Taxonomic, Genetic and Ecological Status of the Daikoku Vole

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ABSTRACT

Based on their skull morphology voles found on Daikoku Island have been regarded as a distinct form of Clethrionomys rufocanus (Sundevall, 1846) but their taxonomic status has been controversial: the vole population on this island was once identified to be a subspecies of Neoashizomys sikotanensis Tokuda, 1935, then named N. s. akkeshii [1], but Ota (1956) insisted that the voles there are C. rufocanus [2]. Their ecological features are also fascinating; their population abundance greatly fluctuates from extremely low to very high densities. Because of these unique morphological and ecological characteristics distinct genetic features are also expected compared with those of the mainland voles. We thus compared the ecological, morphological, and genetic features of the voles from Daikoku Island with populations from mainland Hokkaido. Although the Daikoku population largely fluctuated from low to high density, the amplitude was within the range that has been frequently observed in the eastern part of Hokkaido. A single unique mtDNA haplotype was observed in Daikoku, which was different from the 145 haplotypes observed in the mainland and other island populations, though they were closely related each other. Genetic diversity indices of the Daikoku population were greatly lower than those of mainland populations. Our results on the cranial measurements were consistent with those of Ref. 4, 5 that the Daikoku voles, generally, have larger skull dimensions than those of the mainland. Principal Component Analysis indicated morphological differentiation between the Daikoku voles and those of the mainland. Our genetic and morphological results confirm that the Daikoku vole is a local form of the gray-sided vole, Clethrionomys rufocanus, which relatively recently originated from mainland Hokkaido.

Keywords: Clethrionomys rufocanus, Daikoku Island, mtDNA, The gray-sided vole

INTRODUCTION

Voles that inhabit the Daikoku Island, Hokkaido, Japan show some distinct features in morphology and ecology. Their skull is large and relatively wide.

Based on their morphological features they have been regarded as a distinct form of Clethrionomys rufocanus (Sundevall, 1846), but their taxonomic status has been controversial. They were once identified to be a subspecies of Neoashizomys sikotanen-
Sample collection
In addition to the samples \((n = 81)\) analyzed by Ref. 8, we collected samples from Haboro, Shiretoko and from the islands of Rebun, Teuri, and Yagishiri. Samples provided by the Hokkaido Regional Office, Forestry Agency of Japan were also analyzed. In total 351 samples from 44 populations were used for the genetic analysis and 87 adult individuals from 16 populations were included for the morphological analysis (Fig. 1).

Genetic and morphological analyses
Mitochondrial DNA control region and flanking regions were analyzed. Procedures for analyses are given by Ref. 8. We also followed Ref. 8’s methods for skull morphology. In addition microsatellite DNA was also analyzed to compare genetic diversity among islands. Procedures for microsatellite DNA analysis are given by Ishibashi and Saitoh (in press).

RESULTS AND DISCUSSION

Population dynamics
Figure 2 illustrates the dynamics of the Daikoku vole population. Since the data were fragmented, the fluctuation pattern was not characterized. To compare the feature of dynamics with other populations in the mainland, \(s\)-index which indicates the amplitude of fluctuation [10] was calculated. The \(s\)-index of the Daikoku population was 0.46. This value was within the variation of the \(s\)-index for the mainland populations (Fig. 3) in which \(s\)-index of around 0.4 was most common. Although the \(s\)-index of the Daisis Tokuda, 1935, then named \(N. s. akkeshii\) (see [1]). Ota (1956), however, insisted that the voles there are \(C. rufocanus\) [2]. Wakana et al. (1996) confirmed through Restriction Fragment Length Polymorphisms (RFLP) analysis that voles on Daikoku Island and those of other regions in mainland Hokkaido have identical types of rDNA and mtDNA [3]. The morphological studies of Abe (1968, 1973a, b) found significant divergence of Daikoku samples with that from the mainland (Sapporo) and identified them as the Daikoku form (the D-form) [4-6].

Ecological features of the Daikoku vole are also fascinating; the population greatly fluctuates in abundance from extremely low to very high densities [7]. The Daikoku population has been regarded as one of the cyclic populations in Hokkaido.

de Guia et al. (2007) compared the morphology and genetic features between samples from the Daikoku Island and those from eastern parts of Hokkaido [8]. They concluded that a single vole species is present on Daikoku Island, \(Clethrionomys rufocanus\) (Sundevall, 1846), based on a single unique mtDNA haplotype and indications of morphological differentiation of cranial measurements between Daikoku and the mainland (Akkeshi and Shibeche). However, because of insufficient sampling in mainland Hokkaido, they pointed out that the morphological and genetic differentiation detected with the Daikoku voles should be interpreted in the full context of morphological and genetic variation including other mainland populations.

This paper is a follow-up report of de Guia et al. (2007) using additional samples from mainland Hokkaido and its related islands. The purpose of this study is to examine the ecological, morphological and genetic features of the Daikoku voles in the full context of variation in the mainland populations.

MATERIALS AND METHODS

Population dynamics
Abundance of the Daikoku vole has been investigated by several researchers. Those investigations were systematically organized in some cases, while fragmented in other cases. Ota (1984) published census results from 1959 to 1972 [7]. We standardized those data as individuals per 150 trap-nights in autumn (September-November) including unpublished data provided by M. Yoneda. Abundance and amplitude of the dynamics of the Daikoku population were compared with those of mainland populations. The data on mainland populations were obtained from Ref. 9.
The Daikoku population had conspicuously high abundance. Average abundance of the Daikoku population was extremely high (54.9), beyond the observed range of average abundance of mainland populations (Fig. 4).

To summarize the population dynamics of the Daikoku population, 1) although it seemed largely fluctuating, its periodicity is unknown, 2) amplitude is regarded as average, and 3) mean abundance is extremely high.

**Skull morphology**

Following Ref. 8, a principal component analysis (PCA) was carried out using 18 cranial measurements to determine which characters contributed to the differences among populations (Fig. 5). The first two principal components accounted for 63% of the total variation present in the original variables. The first principal component explained 51% and the second principal component explained 12% of the variances. The first principal component was sufficiently explained by five of the 18 cranial measurements (CBL, PL, LB, ZW and D; see Ref. 8 for the abbreviation). The second principal component was sufficiently explained by three cranial measurements (PLN, NW, and IW). The first principal component characterizes the skull as long and wide due to the longer Condylobasal Length (CBL) and wider Zygomatic Width (ZW). The second principal compo-
ent, on the other hand, characterizes the skull as also wider but due to the wider Interorbital Width (IW) and nasals (NW).

PCA showed five morph groups (Fig. 5). The Daikoku population was the only population which showed a distinct identity and exclusivity of cranial characters. This single population comprised group E.

Group A formed the biggest cluster consisting of the most number of individuals from various populations stretching from the southwestern region (Date, Tomakomai and Tobetsu), the northwestern islands (Rebun and Yagishiri), central area of Iwamatsu, northeastern region of Shiretoko and the island of Kunashiri. A smaller cluster, group B, was formed by populations that included southwestern and another northwestern island population. Group C was formed by populations from Akkeshi and Shibecha which was restricted to the eastern part of mainland Hokkaido. Group D was formed primarily by the northwestern populations of Rishiri Island, Teshio, Haboro and Teuri Island and some individuals from Kunashiri Island.

The Interorbital Width (IW) was smaller in group C and E than in group A, B and D (Wilcoxon test, $Z = 6.87$, $P < .001$). Group E almost equally had longer skull as group D but was longer than those of group C. Group C was as long as groups A and B. The condylobasal length (CBL) of group E was significantly longer than that of C (Wilcoxon test, $Z = 3.84$, $P < .0001$). Therefore, group E can be characterized as voles with longer and wider skull in general, but their IW is smaller.

**Mitochondrial DNA haplotypes**

de Guia et al. (2007) compared the control region of mtDNA between the Daikoku population and two populations in eastern Hokkaido (Akkeshi and Shibecha) [8]. They found 27 distinct haplotypes from 81 samples from the three populations. The Daikoku population possessed a single unique haplotype. The divergence rates ranged from 0.001–0.018. Between the Daikoku haplotypes (Crmt25) and the 26 other *C. rufocanus* haplotypes, the computed mean pairwise divergence rate was 0.013. Among the 26 other *C. rufocanus* (without Crmt25), the computed mean pairwise divergence rate was 0.011.

de Guia et al. (2007) showed that the Daikoku haplotype (Crmt25) was found at the periphery of the median-joining network and was most closely related to haplotype Crmt26 found in Shibecha [8]. They concluded that the mtDNA haplotype which was unique to Daikoku may have originated from the mainland, and had been lost in the mainland like Akkeshi or Shibecha, or may possibly be still present in other unsampled eastern regions or other ar-
Including the samples analyzed by Ref. 8 we analyzed 351 samples from various locations (Fig. 1). Although 146 different haplotypes were obtained from the 44 populations, the Daikoku haplotype (Crm25) was not shared with any other island or mainland populations. However, this haplotype is included within the C. rufocanus variation and is phylogenetically close to mainland populations. Thus, we can conclude that the Daikoku population possesses a single unique haplotype closely related to haplotypes found in the mainland populations.

**Microsatellite DNA**

The fact that the Daikoku population possessed a single mtDNA haplotype indicates that genetic diversity of the Daikoku vole was very low. Is genetic diversity of the Daikoku population uniquely low? Or can it be interpreted in the general context that genetic diversity of small island populations is low? To answer these questions we compared genetic diversity measured by microsatellite DNA among island populations.

We collected 134 samples from six islands (Daikoku, Rishiri, Rebun, Teuri, Yagishiri and Kunashiri) with various sizes and isolation period. Daikoku Island is the smallest (1.1 km²) among the sampled islands and has been isolated from the mainland since about 5000 years ago. Lower genetic diversity of populations on smaller islands is expected because of founder effects and genetic drift due to small effective population size, while genetic diversity of populations on islands with longer isolation periods is expected to be lower, because they have been subjected to genetic drift for longer periods.

Following the first prediction, genetic diversity, shown by allelic richness, positively correlated with island sizes (Fig. 6; Spearman’s rank correlation test, $r_s = 0.94$, $P = 0.0167$). The smallest island, Daikoku, had the lowest genetic diversity. Contrary to the other prediction, the relationship between genetic diversity and isolation period was unclear ($r = 0.38$, $P = 0.4542$). Although the Daikoku population had the shortest isolation period and should show higher genetic diversity, it had the lowest. Therefore, our results suggest that isolation period may have little effect on genetic diversity when there are only slight variations among the isolation periods of islands.

**CONCLUSIONS**

1. The Daikoku population largely fluctuated, but its periodicity was unknown. Amplitude of the fluctuation was regarded as average while the mean abundance was extremely high in comparison with populations in the mainland.
2. The Daikoku vole was distinguishable based on some skull measurements. Generally, they were characterized as voles with longer and wider skulls.
3. The Daikoku population possessed a single unique mtDNA haplotype that was closely related to haplotypes found in the mainland populations.
4. Low genetic diversity of the Daikoku population can be interpreted in the context of lower genetic diversity in small island populations.
5. From these results the Daikoku vole should be considered as a local form (the D-form) of Clethrionomys rufocanus (Sundevall, 1846).

**ACKNOWLEDGMENTS**

We are indebted to the Forestry Agency of Japan for providing the samples analyzed in this paper. We thank the Hokkaido University Botanic Garden Museum for allowing us to examine and measure the skulls used in this study. We also thank Dr. H. Abe and the National Science Museum for allowing us to confirm the identification of the type specimen cited in this study. H. Ito and J. Ohsawa provided assistance during the collection of samples from Akkeshi and Daikoku Island. This study was partly supported by the Grant-in-Aid from Japan Society for the Promotion of Science. This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (B), No. 17370006 to TS.

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