S2-10 **Research on Estimating Population Size of Bears in Japan**
（**Abstract of the Interim Report**）

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[Abstract]
Population size and its trend of wildlife are essential information for management planning and implementation of target species. Two species of bears (*Ursus arctos* and *U. thibetanus*) are game animals in Japan. Population sizes of the bears are comparatively smaller than other game animals. Several local populations of the bears are designated as threatened local populations under national Red List. Careful managements are necessary for the bears based on scientific studies and planning. But, method for accurate estimation of the bear population with high cost performance is not yet developed. This research aims to provide suitable method for estimating population size of the bears for local governments for their management planning and monitoring. Hair trap method using DNA marker for population estimation has been developed in a last decade. Reviews on the hair trap were conducted to apply the method for bear study in Japan. A large scale study area (607 km^2) for full-scale hair trap study in FY2010 was established in Kitakami Mountains. DNA extraction and sequence from small quantities of hair roots are necessary for microsatellite DNA marker analysis. Suitable microsatellite markers were selected for the bears in Japan, and a standard protocol of the DNA analysis was prepared for full-scale study in FY2010. Sample collection rate per trap day was high in early summer. 'Moon mark' on breast and spot on mandible of the Asian black bears are suitable markers for individual identification by camera trap as an alternative/additional method. Microsatellite DNA extracted from chewing remains of the black bears can be also used for individual identification and sex determination. Spatially-explicit simulation model for the hair trap study has been developed and applied for the large scale study area in the Kitakami Mountain. Trap density and distribution plan are justified by the model.

1. Introduction
Bears are game animals which need careful management from view points of biological feature, social request for damage control and local population conservation in Japan. Estimating population size and/or population trend of target species are essential information for wildlife

management planning and implementation. The Wildlife Protection and Hunting Law of Japan set up a specific wildlife management system for conservation and management of species specified. Sixteen prefectural governments have made management plans of the Asian black bear under the specific wildlife management system of the law. The management system asks management planning including harvest number based on appropriate census of the target species. Status study of the bears is also necessary in other prefectural governments which have not yet made the management plans under the specific wildlife management system, especially in distribution areas of threatened local populations of the bears designated by national Red List. Several methods were applied for estimating bear population, including direct count, capture-mark-recapture and hunting records analysis. But, method for accurate estimation of bear population or its trend with high cost performance is not yet developed. Appropriate management of the bears based on reliable estimation of population size and trends is requested for conservation and game control.

2. Research Objective

The Wildlife Protection and Hunting Law of Japan ask the prefectural government to prepare management plan of game animals based on their status study. The objective of this research is to provide suitable standard method for the estimating bear population for the local governments and other relevant organizations. The hair trapping is a non-invasive DNA tagging method for the bear population study using microsatellite DNA marker (Fig. 1). But, several issues, such as hair trap distribution design, effective DNA analysis and population estimation from the DNA marker-recapture, are remained for standardization of the hair trap method. We set up 4 research subjects for the bear population study in Japan; (1) development of hair trap study, (2) DNA marker analysis, (3) development of alternative/additional study method, and (4) population modeling and monitoring.

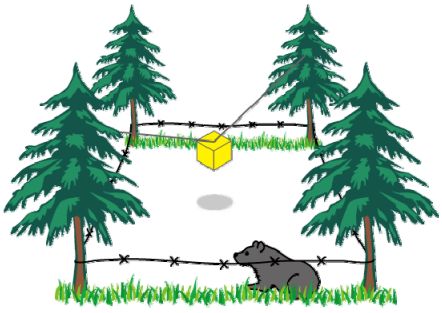

Figure 1 Hair trap

3. Methods and study area

(1) Development of hair trap study

We reviewed papers and reports on the hair trap study, focusing on the suitable trap design, effective allurement, spatial arrangement, trap density and trapping area. A large scale hair trap study area (607 km^2) was established in Kitakami Mountain where the Asian black bears inhabit as a semi-closed local population. Trapping sites of 262 traps in the study area were established for full-scale study in FY2010.

(2) DNA marker analysis

Fifty loci were listed from reviews on genetic markers of bears for hair trap study. We analyzed the 50 microsatellite markers of 42 samples of the Asian black bears captured in Yamagata for selection of effective microsatellite markers using Pid (Probability of Identity) and number of allele. The optimum DNA analysis and PCR procedure were studies for development of standard protocol for the DNA analysis in full-scale study in FY2010. Seasonal variation of trap success and

successful DNA analysis was investigated from May to December 2009 in the Gomyouzin experimental forest of Iwate University.

(3) Development of alternative and additional study method

Four potential markers, skull shape, lines of nose face, 'moon mark' on breast and spot on mandible of the Asian black bears, were studies for selection of effective individual identification marker from camera trap. Skull collection of the bears of the Forest Research Institute and photos taken at Ani and Okuhida bear farms were used for the study. Study on genetic markers extracted from chewing remains of the black bears was reviewed for individual identification and sex determination of the animals.

(4) Population modeling and monitoring

We developed a spatially-explicit simulation (SES) model for the hair trap study based on Gardner et al. (2009). Dummy data drawn from the known home range size and known population density of the black bears in Iwate Prefecture were applied to the large scale hair trap study area in the Kitakami Mountain using the simulation model.

4. Results and Discussion

(1) Development of hair trap study

Hair trap study is not so high cost-performance method, but it provides more individuals identification in a study area and in certain study period comparing with barrel trapping or direct census. Five prefectural governments in 16 ones which made bear management plan under the law applied the hair trap method for the census of the bears. From the review of hair trap studies, effective hair trap design is shown as followings; barbed wire enclosure of side about 4 m above 40 cm with inside diagonal wire lines, honey bait for the Asian black bear and fishes or deer meat for the brown bear. For the full scale study in FY2010, 262 hair trap sites were located in the large scale hair trap study area in the Kitakami Mountain (Fig. 2). Two trap densities, 1 trap/1-km cell and 1 trap/2-km cell, were set up in the study area. Detailed study plan in FY2010 was prepared through preliminary hair trap study in October – November 2009.

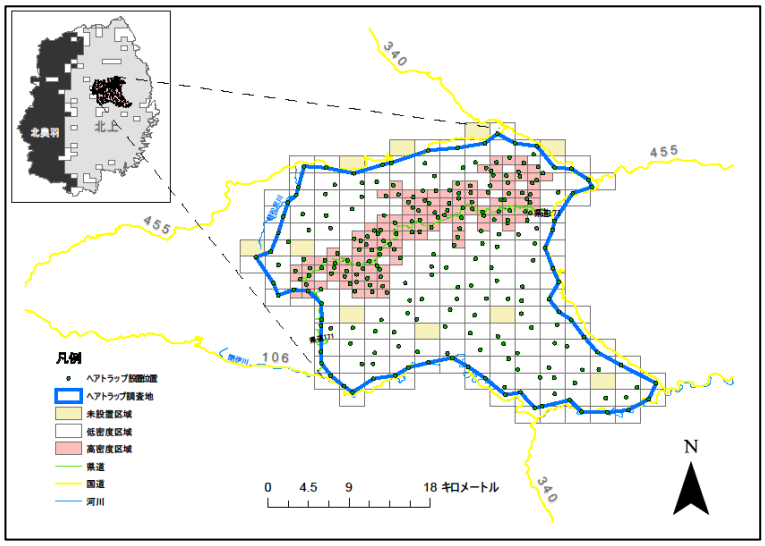

Figure 2 Large-scale study area in Kitakami Mountain

(2) DNA marker analysis

The Pid of 23 microsatellite markers in the 50 markers analyzed were <0.25 in the 42 samples of the Asian black bears. Four bases repeat loci of the microsatellite markers of the black bears and brown bears showed more polymorphism than two bases repeat ones. Fig. 3 shows proposed

standard protocol for the DNA marker analysis, using multiplex PCR. Amelogenin locus is a suitable marker for sex determination. Procedure using standard samples was prepared for calibration of DNA sequencer among different institutions to reduce genotyping error. Hair capture per trap days and success rate of DNA analysis showed seasonal variation, and were high in early summer in the study area in Iwate. Successive sampling is necessary for monitoring of effective population size (Ne) from genetic analysis of a local population.

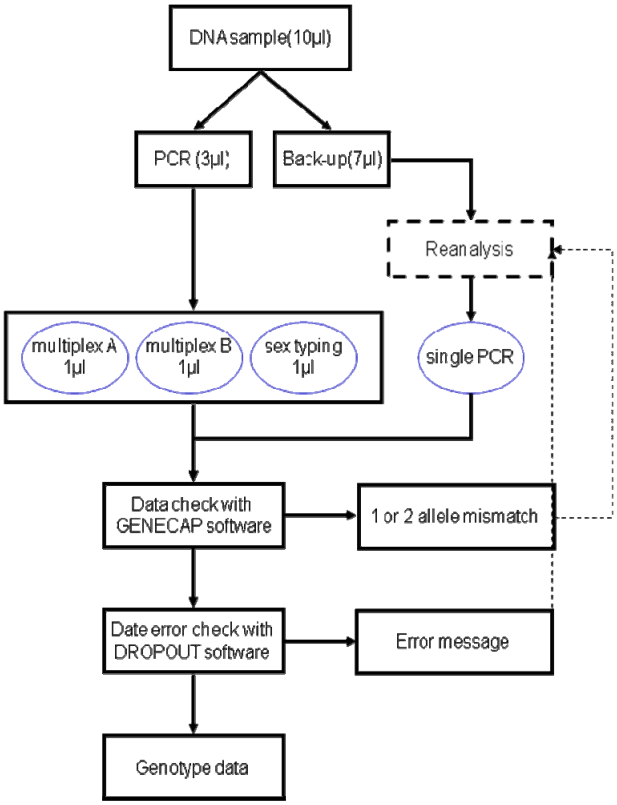

Figure 3 Procedure of DNA marker analyses

(3) Development of alternative and additional study method

'Moon mark' on breast and spot on mandible of the black bears showed individual variation (Fig. 4), but skull and lines of nose face were not recognizable part of individual distinction from camera trap. Microsatellite DNA extracted from chewing remains of the Asian black bears can be used for individual identification and sex determination.

Figure 4 Moon mark variation of black bears

(4) Population modeling and monitoring

The SES model for the hair trap study in the Kitakami Mountain reduced negative effect on estimation of population even if the hair traps were arranged un-equal distance in the area. The SES model can also directly estimate the population density of the bears without buffer setting around trapping area. Results of the SES model applied dummy data to the Kitakami Mountain study area showed more accurate population estimation than non spatial model. Excluding of one capture individual from the SES model for reducing genotyping error will result underestimation of the population size.

References

Gardner, B.; Royle, J. A.; Wegan, M. T. (2009) Hierarchical models for estimating density from DNA mark-recapture studies. Ecology 90(4):1106-1115.

Major Publications

Not yet published in academic journals as of April 2010