

### F-3 Research for genetic conservation of endangered animals (Final Report)

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Present status of genetic variability between and within local populations of three birds (*Grus japonensis*, *Ketupa blakistoni*, *Lagopus mutus*) and five mammal species (*Dryocopus martius*, *Dendrocops major*, *Mayailurus inriomotensis*, *Cervus nippon keramae*) listed in the Red Data Book of Japan was studied to contribute their conservation. Genetic variability in *Grus japonensis* was found to be smaller in Japanese population (Kushiro) than that in China. Genetic variability among individuals examined at Cyt-b, 12 SrDNA and D-loop was found to be almost scanty in *Ketupa blakistoni*. RAPD-PCR showed *Cervus nippon* population in Tsushima and Kinkazan (both isolated) showed a smaller genetic variability than those in other populations in Tohoku and Hokkaido districts.

Using two hypervariable DNA makers, we examined spatial genetic structures and the mating system of the grey-side vole, *Clethrionomys rufocanus*. Four clusters of related females in three out of 13 matrilineal lineages were detected. Random mating was also suggested. A present distribution and troop size of Japanese macaques, *Macaca fuscata*, were investigated in Tohoku District by radio-tracking. We also examined mtDNA variations by RFLP analysis to assess genetic variability. The degree of differentiation was small between groups within local populations but large between local populations. We have studied the present distribution and habitat destruction of black woodpecker, *Dryocopus martius*, and Amami rabbit, *Pentalagus furnessi*, and collected some samples for extracting DNA. Demographic MVP (minimum viable population) size for Asian black bear (*Ursus thibetanus*) population was calculated from simulation model based on reviewing its life history traits.

In the process of population decrease, populations may suffer a significant genetic deterioration, namely a decrease in genetic variability, which may lead to a corresponding decrease in fitness (survival and reproduction). As fitness can often be measured as fluctuating asymmetry (FA), we analysed FA in relation to various fitness-related characters, such as survival, growth rate, territoriality and/or reproductive success in several animals. We found negative correlation between mating success and FA in insects including dragonflies, butterflies and midges. FA measurements on several passerine birds revealed that population density and the distribution ranges were negatively correlated with population mean of FA.

Analysing data of Japanese quail inbred during 20 years, we found declines in fitness-related parameters with generations, including fecundity, egg hatchability and fertilisation. A simulation study based on these data suggests that the fitness of this population reduced in a stepwise (bottleneck) rather than in a gradual manner.

Dispersed cells of inner organs of a Japanese male of the Japanese crested ibis, *Nipponia nippon*, and those of a Chinese male of the same species were cryo-preserved. The preserved cells of the former individuals were cultured and revealed to proliferate over several generations. Tissue pieces of the former bird were frozen and preserved in liquid nitrogen. To monitor gonadal developmental conditions, we devised a non-invasive method in which sex steroid hormone concentrations in faeces are used as the index. We also developed a method to induce oocyte growth, ovulation and oviposition by hormone administration. By means of the implantation of primordial germ cells, a cimeric gonads of the domestic fowl and the Japanese quail could be produced, but no chick of the heterologous species has been produced.

The Primordial Germ Cells (PGCs) from quail and chickens could be preserved in liquid nitrogen with high viability. Sperm of Shika-deer and Japanese serow was well preserved with the techniques developed to preserve bull sperm.