



HOKKAIDO UNIVERSITY

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Title of Doctoral Dissertation

Molecular evolutionary study on the Japanese weasel (*Mustela itatsi*) and the Siberian weasel (*M. sibirica*), based on complete mitochondrial genome sequences

（ミトコンドリアゲノム全配列に基づくニホンイタチ(*Mustela itatsi*)および
シベリアイタチ(*M. sibirica*)の分子進化学的研究）

In the present study, molecular evolution of the endemic Japanese weasel (*Mustela itatsi*) and the continental allopatric species, the Siberian weasel (*M. sibirica*), was investigated intensively using complete mitochondrial genome sequences. This dissertation on the study is divided into three chapters. In the first chapter, complete mitochondrial genome sequences for 26 individuals of *M. itatsi* and 20 individuals of *M. sibirica* were analyzed. The divergence time between *M. itatsi* and *M. sibirica* estimated from the sequence data were 2.36 million years ago (Mya), corresponding with the Early Pleistocene. This divergence time is close to that of most of other Japan-endemic/continental mammalian species pairs previously reported. *Mustela itatsi* comprised two haplotype clades that diverged an estimated 1.64 Mya, in the Middle Pleistocene: a northern (Honshu) clade comprising geographically distinct basal, northern, and eastern subclades, and a western paraphyletic group; and a southern clade comprising geographically distinct sub-clades on Kyushu, Shikoku, and adjacent small islands. The results indicate a single migration of an ancestral population from the Korean Peninsula to southern Japan across a Late Pliocene or Early Pleistocene land bridge, followed by allopatric speciation of *M. itatsi* in Japan. The southern lineage appears to have remained in place, whereas the range of the northern lineage expanded stepwise from southwestern to northern Honshu between 0.68 and 0.27 Mya. By contract, *M. sibirica* also comprised two main clades that diverged an estimated 1.57 Mya: one containing haplotypes from continental Russia and Tsushima Island (Japan), and the other containing haplotypes from Korea, China and Taiwan. The *M. sibirica* population on Tsushima Island is likely a relict from the continental Russian population. The estimated divergence times indicated that both *Mustela* species were the early colonists of the Japanese islands and continental Eurasia, respectively. The present study based on the complete mitochondrial genome sequences provides a higher resolution of the phylogeographic relationships between and within these closely related insular and continental species of *Mustela*, compared with previous studies using single or a few mitochondrial gene loci.

In the second chapter, the obtained complete mitochondrial (mtDNA) genome sequences from the previous chapter were used to compare sequence variations among different genes of mtDNA in *M. itatsi* and *M. sibirica*. The ratio between parsimoniously informative sites (Pi) and the length in base-pairs (bp) (Pi/length) were calculated for each gene locus. The results showed that the control region (D-loop) has the highest Pi/length ratio among gene loci. The protein-coding gene commonly used in phylogenetic studies, cytochrome *b*, did not show the second highest Pi/length in both species

following the control region, but other genes like the protein coding genes CO3 for *M. itatsi* and ND4 for *M. sibirica* and ND2 for both species showed the higher values. The result indicated that a combination of control region/ ND2/ ND4/ cytochrome *b* genes could lead to better resolution of the phylogenetic relationships between mustelid species.

In the third chapter, the variation of the mtDNA control region including the C/T indel sites and the specific tandemly repeated sequences were investigated in the *M. sibirica* population native to Tsushima Island, located between the Japanese islands and the Korean Peninsula. From 31 animals examined, variants of 17 C/T insertion/deletion (indel) sites and seven different patterns of tandem repeats were detected. The tandem repeats consisting of less than 10-bp units were almost identified, although nucleotide sequences of the other parts in the mtDNA control region were identical among all of them. The repetitious patterns of tandem repeats all shared the same starting and ending repeat units, but were different in the number of the core repeat compound units of 10 bp. Compared with repeat units of non-insular carnivores published previously, the repetitious sequences found in the present study were remarkably highly polymorphic. In addition, one nucleotide deletion at the 3' end of the last repeat unit occurred in all animals, whereas the 3' end of a previously reported unit in carnivorans was not deleted. The number variation of the compound units in the core region together with the occurrence of the particular last unit with a nucleotide deletion could have been formed by the continuous step-wise slippage. Even among the individuals sampled from the same geographic location during one year within the island, the repeat tandem repeats unit numbers were highly variable, suggesting the remarkably rapid evolution of the repeat units in the control region of *M. sibirica*. Combining the detected seven patterns of tandem repeats together with the 17 variants of C/T indel sites yielded 27 different variants among the studied 31 individuals, showing remarkably high mtDNA diversity in such a small insular population of *M. sibirica*.