

## **F-1.4.2 Studies on minimum viable population size(MVP) in endangered wildlife**

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### **1) Population Viability Analysis of a Japanese Black Bear Population**

Key words: PVA, Japanese black bear, simulation model

#### Research objects

This study was aiming at determining an MVP (Minimum Viable Population size), and performing a PVA (Population Viability Analysis) on the Shimokita black bear population, in order to compile a bear conservation plan.

#### Material and Method

An individual-based model (IBM) of bear population was constructed. The parameters the model uses are: initial numbers of individuals and survival rate of each age and sex, and, if female, reproduction rate and mean litter size of each age for a given level of mast production of the year. The mast production in the model is randomized to fluctuate from year to year with the observed mean frequencies of good and fair mast years.

Numerical experiments were made to examine the sensitivity of carrying capacity and hunting level to the population dynamics.

#### Results

If the carrying capacity will not decrease, the extinction risk after 100 years will be 5.1%. But if it will decrease by 50%, the extinction risk will be almost twice as large as this.

The actual hunting level on the Shimokita bear population is roughly equivalent to additional mortality of 6%. The model simulation shows that if the hunting level will not be lowered from the present level, we can lose the population in the next century at a very high provability (nearly 90%).

#### Discussion

Now the Shimokita bear population has become quite sensitive to decrease of carrying capacity. We have to prevent further decrease and fragmentation of their habitat. The population is much more sensitive to the hunting pressure. We can't help predicting that we can lose the population by the end of 21st century if consideration is not given to reducing the hunting pressure.

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## 2) A mechanism of change in genetic diversity: a model study using a enclosed vole population.

Key words: the grey-sided vole, heterozygosity, mating system.

### Research objects

This study is aimed to monitor changes in genetic diversity in an experimental population of the grey-sided vole and investigate their mating system as a mechanism determining genetic diversity.

### Methods

This study was conducted within a 3-ha outdoor enclosure (200 x 150 m) in a natural broad-leaved forest (42°59'N, 141°23'E) in Hokkaido, the northernmost island of Japan. Forty-six, unrelated gray-sided voles (22 males and 24 females) were released into the enclosure on 29 September 1992. They were selected to maximize heterozygosities at three microsatellite loci<sup>1)</sup> so as to facilitate the determination of parentage for each of the juveniles captured within the enclosure. Genotypes were determined with the polymerase chain reaction (PCR) technique at three highly variable microsatellite loci<sup>1),2),3)</sup>. The number of individuals, reproductive condition, and spacing behavior were observed by the CMR-technique.

### Results

Six generations were observed in one and half year study period. Genetic diversity (heterozygosity) gradually decreased, but it kept a high level. 215 families were determined by DNA analyses. Inbreeding was very rare (only 7 of 215 families). Male-biased dispersal seemed to function as a inbreeding avoidance mechanism. The population was polygynous in a low density condition, whereas it was promiscuous in a high density condition. Competition for mates among males seemed important for the change in the mating system.

### Discussion

Most mammal species are polygynous or promiscuous and males tend to disperse more than females. The resent results indicate such male-biased dispersal may be important to avoid inbreeding, so that to maintain high genetic diversity. Recently many forests have been fragmented by human activity. It is important to connect these fragmented habitats to keep genetic diversity and to conserve mammalian populations.

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### 3) Behavioral ecology and genetic variability of the endangered Amami rabbit

Key words: Amami rabbit, Conservation biology, Home range size, molecular phylogeny

#### Research objects

The Amami rabbit *Pentalagus furnessi*, one of the most primitive lagomorphs, is found only on Japan's Amami Island (710 ) and Tokuno Island (250 ) 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.

#### Materials and Methods

This study was conducted on Kawauchi district (28°00'N, 129°25'E, 300-400 m above sea-level), Sumiyo village in Amami Island during August 1995 to March 1999. The rabbits were trapped by live traps (Tomahawk, U.S.A.) baited by sweet potatoes, apples, and carrots. All rabbits caught were weighed and fitted with a radio collar (30 g in weight, TELEVILT, Sweden) and released at the capture places. Samples of DNA of the Amami rabbit were obtained from skins of each ear of the rabbits.

#### Results and Discussion

The average body weight of the 7 rabbits captured in this study was 2,226 g (range, 2,030 to 2,675 g) for males and 2,477 g (range, 2,550 to 2,880 g) for females. The average size of home range of the Amami rabbit was small (1.3 ha for males and 1.0 ha for female). The size of male was a little bit larger than that of female. Seven rabbits lived in 30 ha in the forest. The size of home range of the rabbit is almost equivalent to the range of the European rabbit<sup>5)</sup> and the species of cottontail<sup>2,4)</sup>, but was smaller than the range size (8.2 ha for male and 2.8 ha for female) of *Caprolagus hispidus*<sup>1)</sup>, and the range size (20 ha for male and 13 ha for female) of *Bunolagus monticularis*<sup>3)</sup>. The range of the three females of the Amami rabbit did not overlap each other, however, male's ranges fell within the females' and other males'. Therefore, the mating system of the Amami rabbits seems to be promiscuous as well as other Leporidae species. The Amami rabbits became active mainly during the nighttime, and moved to eat food and to drop their pellets at open area 100-200 m from their burrows located in small valley covered by dense forest. We determined sequences of the mitochondrial 12S ribosomal RNA gene (831 bp) and the cytochrome *b* gene (1140 bp) in *P. furnessi*, three other native lagomorphs in Japan, and the Volcano rabbit *Romerolagus diaz* from Mexico and compared with those of species of genera *Lepus* and *Sylvilagus* obtained from database. The results suggest that lineage establishment of *Pentalagus* was so ancient as in those of the other leporids genera through a radiation event probably at Miocene era, 10-20 million years ago. More Ecological and genetic studies are necessary to conserve the

Amami.

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#### 4) Genetic relationship of black woodpecker populations between Hokkaido and Tohoku in Japan

Key words: Genetic relationship, black woodpecker, mtDNA

##### Research objects

We examine the genetic relationship between Black Woodpecker in Hokkaido and northern Honshu, in order to investigate the mechanism through which the population size and the isolation (or immigration) determine the genetic diversity of the present population.. So we tried to extract the DNA from the fecal samples or tissue of Woodpeckers, to amplify by the PCR used the primer sets designed from the Chicken mitochondrial DNA , and to determine the partial sequences of Woodpeckers.

##### Materials and Methods

We used the fecal samples of Black Woodpeckers, the blood of Great Spotted Woodpeckers, and the tissue(the wing or specimens or the liver) of Black Woodpeckers, Great Spotted Woodpeckers, White-Backed Woodpeckers and Japanese Green Woodpeckers. DNA was extracted from fecal samples as follows: fecal was added to STE buffer and mixed, tissue was added to STE buffer and homogenized . So we extracted DNA according to the conventional Proteinase K /phenol/chloroform method.<sup>1)</sup> One microliter of the product was then electrophoresed in a 1% agarose gel, visualized with ethidium bromide staining.

Primers LC01725 (5'-AAA CTG GGA TTA GAT ACC CCA CTA-3') and HC02152 (5'-GAA GAG GGT GAC GGG CGG TAT GT-3'), LC01346 (5'-TGC AAG TAT CCG CAT CCC AGT G-3') and HC01820 (5'-GCG TTT GTG CTC GTA GTT CT-3'), LC01801 (5'-AGA ACT ACG AGC ACA AAC GC-3') and HC02152 (5'-GAA GAG GGT GAC GGG CGG TAT GT-3'), LC049 (5'-GCAAGTATC CGC ATC CCA GTG A-3') and HC431(5'-GGG TCC TAG CTT TCG TGG GTT A-3')for the 12S rRNA gene were designed by referring to the published sequences of Chicken mitochondrial DNA.<sup>2)</sup> Primers LC14990 (5'-CCA TCC AAC ATC TCT GCT TGA TGA AA-3') and HC15304 (5'-TGG CCC CTC AGA ATG ATA TTT G-3'), LC15362 (5'-TTG GAC ACA CCC TAG TAG AG-3') and HC15907 (5'-TCT ACT GGT TGG CTT CCG AT-3') for the cytochrome b gene were designed by referring to the published sequences of Chicken mitochondrial DNA.<sup>2)</sup> In order to produce double-stranded DNAs, symmetric PCR were performed with a GeneAmp PCR reagent kit (Perkin-Elmer) according to the manufacture's instruction. The step programs for PCR were as follows: denaturing at 94°C for 1 min, annealing at 60°C for 1min, and extending at 72°C for 1min. The cycle was repeated 30–50 times followed by the reaction completion at 72°C