Comparative Phylogeography between the Ermine Mustela erminea and the Least Weasel M. nivalis of Palaearctic and Nearctic Regions, Based on Analysis of Mitochondrial DNA Control Region Sequences

Authors: Kurose, Naoko, Abramov, Alexei V., and Masuda, Ryuichi

Source: Zoological Science, 22(10) : 1069-1078

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.22.1069

ZOOLOGICAL SCIENCE **22**: 1069–1078 (2005)

Comparative Phylogeography between the Ermine *Mustela erminea* and the Least Weasel *M. nivalis* of Palaearctic and Nearctic Regions, Based on Analysis of Mitochondrial DNA Control Region Sequences

Naoko Kurose[1], Alexei V. Abramov[2] and Ryuichi Masuda[3*]

[1]*Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
[2]*Zoological Institute, Russian Academy of Sciences, Saint-Petersburg 199034, Russia*
[3]*Laboratory of Genetic Diversity, Division of Genome Dynamics, Creative Research Initiative "Sousei", Hokkaido University, Sapporo 060-0810, Japan*

ABSTRACT—Phylogeography of the ermine *Mustela erminea* and the least weasel *M. nivalis* from Palaearctic and Nearctic regions were investigated based on mitochondrial DNA control region sequences. *Mustela erminea* exhibited a very low level of genetic variation, and geographic structures among populations were unclear. This may indicate that *M. erminea* recently reoccupied a wide territory in Eurasia following the last glacial retreat. In comparison with *M. erminea*, genetic variations within and among populations of *M. nivalis* were much greater. Molecular phylogenetic relationships showed that two lineages of *M. nivalis* occurred in the Holarctic region: one spread from the Eurasian region to North America, and the other occurred in south-eastern Europe, the Caucasus and Central Asia. The results suggest either mitochondrial DNA introgression among populations of south-eastern Europe, the Caucasus and Central Asia, or ancestral polymorphisms remaining in those populations. Contrastive phylogeographic patterns between the two mustelid species could reflect differences of their migration histories in Eurasia after the last glacial age.

Key words: ermine, *Mustela erminea*, least weasel, *Mustela nivalis*, mitochondorial DNA, phylogeography, Eurasia

INTRODUCTION

The ermine *Mustela erminea* Linnaeus, 1758, inhabits the tundra, boreal forest and deciduous forest zones of the Palaearctic and Nearctic regions: Europe including Britain and Ireland but excluding most parts of Mediterranean zones; Siberia and Asia including the Caucasus, Himalayas, Mongolia, Manchuria, and Far East including Sakhalin, Hokkaido and Honshu of Japan. This species is also distributed across North America, south to California in the west and to Maryland in the east (Corbet, 1978; Nowak, 1991; Wozencraft, 1993). Although many subspecies of *M. erminea* have been recognized in Eurasia and North America (Ellerman and Morrison-Scott, 1951; Hall, 1951; Heptner *et al*., 1967; King, 1983), an unequivocal taxonomic classification has not yet been made. Corbet (1978) recognized *M. erminea* throughout Eurasia and North America as one subspecies, and only insular populations from Ireland and the Honshu Island of Japan as separate subspecies.

The least weasel *Mustela nivalis* Linnaeus, 1766, also has a circumboreal range throughout the Holarctic region, including Europe, North Africa, and a major part of Asia and North America (Corbet, 1978; Nowak, 1991). Over this wide area, *M. nivalis* displays such a high degree of morphological variations that the systematic relationships among different geographic forms are unresolved. The most recent revisions of the morphometric variation of *M. nivalis* were carried out by van Zyll de Jong (1992), Reig (1997) and Abramov and Baryshikov (2000).

Thus, *Mustela erminea* and *M. nivalis* are largely sympatrically distributed over the major parts of Eurasia and North America. Paleontological evidences indicate that the two species have been typical small predators of the same

* Corresponding author. Phone: +81-11-706-3588;
Fax : +81-11-706-3588;
E-mail: masudary@ees.hokudai.ac.jp

Table 1. Profiles of ermines *Mustela erminea* (MER) and least weasels *Mustela nivalis* (MNI) examined in the present study

Species	Sample code	Haplotype No.*	Sampling locality (Specimen No.**)	Locality No.#	Accession No.##
Mustela erminea	RBEL1	E12	Grodno Distr., Belarus (ZIN C.61856)	1	AB049777
	RNOV1	E14	Novgorod Prov., Russia (ZIN C.61839)	2	AB049778
	RNOV2	E4	Novgorod Distr., Russia (ZIN C.61838)	2	AB049779
	RSEK1	E16	West Karakol, Kirghizia (ZIN C.61868)	16	AB049780
	RSEK2	E15	Kopal, Kazakhstan (ZIN C.56459)	3	AB049781
	RSEK4	E5	Kopal, Kazakhstan (ZIN C.56457)	3	AB049782
	NWM2	E19	Sugnur, Mongolia (ZIN C.83322)	4	AB049783
	RALD1	E13	Aldan, Russia (ZIN C.17678)	5	AB049784
	RKOL1	E6	Kolyma, Russia (ZIN C.83035)	6	AB049785
	RANA1	E17	Anadyr, Russia (ZIN C.83323)	7	AB049786
	RANA2	E7	Anadyr, Russia (ZIN C.83324)	7	AB049787
	RKAM1	E10	Kamchatka, Russia (ZIN C.83326)	8	AB049788
	1(JPN1)	E1	Hokkaido, Japan	9	AB006730@
	CH1	E3	Hokkaido, Japan	10	AB006731@
	IWA1	E2	Honshu, Japan	11	AB006732@
	YAM1	E11	Honshu, Japan	12	AB006729@
	TOG1	E11	Honshu, Japan	12	AB006733@
	US3	E18	Minnesota, U.S.A (ZIN C.79034)	15	AB061215
	US4	E8	Kodiak, Alaska, U.S.A (ZIN C.3872)	13	AB061216
	US10	E9	Kenai, Alaska, U.S.A (ZIN C.3377)	14	AB061217
Mustela nivalis	RCAU1	N1	Lagodehi, Georgia (ZIN C.84102)	▲ 1	AB049764
	RTUR2	N1	Murgab, Turkmenistan (ZIN C.83514)	▲ 1	same as RCAU1
	RASK1	N3	Askania-Nova, Ukraine (ZIN C.9621)	▲ 2	AB049765
	RMOS1	N6	Moscow Prov., Russia (ZIN C.83315)	● 1	AB049766
	RCRY1	N17	Crimea, Ukraine (ZIN C.83377)	■ 1	AB049767
	RASK2	N2	Askania-Nova, Ukraine (ZIN C.9618)	▲ 3	AB049768
	RALT1	N7	Altai, Russia (ZIN C.83325)	● 2	AB049769
	RCAC1	N4	Tbilisi Distr., Georgia (ZIN C.83516)	▲ 4	AB049770
	RCAU2	N18	Lagodehi, Georgia (ZIN C.83526)	■ 2	AB049771
	RROS1	N2	Rostov, Russia (ZIN C.83517)	▲ 3	same as RASK2
	RIND1	N8	Indigirka, Russia (ZIN C.83523)	● 3	AB049772
	RKAR1	N19	SE Karakum, Turkmenistan (ZIN C.51347)	■ 3	AB049773
	RKAR2	N16	SE Karakum, Turkmenistan (ZIN C.51346)	★	AB049774
	RJIL6	N20	Gurjev Prov., Kazakhstan (ZIN C.45721)	■ 4	AB049775
	RUMA1	N7	Kiev Prov., Ukraine (ZIN C.83317)	● 2	same as RALT1
	RETU1	N5	Kars Prov., Turkey (ZIN C.84099)	▲ 5	AB049776
	RLEN3	N7	Leningrad Prov., Russia (ZIN O.35085)	● 2	same as RALT1
	5(JPN5)	N12	Hokkaido, Japan	◆ 1	AB006717@
	S13	N10	Hokkaido, Japan	◆ 2	AB006721@
	N32	N13	Hokkaido, Japan	◆ 3	AB006727@
	HIT1	N11	Hokkaido, Japan	◆ 4	AB006722@
	AKI1	N9	Honshu, Japan	▼	AB006728@
	IWA1	N9	Honshu, Japan	▼	same as AKI1
	IWA2	N9	Honshu, Japan	▼	same as AKI1
	US1	N14	Michigan, U.S.A (ZIN C.77164)	# 1	AB061213
	US2	N15	Michigan, U.S.A (ZIN C.39879)	# 2	AB061214

* Haplotype numbers correspond to those of Figs. 2 and 3.
** Specimen numbers registered in Zoological Institute, Russian Academy of Sciences.
# Locality numbers correspond to those of Figs. 2 and 3.
Sequence data will appear in the DDBJ nucleotide sequence database with three accession numbers.
@ Cited from Kurose *et al.* (1999).

biotic association since the Middle Pleistocene (Kurten, 1968; Kurten and Andersen, 1980). The two species are morphologically and ecologically very similar to each other. Therefore, both species were believed to be closely related and they were formerly placed in one subgenus *Mustela* (Ellerman and Morrison-Scott, 1951; Youngman, 1982; Nowak, 1991; Wozencraft, 1993). Their common circumpolar distribution suggests that they might have experienced the similar history in the post glacial recolonization. However, comparative analyses of geographical variations in morphological characters show differences between the two species: the geographic variability of morphology in *M. nivalis* is larger than that of *M. erminea* (Heptner *et al*., 1967; Ralls and Harvey, 1985; Meia 1990; Meia and Mermod, 1992).

Recently, Kurose *et al.* (1999) found nucleotide sequence differences in the mitochondorial DNA (mtDNA) control region between the Hokkaido and Honshu populations of *M. erminea* and between those of *M. nivalis* in Japan. The mtDNA (Kurose *et al*., 1999) and karyotypic (Obara, 1991) differentiations of *M. nivalis* could have been fixed to the Honshu Island population, after their geographic isolation in the Middle Pleistocene. Meanwhile, the mtDNA control region sequences did not show any clear divergence between the Hokkaido and Honshu populations of *M. erminea* (Kurose *et al*., 1999). Fleming and Cook (2002) examined DNA sequence variations in the mitochondrial cytochrome *b* gene of *M. erminea* in Alaska and found three lineages. One lineage includes southeast Alaskan endemics and ermines from western Canada and the coterminous United States. The second lineage appears restricted to the Prince of Wales Island complex in southeast Alaska, the Graham Island and British Columbia. The third lineage has a Beringian distribution including Alaska and north-eastern Russia (Magadan).

In the present study, we compared phylogeographic patterns of mtDNA control region sequences between the two sympatric mustelid species across Eurasia and North America, and then discuss their post glacial history and the subspeciation.

MATERIALS AND METHODS

Animals and DNA extraction

Profiles and sampling localities of animals examined in the present study are described in Table 1 as well as Figs. 2a and 3a. All samples were muscle tissues preserved in 70% ethanol for approximately 10–160 years. Total DNAs were extracted from homogenates of tissues (approximately 1×1×1 cm), according to the phenol/proteinase K/sodium dodecyl sulphate method of Sambrook *et al.* (1989) with some modifications (Masuda and Yoshida, 1994). DNA extracts were concentrated using Centricon-30 microconcentrators (Amicon), because DNAs contained in long-preserved tissues were fragmented. The STE buffer (100mM NaCl/10mM Tris pH 7.5/1mM EDTA) treated by the same procedure was used as a negative control in subsequent polymerase chain reaction (PCR) amplification. MtDNA control region sequences (Accession numbers AB006717, AB006721, AB006722, AB006727–AB006733) from Japanese populations of *M. nivalis* and *M. erminea* (Kurose *et al*., 1999) were added to the present analysis. The sequence (AB007327) of *Mustela itatsi* was used as an outgroup, because

Table 2. Primers for amplification and sequencing of the mtDNA control region, which were newly designed in the present study

Primer name	Strand	Sequence (5' to 3')
DS2	L	CCGCCATCAGCACCCAAAGCTG
DS3	L	GCTGACATTCTAACTAAACTATTCC
MNE-DR3	H	GAGGCATGGTGATAAAGCTCGTG
MNE-D3	L	TGCCCCATGCATATAAGCATGTAC
MNE-DR4	H	GGTTAGTAGCATTGGATTGAGGA
MNE-D4	L	TTCTCGCTCCGGGCCCATCAA
MNE-DR5	H	TAGCTGAGTGATACCAAGTTCTC
MNE-D5	L	GGACTAATGACTAATCAGCCCATG
MSD2	H	TATGTCCTGTGACCATTGACTGAA
MSD3	H	CCTGTGACCATTGACTGAATAACAC
MNE-D8	L	GCCCCATGCATATAAGCATGTA
MNE-D9	L	GGCATCTGGTTCTTACTTCAGGG

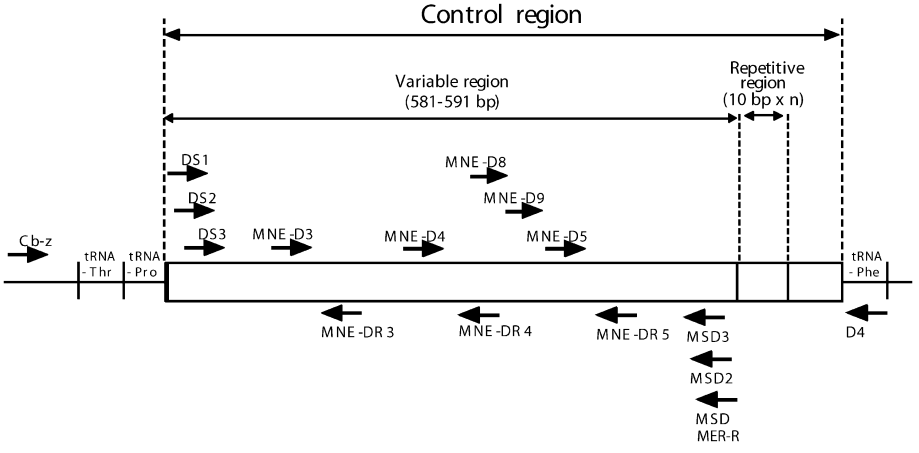


Fig. 1. Schematic diagram of the mtDNA control region of *Mustela erminea* and *M. nivalis*. Arrows show primers used for PCR amplification and sequencing. Detailed information is described in 'MATERIALS AND METHODS' in the text and Table 1.

this species is phylogenetically closer to *M. erminea* and *M. nivalis* in the genus *Mustela* (Kurose *et al.*, 2000).

PCR, sequencing and data analysis

Since DNAs in long-preserved tissues were fragmented, four portions of the mtDNA control region were amplified separately using two primers from those described in Table 2 and Fig. 1, except for MNE-D8 and MNE-D9 which were used for the following sequencing. From sequences reported by Shield and Kocher (1991) and Árnason and Johnsson (1992), primer Cb-z was modified and primer D4 was designed, respectively (Kurose *et al.*, 1999). Primers DS1, MSD and MER-R were cited from Kurose *et al.* (1999). The other primers (Tasble 1) were newly designed in the present study based on control region sequences of Japanese populations of *M. nivalis* and *M. erminea* , previously reported by Kurose *et al.* (1999). Combination of the four portions yielded approximately 600 base-pairs (bp) of the 5’ end of the mtDNA control region, which were reported to be polymorphic in Japanese populations of these species (Kurose *et al.*, 1999). Symmetric PCR amplification to generate double-stranded DNAs was done in a total volume of 50 μl of the reaction mixture including 20 mg of bovine serum albumin (Boehringer). PCR included 35 cycles using a DNA thermal cycler (PJ2000, Perkin-Elmer Cetus) as follows: 94°C, 1 min denaturing; 50°C, 1 min annealing; 72°C, 1 min extension, and then the reaction was completed at 72°C for 10 min. To varify positive amplification, an aliquot of 10 μl from the PCR products was electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under an ultraviolet illuminator. The remaining 40 μl of the PCR product was purified with the QIAquick purification kit (Qiagen). Purified PCR products were labelled using the dye-terminator pre-mix kit (Amersham) on a DNA thermal cycler (PCR system 9700, Perkin-Elmer) following the manufacturer’s instruction and sequenced using the ABI Prism™ 377 DNA sequencing system (Perkin-Elmer). For sequencing, all PCR primers except for Cb-z and D4 were used.

Sequence alignment was done using GeneWorks (Intelligenetics). Insertions and/or deletions (indels) were compensated through observation by eyes and eliminated for sequence analysis. Neighbor-joining trees (Saitou and Nei, 1987) using MEGA (Kumar *et al.*, 1993), based on genetic distances of Kimura's (1980) two-parameter model, were constructed. The maximum parsimonious method was done using PAUP* 4.0b10 (Swofford, 1998).

Samples with successful sequencing results are listed in Table 1. However, the table does not include other samples, where no PCR products were obtained because of DNA degradation in aged specimens.

RESULTS

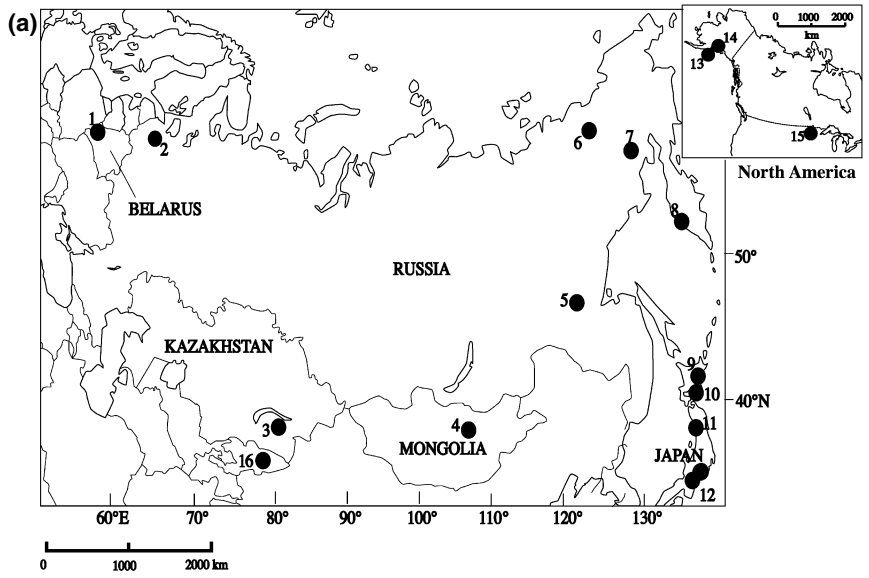
Sequence variations in *Mustela erminea*

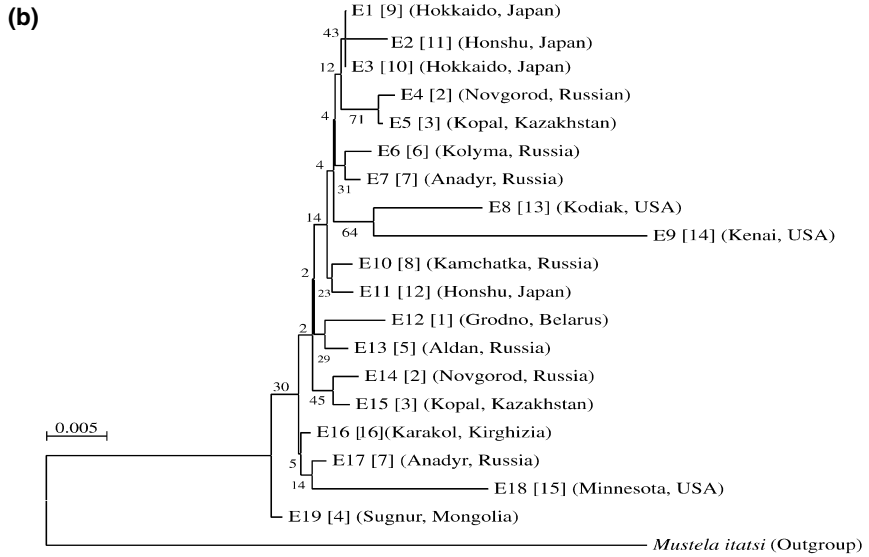
An alignment of the mtDNA control region sequences of *Mustela erminea* (Table 3) showed 40 sites to be variable among 579 bp (excluding one indel at nucleotide site 71) with the transitional bias. Intraspecific sequence diversity (percentage differences) varied from 0.17% to 4.32% (1.16% in average). The neighbor-joining phylogenetic tree

Table 3. Sequence variation of the mtDNA control region (581-582 bp) in the ermine *Mustela erminea*

	Variable site																																							
							1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	5	5	5	5	5
		1	7	7	8	8	0	0	2	3	3	5	6	7	7	0	1	1	1	1	1	6	6	1	2	4	4	4	6	6	1	7	7	8	8	0	4	5	6	6
Haplotype	5	1	1	6	3	8	6	7	8	4	9	9	0	0	7	1	1	2	3	5	9	5	6	2	6	1	5	8	2	3	5	0	4	3	8	8	2	8	2	5
E12	C	T	T	C	T	A	C	C	T	T	A	T	C	T	C	C	T	G	C	C	C	C	T	A	C	A	G	C	A	A	T	C	T	A	T	T	C	T	G	C
E14	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
E4	.	C	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E16	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
E15	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	G	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
E5	.	C	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E19	.	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
E13	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
E6	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	G	G	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E17	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
E7	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.
E10	.	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.
E1	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E3	.	.	–	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E2	.	.	–	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	T	C	.	.
E11	.	.	C	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.
E18	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	T	C	G	.	.	.	.	.	.	C	.	C	.	C	.	.	C	.	.
E8	T	.	.	.	.	.	.	T	.	.	.	.	T	.	T	T	.	C	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	C	.	C	.	.
E9	T	.	.	.	.	T	G	.	A	.	.	.	T	.	T	A	.	.	.	.	.	.	.	G	G	G	A	G	C	C	.	T	.	G	.	.	T	C	A	G

Dots indicate identical nucleotides with those of haplotype E12.
Dashes at site number 71 show an indel.





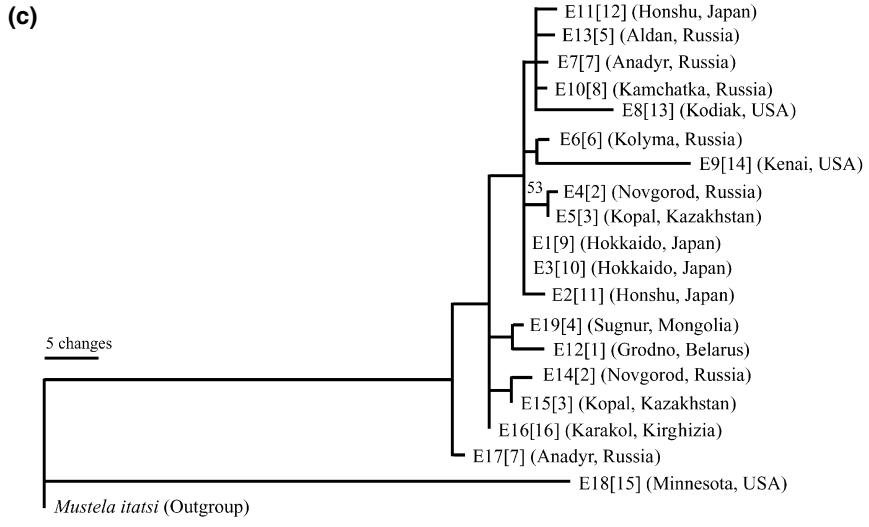


Fig. 2. **(a)** Sampling localities of *Mustela erminea* in Eurasia. The small map at the upper right shows the sampling localities in North America. Locality numbers refer to those in Table 1. **(b)** Neighbour-joining relationships among haplotypes (581–582 bp) of *M. erminea* mtDNA control region. The homologous sequence of *M. itatsi* was used as an outgroup. The scale indicates genetic distance estimated with Kimura's (1980) two-parameter model, excluding indel sites. Numbers (%) on internal branches are bootstrap values derived from 1,000 replications. Haplotype numbers, locality numbers in brackets and locality names in parentheses refer to those in Table 1 and Fig. 2a. **(c)** Maximum parsimony tree using haplotypes of *M. erminea*. The sequence of *M. itatsi* was used as outgroup. The number '53' on the branch indicates a bootstrap value from 100 replications, however, low bootstrap values less than 50% are not shown. The horizonal branch length is proportional to distances as shown by the scale bar (number of substitutions). Haplotype numbers, locality numbers in brackets and locality names in parentheses refer to those in Table 1 and Fig. 2a.

(Fig. 2b) clearly showed a low level of genetic differentiation between populations of *M. erminea* in Eurasia. Most of the clusters of haplotypes were not supported with bootstrap values greater than 71% (Fig. 2b). Two haplotypes E15 (RSEK2) and E5 (RSEK4) from eastern Kazakhstan (locality No. 3 in Fig. 2a) were positioned separately from each other. The two haplotypes E17 (RANA1) and E7 (RANA2) from north-eastern Siberia were also remotely related to each other (Table 1, Fig. 2b and 2c). The haplotype E11 (TOG1 and YAM1) from the Honshu Island of Japan (locality No. 12 in Fig. 2a) was most closely related to the haplotype E10 (RKAM1) from Kamchatka (locality No. 8 in Fig. 2a), although the relationship between them was relatively weak (23% bootstrap value). In addition, the haplotype E18 (US3) from North America (locality No. 15 in Fig. 2a) was closer to E17 (RANA1) from north-eastern Siberia (locality No. 7 in Fig. 2a). Thus, the phylogenetic relationships among haplotypes did not relate closely to their geographic distribution throughout Eurasia. Genetic distances of North American individuals after divergence were much larger than those observed in Eurasian populations (Fig. 2b). The phylogenetic relationships of haplotypes by maximum parsimony (Fig. 2c) were similar to those by the neighbor-joining relationships (Fig. 2b).

Sequence variations in *Mustela nivalis*

A sequence alignment of the mtDNA control region sequences of *Mustela nivalis* (Table 4) showed 61 sites to be variable among 579 bp (excluding seven indels) with the transitional bias. Intraspecific sequence differences varied from 0.17% to 6.04% (2.91% in average). The neighbor-joining tree indicated that individuals of *M. nivalis* were divided into seven clusters A–G with 55–100% bootstrap values (Fig. 3b).

Sequence differences in Cluster A with a 55% bootstrap value were between 0.17% to 1.21%. Cluster A includes haplotypes N1 (RCAU1), N2 (RROS1), N4 (RCAC1) and N5 (RETU1) from the Caucasus and haplotypes N3 (RASK1) and N2 (RASK2) from southern Ukraine and haplotypes N1 (RTUR2) from Turkmenistan. Cluster B with a 79% bootstrap value showed sequence differences varying from 0.17% to 0.35%. Three specimens (RALT1, RUMA1, and RLEN3) shared an identical haplotype N7, despite these individuals originating from distant localities (see Fig. 3a). As reported by Kurose *et al.* (1999), cluster C comprising the Honshu Island population of Japan was defined by one haplotype N9, which was shared by three individuals (AKI1, IWA1, and IWA2), and cluster D (the Hokkaido Island population of Japan; 87% bootstrap value) showed intra-cluster

Table 4. Sequence variation of the mtDNA control region (581–591 bp) in the least weasel *Mustela nivalis*

Haplotype	Variable site
	1111111111222222223333444445555555
	1444555666666777777888880034444669012245671234236690233449
	3456258137135679123679012375983789347176764504409406929978160
N1	T–CTTATCCATTCTCTTT–––CCTCCTACTGATTCTCACGTCTACTTGGAAAACTCTGACC
N3	.–................–––..........GC..............C.............
N6	.C................–––............CT.........TA.C...GG..T...T.
N17	.T.CCG..G...TCTCCCCCCTT..T.CT......C..G.C.ATTCCC.TG...C.CAGT.
N2	.–.–..............–––..........................C.............
N7	.–................–––............CT..........A.....GG..T...T.
N4	.–........C.......T–––.C..C.GC..C..............C.............
N18	.T–CCG.TGG..TC.CCCCTTTT..T.CT......C...AC.AT.C.C.TG...C.CA.TG
N8	.–................–––............CT..........A.C...GG..T...T.
N19	.TTC.G.TGG..TC.CCCCTTTT..T.GT......C...AC.AT.CCC.TG...C.CAGT.
N16	.–.C........T.....–––..C..............................C.CA.T.
N20	.TTCCG.TGG..TC.CCCCTTTT..T.CT..........AC.AT.CCC.TG...C.CAGT.
N5	.–................–––..........................C.....T.......
N12	CC................––––.CT...T.A..C...........A.C...G.T.T.A.TG
N10	CC................–––.......T.A..CT..........A.C...G.T.T.A.TG
N13	CC................––––.CT.C...A..C...........A.C.....T.T.A.TG
N11	CC.........C......–––.......T.A..C...........A.C...G.T.T.A.TG
N9	.C....C...........–––............C..........TA.C..TGG.......G
N14	.C................––––.CT...........GG...A..TA.CC.G..T.T...T.
N15	.C................––––.CT...................TA.C..G..T.T...T.

Dots indicate identical nucleotides or indels with those of haplotype N1.
Dashes show indel sites

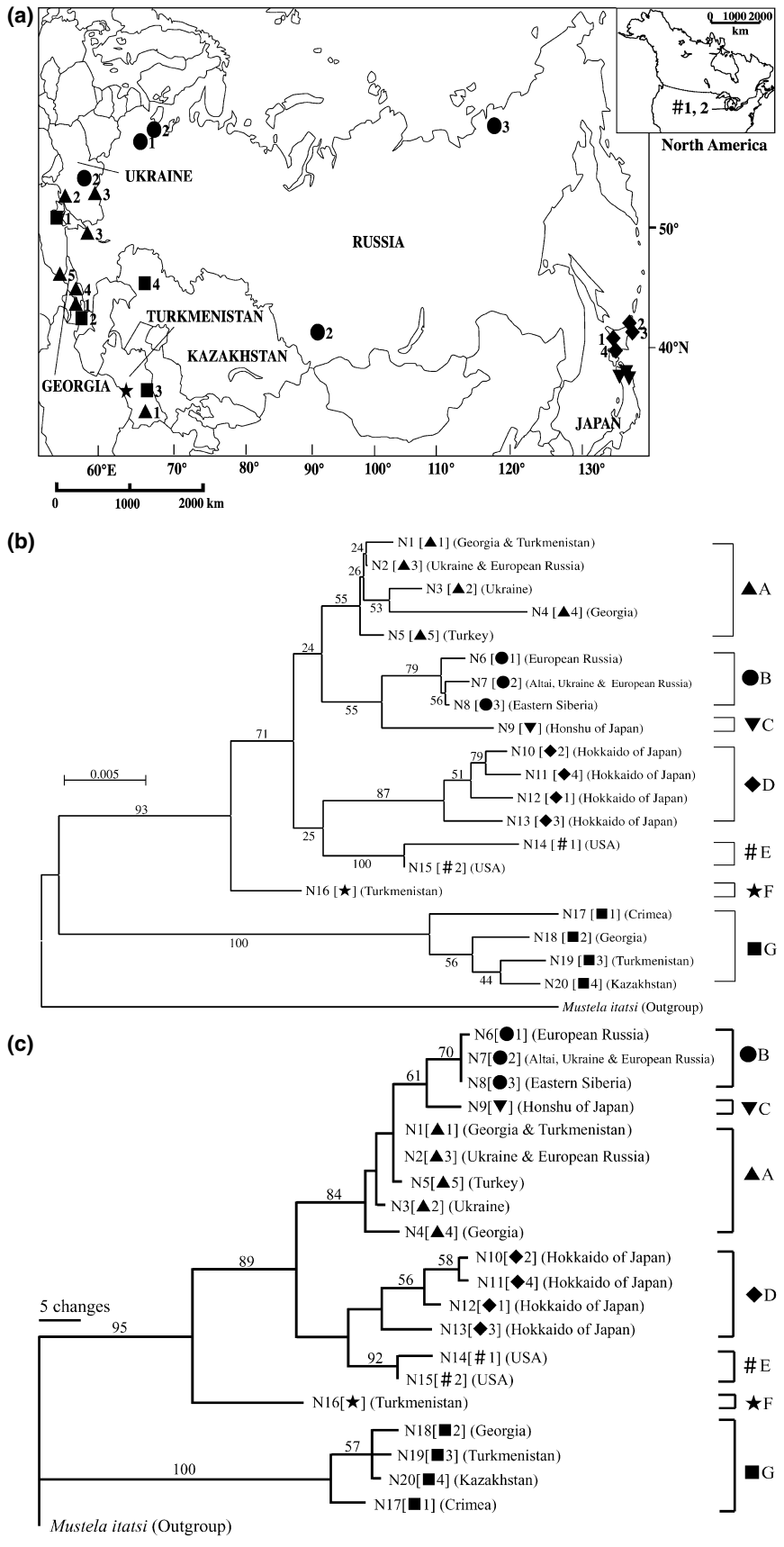


Fig. 3. **(a)** Sampling localities of *Mustela nivalis* in Eurasia. The small map at the upper right shows the sampling localities in North America. Locality numbers refer to those in Table 1. **(b)** Neighbour-joining relationships among haplotypes (581–591 bp) of the *Mustela nivalis* control region sequences. The sequence of *M. itatsi* was used as an outgroup. The scale indicates genetic distance estimated with Kimura's (1980) two-parameter model, excluding indel sites. Numbers (%) on internal branches are bootstrap values derived from 1,000 replications. Haplotype numbers, locality numbers in brackets and locality names in parentheses refer to those in Table 1 and Fig. 3a. ▲, Cluster A; ●, Cluster B; ▼, Cluster C; ◆, Cluster D; #, Cluster E; ★, Cluster F; ■, Cluster G. **(c)** Maximum parsimony (MP) tree reconstructed using haplotypes of *M. nivalis*. The homologous sequence of *M. itatsi w*as used as outgroup. Numbers indicate bootstrap values from 100 replications, however, low bootstrap values less than 50% are not shown. The horizonal branch length is proportional to distances as shown by the scale bar (number of substitutions). Haplotype numbers, locality numbers in brackets and locality names in parentheses refer to those in Table 1 and Fig. 3a.

sequence differences varying from 0.35% to 1.04%. Cluster E includes haplotypes N14 (US1) and N15 (US2) from North America with a 100% bootstrap value. Cluster F (one haplotype N16; RKAR2 from Turkmenistan) was split from clusters A–E. Cluster G, with a 100% bootstrap value, showed intra-cluster sequence differences varying from 0.52% to 1.55% and consisted of individuals from distant localities: N17 (RCRY1) from Crimea, N18 (RCAU2) from the Caucasus, N19 (RKAR1) from Turkmenistan and N20 (RJIR6) from western Kazakhstan (Fig. 3b). Within-population nucleotide diversities of *M. nivalis* were higher than those of *M. erminea* mentioned above. For instance, the population from eastern Turkmenistan contained very distinct haplotypes N19 (RKAR1), N16 (RKAR2) and N1 (RTUR2) that belonged to three clusters A, F and G, respectively. Two haplotypes N1 (RCAU1) and N18 (RCAU2) of the morphologically similar specimens from the same locality in the Caucasus were included in different clusters, A and G, respectively. The phylogenetic relationships analyzed by maximum parsimony (Fig. 3c) were similar to those analyzed by the neighbor-joining method (Fig. 3b).

DISCUSSION

Genetic population structure of *Mustela erminea*

Genetic differentiation among populations of *Mustela erminea* was relatively lower than that of *M. nivalis*. Phylogenetic relationships based on sequence differences did not correspond with geographic distances of sampling localities (Fig. 2b and 2c). For instance, Japanese haplotypes were separated into two groups and positioned with clusters of the Eurasian continental haplotypes. North American haplotypes were also clustered into two groups and separately positioned with Eurasian haplotypes.

Phylogenetic data based on cytogenetic (Graphodatsky *et al.*, 1976; Obara, 1991) and mtDNA (Kurose *et al.*, 2000) analyses suggest that *M. erminea* was first split from other species of the genus *Mustela* and that this species has a more ancestral form among the genus *Mustela*. Meanwhile, *M. erminea* likely appeared in central Europe in the early Middle Pleistocene and it was one of the common small carnivores in central and eastern Europe during the Middle Pleistocene and later (Kurten, 1968). The original European population of *M. erminea* could have colonized eastern parts of the Palaearctic region and crossed the Bering land-bridge into North America, where the earliest known remains are of the Illinoian age (Kurten and Andersen, 1980). Although recent *M. erminea* slightly differs from the Early Pleistocene and Late Pliocene ermine-like forms, the geographical variability in cranial and external characters of the Palaearctic and Nearctic ermines is also very limited (Ralls and Harvey, 1985; Eger, 1990; Meia, 1990; Meia and Mermod, 1992). The results of the present study may indicate that the intercontinental genetic difference between Eurasian and North American populations is not large, in congruence with the low level of intra-specific variation obtained in previous morphological studies. One explanation for the low variation in *M. erminea* is that colonization of Eurasia might have occurred quickly during a short period after the last glacial age. After the original colonization by the ancestral populations of eastern Palaearctic regions including Japan, there may have been continuous exchanges among populations, resulting in homogenization of mtDNA. Meanwhile, it was reported that populations of the gray wolf *Canis lupus* showed a similar pattern of mtDNA control region sequences, that is, low genetic variations and no geographic structures in Eurasia (Tsuda *et al.*, 1997; Vila *et al.*, 1997). It was suggested that mtDNA genotypes were randomly fixed to be monomorphic because of decreasing and fracturing of available habitats for wolves and a decrease in the size of their populations. *Mustela erminea* might also have experienced the similar population history.

Meanwhile, the phylogenetic trees indicate that the mutation rates of the North American haplotypes (E8, E9 and E18) are likely more rapid than those of Eurasian haplotypes (Fig. 2b and 2c). North American haplotypes were positioned not outside but within clusterings of the Eurasian haplotypes. The future examination by comprehensive sampling in North America may reveal the genetic specificity of the mtDNA control region of North American *M. erminea*. The differences found in the present study between North American ermines from Alaska (Kodiak and Kenai) and inner USA (Minnesota) correspond with data of Fleming and Cook (2002). These authors found three distinct lineages in North America – Beringian (includes specimens from north-eastern Alaska and north-eastern Russia), the Continental (distributed from the south-eastern Alaska throughout western Canada and across the coterminous USA), and the Island lineage (found on a few islands of southeastern Alaska).

Genetic population structure of *Mustela nivalis*

In comparison with *M. erminea*, the genetic variation within and among populations of *M. nivalis* was much larger. Both neighbor-joining and parsimonious methods produced the similar phylogenetic relationships among haplotypes (Fig. 3b and 3c). There are clearly two distinct mtDNA lineages for *M. nivalis*: one lineage comprizing clusters A–F with 93–95% bootstrap values (Fig. 3b and 3c) is distributed across Eurasia including Japan and North America, and the other lineage (cluster G) with a 100% bootstrap value (Fig. 3b and 3c) was found in some populations of the Caucasus, Central Asia (Turkmenistan, Kazakhstan) and south-eastern Europe (southern Ukraine).

Recently, Abramov and Baryshnikov (2000) investigated cranial characters, sizes, proportions of the body and tail, and the coloration of pelage, and reconstructed subspecific taxonomy of *M. nivalis* as well as possible phylogenetic relationships. The results of the present study mostly support their phylogeny.

According to the above-mentioned taxonomy, most parts of northern Eurasia (including the Hokkaido Island of

Japan) were occupied by the small-sized subspecies *M. n. nivalis*. All specimens from clusters B and D (European parts of Russia, Altai, Eastern Siberia, Hokkaido) belong to this form.

Obara (1991) reported the occurrence of the Robertsonian translocation of chromosomes in the Honshu Island population of Japan (2n=38), which distinguishes this population from the other Eurasian (including Hokkaido Island of Japan, 2n=42) and North American populations (2n=42). Based on the karyological difference, Obara (1991) considered the Honshu Island population as a distinct species *M. namiyei* Kuroda, 1921. However, genetic distances between the two populations estimated from the mtDNA cytochrome *b* (Masuda and Yoshida, 1994) and control region (Kurose *et al*., 1999) did not support the specific level of divergence. The molecular phylogeny obtained in the present study again supports that the Honshu population is genetically differentiated but is not more than the subspecific level. Interestingly, the population from Honshu Island (cluster C) was closer to the continental *M. n. nivalis* (cluster B) (with 55–61% bootstrap value) than the Hokkaido Island group (cluster D) (Fig. 3b and 3c).

Moreover, the Hokkaido Island group (cluster D) was closely related to the North American group (cluster E), while this was supported with <50% bootstrap values (Fig. 3b and 3c). North American specimens used in the present study were classified as *M. n. allegheniensis*, which is morphologically closer to the nominotypical subspecies *M. n. nivalis* that also includes weasels from Hokkaido (Abramov and Baryshnikov, 2000). Based on craniological data, Reig (1997) considered the North American least weasel as distinct species *M. rixosa* (Bangs, 1896). However, the present molecular data (Fig. 3b and 3c) as well as the results of previous morphological analysis (Abramov and Baryshnikov, 2000) do not support the specific level of divergence between Eurasian and North American least weasels.

The other three clusters (A, F and G) consisted of medium-sized weasels from southern Ukraine (subspecies *vulgaris*) and the Caucasus (subspecies *caucasica*), and large-sized weasels from Turkmenistan and western Kazakhstan (subspecies *heptneri*) (subspecific ranks according to Abramov and Baryshnikov, 2000). The clustering of haplotypes in the phylogenetic tree (Fig. 3b and 3c) did not correspond with geographically and morphologically expected relationships between populations. Nor did the clustering always relate to body size and color patterns. The least weasels from these localities shared the haplotypes from two different lineages. The results of the present study indicate that mtDNA introgression may have occurred between populations distributed in the Caucasus, southern Ukraine and Central Asia, or polymorphic status of their ancestral populations still remains in those areas. Hewitt (1999) reported that many animal species of Europe show lower genetic diversity in northern populations that have expanded rapidly. By contrast, southern populations in refugial regions show relatively greater diversity.

The ancestry of the recent *M. nivalis* can be traced back to *M. praenivalis* of the Late Pliocene and Early Pleistocene in central Europe (Kurten, 1968). Probably more ancestral forms of *M. nivalis* were large long-tailed weasels of northwestern Africa, southern Spain and Mediterranean (form *numidica*) (Frank, 1985; Abramov and Baryshnikov, 2000). In the past, these large weasels were more widely spread in Europe and might also penetrate western parts of Asia. Small weasels of the form *nivalis* appeared later, probably in the boreal regions of the Palaearctic. The sympatric occurrence of fossil remains of large and small weasels in cave layers is known for the Late Pleistocene sites of the Caucasus, Poland, Slovakia and Crimea (see Abramov and Baryshnikov, 2000 and references therein). These small weasels could have penetrated North America through Beringia. Our results suggest that the form *nivalis* might have colonized the most parts of the Eurasian continent during a short period, probably after the last glacial age. The medium-sized weasel (*vulgaris-boccamela*) was evidently formed in eastern parts of the Mediterranean region during the Late Pleistocene. From southern refugia, weasels of this form could have recolonized most parts of Europe, displacing *nivalis* to the north and east (see Kratochvil, 1977). To examine this process, further studies are required by using more specimens from the different areas including the Mediterranean and western Europe.

The present study revealed that the genetic variations between Palaearctic populations were much larger in *M. nivalis* than in *M. erminea*. This contrasting result is in congruence with the variability of cranial and external characters of *M. nivalis* which is much larger than that of *M. erminea* (Heptner *et al*., 1967; Ralls and Harvey, 1985; Meia 1990; Meia and Mermod, 1992). These findings reflect that *M. nivalis* has a wider distribution and inhabits more diverse biotopes and possibly has a higher adaptability to environments than *M. erminea*.

ACKNOWLEDGMENTS

We are grateful to the Institute of Low Temperature Science of Hokkaido University for technical support. This study was supported in part by Grants-in-Aid for Scientific Research and the 21th Century COE Program “Neo-Science of National History” at Hokkaido University financed from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

Abramov AV, Baryshnikov GF (2000) Geographic variation and intraspecific taxonomy of weasel *Mustela nivalis* (Carnivora, Mustelidae). Zoosystematica Rossica 8: 365–402

Árnason U, Johnsson E (1992) The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina*. J Mol Evol 34: 493–505

Corbet GB (1978) The Mammals of the Palaearctic Region: A Taxonomic Review. Cornell Univ Press, London

Eger JL (1990) Patterns of geographic variation in the skull of Nearctic ermine (*Mustela erminea*). Can J Zool 68: 1241–1249

Ellerman JR, Morrison-Scott TCS (1951) Checklist of Palaearctic and Indian Mammals (1758 to 1946). Trustees of British Museum (Natural History), London

Fleming MA, Cook JA (2002) Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. Mol Ecol 11: 795–807

Frank F (1985) Zur Evolution und Systematik der kleine Wiesel (*Mustela nivalis* Linnaeus, 1766). Z Saugetierkd 50: 208–225 (in German with English abstract)

Graphodatsky AS, Volobuev VT, Ternovsky DV, Radjabli SI (1976) G-banding of the chromosomes in seven species of Mustelidae (Carnivora). Zoologicheskii Zhurnal 11: 1704–1709 (in Russian with English abstract)

Hall ER (1951) American weasels. Sci Pap Univ Kansas Nat Hist Mus 4: 1–466

Heptner VG, Naumov NP, Yurgenson PB, Sludskiy AA, Chirkova AF, Bannikov AG (1967) Mlekopitayushchie Sovetskogo Souza [Mammals of Soviet Union], Vol.2 (1) Sea Cows and Carnivores. Vyshaya Shkola, Moscow (in Russian)

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biol J Linn Soc 68: 87–112

King CM (1983) *Mustela erminea*. Mamm Species 195: 1–8

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120

Kratochvil J (1977) Sexual dimorphism and status of *Mustela nivalis* in Central Europe (Mamm., Mustelidae). Acta Scientarum Naturalium Academiae Scientarum Bohemoslovacae (Brno) 11: 1–42

Kumar S, Tamura K, Nei M (1993) MEGA: Molecular Evolutionary Genetics Analysis Version 1.01. Pennsylvania State Univ, Pennsylvania

Kurose N, Masuda R, Yoshida MC (1999) Phylogeographic variation in two mustelines, the least weasel *Mustela nivalis* and the ermine *M. erminea* of Japan, based on mitochondrial DNA control region sequences. Zool Sci 16: 971–977

Kurose N, Abramov AV, Masuda R (2000) Intrageneric diversity of the cytochrome *b* gene and phylogeny of Eurasian species of the genus *Mustela* (Mustelidae, Carnivora). Zool Sci 17: 673–679

Kurten B (1968) Pleistocene Mammals of Europe. Aldine, Chicago

Kurten B, Andersen E (1980) Pleistocene Mammals of North America. Columbia Univ Press, New York

Masuda R, Yoshida MC (1994) A molecular phylogeny of the family Mustelidae (Mammalia, Carnivora), based on comparison of mitochondrial cytochrome *b* nucleotide sequences. Zool Sci 11: 605–612

Meia JS (1990) Étude de la variation de taille de l'hermine (*Mustela erminea* L.) et de la belette (*Mustela nivalis* L.) en Europe. Bulletin de la Societe Neuchâteloise des Sciences Naturelles 113: 307–318 (in French with English abstract)

Meia JS, Mermod C (1992) Taxonomie et variation de taille des hermines et des belettes en Europe: revue de littérature et commentaire. Revue Suisse Zoology 99: 109–118 (in French with English abstract)

Nowak RM (1991) Walker's Mammals of the World. 5th ed., Johns Hopkins Univ Press, Baltimore

Obara Y (1991) Karyosystematics of the mustelid carnivores of Japan. Honyurui Kagaku 30: 197–220 (in Japanese with English summary)

Ralls K, Harvey PH (1985) Geographic variation in size and sexual dimorphism of North American weasels. Biol J Linn Soc 25: 119–167

Reig S (1997) Biogeographic and evolutionary implications of size variation in North American least weasel (*Mustela nivalis*). Can J Zool 75: 2036–2049

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd ed, Cold Spring Harbor Laboratory Press, New York

Shields GF, Kocher TD (1991) Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. Evolution 45: 218–221

Swofford DL (1998) Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4, Sinauer Associates, Sunderland

Tsuda K, Kikkawa Y, Yonekawa H, Tanabe Y (1997) Extensive interbreeding occurred among multiple matriarchal ancestors during the domestication of dogs: evidence from inter- and intraspecies polymorphisms in the D-loop region of mitochondrial DNA between dogs and wolves. Genes Genet Sys 72: 229–238

van Zyll de Jong CG (1992) A morphometric analysis of cranial variation in Holarctic weasels (*Mustela nivalis*). Z Saugetierkd 57: 77–93

Vila C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK (1997) Multiple and ancient origins of the domestic dog. Science 276: 1687–1689

Wozencraft WC (1993) Order Carnivora. In "Mammal Species of the World: A Taxonomic and Geographic Reference, 2nd ed" Ed by DE Wilson, DM Reeder, Smithsonian Inst Press, Washington, pp 279–348

Youngman PM (1982) Distribution and systematics of the European mink *Mustela lutreola* Linnaeus 1761. Acta Zoologica Fennica 166: 1–48

(Received May 25, 2005 / Accepted August 31, 2005)