

F-3.1.2 Mechanism of Reduction of Genetic Variation in Endangered Wildlife

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Abstract 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. Using two hypervariable DNA markers, 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. 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. MVP size was estimated to be >100 individuals for avoiding extinction at 50 years later with <10% probability.

Key Words Endangered Species, Genetic Variation, Habitat, Minimum Viable Population Size

(1) Distribution and habitat of Japanese macaque populations in the Tohoku District

A questionnaire survey revealed existence of the Goyosan population, the Shiragami population, and the Hachimantai population in Iwate and Akita Prefecture of northern Tohoku where isolation and shrinkage of populations appeared to be conspicuous. And, two populations in Iwate, and three populations in Akita which were found in 1978 (Kankyocyo, 1979) might have been extinct or might have been missed because of extremely small size. Solitary males were sighted everywhere excepting in large plains and basins where towns and cultivated fields spread continuously and widely. The effects of the isolation and the size of populations on dispersal of solitaries should be investigated next. The size Ohsawa Troop which was comprised in the Goyosan population (four or five troops, 70 to 100 individuals) was 20 individuals, and stable from 1991 through 1995. The troop included six mature females,

and the birth rate was more than 0.33. The home range was 7.3 km², and 84% of the area was composed of relatively-undisturbed old broad-leaved forests and mixed forests. The home range size per capita was 29ha, and was the widest among those reported previously in snowy and/or cold habitat (8-24ha). The fine comparison of vegetation among the sites would explain the variations of per capita home range size. As the mast and the length of snowy season are known to affect birth and mortality rates dramatically, we should develop models which could simulate the population dynamics with the influences of such fluctuation of environments, in order to evaluate viability of the critical populations in terms of conservation. Analysis of genetic viability of those populations are also important. However, collection of blood samples for DNA analysis from live animals of declining small populations are quite difficult. Thus, I recommend to develop techniques to analyze DNA from feces which could be easier to obtain.

(2) Genetic assessment of local populations of Japanese macaques in the Tohoku District

Intraspecific variations of mitochondrial DNAs (mtDNAs) were examined for Japanese macaques (*Macaca fuscata*) in order to quantify genetic variations within and between local populations and to evaluate the causes of them. Restriction patterns of 70 samples collected from 24 localities in east Japan were compared using 31 kinds of enzymes. From the typing of mitochondrial DNA haplotypes, genetic variability within local populations and genetic differentiation between local populations were evaluated. Electrophoretic examination detected 14 haplotypes. The degrees of genetic variability within local populations were generally very low. Polymorphism found in a social group or a local population was often related to adult males. There are two major clusters of mtDNA haplotypes in the study populations. The patterns of geographic distribution of mtDNA variants were different among the districts in east Japan. The results of present study suggest that low genetic variability within social groups or local populations of monkeys result from female philopatry and matrilineal group fission. Since geographic distribution of mtDNA variants may reflect the history of group division in the past, the two mtDNA clusters found in east Japan suggest that there was geographic isolation of different maternal lineage during the speciation of Japanese macaques. Then, postglacial changes in vegetation pattern and subsequent expansion of habitats may rule the present-day distribution of mtDNA variants in Japanese macaques.

(3) Present status of the Amami rabbit populations and its genetic variation

The Amami rabbit *Pentalagus furnessi*, one of the most primitive lagomorphs, is found only on Japan's Amami Island and Tokuno-Shima Island which belong to the Ryukyu Island Chain in the most southern part of Japan. The rabbit is designated as a special symbol of natural heritage by the government and is classified as endangered by the IUCN. Little

attention has been paid to the conservation and management of its habitat. The demographic properties and genetic variability in the Amami rabbit population were investigated in this study. The population size of the rabbit has decreased and its distribution has been fragmented recently on the two islands. Two rabbits were captured, tagged by radio-transmitter and released. The range size of the rabbits was about 10ha in forest, and one rabbit moved mainly during the nighttime 100-200m from its burrow located in small valley. DNA were extracted from the rabbits and were compared with the Japanese hare *Lepus brachyurus* to estimate genetic distance and diversity. There were some difference in nucleotide sequences of mtDNA between Amami rabbit and Japanese hare. More Ecological and behavioral studies are necessary to conserve the Amami rabbit including to find the sustainable development in the two islands.

(4) Genetic structure of the grey-sided vole population by DNA markers as a model of endangered wildlife populations

Two DNA analysis techniques (microsatellite DNA typing based on a single locus and direct sequencing mtDNA) were developed and applied to a field population voles in the present study. These two techniques are necessary to investigate ecological and genetic features in endangered species. The gray-sided vole, *Clethrionomys rufocanus*, was used for this study. Home ranges and activity centers of individual voles (N=81) were estimated using the catch-mark-release method. The toes clipped for individual identification were collected and DNA was extracted from them. By amplifying DNA with polymerase chain reaction (PCR), the sequences of a part of mtDNA were determined directly for all the voles captured in the population, based on which matrilineal genealogy was estimated. In addition to this, relatedness between voles were assessed on the basis of genotypes at four clusters of related females were observed, though female's home ranges seemed to evenly distribute. On the other hand, closely related males did not form such a cluster probably owing to the male-biased natal dispersal in this vole. The sex-biased dispersal pattern may also have provided both incest avoidance and random mating in the population of gray-sided voles. Thus these results suggest that males' natal dispersal is important to maintain genetic diversity in a population.

(5) Minimum viable population size of the Japanese black bear by demographic analysis

The Japanese black bear (*Ursus thibetanus*) is one of two ursine species in Japan. It occurs on three of the four major islands of Japan. The bear is exploited as a natural resource and large number of bears are hunted every year. The distribution of the bear shows good parallelism with that of beach and oak forests which provided food in the form of beech nuts and acorns. These forests have been decreasing since the 1950' mainly due to forestry activity. The decrease in the bears' habitat has led to fragmentation and isolation of bear populations. We have developed a computer simulation model to estimate the survival rate of an isolated

population. This model is individual-based. The parameters are initial numbers of individuals and survival rate of each age and sex, and if female, pregnancy rate and mean letter size of each age for a given crop of acorns produced in the year. The acorn production is made to fluctuate randomly from year to year. When the most realistic parameters were given, a population that initially had 50 bears would survive for 50 years at a probability of 63% and for 100 years at a probability of 16%. We predict that we can lose the black bear in the next century if further decrease and fragmentation of their habitat is not prevented, and if consideration is not given to curbing hunting by litigation.

(6) Genetic distance of black woodpecker populations by DNA markers

Black Woodpecker inhabits in Hokkaido and northern Honshu of Japan, and the population is quite smaller in the latter region. It is important to examine whether the Honshu population is isolated or not, and the genetic relationship between the two populations, in order to investigate the mechanism through which the population size and the isolation (or immigration) determine the genetic diversity of the present population, and make policy of the conservation by identifying the unit of conservation. For these purposes, we adopted the genetic analysis using mtDNA which was appropriate to examine inter-population genetic differences. We have succeeded in extracting a large quantity of DNA from the blood of Great Spotted Woodpecker, and a little DNA from the richness of Great Spotted Woodpecker, droppings of Black Woodpecker, and richness of the stuffed Black Woodpecker. Furthermore, we designed the primers for polymerase chain reaction from the code region of 12S rRNA and cytochrome b, and successfully amplified the DNA which was extracted from the blood of Great Spotted Woodpecker by the polymerase chain reaction. These results would promise the future success of genetic research of the Honshu population of Black Woodpecker.