

Alien and native *Gekko* species interaction in Yakushima Island

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ABSTRACT

As a result of human activity, *Gekko hokouensis* introduced to the Yakushima Island where it came to contact with the morphologically similar native species *G. yakuensis*. In this work we investigated the distribution and the hybridization status of these species to evaluate the interaction between the two species. First we conducted a survey around this island and compared to a distributional data acquired seven years earlier in order to assess if populations range changed through time. Second, we conducted genetic analysis in order to assess the putative hybridization and the genetic structures of the populations. The present survey showed that neither the native nor the alien species had expanded their range. Third, We compared their distribution in habitat use *of these species* and genetic structures. Although it appeared that the frequency of occurrence of hybridization is low, moderate genomic introgression is implicated from the microsatellite data. However, the degree of interbreeding seemed much lower than it was expected. The alien species is well adapted to human disturbed areas, while it is unable to expand its range through the wild environment where is likely to be outcompeted by the native species. Since this habitat partitioning seems to be unlike to explain, alone, the low hybridization rate we believe some reproductive isolation mechanism is playing a central role in preventing the breakup of the species boundaries.

INTRODUCTION

Alien species are defined as the species introduced, deliberately or accidentally, to outside their native range (Lodge, 1993). Although these species may bring negative consequences to the ecosystem such as reduction in native biodiversity (Wilcove et al., 1998), most of the alien species do not experience a steep increase in their geographical range and population growth to become pests (Williamson, 1996) and most invasions fail (Veltman et al., 1996). Biotic resistance may pose a major impediment to the alien species population growth, especially when the new occupied environment bears a locally adapted closely-related species (Strauss et al., 2006). On the other hand, the contact of two closely-related species may lead to hybridization and genetic introgression, especially in cases when the two species do not possess reproductive isolation mechanism (Rassmann et al., 1997).

Species of the genus *Gekko* from Japan comprise a monophyletic group of six formally described species (*Gekko japonicus*, *G. tawaensis*, *G. yakuensis*, *G. shibatai*, *G. vertebralis*, *G. hokouensis*) and two putative undescribed species (Shibaike et al., 2009). In Yakushima Island, two species, *G. hokouensis* and *G. yakuensis*, inhabit and they have very similar morphological characteristics (Toda et al., 2001). These species can be distinguished only by the numbers and shape of some scales and the subtle differences of body color and pattern (Tokunaga, 1988; Toda et al., 2001). A recent study strongly suggests that the current Yakushima Island populations of *G. hokouensis* have originated from accidental artificial introductions from the outside of the island, and only *G. yakuensis* is native to the island (Toda and Hikida, 2011). Allozyme data have revealed that *G. hokouensis* and *G. yakuensis* are hybridizing after the secondary contact on this island (Kawai and Toda, personal communication).

In this study, we evaluated the temporal change of the fine-scale distribution of the two species. Moreover, we reassessed the hybrid formation formerly reported in Toda et al., (2001) using genetic techniques. We compared their distribution with the habitat use of these species and genetic structures.

MATERIALS AND METHODS

Sampling of geckos

Fieldwork was conducted from September 8th to 12th 2012 in Yakushima Island, south of Kyushu, Japan. Because the geckos inhabit lower than about 400m asl in Yakushima Island (Toda and Hikida, 2011), geckos were captured along the vicinity of Routes 78, the outer perimeter road of Yakushima Island, and their branches. Altitude of the survey area was lower than 300m asl.

We conducted field survey at night. We searched geckos by carefully looking about their microhabitats, such as tree trunks, rocks, artificial building walls, guardrails in the side of roads, electric poles, bridge parapets and derelict buildings. Geckos were captured by hand carefully not to autotomize their tails, and they kept in a plastic bag. Fishing rods were used as adjunctively for dropping geckos to low place to capture easily. Immediately after its capture, surrounding environment and location of captured sites were recorded. We categorized the surrounding environment as forest or village. Each gecko was released at the original sampling site after having been taken a tip of tail, or otherwise fixed by 10% formalin after having been taken from a piece of the liver. Fixed geckos were deposited in the Zoological Collection of the Kyoto University Museum (KUZ R68933–68999, 69400–69413) and stored at 75% ethanol. Tissues were stored at the 75% ethanol for DNA analysis.

Identification of geckos

In this survey, we found *G. yakuensis* and *G. hokouensis*. These species were distinguished by the size of internasal scale and the number of tail tubercles following Toda et al. (2001): enlarged internasal in *G. yakuensis*, not enlarged in *G. hokouensis*, paired tubercles continuously existing from the base to beyond the 12th caudal groove in *G. yakuensis*, lacking or existing around the basal portion but not extending beyond the 12th caudal groove in *G. hokouensis* (Fig. 1). When there was a discrepancy between the two character states in an individual, we regarded the gecko as a hybrid of the two species following Toda et al. (2001).

Fig. 1

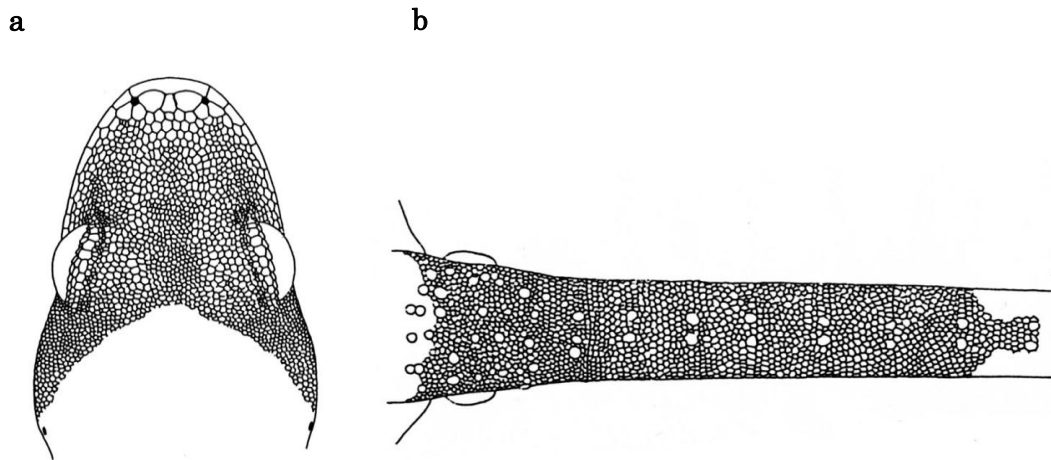


Fig. 1. Two diagnostic morphological characters using for identifying *G. yakuensis*

The illustration of *Gekko yakuensis* from Matsui and Okada (1968). (a) The head scutellation. The internasal scales are larger than following scales. (b) The tail scales. The paired tubercles are existing from tail base to tip, but regenerated tail lacks them.

Analysis for distribution and habitat data

We made distribution map of two species of geckos using positional data and to compare their habitat this year and 2005. We also performed Chi2 test using the number of captured geckos in sampling site where each species was found between 2012 and 2005 to evaluate the change of the fine-scale distribution of *G. yakuensis* and *G. hokouensis*.

DNA analysis

We extracted DNA of 96 geckos from their tail or liver tip using DNeasy Blood & Tissue Kit .Using these DNA samples, we analyzed mitochondrial sequence and microsatellite loci.

In the mitochondrial DNA analysis, we amplified and sequenced a part of cytochrome *c* oxidase I gene (COI). Polymerase chain reaction (PCR) was done using TaKaRa Ex Taq kit. We used the primer pair (LCO1490 and HCO2198) and PCR program from Hebert et al. (2003). The PCR products were checked by 1% Agarose S and purified by ExoSAP-IT. Sequencing reactions were performed using BidDye Terminator v3.1 Cycle Sequencing Kit and the same primers used in the PCR. The products were cleaned by ethanol precipitation and sequenced on an ABI 3130xl Genetic Analyzer. We checked the sequences using the FinchTV 1.4.0 (<http://www.geospiza.com/Products/finchtv.shtml>), and reconstructed an unrooted phylogenetic tree using neighbor-joining method (Saitou and Nei, 1987) by MEGA5 (Tamura et al., 2011).

In the microsatellite analysis, we amplified the six loci (Gs112, Gs206, Gs214, Gs215, Gs217, GS223; Li and Zhou, 2007). The PCR was done using Multiplex PCR kit, the locus specific primer pairs, and the fluorescently tagged universal primer (Schuelke, 2000; Blacket et al., 2012). The PCR condition as follows: 15 min at 95°C; 35 cycles of 94°C for 30 s, 54°C for 1 min 30 s, and 72°C for 1 min followed by 30 min at 60°C. The PCR products were measured with GeneScan 600 LIZ size standard on ABI 3130xl Genetic Analyzer and analyzed by Peak Scanner software for decision of the genotypes. To know the frequency of crossbreeding between *Gekko yakuensis* and *Gekko hokouensis*, we analyzed genotype data of these loci with 2 methods. First is principal coordinate analysis based on the genetic distances between individuals with GenAlEx 6.41 (Peakall and Smouse, 2006). Second is STRUCTURE analysis with STRUCTURE 2.3.4 (Pritchard, 2000).

RESULTS

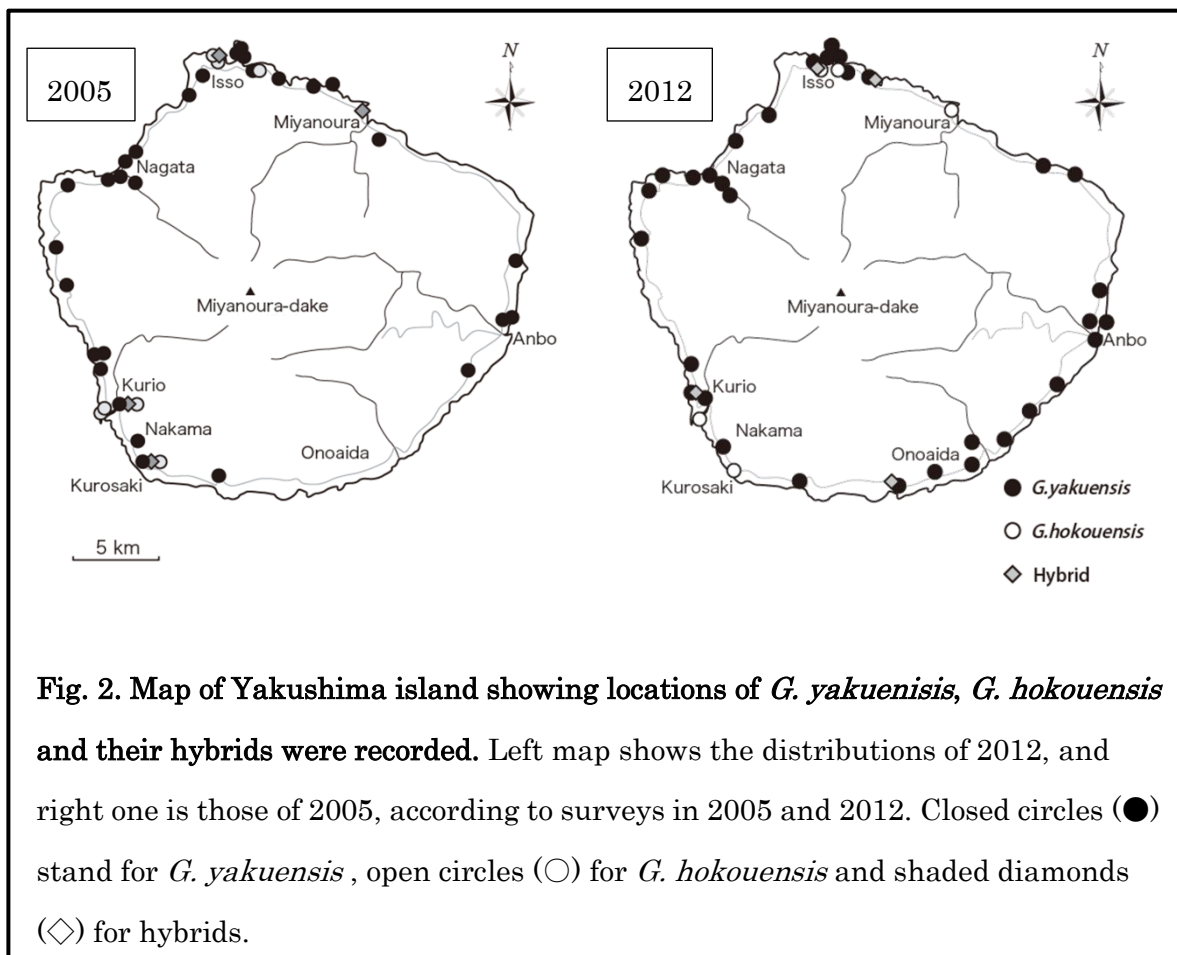
The distribution patterns of *G. yakuensis* and *G. hokouensis* did not change in 7 years

A total of 126 individuals were recorded, of which 73, 46, five were identified as *G. yakuensis*, *G. hokouensis* and their hybrids, respectively, by using morphological characters. The remaining one individual could not be identified because of its intermediate-sized internasal scales and the lack of original tails. Among these identified individuals, 15 *G. yakuensis* and six

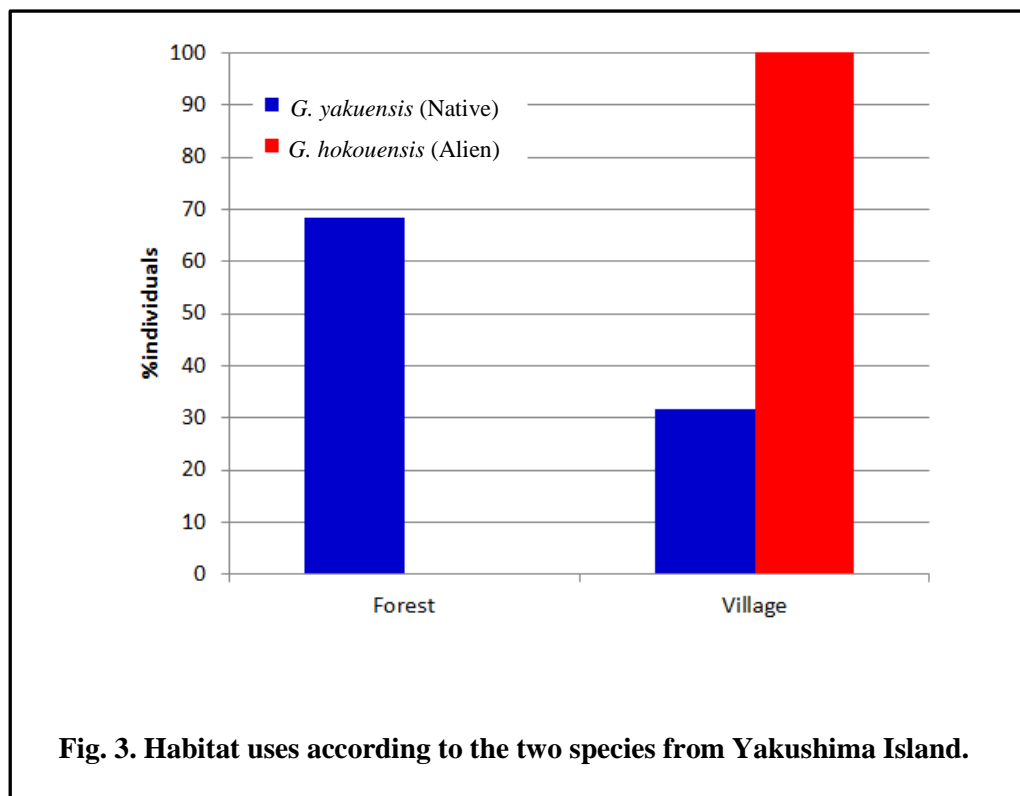
G. hokouensis were identified solely by the internasal condition because they have regenerated tails.

While *G. yakuensis* were found at 33 sites that were interspersed throughout an entire region of lowland Yakushima, *G. hokouensis* were found only at three sites, Isso, Miyanoura, and Kurio-Kurosaki, and hybrids were found at Isso, Kurosaki and Onoaida.

By comparing the distribution map of 2005 and 2012 about *G. yakuensis*, *G. hokouensis* and their hybrids, the distribution patterns did not change so much and the hybrids did not extend their distribution areas (Fig. 2).

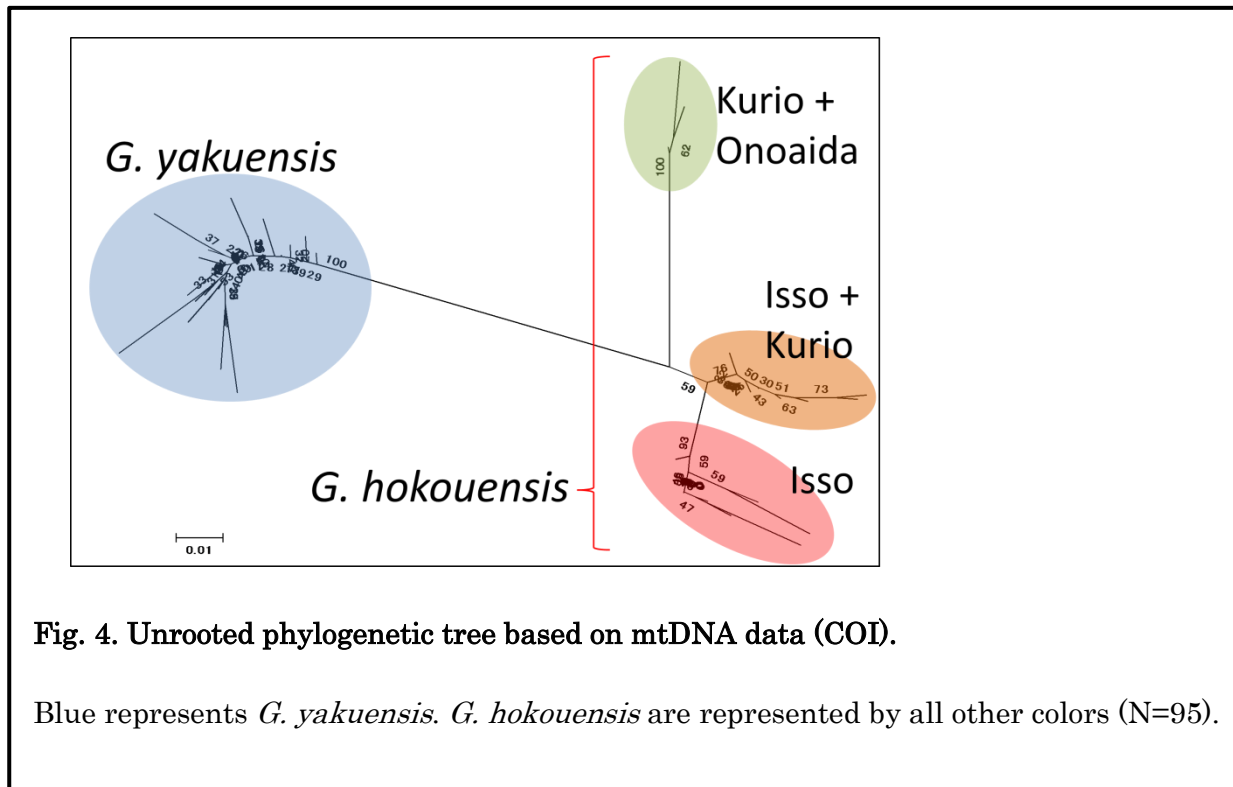


Moreover, the data of surrounding environment of capturing site suggested that *G. hokouensis* inhabited exclusively in urban areas, whereas *G. yakuensis* inhabited mainly in forested areas (Fig. 3).



mtDNA analysis

We used 626 bp of COI gene decided at 95 geckos for phylogenetic reconstruction. The unrooted phylogenetic tree shows that there were two main clades. One consisted of the samples of *G. yakuensis*, and the other is *G. hokouensis* (Fig. 4). *G. hokouensis* are further divided into three subclades of different origins (Fig. 4). The first subclade only consists of individuals from Isso area, the northern part of the island, the second consisted of Isso and Kurio, the last consists of Kurio and Onoaida, the southern part of the island. The subclade of Kurio and Onoaida are composed of only three individuals.



Microsatellite analysis

We could decide the genotypes of 92 samples at five of six loci. GS223 was not amplified clearly. Therefore, We used the genotypes of 92 samples at the remaining five loci for the analysis.

The result of the principal coordinate analysis (Fig. 5) showed that the genetic distances between individuals were divided them into two main groups; one comprised *G. yakuensis* and the other comprised *G. hokouensis*. The group of *G. hokouensis* exhibits higher genomic diversity than the group of *G. yakuensis* However, some individuals were plotted between these two groups.

The results of the STRUCTURE analysis at $K = 3$ showed that one of the three clusters corresponded to the samples of *G. yakuensis* (blue in Fig. 6), and the remaining two clusters corresponded to the samples of *G. hokouensis* (red and green in Fig. 6). The samples of hybrids showed genetic admixture between these two species. However, even within the

individuals identified as *G. yakuensis* or *G. hokouensis*, some individuals showed the assignment to another cluster or some genetic admixture level.

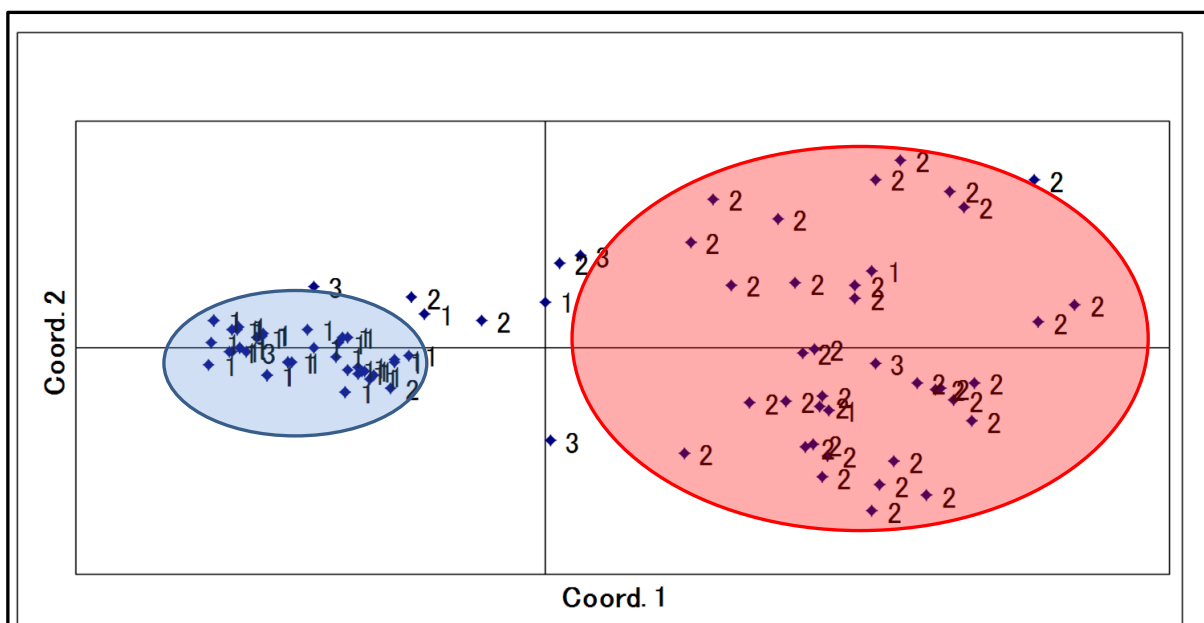


Fig. 5. Principal coordinates analysis of 92 individuals of *Gekko yakuensis*, *G. hokouensis*, and their hybrids based on the genetic distances between individuals from microsatellite data. The numbers represent identification based on the morphological characters in the field: 1) *G. yakuensis* , 2) *G. hokouensis* and 3) hybrids.

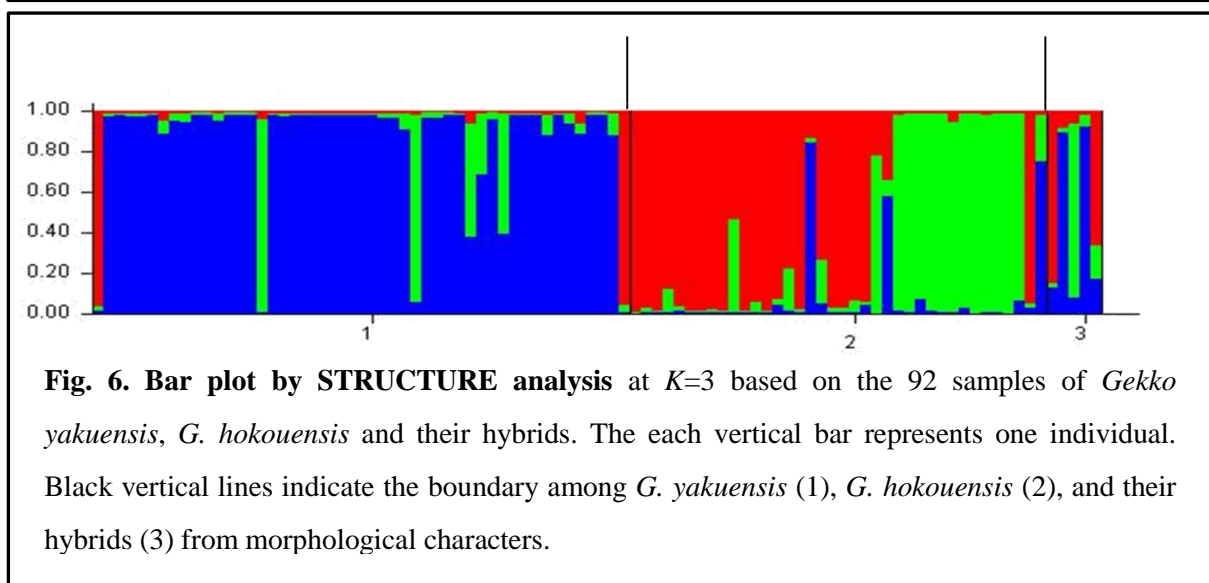


Fig. 6. Bar plot by STRUCTURE analysis at $K=3$ based on the 92 samples of *Gekko yakuensis*, *G. hokouensis* and their hybrids. The each vertical bar represents one individual. Black vertical lines indicate the boundary among *G. yakuensis* (1), *G. hokouensis* (2), and their hybrids (3) from morphological characters.

DISCUSSION

The distribution map suggests that the distributions of neither *G. yakuensis* nor *G. hokouensis* in Yakushima Island changed so much for the seven years. While *G. yakuensis* was found almost entire region around the Yakushima Island, *G. hokouensis* was found at the limited areas.

In the previous work of Toda and Hikida (2011), it was mentioned that the distribution of *G. hokouensis* was restricted to the vicinity of human settlements, and these restriction may came from their habitat preference. Considering their limitation of habitats, *G. hokouensis* would not be able to expand their distribution because the urban areas are interspersed and surrounded by forests. Our data also supported the idea that two species segregate their habitats by their preference. So, it seemed that distribution of two species had become equilibrium states already.

However, some area also existed that both *G. yakuensis* and *G. hokouensis* were recorded, or their hybrids were found. Then, specimens were collected and DNA samples of mitochondrial DNA and microsatellite markers were extracted to analyze how deep hybridization progressed. The DNA analysis, suggested that *G. hokouensis* have at least two differentiated genetic groups in Yakushima Island.

Previous work of Toda and Hikida (2011) also showed that there were high correlations between the villages inhabited by *G. hokouensis* and the presence of the port with regular vessel in that villages, either at past or present. This means the artificial introductions of *G. hokouensis* from outside of Yakushima Island through the ports. From this point of view, the diversity of mtDNA and microsatellite data of *G. hokouensis* in Yakushima Island indicate that each genetic groups of *G. hokouensis* had come from more than two different places. Comparing DNA data of *G. hokouensis* in Yakushima Island with that of other island populations will reveal where these genetic groups of *G. hokouensis* came from.

In the STRUCTURE analysis, some admixture levels between *G. yakuensis* and *G. hokouensis* were observed in individuals not only identified as hybrids but also *G. yakuensis* and *G. hokouensis*. This suggests that the genetic introgression between these species progressed moderately. By the work of Jono (2012), the male of *G. yakuensis* and

G.hokouensis use different patterns of courtship calls, suggested the behavioral isolation mechanism may work as the reproductive barrier and relieve the genetic introgression.

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