

F-3.1.1 Measurement of Genetic Variation of Threatened Animals in Japan (Final Report)

Contact person Masaaki Yoneda

Japan Wildlife Research Center (JWRC)

Yushima 2-29-3, Bunkyo-ku, Tokyo 113 Japan

Tel. : +81-3-3813-8897, Fax. : +81-3-3813-8898

E-mail: KYM00063@niftyserve.or.jp

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Abstract Genetic variation between and within local populations of three bird species and five mammal species listed in the Red Data Book of Japan was studied to contribute the species conservation. Subject species were Blakiston's fish-owl (*Kepta blakistoni*), Japanese crane (*Grus japonensis*), rock ptermigan (*Lagopus mutus*), Iriomote cat (*Felis bengalensis iriomotensis*) and Tsushima cat (*F. b. euphilura*), Ryukyu and Bonin flying foxes (*Pteropus dasymallus* and *P. pselaphon*), and sika deer (*Cervus nippon*). No variation of mtDNA sequences was found within the populations of *K. blakistoni*, *L. m. japonensis*, *F. b. iriomotensis* and *P. dasymallus*, suggesting little genetic variety. The results were correlated with ecological characteristics of the subject species, and the research and conservation plan was considered.

Key Words Genetic Variation, Threatened Animals

1. Introduction

Aims of this study were to clarify the unit for conservation, to evaluate the importance of conservation and to estimate the viability, by revealing the genetic variation between and within local populations of threatened animal species in Japan. Among the animal species listed in the Red Data Book of Japan, the following three bird species and five mammal species were selected as the study subject: Blakiston's fish-owl (*Kepta blakistoni*), Japanese crane (*Grus japonensis*), rock ptermigan (*Lagopus mutus*), Iriomote cat (*Felis bengalensis iriomotensis*) and Tsushima cat (*F. b. euphilura*), Ryukyu and Bonin flying foxes (*Pteropus dasymallus* and *P. pselaphon*), and sika deer (*Cervus nippon*).

2. Materials and Methods

Variation of nucleotide sequences of mitochondrial 12S rRNA and cytochrome b genes and D-loop region was examined newly developed CAP-PCR/DNA fingerprinting for 25 individuals of *Kepta blakistoni* from eastern Hokkaido. Inter- and intraspecific variations of mt DNA were investigated for 71 individuals of 11

crane species. To investigate genetic variation of *Lagopus mutus japonicus*, using the feathers found in five locations of the Japan Alps to extract DNA, direct sequencing of the control region of mtDNA was done. Regarding the two Japanese wildcats, mt DNA was sequenced and compared with that of other feline species. Two Japanese species of fruit bats were genetically characterized based on the variation of mtDNA, rDNA and Y-linked Sry. Threatened populations of *Cervus nippon* in Kerama and Tsushima were subjected to the analysis of cytochrome b gene, and genetic diversity was examined by RAPD method for five local populations.

3. Results and Discussion

For *Kepta blakistoni* no inter-individual variation in the nucleotide sequences of 12S rRNA and cytochrome b genes and D-loop region was detected among the examined individuals. Some of them, however, showed genomic polymorphism by the DNA fingerprinting. Both cytochrome b and 12S rRNA sequence data implied close relationship among *G. japonensis*, *G. grus* and *G. monacha*. Regarding the genetic difference between Japanese (Kushiro, Hokkaido) and Chinese populations of *Grus japonensis*, analysis of D-loop region sequences revealed three and nine haplotypes in 19 Kushiro and 20 Chinese individuals respectively, although no uncommon haplotypes were found between the two groups. The findings inferred that the observed genetic divergence between the two local populations is not to discriminate them. For *Lagopus mutus japonicus* no polymorphic sites were found among the 15 samples from 5 locations, and it was suggested that the ptarmigan has very little genetic variability. Phylogenetic trees reconstructed from the mtDNA sequence data indicated that Iriomote cat and Tsushima cat are very closely related to *Felis bengalensis* and it is reasonable for the two Japanese wild cats to be classified as distinct subspecies of *F. bengalensis*. No variation of mtDNA sequences within the Iriomote population was found, suggesting reduction of the genetic variety. DNA sequence of the two Japanese fruit bats were distinct each other. However, there were no variations in mtDNA (cytochrome-b) among the individuals from various island populations of *P. dasymallus*. The results on *Cervus nippon* indicated that both are phylogenetically distinct from other sika deer populations. The genetic diversity of the five local populations analyzed by RAPD method was

estimated as the following order: Goyozan > Ashoro > Tsushima > Kinkazan populations. The above results were correlated with ecological characteristics of the subject species, and the future research and conservation plan was considered.