Jpn. J. Vet. Res. **42**(3-4): 127-136 (1994)

ESTIMATE OF GENETIC VARIATIONS IN HOKKAIDO BROWN BEARS (*Ursus arctos yesoensis*) BY DNA FINGERPRINTING

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(Accepted for publication: Nov. 30, 1994)

ABSTRACT

Genetic variations within and between local populations of Hokkaido brown bears, *Ursus arctos yesoensis*, were quantified by means of DNA fingerprinting using a minisatellite DNA probe. The estimates of the average heterozygosity (gene diversity) H were 0.302 and 0.241 for the populations on the southwestern part of the Oshima peninsula and the Shiretoko peninsula, respectively. These values suggest that local populations studied in this study have low genetic variability compared with those for other animals. The degree of genetic differentiation between the populations, measured by the coefficient of gene diversity (G_{ST}), was 7.9 percent and 19.5 percent. These results indicate a low degree of genetic differentiation between the local populations. The results obtained are discussed in relation to a population bottleneck in the ancestors and subsequent expansion of their habitat.

Key words: BROWN BEAR, GENE DIVERSITY, DNA FINGERPRINT

INTRODUCTION

The distribution of the brown bear, *Ursus arctos*, is known only in Hokkaido island in Japan. The population covers nearly the whole island, but the habitat tends to decrease and local populations are becoming isolated in recent years. Thus, the conservation biology of this wildlife is a matter of great importance[3].

In general, little is known about the genetic diversity in bear populations. There are fragmentary data on molecular variations in blood proteins and mitochondrial DNAs suggesting low genetic variability in the bear populations in America and Europe[1,14].

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DNA fingerprinting by hypervariable minisatellites is unique to an individual and has been applied for individual identification and paternity determination in humans[4,5,7-9]. Recently, this method has been applied to estimate genetic diversity in wild animals[2], and for individual identification of Hokkaido brown bears[24].

In this report, we applied the technique of single probe multilocus DNA fingerprinting in order to quantify the amounts of genetic variations within and between bear populations in Hokkaido. Simultaneous screening of many polymorphic loci in the genome provides an effective tool for detecting individual variations and an original source of information to observe the gene constitution of the wildlife population.

MATERIALS AND METHODS

Samples were obtained from both wild and captive animals. Figure 1 shows the location of the capture sites where a total of 26 bears were caught alive; 13 from the southwestern part of Oshima peninsula and 13 from Shiretoko peninsula. The population on Oshima peninsula is thought to be isolated from the population of central Hokkaido[10], and it has tended to decrease in total number recently[15]. The population on the Shiretoko peninsula is considered to be nearly isolated from the population of central Hokkaido[3].

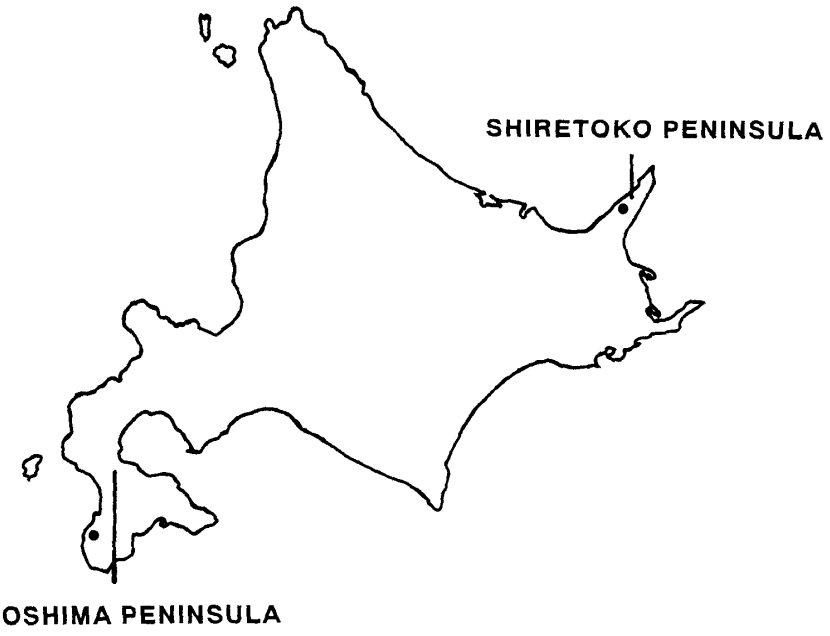

Fig. 1. • : Location of capture sites where 26 bears were caught alive (13 from the southwestern part of the Oshima peninsula and 13 from the Shiretoko peninsula).

Fig. 2. • : The origins of bears kept at Noboribetsu bear park.

To assess the population subdivisions, six individuals of known origin (Fig. 2) kept at the Noboribetsu bear park and one each from the Oshima peninsula and the Shiretoko peninsula, were simply pooled to measure the total gene diversity of bear populations in Hokkaido.

Preparation of DNA: To extract DNA, blood was collected from bears under anesthesia using a Venoject (Terumo) with an anticoagulant, and white blood cells (WBCs) were separated by centrifugation. WBCs were resuspended in TNE buffer (10mM Tris, 100mM NaCl, 5mM EDTA) containing 0.5% w/v sodium dodecyl sulfate (SDS) and 50 μg/ml of proteinase K, and incubated overnight at 37°C. After three treatments with phenol and one with chloroform, DNA was extracted, and precipitated by addition of three volumes of ethanol.

DNA digestion and electrophoresis: Ten micrograms of each DNA sample was digested with *Hin*fI restriction enzyme. After digestion, the DNA was subjected to electrophoresis in 15cm length of 0.8% agarose gel at 30V for 21 to 24 hours, denatured and then transferred to a nitrocellulose membrane filter[21].

DNA probe: The minisatellite Myo probe for hybridization was labeled with [^{32}P]-dCTP using the Multiprime DNA labelling system (Amersham). The consensus sequence of the Myo was 5'-GACCGAGGTCTAAAGCTGGAGGTGGGCAGGAAG-3'[4].

Hybridization and washing: The filters were prehybridized for over 4 hours in 5× SSC, 20mM Tris-HCl (pH 8.0), 1mM EDTA, 1% SDS and 10 μg/ml yeast tRNA at 55°C, and hybridized overnight after addition of the probe (2.0×10^6cpm/ml). Washing

was performed in 1×SSC at 42°C for 30 minutes, and then the filters were autoradiographed at −80°C for 3 days.

Calculation of genetic indices : Bias-corrected estimates of the average heterozygosity (gene diversity) H and the coefficient of gene diversity GST were calculated according to the methods derived by Stephens et al.[22] and Jin and Chakraborty[11,12]. Since all bands (alleles) observed in the gel are considered to follow a dominant mode of inheritance, the estimation of the frequency of the kth allele was given by

$$P_k=(1-\sqrt{1-S_k}),$$

where S_k is the frequency of occurrence of the kth band.

The estimation of the bias-corrected number of loci (L) and average heterozygosity or gene diversity (H) were given by

$$L=Lm+\sum_{k=1}^{Ap}(1-\sqrt{1-S_k})-\frac{\sum_{k=1}^{Ap}S_k/\sqrt{1-S_k}}{8n}$$

and

$$H=(\sum_{k=1}^{A}S_k/L)-1,$$

where Ap is the total number of alleles at all polymorphic loci, A is the total number of alleles, Lm is the number of monomorphic loci and n is the number of sample individuals[12].

The coefficient of gene diversity (GST) given by Nei[16] is

$$GST=\frac{HT-HS}{HT},$$

where $Hs=\Sigma_k HK/s$, in which HK is the gene diversity of the kth population and s is the number of populations, and HT is total gene diversity. In this report, we used two methods to calculate HT. In the first, allele frequencies were estimated from the samples collected from the eight sites shown in Figures 1 and 2. In the second, they were estimated by pooling the data from the two survey sites shown in Figure 1.

RESULTS

Figure 3 shows DNA fingerprints obtained from bear populations of the southwestern part of Oshima peninsula (a) and the Shiretoko peninsula (b). To caluculate genetic indices, DNA fragments ranged from 2.0 to 23.1 kbp were scored. When fragments detected in each lanes were compared, they were scored as the identical allele of the same locus if showing the same mobility and similar intensities. In the case that fragments that had a faint autoradiographic band intensity in some individuals showed the same mobility as those that had a strong band intensity in other individuals, the former were typed as recessive homozygotes. Table 1 presents the allele frequencies, numbers of loci and average heterozygosities (gene diversities) of the two populations. An allele that was present in one population but absent in the

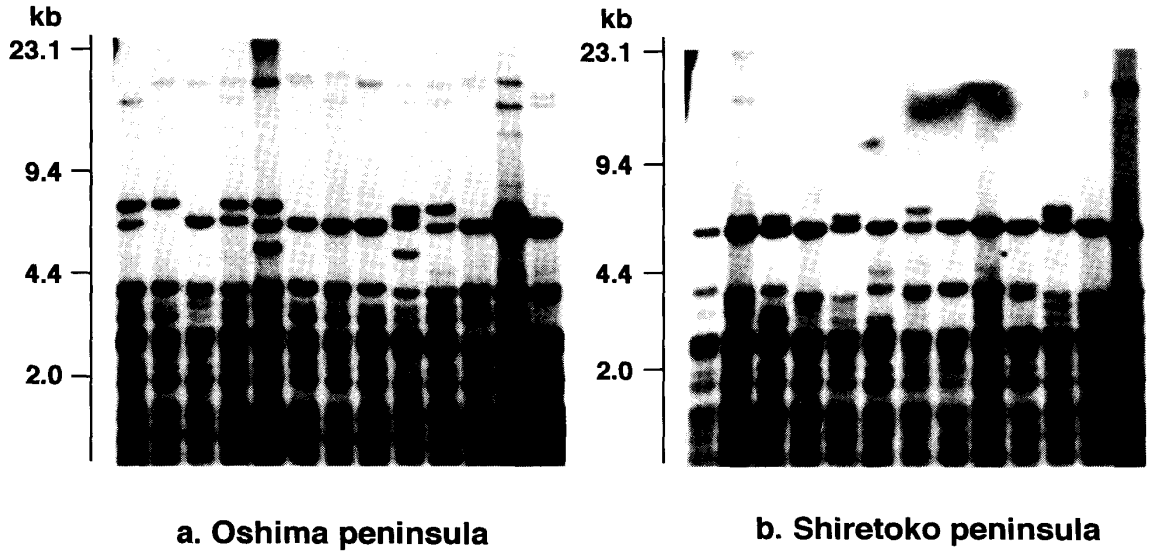

Fig. 3. DNA fingerprints of bears from the southwestern part of the Oshima peninsula (a) and the Shiretoko peninsula (b). *Hin*d III-digested lambda DNA was used as a size marker.

other population was not listed in the table. The estimate of average heterozygosity (gene diversity) of the population on the Shiretoko peninsula was smaller than that of the Oshima peninsula. This suggests relatively low genetic variability in the bears inhabiting the Shiretoko peninsula.

Estimates of the coefficients of gene diversity are summarized in Table 2. *HT* is the heterozygosity (gene diversity) of the total population and *Hs* is the mean heterozygosity (gene diversity) within local populations. The gene diversity between local populations was estimated to be 7.9 and 19.5 percent of the total population. The results of quantification were different in two cases of definition of the total population. The estimate of the gene diversity index *GST* in case 2, which defined the total population by pooling the data of the Oshima and Shiretoko populations, was larger than that in case 1.

Table 1. Estimation of number of loci and average heterozygosity (gene deversity)

Allele	Frequencies of kth allele (P_k)	
	S. O. P. [1]	Shiretoko
1	0.445	0.445
2	0.321	0.226
3	0.080	0.039
4	0.321	0.080
5	0.080	0.168
6	0.723	1.000
7	1.000	0.380
8	0.216	0.445
9	0.723	0.445
10	0.723	0.380
11	0.723	1.000
12	0.520	1.000
13	0.168	0.168
14	1.000	1.000
15	1.000	1.000
L [2]	7.858	7.746
H [3]	0.302	0.241

1) : southwestern part of the Oshima peninsula
2) : number of loci
3) : average heterozygosity (gene diversity)

Table 2. Estimate of coefficient of gene diversity

Condition	Hs	HT	GST
Case 1 [1]	0.272	0.295	0.079
Case 2 [2]	0.281	0.349	0.195

1) HT was estimated from samples collected from the eight sites shown in Figures 1 and 2.
2) HT was estimated from samples collected from the two sites shown in Figure 1.

Discussion

The value of heterozygosity (gene diversity) that quantified the genetic variability within the bear population on the Shiretoko peninsula suggested a relatively low degree of genetic variation. This may be caused by geographic isolation from other bear populations and/or the small effective size of the studied population. Actually, the bear population on the Shiretoko peninsula is considered to be nearly isolated from a large population distributed in the central area of Hokkaido[3].

The estimate of gene diversity within populations from single probe multilocus DNA fingerprinting data is 0.435 for domestic cats and that from imitated multilocus DNA fingerprinting data is 0.710 for humans (mean of values for seven loci from three populations)[11,12,22]. The obtained values of 0.302 and 0.241 for the bear populations in this study are considered to be showing a low level of genetic variability within the species, which corresponds to the results of previous studies on the bear populations in America and Europe[1,14].

Jin and Chakraborty (1994) found that values of gene diversity obtained from imitated multilocus fingerprinting data consist with those estimated from short tandem repeat loci[12]. If the estimates of genetic variability obtained from microsatellite DNA data are comparable to the present estimation, the genetic features of the bear population can be evaluated further. A population study of the hairy-nosed wombat (*Lasiorhinus krefftii*), which has passed a small bottleneck that has caused a decrease in their genetic variability, gives an insight into the present data. In the study, the value of gene diversity estimated by molecular variations in short tandem repeat loci was 0.28, which is similar to values obtained in this study[23]. Thus, from this comparison as well, genetic variability within the bear populations is thought to be low. However, it is difficult to explain this low genetic variability found in Hokkaido brown bears and further research on their breeding structure must be performed.

As for the coefficient of gene diversity, there was a difference between the two cases used for comparison in Table 2. The larger estimate of G_{ST} in case 2 seems to reflect genetic differentiation between the populations on the Oshima and Shiretoko peninsulas. It is of interest to compare the obtained estimates with those reported for other land mammals. The estimates of G_{ST} obtained from blood protein polymorphism data for Japanese mammals and monkey species in Indonesia are shown in Table 3. For Japanese people and cats, large genetic variability as a whole population (large H_T) is observed with less local differentiation (small G_{ST}). In contrast, the results for bears are similar to that obtained for the Japanese serow. In these cases, the total population shows low variability (small H_T) and less differentiation (small G_{ST}). In the case of the Japanese serow, the low degree of intra- and interlocal genetic differentiation is discussed in relation to a recent bottleneck in the ancestral populations and a subsequent expansion of habitat. This may have occurred with the

Table 3. *GST* values of other mammals

Population	No. of loci	*HT*	*HS*	*GST*	References
Japanese people	30	0.0915	0.0912	0.0029	6,20
Japanese cat	31	0.0802	0.0793	0.0112	18
Japanese serow	30	0.0294	0.0261	0.1122	17
Macaca fuscata (Japan)	32	0.0315	0.0215	0.3175	19
Macaca fascicularis (Indonesia)	33	0.0827	0.0384	0.5356	13

bear populations in Hokkaido as well.

Consequently, it is considered that the genetic diversity of the Hokkaido brown bear is generally low as determined by quantification of genetic variability within local populations and the level of genetic differentiation between local populations. The sample size of the population in this study was small and the sampling sites were geographically biased as well. Thus, to ascertain whether the results of this study reflect the proper gene constitution of bear populations in Hokkaido, further study should be done by increasing sample numbers in the future.

When the gene constitution of a wildlife population is investigated, information about the number of loci and locus affiliations of alleles is required. Though DNA fingerprinting does not fill these requirements completely, it can be a useful tool when the objective species or population is rare and the samples are limited, as shown in this study.

ACKNOWLEDGMENTS

We would like to express our sicere gratitude to S. Ise of the Noboribetsu Bear Park who provided bear samples and also thank Dr. Y. Kawamoto of Primate Research Institute, University of Kyoto for his valuable advice.

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