.66
			S:252–258	0.50	S:4	S:0.60	S:0.74
ArE12F_C06R	F: **HEX**-GAGGCTATTTCAGAGTGCCA	$(TC)_6(TC)_{11}$	WA:253–259	0.40	WA:4	WA:0.47	WA:0.71*
	R: TTTTGAGTTCCTGTGGCTTC		C:247–259	0.50	C:2	C:0.13	C:0.13
			S:247–259	0.50	S:2	S:0.29	S:0.26
ArB07F_E04R	F: **FAM**-CTGTAGCAGAAAGGGCATGT	$(CT)_{13}$	WA:372–380	0.35	WA:5	WA:0.67	WA:0.66
	R: TTTTAGGGCTGTAAACCATTTT		C:362–370	0.60	C:3	C:0.63	C:0.69
			S:364–370	0.60	S:3	S:0.10	S:0.19
ArG05F_H06R	F: **FAM**-CAGTGTTTCTCAACAACTTTCTTT	$(AC)_{23}$	WA:155–185	0.80	WA:9	WA:0.83	WA:0.88
	R: ATAAGCACTGAGCCACCACT		C:162–174	0.60	C:5	C:1.00	C:0.80
			S:162–172	0.60	S:4	S:0.60	S:0.78
Panel B:							
ArA08F_A08R	F: **FAM**-GCCTGACACCAGCATAGATT	$(CA)_{16}$	WA:252–258	0.40	WA:4	WA:0.57	WA:0.59
	R: GACTGCAAGCAAGTTTGGAT		C:248	0.50	C:1	C:--	C:--
			S:246–254	0.50	S:3	S:0.22	S:0.22
ArH04F_H04R	F: **HEX**-TTTATAACCCATGCCTTCCA	$(GT)_{13}$	WA:281–285	0.45	WA:3	WA:0.18	WA:0.17
	R: CCAAACTGAAACTTTGTGCC		C:273	0.50	C:1	C:--	C:--
			S:273	0.50	S:1	S:--	S:--
ArC09F_C09R	F: **NED**-CTCTCCCTCTCTCGCTCTCT	$(TG)_7$	WA:339–355	0.50	WA:6	WA:0.71	WA:0.71
	R: GAGCCCCAAATACATTTCCT		C:330	0.50	C:1	C:--	C:--
			S:330	0.50	S:1	S:--	S:--
Panel C:							
ArG08F_G08R	F: **FAM**-TCCTCTCTGCTAGGAAGGGT	$(TC)_{13}$	WA:232–236	0.25	WA:4	WA:0.38	WA:0.56*
	R: GCTGGAGACCTTCAAACTCA		C:225–227	0.50	C:2	C:0.25	C:0.40
			S:225–227	0.50	S:2	S:0.22	S:0.47
ArD10F_F05R	F: **HEX**-GAAAGAGTTCAGACAGGGCA	$(CA)_{12}$	WA:310–316	0.60	WA:4	WA:0.59	WA:0.60
	R: TGGTTTCAGACATCCACTGA		C:308	0.50	C:1	C:--	C:--
			S:308	0.50	S:1	S:--	S:--
ArC10F_A05R	F: **NED**-AGAGGGTGGGAAGAGAGAAA	$(GA)_{17}$	WA:150–166	0.60	WA:7	WA:0.83	WA:0.76
	R: CCAGGAACACACACACAACT		C:142–156	0.60	C:6	C:0.75	C:0.78
			S:142–152	0.60	S:5	S:0.89	S:0.76

GenBank Accession nos EU743747–EU743756; N_A, mean number of alleles per locus; WA, Washington population; C, Chilliwack, British Columbia population; S, Sumas, British Columbia population; H_O, observed and H_E, expected heterozygosities; null allele frequencies are based on Brookfield2 estimates from Micro-Checker software; * indicates significant evidence of null alleles with 95% confidence intervals. Null allele estimates for two loci in the Washington population were moderate (ArE12F_C06R = 0.1375; ArG08F_G08R = 0.1969).

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Table 2 Panels 1, 2, and 3 are multiplex panels we developed from previously developed markers (Pilgrim *et al*. 2006). These panels were only tested in the Washington population

Multiplex: loci	Size range (bp)	PCR primer quantity using 1 μM primer (μL)	N_A Total	H_O	H_E
Panel 1:					
AruA1	113–123	0.70	6	0.87	0.77
AruA104	76–100	0.40	6	0.77	0.79
AruA12	72–74	0.65	2	0.27	0.24
Panel 2:					
AruA114	143–151	0.30	5	0.73	0.75
AruB12	89–99	0.35	5	0.63	0.69
AruC6	75	0.90	1	—	—
Panel 3:					
AruB8	n/a	n/a	n/a	n/a	n/a
AruD101	74	0.25	1	—	—
AruC3	105–121	0.30	5	0.73	0.68

N_A, mean number of alleles per locus; H_O, observed and H_E, expected heterozygosities; AruB8 from Panel 3 was dropped from analyses of *A. r. rufa* populations due to multiple alleles but is left to show how it can be multiplexed.

2006) was dropped due to multiple alleles in *A. r. rufa*. The number of alleles per locus ranged from monomorphic to nine (Tables 1 and 2). No loci demonstrated significant deviations from HWE (Tables 1 and 2) after sequential Bonferroni correction (Rice 1989). The addition of these 10 markers to the previously published nine (Pilgrim *et al*. 2006) provides a powerful tool for researchers in the study of *A. r. rufa* populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 PCR chemistry and conditions for each panel for use on ABI 3130/3130Xl genetic analyzer and for primers for use on licor42005

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