2007

Characterization of eight microsatellite loci in Steller sea lions (*Eumetopias jubatus*)

Ryan M. Huebinger
Texas A&M University, College Station

Edward E. Louis Jr.
Center for Conservation and Research, Henry Doorly Zoo

Thomas Gelatt
National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service

Lorrie D. Rea
Marine Mammals Section, Division of Wildlife Conservation, Alaska Department of Fish and Game, University of Alaska Fairbanks

John W. Bickham
Texas A&M University, College Station

Molecular Ecology Notes (2007) 7, 1097–1099 doi: 10.1111/j.1471-8286.2007.01790.x

PRIMER NOTE

Characterization of eight microsatellite loci in Steller sea lions (*Eumetopias jubatus*)

RYAN M. HUEBINGER,*¶ EDWARD E. LOUIS JR,† THOMAS GELATT,‡ LORRIE D. REA§ and JOHN W. BICKHAM*¶

*Department of Wildlife and Fisheries, Texas A&M University, College Station, TX 77843, USA, †Center for Conservation and Research, Henry Doorly Zoo, 3701 South 10th Street, Omaha, NE 68107, USA, ‡National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way, NE, Seattle, WA 98115-6349, USA, §Marine Mammals Section, Division of Wildlife Conservation, Alaska Department of Fish and Game, University of Alaska Fairbanks, 245 O'Neill Building, Fairbanks, AK 99775-7220, USA, ¶Current address: Centre for the Environment, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA

Abstract

Steller sea lions (*Eumetopias jubatus*) are listed as an endangered species in western Alaska and have exhibited a significant population decline throughout their range. Eight microsatellite loci were isolated from genomic DNA libraries. In addition, all these markers were found to be variable in nine individuals of the California sea lion (*Zalophus californicus*). This panel of markers was developed to analyse population structure in Steller sea lions throughout their range.

Keywords: *Eumetopias*, microsatellites, Steller sea lion

Received 6 August 2006; revision accepted 12 March 2007

The Steller sea lion (*Eumetopias jubatus*) is distributed throughout the North Pacific Ocean and Bering Sea. Rookeries occur along the North Pacific Rim from the Sea of Okhotsk and Kuril Islands; north to the Commander Islands; east through the Aleutian Island chain to mainland Alaska; and south to California (Loughlin *et al*. 1987). This species has experienced a marked decline from an estimated 240 000–300 000 individuals in the 1960s (Kenyon & Rice 1961) to an estimated 116 000 individuals in 1989 (Loughlin *et al*. 1992). Population numbers in the USA have declined by about 75% over the past 20 years (Calkins *et al*. 1999) with most of the decline occurring in the western portion of their range. Several studies have investigated the decline of Steller sea lions, but the exact cause remains elusive (Loughlin 1998). Suggested causes include reduced prey availability, historical sea lion pup harvest, diseases, predation, and environmental changes. Genetic studies of Steller sea lions have been conducted to investigate the phylogeographic structure of the populations, to identify management units, and to document the potential loss of genetic diversity resulting from the decline using mtDNA markers (Bickham *et al*. 1998; Baker *et al*. 2005). In addition, studies utilizing microsatellite data, mainly derived from loci described from distantly related pinnipeds, across the range of Steller sea lions have been conducted (Hoffman *et al*. 2006).

Genomic DNA was isolated from Steller sea lion and northern fur seal (*Callorhinus ursinus*) flipper punches using a standard protocol (Sambrook *et al*. 1989). A procedure for creation of a genomic library enriched for microsatellites was carried out as described in An *et al*. (2004). A brief overview of the enrichment protocol follows. Genomic DNA was digested with *Sau*3AI and then size selected utilizing Chroma Spin columns (Clontech Laboratories, Chroma Spin + TE-400). Fragments were then ligated to linker molecules described in An *et al*. (2004). Enrichment for CA repeats was accomplished utilizing biotinylated oligoprobe and Vectrex Avidin D (Vector Laboratories). Enriched fragments were then ligated into pCR 2.1 TOPO vector (Invitrogen). Ligated DNA was transformed into *Escherichia coli*. Colonies that contained inserts were hybridized to a $(CA)_{10}$ oligoprobe. Screening of 1000 clones yielded 19 positives. Primers for polymerase chain reaction (PCR) amplification were designed from the

Correspondence: John W. Bickham, Centre for the Environment, Purdue University, West Lafayette, IN 47907-2966, USA. Fax: (765)496-1369; E-mail: bickham@purdue.edu

Table 1 Primer sequences, allele characteristics, sizes, and number (*k*) of eight *Eumetopias jubatus* microsatellites, observed and expected heterozygosities with Hardy–Weinberg probabilities, as screened across 20 Steller sea ions

Locus	Primer sequence (5′ to 3′)	Repeat motif	Annealing temp. (°C)	GenBank accession No.	Fragment size (bp)	k	H_O	H_E	Prob HWE
13HDZ462	F-AGG CTT AAA ATC ACA AAA CAT CAG R-TTA CCA GAC AGT GTT ATT GAC GC	$(CA)_{19}$	54	DQ777851	125–131	3	0.35	0.432	0.0345*
71HDZ2x	F-GTC ACA CCA TAT GTT TTG TGT AAT TAC R-HEX ATA TTT ATA AAA TGT TAG ACT AAT GTA GCT TC	$(CA)_4AA(CA)_{13}$	52	DQ777840	139–141	2	0.526	0.512	1.0
71HDZ301	F-GTG AAA GAA ACC TGA TGC TGC R-HEX AAG TCA TAG CCA GTC TCC TTA CAG	$(CA)_{14}$	53	DQ777847	255–259	3	0.5	0.575	0.6208
71HDZ 441	F-HEX ACC TTT TCA TTT GCC TCC TC R-GCC TCA GAG AGA CCA GTG TGC	$(CA)_{13}$	58	DQ777849	304–306	2	0.158	0.149	1.0
71HDZ 529	F-6-FAM GAT CCA TTA CTA GAC AGA CAG ACA GAC R-GTT TTG CTT CTT CAC ACC ATA TAC	$(CA)_{15}$	50	DQ777850	201–211	5	0.684	0.648	0.3951
71HDZ 5A	F-6-FAM GTG GGG CAT AAC ACA ATC TG R-TCT TCC TAC CTC TGC TCT CTC C	$(GT)_{25}$	54	DQ777853	160–168	4	0.235	0.401	0.0449*
71HDZ 5x	F-6-FAM CTT TAG ACC ATT GCT TTT AGA CTT C R-GTT GCC ATG ACA TTT AGC TTT G	$(CA)_{18}$	50	DQ777841	304–310	3	0.474	0.421	0.5198
71HDZ 15	F-HEX AGA AAG GTA ACT GGG GAG AGG R-ACA AAG TGG GAA AGT GAG TGC	$(GT)_{15}$	54	DQ777852	178–186	5	0.7	0.587	0.5243

*denotes significant deviation from Hardy–Weinberg equilibrium $P < 0.05$.

Table 2 Cross-species amplification results using the primers developed for Steller sea lion. The number of alleles for each locus from 12 California sea lions (*Zalophus californicus*) are shown in the first row. Allele size range for each locus is *Z. californicus* are listed in the second row. 71HDZ15 was not tested with regards to cross amplification

Species	13HDZ462	71HDZ2x	71HDZ301	71HDZ 441	71HDZ 529	71HDZ 5A	71HDZ 5x
Z. californicus	5	4	4	2	7	9	3
	125–149	139–147	259–267	300–302	203–217	143–176	300–304

flanking regions, which were selected for analysis based on a high repeat number using MACVECTOR 6.5.3 (Oxford Molecular Group). Loci were determined to be polymorphic based on PCR fragment size when screened across 20 *Eumetopias* and nine California sea lions (*Zalophus californicus*). Loci were screened for polymorphism by electrophoresing on 3% agarose gels. One of the eight loci (13HDZ462) was developed from the sequence of a clone containing a northern fur seal insert and the remaining seven were obtained from Steller sea lion inserts.

Genomic DNA was isolated from tissue samples and PCR amplification was performed in a 25-μL reaction volume using an ABI 2700 thermocycler (PerkinElmer) with approximately 50 ng of genomic DNA as template. Final amplification conditions consisted of 12.5 pmol unlabelled reverse primer, 12.5 pmol fluorescently labelled forward primer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 0.5 U of *Taq* DNA polymerase (Promega). The PCR amplification profile was 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, a primer-specific annealing temperature for 30 s (Table 1), 72 °C for 30 s, and ending with a single extension at 72 °C for 10 min.

Allele sizes were determined by fragment separation on a 7% polyacrylamide gel electrophoresed on an ABI 377 DNA Analyser (Applied Biosystems, Inc.). Fragment lengths were assigned by the GENESCAN software program (Applied Biosystems, Inc.) using GeneScan-400 [ROX] size standard. Heterozygosity values for each locus were calculated, and tests for Hardy–Weinberg equilibrium of the genotypic frequencies were carried out using GENEPOP version 3.1 software package (Raymond & Rousset 1995). Additionally, loci were tested for linkage disequilibrium in GENEPOP version 3.1 and no significant linkages were detected among these loci. Primer sequences, annealing temperature, repeat motif, GenBank accession number for each locus, number and size of alleles, and the heterozygosity values for each of the markers are presented in Table 1. Two loci, 13HDZ462 and 71HDZ5A, differed significantly from Hardy–Weinberg equilibrium ($P < 0.05$). The excess of homozygotes detected as deviations from Hardy–Weinberg equilibrium could be created as an effect of sampling error and sample size. The number of alleles found ranged from two to five alleles per locus within Steller sea lions. In addition, the nine Californian sea lions had an equal or greater number of alleles at each locus when compared to the Steller sea lions, with the range of alleles per locus being two to nine alleles (Table 2).

The suite of markers will be utilized to analyse a more extensive collection of Steller sea lion samples throughout their range.

Acknowledgements

The National Marine Fisheries Service, the Alaska Department of Fish and Game, and the Henry Doorly Zoo provided funding for this study. Specimens were humanely treated and collected under Marine Mammal Protection Act permits 782-1532-02 and 358-1546.

References

An J, Sommer JA, Shore GD, Williamson JE, Brenneman RA, Louis EE Jr (2004) Characterization of 20 microsatellite marker loci in the West Indian rock iguana (*Cyclura nubila*). *Conservation Genetics*, **5** (1), 121–125.

Baker AR, Loughlin TR, Burkanov V *et al*. (2005) Variation of mitochondrial control region sequences of Steller sea lions, *Eumetopias jubatus*: The three-stock hypothesis. *Journal of Mammalogy*, **86**, 1075–1084.

Bickham JW, Loughlin TR, Wickliffe JK, Burkanov VN (1998) Geographic variation in the mitochondrial DNA of Steller sea lions: haplotype diversity and endemism in the Kuril Islands. *Biosphere Conservation*, **11**, 107–117.

Calkins DG, McAllister DC, Pitcher KW, Pendleton GW (1999) Steller sea lion status and trend in southeast Alaska: 1979–97. *Marine Mammal Science*, **15**, 462–477.

Hoffman JI, Matson CW, Amos W, Loughlin TR, Bickham JW (2006) Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lions *Eumetopias jubatus*. *Molecular Ecology*, **15**, 2821–2832.

Kenyon KW, Rice DW (1961) Abundance and distribution of the Steller sea lion. *Journal of Mammalogy*, **42**, 223–234.

Loughlin TR (1998) The Steller sea lion: a declining species. *Biosphere Conservation*, **1**, 91–98.

Loughlin TR, Perez MA, Merrick RL (1987) *Eumetopias jubatus*. *Mammalian Species*, **283**, 1–7.

Loughlin TR, Perlov AS, Vladimirov VA (1992) Range-wide survey and estimation of total number of Steller sea lions in 1989. *Marine Mammal Science*, **8**, 220–239.

Raymond M, Rousset F (1995) GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Sambrook J, Fritch EF, Maniatus T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Press, New York.