A note on the genetic status of the dark red-backed vole, Myodes rex, in Hokkaido, Japan

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The dark red-backed vole, *Myodes* (formerly *Clethrionomys*) *rex*, was first described from specimens collected on Rishiri Island, Hokkaido, northernmost Japan, by Imaizumi (1971) as a new species on the basis of the cranial characteristics, which differ from those of the gray red-backed vole, *M. rufocanus*, in the Hokkaido area (including Hokkaido and the peripheral islands), Japan. *Myodes rex* is thought to be rarer than the other *Myodes* voles, *M. rufocanus* and *M. rutilus*, as shown by the collection of records of *M. rex*, which is quite limited, according to Nakata (2000). In addition, *M. rex* is ranked as NT (Near Threatened) by the Ministry of the Environment of Japan (Ohdachi et al. 2009).

Kaneko and Sato (1993) and Nakata (1995, 2000) reported that *M. rex* prefers grass fields, abandoned cultivated lands near grass fields, valley bottoms, and higher altitude forests as its habitat. In contrast, *M. rufocanus* prefers a habitat of *Sasa* dwarf bamboo, which is extensively distributed throughout Hokkaido (Toyooka et al. 1983). Such habitat preferences of the two species are more strongly observed on Rishiri Island than on Hokkaido Island. On the other hand, *M. rutilus* is also dominant in coniferous forests with sparse *Sasa* bamboo throughout Hokkaido Island, but *M. rutilus* and *M. rufocanus* show microhabitat segregation at sympatric areas, particularly in the east (Ota 1984; Abe 1986). Therefore, *M. rex* is not dominant and is rare in the Hokkaido area. To consider the conservation of such a rare species, fundamental biological data that include ecological and genetic information are needed.

To date, Kashiwabara and Onoyama (1988) and Wakana et al. (1996) have analyzed the chromosome constitutions and genetic differentiation of *M. rex* interspecifically with other vole species, respectively, but the degree of intraspecifically genetic divergence in *M. rex* has not been analyzed and compared with those of the other *Myodes* voles in the Hokkaido area (Iwasa et al. 2000, 2002). In this study, to preliminarily evaluate the genetic diversity in *M. rex*, we analyzed the intraspecific sequence divergence of the mitochondrial cytochrome b (*Cytb*) gene of *M. rex* and compared it with that of two *Myodes* species, *M. rufocanus* and *M. rutilus* in the Hokkaido area.

Materials and methods

In total, 11 samples of *M. rex* were analyzed from six localities shown in Fig. 1 and Table 1 in this study. All samples were species-identified by cranial characteristics according to Kaneko et al. (1998) and preserved in the author’s laboratory as private collections.

Total genomic DNA was extracted from liver tissues using the conventional phenol-chloroform method. *Cytb* (1,140 base pairs) was amplified by PCR with the primer set L14724/H15915 (Irwin et al. 1991). PCR was carried out for 40 cycles, each consisting of 30 sec at 96°C, 60 sec at 50°C, and 60 sec at 72°C. The reaction mixtures (20 μl) contained 0.1 mM dNTPs, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl2, 0.05 μM primers, and 0.5 units of ExTag® polymerase (Takara). The PCR products were sequenced using an automated sequencer (model 310, ABI) using the BigDye Terminator Cycle Sequencing Kit ver. 3.1 (ABI). All the sequences for the *Cytb* region from the *M. rex* samples determined in this study have been deposited in the GenBank/EMBL/DBJ nucleotide sequence databases with accession numbers AB031582 and AB565454–AB565461.

We calculated the haplotype diversity, mean number of pairwise differences, genetic distances, and nucleotide diversity (π) using Arlequin (Schneider et al. 2000) with the Tamura–Nei model (Tamura and Nei 1993). For a comparison, the *Cytb* data of *M. rufocanus* (n = 14) and *M. rutilus* (n = 14) in Hokkaido previously reported by Iwasa et al. (2000, 2002) were used with the same calculation. We also constructed a neighbor-joining tree (Saitou and Nei 1987) with the *Cytb* sequences on