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ORIGINAL ARTICLE

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- 5 Population genetic structure of *Sorex unguiculatus*
and *S. caecutiens* (Soricidae, Mammalia) in
Hokkaido, based on microsatellite DNA
polymorphism

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Abstract

We investigated the genetic structure of *Sorex unguiculatus* and *S. caecutiens* populations in Hokkaido, Japan, using hypervariable

microsatellite DNA markers. We used 5 microsatellite loci to type

5 475 of *S. unguiculatus* individuals from 20 localities on Hokkaido

mainland and 4 localities from each of four offshore islands (and 11

shrews from one locality in southern Sakhalin for a particular

analysis). We used 6 microsatellite loci to type 240 of *S. caecutiens*

individuals from 13 localities on Hokkaido mainland. Genetic

10 variation was high in mainland populations of both species and low

in the island populations of *S. unguiculatus*. Allelic richness and

island size were positively correlated for *S. unguiculatus*, suggesting

that genetic drift occurred on those islands due to small population

size. In addition, 4 insular populations of *S. unguiculatus* were

15 genetically differentiated from the mainland populations, although

clear phylogeographic clustering was not confirmed among

populations on Hokkaido mainland both for *S. unguiculatus* and *S. caecutiens*. Heterozygosity excess was observed in more than half of populations including the mainland populations in the two species, suggesting recent bottleneck events in those populations. Population dynamics of the shrews might be explained under metapopulation scheme. According to autocorrelation analysis, the extent of nonrandom spatial genetic structure was approximately 100 km. Isolation by distance was observed in *S. unguiculatus*, but not in *S. caecutiens* although there is a positive trend. The no correlation in *S. caecutiens* might have been caused due to small sample size. Thus, no obvious differences in population genetic structure were found between the two species on the Hokkaido mainland in the present study, while previous investigations using mitochondrial DNA sequences inferred those two species might have rather different biogeographic histories.

Key words Isolation by distance • Autocorrelation • Microsatellite

DNA • Shrew • bottleneck • metapopulation

Introduction

Change in geographic distribution of organisms is one of the major subjects in biogeographic studies. Major influences on such changes are the connection or disconnection of land masses and/or the
5 vegetational changes. These drastically alter species distributions and prompt important evolutionary events, including genetic change and speciation. The Pleistocene glaciation had particularly contributed to structuring of extant biota. In Europe many phylogeographical studies reveal that organisms expanded their
10 range after the last glacial age, which resulted in change in genetic variation and/or genetic structure of a population (e.g., Avise 2000; Brunhoff et al. 2003; Hewitt 2000; Queney et al. 2001; Triantafyllidis et al. 2002). Although northeastern Asia was not covered with continental ice sheets during the last glacial period, sea level change,
15 which caused connection/disconnection between islands and the continent, and vegetational change owing to glaciation affects faunal

dynamics in East Asia (Japan Association for Quaternary Research 1987; Masuda 1999). In northeastern Asia, nine species of *Sorex* shrews inhabit (Dolgov 1985; Nesterenko 1999), and diversity of *Sorex* species is highest in Eurasia. In addition, there are many

5 islands close to the continent. Thus, the *Sorex* shrew community in northeastern Asia are a good model to investigate the relationships between biogeography and genetic structure.

Ohdachi et al. (1997, 2001, 2003) and Ohdachi (2005) studied intra- and interspecific phylogeny of six soricine shrew species in the
10 Japanese archipelago and neighbouring regions based on nucleotide sequences of the mitochondrial cytochrome *b* gene (mtDNA *cytb*) and discussed the history of range expansion of those species.

Among the six species, *S. unguiculatus* Dobson and *S. caecutiens* Laxmann are abundant species in northeastern Asia and showed
15 contrasting results of phylogeography. In *S. unguiculatus*, there was no concordance between the phylogenetic positions and the

geographical origins of specimens throughout its range.

Furthermore, no positive correlation was found between genetic and geographic distances. Thus, this species is considered to have spread so recently, forming no local genetic divergence. In contrast,

5 phylogeny of *S. caecutiens* is clearly separated between Hokkaido and Sakhalin-Eurasia populations, suggesting that it colonised Hokkaido earlier than *S. unguiculatus*. However, their genealogy was based solely on mtDNA *cytb* sequences, and this analysis is insufficient to detect biogeographical history among populations of
10 these shrew species.

In this paper, we used microsatellite DNA markers to try to trace biogeographical history of populations of *S. unguiculatus* and *S. caecutiens* in Hokkaido, because they often can be used for population genetics (Goldstein and Schlotterer 1999; Balloux and
15 Lugon-Moulin 2002). They can reveal detailed histories of range expansion, reduced population sizes, or genetic differentiation (e.g.

Goodman et al. 2001; Hänfling et al. 2002; Lugon-Moulin and Hausser 2002). Herein, genetic structures of populations based on hypervariable microsatellite DNA makers were compared between the two shrew species to investigate their biogeographical history within Hokkaido.

Methods

Animals examined and microsatellite DNA analysis

Specimens of *S. unguiculatus* and *S. caecutiens* captured on

Hokkaido mainland and adjacent islands from 1994 to 2002 were used for analysis (Tables 1 and 2; Fig. 1). A total of 475 individuals of *S. unguiculatus* from 20 locations on the mainland and four locations on four offshore islands and 240 individuals of *S. caecutiens* from 13 locations on the mainland were analysed (Tables 1 and 2; Fig. 1).

Five primer sets of microsatellite DNA loci (A6, B4, D2, L57 and

L62) were used for *S. unguiculatus* and six primer sets (A6, A10, B4, D2, D8, and E1) for *S. caecutiens*, based on Wyttenbach et al. (1997) and Naitoh et al. (2002).

Total DNA was extracted from liver or muscle tissues preserved
5 in 70-90% ethanol by the proteinase K/phenol/chloroform
(Sambrook et al. 1989) or Chelex-100 method (Walsh et al. 1991).

Polymerase chain reaction (PCR) amplification was carried out
according to Masuda and Yoshida (1994) or Naitoh et al. (2002), or
the method described as follows. The PCR was conducted in a 15 μ L

10 reaction mixture containing 1 mM MgSO_4 , 1 $\times$ PCR Buffer for KOD-
plus-, 0.2 mM dNTPs, 0.3 μ M of each primer, 0.3 U KOD -plus-
(Toyobo, Osaka) and 10 - 100 ng of total DNA using iCycler (BIO-RAD,
Hercules, CA). After denaturation at 94 $^{\circ}\text{C}$ for 2 minutes, and PCR
was performed for 35 cycles under following condition: 15 seconds
15 at 94 $^{\circ}\text{C}$, 30 seconds at 55 $^{\circ}\text{C}$, 30 seconds at 68 $^{\circ}\text{C}$. The PCR products
were sized by an ABI PRISM 310 Genetic Analyser, using

GENESCAN-500™ TAMRA (Applied Biosystems, Foster City, CA) or
GENOTYPE™ TAMRA 50-500 DNA Ladder (Invitrogen, Carlsbed, CA)
as a size standard.

5 Statistical analysis

Population genetic analyses were performed using GENEPOP 3.1c
(Raymond and Rousset 1995) and FSTAT 2.9.3 (Goudet 2001).

Genotypic linkage disequilibrium, departure from Hardy-Weinberg
equilibrium with Fisher's exact test, the number of alleles, and

10 expected heterozygosity were calculated by GENEPOP 3.1c. The allele
diversity among populations with unequal sample size was

compared by using allelic richness, which is the expected number of
alleles in 2N genes ("N" is fixed as the smallest number of sampled
individuals among compared populations in FSTAT; El Mousadik and

15 Petit 1996). To examine the relationship between island sizes and
genetic diversities, correlation between island sizes and allelic

richness or heterozygosity per site was tested by the Spearman rank correlation test. The degree of differentiation across all populations was quantified using estimator (θ) of F-statistics of Weir and Cockerham (1984) by FSTAT. Values of F_{ST} were tested for significant
5 departure from zero using 10,000 permutations. Standard errors of F_{ST} (θ) were calculated by the jackknife method over populations and loci, and a 95% confidence interval was generated by bootstrapping with 15,000 replications over loci. To show the genetic relationships among local populations, unrooted neighbour-joining trees on chord
10 distance (Cavalli-Sforza and Edwards 1967) were constructed by PHYLIP 3.6 (Felsenstein 2002).

To detect a recent bottleneck event in each local populations of the two shrew species, we used BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). Infinite allele model (IAM), stepwise mutation model
15 (SMM), and two-phased model of mutation (TPM) with values of 30 for the variance for TPM and 70% for the proportion of SMM in TPM

were applied as locus evolution models. Iteration time was 1,000.

Wilcoxon sign-rank test was conducted to test heterozygosity excess (Cornuet and Luikart 1996), because the test is most appropriate for the present data set (Luikart et al. 1998). Also, note that

5 microsatellite data generally better fit TPM than SMM or IAM (Di Rienzo et al 1994).

To know genetic structure of shrew populations, we inferred K (numbers of clusters or populations) by STRUCTURE 2.1 (Pritchard et al. 2000) using all microsatellite loci. For MCMC simulation,

10 Burn-in period and number of simulation were 10,000 and 100,000, respectively, following Pritchard et al. (1994). Several trials of computation with different parameters were conducted. Proportion of membership of each predefined local populations in each of K clusters was computed by STRUCTURE. In this analysis, 11 *S.*

15 *unguiculatus* individuals from southern Sakhalin (Nevel'sk) were also included. To estimate the spatial extent of a genetically related

population, spatial genetic autocorrelation analysis was conducted by GenAlEx version 5.1 (Peakall and Smouse 2001). Permutations were 999 and bootstrap replications were 1,000.

Correlation between genetic and geographic distances was tested
5 for mainland populations of both species by Mantel test (Mantel
1967). In general we used straight-line distance between a pair of
localities. However, distances between localities in southern
Hokkaido (#1 and #I, Mt. Daisengen; #2, Kaminokuni; #3, Mt.
Komagatake; Tables 1 and 2) and the others were measured via
10 Kuromatsunai (#4) to avoid underestimation. For example, distance
between #1 and #13 were calculated as the sum of that between #1
and #4 and that between #4 and #13. We derived genetic differences
as $F_{ST}/(1 - F_{ST})$ suggested by Rousset (1997) between all pairs of local
populations. We also used simple straight-line distances for analysis
15 but result was fundamentally the same as in the procedure above.

Results

General description of population genetics parameters

All pairs of loci were at genotypic linkage equilibrium in both species (*S. unguiculatus*, $P > 0.45$; *S. caecutiens*, $P > 0.87$). Genetic variation

5 was high on Hokkaido mainland; average numbers of allele per locus

and average expected heterozygosities were 11.5 and 0.90,

respectively in *S. unguiculatus* (Table 1) and 11.7 and 0.89 in *S.*

caecutiens (Table 2). In contrast, four island populations (Daikoku,

Teuri, Rebun, and, Rishiri Islands) of *S. unguiculatus* had lower

10 genetic variation than those of mainland populations: 4.4 alleles per

locus and average expected heterozygosity of 0.52 (Table 1).

Especially, loci D2 and L62 were monomorphic on Daikoku Island.

No unique alleles were observed in the four island populations.

The allelic richness showed a significant (but marginal) positive

15 correlation with island area size ($r = 1.0$, $P = 0.0455$; Fig. 2).

Correlation between heterozygosity and island size was positive,

though statistically non-significant but marginal ($r = 0.9$, $P = 0.0719$; Fig. 2).

There was significant genetic differentiation of local populations in *S. unguiculatus* and *S. caecutiens* for each and overall locus

5 (Tables 3 and 4). Genetic differentiation, $F_{ST}(\theta)$, over all loci was 0.019 among mainland localities of *S. unguiculatus* (Table 3). When insular populations were included, the value increased to 0.088 (Table 3). Among mainland populations of *S. caecutiens*, $F_{ST}(\theta)$ estimated over all loci was 0.022 and was similar to that on mainland
10 of *S. unguiculatus*.

Neighbor-joining trees

Concordance between the unrooted neighbour-joining (NJ) tree and geographic positions of populations was not clear in either species

15 (Figs. 1 and 3). In the NJ tree of *S. unguiculatus*, southern populations (Mt. Daisengen, Kaminokuni, Mt. Komagatake, Rankoshi,

and Abuta; Fig. 1 and Table 1) except Kuromatsunai were clustered but bootstrap value was rather low (57 %) (Figs. 1 and 3-A). The four island populations of *S. unguiculatus* were not connected directly with any of the nearest mainland populations examined (Fig. 3-A).

5 For instance, populations from Akkeshi (#17) and Daikoku Island (#21) were not directly connected. The neighbouring *S. caecutiens* populations in southern and central Hokkaido, Daisengen (#I) and Sapporo (# II), and Bibai (#IV) and Furano (#VII) were clustered together in NJ tree with very low (53 %) bootstrap value (Figs. 1 and
10 3-B).

Bottleneck analysis

In *S. unguiculatus*, heterozygosity excess with higher probability (>5%) was observed in all populations except Kuromatsunai and
15 Hokuryu under at least one of the three mutation models (Table 5).
In addition to the two populations from Kuromatsunai and Hokuryu,

four populations (Bibai, Haboro, Obihiro, and Shiretoko) showed non-significant heterozygosity excess under both of IAM and TPM mutation models (Table 5).

In *S. caecutiens*, significant heterozygosity excess was
5 observed (>5%) in 9 out of 13 populations under at least one of the three mutation models (Table 6). Four populations (Sapporo, Bibai, Akkeshi, and Shiretoko) did not show heterozygosity excess. In addition to the four, two populations (Monbetsu and Furano) showed non-significant heterozygosity excess under both of IAM and
10 TPM (Table 6).

Inferred numbers of clusters

Log likelihood values attained asymptote when K (inferred number of clusters) was approximately 14 for *S. unguiculatus* populations
15 including insular and Sakhalin populations, 10 for *S. unguiculatus* populations on Hokkaido mainland, and 9 for *S. caecutiens*

population (Fig. 4). The first value of K for asymptote of log likelihood denotes the number of clusters (populations) recognized statistically (Pritchard et al. 2000). Thus, inferred numbers of populations were 14, 10, and 9 for *S. unguiculatus* examined from Hokkaido and southern Sakhalin, *S. unguiculatus* from Hokkaido mainland, and *S. caecutiens* from Hokkaido, respectively, although these numbers are not strict ones.

In *S. unguiculatus*, proportions of membership of 25 predefined local populations in Hokkaido and southern Sakhalin in the 14 inferred clusters were calculated (Table 7). Large proportion (>69 %) of shrews from each of four offshore islands tended to be included in a unique cluster, respectively. Shrews from four predefined populations from the southern part (Mt. Daisengen, Mt. Komagatake, Rankoshi, and Abuta; refer to Fig. 1 and Table 1) tended to be included in one cluster (# 8) with relatively high proportion (>17%). However, shrews from other local populations

did not show clear clustering (Table 7).

In *S. caecutiens*, proportions of 13 predefined local populations in the 9 inferred clusters were calculated (Table 8). Many shrews (>20%) from Horonobe (#V) and Akkeshi (#XI) and those from Yufutsu (#III) and Akkeshi (#XI) were included in cluster # 2 and # 6, respectively. In general, however, explicit regional clustering was not found in *S. caecutiens* in Hokkaido (Table 8).

Spatial genetic autocorrelation

When distance size class was 50 km, autocorrelation coefficient was significant (95% confidence) for 50 km in both species and the first x-intercept was 151.2 km for *S. unguiculatus* and 92.6 km for *S. caecutiens* (Fig. 5). However, in *S. unguiculatus*, autocorrelation coefficient for 100 km was not significantly different from zero (95% confidence). Hence, for both species, significant autocorrelation was observed less than 100 km distance between local populations.

When distance size class was 100 km, the coefficient was significant for 100 km in both species, and the first x-intercept was 191.7 km for *S. unguiculatus* and 191.6 km for *S. caecutiens* (Fig. 5). Shortly summarized above, significant positive spatial autocorrelation was
5 observed less than approximately 100 km for both species.

Correlation between geographic and genetic distance

A significant positive correlation, though small, was observed between genetic and geographic distances in *S. unguiculatus* ($r =$
10 0.222; Mantel test, $P = 0.003$; Fig. 6), but not in *S. caecutiens* ($r =$
0.172; Mantel test $P = 0.1225$; Fig. 6).

Discussion

The genetic diversity based on the microsatellite markers that we
15 used in *S. unguiculatus* and *S. caecutiens* on Hokkaido mainland
(Tables 1-4) was similar to or higher than those of other

microsatellite markers in some other small mammals: e.g.,

Apodemus argenteus and *Clethrionomys rufocanus* (Ohnishi 2002),

Lepus americanus (Burton et al. 2002), and *Sorex araneus* (Lugon-

Moulin et al. 1999). Genetic diversification of the microsatellite DNA

5 was significant among local populations for the two shrew species in
Hokkaido (Tables 3 and 4).

Populations of *Sorex unguiculatus* on Hokkaido mainland were

not genetically structured in general (Table 7) although some

southern populations tended to be clustered together (Fig. 3). In

10 addition, nearby local populations were not always genetically close

(Fig. 3). It is somewhat unexpected, because *S. unguiculatus* is

abundant and its habitat is continuous and ubiquitous in Hokkaido

(Ohdachi and Maekawa 1990), suggesting frequent immigrations

may occur between nearby populations. Each local population might

15 be maintained by frequent extinctions and re-immigrations by a few

individuals from nearby populations, and thus genetic structure

might be different even between nearby populations due to genetic drift. Metapopulation scheme (Whitlock 2004) seems appropriate to investigate of genetic structure dynamics of *S. unguiculatus* population in Hokkaido.

5 In *S. unguiculatus* of Hokkaido mainland, the extent of positive (non-random) genetic structure was estimated as approximately 100 km (Fig. 5-A). In addition, a significant positive correlation was observed between geographic and genetic distances (Fig. 6-A). Thus, isolation by distance (IBD, Wright 1943) was observed to some
10 extent, which means that the degree of gene exchange decreases as distance between local populations increases. Isolation by distance arises from an equilibrium between gene flow and genetic drift in local populations (Hutchison and Templeton 1999).

 Populations of *Sorex caecutiens* in Hokkaido were not genetically
15 structured in general (Table 8), although shrews from the central and southern parts of Hokkaido showed weak clustering (Fig. 3-B) as

in *S. unguiculatus* populations in southern Hokkaido (Fig. 3-A).

Southern Hokkaido might have played a special role (e.g. refugium)

in biogeographic history of the two species. *Sorex caecutiens* showed

rather patchy distribution and is a predominant species only in

5 habitats with sandy soils or volcanic ashes on Hokkaido mainland

while *S. unguiculatus* occurs ubiquitously and is a predominant in

most habitat types (Ohdachi and Maekawa 1990). Thus, it is

expected that *S. caecutiens* had larger genetic differentiation

between local populations and higher F_{ST} values than *S. unguiculatus*.

10 However, F_{ST} was not different from that of *S. unguiculatus* (Tables 3

and 4). Immigration rate among local populations of *S. caecutiens*

might be higher than we expected from the distribution pattern, and

thus it has rather even genetic structures among localities in

Hokkaido.

15 In *S. caecutiens*, the extent of positive (non-random) genetic

structure was estimated as approximately 100 km (Fig. 5-B).

However, isolation by distance was not confirmed although there was a tendency of positive correlation (Fig. 6-B). The small sample size (13 localities) for this species might have resulted in non-significant correlation.

5 Heterozygosity excess with high probability ($>5\%$) was observed in a majority of populations for both of *S. unguiculatus* and *S. caecutiens* in Hokkaido (Tables 5 and 6). A population that has experienced a recent reduction of effective population size exhibits reduction of both allele numbers and heterozygosity but the allele
10 numbers is reduced faster than the heterozygosity (Cornuet and Luikart 1996). Thus, observed heterozygosity is higher than expected equilibrium heterozygosity in a bottlenecked population. In other words, a population with heterozygosity excess is supposed to have experienced a recent bottleneck event when mutation-drift
15 equilibrium is assumed (Luikart et al 1998). Therefore, it is suggested that not only insular populations of *S. unguiculatus*, but

also many populations of the two shrew species on Hokkaido mainland have experienced recent population reduction. In populations of *S. unguiculatus* and *S. caecutiens*, bottleneck events may frequently occur in Hokkaido. Hence, populations of the shrews in Hokkaido might be maintained by frequent local extinctions and re-immigrations of a few individuals from nearby habitats.

Metapopulation theory (Hanski 1999) could be applied to describe population dynamics of these shrews.

Based on haplotype of mtDNA *cytb* (Ohdachi et al. 2001, 2003)

and repetype (repetitive type) of restriction fragment length polymorphism of the nuclear rDNA spacer region (Naitoh et al. 2005), Hokkaido population of *S. caecutiens* is genetically distant from Eurasian and Sakhalin populations, while the genetic variation within the Eurasian Continent is very small in the mtDNA and nuclear rDNA analyses. On the other hand, *S. unguiculatus* shows no regional differentiation of the mtDNA haplotype among populations

throughout its whole range (Hokkaido to Northeastern Asian
Continent) (Ohdachi et al. 2001). This suggests that *S. caecutiens*
colonised into Hokkaido rather earlier than *S. unguiculatus*. Thus,
we expected that *S. caecutiens* genetically more structured than *S.*
5 *unguiculatus* as the former species occurred in Hokkaido earlier
than the latter and that clear local genetic clustering was observed in
S. caecutiens. The microsatellite analysis of the present study,
however, indicates that genetic structure was not substantially
different between *S. caecutiens* and *S. unguiculatus* and both species
10 did not have clear local clustering among populations within
Hokkaido mainland. Therefore, the results of the microsatellite DNA
analysis did not support the prediction based on mtDNA, although
there was no contradiction between them. The microsatellite
markers seem not to be good indicators to investigate to trace
15 biogeographic history for the two *Sorex* species in Hokkaido
although they are useful to describe their population genetic

dynamics.

Genetic structure was not significantly different even between populations on Hokkaido mainland and a Sakhalin population in *S. unguiculatus* (Table 7). However, each of the four offshore island

5 populations in *S. unguiculatus* had unique genetic structure and was genetically quite distant from those on Hokkaido mainland (Fig. 3-A). This is mainly caused by loss of genetic diversity on the small insular populations (Tables 1 and 3). In addition, there was a positive correlation between island size and allelic richness (Fig. 2).
10 The larger the island size is, the greater the genetic diversity.

The latest isolation periods of some islands from Hokkaido mainland were estimated by Ohshima (1990) and Igarashi (2000), based on present sea bottom depth and sea level change (Table 9). In addition, we also estimated the isolation periods for some other

15 islands based on the maximum depth of sea bottom (Japan Hydrograph Association, 2005 version, Tokyo; chart codes, W1045,

W1040, W26) and sea level change (Shackleton 1987). At a glance, there seems to be a positive correlation between genetic diversity of *S. unguiculatus* and isolation period from Hokkaido mainland (Tables 1 and 9). However, this is an apparent correlation because
5 smaller islands tend to separate more recently than larger ones (Table 9). The microsatellite loci lose allele numbers very rapidly if population size is small although isolation period is short (e.g., see Daikoku Island).

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FIGURE LEGENDS

Fig. 1 Sample locations for *S. unguiculatus* (A) and *S. caecutiens* (B) in Hokkaido. Location numbers are indicated by Arabic and Roman numerals (see Tables 1 and 2). Shaded region indicates the distribution of each species.

Fig. 2 Correlations between area of Hokkaido mainland and adjacent four islands and genetic diversity (allelic richness, A , and heterozygosity, H_E) in *Sorex unguiculatus*. Average number of allele and average heterozygosity of the mainland is the values of all sites combined. Results of Spearman's rank correlation test are shown in each graph.

Fig. 3 Unrooted neighbour joining tree based on Cavalli-Sforza chord distance, analysing five microsatellite loci in *Sorex unguiculatus* (A) and six microsatellite loci in *S. caecutiens* (B) in Hokkaido (see Tables 1 and 2 for locality numbers). The length of tree branches is relative to the genetic distances (note scale).

Bootstrap values are indicated for nodes for $\geq 50\%$ support (100 replicates).

Fig. 4 Relationship between log likelihood and the value of K , the number of populations, for *Sorex unguiculatus* from Hokkaido mainland, 4 offshore islands, and Sakhalin (A), *S. unguiculatus* on Hokkaido mainland (B) and *S. caecutiens* on Hokkaido mainland (C), based on microsatellite loci. Arrows denote the first values of K for asymptotes, thus inferred numbers of populations (Pritchard et al. 2000).

Fig. 5 Correlograms showing r (autocorrelation coefficient) as a function of distance between populations (km), 95% CI about the null hypothesis of a random distribution, and 95% confidence bars calculated by bootstrapping (1000 replications) for *Sorex unguiculatus* (A) and *S. caecutiens* (B) on Hokkaido mainland, based on microsatellite loci. The first x-intercepts,

which provide estimates of the extent of nonrandom genetic structure (Peakall et al. 2003), were indicated.

Fig. 6 Correlation between geographic ($\ln$ km) and genetic distances ($F/(1-F)$) among local populations in *Sorex unguiculatus* (A) and *S. caecutiens* (B) on Hokkaido mainland. Results of Mantel tests are shown top-right on each graph.

5

Table 1 Genetic diversity of *Sorex unguiculatus*. Five loci (A6, B4, D2, L57, and L62) were used. Sampling locality (#), number of shrews analysed (N), average number of alleles per locus (A) and average expected heterozygosity (H_E) are given. Ranges are indicated in parentheses for average number of alleles and heterozygosity

#	Locality	N	Sampling year	A	H_E
Mainland					
1	Mt.Daisengen	14	2001	10.2 (7-11)	0.89 (0.84-0.92)
2	Kaminokuni	26	2001	12.2 (11-13)	0.88 (0.87-0.89)
3	Mt. Komagatake	25	2002	13.0 (11-16)	0.89 (0.83-0.94)
4	Kuromatsunai	9	1997	8.4 (7-9)	0.90 (0.89-0.90)
5	Rankoshi	25	2002	13.4 (12-15)	0.89 (0.87-0.91)
6	Sapporo	21	1996	12.6 (11-14)	0.87 (0.79-0.91)
7	Abuta	25	2001	11.6 (11-13)	0.86 (0.81-0.90)
8	Bibai	49	1998	15.8 (13-18)	0.90 (0.88-0.92)
9	Hokuryu	22	1997	10.8 (10-11)	0.90 (0.88-0.91)
10	Haboro	15	1997	10.0 (7-13)	0.88 (0.79-0.93)
11	Sarobetsu	15	1994, 1995, 1998	11.2 (9-15)	0.88 (0.85-0.91)
12	Furano	26	1997	14.4 (13-15)	0.90 (0.87-0.92)
13	Samani	14	1997	10.2 (8-12)	0.89 (0.87-0.91)
14	Obihiro	25	2002	12.6 (10-15)	0.89 (0.85-0.91)
15	Bihoro	20	1999	11.6 (11-13)	0.89 (0.83-0.92)
16	Bihoro-Tohge	11	1999	10.2 (8-12)	0.90 (0.86-0.94)
17	Akkeshi	15	1998	10.6 (9-13)	0.89 (0.86-0.91)
18	Hamanaka	15	2001	9.0 (7-12)	0.85 (0.79-0.90)
19	Shiretoko	15	1995	11.0 (7-13)	0.91 (0.87-0.92)
20	Nemuro	15	1997, 1999	11.4 (8-14)	0.89 (0.83-0.93)
subtotal	All sites combined	402		22.6 (19-26)	0.90
	Average	20.10		11.5 (7-18)	0.90
Island					
21	Daikoku Is.	13	1995, 1999	2.2 (1-4)	0.46 (0.00-0.70)
22	Teuri Is.	20	1996, 1997, 1998	2.6 (2-4)	0.37 (0.05-0.53)
23	Rebun Is.	20	1996, 1997	4.8 (2-7)	0.52 (0.05-0.83)
24	Rishiri Is.	20	1994, 1995	7.8 (6-10)	0.73 (0.64-0.84)
subtotal	All sites combined	73		10.0 (6-13)	0.79
	Average	18.25		4.4 (1-10)	0.52
Total					
	All sites combined	475		22.6(19-29)	0.90
	Average	19.79		10.3 (1-18)	0.83

Table 2 Genetic diversity of *Sorex caecutiens*. Six loci (A6, A10, B4, D2, D8, and E1) were used. See Table 1 for further explanation

#	Locality	N	Sampling year	A	HE
I	Mt.Daisengen	15	2001	13.0 (12-14)	0.92 (0.90-0.93)
II	Sapporo	13	1996	10.3 (9-14)	0.90 (0.86-0.94)
III	Yufutsu	24	1997	12.8 (10-16)	0.88 (0.87-0.90)
IV	Bibai	42	1998	17.8 (15-27)	0.92 (0.91-0.95)
V	Horonobe	9	1998	7.0 (5-9)	0.82 (0.72-0.91)
VI	Sarobetsu	12	1994, 1995, 1998	10.2 (8-14)	0.89 (0.83-0.93)
VII	Furano	27	2001	15.0 (12-21)	0.92 (0.89-0.95)
VIII	Obihiro	17	2002	10.0 (6-14)	0.87 (0.80-0.92)
IX	Monbetsu	13	2002	11.2 (9-13)	0.91 (0.87-0.95)
X	Bihoro	10	1999	9.8 (7-12)	0.90 (0.83-0.94)
XI	Akkeshi	11	1998	8.5 (7-10)	0.88 (0.81-0.92)
XII	Shiretoko	13	1995	11.8 (8-14)	0.92 (0.87-0.95)
XIII	Nemuro	34	2001	14.8 (12-17)	0.90 (0.88-0.92)
	All sites combined	240		24.8 (21-32)	0.92
	Average per site	18.46		11.7 (5-27)	0.89

Table 3 Genetic differentiation in *Sorex unguiculatus* in Hokkaido. Each locus and overall $F_{ST}(\theta)$ and standard error (in parentheses) are given, with 95% confidence interval (CI) of the overall value

	Excluding Islands	Including Islands
Locus	$F_{ST}(\theta)$	$F_{ST}(\theta)$
A6	0.009** (0.004)	0.047** (0.020)
B4	0.027** (0.005)	0.092** (0.034)
D2	0.025** (0.007)	0.117** (0.051)
L62	0.026** (0.006)	0.114** (0.047)
L57	0.008** (0.004)	0.063** (0.033)
Overall	0.019** (0.004)	0.088** (0.014)
95% CI	0.012-0.026	0.064-0.113

Results of permutation testing of significant departure from zero are also given (** $p < 0.001$)

Table 4 Genetic differentiation in *Sorex caecutiens* in Hokkaido. See Table 3 for further explanation

Locus	$F_{ST} (\theta)$
A6	0.013** (0.007)
A10	0.011** (0.007)
B4	0.023** (0.008)
D2	0.035** (0.013)
D8	0.022** (0.004)
E1	0.026** (0.011)
Overall	0.022** (0.004)
95% CI	0.016-0.029

Results of permutation testing of significant departure from zero are also given (** $p < 0.001$)

Table 5 Probability of heterozygosity excess by Wilcoxon sign-rank test under tree mutation models in *Sorex unguiculatus* from Hokkaido. Locality numbers correspond with those in Fig. 1 and

Table 1. IAM: Infinite allele model. SMM: Stepwise mutation

model. TPM: Two-phased model of mutation

		IAM	SMM	TPM
1	Mt.Daisengen	0.031	0.313	0.109
2	Kaminokuni	0.016	0.922	0.078
3	Mt. Komagatake	0.031	0.688	0.406
4	Kuromatsunai	0.015	0.031	0.016
5	Rankoshi	0.016	0.984	0.109
6	Sapporo	0.313	1.000	0.688
7	Abuta	0.078	0.891	0.313
8	Bibai	0.016	0.984	0.016
9	Hokuryu	0.016	0.016	0.016
10	Haboro	0.016	0.688	0.016
11	Sarobetsu	0.078	0.953	0.594
12	Furano	0.016	0.984	0.406
13	Samani	0.047	0.594	0.109
14	Obihiro	0.016	0.922	0.047
15	Bihoro	0.031	0.500	0.313
16	Bihoro-Tohge	0.047	0.500	0.109
17	Akkeshi	0.109	0.594	0.313
18	Hamanaka	0.109	0.953	0.594
19	Shiretoko	0.016	0.078	0.031
20	Nemuro	0.078	0.688	0.406
21	Daikoku Is.	0.188	0.875	0.188
22	Teuri Is.	0.313	0.594	0.406
23	Rebun Is.	0.500	0.969	0.891
24	Rishiri Is.	0.500	1.000	1.000

Table 6 Probability of heterozygosity excess by Wilcoxon sign-rank test under tree mutation models in *Sorex caecutiens* from Hokkaido. Locality numbers correspond with those in Fig. 1 and Table 2. IAM: Infinite allele model. SMM: Stepwise mutation model. TPM: Two-phased model of mutation

		IAM	SMM	TPM
I	Mt.Daisengen	0.008	0.781	0.078
II	Sapporo	0.008	0.039	0.008
III	Yufutsu	0.008	0.977	0.422
IV	Bibai	0.008	0.008	0.008
V	Monbetsu	0.008	0.422	0.023
VI	Horonobe	0.016	0.719	0.078
VII	Sarobetsu	0.281	0.578	0.500
VIII	Furano	0.008	0.281	0.008
IX	Obihiro	0.008	0.281	0.061
X	Bihoro	0.039	0.656	0.295
XI	Akkeshi	0.008	0.039	0.008
XII	Shiretoko	0.008	0.008	0.008
XIII	Nemuro	0.008	0.977	0.281

Table 7 Proportion of membership of each of 25 predefined local population in each of 14 inferred clusters in *Sorex unguiculatus* from Hokkaido and one locality in Sakhalin. Locality numbers correspond with those in Fig. 1 and Table 1. Eleven individuals from Nevel'sk (southern Sakhalin) were included in this analysis. Values with single underlines are of relatively high probability (>15%) and those with double underlines of small island populations

inferred cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14
locality														
1 Mt.Daisengen	0.058	0.060	0.098	0.067	0.017	0.068	0.058	<u>0.198</u>	0.033	0.015	0.049	0.058	0.049	0.173
2 Kaminokuni	0.092	0.102	0.073	0.138	0.014	0.101	0.021	0.093	0.011	0.023	0.084	0.094	0.090	0.062
3 Mt. Komagatake	0.057	0.071	0.073	0.066	0.030	0.075	0.089	<u>0.207</u>	0.015	0.022	0.061	0.062	0.066	0.105
4 Kuromatsunai	0.093	0.094	0.097	0.107	0.011	0.088	0.029	0.054	0.038	0.013	0.118	0.092	0.106	0.062
5 Rankoshi	0.067	0.069	0.095	0.073	0.022	0.075	0.048	<u>0.176</u>	0.017	0.019	0.063	0.065	0.065	0.146
6 Sapporo	0.097	0.085	0.091	0.094	0.015	0.104	0.025	0.078	0.040	0.023	0.098	0.088	0.090	0.072
7 Abuta	0.085	0.089	0.073	0.086	0.027	0.095	0.022	<u>0.208</u>	0.007	0.018	0.071	0.073	0.078	0.067
8 Bibai	0.099	0.095	0.100	0.082	0.017	0.096	0.025	0.061	0.016	0.026	0.096	0.098	0.097	0.090
9 Hokuryu	0.100	0.092	0.090	0.087	0.022	0.091	0.060	0.041	0.024	0.038	0.092	0.100	0.092	0.072
10 Haboro	0.099	0.098	0.073	0.069	0.022	0.099	0.033	0.104	0.025	0.020	0.106	0.099	0.105	0.049
11 Sarobetsu	0.098	0.086	0.093	0.080	0.018	0.093	0.034	0.078	0.032	0.019	0.097	0.099	0.094	0.080
12 Furano	0.092	0.093	0.092	0.079	0.026	0.090	0.033	0.070	0.021	0.020	0.102	0.099	0.095	0.089
13 Samani	0.097	0.105	0.091	0.099	0.014	0.092	0.024	0.048	0.014	0.050	0.104	0.098	0.103	0.061
14 Obihiro	0.083	0.088	0.086	0.116	0.035	0.085	0.045	0.099	0.012	0.011	0.093	0.084	0.087	0.075
15 Bihoro	0.074	0.082	0.113	0.070	0.020	0.075	0.048	0.076	0.031	0.013	0.080	0.086	0.082	0.152
16 Bihoro-Tohge	0.089	0.085	0.099	0.075	0.049	0.086	0.027	0.057	0.034	0.016	0.092	0.088	0.090	0.112
17 Akkeshi	0.098	0.087	0.096	0.081	0.021	0.088	0.043	0.045	0.025	0.034	0.097	0.091	0.089	0.104
18 Hamanaka	0.092	0.096	0.079	0.110	0.032	0.085	0.038	0.053	0.060	0.024	0.086	0.095	0.095	0.055
19 Shiretoko	0.101	0.099	0.086	0.101	0.020	0.094	0.036	0.037	0.041	0.017	0.099	0.108	0.102	0.056
20 Nemuro	0.102	0.092	0.082	0.091	0.045	0.092	0.038	0.032	0.055	0.031	0.090	0.094	0.093	0.064
21 Daikoku Is.	0.006	0.006	0.006	0.006	0.004	0.006	0.006	0.006	<u>0.924</u>	0.005	0.006	0.006	0.006	0.006
22 Teuri Is.	0.006	0.006	0.006	0.006	0.010	0.006	0.006	0.006	0.007	<u>0.918</u>	0.006	0.006	0.006	0.006
23 Rebun Is.	0.007	0.008	0.008	0.007	<u>0.862</u>	0.007	0.045	0.008	0.009	0.009	0.007	0.007	0.008	0.008
24 Rishiri Is.	0.013	0.013	0.014	0.013	0.058	0.013	<u>0.692</u>	0.010	0.088	0.032	0.014	0.014	0.014	0.013
25 Nevel'sk (Sakhalin)	0.090	0.082	0.073	0.074	0.024	0.090	0.112	0.045	0.071	0.016	0.094	0.088	0.089	0.053

Table 8 Proportion of membership of each of 13 predefined local population in each of 9 inferred clusters in *Sorex caecutiens* from Hokkaido. Locality numbers correspond with those in Fig. 1 and Table 2

		inferred cluster								
		1	2	3	4	5	6	7	8	9
locality										
I	Mt.Daisengen	0.091	0.075	0.122	0.118	0.134	0.080	0.134	0.119	0.128
II	Sapporo	0.080	0.093	0.118	0.120	0.123	0.082	0.130	0.126	0.128
III	Yufutsu	0.065	0.109	0.099	0.084	0.105	0.209	0.141	0.098	0.090
IV	Bibai	0.084	0.099	0.121	0.105	0.128	0.108	0.115	0.128	0.113
IX	Monbetsu	0.145	0.070	0.121	0.106	0.122	0.115	0.109	0.107	0.104
V	Horonobe	0.085	0.294	0.075	0.119	0.054	0.112	0.067	0.092	0.101
VI	Sarobetsu	0.116	0.129	0.101	0.117	0.111	0.110	0.107	0.099	0.111
VII	Furano	0.110	0.076	0.126	0.111	0.127	0.091	0.106	0.125	0.127
VIII	Obihiro	0.066	0.192	0.117	0.176	0.083	0.097	0.122	0.080	0.067
X	Bihoro	0.198	0.082	0.113	0.108	0.107	0.062	0.091	0.104	0.135
XI	Akkeshi	0.048	0.200	0.082	0.079	0.081	0.235	0.105	0.098	0.072
XII	Shiretoko	0.110	0.120	0.118	0.105	0.122	0.111	0.105	0.117	0.092
XIII	Nemuro	0.213	0.077	0.102	0.113	0.088	0.062	0.094	0.105	0.147

Table 9 Estimated periods of the latest separation of 11 islands from Hokkaido mainland (10^3 years BP), maximum depth (m) of the present sea bottom from Hokkaido mainland, and area (km^2). Islands in italic are those without *Sorex unguiculatus*

	isolation period (10^3 YBP)	max depth (m)	area size (km^2)
<i>Okushiri</i>	>200 ?	400	142.9
<i>Honshu</i>	150-140	140	227,415.0
Rebun & Rishiri*	13	85	81.0 & 182.1
Sakhalin (= Karafuto)	12-11	70	76,405.0
Teuri & <i>Yagishiri</i> *	11	55	5.5 & 5.2
Shikotan & Habomai arc.	8	35	255.1 & -
Kunashiri	7	20	1,498.5
Daikoku*	5	5	1.1
Hokkaido mainland	-	-	78,422.7

*estimated from the present depth of sea bottom and sea level change by Shackleton (1987). Estimation for other islands was based on Ohshima (1990) and Igarashi (2000).

Fig. 1. Naitoh & Ohdachi

A. Allelic richness

B. Average heterozygosity

A. *S. unguiculatus*

B. *S. caecutiens*

Fig. 3. Naitoh & Ohdachi

A. *S. unguiculatus* including 4 insular populations and one Sakhalin population

B. *S. unguiculatus* on Hokkaido mainland

C. *S. caecutiens*

A. *S. unguicualtus*

1. Distance size class = 50 km

2. Distance size class = 100 km

B. *S. caecuetiens*

1. Distance size class = 50 km

2. Distance size class = 100 km

A. *S. unguiculatus*

B. *S. caecutiens*

Geographic distance ($\ln$ km)

Table 1 Genetic diversity of *Sorex unguiculatus*. Five loci (A6, B4, D2, L57, and L62) were used. Sampling locality (#), number of shrews analysed (N), average number of alleles per locus (A) and average expected heterozygosity (H_E) are given. Ranges are indicated in parentheses for average number of alleles and heterozygosity

#	Locality	N	Sampling year	A	H_E
Mainland					
1	Mt.Daisengen	14	2001	10.2 (7-11)	0.89 (0.84-0.92)
2	Kaminokuni	26	2001	12.2 (11-13)	0.88 (0.87-0.89)
3	Mt. Komagatake	25	2002	13.0 (11-16)	0.89 (0.83-0.94)
4	Kuromatsunai	9	1997	8.4 (7-9)	0.90 (0.89-0.90)
5	Rankoshi	25	2002	13.4 (12-15)	0.89 (0.87-0.91)
6	Sapporo	21	1996	12.6 (11-14)	0.87 (0.79-0.91)
7	Abuta	25	2001	11.6 (11-13)	0.86 (0.81-0.90)
8	Bibai	49	1998	15.8 (13-18)	0.90 (0.88-0.92)
9	Hokuryu	22	1997	10.8 (10-11)	0.90 (0.88-0.91)
10	Haboro	15	1997	10.0 (7-13)	0.88 (0.79-0.93)
11	Sarobetsu	15	1994, 1995, 1998	11.2 (9-15)	0.88 (0.85-0.91)
12	Furano	26	1997	14.4 (13-15)	0.90 (0.87-0.92)
13	Samani	14	1997	10.2 (8-12)	0.89 (0.87-0.91)
14	Obihiro	25	2002	12.6 (10-15)	0.89 (0.85-0.91)
15	Bihoro	20	1999	11.6 (11-13)	0.89 (0.83-0.92)
16	Bihoro-Tohge	11	1999	10.2 (8-12)	0.90 (0.86-0.94)
17	Akkeshi	15	1998	10.6 (9-13)	0.89 (0.86-0.91)
18	Hamanaka	15	2001	9.0 (7-12)	0.85 (0.79-0.90)
19	Shiretoko	15	1995	11.0 (7-13)	0.91 (0.87-0.92)
20	Nemuro	15	1997, 1999	11.4 (8-14)	0.89 (0.83-0.93)
subtotal	All sites combined	402		22.6 (19-26)	0.90
	Average	20.10		11.5 (7-18)	0.90
Island					
21	Daikoku Is.	13	1995, 1999	2.2 (1-4)	0.46 (0.00-0.70)
22	Teuri Is.	20	1996, 1997, 1998	2.6 (2-4)	0.37 (0.05-0.53)
23	Rebun Is.	20	1996, 1997	4.8 (2-7)	0.52 (0.05-0.83)
24	Rishiri Is.	20	1994, 1995	7.8 (6-10)	0.73 (0.64-0.84)
subtotal	All sites combined	73		10.0 (6-13)	0.79
	Average	18.25		4.4 (1-10)	0.52
Total					
	All sites combined	475		22.6(19-29)	0.90
	Average	19.79		10.3 (1-18)	0.83

Table 2 Genetic diversity of *Sorex caecutiens*. Six loci (A6, A10, B4, D2, D8, and E1) were used. See Table 1 for further explanation

#	Locality	N	Sampling year	A	HE
I	Mt.Daisengen	15	2001	13.0 (12-14)	0.92 (0.90-0.93)
II	Sapporo	13	1996	10.3 (9-14)	0.90 (0.86-0.94)
III	Yufutsu	24	1997	12.8 (10-16)	0.88 (0.87-0.90)
IV	Bibai	42	1998	17.8 (15-27)	0.92 (0.91-0.95)
V	Horonobe	9	1998	7.0 (5-9)	0.82 (0.72-0.91)
VI	Sarobetsu	12	1994, 1995, 1998	10.2 (8-14)	0.89 (0.83-0.93)
VII	Furano	27	2001	15.0 (12-21)	0.92 (0.89-0.95)
VIII	Obihiro	17	2002	10.0 (6-14)	0.87 (0.80-0.92)
IX	Monbetsu	13	2002	11.2 (9-13)	0.91 (0.87-0.95)
X	Bihoro	10	1999	9.8 (7-12)	0.90 (0.83-0.94)
XI	Akkeshi	11	1998	8.5 (7-10)	0.88 (0.81-0.92)
XII	Shiretoko	13	1995	11.8 (8-14)	0.92 (0.87-0.95)
XIII	Nemuro	34	2001	14.8 (12-17)	0.90 (0.88-0.92)
	All sites combined	240		24.8 (21-32)	0.92
	Average per site	18.46		11.7 (5-27)	0.89

Table 3 Genetic differentiation in *Sorex unguiculatus* in Hokkaido. Each locus and overall $F_{ST}(\theta)$ and standard error (in parentheses) are given, with 95% confidence interval (CI) of the overall value

	Excluding Islands	Including Islands
Locus	$F_{ST}(\theta)$	$F_{ST}(\theta)$
A6	0.009** (0.004)	0.047** (0.020)
B4	0.027** (0.005)	0.092** (0.034)
D2	0.025** (0.007)	0.117** (0.051)
L62	0.026** (0.006)	0.114** (0.047)
L57	0.008** (0.004)	0.063** (0.033)
Overall	0.019** (0.004)	0.088** (0.014)
95% CI	0.012-0.026	0.064-0.113

Results of permutation testing of significant departure from zero are also given (** $p < 0.001$)

Table 4 Genetic differentiation in *Sorex caecutiens* in Hokkaido. See Table 3 for further explanation

Locus	$F_{ST} (\theta)$
A6	0.013** (0.007)
A10	0.011** (0.007)
B4	0.023** (0.008)
D2	0.035** (0.013)
D8	0.022** (0.004)
E1	0.026** (0.011)
Overall	0.022** (0.004)
95% CI	0.016-0.029

Results of permutation testing of significant departure from zero are also given (** $p < 0.001$)

Table 5 Probability of heterozygosity excess by Wilcoxon sign-rank test under three mutation models in *Sorex unguiculatus* from Hokkaido. Locality numbers correspond with those in Fig. 1 and Table 1. IAM: Infinite allele model. SMM: Stepwise

mutation model. TPM: Two-phased model of mutation

		IAM	SMM	TPM
1	Mt.Daisengen	0.031	0.313	0.109
2	Kaminokuni	0.016	0.922	0.078
3	Mt. Komagatake	0.031	0.688	0.406
4	Kuromatsunai	0.015	0.031	0.016
5	Rankoshi	0.016	0.984	0.109
6	Sapporo	0.313	1.000	0.688
7	Abuta	0.078	0.891	0.313
8	Bibai	0.016	0.984	0.016
9	Hokuryu	0.016	0.016	0.016
10	Haboro	0.016	0.688	0.016
11	Sarobetsu	0.078	0.953	0.594
12	Furano	0.016	0.984	0.406
13	Samani	0.047	0.594	0.109
14	Obihiro	0.016	0.922	0.047
15	Bihoro	0.031	0.500	0.313
16	Bihoro-Tohge	0.047	0.500	0.109
17	Akkeshi	0.109	0.594	0.313
18	Hamanaka	0.109	0.953	0.594
19	Shiretoko	0.016	0.078	0.031
20	Nemuro	0.078	0.688	0.406
21	Daikoku Is.	0.188	0.875	0.188
22	Teuri Is.	0.313	0.594	0.406
23	Rebun Is.	0.500	0.969	0.891
24	Rishiri Is.	0.500	1.000	1.000

Table 6 Probability of heterozygosity excess by Wilcoxon sign-rank test under tree mutation models in *Sorex caecutiens* from Hokkaido. Locality numbers correspond with those in Fig. 1 and Table 2. IAM: Infinite allele model. SMM: Stepwise mutation model. TPM: Two-phased model of mutation

		IAM	SMM	TPM
I	Mt.Daisengen	0.008	0.781	0.078
II	Sapporo	0.008	0.039	0.008
III	Yufutsu	0.008	0.977	0.422
IV	Bibai	0.008	0.008	0.008
V	Monbetsu	0.008	0.422	0.023
VI	Horonobe	0.016	0.719	0.078
VII	Sarobetsu	0.281	0.578	0.500
VIII	Furano	0.008	0.281	0.008
IX	Obihiro	0.008	0.281	0.061
X	Bihoro	0.039	0.656	0.295
XI	Akkeshi	0.008	0.039	0.008
XII	Shiretoko	0.008	0.008	0.008
XIII	Nemuro	0.008	0.977	0.281

Table 7 Proportion of membership of each of 25 predefined local population in each of 14 inferred clusters in *Sorex unguiculatus* from Hokkaido and one locality in Sakhalin. Locality numbers correspond with those in Fig. 1 and Table 1. Eleven individuals from Nevel'sk (southern Sakhalin) were included in this analysis. Values with single underlines are of relatively high probability (>15%) and those with double underlines of small island populations

inferred cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14
locality														
1 Mt.Daisengen	0.058	0.060	0.098	0.067	0.017	0.068	0.058	<u>0.198</u>	0.033	0.015	0.049	0.058	0.049	0.173
2 Kaminokuni	0.092	0.102	0.073	0.138	0.014	0.101	0.021	0.093	0.011	0.023	0.084	0.094	0.090	0.062
3 Mt. Komagatake	0.057	0.071	0.073	0.066	0.030	0.075	0.089	<u>0.207</u>	0.015	0.022	0.061	0.062	0.066	0.105
4 Kuromatsunai	0.093	0.094	0.097	0.107	0.011	0.088	0.029	0.054	0.038	0.013	0.118	0.092	0.106	0.062
5 Rankoshi	0.067	0.069	0.095	0.073	0.022	0.075	0.048	<u>0.176</u>	0.017	0.019	0.063	0.065	0.065	0.146
6 Sapporo	0.097	0.085	0.091	0.094	0.015	0.104	0.025	0.078	0.040	0.023	0.098	0.088	0.090	0.072
7 Abuta	0.085	0.089	0.073	0.086	0.027	0.095	0.022	<u>0.208</u>	0.007	0.018	0.071	0.073	0.078	0.067
8 Bibai	0.099	0.095	0.100	0.082	0.017	0.096	0.025	0.061	0.016	0.026	0.096	0.098	0.097	0.090
9 Hokuryu	0.100	0.092	0.090	0.087	0.022	0.091	0.060	0.041	0.024	0.038	0.092	0.100	0.092	0.072
10 Haboro	0.099	0.098	0.073	0.069	0.022	0.099	0.033	0.104	0.025	0.020	0.106	0.099	0.105	0.049
11 Sarobetsu	0.098	0.086	0.093	0.080	0.018	0.093	0.034	0.078	0.032	0.019	0.097	0.099	0.094	0.080
12 Furano	0.092	0.093	0.092	0.079	0.026	0.090	0.033	0.070	0.021	0.020	0.102	0.099	0.095	0.089
13 Samani	0.097	0.105	0.091	0.099	0.014	0.092	0.024	0.048	0.014	0.050	0.104	0.098	0.103	0.061
14 Obihiro	0.083	0.088	0.086	0.116	0.035	0.085	0.045	0.099	0.012	0.011	0.093	0.084	0.087	0.075
15 Bihoro	0.074	0.082	0.113	0.070	0.020	0.075	0.048	0.076	0.031	0.013	0.080	0.086	0.082	0.152
16 Bihoro-Tohge	0.089	0.085	0.099	0.075	0.049	0.086	0.027	0.057	0.034	0.016	0.092	0.088	0.090	0.112
17 Akkeshi	0.098	0.087	0.096	0.081	0.021	0.088	0.043	0.045	0.025	0.034	0.097	0.091	0.089	0.104
18 Hamanaka	0.092	0.096	0.079	0.110	0.032	0.085	0.038	0.053	0.060	0.024	0.086	0.095	0.095	0.055
19 Shiretoko	0.101	0.099	0.086	0.101	0.020	0.094	0.036	0.037	0.041	0.017	0.099	0.108	0.102	0.056
20 Nemuro	0.102	0.092	0.082	0.091	0.045	0.092	0.038	0.032	0.055	0.031	0.090	0.094	0.093	0.064
21 Daikoku Is.	0.006	0.006	0.006	0.006	0.004	0.006	0.006	0.006	<u>0.924</u>	0.005	0.006	0.006	0.006	0.006
22 Teuri Is.	0.006	0.006	0.006	0.006	0.010	0.006	0.006	0.006	0.007	<u>0.918</u>	0.006	0.006	0.006	0.006
23 Rebun Is.	0.007	0.008	0.008	0.007	<u>0.862</u>	0.007	0.045	0.008	0.009	0.009	0.007	0.007	0.008	0.008
24 Rishiri Is.	0.013	0.013	0.014	0.013	0.058	0.013	<u>0.692</u>	0.010	0.088	0.032	0.014	0.014	0.014	0.013
25 Nevel'sk (Sakhalin)	0.090	0.082	0.073	0.074	0.024	0.090	0.112	0.045	0.071	0.016	0.094	0.088	0.089	0.053

Table 8 Proportion of membership of each of 13 predefined local population in each of 9 inferred clusters in *Sorex caecutiens* from Hokkaido. Locality numbers correspond with those in Fig. 1 and Table 2

		inferred cluster								
		1	2	3	4	5	6	7	8	9
locality										
I	Mt.Daisengen	0.091	0.075	0.122	0.118	0.134	0.080	0.134	0.119	0.128
II	Sapporo	0.080	0.093	0.118	0.120	0.123	0.082	0.130	0.126	0.128
III	Yufutsu	0.065	0.109	0.099	0.084	0.105	0.209	0.141	0.098	0.090
IV	Bibai	0.084	0.099	0.121	0.105	0.128	0.108	0.115	0.128	0.113
IX	Monbetsu	0.145	0.070	0.121	0.106	0.122	0.115	0.109	0.107	0.104
V	Horonobe	0.085	0.294	0.075	0.119	0.054	0.112	0.067	0.092	0.101
VI	Sarobetsu	0.116	0.129	0.101	0.117	0.111	0.110	0.107	0.099	0.111
VII	Furano	0.110	0.076	0.126	0.111	0.127	0.091	0.106	0.125	0.127
VIII	Obihiro	0.066	0.192	0.117	0.176	0.083	0.097	0.122	0.080	0.067
X	Bihoro	0.198	0.082	0.113	0.108	0.107	0.062	0.091	0.104	0.135
XI	Akkeshi	0.048	0.200	0.082	0.079	0.081	0.235	0.105	0.098	0.072
XII	Shiretoko	0.110	0.120	0.118	0.105	0.122	0.111	0.105	0.117	0.092
XIII	Nemuro	0.213	0.077	0.102	0.113	0.088	0.062	0.094	0.105	0.147

Table 9 Estimated periods of the latest separation of 11 islands from Hokkaido mainland (10³ years BP), maximum depth (m) of the present sea bottom from Hokkaido mainland, and area (km²). Islands in italic are those without *Sorex unguiculatus*

	isolation period (10 ³ YBP)	max depth (m)	area size (km ²)
<i>Okushiri</i>	>200 ?	400	142.9
<i>Honshu</i>	150-140	140	227,415.0
Rebun & Rishiri*	13	85	81.0 & 182.1
Sakhalin (= Karafuto)	12-11	70	76,405.0
Teuri & <i>Yagishiri</i> *	11	55	5.5 & 5.2
Shikotan & Habomai arc.	8	35	255.1 & -
Kunashiri	7	20	1,498.5
Daikoku*	5	5	1.1
Hokkaido mainland	-	-	78,422.7

*estimated from the present depth of sea bottom and sea level change by Shackleton (1987). Estimation for other islands was based on Ohshima (1990) and Igarashi (2000).

Fig. 1. Naitoh & Ohdachi

A. Allelic richness

B. Average heterozygosity

A. *S. unguiculatus*

B. *S. caecutiens*

Fig. 3. Naitoh & Ohdachi

A. *S. unguiculatus* including 4 insular populations and one Sakhalin population

B. *S. unguiculatus* on Hokkaido mainland

C. *S. caecutiens*

A. *S. unguicualtus*

1. Distance size class = 50 km

2. Distance size class = 100 km

B. *S. caecuetiens*

1. Distance size class = 50 km

2. Distance size class = 100 km

A. *S. unguiculatus*

B. *S. caecutiens*

Geographic distance ($\ln$ km)