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Short communication

Lack of sequence variation of Y chromosome-linked loci in Steller's sea lions (*Eumetopias jubatus*) from Iony Island and the Kuril Islands

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The Y chromosome-linked (Y-linked) genetic markers are important for understanding historical patterns of male dispersal (e.g., Tucker and Lundrigan 1996). Especially, the sex-determining region of the Y gene (SRY) is used as a marker to resolve phylogeography, population structure, and population dynamics of wild mammals (Hurles and Jobling 2001; Petit et al. 2002). For instance, Iwasa and Suzuki (2002) reported that SRY variation in Japanese red-backed moles (*Eothenomyce andersonii*) showed substantial geographic distribution. Geraldes et al. (2005) found high levels of nucleotide diversity in the SRY of European rabbits (*Oryctolagus cuniculus*). Moreover, Y-linked introns such as the DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide of the Y chromosome (DBY) and the histocompatibility Y antigen gene (SmcY) may be effective markers in phylogeographical study (e.g., Underhill et al. 2001).

Steller's sea lion *Eumetopias jubatus* (Schreber, 1776), a highly vagile marine mammal breeding in rookeries (e.g., Nowak 1999), is distributed along rocky continental coasts and near-shore islands from Northern California, through the Gulf of Alaska, along the Aleutian Islands, to the Kamchatka Peninsula, Kuril Islands, and the Sea of Okhotsk (Loughlin et al. 1992; Abe et al. 2005; Burkanov and Loughlin 2005; Wilson and Reeder 2005). Pups disperse from their natal rookeries within a year of their birth. Most adults, however, generally remain within 500 km of their natal rookeries (Raum-Suryan and Pitcher 2002). Female Steller's sea lions show strong enough philopatry to create a matrilineal substructure, as shown by mitochondrial DNA (mtDNA) lineage (Bickham et al. 1998), similar to that of the Atlantic walrus *Odobenus rosmarus* (Anderson et al. 1998) and the harbor seal *Phoca vitulina* (Stanley et al. 1996). In fact, there are three main geographical mtDNA lineages of the Steller's sea lion: 'eastern' (California to the southeastern Gulf of Alaska), 'western' (Prince William Sound to the Commander Islands), and 'Asian' (Kamchatka Peninsula, Kuril Islands, and Sea of Okhotsk) (Bickham et al. 1998; Harlin-Cognato et al. 2006). Within local populations, genetic divergences of mtDNA sequence among rookeries are also recognized (Baker et al. 2005). Unlike mtDNA phylogeography, a study (Hoffman et al. 2006a) using autosomal microsatellites found that there was no clear genetic divergence between western and Asian stocks, suggesting that the phylogeographical difference between mtDNA and microsatellites studies was the result of higher dispersal rates by males. Male Steller's sea lions tend to disperse longer distances from their natal rookeries (Raum-Suryan and Pitcher 2002). Consequently, greater male-mediated gene flow would explain the differences in pattern. Therefore, Y-chromosomal markers would provide an enhanced description of male-mediated gene flow. On the other hand, since Steller's sea lion is gregarious and polygynous (e.g., Nowak 1999), the many offspring of a

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Table 1. Specimens of 53 male Steller's sea lions (*Eumetopias jubatus*) examined for variation in SRY and Y-linked introns (DBY5, DBY7, and SMCY7) in this study

Locality	SRY		Y-linked introns	
	Identity number	Number of individuals	Identity number	Number of individuals
Antsiferov	32, 33, 40, 41, 110	5	25*, 379*	2
Lovushuki	149, 161, 170, 178, 183, 461, 470, 479	8	149, 470	2
Raykoke	14, 118, 119, 120, 133, 137, 487, 524, 527, 534, 538	11	138*, 524, 527	3
Srednego	45, 61, 404, 407, 411, 415, 419, 426	8	61, 404, 415	3
Brat Chiripoev	19, 21, 69, 70, 83, 86, 89, 296	8	70, 83, 296	3
Iony	7, 15, 240, 244, 245, 250, 312, 325, 329	9	250, 312, 314*	3
Total		49		16

Asterisks mean individuals examined only Y-linked introns. Identity numbers correspond to those in our ecological research (Joint survey project of Steller sea lions among American, Japanese and Russian researchers in the Kuril Islands in 2001).

dominant male may share his Y chromosome, resulting in low genetic diversity of Y chromosomal markers within a local population. We tested these conjectures for Asian Steller's sea lions by examining sequences of SRY, two DBY introns (DBY5 and DBY7), and one SmcY intron (SMCY7).

Materials and methods

Specimens

We examined a total of 53 male Steller's sea lion specimens from Iony Island (n = 10) in the Sea of Okhotsk and five rookeries in the Kuril Islands: Antsiferov Island (n = 7), Lovushki Island (n = 8), Raykoke Island (n = 12), Srednego Island (n = 8), and Brat Chiripoev Island (n = 8) (Fig. 1 and Table 1). From 29 June to 6 July 2001, we collected skin samples by punching hind flippers as described in Bickham et al. (1996). These samples were preserved in 70% ethanol.

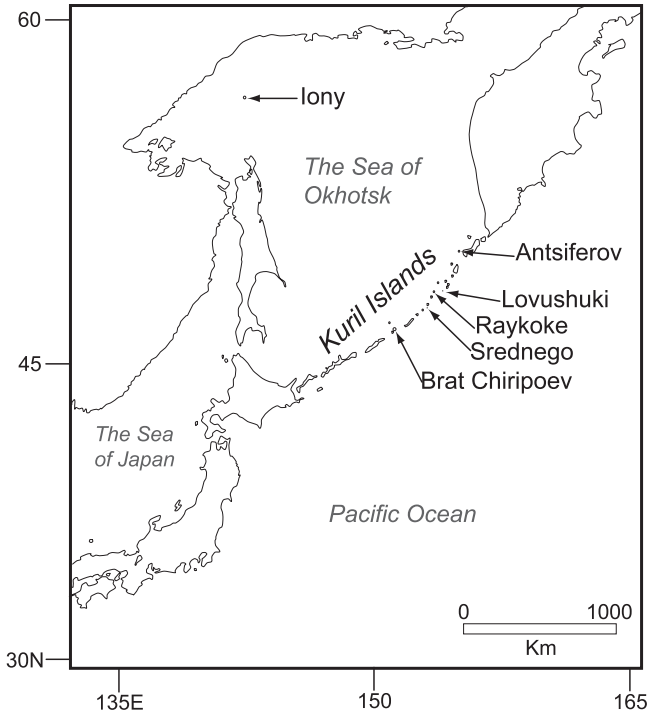

Fig. 1. Sampling localities of the Steller's sea lion (*Eumetopias jubatus*) males examined in the present study.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from preserved skin tissue with the modified phenol-chloroform method (Sasaki et al. 1995) and suspended in Tris-EDTA buffer. Partial SRY sequences (520 bp) of 49 males (Table 1) were amplified with a set of new primers: F13 (5'-AACAAT TCACAATCTTTCCT-3') and R11 (5'-CGACCACAC CATGAATGCGT-3'). These primers were designed based on submitted DNA sequence (accession number AY424649). The 50 µl reaction mixture contained 40 ng of genomic DNA, 25 µM of each primer, 0.2 mM dNTPs, 1 × polymerase chain reaction (PCR) buffer, 50 mM KCl, 0.025 µM $MgCl_2$, and 1.5 units of *Taq* DNA polymerase (Ampli *Taq* Gold, Applied Biosystems). Amplification was with a PE9700 thermocycler (Perkin-Elmer). Cycling conditions consisted of an initial denaturation for 10 min at 95°C followed by 30 cycles at 94°C for 30 sec and annealing at 55°C for 30 sec and at 72°C for 1 min, followed by a final extension of 72°C for 2 min. The PCR products were purified using the

QIAQuick PCR purification kit (Qiagen) and eluted in 30 µl dH_2O. In all 49 males, the 419 bp of this fragment were sequenced with two newly designed internal sequence primers (SSL-F14: 5'-CCTGCACCTTCCTC CTTTGA-3' and SSL-R12: 5'-TTCTGGCCGCTGTC CCTACCA-3') using an automated sequencer (ABI3100, Applied Biosystems) and the Big dye terminator, version 3.1 (Applied Biosystems).

We followed the methods of Hellborg and Ellegren (2003) to amplify and sequence in 16 males (Table 1) the 1,700 bp DNA fragments from three Y-linked introns, DBY5, DBY7, and SMCY7. The PCR mixtures contained 40 ng of genomic DNA, 0.2 µM each primer, 0.2 mM dNTPs, 1 × PCR buffer, 0.025 µM $MgCl_2$, and 1.5 units *Taq* DNA polymerase (Ampli *Taq* Gold, Applied Biosystems) in a total volume of 50 µl. Cycling conditions, with touch-down annealing steps in the first 20 cycles, was: initial denaturation at 95°C for 5 min, followed by 20 cycles of 95°C for 30 sec, decreasing by 0.5°C/cycle using the lowest annealing temperature. A final extension was at 72°C for 10 min. The PCR products were purified using the QIAQuick PCR purification kit (Qiagen), and eluted in 30 µl dH_2O. The DBY5 intron was sequenced using the same primer set. To sequence the other loci, we used newly designed internal primers: DBY7seqF1: 5'-TGAAGGCTGTGAAGG-3', DBY7seqF2: 5'-GGCTGTGAAGGTAAA-3', SMCY7seqF: 5'-TCAT GGAGGTATGAC-3', and SMCYseqR: 5'-TGGGTAG CCTGCTCAAA-3'.

Sequence and phylogeographic analyses

Sequence alignment was with the software program CLUSTAL W (Thompson et al. 1994). Accession numbers of these sequences are AB364235 (SRY), AB364236 (DBY5), AB364237 (DBY7), and AB364238 (Smcy 7).

Results and discussion

Partial SRY sequences (419 bp) of 49 males were identical. There were no sequence differences in the three Y-linked intron sequences (1,700 bp) of 16 males. We found only three nucleotide substitutions in the SRY sequences compared to those submitted previously (accession number AY424649).

In fact, the Y-linked loci do not tend to be highly polymorphic because of the clonal nature of inheritance of Y chromosomes through paternal lineages (Hellborg and Ellegren 2003). As all Y-linked sequences used in our study were completely identical, males from each rookery may also have the same paternal lineage and a common ancestral stock. Assuming they have the same paternal linage, two factors may explain the lack of variation in the Y-linked sequence of male Steller's sea lions in the Sea of Okhotsk (Iony Island) and in the Kuril Islands. First, this area may have high gene flow by males. Young male Steller's sea lions can disperse 1,000 km from their natal rookeries (Raum-Suran and Pitcher 2002). This distance is enough to cover the entire study area. Second, the effective population size of Steller's sea lions in this area may be small. Since Steller's sea lions are highly polygynous (Gisiner 1985), some Y-specific sequences may have spread over the entire distribution of this population.

Further study of the paternal lineage of Steller's sea lions in Iony Island and the Kuril Islands should include analysis of hypervariable Y-linked genetic markers (such as microsatellites) from more specimens from more rookeries. Analysis of multilocus microsatellites, used to study mate choice, mating strategy, and reproductive success in pinnipeds (Gemmel et al. 2001; Hoffman et al. 2006b, 2007; Lancaster et al. 2007), would clarify patterns of paternal lineage in Steller's sea lion.

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