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TECHNICAL NOTE

Characterization of eight microsatellite loci in Sea Otter, *Enhydra lutris*, and cross-species amplification in other *Mustelidae*

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Abstract Herein we describe the development of eight microsatellite markers for the northern sea otter, *Enhydra lutris kenyoni*. A total of 45 primer pairs were developed and screened from enriched AAAT, CATC, TACA, and TAGA libraries derived from genomic DNA of *E. lutris kenyoni*. Of these, eight amplified successfully. The average observed heterozygosity, expected heterozygosity, and number of alleles per locus was 0.506, 0.537, and 3.4, respectively. These eight loci were tested across three additional genra; *Vulpes lagopus*, *Martes americana*, and *Mustela nivalis*. Based on the success of our results these loci will be useful for future studies across all subspecies of *E. lutris*.

Keywords Sea otter · *Enhydra lutris* · *E. lutris kenyoni* · Microsatellites · Inbreeding depression · Keystone species

The Sea Otter, *Enhydra lutris*, is a marine mammal in the family *Mustelidae*. There are three recognized subspecies of *E. lutris*: *E. lutris nereis* ranging along the coast of California (CA); *E. lutris kenyoni* ranging from Washington (WA) to southwest Alaska (SWAK); and *E. lutris lutris* ranging from the Aleutian Islands to the Kamchatka Peninsula in Russia (Wilson et al. 1991). Sea otters are considered a keystone species, strongly influencing the species composition and diversity of nearshore communities of the North Pacific Ocean (Estes et al. 1978).

Toward the end of the 19th century, sea otters went through a major bottleneck. Russian and American fur hunters reduced the entire species to only 1,000–2,000 animals (Kenyon 1969). In 1911, the International Fur Seal Treaty was created to protect sea otters in response to the hunting pressure along the Pacific Rim. By the late 1970s, the sea otter population, as a whole, had recovered to pre-exploitation levels (Kenyon 1969). However, in recent years there has been a sharp decline of *E. lutris kenyoni* along the coast of SWAK (Doroff et al. 2003) despite efforts to re-introduce remnant populations to this region. The continuing decline of sea otters in this region caused the United States Fish and Wildlife Service (USFWS) to "determine threatened status for the southwest Alaska distinct population segment of the northern sea otter (*Enhydras lutris kenyoni*)" in August 2005 (USFWS 2005).

Evidence from microsatellite data has revealed a significant loss in genetic variation across all extant populations of *E. lutris* since the fur trade era, indicating possible inbreeding depression (Larson et al. 2002). In an effort to help monitor the potential long-term negative effects associated with the loss of genetic variation, we have developed eight microsatellite loci that can be added to existing markers for genetic analysis in *E. lutris*.

Genetic Identification Services, Inc. (GIS; Chatsworth, CA) constructed libraries enriched for the repeat motifs AAAT, CATC, TACA, and TAGA. The genomic DNA used for these libraries was isolated from a single *E. lutris kenyoni* by the USFWS Conservation Genetics Laboratory (CGL) using the Qiagen, Inc. (Qiagen, Inc., Valencia, CA) DNeasy® protocol. The genomic DNA was digested using the *Hind* III restriction enzyme and the fragments were ligated into the pUC19 plasmid at the *Hind* III site. The *Hind* III sites were flanked with M13 primer sequences for downstream PCR analysis. The recombinant plasmid was then electroporated into *Escherichia coli* DH5α. The clones ($N = 103$) were screened for the four

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Table 1 Characterization of eight microsatellite loci from Sea Otters (*E. lutris*) in Alaska

Locus	Primer sequence (5′–3′)	Repeat sequence	GenBank number	Annealing (°C)	No. of alleles	Allele size range (bp)	*N*	H_E	H_O	*P*
Elu1	F: AgC CAT TgT CAC CTg TAA AC R: CTT TgC TTg TgC TCT CTC TC	$(ATTT)_7$	EU445511	54	4	176–212	22	0.570	0.500	0.33
Elu2	F: gCC TCT CTg CCT ACT TgT g R: CCA CTg TgA Agg gAA TAg C	$(TC)_7(AAAT)_{12}$	EU445512	52	2	172–204	17	0.478	0.765	1.00
Elu3	F: CTg gAg ggA ACA CTC TTC TC R: CTC ACT CTT TCg CTC TCT CAC	$(ATTT)_8$	EU445513	54	4	179–195	20	0.657	0.850	1.00
Elu5	F: Agg CAA AAT AgT gAA Tgg g R: CTg CTT CTC TCT CCg TCT C	$(ATTT)_8$	EU445515	52	5	129–161	20	0.607	0.600	0.60
Elu6	F: CTC CCT CTg ACC TTC TCC C R: TCC CCA ggA Cag TCT gAT g	$(TC)_2ATGC(TC)_7(TAAA)_6$	EU445516	54	2	94–102	14	0.071	0.071	1.00
Elu7	F: gCA TgA Tgg TAg CTC ATT CAC R: ggg TAT CTg CTT gAg ATg CTC	$(ATTT)_{10}$	EU445517	52	3	259–267	19	0.544	0.368	0.06
Elu9	F: ACC AgC TTT gCT CTT gTT Tg R: Cgg TgT Cag Agg AAg ATg Ag	$(ATGT)_{11}(ATTT)_2(GA)_3CA(GA)_4$	EU445519	54	4	261–277	13	0.734	0.846	0.93
Elu10	F: TgC CTC TCT gCC TAC TTg Tg R: ggg Tgg gAC CAT ACT TTT TC	$(CT)_8(TAGA)_7$	EU445520	54	3	134–146	17	0.386	0.294	0.14

Primer sequences, sequence motifs, GenBank accession numbers, anneal temperatures, number of observed alleles, allele size range, N = Number of samples per locus, H_E = Expected heterozygosity, H_O = Observed heterozygosity, and P = P-value for the HW equilibrium tests

motifs mentioned above using X-gal/IPTG/ampicillin agar plates. This resulted in the identification of 45 potential microsatellites that were screened by CGL. Seventeen of the 45 loci were amplified after eliminating those containing imperfect repeats or problematic sequences. Finally, eight loci were finally chosen because of their lack of stutter and ease of scoring (Table 1).

Genomic DNA was isolated from sea otter muscle tissue acquired from carcasses ($N = 24$) collected by the USFWS. The sample distribution ranged from Baranof Island in southeast Alaska to Unimak Island in southwest Alaska. The Qiagen, Inc. 96-Well DNeasy® protocol was used for DNA isolations. Polymerase chain reaction amplifications in a 10 ul total volume contained the following components: 30 ng template DNA, 0.4 uM unlabeled/labeled forward primer, 0.4 uM reverse primer, 1 M Betaine, 0.5 U *Taq* DNA Polymerase (Promega, Madison, WI), 0.8 mM dNTPs, 2.5 mM $MgCl_2$. Amplifications were performed on the MJ Research™ PTC-225 DNA Engine® using the following protocol: initial denaturation at 92°C for 2 min, 25 cycles of 92°C for 30 s, annealing temperature ranged 52–54°C (Table 1) for 15 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Amplification success varied across loci due to the poor quality of the muscle tissue acquired from the necroscopies (Table 1).

The program GENEPOP'007 (Rousset 2008) was used to test the null hypothesis of genotypic equilibrium between all pairs of loci. The *P*-value for one test (*Elu5* × *Elu10*, $P = 0.04$) was below 0.05, however, the result was not considered statistically significant when the α-level was adjusted ($\alpha = 0.0018$) for 28 simultaneous tests (Rice 1989). The program FSTAT (Goudet 2001) was used to estimate the observed and expected heterozygosity (H_O, H_E) for each locus and to test the null hypothesis of Hardy Weinberg equilibrium (HWE; Table 1). There was no evidence of deviations from HWE at any locus. The average H_O, H_E and number of alleles per locus was 0.506, 0.537, and 3.4, respectively (Table 1).

The same eight loci were amplified in *Vulpes lagopus* (N = 4), *Martes americana* (N = 2), and *Mustela nivalis* (N = 6) using identical reaction conditions and temperature ranges. The cross-amplification results are reported in Table 2.

Table 2 Cross-species amplification in Arctic Fox (*V. lagopus*), Martin (*Martes americana*), and Least Weasel (*Mustela nivalis*)

Locus	*V. lagopus*	*M. americana*	*M. nivalis*
Elu1	P	P	P
Elu2	na	P	na
Elu3	na	M	M
Elu5	M	na	na
Elu6	M	P	M
Elu7	na	M	na
Elu9	na	na	na
Elu10	na	na	P

Results are reported as Polymorphic (P), Monomorphic (M), or no amplification (na)

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