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Intrageneric Diversity of the Cytochrome *b* Gene and Phylogeny of Eurasian Species of the Genus *Mustela* (Mustelidae, Carnivora)

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ABSTRACT—To illuminate molecular phylogenetic relationships among Eurasian species of the genus *Mustela* (Mustelidae, Carnivora), we determined nucleotide sequences of the complete mitochondrial cytochrome *b* gene region (1,140 base pairs). Molecular phylogenetic trees, constructed using the neighbor-joining and the maximum likelihood methods, showed the common topology of species relationships to each other. The American mink *M. vison* first branched off and was positioned very remotely from the other species of *Mustela*. Excluding *M. vison*, the ermine *M. erminea* first split from the rest of the species. Two small body-sized weasels, the least weasel *M. nivalis* and the mountain weasel *M. altaica*, comprised one cluster (named "the small weasel group"). The other species formed another cluster, where the remarkably close relationships among the domestic ferret *M. furo*, the European polecat *M. putorius*, and the steppe polecat *M. eversmanni* were noticed with 87–94% bootstrap values (named "the ferret group"), supporting the history that the ferret was domesticated from *M. putorius* and/or *M. eversmanni*. The European mink *M. lutreola* was the closest to the ferret group. The genetic distance between the Siberian weasel *M. sibirica* and the Japanese weasel *M. itatsi* corresponded to differences of interspecific level, while the two species were relatively close to *M. lutreola* and the ferret group. These results provide invaluable insight for understanding the evolution of *Mustela* as well as for investigating the hybridization status between native and introduced species for conservation.

INTRODUCTION

The family Mustelidae, which consists of more than 65 species, has been most diversified in the order Carnivora (Nowak, 1991). In this family, the genus *Mustela* including 17 species is a group of small or middle-sized weasels and distributed in a worldwide scale (Wozencraft, 1993; Abramov, 1999a). In Eurasia, 12 species belonging to *Mustela* are known: the mountain weasel *M. altaica* Pallas, 1811; the ermine *M. erminea* Linnaeus, 1758; the least weasel *M. nivalis* Linnaeus, 1766; the Siberian weasel *M. sibirica* Pallas, 1773; the European polecat *M. putorius* Linnaeus, 1758; the steppe polecat *M. eversmanni* Lesson, 1827; the European mink *M. lutreola* (Linnaeus, 1761); the Japanese weasel *M. itatsi* Temminck, 1844; the yellow-bellied weasel *M. kathiah* Hodgson, 1835; the back-striped weasel *M. strigidorsa* Gray, 1853; the Malaysian weasel *M. nudipes* Desmarest, 1822; the Indonesian weasel *M. lutreolina* Robinson and Thomas, 1917. In addition, the American mink *M. vison* Schreber, 1777 was introduced to the Old World from North America and naturalized in Europe, Siberia, and Hokkaido Island of Japan.

Some researchers reported the phylogeny of *Mustela* based on previous morphological data (Youngman, 1982; Anderson, 1989; Wozencraft, 1989; Dunstone, 1993). However, the overall phylogeny of *Mustela* is still controversial, because there are few comparative studies between European and Asian species. On the other hand, based on genetic data, some phylogenetic relationships of *Mustela* were reported as follows. Graphodatsky *et al*. (1976) studied karyotaxonomy among seven species of *Mustela*, and considered that *M. vison* first split from the other *Mustela* species. The close relationship between *M. putorius* and *M. eversmanni* was indicated, and their ancestral characters were seen in *M. lutreola* and *M. sibirica*. *Mustela altaica* and *M. nivalis* were closer to each other, and *M. erminea* retained ancestral characters for the other species except *M. vison*.

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Masuda and Yoshida (1994) reported the molecular phylogeny of Mustelidae living around Japan using partial sequences of the mitochondrial DNA (mtDNA) cytochrome *b* gene. Also there, *M. vison* was remote from the other *Mustela* species. The large genetic difference between *M. itatsi* and *M. sibirica* was found, supporting the original taxonomical treatment that *M. itatsi* Temminck, 1844 is a distinct species.

Especially, the phylogenetic position and genetic characteristics of *M. vison* are required to be elucidated. At present, the precise evolutionary distance between *M. vison* and other species is still unclear, although the common opinion is that *M. vison* is phylogenetically positioned out of the other *Mustela* species (Graphodatsky *et al*., 1976; Belyaev *et al*., 1980; Lushnikova *et al*., 1989; Taranin *et al*., 1991; Masuda and Yoshida, 1994).

The domestic ferret *M. furo* is generally thought to be domesticated from *M. putorius*, or the congener *M. eversmanni* which has a more similar cranial morphology (Blandford, 1987). Davison *et al*. (1999) investigated the mtDNA phylogeny among *putorius*, *eversmanni*, and *furo*, and found two geographically distinct lineages of *putorius* in Britain. The population expansion may be mediated by dispersing male *putorius* hybridizing with female feral ferrets (Davison *et al*., 1999). The hybridization between the ferret and polecats has been anticipated as a threat to the genetic integrity of native polecat populations in Europe. Thus, it is now much required to clarify the genetic relationships within *Mustela*.

In the present study, we determined the complete nucleotide sequences of the cytochrome *b* gene for ten *Mustela* species distributed in Eurasia, including the domestic ferret and the American mink. Based on sequence diversity, we here present the molecular phylogeny within this genus and discuss the genetic relationships between species.

MATERIALS AND METHODS

Samples and DNA extraction

Species of the genus *Mustela* examined in the present study were listed in Table 1. Sequence data of the Japanese marten *Martes melampus* and the sable *Martes zibellina* as outgroups were quoted from our previous report (Kurose *et al*., 1999). Muscle tissues from animals were preserved in 70–100% ethanol at room temperature until use. Total DNAs were extracted from muscles using the phenol/ proteinase K/sodium dodecyl sulfate (SDS) method of Sambrook *et al*. (1989) with some simplified modifications as indicated by Masuda and Yoshida (1994). DNA extracts of *M. lutreola* (muscle tissues preserved in 70% ethanol for 30 years), and *M. altaica* (muscle tissues preserved in 70% ethanol for 108 years) were concentrated to approximately 100 fold using Centricon-30 microconcentrators (Amicon), because these tissues were preserved so long that the DNAs contained were fragmented. Hair DNA was extracted only from *M. furo* using the method of Walsh *et al*. (1991). An aliquot (1–10 µl) of each DNA extract was used as template of subsequent polymerase chain reaction (PCR).

PCR amplification and direct sequencing

The complete (1,140 base pairs, bp) or partial (approximately 450 bp) region of the cytochrome *b* gene was PCR-amplified using any pair of the following primers, all of which were newly designed in the present study (Fig. 1): Cb-M1 5'-CTCACATGGAATCTAACCATGAC-3'; Cb-M6 5'-AGACGTCAACTACGGCTGAAT-3'; Cb-M7 5'-GAATGAATCTGAGGCGGATTTTC-3'; Cb-M8 5'-CAACCCCCTCAACACACCT-3'; Cb-M9 5'-CTATTAGTATTATTCTCACCCGA-3'; Cb-M11 5'-CGATTCTTCGCTTTCCACTT-3'; Cb-MR1 5'-TCTTCCTTGAGTCTTAGGGAG-3'; Cb-MR5 5'-GGCTAGGACTAGGATGGAGA-3';

Table 1. Profiles of samples examined in the present study

Species	Common name	Code (individual No.)	Chromosome No. (2n)[#]	Tissue	Sampling locality if known	Accession Number*
Mustela nivalis	Least weasel	MNI(5)	42[#] (38[$])	muscle	Hokkaido, Japan	AB026106
Mustela altaica	Mountain weasel	MAL(RMNG1)	44[#]	muscle	Great Hingan, Mongolia (ZIN C.83033)[@]	AB026100
Mustela erminea	Ermine	MER(1)	44[#]	muscle	Hokkaido, Japan	AB026101
Mustela itatsi	Japanese weasel	MIT(MR1)	38[$]	muscle	Iwate, Japan	AB026104
Mustela sibirica	Siberian weasel	MSI(KYO1)	38[#]	muscle	Kyoto, Japan	AB026108
Mustela eversmanni	Steppe polecat	MEV(RURA1)	38[#]	muscle	Ural Mountains, Russia (ZIN O.34843)[@]	AB026102
Mustela putorius	European polecat	MPU(RLEN1)	40[#]	muscle	Leningrad Province, Russia (ZIN O.34838)[@]	AB026107
Mustela furo	*Ferret*	MFU(2)	40[!]	hair	Domestic	AB026103
Mustela lutreola	European mink	MLU(RPSK1)	38[#]	muscle	Pskov Province, Russia (ZIN C.55065)[@]	AB026105
Mustela vison	American mink	MVI(1)	30[#]	muscle	Domestic	AB026109
Martes melampus	Japanese marten	MME(1)	38[$]	muscle	Iwate, Japan (outgroup)	AB012351**
Martes zibellina	Sable	MZI(1)	38[+]	muscle	Hokkaido, Japan (outgroup)	AB012360**

[#] Cited from Graphodatsky *et al*. (1976).
[$] Obara (1991) reported 38 chromosomes specific to the population of Honshu main island of Japan.
[+] Cited from Graphodatsky *et al*. (1977).
[!] Cited from Fredga and Mandahl (1973).
[@] Specimen No. of Zoological Institute of Russian Academy of Sciences, St. Petersburg (ZIN).
* The nucleotide sequence data reported in the present study will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with these accession numbers.
** Cited from Kurose *et al*. (1999).

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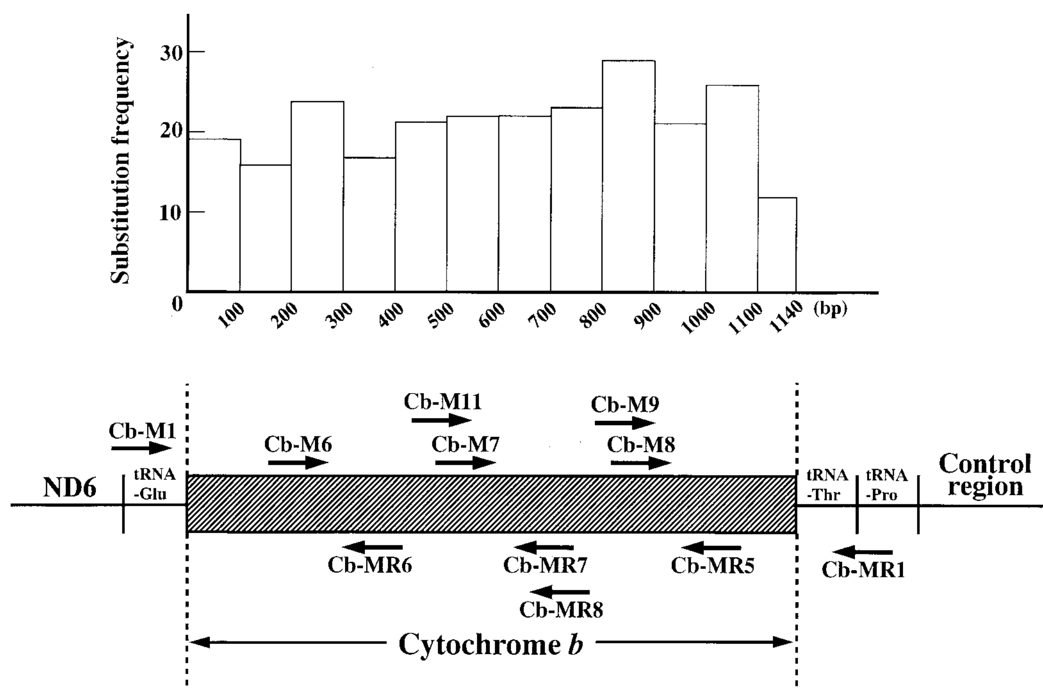

Fig. 1. Schematic diagram of the complete sequence region (1,140 bp) of the cytochrome *b* gene of the genus *Mustela*. The above histogram indicates the substitution frequency in every 100 bp fragment. Arrows (Cb-M1, Cb-M6, Cb-M7, Cb-M8, Cb-M9, Cb-M11, Cb-MR1, Cb-MR5, Cb-MR6, Cb-MR7, and Cb-MR8) in the lower figure show the primer positions used for PCR amplification and/or sequencing.

Cb-MR6 5'-GCCGATGTTTCATGTTTCGG-3'; Cb-MR7 5'-GATCCTG-TTTCGTGGAGGAATA-3'; Cb-MR8 5'-GATCGTAGAATAGCATAT-GCGAA-3'. Procedures of symmetric PCR and direct sequencing were the same as those reported by Kurose *et al.* (1999).

Sequence analysis

Sequence alignment was done using GeneWorks computer software (Intelligenetics). The neighbor-joining tree (Saitou and Nei, 1987) using Kimura's (1980) two-parameter distance was constructed by Mega computer software (Kumar *et al.*, 1993). The maximum likelihood tree (Felsenstein, 1981) was constructed using PHYLIPS computer software (Felsenstein, 1993). Numbers of transitions and transversions were calculated using Clustal V computer software (Higgins *et al.*, 1992). The divergence time between species was estimated using the transversional substitution rate (0.5% per million years, Myr) at the third codon positions of the mammalian cytochrome b gene (Irwin *et al.*, 1991).

RESULTS

Figure 1 shows nucleotide substitution frequencies in every 100-bp segment of cytochrome *b* among the *Mustela* species. It is obvious that nucleotide substitutions within *Mustela* appeared almost constantly throughout the gene region. Transitions occurred more dominantly than did transversions (Table 2).

In order to assess multiple substitutions in cytochrome *b* of *Mustela*, transitions per site were plotted against trans-

Table 2. Percentage differences (above diagonal) and numbers of transitions/transversions (below diagonal) for the complete cytochrome *b* gene sequence (1,140 bases) of genera *Mustela* and *Martes* (outgroup)

Code*	MER	MNI	MAL	MPU	MFU	MEV	MLU	MSI	MIT	MVI	MZI**	MME**
MER	—	7.89	6.93	8.25	8.33	8.25	7.98	7.98	7.98	11.32	12.19	11.32
MNI	81/9	—	7.28	8.07	8.16	8.07	7.98	8.33	8.33	13.77	13.51	12.28
MAL	69/10	72/11	—	7.81	7.89	7.81	8.33	8.25	10.00	12.98	12.98	12.11
MPU	85/9	82/10	78/11	—	0.61	0.35	1.14	3.95	5.53	12.28	12.72	11.84
MFU	86/9	83/10	79/11	5/2	—	0.26	1.05	3.68	5.70	12.46	13.16	12.28
MEV	86/8	83/9	79/10	3/1	2/1	—	0.79	3.60	5.61	12.37	13.07	12.19
MLU	83/8	82/9	85/10	12/1	11/1	9/0	—	3.51	5.18	12.46	13.42	12.54
MSI	83/8	86/9	84/10	42/3	39/3	39/2	38/2	—	5.26	12.46	14.21	12.81
MIT	81/10	84/11	102/12	60/3	62/3	62/2	57/2	56/4	—	12.02	14.04	13.51
MVI	107/22	132/25	124/24	119/21	121/21	121/20	122/20	120/22	115/22	—	15.35	15.35
MZI**	105/34	121/33	114/34	114/31	117/33	117/32	121/32	128/34	126/34	141/34	—	3.95
MME**	93/36	105/35	102/36	102/33	105/35	105/34	109/34	110/36	118/36	139/36	43/2	—

* Codes refer to those in Table 1.
** Two species (MME and MZI) of *Martes* were used for outgroups.

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versions per site (Fig. 2). The relationships between transitions and transversions were almost proportional within the genus *Mustela* as well as between *Mustela* and *Martes* (outgroup) (Fig. 2), indicating that transitional substitutions among cytochrome *b* genes of these species have not yet been saturated and that genetic distances calculated from sequences comparisons certainly provide confident phylogenetic relationships. Percentage differences within *Mustela* were 0.3–13.8% and those between *Mustela* and *Martes* (outgroup) were 11.3–15.4% (Table 2).

Molecular phylogenetic trees constructed using the neighbor-joining (NJ) and the maximum likelihood (ML) methods showed the almost same topology of the species relationships (Fig. 3). *M. vison* first split from the other species. Then, *M. erminea* was separated from the rest of the species. *M. altaica* and *M. nivalis* were clustered with 57%/72% (NJ/ML) bootstrap values, and the cluster was named “the small weasel group”.

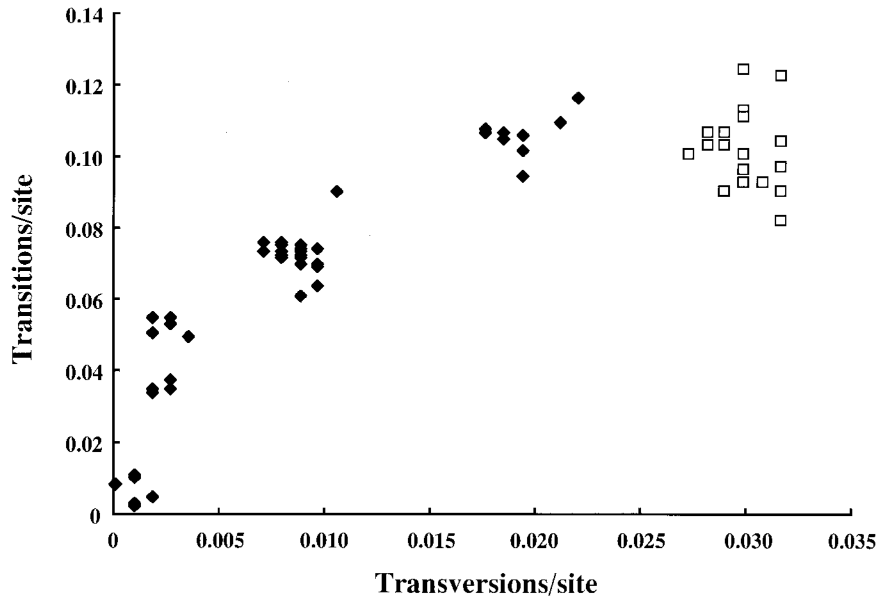

Fig. 2. Transitions per site were plotted against transversions per site from pairwise comparisons of the cytochrome *b* nucleotide sequences (1,140 bp). Closed squares indicate pairwise comparisons within *Mustela* and open ones do those between *Mustela* and *Martes* (outgroup).

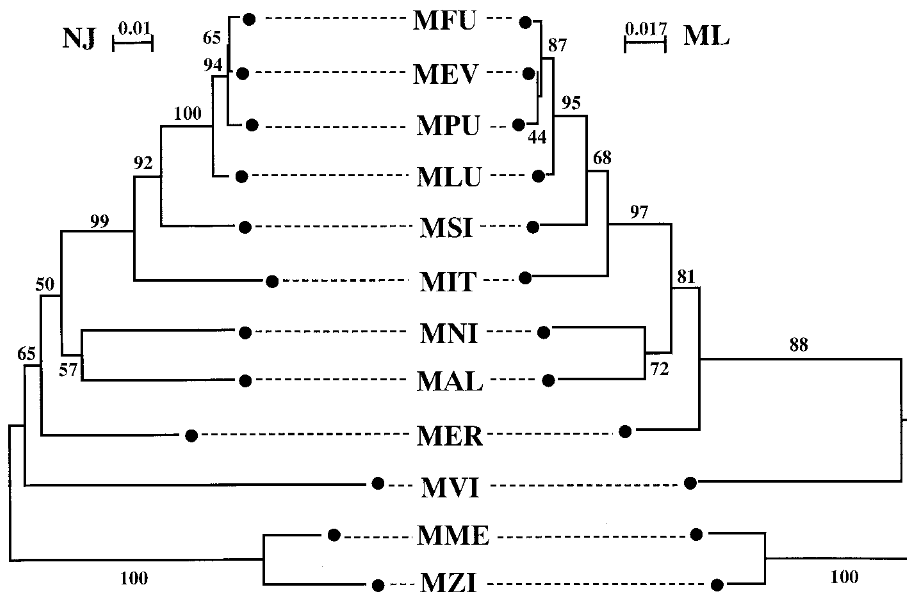

Fig. 3. Phylogenetic trees of the complete cytochrome *b* nucleotide sequences constructed using the neighbor-joining method with Kimura’s two-parameter distances (NJ, left) and the maximum likelihood method (ML, right). Codes refer to those of Table 1. The scale bars indicate the genetic distance as substitutions per site. Numbers (%) near internal branches are bootstrap values derived from 1,000 (NJ) and 100 (ML) replications.

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In another cluster, *M. putorius*, *M. eversmanni*, and *M. furo* were remarkably close to each other (named “the ferret group”) with 94%/87% bootstrap values, having small genetic distances (0.3–0.6% sequence difference, Table 2) among them. NJ tree showed that *M. eversmanni* was closer (65% bootstrap value) to *M. furo* than to *M. putorius*, while ML tree indicated that *M. eversmanni* was closer to *M. putorius* (44% bootstrap value) than to *M. furo* (Fig. 3), although the bootstrap values were not so high.

Mustela lutreola was closer to, but out of, the ferret group (100%/95% bootstrap values). The genetic distance (5.3% sequence difference, Table 2) between *M. itatsi* and *M. sibirica* corresponded to differences between other distinct species, while the two species were separated from the ferret group and *M. lutreola*.

DISCUSSION

The overall species relationships (Fig. 3) obtained in the present study are partially in concordance with previous morphological classification and karyotaxonomy (Graphodatsky *et al*., 1976; Wozencraft, 1989; Dunstone, 1993). Moreover, our data well answered some questions concerning the *Mustela* phylogeny as follows.

Phylogenetic positions of two minks, *M. vison* and *M. lutreola*

The phylogenetic relationship between *M. vison* and *M. lutreola* had long been unclear. Many researchers believed that the two minks were conspecific or at least closely related to each other. Then, the presence of some remarkable differences in cytogenetic and molecular characters between *M. vison* and the other *Mustela* species was reported (Graphodatsky *et al*., 1976; Belyaev *et al*., 1980; Lushnikova *et al*., 1989; Taranin *et al*., 1991; Masuda and Yoshida, 1994). For example, the diploid chromosome number of *M. vison* is 30 (2n=30, Table 1), while the other *Mustela* species have a range between 38 and 44 chromosomes (Ewer, 1973). The present study also demonstrated that *M. vison* is clearly separated from the other *Mustela* species. The genetic distances (11.3–13.8%) which *M. vison* had with the other *Mustela* species were much higher than interspecific differences among the other *Mustela* species, and nearly corresponded to the differences between the other *Mustela* species and another genus *Martes* (Table 2, Fig. 3). However, the frequencies of transversions at the third codon positions (Table 3) clearly show that the two species of *Martes* are remoter than *Mustela* vison from the other *Mustela* species. *M. vison* was estimated to have diverged from the other *Mustela* species approximately 8–11 Myr ago (Table 3).

Previous morphological classification (Youngman, 1982; Wozencraft, 1989; Dunstone, 1993) as well as karyotaxonomy (Graphodatsky *et al*., 1976) supported the closer relatedness in the *lutreola-sibirica* lineage. The molecular phylogeny of the present study also exhibited that *M. lutreola* has close relationship with *M. sibirica* as well as with the ferret group (but not included in this group cluster). The comparative analysis of the antigenic structure of the immunoglobulin chain in Mustelidae (Taranin *et al*., 1991) showed that *M. lutreola* is closer to the ferret group. Meanwhile, Davison *et al*. (1999) reported that *M. lutreola* was included in the ferret group cluster comprising *M. furo*, *M. putorius*, and *M. eversmanni*, based on the molecular phylogeny using partial cytochrome *b* sequences (337 bp). The partial sequence (Accession No. AF068544) reported by Davison *et al*. (1999) was identical with the homologous region of the complete cytochrome *b* sequences obtained in the present study. The difference on *M. lutreola*’s position may be ascribed to the shorter informative sequence (337 bp) used by Davison *et al*. (1999). Otherwise, the intraspecific variations of *M. putorius* and *M. eversmanni* might be so large that the position of *M. lutreola* was included in them.

Now, it is obvious that *M. vison* and *M. lutreola* are phylogenetically much differentiated from each other, although both

Table 3. Percentage differences of transversions at the third codon positions of the cytochrome *b* sequences (above diagonal) and the estimated divergence time (Myr, below the diagonal) using the transversional substitution rate (0.5%/Myr) at the third codon positions of mammalian cytochrome *b* reported by Irwin *et al*. (1991)

Code*	MER	MNI	MAL	MPU	MFU	MEV	MLU	MSI	MIT	MVI	MZI	MME
MER	—	2.37	2.63	2.11	2.11	1.84	1.84	1.84	2.11	5.00	7.89	8.42
MNI	4.74	—	2.89	2.37	2.37	2.11	2.11	2.11	2.37	5.79	7.63	8.16
MAL	5.26	5.78	—	2.63	2.63	2.37	2.37	2.37	2.63	5.53	7.89	8.42
MPU	4.22	4.74	5.26	—	0.53	0.26	0.26	0.79	0.53	4.47	6.84	7.37
MFU	4.22	4.74	5.26	1.06	—	0.26	0.26	0.79	0.53	4.47	7.37	7.89
MEV	3.68	4.22	4.74	0.52	0.52	—	0.00	0.53	0.26	4.21	7.11	7.63
MLU	3.68	4.22	4.74	0.52	0.52	0	—	0.53	0.26	4.21	7.11	7.63
MSI	3.68	4.22	4.74	1.58	1.58	1.06	1.06	—	0.79	4.74	7.63	8.16
MIT	4.22	4.74	5.26	1.06	1.06	0.52	0.52	1.58	—	4.47	7.37	7.89
MVI	10.00	11.58	11.06	8.94	8.94	8.42	8.42	9.48	8.94	—	8.16	8.68
MZI**	15.78	15.26	15.78	13.68	14.74	14.22	14.22	15.26	14.74	16.32	—	0.53
MME**	16.84	16.32	16.84	14.74	15.78	15.26	15.26	16.32	15.78	17.36	1.06	—

* Codes refer to those in Table 1.

** Two species (MME and MZI) of *Martes* were used for outgroups.

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animals are commonly called ‘mink’.

Parental species of the domestic ferret *M. furo*

Our results showed the closer relationships among the three morphological similar polecats, *M. putorius*, *M. eversmanni*, and *M. furo* (Table 2 and Fig. 3). The sequence differences (0.3–0.6%) were very similar to the level of intraspecific variations of the *Martes* species reported by Kurose *et al*. (1999). The divergence among these three species was estimated to have occurred within one Myr ago (Table 3). The diploid chromosome number is 2n=40 for *M. putorius* as well as *M. furo*, while 2n=38 for *M. eversmanni* (Table 1). The former two species have morphologically identical chromosome sets, but the karyotype of *M. eversmanni* differs from those of the former by a single Robertsonian rearrangement (Fredga and Mandhal, 1973; Volobuev *et al*., 1974). A series of embryological characteristics of *M. furo* was reported to be more similar to *M. putorius* than to *M. eversmanni* (Ternovsky, 1977). Experimental hybridization among *M. furo*, *M. putorius*, and *M. eversmanni* was found to be possible, and all the hybrids were fertile (Ternovsky, 1977). In the present study, we found the very close molecular phylogenetic relationships among the three species, supporting the parental species of the domestic ferret was the polecats. Which polecat is closer to *M. furo* was not concluded in the present study, because the relationships among them were not supported with high confidence (Fig. 3). Further analyses of karyotypes, mtDNA and nuclear DNA, and morphology of the ferrets with different lineages as well as the wild populations of polecats would illuminate more precise history of the ferret’s domestication.

Phylogenetic positions of the Japanese weasel *Mustela itatsi* and the Siberian weasel *M. sibirica*

The taxonomical relationship between *M. itatsi* and *M. sibirica* has been obscure for a long time. Very often was *itatsi* considered as conspecific to *sibirica* (Ellerman and Morrison-Scott, 1951; Corbet and Hill, 1992; Wozencraft, 1993; Pavlinov *et al*., 1995). Imaizumi (1960) also regarded *itatsi* as a subspecies of *M. sibirica*, showing that the ratio of the tail length to the head-body length is more than 50% for *sibirica* of Korean Peninsula and around 40% for *itatsi* of Japanese islands. The present study followed this classification of the two weasels. Recently, Abramov (1999b) reported the presence of a clear craniological difference between the two species. Masuda and Yoshida (1994) also supported the idea that *itatsi* is an independent species, based on partial cytochrome *b* sequence data. Table 2 shows that *itatsi* has the relatively closer genetic distance (5.3%) with *sibirica*, while this value refers to the sequence difference between the other *Mustela* species. The two weasels are still phylogenetically closer to each other than to other species (Fig. 3). We recently found the karyotypical difference of G- and C-banding patterns between the two weasels (unpublished data), both of which share the identical diploid chromosome number (2n=38, Table 1). The transversional difference of the third codon positions (0.8%, Table 3) between *itatsi* and *sibirica* refers to approximately 1.6 Mry ago of the divergence time. The ancestor of *itatsi* might have been derived from *sibirica* probably in the continent in the early Pleistocene. After that, a certain ancestral population of *itatsi* might have immigrated to Japanese islands, and then it could have been isolated on the islands through the strait formation. The other ancestral populations remaining in the continent might have been extinct. Otherwise, from an ancestor common to *sibirica*, *itatsi* might have evolved independently on Japanese islands after separation from the continent.

Phylogeny of the small weasel group

The closer relationship between *M. altaica* and *M. nivalis* indicated in the present study was also supported by classic morphological classification (Heptner *et al*., 1967; Youngman, 1982; Wozencraft, 1989; Dunstone, 1993) and karyotaxonomy (Graphodatsky *et al*., 1976). *M. altaica* is distributed from the Altai mountains to the Himalayan region and some mountainous areas of Asia, while *M. nivalis* is widespread in Eurasia and North America. The two species were estimated to have diverged over five Myr ago (in the end of Miocene) (Table 3). The present study first reported mtDNA sequence data of *M. altaica* and exhibited the molecular phylogenetic position.

Mustela erminea is widespread in Eurasia and North America. The divergence time between *M. erminea* and the other *Mustela* species except *M. vison* was estimated around three to five Myr ago (Table 3). Thus, the species diversification within *Mustela* might have started from the end of Miocene to Pliocene. Our data that *M. erminea* first split from the other *Mustela* species is supported by the karyological study (Graphodatsky *et al*., 1976; Obara, 1991) that *M. erminea* is the more ancestral form among *Mustela*.

The present study revealed the phylogenetic relationships among Eurasian species of the genus *Mustela*. Our results provide not only invaluable insight to reconstruct the taxonomy of this carnivore group, but also useful information to survey genetic characteristics and hybridization problems between native and introduced populations for species conservation. In addition, further study involving other *Mustela* species of Eurasia and America would illuminate the worldwide evolution of *Mustela*.

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