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Author(s)	Naitoh, Y.; Ishibashi, Y.; Abe, S.; Ohdachi, S. D.
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**Isolation and characterization of polymorphic microsatellite DNA markers in two shrew species, *Sorex unguiculatus* and *S. caecutiens***

Y. NAITOH,<sup>\*</sup> Y. ISHIBASHI,<sup>†</sup> S. ABE,<sup>‡ ¶</sup> and S. D. OHDAKI<sup>\*</sup>

5      <sup>\*</sup>*Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan,*

<sup>†</sup>*Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo*

*062-8516, Japan, <sup>‡</sup>Laboratory of Animal Cytogenetics, Center for Advanced Science*

*and Technology, <sup>¶</sup>Laboratory of Cytogenetics, Graduate School of Environmental Earth*

*Science, Hokkaido University, Sapporo 060-0810, Japan.*

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Correspondence: Satoshi D. Ohdachi

Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan.

Fax: (+81) 11 706 7142; E-mail: ohd@pop.lowtem.hokudai.ac.jp

20      Running title: Microsatellites in two *Sorex* species

**Abstract**

We isolated six microsatellite markers from the partial genomic libraries of two *Sorex* shrews, *S. unguiculatus* and *S. caecutiens*, and examined their allelic variation. All loci showed high allelic variation ranging from 15 to 19 alleles and all but one locus 5 conformed to Hardy-Weinberg expectations in the species where the loci were isolated. Cross-species amplifications showed that all primers derived from *S. unguiculatus* were useful for *S. caecutiens*, while among primer sets derived from *S. caecutiens*, only one was useful for *S. unguiculatus*. Accordingly, at least five microsatellite markers were useful in *S. caecutiens* and three in *S. unguiculatus*.

To infer biogeographical history of an organism in detail, comparison of genetic structure among various local populations is essential. Recently, microsatellite markers have been routinely used to investigate genetic structure of natural populations in many species (Balloux and Lugon-Moulin 2002). With regard to soricine shrew some

5 microsatellite primers have been designed for *S. araneus* (Wytttenbach *et al.* 1997).

However, according to our preliminary study, only a few of their markers were useful for two *Sorex* species in north eastern Asia, *S. unguiculatus* and *S. caecutiens*, which are common in Hokkaido, the northernmost island of Japan (Ohdachi and Maekawa 1990).

Here, we isolated new microsatellite loci from the genomes of these two Asian *Sorex*

10 species and assessed their allelic variation in a natural population of each species.

Cross-species amplification was also performed using the primer sets designed for the species where the loci were originally isolated.

Total genomic DNAs were extracted from liver tissues preserved in 90% ethanol in one *S. unguiculatus* and one *S. caecutiens* captured in Sarobetsu moor, Hokkaido, by

15 the proteinase K/phenol/chloroform method (Sambrook *et al.* 1989). Partial genomic

libraries were constructed from *Sau3A* I-digested genomic DNA, in which size-selected

DNA fragments ranging from 0.5 to 1 kb were ligated into pBluescript II. For each

shrew, approximately 8 000 transformed colonies were screened with a <sup>32</sup>P-5'

end-labelled (CA)<sub>8</sub> probe. A second screening of the positive clones was performed,

20 from which 18 positive clones for *S. unguiculatus* and 23 positive clones for *S.*

*caecutiens* were isolated. These isolated clones were sequenced using ABI PRISM

dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with an ABI PRISM

377 DNA Sequencer. Six and 11 primer sets for PCR amplification were designed for *S.*

*unguiculatus* and *S. caecutiens*, respectively, based on the nucleotide sequences of the

clones containing a CA/GT-repeat.

Allelic variation and other characteristics at the cloned microsatellite loci were examined in both shrew species captured at Bibai in Hokkaido (*S. unguiculatus*;  $N = 49$ ,

and *S. caecutiens*;  $N = 42$ ). Genomic DNA was extracted from liver or muscle tissue by

5 the proteinase K/phenol/chloroform method or the Chelex-100 method (Walsh *et al.*

1991). PCR amplification was carried out following Masuda and Yoshida (1994), or

using icycler (BIO-RAD) in a 15  $\mu\text{l}$  reaction mixture containing 50 mM KCl, 1.5 mM

MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 8.3), 0.1 % Triton X-100, 0.2 mM dNTPs, 0.5  $\mu\text{M}$  of each

primer, 0.5 U rTaq DNA polymerase (TOYOBO) and 10 - 100 ng of genomic DNA.

10 After denaturation at 94 °C for 1 minute, cycling was performed for 30 - 35 cycles

under the following conditions: 2 minutes at 94 °C, 1 minute at 55 or 58 °C and 1

minute at 72 °C. The PCR products were analysed on an ABI PRISM 310 Genetic

Analyzer. Hardy-Weinberg equilibrium was tested at each of the loci using the program

GENEPOP 3.1c (Raymond & Rousset 1995).

15 Among the synthesized primer sets, three sets for *S. unguiculatus* and eight sets

for *S. caecutiens* were useless because they showed monomorphic or unclear peak

patterns in the species where the primer sets were developed. As a result, three primer

sets were useful for both species. These six loci showed allelic variation ranging from

15 - 19 alleles in the species where the loci were isolated. Observed heterozygosities

20 were very high except for a locus, A10 (mean 0.787 for *S. unguiculatus* and 0.857 for *S.*

*caecutiens*). In each species, all but one locus conformed to Hardy-Weinberg

expectations (Table 1). In *S. unguiculatus*, non-amplification occurred in one of the 49

individuals at A10. Since the other two loci were amplified successfully in this

individual, a null allele may exist at the locus A10 with a relatively high frequency in

the examined population. In *S. caecutiens*, the observed genotype frequencies deviated slightly from Hardy-Weinberg equilibrium at D2 ( $P = 0.041$ ). Since both the observed and expected heterozygosities showed very high and similar values for this locus (Table 1), a sampling bias due to the small sample size in the present study might cause the 5 deviation from Hardy-Weinberg equilibrium, although this remains to be clarified.

For the primer sets derived from *S. caecutiens*, only a locus, D2, showed high allelic variation and conformed to Hardy-Weinberg expectation in *S. unguiculatus* (Table 1). However, in *S. unguiculatus* PCR products of E1 showed monomorphic and those of D8 could not be typed because of a complex peak pattern. On the other hand, 10 all the primer sets derived from *S. unguiculatus* provided analysable PCR products with high allelic variation ranging from 16 to 25 alleles and high observed heterozygosities (mean 0.928) in *S. caecutiens*, and all loci conformed to Hardy-Weinberg expectations (Table 1). Thus, among the isolated microsatellite markers, at least five will be useful in assessing population genetic structure in *S. caecutiens* and three in *S. unguiculatus*.

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Table 1 Characterization of six microsatellite loci isolated from *Sorex unguiculatus* and *S. caecutiens*. Repeat motifs are derived from the sequenced clones. Product size is the range of allele size detected in the origin species. Italicized letters are the results of cross-species amplification. Accession, EMBL/GenBank/DDBJ accession number; A, number of alleles; H<sub>O</sub>, observed heterozygosity; H<sub>E</sub>, expected heterozygosity; NA, not available.

5	Locus (Accession)	Origin	Primer sets (5'-3')	Fluorescent	Repeat motif	Product size (bp)	Annealing temp. (°C)	<i>S. unguiculatus</i> (N = 49)			<i>S. caecutiens</i> (N = 42)		
								A	H <sub>O</sub>	H <sub>E</sub>	A	H <sub>O</sub>	H <sub>E</sub>
A6 (AB084159)	<i>S. unguiculatus</i>	GGCAGCTTCACTGTTACAGG	TET	(AC) <sub>25</sub>	219-249	55		15	0.816	0.887	17	0.952	0.912
A10 (AB084160)	<i>S. unguiculatus</i>	AGATTGCTGAAGGAGCCAGG	-										
A10 (AB084160)	<i>S. unguiculatus</i>	CCTACATCTGGTTGAAGAGGC	6-FAM	(AC) <sub>29</sub>	181-267	55		19	0.646*	0.939	16	0.881	0.932
B4 (AB084161)	<i>S. unguiculatus</i>	TGGCTTGTCACTGGGTTC	-										
B4 (AB084161)	<i>S. unguiculatus</i>	GCCTCTGTAAACACTGAACG	TET	(AC) <sub>25</sub>	168-205	55		18	0.898	0.895	25	0.952	0.948
D2 (AB084162)	<i>S. caecutiens</i>	GACACATCACGATAACAAGGG	-										
D2 (AB084162)	<i>S. caecutiens</i>	AAGTACTCCACAGCTGATGGG	HEX	(GT) <sub>23</sub>	204-236	58		18	0.878	0.912	15	0.905**	0.906
D8 (AB084163)	<i>S. caecutiens</i>	TGCATCTGGTACGTCATCGG	-										
D8 (AB084163)	<i>S. caecutiens</i>	GTGTGGTTAAGGGTGAGCC	HEX	(CA) <sub>16</sub>	174-206	55		NA	-	-	15	0.881	0.917
E1 (AB084164)	<i>S. caecutiens</i>	GCCAGAGACCAAACTAGTCGC	-										
E1 (AB084164)	<i>S. caecutiens</i>	AGCACGGAGAACCTATAGGAC	6-FAM	(CA) <sub>28</sub>	248-284	55		1	0	0	17	0.786	0.920

\*, \*\* Not conformed to Hardy-Weinberg equilibrium (\*P < 0.001, \*\*P < 0.05).