

Geographic divergence pattern and morphological variability of the Japanese greater horseshoe bat

Yugo IKEDA

Abstract

Background

The Japanese greater horseshoe bat, *Rhinolophus nippon* Temminck, 1835, is widely occurring from the Japanese Archipelago through the Korean Peninsula, to northeastern and southwestern China, Sikkim, Nepal, and northern India. Previous phylogenetic studies revealed that *R. nippon* is a sister species of *R. ferrumequinum* (Schreber, 1774) occurring from England through northeastern Africa, western to eastern Europe, Crete, northeastern Iraq, Iran, Turkmenistan, Afghanistan, southern Kazakhstan, to Kashmir. However, no qualitative and quantitative discriminant character between two species has revealed. The current phylogenetic study of *R. nippon* revealed that Northeast Asian lineage including populations from the Japanese Archipelago, the Korean Peninsula, northeastern China, and those peripheral islands is the most recently diverged lineage, and the Japanese Archipelago population has been considered the monophyletic clade on the basis of phylogenetic tree constructed by cytochrome *b* and D-loop mitochondrial DNA (mtDNA). However, all Japanese specimens were collected from eastern Japan and only sequenced cytochrome *b* region, and the specimens from western Japan and Kyushu and other mtDNA region such as D-loop were lacked, so that the monophyly of the Japanese Archipelago population has not been clear. Moreover, *R. nippon* in the Japanese Archipelago has been reported the variation of echolocation call frequency of maximum energy (PF) increasing from northern to southern Japan and peripheral island populations. In order to answer this echolocational variation, previous study hypothesized the negative correlation between PF and the body size, however, the other morphological study indicated that nasal capsule height and pinna size is more suitable for the indicator of PF than forearm length, thus the PF variation is considered to be promoted by the vocalization-related morphological variations.

In this dissertation, the author investigates three steps to solve taxonomic and phylogenetic problems and to reveal the morphological variation of the unique cranium of *R. nippon*. First, finding the morphological difference between *R. nippon* and *R. ferrumequinum*. Second, revealing the monophyly of the Japanese Archipelago population of *R. nippon*. Third, revealing the echolocation-related morphological variation and its variability, especially the variation on nasal swellings which contains smooth and ridge structures and have been poorly understood.

Materials and Methods

In Chapter 2, the author conducts three morphological methods on their skulls: linear measurement of nine cranial character; geometric morphometrics; evaluating diagnostic characters for the two species by comparing skull photographs. In Chapter 3, the author carries out the field survey in western Japan,

and reconstructs a time-calibrated phylogenetic tree of *R. nippon* of Northeast Asian lineage adding cytochrome *b* and D-loop sequences from western Honshu and Kyushu. In Chapter 4, the author constructed the three-dimensional (3-D) mesh model of *R. nippon* from the Japanese Archipelago, the Korean Peninsula, and the peripheral islands, and of *R. cornutus* from the Japanese Archipelago and the peripheral island, and conducts 3-D geometric morphometrics using patch-based semi-landmarking method.

Results

Results of Chapter 2 revealed that the clear cranial shape differences between *R. nippon* and *R. ferrumequinum*, and defined the character traits of diagnostic characters for species discrimination. Additionally, results also revealed that the morphological shape variation within *R. nippon* consistent with genetic divergence pattern, and the increase of the cranium size of the Japanese Archipelago population and decrease of the cranium size of Jeju Island population.

Results of Chapter 3 indicated that the Japanese Archipelago population diverged into three clades of eastern Japan / western Japan / southern Kyushu clades, and southern Kyushu clade includes the populations from the Korean Peninsula, Jeju Island, and northeastern China. Results denied the monophyly of the Japanese Archipelago population, and proposed the reverse colonization hypothesis. Additionally, results indicated that the genetic divergence pattern between eastern and western clades is consistent with middle and large mammals.

Results of Chapter 4 detected the variation of posterior median swellings of nasal bone between *R. nippon* and *R. cornutus* and the variation of anterior median swellings of nasal bone between Kyoto and Jeju Island populations of *R. nippon*, and forearm length (FA) was selected for former and frequency of maximum energy of the pulse (PF) was selected for latter best model by Generalized Linear Model. Thus, it is indicated that the variability differs depending on the position of nasal swellings, and high variability of anterior median swellings possibly involves in the intraspecific variation in PF occurring in island populations.

Discussion

Based on the results of Chapter 2 to 4, the author discussed the possibility that geographical barriers such as mountainous ranges, mountains, and straits did not work on bats effectively in maintaining unique haplotypes, but the vocalization-related morphological variation such as highly variable nasal bone accumulated with population isolation to multiple refugia during glacial periods. Then, it causes the discrepancy in vocalization communication among the local populations. The dissertation suggests that vocalization-related morphological characters specific to the family Rhinolophidae promote early population divergence, and this finding might lead to the clarification of the speciation mechanism that produces high species diversity in the family Rhinolophidae.