博士論文

History rewritten by the Victors: the truth of the origins and human-triggered competition of Japanese Geckos
（勝者が書き換えた歴史：日本産ヤモリ相の起源とヒトに起因する競争の真相）

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Contents

Chapter 1:
General introduction

How humans have impacted the ecosystem or biodiversity over a long history is a vital question. In today's world, where there is a strong demand to conserve biodiversity, it is essential to understand not only the current state of biodiversity but also its historical development and origins. This understanding is a critical foundation for formulating conservation strategies. In this context, countless studies have been performed on the relationship between humans and other organisms. However, despite the tens of thousands of years of human civilization history, many of these studies are focused on recent cases, and studies on how human activities have historically affected organisms are relatively scarce (Carlton, 1989; Richardson *et al.*, 2011; Yeakel *et al.*, 2014; Lefebvre *et al.*, 2022).

Geckos serve as excellent models for exploring this theme because they are synanthropes that prefer artificial environments and are considered to have been affected by human activities for a long time. Indeed, in recent years, various gecko species have been dispersed around the world through human logistics and transportation networks, and have even been found across the Pacific Ocean unintentionally (e.g. Fisher, 1997; Rödder *et al.*, 2008; Kwon *et al.*, 2023). Such human-mediated dispersal might also have occurred in the past by taking advantage of historical human activity (trades, wars, building and more). In this study, I have uncovered the hidden events behind the history surrounding the Japanese people and three gecko species in Japan.

In Chapter 2, which examines a recent case, I analyzed the mitochondrial DNA of *Gekko hokouensis* collected from the Izu Islands (maybe an artificial introduced population) and the Nansei Islands (native population), both in Japan. *G. hokouensis* is a widely distributed gecko species that inhabits Kyushu and the Nansei Islands in Japan, southeastern China, and Taiwan. Molecular phylogenetic analysis suggests that *G. hokouensis* of Japan belongs to a cryptic monophyletic group different from that of the currently discovered sample of China. The Japanese clade of *G. hokouensis* is further divided into two subclades (Clade 1 and Clade 2 in this paper). In the Nansei Islands, these two subclades exhibit a complex nested-distribution pattern and do not coexist on any islands, while in the Izu Islands, both clades appear to coexist. High genetic diversity is observed in both clades in the Nansei islands, the source population. Surprisingly, high genetic diversity is also maintained in each clade in the Izu Islands, the introduced population. Analysis Of Molecular Variance (AMOVA) has also revealed that the genetic differentiation between the populations in the Izu Islands and the Nansei Islands was not significant in each clade. These results suggest that the population of the Izu Islands is now experiencing secondary contact between the two clades due to multiple migrations from various regions of the Nansei Islands.

In Chapter 3, which examines a historical case, I conducted a study to clarify the dispersal history of *Gekko japonicus* in the Japanese archipelago thought to have been influenced by human activity over a long-term period. *G. japonicu*s is the most common and popular gecko species in Japan. I hypothesized that ancient urban development and transitions had a non-negligible effect on species distribution. Inferring the impact of past human activity on ecosystems from ancient literature and verifying that impact by genetic analysis and human history is an effective means of tackling this problem. I performed this combination approach for *G. japonicus* using ddRAD-seq and verification of ancient literature and human history. This approach revealed that *G. japonicus* migrated from China to the western Japanese archipelago before Christ and dispersed from west to east over thousands of years. The dispersal history of *G. japonicus* shows many synchronizations with the development of human society, suggesting that humans have repeatedly influenced its distribution throughout history.

In Chapter 4, which examines a past-to-future scenario, I explored whether *G. japonicus*, now recognized as an ancient nonnative species, was “invasive”. The study focused on its current and past competitiveness with *Gekko* sp. (Nishiyamori in Japanese; an undescribed endemic species) and *G. hokouensis*, both co-distributed with *G. japonicus*. Although *G. japonicus* appears neutral currently, I hypothesized that it was invasive upon its initial introduction. To examine the past invasiveness of *G. japonicus*, I performed niche analysis and population genetics using ddRAD-seq, comparing them between the areas with and without interspecific competition. Niche analysis on the Goto Islands, Hiradojima Island (colonized by *G. japonicus*), and the Koshikishima Islands (not colonized by *G. japonicus*) revealed that the microhabitat use of endemic *Gekko* sp. change in response to invasions by other introduced gecko species, although its potential suitable habitat and microhabitat use are quite similar to those of others. Population genetic analysis using ddRAD-seq showed that while the population of *Gekko* sp. in areas where other gecko species had been introduced experienced significant declines (bottleneck) after their introduction, the population of *Gekko* sp. in competition-free areas did not experience any bottlenecks. These results suggest a potential three-way competitiveness among the gecko species, with invasion of *G. japonicus* and *G. hokouensis* localizing the distribution of *Gekko* sp.. In other words, *G. japonicus* may have been an invasive species in the past.

Finally, I summarized Chapters 2-4, how to comprehend and address the history that has come to light. The ability to learn from history and use that knowledge for future decision-making is a unique human strength. Emphasizing this, I highlighted the importance of studying the historical formation and origins of current biodiversity as fundamental research.

Chapter 2:

Secondary contact of two cryptic Hokou gecko groups in the Izu Islands, Japan

Introduction

Species distributions are limited by geographical barriers such as oceans, rivers, or mountain ranges. These geographical barriers can impede gene flow between populations and promote speciation (Slatkin, 1987). However, human-mediated dispersal can facilitate breaking through these geographical barriers and allow for rapid, long-distance dispersal that far exceeds the potential migratory capacity of a species (Wilson *et al.*, 2009; Gippet *et al.*, 2019). When two species with similar niches coexist, competitive exclusion often leads to the regional extinction of one of the species. However, shifts in the niches of one or both species may allow them to coexist. In addition, when two species are phylogenetically close and speciation is incomplete, hybridization may inhibit differentiation. Conversely, the creation of new genetic traits may promote differentiation (Abbott *et al.*, 2013).

Evolution had traditionally been regarded as a process that cannot be directly confirmed, because it occurs on an extremely long time scale. However, in recent years, it has been suggested that adaptive evolution and speciation can occur within a very short time span that I can observe directly (e.g. Meyer *et al.*, 2012). The influence of human activity such as dispersal and/or local adaptation to the urban environment may also drive these rapid evolutions (Byrne & Nichols, 1999; Winchell *et al.*, 2016).

Geckos, which are synanthropes that prefer to use artificial environments, have been affected by human activities for a long time (Chiba *et al.*, 2022). Some geckos that live in pristine natural environments have diversified due to habitat fragmentation by geographical barriers (Yan *et al.*, 2010; Nguyen *et al.*, 2019). However, many geckos prefer artificial environments such as buildings and material cargo as habitats, while some other species use both artificial and natural environments (Kim *et al.*, 2018; Sabath, 1981). Therefore, geckos are a suitable model system for testing the effects of human activity on speciation.

Gekko hokouensis Pope, 1928 (Reptilia: Squamata: Gekkonidae), is a widely distributed gecko that inhabits Kyushu and the Nansei Islands in Japan, southeastern China, and Taiwan (Zhou, Liu & Li, 1982). However, it has been suggested that *G. hokouensis*, which is currently described as a single species of gecko, may contain a large number of cryptic species as in the following research examples: Protein electrophoresis data suggests that the genetic structure of *G. hokouensis* is divided into northern and southern populations by the boundary between Yokoatejima Island and Amami- Oshima Island in the Nansei Islands (Toda, Hikida & Ota, 1997). Furthermore, allozyme data has revealed another genetically differentiated population on the main island of Okinawa and surrounding areas. (Toda, Hikida & Ota, 2001). Thus, the species status of *G. hokouensis* with regard to their phylogenetic relationships remains unclear.

Then, *G. hokouensis* has first been reported as inhabiting on Hachijojima Island in the Izu Islands, a volcanic archipelago located in the southern part of Honshu in the Japanese Archipelago in 1995 (Ota, Fusure & Yagishita, 1995). I believe that *G. hokouensis* did not inhabit the Izu Islands before 1995 and is a recently introduced population. There are traces of human settlement on the Izu Islands going back thousands of years (e.g. Ichikawa *et al.*, 2019), and with continuous inhabitation since at least 600s A.D (e.g. Prince Toneri & Kodera, 720). Geckos inhabits in human houses and are highly visible organisms, so if geckos had inhabited, their existence must have been recognized. Despite this, in the flora and fauna lists recorded on Hachijo Island in 1968 and Aogashima in 1984, it was written that only one skink species (*Plestiodon latiscutatus*) was inhabited there in the lizard family, and there was no record of any geckos (Kasai, 1968; Aogashima Village Board of Education & Aogashima Village, 1984). Okada's blue-tailed skink *Plestiodon latiscutatus* (former *Eumeces okadae*), the only native skink inhabiting the Izu Islands, was reported in 1907 (Stejneger, 1907), and a survey of this lizard conducted in the Izu Islands in the 1970s also clearly stated that geckos were not found on the island there at that time (Hara, 1976). Since 1995, little research on geckos in the Izu Islands has been done but *G. hokouensis* was recorded for the first time on Oshima in the Izu Islands in 2021 (Nakamura & Marunouchi, 2023). As many materials and tourists are regularly transported to the Izu Islands by cargo ships (https://www.tokaikisen.co.jp/boarding/searoute/), and geckos have been observed by islanders and tourists in recent years, it is believed that the distribution of geckos is now expanding due to human dispersal.

The purpose of this study was to clarify the status of *G. hokouensis* in the Izu Islands, whose distribution is believed to be artificially expanding, and its phylogenetic relationship with native populations. I compared the mitochondrial DNA of samples collected from the Izu and Nansei Islands with samples from China registered in GenBank, and performed a molecular phylogeny analysis.

Materials & Methods

1. Sampling

Sampling was conducted from the spring to summer of 2019 on Hachijojima Island (HC), Aogashima Island (AO), and Miyakejima Island (MY) in the Izu Islands (upper right of Fig. 2-1). Hachijojima Island is an island formed by two connected cone-shaped volcanoes. There, it was confirmed that the gecko inhabited almost all areas except the steep mountainous regions but mainly found in the villages. Aogashima Island is a volcanic island with a large caldera surrounded by cliffs. There is a small village outside the caldera, and agricultural and salt industry activities are carried out inside the caldera. In addition to being abundant in the villages, *G. hokouensis* was confirmed to inhabit the locally artificial environment of the harbor and the caldera there. A total of 74 *G. hokouensis* individuals were collected from Hachijojima Island and Aogashima Island. On Miyakejima Island, one *G. hokouensis* was observed, but not collected. Details about the sampling on Miyakejima Island are provided in the supplementary (Additional discussion S2-1). In the Nansei Islands, sampling was conducted on Yakushima Island (YK), Kakeromajima Island (KK), Yoroshima Island (YR), Okinoerabujima Island (OB), Yoronjima Island (YN), and Okinawajima Island (OW) from the spring to autumn of 2020 (bottom right of Fig. 2-1), with a total of 15 *G. hokouensis* collected. Sampling was conducted on the mainland of Kyushu in 2023, and one individual was collected from Ibusuki (IB). The collected samples were stored in 100% ethanol after euthanasia, or in some cases, only the tail tissue was autotomized and stored, and the individuals were released. All samples are listed in Table S2-1, and the sequences used for the analysis have been deposited in the DDBJ Sequence Read Archive (https://www.ddbj.nig.ac.jp/dra).

2. Laboratory protocol

Genomic DNA was extracted from the tail tissue of the samples using the Nucleospin Tissue kit (TaKaRa, Shiga Pref., Japan). A ~960 bp region of mitochondrial DNA, from 12S rRNA to 16S rRNA across tRNA V, was amplified using squamate-specific primers r12S-1L (AGGATTAGATACCCTACTA) and r16S-5H (TTTATYRRGYAACCAGCTATC; Kumazawa & Endo, 2004). The PCR reactions and sequencing were performed according to a previously published protocol (Hirano, Saito & Chiba, 2015), the only difference being an annealing temperature of 40 °C.

3. Mitochondrial DNA analyses

A contig was created from the obtained forward and reverse sequences using GeneStudio v 2.2.0.0. The alignment was performed using MUSCLE v3.8 (Edgar, 2004), and a consensus sequence of about 800 bp (including a gap) was created by trimming the primer sequences at both ends to remove regions with low-probability base calling. Haplotypes were defined as having one base difference from any other sequence. A Bayesian phylogeny was estimated using MrBayes v

3.2.7 (Ronquist & Huelsenbeck, 2003). Evolutionary models were tested by maximum likelihood using MEGA6 (Tamura *et al.*, 2013) and GTR+G was adopted based on BIC. Two simultaneous runs were used, consisting of four simultaneous chains for 25 million generations, and trees were sampled every 1000 generations. The first 1 million generations were excluded as burn-in. *G. japonicus* from Korea (GenBank Accession No. KR996131.1; Kim *et al.*, 2016), *G. hokouensis* collected from China (GenBank Accession No. KT005801.1), *G. chinensis* from China (GenBank Accession No. NC_027191.1; Hao, Ping & Zhang, 2016), *G. swinhonis* from China (GenBank Accession No. NC_018050.1; Li *et al.*, 2013), *G. yakuensis* from the Nansei Island (GenBank Accession No. LC633667.1) and *G. shibatai* (collected by authors from the Nansei Islands and now submitting) samples were added to the analysis to show the phylogenetic relationships among related species. In addition, another genus gecko *Hemidactylus frenatus* was used as the outgroup. The genetic distances between haplotypes were calculated for the Japanese and Chinese *G. hokouensis* samples (K2P, bootstrap: 100). The genetic distance between clades is the average value of the pairwise genetic distance between each included haplotype. A haplotype network based on median joining ($\varepsilon = 0$; McTavish, 2018) was constructed using PopART v 1.7 (Leigh & Bryant, 2015) for the Japanese *G. hokouensis* population. Analysis of molecular variance (AMOVA; K2P, bootstrap: 1000) was performed independently in two clades to calculate the genetic differentiation of the populations in the Nansei Islands and Izu Islands. Using DNaSP v 6.12.3.0 (Rozas *et al.*, 2017), each island was treated as a population unit, and all of them were grouped into either the Nansei Islands or the Izu Islands. Statistical analysis was performed using ALREQUIN v3.5.2.2 (Excoffier & Lischer, 2010).

Results

The results of the molecular phylogenetic analysis and the haplotype network (Fig. 2-2) suggested that *G. hokouensis* inhabiting the islands of Japan formed a clade non-sister to the Chinese outgroup of *G. hokouensis* and that it was further separated into two clades (Clade 1 and Clade 2) that were genetically far apart. Clade 1 contained 15 haplotypes, and Clade 2 contained 10 haplotypes. Of the 15 haplotypes in Clade 1, one was distributed on Yakushima Island, while two different haplotypes were distributed on Okinoerabujima Island. The major haplotype of Okinoerabujima Island was consistent with that of Ibusuki, the mainland of Kyushu. Clade 2 had one haplotype on Kakeromajima Island, two on Yoroshima Island, one on Yoronjima Island, and one on Okinawajima Island. Table 2-1 suggested the average of pairwise genetic distances among each haplotype of Clade 1, 2 and other species of the genus *Gekko*. The average pairwise genetic distance between Clades 1 and 2 was higher than that within each clade. Without considering the positional relationship of the phylogenetic tree, *G. yakuensis* and *G. shibatai*, which are endemic to the Nansei Islands, were the closest to Japanese *G. hokouensis*. The next closest group was *G. hokouensis* of China, and the most far group were other geckos of the continent (*G. japonicus*, *G. chinensi* and *G. swinhonis*). Table 2-2 suggested the genetic diversity of the populations of the Nansei Islands and Izu Islands for Clades 1 and 2, respectively. High haplotype (h) and nucleotide (π) diversities were found for Clades 1 and 2 (Table 2-2). In both clades, although haplotypes were significantly differentiated (p-value < 0.05) by island, no significant difference was found (p-value > 0.05) between the Nansei Islands and the Izu Islands (AMOVA, Table 2-3).

The geographical pattern for each haplotype (Fig. 2-3) suggested that in the Nansei Islands, Clades 1 and 2 formed a complex nested-distribution pattern, but there was no island on which they coexisted. In contrast, in the Izu Islands, 13 haplotypes of Clade 1 and 5 haplotypes of Clade 2 were mixed sympatrically, with both clades having secondary contact.

Discussion

1. Enigmatic phylogenetic relationships and geographical patterns

There was no significant genetic differentiation between the Nansei Islands and the Izu Islands in the results of AMOVA in either clade, so I treat the Nansei Islands and the Izu Islands as one metapopulation. The pairwise genetic distances between the Japanese *G. hokouensis* population and other species of the genus *Gekko* (including the Chinese *G. hokouensis* population; 13.0–22.2%) are roughly corresponding to the general average genetic distance between species of the genus *Gekko* (16.7–37.5%; Zhou & Wang, 2008). In other words, *G. hokouensis* in Japan may be a cryptic species different from that in China. However, this result is only based on mitochondrial DNA, so analysis using more genetic loci and Chinese ancestral populations will be required to conclude that they are a new species. In contrast, the genetic distance between Clades 1 and 2 is relatively short, about 6%. It suggests that the two subclades of the Japanese *G. hokouensis* population are undergoing speciation, although not as much as separate species. In order to confirm what stage of speciation they are currently on, it will be essential to verify whether reproductive isolation has occurred between them.

A comparison of the geographical pattern of the these two clades with the results of a previous study (Toda *et* *al.*, 1997) suggests that Clade 1 corresponds to the group which inhabits the mainland of Kyushu to Yokoatejima Island. And then it suggests that Clade 2 corresponds to the group which inhabits in Amami-Oshima Island and the south. It has been suggested that the *G. hokouensis* population on Yakushima Island may be a recent immigrant population that arrived by artificial transport (Toda & Hikida, 2011). Considering the phylogenetic relationships of this study, the population of Yakushima Island may be derived from neighboring islands. From the above, the distribution patterns of the two clades based on the mitochondrial genomes were roughly consistent with previous studies based on the allozymes (Toda *et al.*, 1997). However, Clade 1 is also distributed on Okinoerabujima Island, south of Amami-Oshima Island, and the two clades are not clearly differentiated in the north and south as in previous studies (Toda *et al.*, 1997), but rather have a complicated nested-distribution pattern.

The herpetofauna of the Nansei Islands is generally considered to have a biogeographic boundary at the Tokara gap between Kodakarajima Island and Akusekijima Island, where it has been divided by a trench since at least the early Pleistocene, when many of the islands of this archipelago were connected (Ota, 1998). It had also been considered that there was a biogeographic boundary between Kodakarajima Island and Akusekijima Island in the previous study mentioned above (Toda *et al.*, 1997). They had questioned that a population (maybe corresponding to Clade 1 in this study) inhabits across this boundary. However, in recent years, it is suspected that there is no clear biogeographic boundary between Kodakarajima Island and Akusekijima Island (Komaki, 2021). So it may not be strange that geckos of two clades exhibit across boundary distribution patterns. But both clades still show a complicated nested-distribution pattern. There are two possible scenarios for forming such geographical pattern. First, in the past, the two clades coexisted when the islands were connected. After the archipelago was divided by the sea level rose, competitive exclusion occurred independently on each island, and one clade became extinct. It is known that when two species with similar niches exist sympatrically, competitive exclusion often leads to the regional extinction of one of the species (Hardin, 1960). There is a similar case of two gecko species explained by this scenario. There are four islands in the Nansei Islands (from the south: Amami-Oshima Island, Takarajima Island, Kodakarajima Island, and Kojima), *G. vertebralis* inhabits Amami-Oshima Island and Kodakarajima Island and *G. Shibatai* inhabits Takarajima Island and Kojima Island. In other words, two gecko species are alternately distributed and never coexist on the same island. This pattern has been thought to be due to competitive exclusion on each island (Toda *et al.*, 2008). Thus, similar events may have occurred in *G. hokouensis* throughout the Nansei islands. The second possibility is that although these clades were originally divided into northern and southern groups by Yokoatejima Island and Akusekijima Island, secondary dispersal in recent years has complicated the distribution pattern. However, if this scenario is true, Clade 2 should have occupied Okinoerabujima island before Clade 1. In that case, a small number of Clade 1 individuals trying to colonize Okinoerabujima island would quickly be eliminated by Clade 2. This competitive exclusion would have made Clade 1 difficult to settle secondary on Okinoerabujima island. Whether Clade 1 is invasive enough to eliminate dominant Clade 2, or whether Clade 1 succeeds in invading when the number of Clade 2 individuals is reduced for some reason, needs to be further examined.

2. Genetic diversity and dispersal pathways in the Izu Islands

In many cases, bottlenecks and founder effects are known to reduce the genetic diversity of populations significantly established on an island (O'Brien & Evermann, 1988; Puillandre *et al.*, 2008). However, there was no significant difference between the Nansei and Izu Islands populations, with those in the Izu Islands also maintaining a high level of genetic diversity. Although geckos live in human houses and are highly visible organisms, until recently there had been no reports that any geckos inhabit the Izu Islands. It is not reasonable to consider the population of the Izu Islands to be native just because they retain high genetic diversity. In addition, considering the phylogenetic relationship that suggests that the population of the Nansei Islands is native, it is rather reasonable that the populations in the Izu Islands have been imported multiple times from the Nansei Islands. The Izu Islands have been invaded by various taxa from the Nansei Islands naturally or artificially. It has been suggested that in ancient times, land snails may have been naturally introduced by the ocean current (Hirano, Kameda & Chiba, 2014; Hirano *et al.*, 2019). In contrast, relatively recent cases such as the blind snake *Indotyphlops braminus* (Ota *et al.*, 1995: Wickramasinghe *et al.*, 2022) and the whip scorpion *Typopeltis stimpsonii* (Karasawa *et al.*, 2015) are believed to have been artificially introduced with horticultural crops in the 1900s. Geckos, which inhabit artificial objects, are also easily dispersed by humans, and there are records of other geckos actually being observed on ferries that operate between remote islands (Takahashi, 2005). In the only area on Miyakejima Island where *G. hokouensis* was observed in this study, there is a container depot where supplies brought in from outside the island are collected. Recently, *G. hokouensis* was also first recorded on Oshima Island in the Izu Islands in a container house near the island's port (Nakamura & Marunouchi, 2023). A similar container collection site exists inside the caldera on Aogashima Island, and it appears that *G. hokouensis* spread from there. Ports and villages located outside the caldera, where the presence of *G. hokouensis* has been confirmed, are separated from this container depot by a high ridge. Considering that it was rarely seen in the mountain areas, it is likely that hitchhiking on cars and cargo has strongly contributed to the movement of individuals on the island. From this, it is inferred that *G. hokouensis* is easily affected by human-mediated dispersal. Therefore, as in conventional theory, I believe that human-mediated dispersal is more likely than ocean current dispersal as the method of import from the Nansei Islands. Historically, Aogashima Island has lacked direct human interaction with the Nansei Islands. However, a regular passenger ship connecting Hachijojima Island and Aogashima Island went into service in 1991, likely allowing distribution to expand from Hachijojima Island.

3. Secondary contact of the two clades in the Izu Islands

The Izu Islands are currently a unique field where two cryptic clades of *G. hokouensis* and many of those haplotypes contact together. Why geckos that cannot coexist in the Nansei Islands can coexist in the Izu Islands is an interesting question, but the results of this study cannot completely answer, and further research will be needed in the future. One of the possible hypotheses, both clades have probably coexisted and been maintained for decades because the timings of their invasions were similar and before one had occupied entirely a niche on the island. It is also possible that some kind of a shift in the niches of one or both species has occurred that avoided competitive exclusion. Although it is not clear how secondary contact between the populations during the mid-speciation stage affects speciation, hybridization may inhibit differentiation or, conversely, new genetic traits may be created that promote differentiation (Abbott *et al.*, 2013). These *G. hokouensis* populations on the Izu Islands could provide important insights into the impact of secondary contact, resulting from human-mediated dispersal, on the speciation of geographically isolated populations.

Conclusions

A series of population genetic analyses have suggested that *G. hokouensis* from the Nansei Islands, which has multiple genetically different populations divided by island, has been introduced to the Izu Islands multiple times and that they are now in secondary contact. In the future, additional high-resolution population genetic analysis using next-generation sequencing will be able to examine the history of the formation of the complicated geographical patterns in the Nansei Islands and the historical distribution and expansion in the Izu Islands. In addition, by collecting detailed ecological information such as the distribution, microhabitat, and morphology of both clades, it will be possible to verify whether the two geckos are causing a rapid shift in niches. *G. hokouensis* in the Izu Islands should be considered good material for studying the impact of secondary contact associated with human-mediated dispersal on the rapid adaptive evolution of organisms. Furthermore, the study of rapid adaptation to new destinations should be emphasized from the viewpoint of conservation biology. Generally, it is known that geckos that are introduced to the islands often become invasive and have a negative impact on native insect or reptile fauna (Cole, Jones & Harris, 2005). Because many endemic insects and reptiles live in the Izu Islands, it is important to obtain basic information on the evolutionary ecology of *G. hokouensis* and to understand its future expansion trends.

Tables

Table 2-1 The average of pairwise genetic distances based on mtDNA (12S, tRNA V, 16S) among each haplotype of Clade 1, 2 and other species.

	G. hokouensis (Clade1)	*G. hokouensis* (Clade2)	*G. hokouensis* (China)	*G. japonicus*	*G. chinensis*	*G. swinhonis*	*G. yakuensis*	*G. shibatai*
***G. hokouensis* (Clade1)**	0.007 (0.003)							
***G. hokouensis* (Clade2)**	0.062 (0.009)	0.021 (0.005)						
***G. hokouensis* (China)**	0.158 (0.015)	0.160 (0.016)	0.000					
G. japonicus	0.209 (0.017)	0.209 (0.017)	0.188 (0.017)	0.000				
G. chinensis	0.215 (0.016)	0.222 (0.018)	0.209 (0.017)	0.212 (0.017)	0.000			
G. swinhonis	0.190 (0.017)	0.191 (0.016)	0.198 (0.017)	0.202 (0.017)	0.208 (0.016)	0.000		
G. yakuensis	0.142 (0.013)	0.131 (0.013)	0.177 (0.016)	0.221 (0.019)	0.204 (0.017)	0.213 (0.018)	0.000	
G. shibatai	0.138 (0.013)	0.130 (0.012)	0.183 (0.015)	0.222 (0.018)	0.208 (0.017)	0.204 (0.018)	0.025 (0.005)	0.000

Numbers in parentheses represent standard error (S.E).

Table 2-2 Genetic diversity based on mtDNA (12S, tRNA V, 16S) calculated for Clade 1 and Clade 2 respectively

	n	N_{Nansei}	N_{Izu}	N_{total}	h_{Nansei}	h_{Izu}	h_{total}	π_{Nansei}	π_{Izu}	π_{total}
Clade1	68	3	13	15	0.5111	0.8082	0.8503	0.0024	0.0067	0.0066
Clade2	22	5	5	10	1.0000	0.7917	0.8918	0.0167	0.0209	0.0211

n, number of individuals; N, number of haplotypes corresponding to each subscript; *h*, haplotype diversity corresponding to each subscript; *π*, nucleotide diversity corresponding to each subscript

Table 2-3 Results of AMOVA based on mtDNA (12S, rRNA V, 16S) calculated for Clade 1 and Clade 2

	Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**	***F* - statistics**	**p-value**
Clade1	between archipelagoes	1	15.605	0.67466 Va	20.83	F_{CT}=0.208	0.185
	Among populations within archipelagoes	3	13.276	0.19021 Vb	5.87	F_{SC}=0.074	**<0.01**
	Within populations	63	149.536	2.37359 Vc	73.29	F_{ST}=0.267	**<0.01**
Clade2	between archipelagoes	1	18.638	0.62576 Va	6.65	F_{CT}=0.067	0.131
	Among populations within archipelagoes	4	48.735	1.73564 Vb	18.44	F_{SC}=0198	**0.011**
	Within populations	16	112.785	7.04907 Vc	74.91	F_{ST}=0.251	**<0.01**

Among Islands; Difference between the Nansei Islands and the Izu Islands, Among population within Islands; Differences among each island in the Nansei Islands / the Izu Islands, Within populations; Differences in each island

Figures

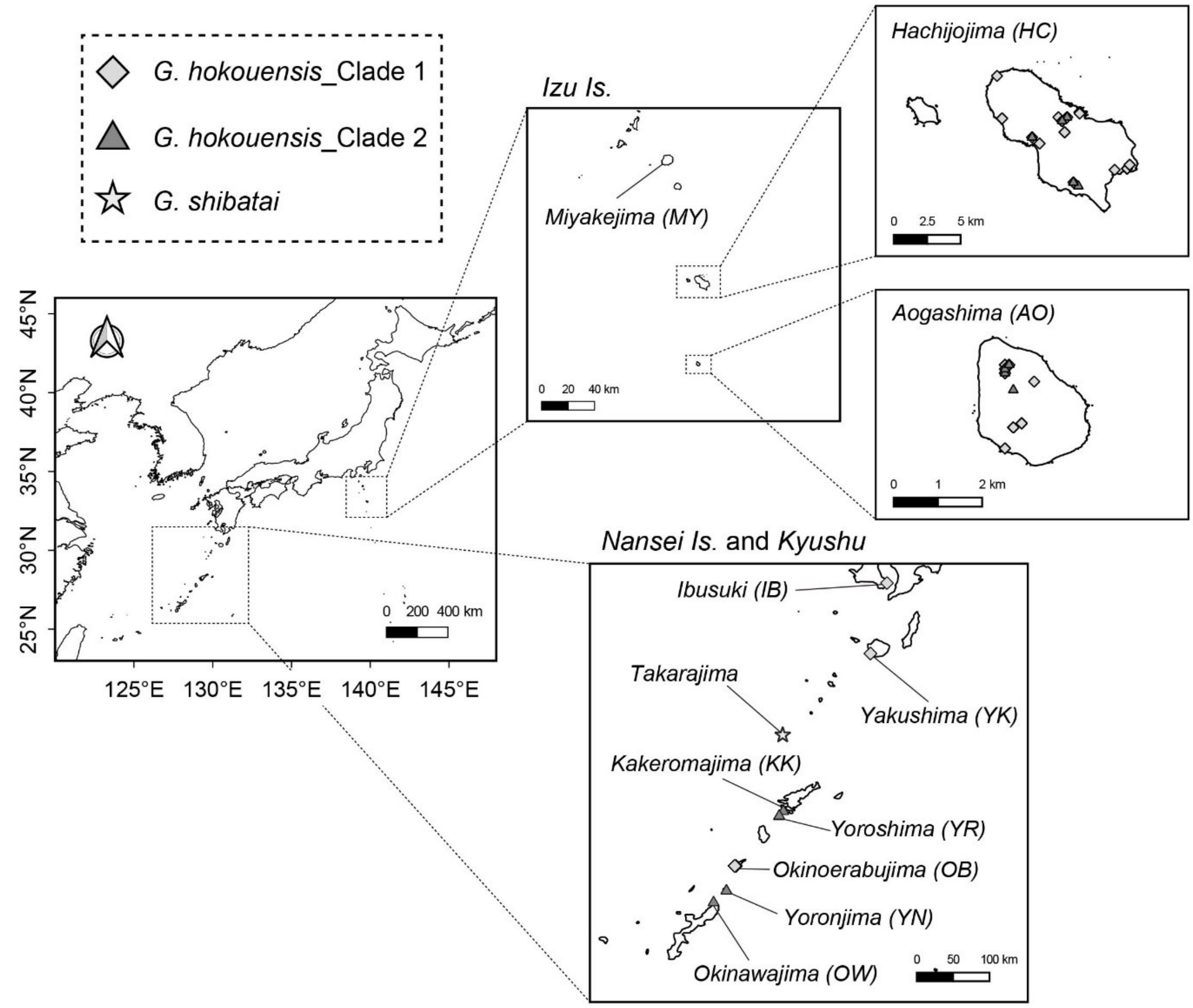

Fig. 2-1 Sampling sites in the Izu Islands (MY, HC, and AO), the Nansei Islands (YK, KK, YR, OB, YN, and OW) and Kyushu (IB).

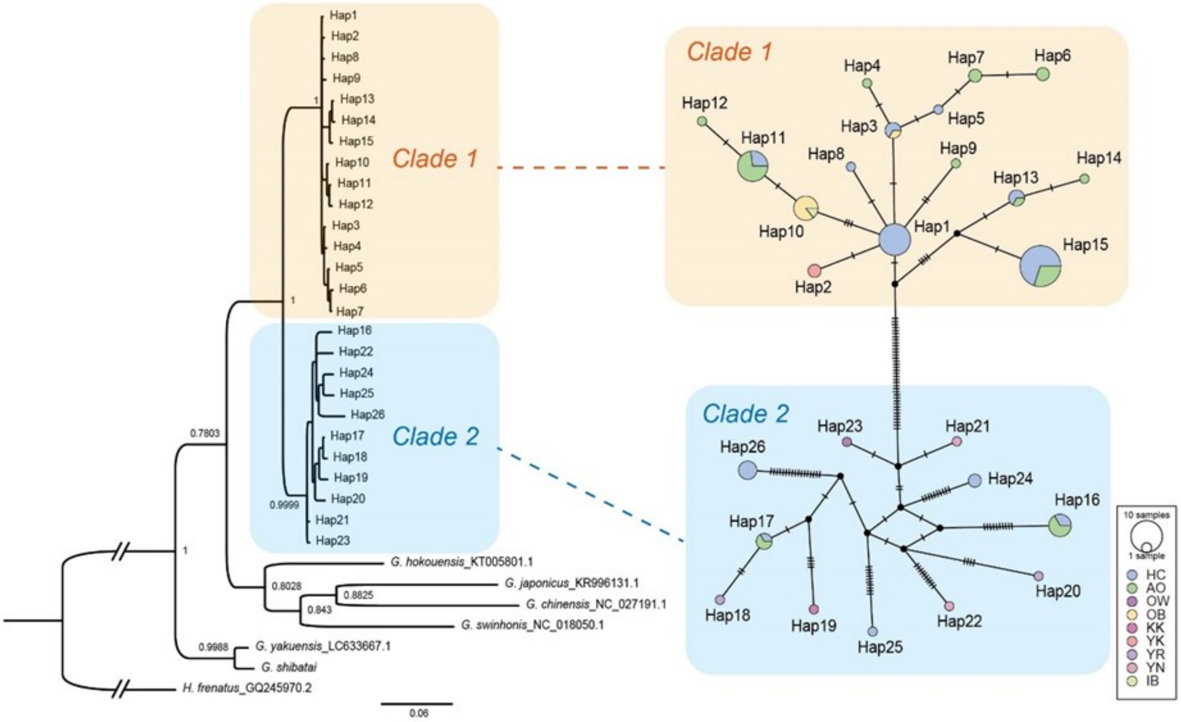

Fig. 2-2 Phylogenetic tree based on Bayesian analysis: The numbers above the tree branches indicate the Bayesian posterior probability. Haplotype network based on Median Joining: Dashes on the network show the distance for each haplotype. HC and AO belong to the Izu Islands, and IB is located on the mainland of Kyushu. The other six islands belong to the Nansei Islands. Both figures were created based on concatenated mtDNA region (12S, rRNA V, 16S).

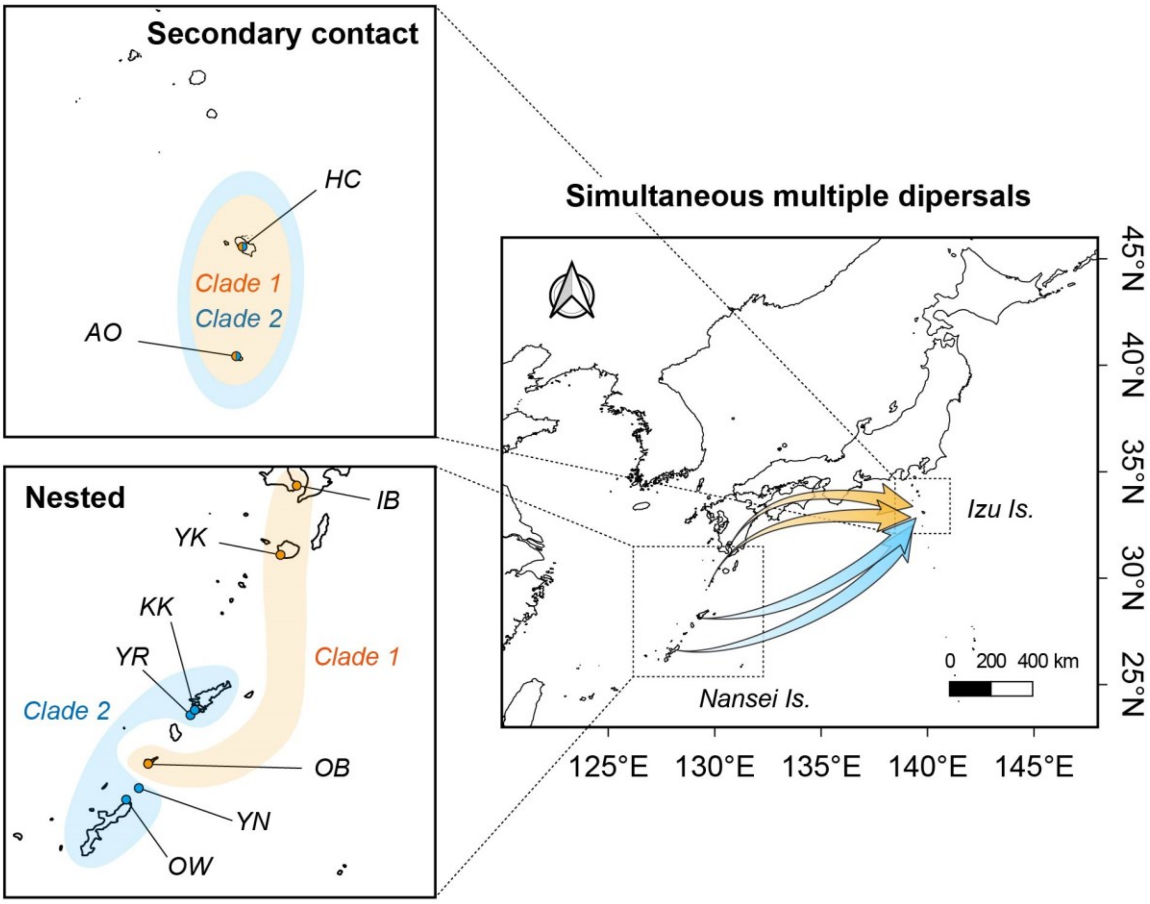

Fig. 2-3 Geographical distribution of Clade 1 and Clade 2. Clade 1 inhabits the area surrounded by the solid line, and Clade 2 inhabits the area surrounded by the broken line.

Chapter 3:
The mutual history of Schlegel's Japanese gecko (Reptilia: Squamata: Gekkonidae) and humans inscribed in genes and ancient literature

Introduction

How has the current distribution of organisms been affected by human activities? The answer to this question is essential to understanding the origins of biodiversity (Bullock *et al.*, 2018). Sometimes, species have been enabled by human-mediated dispersal to travel much faster and further than their natural dispersal ability (Wilson *et al.*, 2009; Gippet *et al.*, 2019). There are countless organisms whose distributions are artificially expanding worldwide (Rödder *et al.*, 2008; Case *et al.*, 1994). However, many of the cases that have received attention are recent events, and there are not many studies from the perspective of evolutionary biology on how human activity has affected organisms over the long history of humans (Carlton, 1989; Richardson *et al.*, 2011; Yeakel *et al.*, 2014).

Schlegel's Japanese gecko, *Gekko japonicus* (Reptilia: Squamata: Gekkonidae) is widely distributed in eastern China, South Korea, and south of the Tohoku region within the Japanese archipelago. Recently, Japanese populations of this species have been considered to be derived from an old immigrant Chinese population. This hypothesis has been supported by morphological and molecular genetic studies (Kim *et al.*, 2019; Kim *et al.*, 2020b). It has been suggested by paleospecies distribution modeling that *G. japonicus* was able to settle in Japan after the middle Holocene when the climate became warmer (Kim *et al.*, 2020b). Due to the habitat preference of *G. japonicus* for artificial environments, such as buildings and cargo (Kim *et al.*, 2018), its migration and expansion from China to Japan are predicted to have been affected by ancient human activities.

Here, I conducted a study to clarify the dispersal history of *G. japonicus* in the Japanese archipelago, thought to have been influenced by human activity over a long-term period. Recently, deciphering historical materials, such as ancient literature, has been emphasized in assessing the effects of humans on past biodiversity and ecosystems (Hayashi, 2014; Kawakatsu *et al.*, 2021). *G. japonicus*, a popular organism living close to humans, is variously recorded in ancient Japanese literature. Therefore, I first comprehensively deciphered ancient Japanese literature and collected descriptions of reptiles and amphibians (it seems that the two were indistinguishable to ancient peoples). Based on this ancient knowledge, I hypothesized that *G. japonicus* was introduced to western Japan by at least the 900s AD, and its distribution expanded to eastern Japan relatively recently, after the 1700s AD. Specimens were then collected from various parts of the Japanese archipelago and China, and a population genetic analysis was performed using genotyping by sequencing

(GBS). The dispersal scenario estimated from population genetics was compared with the hypothesis based on ancient literature. Finally, the validity of my findings was verified from the viewpoint of human history. Through a combined approach of the humanities and biology, I have clarified the long-term effects of humans on the distribution of *G. japonicus*.

Materials & Methods

1. Scrutiny of ancient literature

Using the digital service of the National Diet Library of Japan (https://dl.ndl.go.jp/), in which many originals and manuscripts of ancient Japanese literature are archived, I have scrutinized dictionaries and academic books from various ages, including the most ancient. I comprehensively collected the descriptions of reptiles and amphibians available from these sources.

2. Sampling

From 2018 to 2021, 182 individuals of *G. japonicus* were collected from 37 sites in Japan and one in China (Fig. S3-1). Three other species of the genus, *Gekko* sp. (undescribed species called Nishiyamori in Japanese), *G. tawaensis*, and *G. shibatai*, were collected from three other sites in Japan as outgroups. The individual numbers and locations of the samples used in the analysis are summarized in Table S3-1. To avoid destructive sampling, only autotomized tail tissue was stored in 100% ethanol, and the individuals were released on the spot. It is worth remembering that the tail of the gecko regenerates over time.

3. Laboratory protocol

Total DNA was extracted from tissue pieces of the gecko tails collected according to the protocol using Nucleospin tissue (TaKaRa, Shiga Pref., Japan). The DNA was further subjected to RNase treatment. Then, double digest restricted-site associated DNA sequencing (ddRAD-seq) was conducted following the protocol (Peterson *et al.*, 2012). The library was prepared with 40 ng/µL of gDNA for each sample. Samples were digested with EcoRI, MspI, P1, and P2 adapters, and each fragment was ligated. I then pooled them at equimolar concentrations and purified them using the Nucleospin gDNA clean-up kit (TaKaRa). I then selected 300–500 base pair (bp) fragments using Pippin Prep (Saga Science, MA, USA). The size-selected DNA fragments were amplified in polymerase chain reactions (PCR) for eight cycles using Phusion

PCR reagents (New England Biolabs, MA, USA). Later, I cleaned up the reaction products and removed the PCR primers using the Nucleospin gDNA clean-up kit and Pippin Prep. The tuned ddRAD library was sequenced (150 bp paired-end) using Illumina HiSeqX (Illumina, CA, USA) paired-end sequencing at Macrogen, Japan.

4. De-novo assembly

Demultiplex was performed using ipyrad 0.9.14 (Eaton & Overcast, 2020) from the sequence, read and divided into individual reads. At this time, a barcode sequence mismatch was not allowed. Then, a section from the 3' end to 5 bp of each lead was trimmed as a quality control if the Qscore was lower than 33. The clustering threshold was set to 85%, the read was clustered for each individual, and a consensus sequence was created. The minimum number of depths allowed was six, and the maximum was 10,000. The ratio of an ambiguous site (N) to a heterozygous base arranged in the consensus sequence was 0.05. The number of raw reads obtained for each individual was unexpectedly large. Therefore, only one of the paired ends (R1) was used as a single end. From the consensus sequence created for each individual, clustering was performed with a threshold of 90% to generate a consensus sequence for the entire population. Finally, a sequence with 180/185 shared loci, a bp number by indel lower than five, and a maximum SNP number per locus lower than 0.2 were extracted from this sequence, and an analysis data set (Set.Phylogeny) was created. In addition, the following datasets were prepared for the other analyses. Set.Structure1 was made from only Japanese individuals, excluding three Chinese and three outgroup individuals with 176/182 shared loci. Set.Structure2 was created from individuals from the Shikoku, Kinki, Tokai, and Kanto regions of Japan, with 73/74 shared loci. Set.Divergence was created by excluding individuals that did not form a monophyletic group in the Kyushu region from Set.Phylogeny with 166/169 shared loci.

5. Phylogenetic analysis and population structure

A molecular phylogenetic tree based on the maximum likelihood method was created using iqtree 1.6.8 (Nguyen *et al.*, 2015) from the loci data of Set.Phylogeny. Model selection was performed based on Bayesian information criteria (BIC) using Model Finder Plus (Kalyaanamoorthy *et al.*, 2017), and the Ultrafast Bootstrap was set to 2000. I also calculated the posterior probabilities of each node based on the approximate Bayesian method. The genetic structure of the Japanese population was analyzed using ADMIXTURE (Alexander & Lange, 2011). The data of Set.Structure1 was formatted by PLINK (Purcell *et al.*, 2007) and was used for the analysis. The number of clusters (K) was set to 1–30. The data of the

Set.Structure2 dataset was used to clarify the more detailed population genetic structure in the Shikoku, Kinki, Tokai, and Kanto regions that formed one cluster in the higher-order structure. Then, the data were analyzed similarly using ADMIXTURE (the number of K was set to 1–15).

6. Demographic history and divergence time

The demographic history of each regional population was estimated using Stairway plot v2 (Liu & Fu, 2015). Stairway plot is a population dynamics estimation software similar to Extended Bayesian Skyline Plot (Heled & Drummond, 2008). However, it is performed based on the site frequency spectrum (SFS) and is known to provide a more accurate estimation of recent past dynamics than can be made compared to with the latter. First, the observed SFS values for each regional population were projected from the data of Set.Strucuture1 using easySFS (Isaac, 2022). Individuals were divided into the following 11 groups based on the phylogenetic and population structure analysis results and geographical divisions (partially different from administrative divisions), such as mountains and the sea. The considered regions were 1) Tohoku: Sakata (N = 15); 2) Kanto: Utsunomiya, Noda, Saitama, Tama, and Kawasaki (N = 15); 3) Tokai: Kofu, Kai, Atami, Fujinomiya, Shizuoka, Hamamatsu, and Nagoya (N = 31); 4) Hokuriku: Itoigawa, Takaoka, Kanazawa, Wajima, and Suzu (N = 39); 5) Kinki: Uji, Kyoto, Sayo, and Sakai (N = 16); 6) Chugoku: Izumo (N = 16); 7) Shikoku: Takamatsu, Mitoyo, Kochi, and Muroto (N = 12); 8) Kyushu-T: Nagasaki (only one individual, s158), Fukuoka, Tsushima, Uku, and Iki (N = 16); 9) Fukue Island (N = 6); 10) Kyushu-F: Other individuals of Kyushu 1 and Kyushu 2 (N = 13) and 11) China: Nanjing (N = 3).

A preliminary analysis using all individuals in Kyushu as a single population showed almost unchanged population dynamics. Thus, no bottleneck could be detected in Kyushu, even though other regional populations showed results similar to the true analyses shown in Fig. 3-3B–D. This is the reason why paraphyletic individuals in Kyushu 1 and Kyushu 2 clades with low numbers were excluded, and two monophyletic groups with enough individuals (Kyushu-T and Fukue Island, Fig. 3-2A) from the Kyushu 1 clade were considered as two regional populations for the true analysis. The number of projections was set to 2N-1. Among the parameters in the input file of Stairway plot, SFS (projected value), nseq (2N), and nrand were set at unique values for each regional population. Nrand was specified as four ranges (nseq-2)/4, (nseq-2)/2, 3*(nseq-2)/4, and (nseq-2), as recommended in the manual of Stairway plot software. All other parameters were set to the same values for all regional populations. Following a previous study (Nguyen *et al.*, 2019), the mutation rate values were based on those of *Cnemaspis* geckos (0.025% Myr-1). The exact generation time of *G. japonicus* is not known, but based on data from a closely related species, *G. hokouensis*, it is known that *G. japonicus* individuals take 1–2 years to become sexually mature (Okada, Izawa & Ota, 2002). Based on the above, the mutation rate for *G. japonicus* was converted to 1.5 years per generation (3.75e-8 per site per generation). All other parameters were left as defaults. Analyses were not performed for Kyushu-F, which was removed, or for China, which did not have sufficient populations.

Furthermore, divergence time estimation, based on coalescent theory, was performed from the SNP data of the Set.Divergence dataset using ABCtoolbox (Wegmann *et al.*, 2010). Fastsimcoal2 (fsc26; Excoffier, Foll & Barrett, 2011; Excoffier *et al.*, 2013) was used for the coalescent simulation, and arlsumstat (Excoffier & Lischer, 2010) was used to calculate the number of statistical genetic summaries for the simulated sequence. Fst and ϕ were used as the summary statistics. For the observed data, summary statistics were calculated using arlsumstat from the SNP data of the Set.Divergence dataset. Since the observed data were SNPs, minor allele frequency (MAF) was used as the parameter. As for the other parameters, the number of effective populations and the number of branched generations of each population were used. For each parameter used in the simulation, a non-information prior distribution was set, and random numbers were generated from the initial distribution for each number of simulations to perform the simulation (Table S3-2). In each scenario, 100,000 simulations were performed. Models were selected using the mnlogistic method. The model with the highest probability was adopted. Additionally, the posterior distribution of the parameters was estimated using the rejection method in the adopted scenario. The tolerance rate was set to 0.01 in both cases. The analysis was conducted using the "abc" package (Csilléry, François & Blum, 2012) in R (R Core Team, 2019).

Results

1. Scrutiny of ancient literature

Descriptions of lizards, including geckos, have been found in dozens of ancient documents of various ages, and the most significant examples are shown in Fig. 3-1. As shown in the descriptions in Fig. 3-1A, the Japanese name "tokage," which now means "skink," comes from the word for something that is behind a door and may originally have represented a gecko (Arai, 1717; Kariya, 1827). The word "tokage" had already appeared in the 10th century (Fig. 3-1B), suggesting that its existence has been known for a long time (Fukae, 918). I can see from the examples in Fig. 3-1C, 1D, and 1E that two lizards (skinks and geckos) and one amphibian (newts) were often confused in Japan before 1600 AD, and the notations differed in each document (Minamoto, 931; Sugawara, 1081; Tachibana, 1181; Unknown, 1400a; Unknown, 1400b). “Geckos” were called “yamori” around the 15th century (Unknown, 1603). Since then, the above three have been distinguished, as shown in Fig. 3-1F (Nakamura, 1666; Ono, 1803). On the other hand, it is suggested from Fig. 3-1G that geckos inhabit areas nearby Kyoto and Kyushu (western Japan) but have not been found in the Kanto region (eastern Japan) as of 1697 (Hirano, 1697). This past distribution is shown in Fig. 3-4C.

2. De-novo assembly, phylogenetic analysis and population structure

The average raw reads were Set.Phylogeny: 3360142, Set.Structure1: 3357559, Set.Structure2: 3182293, and Set.Divergence: 3286725. The respective total filtered loci of each dataset were 8464, 8174, 19462, and 8592. The number of single nucleotide polymorphisms (SNPs) was 66991 (2.82% missing sites), 29828 (2.37% missing sites), 50167 (4.15% missing sites), and 30477 (2.43% missing sites), respectively.

The phylogenetic tree, based on maximum likelihood and the approximate Bayesian method, is shown in Fig. 3-2A. Through Model Finder, TVM + F + R10 was selected as the replacement model with the smallest BIC. In most cases, individuals collected from the same site formed a monophyletic group. Looking at the phylogenetic relationships, the individuals in Nanjing, China, were the most ancestral but did not form an independent monophyletic group. The Kyushu population, excluding Beppu, was the eldest ancestor population of the Japanese archipelago. This clade was regarded as Kyushu 1. Only the Beppu population was phylogenetically far away from Kyushu 1, and that was regarded as Kyushu 2. Still, individuals from Kyushu 1 did also not form a single monophyletic group. Also, while the populations of remote islands, such as Fukue Island and Tsushima, were geographically differentiated from island to island, they were not so differentiated on the mainland of Kyushu. Although we could collect from only one site (Izumo) in the Chugoku region, all the individuals collected from this area formed a monophyletic group. Individuals from Kinki, Kanto, Tokai, and Shikoku formed one large clade. Individuals from the Hokuriku and Tohoku regions formed separate monophyly groups and were geographically differentiated. The support for the nodes in each regional clade, which are color-coded in Fig. 3-2A was Bayes posterior probabilities higher than 0.99 and Ultrafast bootstrap posterior probability (UFboot; Minh, Nguyen & Von Haeseler, 2013) higher than 95%.

The results of the ADMIXTURE analysis of the Japanese archipelago are shown in Fig. 3-2B. The lowest value of cross-validation error was at K = 5 (0.39297), and the next lowest values were at K = 4, 6, and 7 (0.39369, 0.39637, and 0.39403). Geographically differentiated genetic structures similar to those in the molecular phylogenetic analysis were found. However, at K = 5, the populations of the Shikoku, Kinki, Tokai, and Kanto regions were all grouped into one cluster. This geographical structure is shown in Fig. 3-2C. As a result of a detailed ADMIXTURE analysis in the Shikoku, Kinki, Tokai, and Kanto regions, K = 1 had the slightest cross-validation error (0.53364), and the next lowest values, as shown in Fig. 3-2D, were at K = 2 (0.53658). The western cluster is the area around the Kofu and Fujinomiya as the eastern end. On the other hand, the western end of the eastern cluster is around Kyoto. In the Tokai region, located between the Kanto and Kyoto regions, it was found that genetic populations were mixed on a gradient.

3. Demographic history and divergence time

The demographic histories of the populations from each region are shown in Fig. 3-3. All regional populations tended to recover effective population sizes after experiencing significant bottlenecks, as shown below—Fukue Island: 3000 years ago (Fig. 3- 3A), Kyushu: 2400 years ago (Fig. 3-3A), Chugoku: 2000 years ago (Fig. 3-3B), Shikoku: 2000 years ago (Fig. 3-3B), Kinki: 2000–800 years ago (Fig. 3-3C), Tokai: 900–300 years ago (Fig. 3-3C), Kanto: 220–100 years ago (Fig. 3-3C), Hokuriku: 600–500 years ago (Fig. 3-3D).

The observed summary statistics are listed in Table S3-3, and the posterior distributions for each parameter simulated with ABCtoolbox are in Table S3-4. Two scenarios (M1, M2) were simulated with ABCtoolbox, and the adopted scenario (M1) is shown in Fig. 3-3E. The rejected one (M2) is shown in Fig. S3-2. The most probable model selected with the multinomial logistic regression (mnlogistic) method was M1 (probability = 0.83). Model M2 (probability = 0.17), in which migration was considered, was rejected. Therefore, migration between regions was considered negligible in subsequent discussions. In model M1, the median age at which each regional group diverged from its ancestral group was as follows: as Nanjing-Fukue Island, 6506 generations; Fukue Island-Kyushu, 3486 generations; Kyushu-Chugoku, 1434 generations; Kyushu-Shikoku, 1380 generations; Shikoku-Kinki, 618.5 generations; Kinki-Hokuriku, 260 generations; Kinki-Tokai, 253 generations; Hokuriku-Tohoku, 100 generations; and Tokai-Kanto, 80 generations.

Discussion

1. Genetic structure in the Japanese archipelago

Previous studies based on mitochondrial DNA and microsatellite analyses have suggested that the Japanese populations of *G. japonicus* have low genetic diversity and poor geographic structure (Kim *et al.*, 2020b). On the other hand, China has more genetic diversity than Japan, even if limited to the east coast. This was explained by the fact that *G. japonicus* is native to China and exotic to Japan, and gene flow between populations may have been facilitated by the modern development of globalized transportation networks. Since *G. japonicus* is widely distributed in eastern China, genetic diversity within China may be quite high, but this is not clear at this time. Although it is a matter of concern that only one site in China is used in this study, it is highly likely that the population on the east coast of China, which is geographically closest to the Japanese archipelago, is a lineage close to the ancestral population.

On the other hand, my high-resolution analysis using genome-wide SNPs showed geographically differentiated genetic structures of *G. japonicus* in the Japanese archipelago. In this study, almost all habitats within the Japanese archipelago were covered, and the reliability of the results for the Japanese archipelago is considered to be high. So, the discrepancy with the results of the previous study is simply due to the resolution of the loci analyzed. As distribution tends to be restricted by temperature, some geckos are geographically isolated by barriers, such as mountain ranges, and genetically differentiated even between relatively close land areas (Nguyen *et al.*, 2019; Yan *et al.*, 2010). In the area of the Japanese archipelago considered here, most of the distribution of *G. japonicus* is in urban areas along the coast. The distribution of the species and its habitat in mountainous regions has not been confirmed (Kim *et al.*, 2020a). Seas and mountains are lined up at the boundaries of each regional group, as shown in Fig. 3-2C and D. Additionally, as the rejected model M2 of divergence time (Fig. 3-3F) shows, the degree of gene flow between each was minimal. Therefore, the pattern is similar to the precedents of other gecko species. The divergence of each regional population occurred hundreds to thousands of years ago, suggesting that the influence of gene flow due to the current transportation networks on the expansion of distribution may not be significant. In other words, although genetic exchanges among each regional population have been promoted by modern transport networks, their impact is thought to be minor at the genome-wide level. It is believed that these gene structures were formed by the founder effect, originating from individuals who accidentally human-mediately broke through geographical barriers and were introduced to each region.

2. Age of migration to the Japanese archipelago and dispersal history

The approximate ages of the bottlenecks of each population were roughly consistent with their divergence times. The demographic history of *Aedes albopictus*, which is a mosquito that is artificially expanding around the world, is similar to that found in this study. It is known that the individuals of this species experienced a steep bottleneck when they invaded each region (Sherpa *et al.*, 2019). Therefore, considering the bottleneck timing as the age of the introduction, the dispersal routes of *G. japonicus* in the Japanese archipelago are estimated with the divergence scenario (Fig. 3-4A and B). It is worth noting that the Tohoku population has experienced two steep bottlenecks in the past. Still, it is thought that the former event reflects the intrusion event into Hokuriku because the individuals from this region are phylogenetically closest to those from the Hokuriku population. Therefore, it is reasonable to think that the actual timing of introduction into Tohoku was the latter. In addition, despite the fact that populations derived from Kyushu in various parts of Japan must have experienced multiple bottlenecks, the dynamics before major bottlenecks were flat in all populations. This can be explained as follows. This estimation was performed based on the genetic information possessed by the current individual. However, much of the genetic information possessed by the ancestral population should have been lost in the most recent major bottleneck. Therefore, it is likely that the dynamics prior to the major bottleneck cannot be plotted in detail. However, the decreasing trend in the starting size of each regional population as one moves to a newer region suggests that the existence of multiple bottlenecks is present.

Based on Japanese human history, I verified whether the human-mediated introduction of *G. japonicus* in the above scenario was possible. An overview of major Japanese transportation networks from ancient to modern times is shown in Fig. 3-4D. The estimated times at which part of the source population in China was introduced to Fukue Island and Kyushu are reasonable. It was suggested that *G. japonicus* became viable in Kyushu about 8,000 years ago (Kim *et al.*, 2020b). The Goto Islands, including Fukue Island, are a volcanic archipelago, and the last eruption of the volcano on Fukue Island occurred between 2400 and 2300 years ago (Nagaoka & Furuyama, 2004). From the results of the demographic history, a pattern was observed in which the population of *G. japonicus* on Fukue Island immediately returned to its original state after a slight decrease in its effective population size during the approximate time of the eruption. This event, considering this dynamic as a temporary effect of the eruption, is a calibration point that strengthens the validity of the calculation method of the introduction age into each region. Located on the west side of Kyushu, the Goto Islands were gateways to biological exchanges with the continent. It may be that the geckos first invaded the Goto Islands a little earlier than they crossed over to the mainland of Kyushu. This era corresponds to the Jomon period (16000–3000 years ago) and the Yayoi period (3000–1700 years ago) in the classification of Japanese human history. Recent archaeological and genetic studies have suggested that rice cultivation had been propagated to Japan from Southeast Asia via China by at least the end of the Jomon period (about 3000 years ago; Shomura *et al.*, 2008). It is suggested that there were human exchanges between the Japanese archipelago and the continent during this period, and *G. japonicus* probably migrated to Kyushu during this time.

The introduction age to the Chugoku region and the Shikoku region corresponds to the middle to late Yayoi period in Japanese human history. Ancient Izumo in the Chugoku region prospered as a major force in Japan at that time, and many archaeological sites have been excavated in the area. Furthermore, ancient Izumo, as the Izumo mythology, appears in the oldest ancient Japanese documents, such as "Kojiki (Ohno, 712)" and "Nihon Shoki (Prince Toneri & Kodera, 720)". It is said that close exchanges with northern Kyushu through maritime traffic across the Sea of Japan were also related to social development in this region (Mizuno, 1975; Mori *et al.*, 1991; Matsuo *et al.*, 2005). Additionally, maritime traffic across the Seto Inland Sea (the inland sea surrounded by the Kyushu, Shikoku, Chugoku, and Kinki regions) from Kyushu to Kinki has developed since ancient times (Obayashi *et al.*, 1991). *G. japonicus* was able to disperse from Kyushu via multiple routes by taking advantage of the ancient development of local communities and cultural exchanges.

This species took a long time to achieve an introduction to the Kinki region, but what kind of insights on that introduction can be obtained from human history? It is said that there was a blank period with almost no records 200–400 AD. Still, trade and transportation continued through the Asuka (592–710 AD), Nara (710–794 AD), and Heian periods (794–1185 AD). These were all periods when the capital was located in the Kinki region. It is reasonable to assume, considering the divergence time estimation scenario, that *G. japonicus* was spread from Shikoku along with the aforementioned maritime traffic in the Seto Inland Sea and the development of human society.

G. japonicus seems to have further expanded into the Tokai region after the introduction to the Kinki region had been mostly achieved. A major arterial road called Tokaido was fully developed during this period (Honda, 2014). This was a land route that ran along the coast of the Tokai region and connected the Kinki and Kanto regions. At the end of 1100s AD, the Shogunate (military government) was established in Kamakura, within the Kanto region. This era was the Kamakura period (1185–1333 AD). At that time, the Tokaido road functioned as an artery connecting the Shogunate with the capital of the Kinki region. From a topographical point of view, there are few geographical barriers, such as large mountains between the Shikoku, Kinki, and Tokai regions. Consequently, slow natural dispersal was active in addition to human-mediated rapid dispersal. Continuous long-term dispersal events could have created a gradual bottleneck in these regions. By 300 years ago, the geckos had completed their expansion into the Tokai region. Still, individuals of the species seem to have been stopped by the mountains that separate the Tokai and Kanto regions (including Mt. Fuji, the highest peak in Japan). It was not until at least 220 years ago, at the end of the Edo period (1603–1868 AD), that the gecko was introduced to the Kanto region. This timeline is consistent with the description in "Honcho Shokkan," in which geckos did not inhabit the Kanto region as of 1697 AD. Then, how did they make their way into the Kanto region? Looking at demographic dynamics, the slope of the bottleneck has become steeper since about 150 years ago, during the Meiji period

(1868–1912 AD). The railway network began to be developed in Japan in the latter half of the 1800s AD. In 1889, the Tokaido Main Line was fully opened, operating a route similar to the Tokaido. Individuals of *G. japonicus* may have gained the ability to break through the geographical barrier presented by the high mountains by riding this modern pipeline connecting the Tokai and Kanto regions.

The age when individuals of *G. japonicus* were introduced to the Hokuriku region from the Kinki region by a route different from their spread to the Tokai and Kanto regions was the so-called "Warring States Period" (1467–1590 AD) in Japanese history. During that time, wars broke out all over the country. However, this period was also when the movement and distribution of people became active, and the monetary economy developed (Miyahara *et al.*, 2012). Possibly, individuals of *G. japonicus* dispersed in the Hokuriku region with the development of the logistics network between the Middle Ages and the early modern period. Subsequently, these individuals reached Sakata in the Tohoku region. A sea route called "Nishimawari-Kaiun" was established in 1672 AD—merchant ships sailed westward along the coast, stopping by port towns from the Kanto to the Tohoku regions. Sakata, one of the port towns that prospered due to the development of this route (Izuta, 1979), functioned as a key route point in the advancement of *G. japonicus* from the Hokuriku region.

Conclusion

G. japonicus migrated from China to Kyushu before prehistoric times, spreading from west to east and south to north in the Japanese archipelago for thousands of years. This becomes clear through an approach in which human history and the natural sciences are integrated. There were many similarities between the spread of *G. japonicus* and the historical development of Japanese society, suggesting that ancient human–organism interactions are an essential factor in understanding the present distribution of these organisms. The past impact on ecosystems by humans, which has often been overlooked, is more significant than previously imagined. It is hoped that organisms worldwide will be examined from various perspectives to understand the mutual history of humans and nature.

Figures

Fig. 3-1 Ancient Japanese literature with a description of lizards. The highlighted descriptions (white) are outlined in the text. (A) Shakuchu Wamyo Ruiju Sho, a document with an annotated dictionary published in the 900s AD. (B) Honzo Wamyo, the oldest Japanese pharmacology dictionary. (C)(D)(E) Ruiju Myogi Sho, Iroha Jirui Sho, and Setsu Yo Shu, are Chinese-Japanese dictionaries from ancient to medieval times. (F) Kinmo Zui, an encyclopedia with pictures published in the early modern period. (G)Honcho Shokkan, a medical book published in the early modern period.

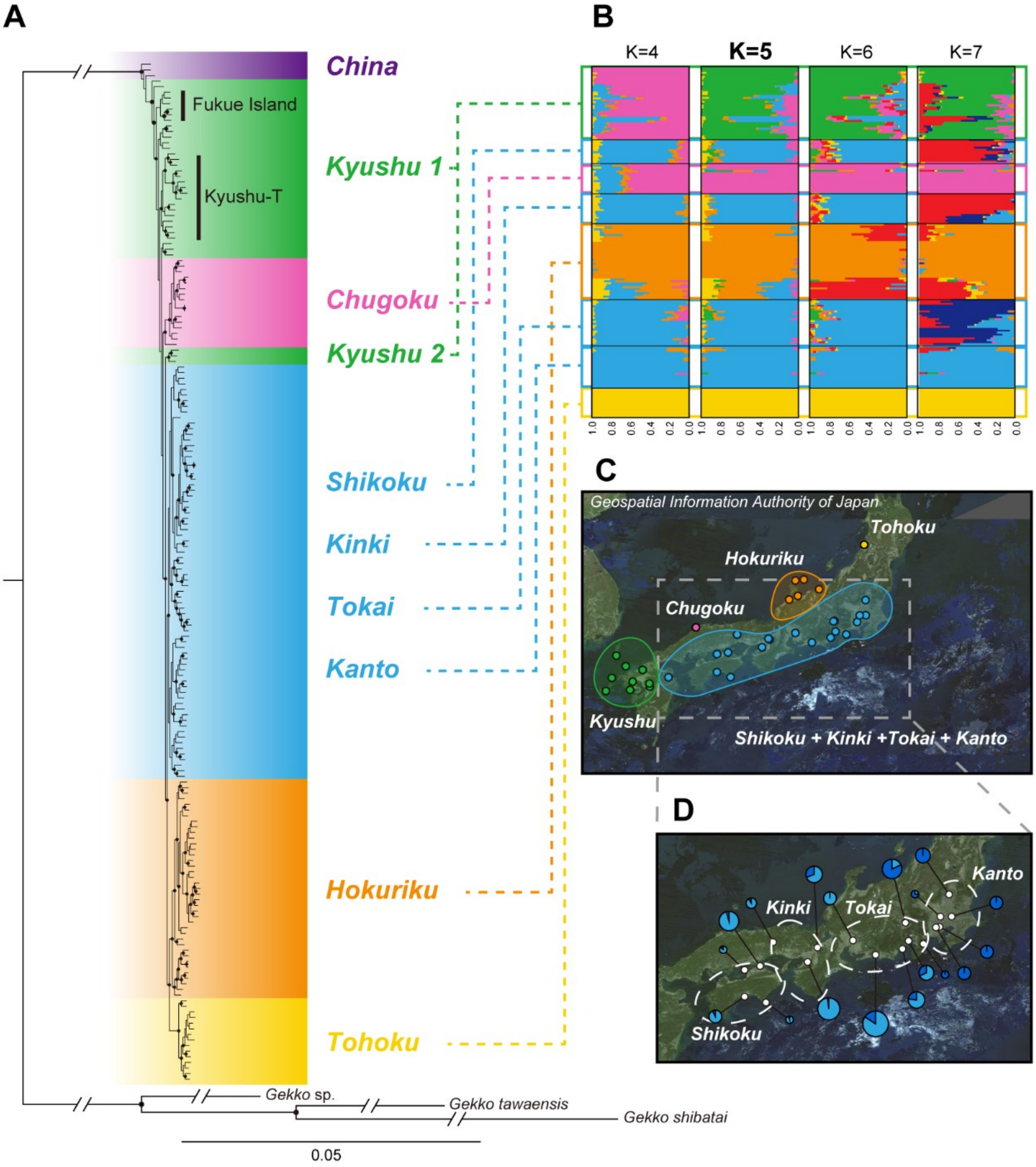

Fig. 3-2 Phylogeographic information of *G. japonicus*. (A) A molecular phylogenetic tree based on the ML method and the approximate Bayesian method. Nodes with a closed circle represent Bayes posterior probabilities higher than 0.99 and UFboot higher than 95%. The Fukue Island and Kyushu-T clades are monophyletic groups that were considered separately in the divergence time and demographic history analyses. (B) Genetic structure of the Japanese populations of *G. japonicus*. Each bar on the horizontal axis represents an individual, and the vertical axis represents the probability that an individual belongs to each cluster (each color). The lowest observed cross-validation error was K = 5. (C) Mapped geographical genetic structure of the Japanese population. (D) Geographical genetic structure of the Shikoku, Kinki, Tokai, and Kanto regions when K = 2. The breakdown of the pie chart represents the probability that an individual belongs to each cluster. The diameter of the pie chart represents the number of individuals at each site.

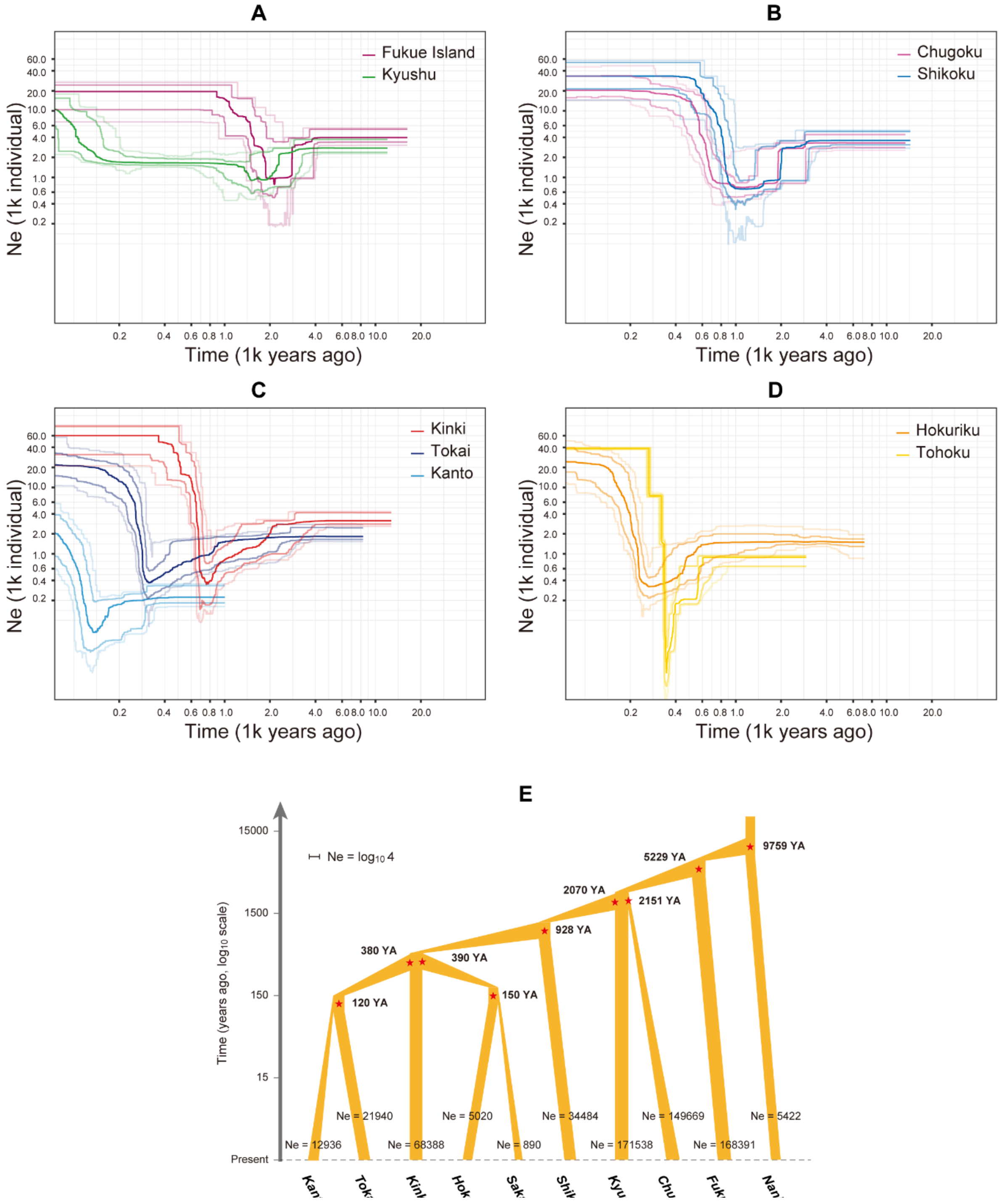

Fig. 3-3 The simulated scenario of the demographic history and divergence time of *G. japonicus*. (A)(B)(C)(D) Demographic history of each regional population. The horizontal axis is the reverse-direction age (1,000 years ago) on the log10 scale. The vertical axis is the number of effective populations (Ne) on the log10 scale. The most apparent lines represent the median population size for the region, and the slightly transparent lines of the same color represent 75% and 95% CI. (E) The adopted scenario, M1. The vertical axis is the reverse direction age (years ago, YA) on the log10 scale. The thickness of each population is the number of effective populations on the log10 scale. The divergence time next to the node, with the star mark and the Ne of each population, represents the median of the estimated range.

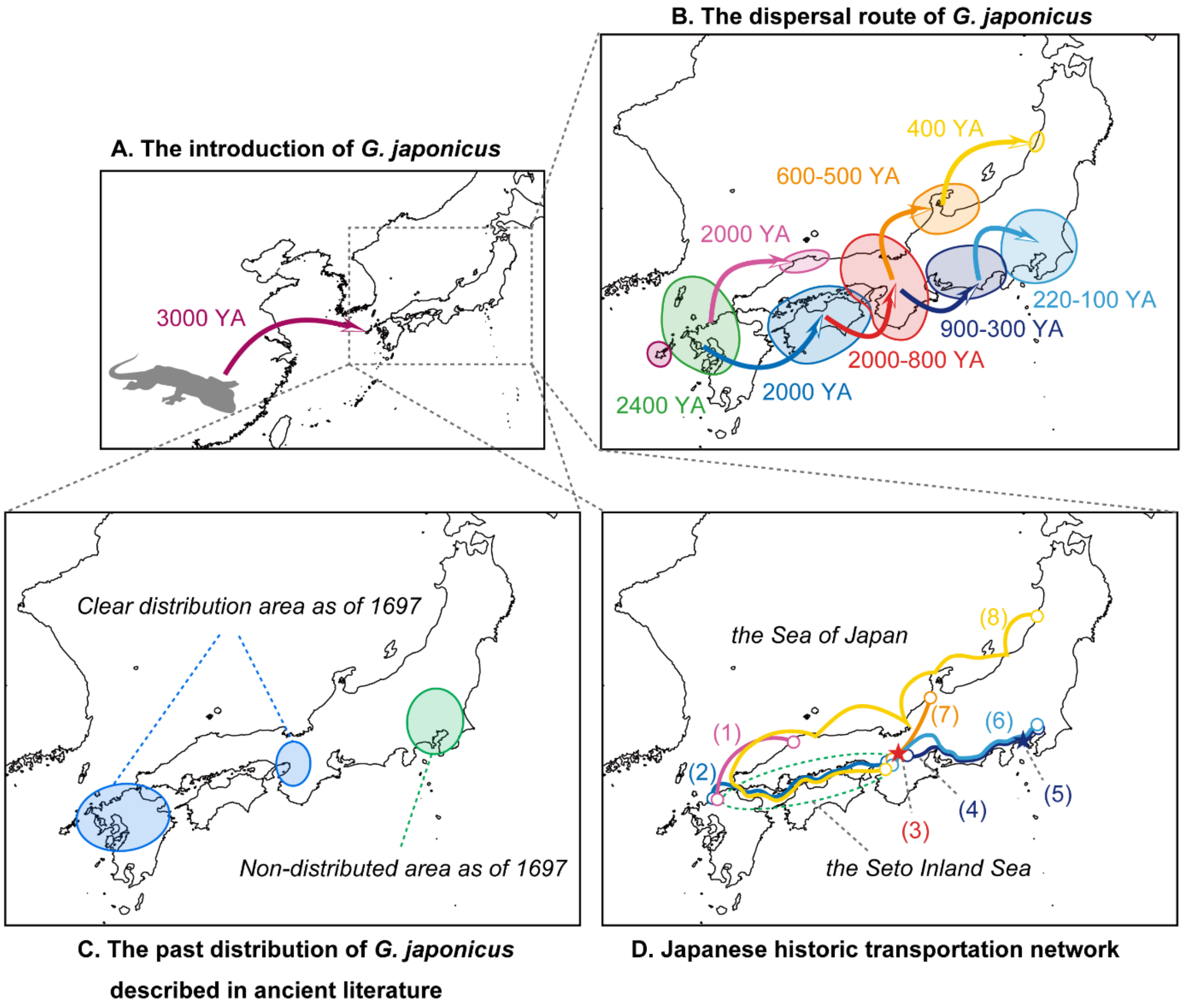

Fig. 3-4 The mutual history of *G. japonicus* and humans. (A) The introduction age of *G. japonicus* from China. (B) The dispersal history of *G. japonicus* was estimated based on the timing at which each regional group experienced a bottleneck. (C) The past distribution of G. japonicus described in ancient literature. (D) An overview of the Japanese historic transportation network. (1)(2) The ancient sea routes of about 2000 years ago. (3) Heiankyo, an ancient capital established in 794 AD. (4) Tokaido, the old arterial road that flourished in the Middle Ages. (5) Kamakura Shogunate, the military government established in 1185 AD. (6) Tokaido Main Line, a railway that opened in 1889 AD. (7) A part of the national transportation network created with the development of the monetary economy in the late Middle Ages. (8) Nishimawari-Kaiun, a merchant ship route that orbits the Sea of Japan and was established in 1672 AD.

Chapter 4:
Hidden invasiveness of nonnative geckos and three-way competition among native and nonnatives in Japan

Introduction

The adage "History is written by the victors" suggests that historical narratives often reflect the perspectives of the winners, potentially overlooking the experiences of the defeated and marginalized, and thereby distorting our understanding of true historical events. This principle extends beyond human endeavors, influencing natural history narratives shaped by the interactions of diverse organisms. The stories of ecological victors may obscure our comprehension of past competitions, leading to misconceptions in scientific knowledge.

Reassessing the impact of naturalized species, those established in new environments for extended periods, is crucial to understanding their true effects on ecosystems. The global expansion of numerous species via human-mediated dispersal has been well-documented (Wilson *et al.*, 2009; Gippet *et al.*, 2019). Yet, research often focuses on recently identified invasive species (e.g. Meyerson & Mooney, 2007; Early *et al.*, 2016; Pyšek *et al.*, 2020). neglecting older introductions presumed non-invasive, possibly due to their perceived lower relevance to current conservation efforts. However, the ongoing displacement of native species by invasive counterparts challenges the benign nature of these ancient introductions. Early invaders, now seen as harmless, may have once been invasive, exerting unnoticed, yet significant, impacts on native ecosystems. The obscured history of these interactions suggests that the ecological 'losers'—significantly impacted native species—might have been silently excluded from our understanding, leading to a modern underestimation of the invasiveness of anciently introduced species.

Schlegel's Japanese gecko, *Gekko japonicus* (Reptilia: Squamata: Gekkonidae) is widely distributed in eastern China, South Korea, and south of the Tohoku region within the Japanese archipelago (Kim *et al.*, 2020a). It is the most common gecko in Japan, long regarded as a native species. It is a familiar organism that prefers to use human dwellings (Kim *et al.*, 2018), and they have deep cultural relationships with the Japanese people. For example, geckos are called "yamori" in Japanese, which means a house guardian. *G. japonicus* even attracts religious belief because it sticks to the walls of human dwellings and appears to protect them. However, it has been revealed that *G. japonicus* is actually an ancient nonnative species that was introduced from China about 3,000 years ago and spread throughout the Japanese archipelago over several thousand years (Chapter 3; Chiba *et al.*, 2022). Nonetheless, few studies are showing its potential invasiveness (Toda *et al.*, 2006), *G. japonicus* is not widely recognized as a harmful invader in the Japanese ecosystem.

However, several other *Gekko* species inhabit the Japanese archipelago, and their distribution areas of four of these species intersect or overlap with that of *G. japonicus*. Among these, *G. tawaensis*, *G. yakuensis*, and *Gekko* sp. (academically undescribed, known as Nishiyamori in Japanese) are considered to be endemic and native to the Japanese archipelago, but their current distribution areas are fragmented and localized (Toda *et al.*, 2003; Okamoto, Tominaga & Toda, 2023). Another species of the same genus, *G. hokouensis*, also inhabits Kyushu and the Nansei Islands in Japan, southeastern China, and Taiwan (Zhou *et al.*, 1982), which has the second widest distribution area in the Japanese archipelago after *G. japonicus*. However, the Japanese population of *G. hokouensis* is genetically distinct from its continental counterparts, suggesting it might be a native cryptic species (Chapter 2; Toda *et al.*, 1997; Toda *et al.*, 2001). This implies that all geckos coexisting with *G. japonicus* and potentially competing with it are likely inhabited Japan before the introduction of *G. japonicus*. If the naturalized *G. japonicus* indeed has have a negative impact on these native geckos as an invader, it would be desirable to formulate a new conservation strategy based on this.

The *Gekko* species, colloquially known as Nishiyamori in Japanese, remains undescribed since its initial discovery in 1986 (Matsuo, Ejima & Matsunaga, 1988). This species is uniquely found along the western coast of Kyushu and its adjacent remote islands, classifying it as endemic to Japan. It primarily inhabits natural settings such as rocky coastal areas, although there are sporadic reports of it dwelling in human-made structures near these locales; these instances, however, lack comprehensive documentation (Matsui & Mori, 2021). Most of its distribution, with the exception of uninhabited islands, coincides with that of *G. japonicus*. Until recently, the Koshikishima Islands in southern Kyushu were the only populated islands believed to be free from *G. japonicus* colonization. A solitary sighting of *G. japonicus* in 2016 was considered accidental, suggesting no established colonization had occurred (Okamoto *et al.*, 2017). This led to the presumption that the Koshikishima Islands were free from interspecific competition, unlike other regions such as the Goto Islands and Hiradojima Island, where competition with *G. japonicus* was evident.

Recent preliminary research, however, has revealed that *G. japonicus* now predominates in a village in the northern Koshikishima Islands. Additionally, *G. hokouensis*, another species native to Japan, has been identified for the first time in this area, dominating two other villages in the northern Koshikishima Islands. Initially classified as a subspecies of *G. japonicus* in 1928, *G. hokouensis* was reclassified as a distinct species in 1982 (Zhou *et al.*, 1982). Its distribution primarily encompasses Kagoshima Prefecture in Kyushu and the Nansei Islands (Toda *et al.*, 1997; Okamoto *et al.*, 2023). Furthermore, there have been instances of *G. hokouensis* being introduced to regions well beyond Kyushu, such as parts of Shikoku and the Izu Islands in recent years (Ota *et al.*, 1995; Chapter 2; Chiba *et al.*, 2024). Consequently, this necessitates a reevaluation of the Koshikishima Islands' status, suggesting that only the southern part may remain free from competition, while other regions inhabited by *Gekko* sp., including the Goto Islands, Hiradojima Island, and now the northern Koshikishima Islands, face interspecies competition.

In this research, I posited that the potential suitable habitats for each endemic gecko species extend beyond their current geographical ranges, and that the encroachment of *G. japonicus* has led to their distributions becoming more localized. To explore this hypothesis, I conducted a comparative analysis of the population genetics and ecological dynamics of native geckos in regions where non-native geckos have been introduced—effectively serving as experimental treatment zones in a large-scale field study—and in regions without such introductions, which acted as control zones. Specifically, we hypothesized that in locales where *G. japonicus* has been introduced, the invasion fragmented the realized niche of *Gekko* sp., leading to a notable reduction in population size (a bottleneck event) following their introduction over 3,000 years ago. Conversely, in areas free from competition, I surmised that *Gekko* sp. occupies a broad range of available realized niches, avoiding any bottleneck events throughout these 3,000 years (refer to Fig. 4-1). Given the challenge of directly observing historical events, distinguishing between correlation and causation in past occurrences is complex. Nevertheless, by systematically evaluating each hypothetical scenario through a comprehensive, multifaceted approach, I aim to uncover plausible insights into the long-term interspecific competition history that has shaped current biodiversity patterns.

Materials & Methods

1. Sampling

Sampling took place across Kyushu from spring 2022 to summer 2023, as depicted in Fig. 4-2. Details regarding the number and locations of samples utilized in this analysis are provided in Table S4-1. To minimize harm, we collected only autotomized tail tissues, preserving them in 100% ethanol, and subsequently released the geckos at their site of capture. It's pertinent to mention that gecko tails regenerate over time. All fieldwork was conducted at night by the same researcher, aligning with the geckos' active period. I meticulously recorded the coordinates of each finding along with the type of substrate, which was categorized as either artificial (including concrete, mortar, wooden, or metal structures) or natural (such as rock, leaves, or natural wood). Although data on gecko hiding spots during daytime were gathered, this information contributed solely to modeling suitable habitats and was excluded from microhabitat usage analysis, which relied strictly on observations of geckos active at night.

2. Suitable habitat modeling for three gecko species

The potential suitable habitats for three gecko species were modeled using MaxEnt (Phillips *et al.*, 2006), building on prior analyses that covered broad areas in Japan and China for *G. japonicus* (Kim *et al.*, 2020a; Kim *et al.*, 2020b). Recent studies, however, have pointed out that *G. japonicus* may exhibit regional adaptations to temperature variations (Li *et al.*, 2022), suggesting that broader modeling might not fully capture localized habitat suitability. Therefore, we refined the habitat suitability model for *G. japonicus* using presence data exclusively from Kyushu, incorporating findings from both previous research (Kim *et al.*, 2020b) and the current study. A kernel density estimation (Parzen, 1962) based on presence data provided the background for density distribution, and pseudo-absence data were generated for 2000 locations randomly.

I selected climate variables—Annual Mean Temperature (Bio 1), Temperature Seasonality (Bio 4), and Annual Precipitation (Bio 12) from World Clim v1.4 (https://www.worldclim.org/)—along with human demographic data from Japan's 2020 National Census (https://www.e-stat.go.jp/gis) as an indicator of urbanization. These environmental variables were mapped onto a 250m square mesh grid, ensuring all combinations were uncorrelated ($-0.7 <$ Pearson's $r < 0.7$, $p < 0.05$) to avoid multicollinearity. The model incorporated categorical variables through various encodings: Logit, Logit-Quadratic, Logit-Quadratic-Hermite, and Hermite transformations, creating 20 models with polynomial degrees from first to fifth. The Complementary Log-Log (cloglog) function served as the link function. The model selection was based on the lowest Akaike Information Criterion corrected (AICc; delta AICc = 0) among the 20 models. To validate the model, we conducted 4-fold cross-validation using 75% of the data for training and the remaining 25% for testing, assessing model accuracy with the Area Under the Curve (AUC) metric. GIS data engineering was performed with QGIS 3.28.1, and MaxEnt modeling was executed in R 4.1.2 (R Core Team, 2019).

For *Gekko* sp., presence data from this study's sampling sites were utilized. Given its localized current distribution, I initially modeled suitable habitats for the Koshikishima Islands, Goto Islands, and Hiradojima Island, then expanded the model across Kyushu to mitigate the bias from extensive pseudo-absence areas. The Kyushu-wide model resulted in a lower AUC value (0.795) compared to the more localized approach. The method for generating pseudo-absence data for *Gekko* sp. mirrored that used for *G. japonicus*, with identical modeling parameters. For *G. hokouensis*, presence data from this study were used, generating pseudo-absence data for 2000 locations under the same conditions as for *G. japonicus*.

The suitability models for the habitats of the three gecko species were comparatively analyzed using the modOverlap function from the R package “FuzzySim” (Barbosa, 2015). This analysis determined the extent of habitat overlap among the species by employing Schoener's D (Schoener, 1968) and the Hellinger Distance (Vaart, 1998). Schoener's D values span from 0 to 1, where values nearing 1 denote a higher degree of habitat overlap between two species. On the other hand, Hellinger Distance values range from 0 to 2, with values approaching 0 indicating a greater similarity between two probability distributions.

To assess the temporal shifts in suitable habitats for *Gekko* sp. and *G. hokouensis*, we projected past habitats using climate data (bio1, bio4, and bio12 from World Clim) from 6,000 and 22,000 years ago. Owing to the absence of historical human population data, the models for current suitable habitats were recalculated for these historical periods using only the bio1, bio4, and bio12 variables, thus excluding human demographic influences. These projections were extended to the two aforementioned time points, maintaining the same conditions and methodologies as outlined in the previous section. It’s noteworthy that modeling for *G. japonicus*' past suitable habitat was omitted due to its absence from the Japanese archipelago 6,000 years ago.

3. Microhabitat use comparison for three gecko species

This study investigated the shifts in microhabitat utilization among three gecko species, focusing on the impact of interspecific competition presence or absence on these shifts. Initially, the collection points for each gecko species were mapped onto a human population mesh (250m square) as described previously. This mesh was categorized into urban (populated areas) and non-urban (unpopulated areas) segments. The substrates identified during the sampling were labeled with urban or non-urban attributes, creating a four-category variable: Urban_artificial, Urban_natural, Non-urban_artificial, and Non-urban_natural. The locations where geckos were collected were further delineated into areas of competition and areas free from competition, based on the gathered data. Specifically, all regions were deemed competitive for *G. japonicus*. For *Gekko* sp., the population on Shimokoshikishima Island was considered to be in a competition-free zone, whereas other locations (the Goto Islands, Hiradojima Island, Kamikoshikishima Island, and Nakakoshikishima Island) were categorized as competitive zones. For *G. hokouensis*, Ibusuki's population was identified as competition-free, with the remaining areas (the Goto Islands, Kamikoshikishima Island, and Nakakoshikishima Island) viewed as competitive zones.

The study then conducted pairwise comparisons of microhabitat use among *G. japonicus* (in competitive areas), *Gekko* sp. (in competitive areas), *Gekko* sp. (in competition-free areas), *G. hokouensis* (in competitive areas), and *G. hokouensis* (in competition-free areas) using Fisher's exact test. This analysis was performed with the R package "RVAideMemoire" (Hervé & Hervé, 2020), and *p*-values were adjusted using the Holm method to account for multiple comparisons.

4. Laboratory protocol

Total DNA was extracted from the tail muscle tissue of each gecko according to the protocol using Nucleospin tissue (TaKaRa, Shiga Pref., Japan). RNA in the extract was degraded by RNase treatment. Double digest restricted-site associated DNA sequencing (ddRAD-seq) was then conducted following protocol (Peterson *et al.*, 2012) twice. The first round of sequencing in 2022 followed a protocol similar to the previous study (Chapter 3; Chiba *et al.*, 2022) for *Gekko* sp. samples. In 2023, due to the discontinuation of the Illumina HiSeqX service (Illumina, CA, USA), the protocol was modified. The library was prepared with 80 ng/µL of gDNA for each sample. Samples were digested with EcoRI and MspI. P1 and P2 adapters and each fragment were ligated. They were pooled at equimolar concentrations and purified using the Nucleospin gDNA clean-up kit (TaKaRa). 300 to 626 base pair (bp) fragments were selected using Pippin Prep (Saga Science, MA, USA). The size-selected DNA fragments were amplified in polymerase chain reactions (PCR) for eight cycles using Phusion PCR reagents (New England Biolabs, MA, USA). Later, the reaction products were cleaned up, and the PCR primers were removed using the Nucleospin gDNA clean-up kit and Pippin Prep. The tuned ddRAD library was sequenced (150 bp paired-end) using DNBSEQ G-400 ((MGI Tech Co Ltd.) paired-end sequencing at BGI Japan (Hyogo Pref., Japan).

Additionally, mitochondrial DNA sequences from various *G. hokouensis* samples were sequenced to facilitate comparisons with reference genomes from other regions and to explore the origins of *G. hokouensis* populations introduced to the Goto Islands and the Koshikishima Islands. Sequencing covered the mitochondrial region from 12S rRNA to 16S rRNA, including tRNA V, using the same primers and protocol as detailed in Chapter 2 and Chiba *et al.* (2024).

5. De-novo assembly

Demultiplexing of each library was conducted using ipyrad version 0.9.14 (Eaton & Overcast, 2020), which facilitated the separation of sequences into individual reads without permitting barcode sequence mismatches. In the quality control process, sequences with a Qscore below 33 underwent trimming from the 3' end to 5 base pairs (bp) into each lead. Reads for each individual were clustered with an 85% similarity threshold to create a consensus sequence. The protocol allowed for a maximum read depth of 10,000 and set a minimum depth threshold of 6. The ratio of ambiguous sites (N) to heterozygous bases in the consensus sequence was capped at 0.05. Due to the unexpectedly high volume of raw reads obtained for each individual, only one side of the paired-end reads (R1) was utilized as a single end in the analysis. The 'branch' feature of ipyrad was employed to generate multiple datasets with varying sample configurations, which were intended for use in further analyses. The details of these datasets, including the rate of shared loci for each, were presented in Table S4-2. Subsequent sections detail the independent analyses conducted for *Gekko* sp. and *G. hokouensis* using the same methodology. The raw sequence data were submitted to the DDBJ Sequence Read Archive (https://www.ddbj.nig.ac.jp/dra/index.html), with submission currently in progress.

6. Phylogenetic analysis and population structure

Two molecular phylogenetic trees were constructed utilizing the maximum likelihood approach through iqtree version 1.6.8 (Nguyen *et al.*, 2015), focusing on datasets specifically prepared for *Gekko* sp. (Set.Phy_S) and *G. hokouensis* (Set.Phy_H). This approach was chosen in light of the incomplete understanding of the phylogenetic relationships among the *Gekko* species native to Japan, thereby necessitating separate analyses for *Gekko* sp. and *G. hokouensis* to elucidate their intra-species phylogenetic relationships. In both instances, an individual of *G. japonicus* was incorporated as an outgroup to provide phylogenetic context.

Model Finder Plus (Kalyaanamoorthy *et al.*, 2017) was employed to identify the optimal model based on the Akaike Information Criterion (AIC). Subsequently, the Ultrafast Bootstrap (UFBoot; Minh *et al.*, 2013) method was applied 2,000 times to assess the robustness of the tree branches. The posterior probabilities of each phylogenetic node were estimated using an approximate Bayesian computation method. Additionally, a molecular phylogenetic tree focusing on mitochondrial DNA from *G. hokouensis* was constructed using the identical methodology, incorporating sequences of *G. hokouensis* from various regions available on GenBank. This analysis aimed to provide further insight into the genetic diversity and phylogenetic positioning of *G. hokouensis* across its range.

To visualize the genetic structure of *Gekko* sp. and *G. hokouensis*, the ADMIXTURE software (Alexander &

Lange, 2011) was utilized. The datasets designated for *Gekko* sp. (Set.Admix_S) and *G. hokouensis* (Set.Admix_H) were processed with PLINK (Purcell *et al.*, 2007) to prepare them for this analysis. The exploration of the number of genetic clusters (K) ranged from 1 to 10, allowing for the identification of potential subpopulations within each species based on their genetic makeup.

7. Demographic history and divergence time

Stairway Plot v2 (Liu & Fu, 2015) was utilized to infer the demographic histories of each regional population of geckos. Initially, the site frequency spectrum (SFS) for each regional population was derived from the Set.Admix_S and Set.Admix_H datasets using easySFS (Isaac, 2022). Based on the outcomes of the phylogenetic tree and population structure analyses, individuals of *Gekko* sp. were categorized into three regional populations: Goto + Hirado (N = 18), Northern Koshiki (N = 29), and Southern Koshiki (N = 23). In parallel, individuals of *G. hokouensis* were distributed into five groups: Ibusuki (N = 11), Goto (N = 20), Northern Koshiki (N = 4), Central Koshiki (N = 9), and the Nansei Islands (N = 5), as specified in Table S4-2.

For the input file of Stairway Plot, distinct values were designated for SFS (projected value), nseq (2N - the sample size doubled to represent alleles), and nrand (number of random samples) for each regional population. The nrand parameter was segmented into four intervals—(nseq-2)/4, (nseq-2)/2, 3*(nseq-2)/4, and (nseq-2)—adhering to the guidelines provided in the software's manual. A mutation rate of 3.75e-8 per site per generation was applied, as documented in several references (Chapter 3; Chiba *et al.*, 2022; Nguyen *et al.*, 2019; Okada *et al.*, 2002), with all other parameters set to their default. Due to the limited number of samples, demographic analyses for the Northern Koshiki and Nansei Islands populations of *G. hokouensis* were not conducted.

Divergence times for regional populations of *Gekko* sp. were estimated using coalescent theory applied to the Set.Admix_S dataset via ABCtoolbox (Wegmann *et al.*, 2010). The individuals were categorized into the same three regional populations identified in their demographic histories. Due to the likely recent introduction of *G. hokouensis* and the small sample size from the Koshikishima Islands and its ancestral population, simulations for *G. hokouensis* could not be conducted effectively. Coalescent simulations were carried out with Fastsimcoal2 (fsc26; Excoffier *et al.*, 2011). Genetic summary statistics, including Fst and ϕ, were derived from these simulations using arlsumstat (Excoffier & Lischer, 2010). For the observed data, summary statistics were similarly calculated from the SNP data within the Set.Admix_S dataset, utilizing minor allele frequency (MAF) among other parameters. These parameters also encompassed the effective population size and the number of generations since divergence for each population group. A noninformative prior distribution was designated for each parameter, as detailed in Table S4-3, from which random numbers for each simulation count were generated. A comprehensive series of 100,000 simulations was conducted for three distinct scenarios (M1, M2, and M3). The identification of the most likely model ensued via the multinomial logistic regression technique (Böhning, 1992). Following this, the posterior distribution of the parameters within the chosen scenario was calculated using the rejection method, adhering to a tolerance level of 0.01. These analyses were conducted using the R package "abc" (Csilléry, François & Blum, 2012).

Results

1. Distribution

Fig. 4-3 illustrates the specific locations where each gecko species was collected or observed within the study region. In areas where *G. japonicus* was introduced (Fig. 4-3A), on Fukuejima Island in the Goto Islands, *G. japonicus* was the sole species found in man-made environments, with an absence of other gecko species. Conversely, on Hisakajima Island, also in the Goto Islands, another species (*Gekko* sp.) was exclusively observed in man-made settings and along the coastline, with no encounters of other gecko species. On Nakadorijima Island, within the Goto Islands, all three species (*G. japonicus*, *Gekko* sp., and *G. hokouensis*) were documented. However, within each human settlement, demarcated by mountains, a single species predominated, and it was uncommon for multiple gecko species to inhabit the same area. Along the coast and in the mountains, either *Gekko* sp. or *G. hokouensis* was dominant, with both species rarely observed together. On Hiradojima Island, only *G. japonicus* was found within human settlements, whereas *Gekko* sp. was identified in man-made areas along the coast and in the mountains. In Kyushu's mainland, urban areas in southern Kagoshima prefecture (Ibusuki) predominantly hosted *G. hokouensis*, while *G. japonicus* was more common in urban locales of northwestern Kagoshima prefecture.

In the Koshikishima Islands, previously considered a competition-free zone (Fig. 4-3B), a village on Kamikoshikishima Island (Segami) was found to be already dominated by *G. japonicus*, contradicting earlier beliefs of its absence in the area. Furthermore, in another village on Nakakoshikishima Island (Taira), *G. hokouensis* was observed to be dominant, marking its first documented presence on the Koshikishima Islands. A few individuals of *Gekko* sp. were collected only near the port and coastline on the outskirts of the village. In the most urbanized area near the harbor on

Kamikoshikishima Island (Sato), man-made environments were predominantly occupied by *G. hokouensis*, while natural settings were chiefly inhabited by *Gekko* sp. Outside these three villages, the broader Koshikishima Islands, encompassing villages, coasts, and mountains, were mainly inhabited by *Gekko* sp., with no detection of other gecko species.

2. Suitable habitat modeling for three gecko species

Fig. 4-4 presents the estimated suitable habitats for the three gecko species, incorporating the total human population mesh. The Area Under the Curve (AUC) value and the percentage contribution of the selected model are summarized in Table 4-1. For *G. japonicus* and *Gekko* sp., human demographics were the most significant contributing environmental variables, at 71.3% and 63.2%, respectively. In contrast, for *G. hokouensis*, Temperature Seasonality (Bio 4) was the predominant factor (52.6%), followed by human demographics (47.4%).

The model overlap test results, using Schoener's D and Hellinger Distance, were as follows: *G. japonicus*-*Gekko* sp.: 0.80/0.28, *G. japonicus*-*G. hokouensis*: 0.80/0.25, and *Gekko* sp.-*G. hokouensis*: 0.70/0.39. These findings suggest that the suitable habitats of the three gecko species generally overlap, with *G. japonicus*, in particular, sharing a highly similar suitable habitat with both *Gekko* sp. and *G. hokouensis*. However, the overlap between the suitable habitats of *Gekko* sp. and *G. hokouensis* was relatively lower compared to that with *G. japonicus*.

Temporal changes in suitable habitats are depicted in Fig. S4-1. The current model, which includes the total human population mesh, exhibited higher AUC values, and thus, it was the sole model employed in this analysis. Consistent with previous research on *G. japonicus*, the study found that the suitable habitats for *Gekko* sp. and *G. hokouensis*, which had significantly contracted during the last ice age, are now expanding as the climate warms.

3. Microhabitat use comparison for three gecko species

Fig. 4-5 displays the ratios of microhabitat use by three gecko species (based on raw data) in both competitive and competition-free areas, along with the outcomes of pairwise comparisons using Fisher's exact test. A significant difference at the $p < 0.05$ level was observed in the use of Urban_artificial versus Non-urban_artificial environments among the following pairs: *G. japonicus* versus *Gekko* sp. (in competitive areas), *Gekko* sp. (in competitive areas) versus *Gekko* sp. (in competition-free areas), *Gekko* sp. (in competitive areas) versus *G. hokouensis* (in competitive areas), and *Gekko* sp. (in competitive areas) versus *G. hokouensis* (in competition-free areas). Specifically, in competition-free areas, *Gekko* sp. showed a preference for Urban_artificial environments, similar to the other two species. However, when competing with other gecko species, *Gekko* sp. significantly increased its use of Non-urban_artificial environments.

Furthermore, while not statistically significant, differences were also noted between Urban_artificial and Urban_natural for the *G. japonicus* versus *Gekko* sp. (in competitive areas) pair ($p = 0.09$), Urban_artificial and Non-urban_natural for the *Gekko* sp. (competitive area) versus *Gekko* sp. (competition-free area) pair ($p = 0.29$), and Urban_artificial and Urban_natural for the *Gekko* sp. (competitive area) versus *Gekko* sp. (competition-free area) pair ($p = 0.08$). These results suggest that in competition-free zones, *Gekko* sp. tends to avoid natural environments, regardless of urban or non-urban settings. Conversely, in competitive environments, *Gekko* sp. may adapt by increasing its use of natural habitats alongside Non-urban_artificial environments. The *p*-values for all other comparisons were close to 1, indicating that the microhabitat use patterns of *Gekko* sp. in competition-free areas are very similar to those of the other two species in competitive settings.

4. De-novo assembly, phylogenetic analysis, and population structure

The total number of filtered loci for each dataset was as follows; Set.phy_S:27857, Set.phy_H:25254, Set.Admix_S:9068, and Set.Admix_H:13707. The number of single nucleotide polymorphisms (SNPs) were Set.phy_S:72580 (3.31% missing sites), Set.phy_H:122223 (4.91% missing sites), Set.Admix_S:6426 (2.02% missing sites) and Set.Admix_H:41767 (2.74% missing sites).

The outcomes of the molecular phylogenetic and population structure analyses are depicted in Fig. 4-6. For *Gekko* sp., the optimal fit was achieved with K = 3, suggesting a division into three distinct populations: the Goto + Hirado population, the Northern Koshiki population (including Kamikoshikishima, Nakakoshikishima, and Northern Shimokoshikishima Islands), and the Southern Koshiki population (encompassing the Southern Shimokoshikishima Island; see Fig. 4-6A). For *G. hokouensis*, the best fit was found with K = 2, illustrating that the populations on the Goto Islands and Kamikoshikishima Island are phylogenetically akin to those on mainland Kyushu and Nakakoshikishima Island (see Fig. 4-6B). The molecular phylogenetic tree for *G. hokouensis*, based on mitochondrial DNA, is presented in Fig. S4-2. All samples from mainland Kyushu, the Goto Islands, and the Koshikishima Islands analyzed in this study are classified under Clade 1 as per Chapter 2 and Chiba *et al.*, 2024. Furthermore, this geographic distribution aligns with prior allozyme studies (Toda *et al.*, 1997), which identified two groups: one located west of the Satsuma Peninsula (in western Kagoshima Prefecture) and another situated east of the Satsuma Peninsula and on the Osumi Peninsula (in eastern Kagoshima Prefecture; see Fig. 4-6B).

5. Demographic history and divergence time

Fig. 4-7 illustrates the demographic histories of each regional population. The *Gekko* sp. population in Goto + Hirado underwent two significant population bottlenecks approximately 1,500 and 1,000 years ago, while the Northern Koshiki population experienced a bottleneck around 500 years ago. Conversely, the Southern Koshiki population did not undergo such demographic reductions. The Ibusuki population of *G. hokouensis* has remained stable in size since the last ice age, without any significant bottlenecks, although the Goto population faced a bottleneck roughly 1,000 years ago. The Central Koshiki population did not exhibit any bottleneck events.

As for the divergence times among regional populations of *Gekko* sp., summary statistics are provided in Table S4-4. The posterior distributions for each parameter, derived from simulations with ABCtoolbox, are detailed in Table S4-5. The most probable scenario, labeled M3, is depicted in Fig. 4-8, while the discarded scenarios (M1 and M2) are presented in Fig. S4-3. According to model M3, the median number of generations for the divergence of the Goto + Hirado population from the Koshikishima Islands population is estimated at 7,618 generations ago, and the split of the Koshikishima Islands population into northern and southern populations occurred around 346 generations ago.

Discussion

1. Current status and origin of domestic nonnative *G. hokouensis*

Although the Koshikishima Islands, part of Kagoshima Prefecture and proximate to the mainland, had not previously confirmed the presence of *G. hokouensis* (Okamoto *et al.*, 2017), recent findings have identified it as the dominant species in two villages on these islands. A 2016 survey (Okamoto *et al.*, 2017) found no geckos in Sato Village (Northern Koshiki), now a stronghold for *G. hokouensis*, and only *Gekko* sp. was observed in Taira (Central Koshiki). These developments suggest that *G. hokouensis* was introduced within the last five years, rapidly overtaking the habitat of *Gekko* sp. The molecular phylogenetic analysis reveals that the *G. hokouensis* populations now predominant in these villages are genetically distinct. Mitochondrial DNA-based phylogenetic relationships indicate the Northern Koshiki population likely originated from the western Satsuma Peninsula, and the Central Koshiki population from the eastern Satsuma Peninsula or the Osumi Peninsula. Sato Village, serving as a ferry gateway to Kyushu's mainland, might have facilitated the introduction of geckos, potentially via hitchhiking on ferries—a phenomenon documented globally. In Central Koshiki, the construction of a 1,533-meter sea bridge connecting Southern and Northern Koshiki (started in 2006 and completed in 2020) could have enabled *G. hokouensis* introduction, perhaps with materials from the eastern Satsuma Peninsula's industrial zone.

In the Goto Islands, numerous *G. hokouensis* samples were collected from the northeast of Nakadorijima Island in this study, marking its presence since at least 1989 and classifying it as a recent domestic nonnative. However, since *G. hokouensis* was not distinguished from *G. japonicus* until 1982, historical literature offers no clues about its introduction timeline to the Goto Islands. Thus, molecular phylogeny and population genetics serve as valuable tools for understanding its origins. Mitochondrial DNA suggests the Goto Islands population stems from the western Satsuma Peninsula. Furthermore, this population underwent a bottleneck approximately 1,000 years ago, hinting at its introduction around that time. It's important to note the limitation of not including the western Satsuma Peninsula population, presumed ancestral, in a genome-wide analysis, leaving open the possibility that this bottleneck affected the ancestral population due to other factors, and recent introduction effects might not be detectable.

2. Character displacement in *Gekko* sp. and tripartite competition among Gecko species

The suitable habitat modeling results for the three gecko species (*G. japonicus*, *Gekko* sp., and *G. hokouensis*) indicate a large overlap in their habitats, heavily influenced by human demographics and potentially extending beyond their current distributions, especially for *Gekko* sp. Furthermore, the microhabitat usage comparison suggests no significant difference in potential habitat use among the three species; however, *Gekko* sp. exhibits a notable shift under interspecific competition. This suggests that the introduction of non-native gecko species may have induced character displacement in *Gekko* sp. as proposed by Brown & Wilson (1956). In urban artificial environments, which all three gecko species potentially favor, the dominance hierarchy appears to be an inverted triangle with *G. japonicus* and *G. hokouensis* being roughly equal and superior to *Gekko* sp. Artificial environments in non-urban areas or natural habitats like coasts, seldom utilized by *G. japonicus*, could serve as refuges for *Gekko* sp. This hierarchy is mirrored in the distribution patterns across Kyushu. In the Goto Islands and Hiradojima Island, Fukuejima Island, the largest and most urbanized, is predominantly occupied by *G. japonicus*, whereas Hisakajima Island, smaller and more natural, is chiefly inhabited by *Gekko* sp. Nakadorijima Island and Hiradojima Island, both of moderate size and urbanization, exhibit a mixed distribution of all three species. In mainland Kyushu, *G. hokouensis* has long been native to urban areas of southern Kagoshima Prefecture, suggesting a balance of dominance with *G. japonicus*, which has spread from northern Kyushu. This balance is now evident around the northern parts of Kagoshima Prefecture. Moreover, the Koshikishima Islands, previously thought to be solely inhabited by *Gekko* sp., are no longer competition-free zones due to recent incursions by *G. japonicus* and *G. hokouensis*. Although this study's sample size is limited, as the invasions are in their initial stages, continued monitoring could provide insights into how this tripartite competition develops a patchy distribution among the three gecko species.

3. Genetic imprints of historical invasions in three Gecko species

The Southern Koshiki population of *Gekko* sp. has not encountered any population bottlenecks, suggesting its long-term habitation without considerable threats from other gecko species, aligning with this study's hypothesis. The concurrence of divergence and bottleneck events in Northern Koshiki might be elucidated by a scenario where *Gekko* sp. was absent from Northern Koshiki over 500 years ago, only to extend its distribution to this northern area around that time. However, definitive evidence to substantiate their absence from Northern Koshiki over 500 years ago, the reasons for this potential absence, and the catalysts for their northward expansion 500 years ago are lacking.

In contrast, the Goto + Hirado populations of *Gekko* sp. diverged approximately 11,000 years ago, not aligning with the timing of the bottlenecks (about 1,500 years ago and 1,000 years ago). Yet, the era of the initial bottleneck coincides with findings from previous research (Chapter 3; Chiba *et al.*, 2022), indicating the introduction of *G. japonicus* to the Goto Islands and mainland Kyushu between 3,000-2,400 years ago, with population recovery commencing between 1,800 to 1,500 years ago. It strongly suggests that the bottleneck *Gekko* sp. experienced in Goto and Hirado 1,500 years ago was likely due to the invasion and proliferation of *G. japonicus*, rather than migration. Moreover, the period of the second bottleneck corresponds with that experienced by the Goto population of *G. hokouensis*. Given the introduction of the Goto population of *G. hokouensis* around this time, as discussed in the initial section, it's plausible that *Gekko* sp., having already been impacted by the prior invasion of *G. japonicus*, further suffered from the encroachment of *G. hokouensis*. This notion is bolstered by the observation that *Gekko* sp.'s microhabitat utilization shifts in reaction to invasions by other gecko species, and its potential suitable habitat and microhabitat usage closely resemble those of the other two species. Considering that competition-driven extinctions usually transpire more slowly than predation-induced ones (Davis, 2003), this could clarify why the bottleneck in *Gekko* sp. occurred slightly after *G. japonicus*' introduction.

Moreover, considering other potential hypotheses, a bottleneck caused by human development contradicts the observed preference for urban artificial environments (Fig. 4-6), and a bottleneck attributed to climate change does not align with the observed trend of habitat recovery following the Last Glacial Period (Fig. S4-1). Thus, it can be inferred that *G. japonicus* (and possibly *G. hokouensis* as well) was indeed an invader in the past, aligning with the hypothesis of this study. This scenario underscores how history is often shaped by those who prevail, leading to the diminishment or neglect of the narratives of those who do not. The long-standing familiarity between the Japanese people and *G. japonicus* may indeed epitomize the adage "History is written by the victors."

All regional populations of *Gekko* sp. examined in this study demonstrated a pattern of swift population recovery following a bottleneck (with the Southern Koshiki population also showing a minor bottleneck at the lower end of the confidence interval before rising). The Stairway plot, utilizing the Site Frequency Spectrum (SFS) as a metric, is better suited for recent demographic history than other methodologies, though it is notably sensitive to the size of the sample (Liu & Fu, 2015; Patton *et al.*, 2019). The accuracy significantly declines with smaller sample sizes, particularly in illustrating population recovery patterns post-bottleneck (Terhorst & Song, 2015). Given that the samples in this study were collected from fragmented habitats on small islands, the limited sample size may have led to an overestimation of the exponential population recovery following the bottleneck.

Conclusion

This study has revealed that long-standing competition among some gecko species, propelled by ancient human-mediated dispersal, has inadvertently jeopardized the native endemic *Gekko* sp. This competitive dynamic is not merely historical but persists into the present, potentially escalating with advancements in modern transportation. The displacement of endemic species by a few widespread species leads to biodiversity homogenization, representing a significant conservation concern (McKinney & Lockwood, 1999). Although *G. japonicus* does not seem to be actively invasive, it has colonized many niches that *Gekko* sp. once occupied, effectively displacing it from its ecological niche. Hence, *G. japonicus* should be reconsidered as not merely neutral, even in the current context. This scenario mirrors other cases in Japan where species previously considered neutral natives were later identified as nonnatives, possibly affecting truly endemic species (Suzuki *et al.*, 2011; Suzuki, Yabe & Hikida, 2014). It's plausible to suggest that human activities have influenced many organisms over thousands of years of civilization. Among these, numerous anciently introduced species might masquerade as neutral natives. In an age where biodiversity conservation is paramount, recognizing the historical backdrop of present-day biodiversity is vital to avoid errors in conservation decision-making.

Tables

Table. 4-1 Adopted suitable habitat model's AUC values and percent contribution of each environmental variables. Bold letters indicate variables with a high degree of contribution.

	AUC	Bio 1	Bio 4	Bio 8	Human demographics
G. japonicus	0.874	0.029	0.259	0	**0.713**
Gekko sp.	0.800	0.235	0.011	0.122	**0.632**
G. hokouensis	0.920	0	**0.526**	0	**0.474**

Figures

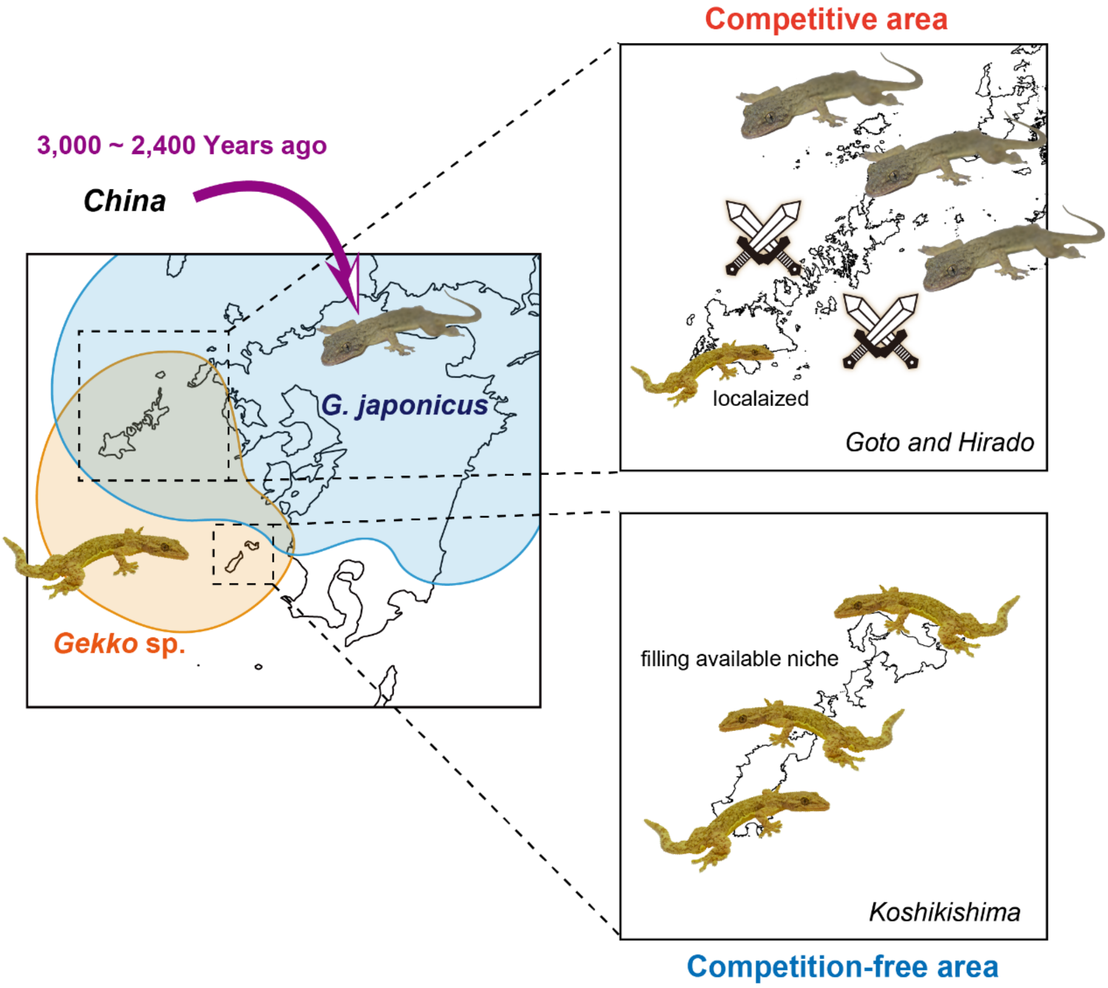

Fig. 4-1 Hypothetical concepts verified in this study. Area surrounded by light blue represent current distribution of *G. japonicus*, and by orange represent that of *Gekko* sp. Where both are overlapping was regarded as a competitive area, and where inhabited only *Gekko* sp. was regarded as a competition-free area.

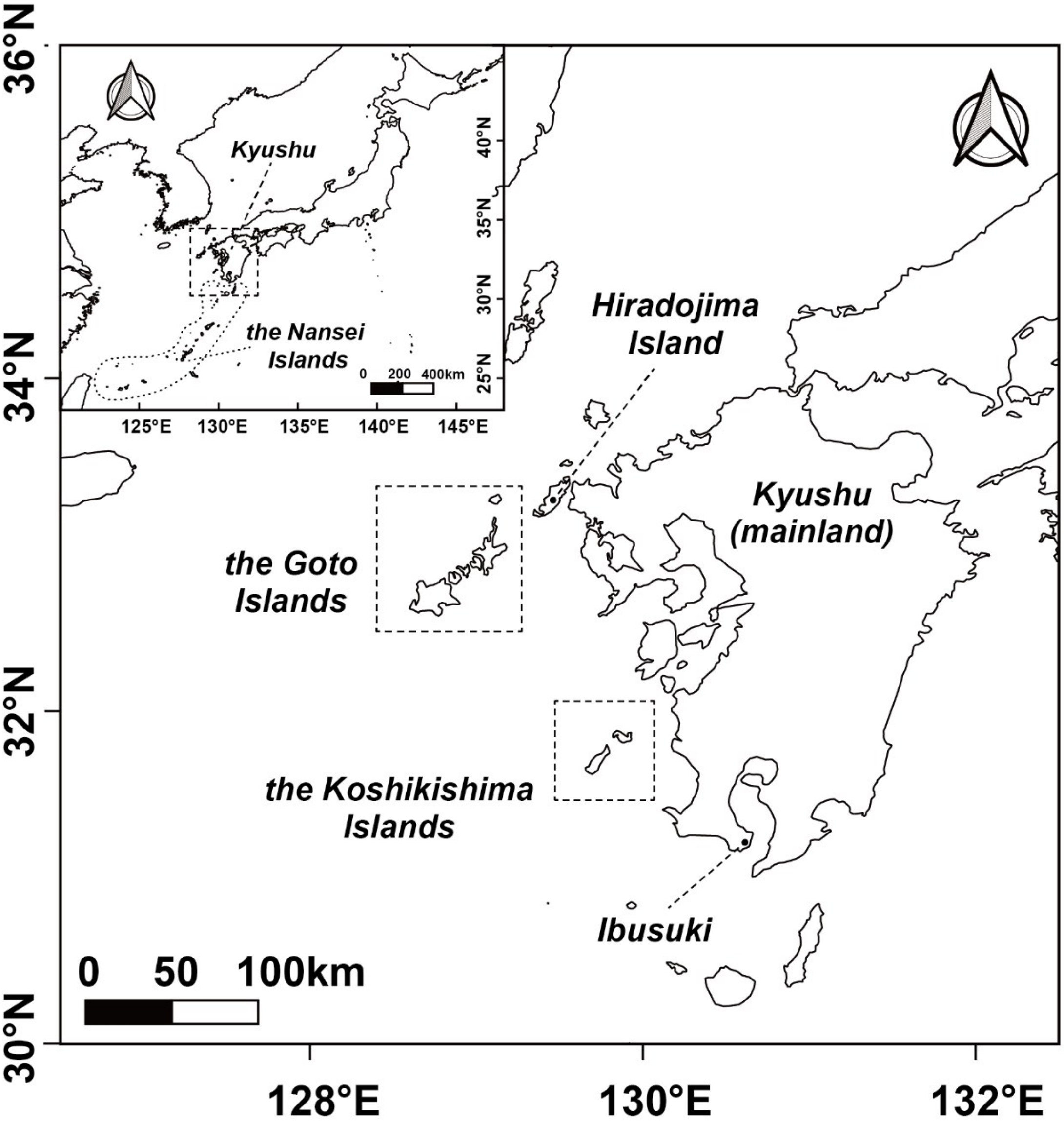

Fig. 4-2 Sampling areas in this study. Kyushu is the most western mainland of the Japanese archipelago. There are thousands of remote islands in the surrounding area, and it is one of the highest areas of the diversity of gecko species in Japan.

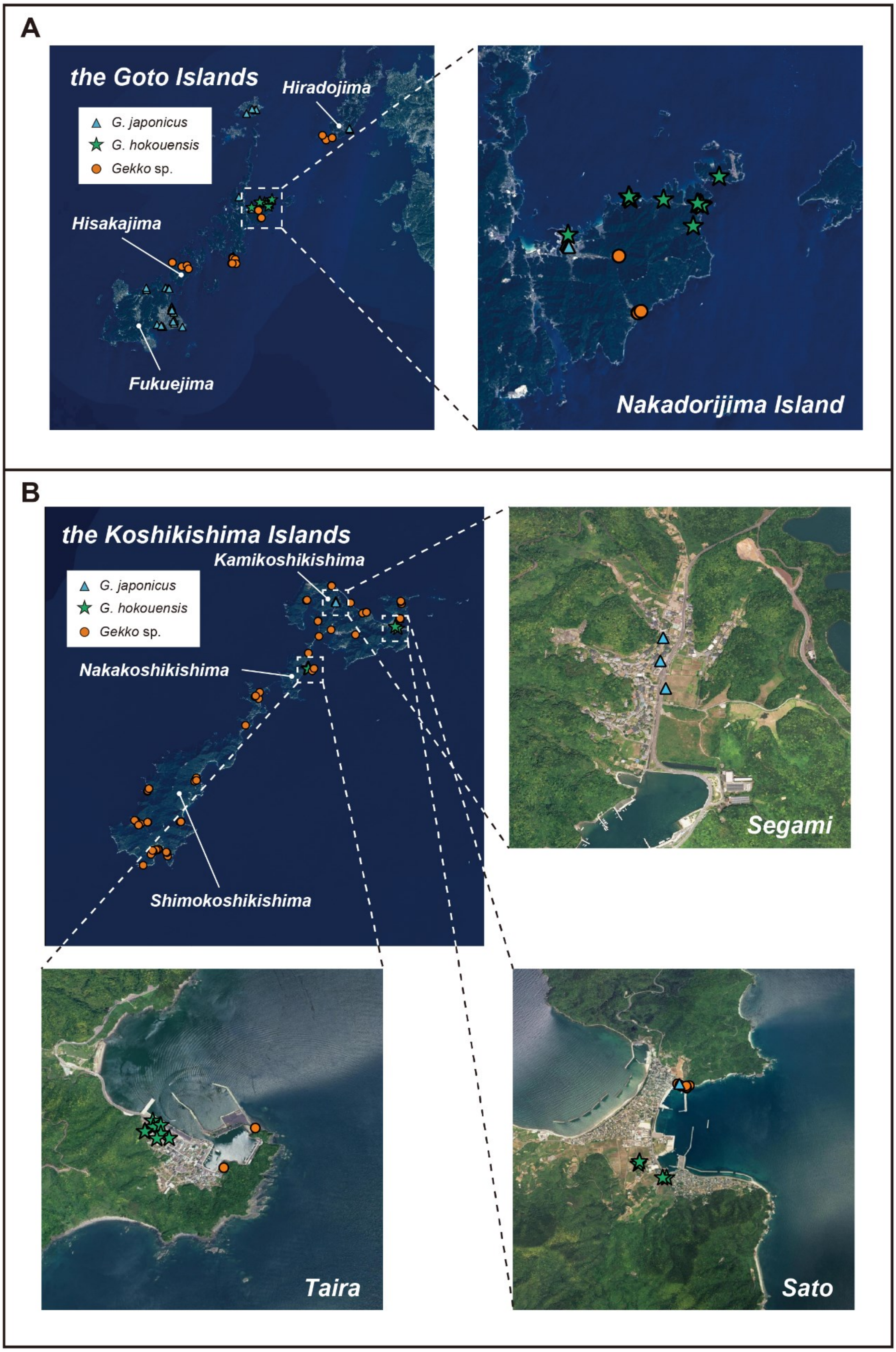

Fig. 4-3 Detail sampling site in the Goto Islands, Hiradojima Island and the Koshikishima Islands.

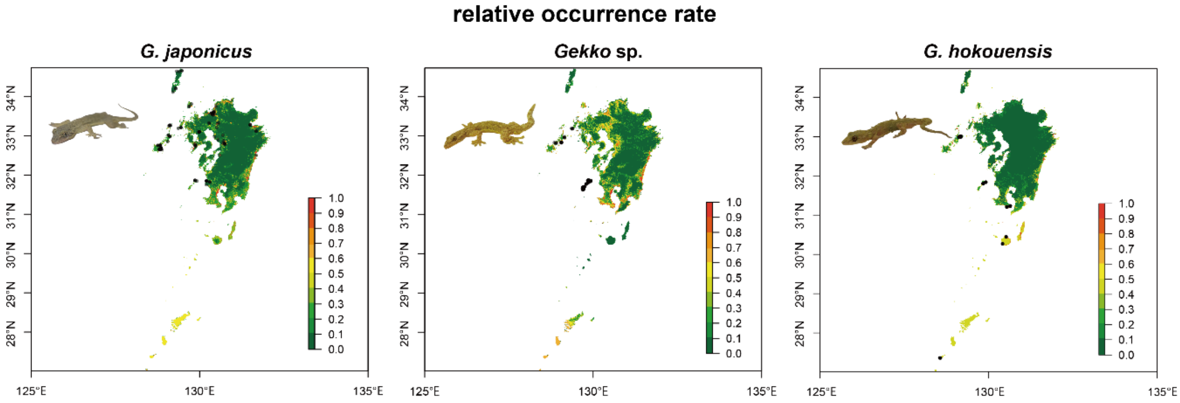

Fig. 4-4 Present suitable habitat of three gecko species. The higher the color temperature, the higher the potential utility.

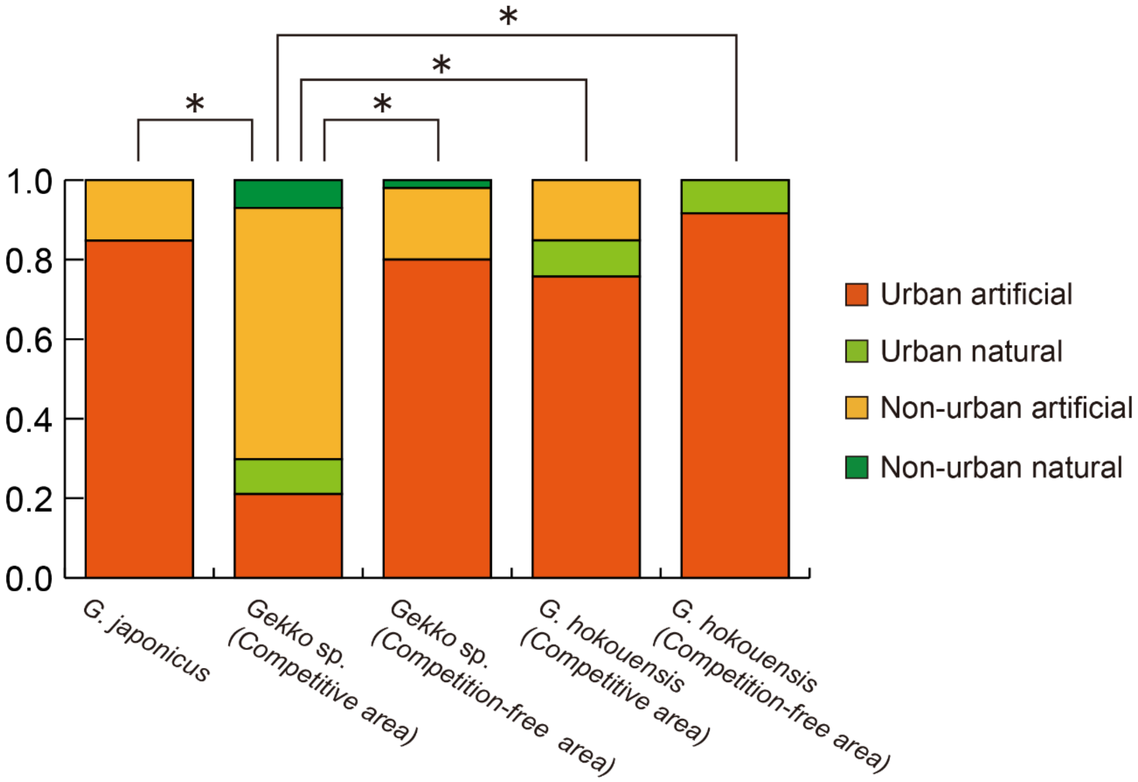

Fig. 4-5 The ratio of microhabitat use and the results of pairwise comparisons using Fisher's exact test among three gecko species. * indicates a significant difference ($p < 0.05$) between groups.

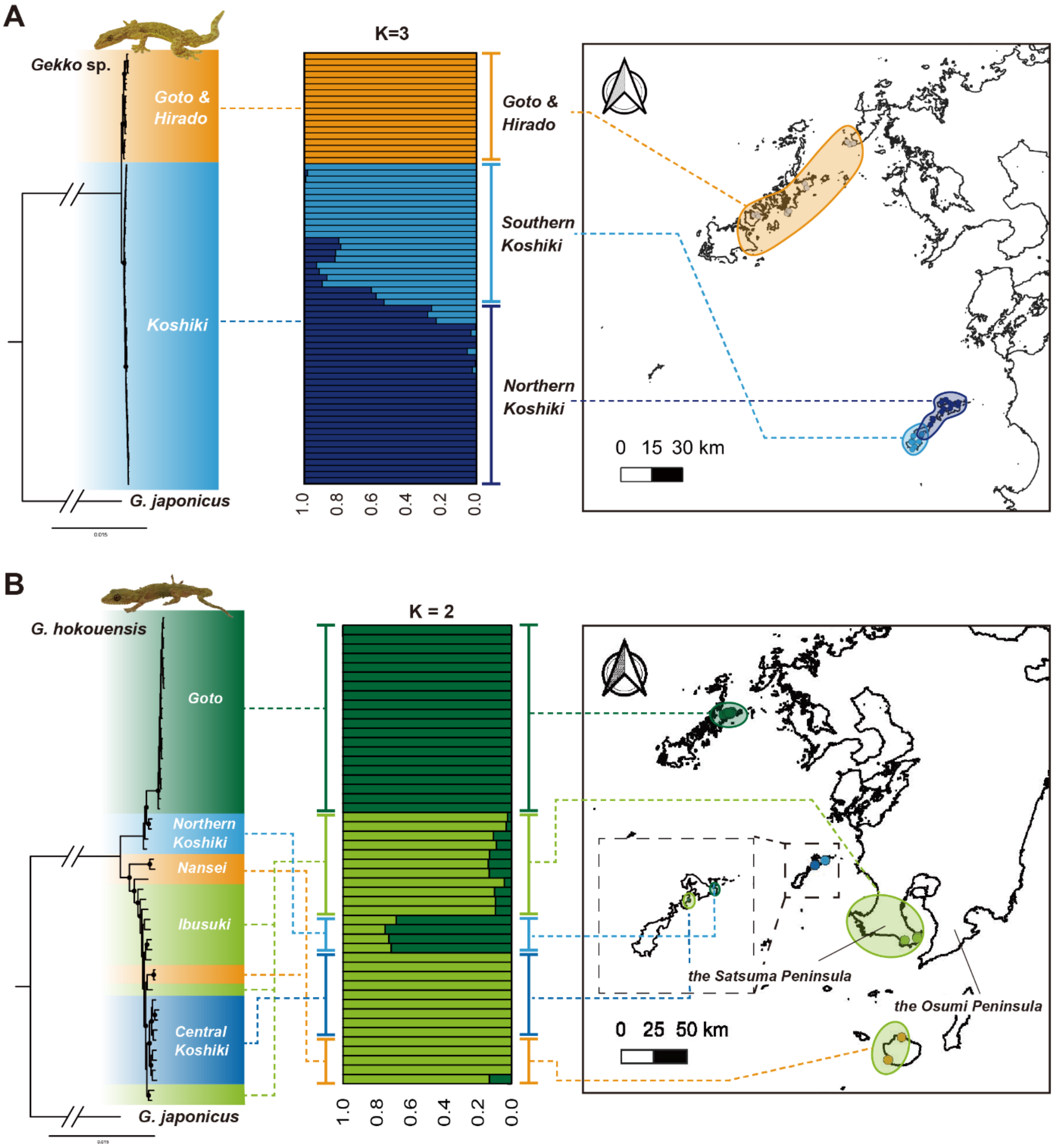

Fig. 4-6 Phylogeography of *Gekko* sp. (A) and *G. hokouensis* (B). Left: A molecular phylogenetic tree based on the ML method and the approximate Bayesian method by ddRAD-seq. Nodes with a closed circle represent Bayes posterior probabilities higher than 0.99 and UFboot higher than 95%. Central: Genetic structure visualized by ADMIXTURE. Right: Geographical mapping for each genetic cluster.

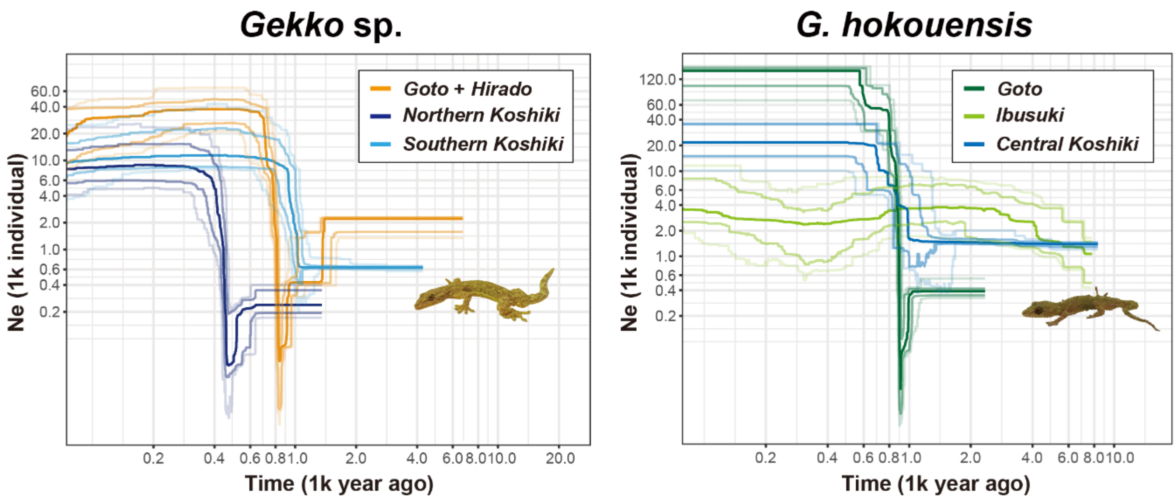

Fig. 4-7 Demographic history of *Gekko* sp. and *G. hokouensis* in each area. The horizontal axis is the reverse-direction age (1,000 years ago) on the log10 scale. The vertical axis is the number of effective populations (Ne) on the log10 scale. The most apparent lines represent the median population size for the region, and the slightly transparent lines of the same color represent 75% and 95% CI.

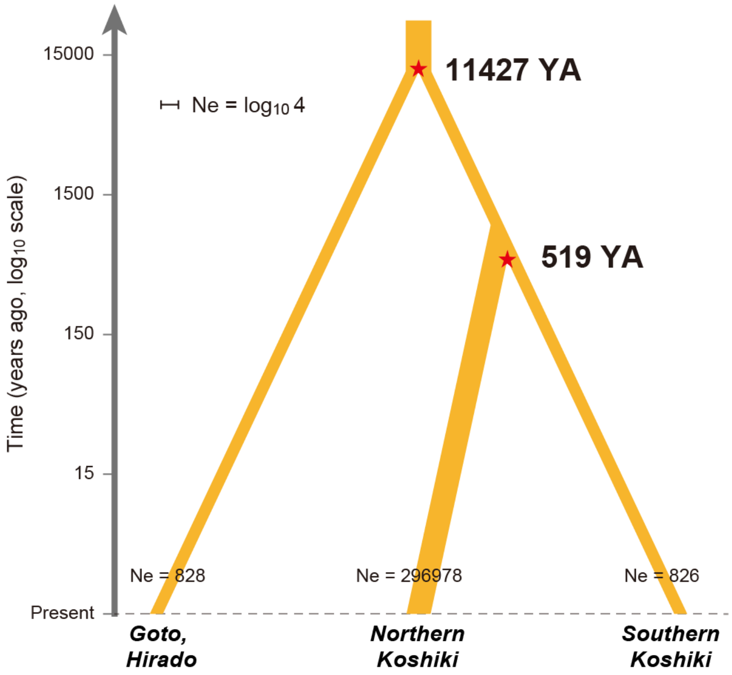

Fig. 4-8 Divergence time of *Gekko* sp. of each area in the adopted scenario. The vertical axis is the reverse direction age (years ago, YA) on the log10 scale. The thickness of each population is the number of effective populations on the log10 scale. In this scenario, first, the population of the Northern Koshiki coalesces into the Southern Koshiki. Next, the population of the Goto + Hirado coalesces into the Koshiki.

Chapter 5:
General discussion

Here, I will summarize and discuss the "interspecific history among Japanese humans and geckos" spotlighted by these studies. Chapter 2 focused on a recent case, illustrating that *G. hokouensis* from the Nansei Islands, which has multiple genetically distinct populations divided by island, has been introduced multiple times to the Izu Islands, leading to secondary contact. This challenges the common theory that genetic diversity in nonnative species is significantly reduced in introduced regions due to the founder effect. It suggests that multiple introductions from diverse origins can maintain genetic diversity akin to the source population. This revelation has implications for conservation biology, as high genetic diversity could imply greater adaptability in new environments for nonnative species. Although *G. hokouensis* of the Izu Islands is likely a domestic nonnative species introduced by humans approximately 30 years ago, it has already become widely dominant across several islands, largely unnoticed. Despite the absence of native gecko species in the Izu Islands, the presence of numerous endemic insects and reptiles makes it imperative to gather basic information on the evolutionary ecology of *G. hokouensis* and to understand its future.

In Chapter 3, which examined a historical case, I demonstrated that *G. japonicus*, the most common gecko species in Japan, is indeed an ancient nonnative species introduced from China about 3000 years ago. It spread throughout Japan over thousands of years, taking advantage of the development of human society. This finding suggests that the influence of humans on the distribution of plants and animals in pre-modern times is often overlooked compared to our influence today, but it might have been more substantial than we realize. The distribution and diversity of organisms that we observe today, as well as the evolutionary patterns of genetic variation in biological populations, may reflect not only natural history but also the long-term influence of human society. In addition, the initial introduction of *G. japonicus* might have exhibited invasiveness comparable to many recently introduced nonnative species. The study shows that biodiversity, traditionally viewed only within the framework of nature, has indeed been closely intertwined with human society for ages, incorporating the history of human activities. This raises the question of determining the conservation value of biodiversity. *G. japonicus,* with its long history interwoven with Japanese society, can be likened to living ancient literature, holding significant cultural conservation value akin to that of historical manuscripts. In the case of nonnative species with a long history, it may not be justifiable to diminish their conservation value based solely on their nonnative characteristics.

In Chapter 4, focusing on a past-to-future scenario, I demonstrated that an ancient alien species *G. japonicus*, now perceived as a neutral native species and the most common gecko in Japan, was historically an invasive nonnative species that displaced the native *Gekko* sp.. This suggests that current neutral appearance of old naturalized species is likely because we are observing them post the completion of native species displacement. Therefore, the influence of ancient nonnative species in shaping current biodiversity should be more thoroughly considered. How then should the Japanese people regard the old neighbor *G. japonicus* moving forward? Given its popularity and cultural significance, as discussed in Chapter 3, *G. japonicus* occupies a complex conservation position. Region-specific conservation strategies should be developed, focusing on control in areas where its impact is substantial.

Chapter 2-4 collectively demonstrate that humans have dispersed several gecko species familiar to the human society not only in the recent past, but throughout their long history, often unintentionally. This human-triggered competition among some gecko species has inadvertently threatened native endemic gecko species. This dynamic is not confined to the past; it continues in the present and may even be intensifying with modern transportation advancements. To prevent repeating historical errors, understanding the formation and origins of current biodiversity is crucial. It is because history rewritten by the victors such as this study might be hidden everywhere in the biodiversity we are seeing today. Recognizing the historical context is a fundamental step in addressing complex issues and making informed decisions.

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References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Väinölä, R., Wolf, J.B.W. & Zinner, D. (2013). Hybridization and speciation. *J. Evol. Biol.* **26**, 229–246.

Alexander, D.H. & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics* **12**.

Aogashima Village Board of Education & Aogashima Village. (1984). *Life and culture in Aogashima Island.*

Aogashima Village.

Arai, H. (1717). Touga. https://dl.ndl.go.jp/info:ndljp/pid/993111

Barbosa, A.M. (2015). fuzzySim: Applying fuzzy logic to binary similarity indices in ecology. *Methods Ecol. Evol.* **6**, 853–858.

Böhning, D. (1992). Multinomial logistic regression algorithm. *Ann. Inst. Stat. Math.* **44**, 197–200.

Brown, W.L. & Wilson, E.O. (1956). Character displacement. *Syst. Zool.* **5**, 49–64.

Bullock, J.M., Bonte, D., Pufal, G., da Silva Carvalho, C., Chapman, D.S., García, C., García, D., Matthysen, E. & Delgado, M.M. (2018). Human-Mediated Dispersal and the Rewiring of Spatial Networks. *Trends Ecol. Evol.*

Byrne, K. & Nichols, R.A. (1999). Culex pipiens in London Underground tunnels: differentiation between surface and subterranean populations. *Heredity (Edinb).* **82**, 7–15.

Carlton, J.T. (1989). Man's Role in Changing the Face of the Ocean: Biological Invasions and Implications for Conservation of Near-Shore Environments. *Conserv. Biol.* **3**, 265–273.

Case, T.J., Bolger, D.T., Petren, K., Case, T.E.D.J., Bolger, D.T. & Petren, K.E.N. (1994). Invasions and Competitive Displacement among House Geckos in the Tropical Pacific Published by : Ecological Society of America Stable. *Ecology* **75**, 464–477.

Chiba, M., Hirano, T., Yamazaki, D., Ye, B., Ito, S., Kagawa, O., Endo, K., Nishida, S., Hara, S., Aratake, K. & Chiba, S. (2022). The mutual history of Schlegel's Japanese gecko (Reptilia: Squamata: Gekkonidae) and humans inscribed in genes and ancient literature. *PNAS Nexus* **1**, 1–10.

Chiba, M., Yamazaki, D., Ito, S., Kagawa, O. & Chiba, S. (2024). Secondary contact of two cryptic Hokou gecko groups in the Izu Islands, Japan. *Mitochondrial DNA Part A* **0**, 1–8.

Cole, N.C., Jones, C.G. & Harris, S. (2005). The need for enemy-free space: The impact of an invasive gecko on island endemics. *Biol. Conserv.* **125**, 467–474.

Csilléry, K., François, O. & Blum, M.G.B. (2012). Abc: An R package for approximate Bayesian computation (ABC). *Methods Ecol. Evol.* **3**, 475–479.

Davis, M.A. (2003). Biotic globalization: Does competition from introduced species threaten biodiversity? *Bioscience* **53**, 481–489.

Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibañez, I., Miller, L.P., Sorte, C.J.B. & Tatem, A.J. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. *Nat. Commun.* **7**.

Eaton, D.A.R. & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics* **36**, 2592–2594.

Edgar, R.C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. & Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists. *Divers. Distrib.* **17**, 43–57.

Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V.C. & Foll, M. (2013). Robust Demographic Inference from Genomic and SNP Data. *PLoS Genet.* **9**.

Excoffier, L., Foll, M. & Barrett, J. (2011). fastsimcoal: a continuous-time coalescent simulator of genomic diversity under arbitrarily complex evolutionary scenarios. *Bioinforma. Appl. NOTE* **27**, 1332–1334.

Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**, 564–567.

Fisher, R.N. (1997). Dispersal and evolution of the pacific basin Gekkonid lizards *Gehyra oceanica* and Gehyra mutilata. *Evolution (N. Y).* **51**, 906–921.

Fukae, S. (918). Honzo Wamyo. https://dl.ndl.go.jp/info:ndljp/pid/2606133

Gippet, J.M., Liebhold, A.M., Fenn-Moltu, G. & Bertelsmeier, C. (2019). Human-mediated dispersal in insects. *Curr. Opin. Insect Sci.*

Isaac, O. (2022).easySFS: Effective selection of population size projection for construction of the site frequency spectrum. Convert VCF to dadi/fastsimcoal style SFS for demographic analysis. https://github.com/isaacovercast/easySFS

Hao, S., Ping, J. & Zhang, Y. (2016). Complete mitochondrial genome of *Gekko chinensis* (Squamata, Gekkonidae). *Mitochondrial DNA Part A DNA Mapping, Seq. Anal.* **27**, 4226–4227.

Hara, K. (1976). Range of distribution of *Eumeces okadae* (Scincidae). *Japanese J. Herpetol.* **6**, 95–98.

Hardin, G. (1960). The competitive exclusion principle. *Science (80-.).*

Hayashi, R. (2014). Past biodiversity: Historical Japanese illustrations document the distribution of whales and their epibiotic barnacles. *Ecol. Indic.* **45**, 687–691.

Heled, J. & Drummond, A.J. (2008). Bayesian inference of population size history from multiple loci. *BMC Evol. Biol.* **8**, 1–15.

Hervé, M. & Hervé, M.M. (2020). Package 'RVAideMemoire'. https://cran.r-project.org/web/packages/RVAideMemoire/index.html

Hirano, H. (1697). Honcho Shokkan. https://dl.ndl.go.jp/info:ndljp/pid/2605286

Hirano, T., Kameda, Y. & Chiba, S. (2014). Phylogeny of the land snails Bradybaena and Phaeohelix (Pulmonata: Bradybaenidae) in Japan. *J. Molluscan Stud.* **80**, 177–183.

Hirano, T., Kameda, Y., Saito, T. & Chiba, S. (2019). Divergence before and after the isolation of islands: Phylogeography of the Bradybaena land snails on the Ryukyu Islands of Japan. *J. Biogeogr.* **46**, 1197–1213.

Hirano, T., Saito, T. & Chiba, S. (2015). Phylogeny of freshwater viviparid snails in Japan. *J. Molluscan Stud.* **81**, 435–441.

Honda, T. (2014). *The Tokaido in Early modern*. Seibundo Publishing.

Ichikawa, S., Matsumoto, T., Nakamura, T. & Kurisaki, T. (2019). Provenance determination of prehistorical pottery from Oshima Island belonging to Izu islands (Tokyo, Japan) using X-ray fluorescence spectrometry. *X-Ray Spectrom.* **48**, 107–125.

Izuta, C. (1979). *Yamagata Prefecture*. Shoheisha.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A. & Jermiin, L.S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods 2017 146* **14**, 587–589.

Karasawa, S., Nagata, S., Aoki, J.I., Yahata, K. & Honda, M. (2015). Phylogeographic Study of Whip Scorpions (Chelicerata: Arachnida: Thelyphonida) in Japan and Taiwan. *Zoolog. Sci.* **32**, 352.

Kariya, E. (1827). Shakuchu Wamyo Ruiju Sho. https://dl.ndl.go.jp/info:ndljp/pid/991791

Kasai, S. (1968). *General inventory of flora and fauna of Hachijojima Island.* Tokyo Metropolitan Board of Education Hachijojima Island Branch.

Kawakatsu, Y., Sakamoto, T., Nakayama, H., Kaminoyama, K., Igarashi, K., Yasugi, M., Kudoh, H., Nagano, A.J., Yano, K., Kubo, N., Notaguchi, M. & Kimura, S. (2021). Combination of genetic analysis and ancient literature survey reveals the divergence of traditional *Brassica rapa* varieties from Kyoto, Japan. *Hortic. Res.* **8**, 132.

Kim, D.I., Choi, W.J., Park, I.K., Kim, J.S., Kim, I.H. & Park, D. (2018). Comparisons of microhabitat use of Schlegel's Japanese gecko (*Gekko japonicus*) among three populations and four land cover types. *J. Ecol.*

Environ. **42**.

Kim, D.I., Park, I.K., Bae, S.Y., Fong, J.J., Zhang, Y.P., Li, S.R., Ota, H., Kim, J.S. & Park, D. (2020a). Prediction of present and future distribution of the Schlegel's Japanese gecko (*Gekko japonicus*) using MaxEnt modeling. *J. Ecol. Environ.* **44**, 5.

Kim, D.I., Park, I.K., Ota, H., Fong, J.J., Kim, J.S., Zhang, Y.P., Li, S.R., Choi, W.J. & Park, D. (2019). Patterns of morphological variation in the Schlegel's Japanese gecko (*Gekko japonicus*) across populations in China, Japan, and Korea. *J. Ecol. Environ.* **43**, 1–9.

Kim, I.H., Park, J., Cheon, K.S., Lee, H.J., Kim, J.K. & Park, D. (2016). Complete mitochondrial genome of schlegel's Japanese gecko *Gekko japonicus* (Squamata: Gekkonidae). *Mitochondrial DNA* **27**, 3684–3686.

Kim, J.S., Park, J., Fong, J.J., Zhang, Y.P., Li, S.R., Ota, H., Min, S.H., Min, M.S. & Park, D. (2020b). Genetic diversity and inferred dispersal history of the Schlegel's Japanese Gecko (*Gekko japonicus*) in Northeast Asia based on population genetic analyses and paleo-species distribution modelling. *Mitochondrial DNA Part A DNA Mapping, Seq. Anal.* **31**, 120–130.

Komaki, S. (2021). Widespread misperception about a major East Asian biogeographic boundary exposed through bibliographic survey and biogeographic meta-analysis. *J. Biogeogr.* **48**, 2375–2386.

Kumazawa, Y. & Endo, H. (2004). Mitochondrial genome of the Komodo dragon: Efficient sequencing method with reptile-oriented primers and novel gene rearrangements. *DNA Res.* **11**, 115–125.

Kwon, H., Yan, J., Jang, H., Park, J., Kim, J., Park, I.K. & Park, D. (2023). First record and origin of the non-native Peking gecko *Gekko swinhonis* Günther, 1864 (Squamata: Gekkonidae), newly discovered in Incheon Metropolitan City, South Korea. *BioInvasions Rec.* **12**, 1157–1171.

Lefebvre, M.J., Ardren, T., Thompson, V.D., Fitzpatrick, S.M. & Ayers-Rigsby, S. (2022). In Support of Sustainability: The Historical Ecology of Vertebrate Biodiversity and Native American Harvest Practices in the Florida Keys, USA. *Sustain. 2022, Vol. 14, Page 6552* **14**, 6552.

Leigh, J.W. & Bryant, D. (2015). popart : full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116.

Li, H.M., Zeng, D.L., Guan, Q.X., Qin, P.S. & Qin, X.M. (2013). Complete mitochondrial genome of *Gekko swinhonis* (Squamata, Gekkonidae). *Mitochondrial DNA* **24**, 86–88.

Li, S., Xu, Z., Luo, L., Ping, J., Zhou, H., Xie, L. & Zhang, Y. (2022). Latitudinal Variation in the Pattern of

Temperature-Dependent Sex Determination in the Japanese Gecko, *Gekko japonicus*. *Animals* **12**, 1–10.

Liu, X. & Fu, Y.-X. (2015). Exploring population size changes using SNP frequency spectra. *Nat. Genet. Vol.* **47**.

Matsui, M. & Mori, A. (2021). *Amphibians and Reptiles of Japan.* Sunrise Publishing.

Matsuo, H., Tanaka, Y., Watanabe, S., Obinata, K., Inoue, H. & Takenaga, M. (2005). *The history of Shimane Prefecture*. Yamakawa Shuppansha.

Matsuo, T., Ejima, M. & Matsunaga, K. (1988). A species of gecko belonging to genus Gekko in Nakadôri-jima Island, Gotô Islands. *Nihon no Seibutsu (The Nat. Hist. Japan)* **2**, 61.

McKinney, M.L. & Lockwood, J.L. (1999). Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends Ecol. Evol.* **14**, 450–453.

McTavish, C.K. (2018). Median-joining networks for inferring intraspecific phylogenies. *Plant Dis.* **102**, 37–48.

Meyer, J.R., Dobias, D.T., Weitz, J.S., Barrick, J.E., Quick, R.T. & Lenski, R.E. (2012). Repeatability and Contingency in the Evolution of a Key Innovation in Phage Lambda **335**.

Meyerson, L.A. & Mooney, H.A. (2007). Invasive alien species in an era of globalization. *Front. Ecol. Environ.* **5**, 199–208.

Minamoto, S. (931). Wamyou Ruiju Sho. https://dl.ndl.go.jp/info:ndljp/pid/2606770?tocOpened=1

Minh, B.Q., Nguyen, M.A.T. & Von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Mol. Biol. Evol.* **30**, 1188–1195.

Miyahara, T., Yamada, K., Fukaya, K., Obinata, S., Yamada, A., Hayakawa, N. & Ishiyama, H. (2012). *History of Japanese society*. Otsuki Shoten.

Mizuno, Y. (1975). *The ancient Izumo and Yamato*. Daiwa Shobo.

Mori, K., Amino, Y., Setodani, A., Yamamoto, K., Nakano, K., Yamanaka, H., Matsumura, K., Wada, A., Monta, S., Ikuta, S., Takahashi, K., Inoue, H., Yunoki, M., Sasaki, T., Takahashi, T. & Noji, T. (1991). *The Sea of Japan and the world of Izumo*. Shogakukan.

Nagaoka, S. & Furuyama, K. (2004). Eruptive History of the Onidake Volcano Group on Fukue Island, Western Japan. *J. Geogr. (Chigaku Zasshi)* **113**, 349–382.

Nakamura, M. & Marunouchi, J. (2023). First record of *Gekko hokouensis* in Izu Oshima Island, Tokyo. *Bull. Herpetol. Soc. Japan* **2023**, 86–88.

Nakamura, T. (1666). Kinmo Zui. https://dl.ndl.go.jp/info:ndljp/pid/11446667

Nguyen, H.N., Lu, C., Chu, J., Grismer, L.L., Hung, C. & Lin, S. (2019). Historical demography of four gecko species specializing in boulder cave habitat: Implications in the evolutionary dead end hypothesis and conservation. *Mol. Ecol.* **28**, 772–784.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**, 268–274.

O'Brien, S.J. & Evermann, J.F. (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *Trends Ecol. Evol.* **3**, 254–259.

Obayashi, T., Amino, Y., Akimichi, T., Makabe, T., Macé, F., Tani, T., Innami, T., Iritani, S., Moriya, T., Yamauchi, Y., Sugawara, M., Tanizawa, A., Mio, Y., Mori, M. & Ogawa, T. (1991). *Ama culture in Setouchi*. Shogakukan.

Ohno, Y. (712). *Kojiki*.

Okada, S., Izawa, M. & Ota, H. (2002). Growth and Reproduction of *Gekko hokouensis* (Reptilia: Squamata) on Okinawajima Island of the Ryukyu Archipelago, Japan. *J. Herpetol.* **36**, 473–479.

Okamoto, K., Otawa, T., Toda, M. & Jono, T. (2017). Gecko fauna on the Koshikijima Islands, Kagoshima Prefecture, Japan, with special reference to new record of *Gekko japonicus*. *Bull. Herpetol. Soc. Japan* **2017**, 43–47.

Okamoto, K., Tominaga, A. & Toda, M. (2023). Demographic imbalance in the hybrid zone led to asymmetric gene flow between two closely related geckos, *Gekko hokouensis* and *Gekko yakuensis* (Squamata: Gekkonidae) . *Biol. J. Linn. Soc.* 1–15.

Ono, R. (1803). Honzo Komoku Keimo. URL https://dl.ndl.go.jp/info:ndljp/pid/2606003?tocOpened=1

Ota, H. (1998). Geographic Patterns of Endemism and Speciation in Amphibians and Reptiles of the Ryukyu Archipelago, Japan, with Special Reference to their Paleogeographical Implications. *Res. Popul. Ecol. (Kyoto).* **40**, 189–204.

Ota, H., Fusure, K. & Yagishita, J. (1995). Colonizations of Two Exotic Reptiles on Hachijojima Island of the Izu Group, Japan. *Biol. Mag. Okinawa* **33**, 55–59.

Parzen, E. (1962). On Estimation of a Probability Density Function and Mode. *Source Ann. Math. Stat.* **33**.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. & Hoekstra, H.E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS One* **7**, e37135.

Prince Toneri & Kodera, K. (720). *Nihon Shoki*.

Puillandre, N., Dupas, S., Dangles, O., Zeddam, J.L., Capdevielle-Dulac, C., Barbin, K., Torres-Leguizamon, M. &

Silvain, J.F. (2008). Genetic bottleneck in invasive species: The potato tuber moth adds to the list. *Biol. Invasions* **10**, 319–333.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J. & Sham, P.C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* **81**, 559–575.

R Core Team. (2019). An introduction to dplR. *Ind. Commer. Train.* **10**, 11–18.

Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Miller, J.T., Robertson, M.P., Rouget, M., Le Roux, J.J. & Wilson, J.R.U. (2011). Human-mediated introductions of Australian acacias - a global experiment in biogeography. *Divers. Distrib.* **17**, 771–787.

Rödder, D., Solé, M., Böhme, W., Forschungsmuseum, Z. & Adenauerallee, A.K. (2008). Predicting the potential distributions of two alien invasive Housegeckos (Gekkonidae: *Hemidactylus frenatus*, *Hemidactylus mabouia*). *J. Zool.*

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. & Sanchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* **34**, 3299–3302.

Sabath, M.D. (1981). Gekkonid lizards of Guam, Mariana Islands: reproduction and habitat preference. *J. Herpetol.* **15**, 71–75.

Schoener, T.W. (1968). The Anolis Lizards of Bimini: Resource Partitioning in a Complex Fauna. *Ecology* **49**, 704–726.

Sherpa, S., Blum, M.G.B., Capblancq, T., Cumer, T., Rioux, D. & Després, L. (2019). Unravelling the invasion history of the Asian tiger mosquito in Europe. *Mol. Ecol.* **28**, 2360–2377.

Shomura, A., Izawa, T., Ebana, K., Ebitani, T., Kanegae, H., Konishi, S. & Yano, M. (2008). Deletion in a gene associated with grain size increased yields during rice domestication. *Nat. Genet.* **40**, 1023–1028.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science (80-.).* **236**, 787–792.

Stejneger, L. (1907). Herpetology of Japan and Adjacent Territory. *Bull. United States Natl. Museum* **58**, 1–25.

Sugawara, K. (1081). Ruiju Myogi Sho. https://dl.ndl.go.jp/info:ndljp/pid/2609857

Suzuki, D., Ota, H., Oh, H.S. & Hikida, T. (2011). Origin of Japanese populations of reeves' pond turtle, *Mauremys reevesii* (Reptilia: Geoemydidae), as inferred by a molecular approach. *Chelonian Conserv. Biol.* **10**, 237–249.

Suzuki, D., Yabe, T. & Hikida, T. (2014). Hybridization between *Mauremys japonica* and *Mauremys reevesii* Inferred by Nuclear and Mitochondrial DNA Analyses. *J. Herpetol.* **48**, 445–454.

Tachibana, T. (1181). Iroha Jirui Sho. https://dl.ndl.go.jp/info:ndljp/pid/1885583

Takahashi, H. (2005). A case of artificial overseas dispersal of *Hemidactylus frenatus*. *Bull. Herpetol. Soc. Japan* **2005**, 116–119.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729.

Toda, M. & Hikida, T. (2011). Possible Incursions of *Gekko hokouensis* (Reptilia: Squamata) into Non-native Area: An Example from Yakushima Island of the Northern Ryukyus, Japan. *Curr. Herpetol.* **30**, 33–39.

Toda, M., Hikida, T., Okada, S. & Ota, H. (2003). Contrasting patterns of genetic variation in the two sympatric geckos *Gekko tawaensis* and *G. japonicus* (Reptilia: Squamata) from western Japan, as revealed by allozyme analyses. *Heredity (Edinb).* **90**, 90–97.

Toda, M., Hikida, T. & Ota, H. (1997). Genetic Variation among Insular Populations of *Gekko hokouensis* (Reptilia: Squamata) near the Northeastern Borders of the Oriental and Palearctic Zoogeographic Regions in the Northern Ryukyus, Japan. *Zoolog. Sci.* **14**, 859–867.

Toda, M., Hikida, T. & Ota, H. (2001). Discovery of sympatric cryptic species within *Gekko hokouensis* (Gekkonidae: Squamata) from the Okinawa Islands, Japan, by use of allozyme data. *Zool. Scr.* **30**, 1–11.

Toda, M., Okada, S., Hikida, T. & Ota, H. (2006). Extensive natural hybridization between two geckos, *Gekko tawaensis* and *Gekko japonicus* (Reptilia: Squamata), throughout their broad sympatric area. *Biochem. Genet.* **44**, 1–17.

Toda, M., Sengoku, S., Hikida, T. & Ota, H. (2008). Description of two new species of the genus *Gekko* (Squamata: Gekkonidae) from the Tokara and Amami Island Groups in the Ryukyu Archipelago, Japan. *Copeia* 452–466.

Unknown. (1400a). Setsuyo Shu. https://dl.ndl.go.jp/info:ndljp/pid/2532120

Unknown. (1400b). Wagoku Hen. https://dl.ndl.go.jp/info:ndljp/pid/2605441

Unknown. (1603). Nippo Jisho dicrionary (Vocabulario da Lingoa de Iapam com a declaração em Portugues).

Vaart, A.W. van der. (1998). *Asymptotic statistics.* , Cambridge series on statistical and probabilistic mathematics.

Cambridge University Press.

Wegmann, D., Leuenberger, C., Neuenschwander, S. & Excoffier, L. (2010). ABCtoolbox: A versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics* **11**.

Wickramasinghe, N., Wickramasinghe, L.J.M., Vidanapathirana, D.R., Tennakoon, K.H., Samarakoon, S.R. & Gower, D.J. (2022). A molecular-genetics perspective on the systematics of the parthenogenetic flowerpot blindsnake *Indotyphlops braminus* (Daudin, 1803) (Squamata: Serpentes: Typhlopidae). *Syst. Biodivers.* **20**, 1–16.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J. & Richardson, D.M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends Ecol. Evol.* **24**, 136–144.

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolón, A.R. & Revell, L.J. (2016). Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution (N. Y).* **70**, 1009–1022.

Yan, J., Wang, Q., Chang, Q., Ji, X. & Zhou, K. (2010). The divergence of two independent lineages of an endemic Chinese gecko, *Gekko swinhonis*, launched by the Qinling orogenic belt. *Mol. Ecol.* **19**, 2490–2500.

Yeakel, J.D., Pires, M.M., Rudolf, L., Dominy, N.J., Koch, P.L., Guimarães, P.R. & Gross, T. (2014). Collapse of an ecological network in Ancient Egypt. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 14475–14477.

Zhou, K., Liu, Y. & Li, D. (1982). Three new species of *Gekko* and remarks on *Gekko hokouensis* (Lacertiformes, Gekkonidae). *Acta Zootaxonomica Sin.*

Zhou, K. & Wang, Q. (2008). New species of *Gekko* (Squamata: Sauria: Gekkonidae) from China: Morphological and molecular evidence. *Zootaxa* **1778**, 59–68.

Supplementary information

Additional discussion S2-1: Details about the sampling on Miyakejima Island

Miyakejima Island is a conical volcanic island with villages built around the outer edge of the island. It is still an active volcano, and a large eruption in 2000 forced the entire island population to evacuate for several years. Only one individual of *G. hokouensis* was observed on Miyakejima Island, and it could not be collected. On Miyakejima Island, the gecko was only observed in a limited area of the island, and it has not yet taken root. In all likelihood, as social life resumed after the lifting of the evacuation of the islanders (due to the eruption in 2000), they have since arrived from Hachijojima Island and are currently expanding their distribution.

Table S2-1 List of samples used for this study. The analyzed sequences were deposited in DDBJ for each haplotype.

Clade	Haplotype	Sample No.	Location	Coordinates	Accession No.
Clade 1	Hap1	HC1	Hachijojima, Izu Islands	33.080'N; 139.851'E	LC795866
		HC5	Hachijojima, Izu Islands	33.080'N; 139.851'E	LC795870
		HC12	Hachijojima, Izu Islands	33.079'N; 139.841'E	LC795877
		HC14	Hachijojima, Izu Islands	33.154'N; 139.747'E	LC795879
		HC19	Hachijojima, Izu Islands	33.105'N; 139.775'E	LC795884
		HC25	Hachijojima, Izu Islands	33.109'N; 139.801'E	LC795890
		HC29	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795894
		HC34	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795899
		HC35	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795900
		HC41	Hachijojima, Izu Islands	33.125'N; 139.813'E	LC795906
		HC42	Hachijojima, Izu Islands	33.125'N; 139.813'E	LC795907
		HC45	Hachijojima, Izu Islands	33.125'N; 139.813'E	LC795910
	Hap2	YK1	Yakushima, Nansei Islands	30.270'N; 130.415'E	LC795921
		YK2	Yakushima, Nansei Islands	30.270'N; 130.415'E	LC795922
	Hap3	HC9	Hachijojima, Izu Islands	33.069'N; 139.808'E	LC795874
		HC44	Hachijojima, Izu Islands	33.125'N; 139.813'E	LC795909
		OB2	Okinoerabujima, Nansei Islands	27.361'N; 128.571'E	LC795914
	Hap4	AO28	Aogashima, Izu Islands	32.447'N; 139.761'E	LC795864
	Hap5	HC2	Hachijojima, Izu Islands	33.080'N; 139.851'E	LC795867
	Hap6	AO12	Aogashima, Izu Islands	32.467'N; 139.761'E	LC795848
		AO13	Aogashima, Izu Islands	32.467'N; 139.761'E	LC795849
	Hap7	AO21	Aogashima, Izu Islands	32.452'N; 139.763'E	LC795857
		AO23	Aogashima, Izu Islands	32.453'N; 139.765'E	LC795859

Hap8	HC30	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795895
Hap9	AO29	Aogashima, Izu Islands	32.447'N; 139.761'E	LC795865
Hap10	OB1	Okinoerabujima, Nansei Islands	27.361'N; 128.571'E	LC795913
	OB3	Okinoerabujima, Nansei Islands	27.363'N; 128.552'E	LC795915
	OB4	Okinoerabujima, Nansei Islands	27.363'N; 128.552'E	LC795916
	OB5	Okinoerabujima, Nansei Islands	27.363'N; 128.552'E	LC795917
	OB6	Okinoerabujima, Nansei Islands	27.363'N; 128.552'E	LC795918
	OB7	Okinoerabujima, Nansei Islands	27.363'N; 128.552'E	LC795919
	IB1	Ibusuki, Kyushu	31.235'N; 130.641'E	LC795911
Hap11	HC26	Hachijojima, Izu Islands	33.109'N; 139.801'E	LC795891
	HC27	Hachijojima, Izu Islands	33.109'N; 139.801'E	LC795892
	HC43	Hachijojima, Izu Islands	33.125'N; 139.813'E	LC795908
	AO4	Aogashima, Izu Islands	32.465'N; 139.761'E	LC795840
	AO5	Aogashima, Izu Islands	32.465'N; 139.761'E	LC795841
	AO6	Aogashima, Izu Islands	32.465'N; 139.761'E	LC795842
	AO7	Aogashima, Izu Islands	32.467'N; 139.762'E	LC795843
	AO8	Aogashima, Izu Islands	32.467'N; 139.762'E	LC795844
	AO11	Aogashima, Izu Islands	32.467'N; 139.761'E	LC795847
	AO17	Aogashima, Izu Islands	32.463'N; 139.768'E	LC795853
	AO24	Aogashima, Izu Islands	32.453'N; 139.765'E	LC795860
Hap12	AO10	Aogashima, Izu Islands	32.467'N; 139.762'E	LC795846
Hap13	HC13	Hachijojima, Izu Islands	33.100'N; 139.781'E	LC795878
	HC40	Hachijojima, Izu Islands	33.124'N; 139.813'E	LC795905
	AO18	Aogashima, Izu Islands	32.452'N; 139.763'E	LC795854
Hap14	AO20	Aogashima, Izu Islands	32.452'N; 139.763'E	LC795856
Hap15	HC3	Hachijojima, Izu Islands	33.080'N; 139.851'E	LC795868
	HC4	Hachijojima, Izu Islands	33.080'N; 139.851'E	LC795869
	HC6	Hachijojima, Izu Islands	33.120'N; 139.751'E	LC795871
	HC15	Hachijojima, Izu Islands	33.120'N; 139.751'E	LC795880
	HC16	Hachijojima, Izu Islands	33.120'N; 139.751'E	LC795881
	HC22	Hachijojima, Izu Islands	33.083'N; 139.853'E	LC795887
	HC23	Hachijojima, Izu Islands	33.083'N; 139.853'E	LC795888
	HC24	Hachijojima, Izu Islands	33.083'N; 139.853'E	LC795889
	HC28	Hachijojima, Izu Islands	33.121'N; 139.796'E	LC795893
	HC31	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795896
	HC32	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795897
	HC36	Hachijojima, Izu Islands	33.121'N; 139.803'E	LC795901

		HC37	Hachijojima, Izu Islands	33.121'N; 139.803'E	LC795902
		HC39	Hachijojima, Izu Islands	33.121'N; 139.803'E	LC795904
		AO14	Aogashima, Izu Islands	32.466'N; 139.761'E	LC795850
		AO19	Aogashima, Izu Islands	32.452'N; 139.763'E	LC795855
		AO22	Aogashima, Izu Islands	32.452'N; 139.763'E	LC795858
		AO25	Aogashima, Izu Islands	32.453'N; 139.765'E	LC795861
		AO26	Aogashima, Izu Islands	32.453'N; 139.765'E	LC795862
		AO27	Aogashima, Izu Islands	32.453'N; 139.765'E	LC795863
Clade 2	Hap16	HC11	Hachijojima, Izu Islands	33.066'N; 139.812'E	LC795876
		HC38	Hachijojima, Izu Islands	33.121'N; 139.803'E	LC795903
		AO2	Aogashima, Izu Islands	32.465'N; 139.761'E	LC795838
		AO3	Aogashima, Izu Islands	32.465'N; 139.761'E	LC795839
		AO15	Aogashima, Izu Islands	32.466'N; 139.761'E	LC795851
		AO16	Aogashima, Izu Islands	32.466'N; 139.761'E	LC795852
	Hap17	HC20	Hachijojima, Izu Islands	33.105'N; 139.775'E	LC795885
		AO1	Aogashima, Izu Islands	32.461'N; 139.763'E	LC795837
		AO9	Aogashima, Izu Islands	32.467'N; 139.762'E	LC795845
	Hap18	YR2	Yoroshima, Nansei Islands	28.039'N; 129.161'E	LC795926
	Hap19	KK1	Kakeromajima, Nansei Islands	28.111'N; 129.221'E	LC795912
	Hap20	YR1	Yoroshima, Nansei Islands	28.046'N; 129.165'E	LC795925
	Hap21	YN1	Yoronjima, Nansei Islands	27.022'N; 128.441'E	LC795923
	Hap22	YN2	Yoronjima, Nansei Islands	27.022'N; 128.441'E	LC795924
	Hap23	OW1	Okinawajima, Nansei Islands	26.862'N; 128.265'E	LC795920
	Hap24	HC7	Hachijojima, Izu Islands	33.069'N; 139.808'E	LC795872
		HC10	Hachijojima, Izu Islands	33.069'N; 139.808'E	LC795875
	Hap25	HC8	Hachijojima, Izu Islands	33.069'N; 139.808'E	LC795873
	Hap26	HC17	Hachijojima, Izu Islands	33.105'N; 139.775'E	LC795882
		HC18	Hachijojima, Izu Islands	33.105'N; 139.775'E	LC795883
		HC21	Hachijojima, Izu Islands	33.105'N; 139.775'E	LC795886
		HC33	Hachijojima, Izu Islands	33.118'N; 139.799'E	LC795898
G. shibatai	-	TKR1	Takarajima, Kagoshima	29.157'N; 129.212'E	LC795927

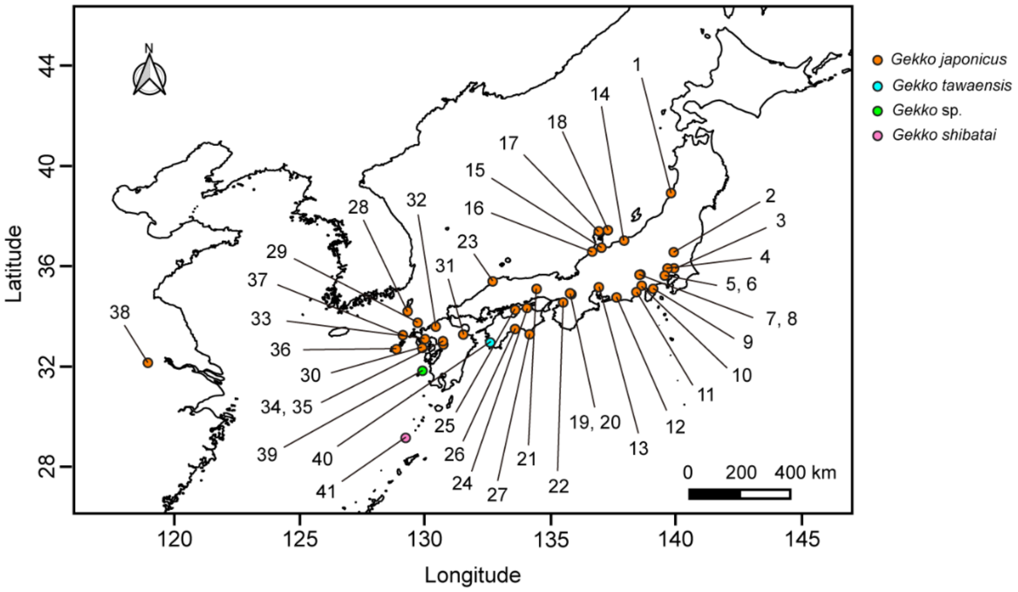

Fig. S3-1. Sampling sites of this study. The orange dots indicate where Gekko japonicus was collected. Dots of other colors indicate where the outgroup geckos were collected.

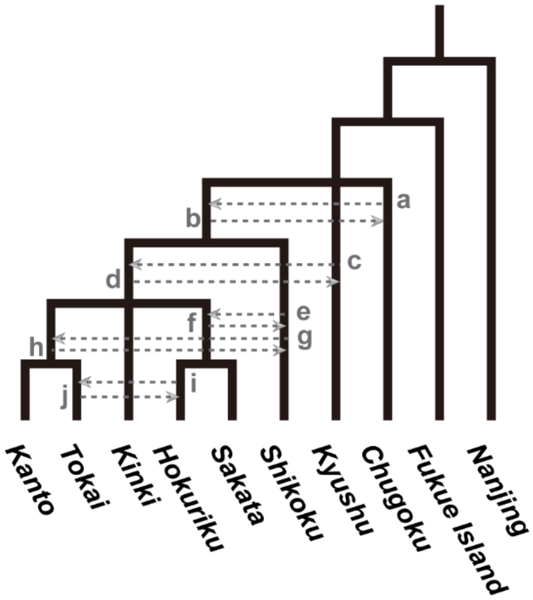

Fig. S3-2. The rejected scenario, M2. Migration between non-adjacent regions was considered, and they are represented by the arrows a~j.

Table S3-1. List of samples used for this study. For each sample number, the scientific name, the city where the sample was collected, the regional group (partially different from the Japanese administrative division) sorted in the analysis, the latitude and longitude are shown.

Sample No.	Accession No.	Species	Cities	Region	latitude	longitude
s1	DRR391249	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.92	139.831
s2	DRR391250	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.92	139.831
s3	DRR391251	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.921	139.831
s4	DRR391252	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.911	139.84
s5	DRR391253	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.906	139.841
s7	DRR391255	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.905	139.842
s8	DRR391256	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.911	139.837
s9	DRR391257	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.913	139.837
s10	DRR391258	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.914	139.84
s11	DRR391259	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.914	139.839
s12	DRR391260	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.92	139.842
s13	DRR391261	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.92	139.832
s14	DRR391262	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.92	139.831
s15	DRR391263	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.919	139.828
s16	DRR391264	*G. japonicus*	1. Sakata,Yamagata	Tohoku	38.918	139.829
s17	DRR391265	*G. japonicus*	2. Utsunomiya,Tochigi	Kanto	36.562	139.886
s18	DRR391266	*G. japonicus*	2. Utsunomiya,Tochigi	Kanto	36.56	139.895
s19	DRR391267	*G. japonicus*	2. Utsunomiya,Tochigi	Kanto	36.562	139.883
s20	DRR391268	*G. japonicus*	2. Utsunomiya,Tochigi	Kanto	36.562	139.885
s21	DRR391269	*G. japonicus*	3. Noda,Chiba	Kanto	35.919	139.9
s22	DRR391270	*G. japonicus*	3. Noda,Chiba	Kanto	35.919	139.9
s23	DRR391271	*G. japonicus*	3. Noda,Chiba	Kanto	35.919	139.9
s25	DRR391273	*G. japonicus*	4. Saitama,Saitama	Kanto	35.917	139.642
s26	DRR391274	*G. japonicus*	5. Tama,Tokyo	Kanto	35.639	139.454
s27	DRR391275	*G. japonicus*	5. Tama,Tokyo	Kanto	35.638	139.453
s28	DRR391276	*G. japonicus*	5. Tama,Tokyo	Kanto	35.634	139.45
s29	DRR391277	*G. japonicus*	6. Kawasaki,Kanagawa	Kanto	35.629	139.529
s30	DRR391278	*G. japonicus*	6. Kawasaki,Kanagawa	Kanto	35.629	139.53
s31	DRR391279	*G. japonicus*	6. Kawasaki,Kanagawa	Kanto	35.628	139.529
s32	DRR391280	*G. japonicus*	7. Kofu, Yamanashi	Tokai	35.676	138.566
s33	DRR391281	*G. japonicus*	7. Kofu, Yamanashi	Tokai	35.665	138.574
s34	DRR391282	*G. japonicus*	7. Kofu, Yamanashi	Tokai	35.663	138.579

s35	DRR391283	*G. japonicus*	7. Kofu, Yamanashi	Tokai	35.663	138.579
s36	DRR391284	*G. japonicus*	7. Kofu, Yamanashi	Tokai	35.659	138.578
s37	DRR391285	*G. japonicus*	8. Kai, Yamanashi	Tokai	35.661	138.525
s38	DRR391286	*G. japonicus*	8. Kai, Yamanashi	Tokai	35.661	138.525
s39	DRR391287	*G. japonicus*	9. Atami,Shizuoka	Kanto	35.1	139.068
s40	DRR391288	*G. japonicus*	10. Fujinomiya, Shizuoka	Tokai	35.227	138.611
s41	DRR391289	*G. japonicus*	10. Fujinomiya, Shizuoka	Tokai	35.221	138.607
s42	DRR391290	*G. japonicus*	10. Fujinomiya, Shizuoka	Tokai	35.221	138.607
s43	DRR391291	*G. japonicus*	10. Fujinomiya, Shizuoka	Tokai	35.223	138.611
s44	DRR391292	*G. japonicus*	11. Shizuoka, Shizuoka	Tokai	34.97	138.401
s45	DRR391293	*G. japonicus*	11. Shizuoka, Shizuoka	Tokai	34.981	138.384
s46	DRR391294	*G. japonicus*	11. Shizuoka, Shizuoka	Tokai	34.978	138.383
s47	DRR391295	*G. japonicus*	11. Shizuoka, Shizuoka	Tokai	34.978	138.383
s48	DRR391296	*G. japonicus*	11. Shizuoka, Shizuoka	Tokai	34.978	138.385
s49	DRR391297	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.757	137.609
s50	DRR391298	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.765	137.614
s51	DRR391299	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.764	137.614
s52	DRR391300	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.763	137.613
s53	DRR391301	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.712	137.726
s54	DRR391302	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.726
s55	DRR391303	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.711	137.726
s56	DRR391304	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.723
s57	DRR391305	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.724
s58	DRR391306	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.724
s59	DRR391307	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.724
s60	DRR391308	*G. japonicus*	12. Hamamatsu, Shizuoka	Tokai	34.713	137.724
s61	DRR391309	*G. japonicus*	13. Nagoya, Aichi	Tokai	35.164	136.904
s62	DRR391310	*G. japonicus*	13. Nagoya, Aichi	Tokai	35.163	136.903
s63	DRR391311	*G. japonicus*	13. Nagoya, Aichi	Tokai	35.154	136.921
s65	DRR391313	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.04	137.863
s66	DRR391314	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.04	137.865
s67	DRR391315	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.041	137.866
s68	DRR391316	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.041	137.866

s69	DRR391317	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.036	137.859
s70	DRR391318	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.032	137.859
s71	DRR391319	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.023	137.866
s72	DRR391320	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.023	137.866
s73	DRR391321	*G. japonicus*	14. Itoigawa, Nigata	Hokuriku	37.023	137.866
s74	DRR391322	*G. japonicus*	15. Takaoka, Toyama	Hokuriku	36.741	137.01
s75	DRR391323	*G. japonicus*	15. Takaoka, Toyama	Hokuriku	36.744	137.011
s76	DRR391324	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.585	136.64
s77	DRR391325	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.58	136.64
s78	DRR391326	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.587	136.649
s79	DRR391327	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.57	136.661
s80	DRR391328	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.569	136.659
s81	DRR391329	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.568	136.657
s82	DRR391330	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.568	136.656
s83	DRR391331	*G. japonicus*	16. Kanazawa, Ishikawa	Hokuriku	36.565	136.655
s84	DRR391332	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.395	136.905
s85	DRR391333	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.397	136.899
s86	DRR391334	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.398	136.897
s87	DRR391335	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.395	136.906
s88	DRR391336	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.395	136.906
s89	DRR391337	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.395	136.906
s90	DRR391338	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.392	136.893
s91	DRR391339	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.393	136.895
s92	DRR391340	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.391	136.897
s93	DRR391341	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.389	136.898
s94	DRR391342	*G. japonicus*	17. Wajima, Ishikawa	Hokuriku	37.386	136.898
s95	DRR391343	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.439	137.264
s96	DRR391344	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.437	137.261
s97	DRR391345	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.437	137.259
s98	DRR391346	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.436	137.261
s99	DRR391347	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.435	137.261
s100	DRR391348	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.429	137.253
s101	DRR391349	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.436	137.264
s102	DRR391350	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.437	137.265
s103	DRR391351	*G. japonicus*	18. Suzu, Ishikawa	Hokuriku	37.437	137.265
s104	DRR391352	*G. japonicus*	19. Uji, Kyoto	Kinki	34.887	135.814

s105	DRR391353	*G. japonicus*	19. Uji, Kyoto	Kinki	34.891	135.81
s106	DRR391354	*G. japonicus*	19. Uji, Kyoto	Kinki	34.888	135.809
s107	DRR391355	*G. japonicus*	19. Uji, Kyoto	Kinki	34.888	135.803
s108	DRR391356	*G. japonicus*	20. Kyoto, Kyoto	Kinki	34.923	135.758
s109	DRR391357	*G. japonicus*	21. Sayo, Hyogo	Kinki	35.098	134.427
s110	DRR391358	*G. japonicus*	21. Sayo, Hyogo	Kinki	35.098	134.427
s111	DRR391359	*G. japonicus*	22. Sakai, Osaka	Kinki	34.56	135.483
s112	DRR391360	*G. japonicus*	22. Sakai, Osaka	Kinki	34.559	135.479
s113	DRR391361	*G. japonicus*	22. Sakai, Osaka	Kinki	34.559	135.479
s114	DRR391362	*G. japonicus*	22. Sakai, Osaka	Kinki	34.56	135.483
s115	DRR391363	*G. japonicus*	22. Sakai, Osaka	Kinki	34.56	135.483
s116	DRR391364	*G. japonicus*	22. Sakai, Osaka	Kinki	34.559	135.483
s117	DRR391365	*G. japonicus*	22. Sakai, Osaka	Kinki	34.559	135.484
s118	DRR391366	*G. japonicus*	22. Sakai, Osaka	Kinki	34.56	135.485
s119	DRR391367	*G. japonicus*	22. Sakai, Osaka	Kinki	34.56	135.483
s120	DRR391368	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.4	132.676
s121	DRR391369	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.4	132.676
s122	DRR391370	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s123	DRR391371	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s124	DRR391372	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s125	DRR391373	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s126	DRR391374	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s127	DRR391375	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s128	DRR391376	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s129	DRR391377	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.398	132.686
s130	DRR391378	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.393	132.687
s131	DRR391379	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.507	132.86
s132	DRR391380	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.365	132.751
s133	DRR391381	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.364	132.756
s134	DRR391382	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.371	132.755
s135	DRR391383	*G. japonicus*	23. Izumo, Shimane	Chugoku	35.374	132.758
s136	DRR391384	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.336	134.041
s137	DRR391385	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.335	134.048
s138	DRR391386	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.347	134.045
s139	DRR391387	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.359	134.104
s140	DRR391388	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.359	134.104

s141	DRR391389	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.359	134.104
s142	DRR391390	*G. japonicus*	24. Takamatsu, Kagawa	Shikoku	34.358	134.102
s143	DRR391391	*G. japonicus*	25. Mitoyo, Kagawa	Shikoku	34.264	133.574
s144	DRR391392	*G. japonicus*	26. Kochi, Kochi	Shikoku	33.499	133.568
s145	DRR391393	*G. japonicus*	26. Kochi, Kochi	Shikoku	33.495	133.57
s146	DRR391394	*G. japonicus*	26. Kochi, Kochi	Shikoku	33.494	133.566
s147	DRR391395	*G. japonicus*	27. Muroto, Kochi	Shikoku	33.288	134.149
s150	DRR391398	*G. japonicus*	28. Tsushima, Nagasaki	Kyushu	34.202	129.292
s151	DRR391399	*G. japonicus*	28. Tsushima, Nagasaki	Kyushu	34.112	129.211
s152	DRR391400	*G. japonicus*	28. Tsushima, Nagasaki	Kyushu	34.204	129.292
s153	DRR391401	*G. japonicus*	29. Iki, Nagasaki	Kyushu	33.749	129.691
s154	DRR391402	*G. japonicus*	29. Iki, Nagasaki	Kyushu	33.746	129.689
s155	DRR391403	*G. japonicus*	29. Iki, Nagasaki	Kyushu	33.814	129.759
s156	DRR391404	*G. japonicus*	30. Nagasaki, Nagasaki	Kyushu	32.742	129.875
s157	DRR391405	*G. japonicus*	30. Nagasaki, Nagasaki	Kyushu	32.741	129.878
s158	DRR391406	*G. japonicus*	30. Nagasaki, Nagasaki	Kyushu	32.752	129.881
s159	DRR391407	*G. japonicus*	31. Beppu, Oita	Kyushu	33.279	131.507
s160	DRR391408	*G. japonicus*	31. Beppu, Oita	Kyushu	33.273	131.506
s161	DRR391409	*G. japonicus*	31. Beppu, Oita	Kyushu	33.288	131.495
s162	DRR391410	*G. japonicus*	32. Fukuoka, Fukuoka	Kyushu	33.593	130.411
s163	DRR391411	*G. japonicus*	32. Fukuoka, Fukuoka	Kyushu	33.59	130.404
s164	DRR391412	*G. japonicus*	32. Fukuoka, Fukuoka	Kyushu	33.596	130.413
s165	DRR391413	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s166	DRR391414	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s167	DRR391415	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s168	DRR391416	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s169	DRR391417	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s170	DRR391418	*G. japonicus*	33. Ureshino, Saga	Kyushu	33.097	129.986
s171	DRR391419	*G. japonicus*	34. Kumamoto, Kumamoto	Kyushu	32.881	130.711
s172	DRR391420	*G. japonicus*	35. Yamaga, Kumamoto	Kyushu	33.012	130.695
s173	DRR391421	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.695	128.847
s174	DRR391422	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.753	128.761
s175	DRR391423	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.753	128.761
s176	DRR391424	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.694	128.844
s177	DRR391425	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.688	128.853
s178	DRR391426	*G. japonicus*	36. Fukue, Nagasaki	Kyushu	32.753	128.761

s179	DRR391427	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.254	129.099
s181	DRR391429	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.254	129.099
s182	DRR391430	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.264	129.129
s183	DRR391431	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.264	129.129
s184	DRR391432	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.265	129.132
s185	DRR391433	*G. japonicus*	37. Uku, Nagasaki	Kyushu	33.268	129.118
s187	DRR391435	*G. japonicus*	38. Nanjing, China	China	32.152	118.948
s188	DRR391436	*G. japonicus*	38. Nanjing, China	China	32.152	118.948
s189	DRR391437	*G. japonicus*	38. Nanjing, China	China	32.152	118.948
s190	DRR391438	*Gekko* sp.	39. Kamikoshikijima, Kagoshima	Kyushu	31.833	129.882
s191	DRR391439	*G. tawaensis*	40. Minamiuwa, Ehime	Shikoku	32.961	132.581
s192	DRR391440	*G. shibatai*	41. Takarajima, Kagoshima	Kyushu	29.157	129.212

Table S3-2. Observed summary statistics. The population number is 1: Nanjing, 2: Fukue Island, 3: Kyushu-T, 4: Chugoku, 5: Shikoku, 6: Kinki, 7: Hokuriku, 8: Tokai, 9: Kanto, 10: Tohoku.

mean_K	0
sd_K	0
tot_K	0
mean_H	0
sd_H	0
tot_H	0
prS_1	0
prS_2	0
prS_3	0
prS_4	0
prS_5	0
prS_6	0
prS_7	0
prS_8	0
prS_9	0
prS_10	0
mean_S	0
sd_S	0
tot_S	30477
mean_D	0
sd_D	0
mean_FS	0
sd_FS	0
Pi_1	149.347
Pi_2	63.1218
Pi_3	385.127
Pi_4	347.186
Pi_5	457.208
Pi_6	479.385
Pi_7	495.967
Pi_8	472.462
Pi_9	532.985
Pi_10	628.467
mean_Pi	401.126
sd_Pi	174.238
FST_2_1	0.264146
FST_3_1	0.175749
FST_3_2	0.033407
FST_4_1	0.175199
FST_4_2	0.108861
FST_4_3	0.068237
FST_5_1	0.159174
FST_5_2	0.048591
FST_5_3	0.018468
FST_5_4	0.062563
FST_6_1	0.232325
FST_6_2	0.189326
FST_6_3	0.121742
FST_6_4	0.116349
FST_6_5	0.149985
FST_7_1	0.165169
FST_7_2	0.076414
FST_7_3	0.03775
FST_7_4	0.064931
FST_7_5	0.037961
FST_7_6	0.112544
FST_8_1	0.153944
FST_8_2	0.158682
FST_8_3	0.107446
FST_8_4	0.082519
FST_8_5	0.098439
FST_8_6	0.070669
FST_8_7	0.088496
FST_9_1	0.171654
FST_9_2	0.235457
FST_9_3	0.161806
FST_9_4	0.1385
FST_9_5	0.143014
FST_9_6	0.133362
FST_9_7	0.126742
FST_9_8	0.05274
FST_10_1	0.363994
FST_10_2	0.362984
FST_10_3	0.320936
FST_10_4	0.278507
FST_10_5	0.299032
FST_10_6	0.274422
FST_10_7	0.266529
FST_10_8	0.188675
FST_10_9	0.232314

Table S3-3. Prior distribution of parameters used for divergence time estimation. All parameters were given a uniform distribution (unif) from the lower bound to the upper bound.

Parameters	**Distribution**	**S1**		**S2**	
		Minimum	**Max**	**Minimum**	**Max**
MAF	unif	0.001	0.5	0.001	0.5
log10_Nanjing	unif	2	6	2	6
log10_Fukuejima	unif	3	7	3	7
log10_Kyushu	unif	3	7	3	7
log10_Chugoku	unif	3	7	3	7
log10_Shikoku	unif	2	6	2	6
log10_Kinki	unif	3	7	3	7
log10_Hokuriku	unif	2	6	2	6
log10_Tokai	unif	2	6	2	6
log10_Kanto	unif	2	6	2	6
log10_Tohoku	unif	2	6	2	6
log10_t (Tokai-Kanto)	unif	1.5	3	1.5	3
log10_t (Hokuriku-Tohoku)	unif	1.5	3	1.5	3
log10_t (Kinki-Tokai)	unif	2	4	2	4
log10_t (Kinki-Hokuriku)	unif	2	4	2	4
log10_t (Kinki-Shikoku)	unif	2	4	2	4
log10_t (Shikoku-Kyushu)	unif	2	4	2	4
log10_t (Kyushu-Chugoku)	unif	2.3	4	2.3	4
log10_t (Fukue-Kyushu)	unif	3	4	3	4
log10_t (Nanjing-Fukue)	unif	3	4	3	4
log10_a	unif	-	-	-15	-11
log10_b	unif	-	-	-15	-11
log10_c	unif	-	-	-15	-11
log10_d	unif	-	-	-15	-11
log10_e	unif	-	-	-15	-11
log10_f	unif	-	-	-15	-11

log10_g	unif	-	-	-15	-11
log10_h	unif	-	-	-15	-11
log10_i	unif	-	-	-15	-11
log10_j	unif	-	-	-15	-11

Table S3-4. Posterior distribution for each parameter simulated by ABC toolbox. The median, standard deviation, and 95% BCI (bottom 2.5% and top 2.5%) are summarized.

Parameters	**S1**				**S2**			
	Median	**sd**	**0.025 BCI**	**0.975 BCI**	**Median**	**sd**	**0.025 BCI**	**0.975 BCI**
MAF	0.2471965	0.1446179	0.0126980	0.4877831	0.2504165	0.1435474	0.01383063	0.48882433
Nanjing	5422	17335.11	129	61194.52	6608.5	17322.71	131	59837.8
Fukuejima	168391	2152355	2235.825	8103024.1	170364.5	2220956	2334.775	8438453.475
Kyushu	171538	2125552	3527.925	8120696.8	159386.5	2102830	3791.9	8085417.4
Chugoku	149669	2107162	1893.9	7960816.2	129445.5	2087710	1692.9	7972953
Shikoku	34483.5	230063.6	851.925	852486.05	35756	226736.6	1001.975	836263.75
Kinki	68388	1955754	1303.95	7622225.2	90661	1984377	1497.825	7642719.05
Hokuriku	5020.5	184499.6	227	748690.9	8213.5	198886.8	257	779911
Tokai	21940	220555.1	501.975	840032.25	23680	218656.4	514.975	824810
Kanto	12935.5	210098.3	175.975	800921.75	13372.5	212157.7	181.975	815196.85
Tohoku	890	155516.4	110	637445.3	1382.5	167506	113	675582.1
t (Tokai-Kanto)	80	108.6256	33	426	83	130.4955	33	528.05
t (Hokuriku-Tohoku)	100	131.4961	34	543.025	106	152.4584	34	629.05
t (Kinki-Tokai)	253	297.9262	105	1168.025	294	435.9764	106	1634.15
t (Kinki-Hokuriku)	260	318.4838	106	1248.3	308	437.1724	108	1660.05
t (Shikoku-Kinki)	618.5	605.4085	185	2457.075	855	921.2764	213	3750
t (Kyushu-Shikoku)	1380	1345.095	325	5463.15	1667.5	1424.605	384	5815.3
t (Kyushu-Chugoku)	1434	1323.038	338.975	5385.05	693.5	1138.353	213	4478.075
t (Fukue-Kyushu)	3485.5	1992.775	1134.975	8372.075	3503	1999.646	1143.975	8338.1

t (Nanjing-Fukue)	6505.5	2294.071	1954.95	9830.025	6430	2317.965	1918.975	9848.025
a	-	-	-	-	1.01E-13	2.06E-12	1.24E-15	7.92E-12
b	-	-	-	-	9.06E-14	2.07E-12	1.24E-15	8.02E-12
c	-	-	-	-	9.76E-14	2.08E-12	1.24E-15	8.14E-12
d	-	-	-	-	1.01E-13	2.03E-12	1.24E-15	7.82E-12
e	-	-	-	-	9.27E-14	2.02E-12	1.25E-15	7.86E-12
f	-	-	-	-	1.07E-13	2.09E-12	1.24E-15	8.08E-12
g	-	-	-	-	1.01E-13	2.04E-12	1.27E-15	7.95E-12
h	-	-	-	-	1.03E-13	2.09E-12	1.28E-15	8.07E-12
i	-	-	-	-	9.81E-14	2.03E-12	1.28E-15	7.80E-12
j	-	-	-	-	1.01E-13	2.08E-12	1.26E-15	7.97E-12

Table S4-1. List of samples used for this study. For each sample number, the scientific name, the location where the sample was collected, the latitude, the longitude and microhabitat which the sample was used are shown. Microhabitats for samples that were not suitable for modeling or accession numbers for samples that were not sequenced are blank.

Sample No.	Accession No.	Species	Location	Latitude	Longitude	Microhabitat
Lib22-1	now submitting	*Gekko japonicus*	Fukue, Nagasaki	32.758	128.825	Urban_artificial
Lib22-2	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.848	129.923	Urban_natural
Lib22-3	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.848	129.923	Urban_natural
Lib22-4	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.848	129.923	Urban_natural
Lib22-5	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.848	129.922	Urban_artificial
Lib22-6	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.862	129.923	Non- urban_artificial
Lib22-7	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.861	129.924	Non-urban_natural
Lib22-8	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.864	129.923	Non- urban_artificial
Lib22-9	now submitting	*Gekko* sp.	Nakakoshiki, Kagoshima	31.816	129.839	Non- urban_artificial
Lib22-10	now submitting	*Gekko* sp.	Nakakoshiki, Kagoshima	31.816	129.839	Non- urban_artificial
Lib22-11	now submitting	*Gekko* sp.	Nakakoshiki, Kagoshima	31.816	129.839	Non- urban_artificial
Lib22-12	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.832	129.849	Non- urban_artificial
Lib22-13	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.833	129.882	Non- urban_artificial
Lib22-14	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.891	Non- urban_artificial
Lib22-15	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.891	Non- urban_artificial
Lib22-16	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.892	Non- urban_artificial
Lib22-17	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.892	Non- urban_artificial
Lib22-18	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.862	129.877	Non-urban_natural
Lib22-19	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.878	129.860	Non- urban_artificial
Lib22-20	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.878	129.860	Non- urban_artificial
Lib22-21	now submitting	*Gekko* sp.	Kamikoshiki, Kagoshima	31.864	129.838	Urban_artificial
Lib22-22	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.639	129.701	Urban_artificial
Lib22-23	now	*Gekko* sp.	Shimokoshiki,	31.639	129.701	Urban_artificial

	submitting		Kagoshima			
Lib22-24	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.700	Urban_artificial
Lib22-25	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.700	Urban_artificial
Lib22-26	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.637	129.700	Urban_artificial
Lib22-27	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.693	Urban_artificial
Lib22-28	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.693	Urban_artificial
Lib22-29	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.664	129.683	Urban_artificial
Lib22-30	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.664	129.682	Non- urban_artificial
Lib22-31	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.664	129.683	Non- urban_artificial
Lib22-32	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.660	129.685	Urban_artificial
Lib22-33	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.624	129.689	Non- urban_artificial
Lib22-34	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.723	Urban_artificial
Lib22-35	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.722	Urban_artificial
Lib22-36	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.691	129.693	Urban_artificial
Lib22-37	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.692	129.694	Non- urban_artificial
Lib22-38	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.692	129.694	Non- urban_artificial
Lib22-39	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.692	129.693	Urban_artificial
Lib22-40	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.693	129.695	Urban_artificial
Lib22-41	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.693	129.694	Urban_artificial
Lib22-42	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.702	129.737	Urban_artificial
Lib22-43	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.703	129.737	Urban_artificial
Lib22-44	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.751	129.782	Non- urban_artificial
Lib22-45	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.751	129.782	Non- urban_artificial
Lib22-46	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.751	129.782	Non- urban_artificial
Lib22-47	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.775	129.794	Non- urban_artificial
Lib22-48	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.781	129.795	Urban_artificial
Lib22-49	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.700	129.736	Urban_artificial
Lib22-50	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.700	129.736	-

Lib22-51	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.700	129.736	Urban_artificial
Lib22-52	now submitting	*Gekko* sp.	Shimokoshiki, Kagoshima	31.701	129.738	-
Lib22-53	now submitting	*Gekko* sp.	Hisakajima, Nagasaki	32.819	128.881	
Lib22-54	now submitting	*Gekko* sp.	Hisakajima, Nagasaki	32.822	128.898	
Lib22-55	now submitting	*Gekko* sp.	Hisakajima, Nagasaki	32.812	128.903	
Lib22-56	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.828	129.049	-
Lib22-57	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.828	129.052	Urban_artificial
Lib22-58	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.827	129.050	Non- urban_artificial
Lib22-59	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.843	129.054	Non- urban_artificial
Lib22-60	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.843	129.054	Non- urban_artificial
Lib22-61	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.843	129.054	Non- urban_artificial
Lib22-62	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.839	129.061	Urban_artificial
Lib22-63	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.979	129.140	Non- urban_artificial
Lib22-64	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.957	129.149	Urban_artificial
Lib22-65	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.957	129.149	Urban_artificial
Lib22-66	now submitting	*Gekko* sp.	Nakadori, Nagasaki	32.958	129.150	Non-urban_natural
Lib22-67	now submitting	*Gekko* sp.	Hirado, Nagasaki	33.179	129.369	Non- urban_artificial
Lib22-68	now submitting	*Gekko* sp.	Hirado, Nagasaki	33.185	129.390	Non- urban_artificial
Lib22-69	now submitting	*Gekko* sp.	Hirado, Nagasaki	33.185	129.390	Non- urban_artificial
Lib22-70	now submitting	*Gekko* sp.	Hirado, Nagasaki	33.192	129.357	Urban_artificial
Lib23-1	now submitting	*Gekko japonicus*	Fukue, Nagasaki	32.758	128.825	Urban_artificial
Lib23-2	now submitting	*Gekko hokouensis*	Kamikoshiki, Kagoshima	31.839	129.920	Urban_artificial
Lib23-3	now submitting	*Gekko hokouensis*	Kamikoshiki, Kagoshima	31.839	129.921	Urban_artificial
Lib23-4	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.803	129.839	Urban_artificial
Lib23-5	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.803	129.839	Urban_artificial
Lib23-6	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.803	129.839	Urban_natural
Lib23-7	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.838	Urban_artificial
Lib23-8	now	*Gekko*	Nakakoshiki, Kagoshima	31.802	129.838	Urban_artificial

	submitting	*hokouensis*				
Lib23-9	now submitting	*Gekko hokouensis*	Kamikoshiki, Kagoshima	31.840	129.918	Urban_natural
Lib23-10	now submitting	*Gekko hokouensis*	Kamikoshiki, Kagoshima	31.841	129.918	Urban_artificial
Lib23-11	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	-
Lib23-12	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	-
Lib23-13	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	-
Lib23-14	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.009	129.186	Urban_natural
Lib23-15	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.990	129.174	Urban_artificial
Lib23-16	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.987	129.117	Urban_artificial
Lib23-17	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.176	Urban_artificial
Lib23-18	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.176	Urban_artificial
Lib23-19	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.176	Urban_artificial
Lib23-20	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.001	129.145	Non- urban_artificial
Lib23-21	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.001	129.145	Non- urban_artificial
Lib23-22	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	Non- urban_artificial
Lib23-23	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	Non- urban_artificial
Lib23-24	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.002	129.145	Non- urban_artificial
Lib23-25	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.177	Urban_artificial
Lib23-26	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	33.001	129.160	Urban_artificial
Lib23-27	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.177	Urban_artificial
Lib23-28	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.998	129.178	Urban_artificial
Lib23-29	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.998	129.178	Urban_artificial
Lib23-30	now submitting	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.176	Urban_artificial
Lib23-31	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.230	130.645	Urban_artificial
Lib23-32	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.235	130.641	Urban_natural
Lib23-33	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.239	130.647	Urban_artificial
Lib23-34	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.208	130.540	Urban_artificial
Lib23-35	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.208	130.540	Urban_artificial

Lib23-36	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-37	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-38	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-39	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-40	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-41	now submitting	*Gekko hokouensis*	Ibusuki, Kagoshima	31.212	130.546	Urban_artificial
Lib23-42	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.838	Urban_artificial
Lib23-43	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.838	Urban_artificial
Lib23-44	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.840	Urban_artificial
Lib23-45	now submitting	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.839	Urban_artificial
Lib23-46	now submitting	*Gekko hokouensis*	Tokunoshima, Kagoshima	27.727	129.018	-
Lib23-47	now submitting	*Gekko hokouensis*	Okinoerabu, Kagoshima	27.363	128.552	-
Lib23-48	now submitting	*Gekko hokouensis*	Okinoerabu, Kagoshima	27.363	128.552	-
Lib23-49	now submitting	*Gekko hokouensis*	Yakushima, Kagoshima	30.27	130.415	-
Lib23-50	now submitting	*Gekko hokouensis*	Okinawa, Okinawa	26.686	128.128	-
NonSeq-1	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.817	130.302	Urban_artificial
NonSeq-2	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.816	130.300	Urban_artificial
NonSeq-3	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.826	130.293	Urban_artificial
NonSeq-4	-	*Gekko japonicus*	Kamikoshiki, Kagoshima	31.848	129.922	Urban_artificial
NonSeq-5	-	*Gekko japonicus*	Nakadori, Nagasaki	32.983	129.117	Urban_artificial
NonSeq-6	-	*Gekko japonicus*	Hirado, Nagasaki	33.188	129.366	Urban_artificial
NonSeq-7	-	*Gekko japonicus*	Hirado, Nagasaki	33.188	129.366	Urban_artificial
NonSeq-8	-	*Gekko japonicus*	Hirado, Nagasaki	33.208	129.450	Urban_artificial
NonSeq-9	-	*Gekko japonicus*	Hirado, Nagasaki	33.210	129.450	Urban_artificial
NonSeq-10	-	*Gekko japonicus*	Hirado, Nagasaki	33.211	129.448	Urban_artificial
NonSeq-11	-	*Gekko japonicus*	Fukue, Nagasaki	32.758	128.825	Urban_artificial
NonSeq-12	-	*Gekko japonicus*	Fukue, Nagasaki	32.758	128.825	Urban_artificial
NonSeq-	-	*Gekko*	Fukue, Nagasaki	32.758	128.825	Urban_artificial

13		*japonicus*				
NonSeq-14	-	*Gekko japonicus*	Fukue, Nagasaki	32.757	128.835	Urban_artificial
NonSeq-15	-	*Gekko japonicus*	Fukue, Nagasaki	32.756	128.837	Urban_artificial
NonSeq-16	-	*Gekko japonicus*	Fukue, Nagasaki	32.664	128.856	Non- urban_artificial
NonSeq-17	-	*Gekko japonicus*	Fukue, Nagasaki	32.653	128.796	Non- urban_artificial
NonSeq-18	-	*Gekko japonicus*	Fukue, Nagasaki	32.756	128.837	Urban_artificial
NonSeq-19	-	*Gekko japonicus*	Fukue, Nagasaki	32.663	128.852	Urban_artificial
NonSeq-20	-	*Gekko japonicus*	Fukue, Nagasaki	32.663	128.852	Urban_artificial
NonSeq-21	-	*Gekko japonicus*	Fukue, Nagasaki	32.663	128.852	Urban_artificial
NonSeq-22	-	*Gekko japonicus*	Fukue, Nagasaki	32.663	128.852	Urban_artificial
NonSeq-23	-	*Gekko japonicus*	Fukue, Nagasaki	32.662	128.851	Non- urban_artificial
NonSeq-24	-	*Gekko japonicus*	Fukue, Nagasaki	32.650	128.810	Urban_artificial
NonSeq-25	-	*Gekko japonicus*	Fukue, Nagasaki	32.705	128.846	Urban_artificial
NonSeq-26	-	*Gekko japonicus*	Fukue, Nagasaki	32.704	128.846	Urban_artificial
NonSeq-27	-	*Gekko japonicus*	Fukue, Nagasaki	32.701	128.845	Urban_artificial
NonSeq-28	-	*Gekko japonicus*	Fukue, Nagasaki	32.662	128.851	Non- urban_artificial
NonSeq-29	-	*Gekko japonicus*	Fukue, Nagasaki	32.664	128.851	Urban_artificial
NonSeq-30	-	*Gekko japonicus*	Fukue, Nagasaki	32.664	128.851	Urban_artificial
NonSeq-31	-	*Gekko japonicus*	Fukue, Nagasaki	32.662	128.854	Non- urban_artificial
NonSeq-32	-	*Gekko japonicus*	Fukue, Nagasaki	32.656	128.854	Non- urban_artificial
NonSeq-33	-	*Gekko japonicus*	Fukue, Nagasaki	32.647	128.816	Urban_artificial
NonSeq-34	-	*Gekko japonicus*	Fukue, Nagasaki	32.648	128.811	Non- urban_artificial
NonSeq-35	-	*Gekko japonicus*	Fukue, Nagasaki	32.649	128.811	Urban_artificial
NonSeq-36	-	*Gekko japonicus*	Fukue, Nagasaki	32.649	128.811	Urban_artificial
NonSeq-37	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.849	130.207	Urban_artificial
NonSeq-38	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.850	130.205	Urban_artificial
NonSeq-39	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.851	130.202	Urban_artificial
NonSeq-40	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.850	130.204	Urban_artificial

NonSeq-41	-	*Gekko japonicus*	Satsumasendai, Kagoshima	31.849	130.205	Urban_artificial
NonSeq-42	-	*Gekko japonicus*	Ichikikushikino, Kagoshima	31.755	130.200	Urban_artificial
NonSeq-43	-	*Gekko japonicus*	Kamikoshiki, Kagoshima	31.863	129.864	Urban_artificial
NonSeq-44	-	*Gekko japonicus*	Kamikoshiki, Kagoshima	31.864	129.864	Urban_artificial
NonSeq-45	-	*Gekko japonicus*	Kamikoshiki, Kagoshima	31.863	129.864	Urban_artificial
NonSeq-46	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.864	129.837	Urban_artificial
NonSeq-47	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.865	129.838	Urban_natural
NonSeq-48	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.853	129.888	Non- urban_artificial
NonSeq-49	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.892	Non- urban_artificial
NonSeq-50	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.863	129.923	Non- urban_artificial
NonSeq-51	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.863	129.923	Non- urban_artificial
NonSeq-52	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.863	129.923	Non- urban_artificial
NonSeq-53	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.863	129.923	Non- urban_artificial
NonSeq-54	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.864	129.923	Non- urban_artificial
NonSeq-55	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.848	129.923	Urban_natural
NonSeq-56	-	*Gekko* sp.	Nakakoshiki, Kagoshima	31.801	129.843	Urban_artificial
NonSeq-57	-	*Gekko* sp.	Nakakoshiki, Kagoshima	31.803	129.844	Non- urban_artificial
NonSeq-58	-	*Gekko* sp.	Nakakoshiki, Kagoshima	31.816	129.839	Non- urban_artificial
NonSeq-59	-	*Gekko* sp.	Nakakoshiki, Kagoshima	31.817	129.839	Non- urban_artificial
NonSeq-60	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.846	129.848	Non-urban_natural
NonSeq-61	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.846	129.848	Non- urban_artificial
NonSeq-62	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.632	129.711	Urban_artificial
NonSeq-63	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.635	129.711	Urban_artificial
NonSeq-64	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.636	129.710	Urban_artificial
NonSeq-65	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.703	Urban_artificial
NonSeq-66	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.702	Urban_artificial
NonSeq-67	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.700	Urban_artificial
NonSeq-	-	*Gekko* sp.	Shimokoshiki,	31.638	129.700	Urban_artificial

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NonSeq-69	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.638	129.701	Urban_artificial
NonSeq-70	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.636	129.698	Urban_artificial
NonSeq-71	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.636	129.698	Urban_artificial
NonSeq-72	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.636	129.698	Urban_artificial
NonSeq-73	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.634	129.696	Urban_artificial
NonSeq-74	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.634	129.696	Urban_artificial
NonSeq-75	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.634	129.696	Urban_artificial
NonSeq-76	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.637	129.698	Urban_artificial
NonSeq-77	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.854	129.892	Non- urban_artificial
NonSeq-78	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.878	129.860	Non- urban_artificial
NonSeq-79	-	*Gekko* sp.	Kamikoshiki, Kagoshima	31.865	129.838	Urban_artificial
NonSeq-80	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.683	Urban_artificial
NonSeq-81	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.665	129.681	Non- urban_natural
NonSeq-82	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.723	Urban_artificial
NonSeq-83	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.663	129.723	Urban_artificial
NonSeq-84	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.693	129.694	Urban_artificial
NonSeq-85	-	*Gekko* sp.	Shimokoshiki, Kagoshima	31.778	129.791	Urban_artificial
NonSeq-86	-	*Gekko* sp.	Nakadori, Nagasaki	32.828	129.051	Urban_artificial
NonSeq-87	-	*Gekko* sp.	Nakadori, Nagasaki	32.843	129.054	Non- urban_artificial
NonSeq-88	-	*Gekko* sp.	Nakadori, Nagasaki	32.845	129.052	Non- urban_artificial
NonSeq-89	-	*Gekko* sp.	Nakadori, Nagasaki	32.839	129.061	Urban_artificial
NonSeq-90	-	*Gekko hokouensis*	Nakakoshiki, Kagoshima	31.802	129.839	Urban_artificial
NonSeq-91	-	*Gekko hokouensis*	Kamikoshiki, Kagoshima	31.840	129.918	Urban_artificial
NonSeq-92	-	*Gekko hokouensis*	Nakadori, Nagasaki	32.999	129.176	Urban_artificial
NonSeq-93	-	*Gekko hokouensis*	Ibusuki, Kagoshima	31.227	130.643	Urban_artificial
NonSeq-94	-	*Gekko hokouensis*	Ibusuki, Kagoshima	31.227	130.643	Urban_artificial

Table S4-2 The list of the loci data set assembled by ipyrad.

Dataset Name	Samples included	Number of samples	Shared locus rate
Set.Phy_S	Lib22-1 to Lib22-70, DRR391438 (Chapter 3)	71	0.96
Set.Admix_S	Lib22-2 to Lib22-70, DRR391438 (Chapter 3)	70	1
Set.Phy_H	Lib23-1 to Lib23-50	50	0.94
Set.Admix_H	Lib23-2 to Lib23-50	49	0.98

Table S4-3 Prior distribution of parameters used for divergence time estimation. All parameters were given a uniform distribution (unif) from the lower bound to the upper bound.

Parameters	Distribution	M1 (rejected)		M2 (rejected)		M3 (adopted)	
		Minimum	Max	Minimum	Max	Minimum	Max
MAF	unif	0.001	0.5	0.001	0.5	0.001	0.5
log10_Northern Koshiki	unif	4.0	7.0	4.0	7.0	4.0	7.0
log10_ Southern Koshiki	unif	2.0	6.0	2.0	6.0	2.0	6.0
log10_Goto + Hirado	unif	2.0	4.0	2.0	4.0	2.0	4.0
log10_t 1 (earlier divergence)	unif	1.0	4.0	1.0	4.0	1.0	4.0
log10_t 2 (older divergence)	unif	2.0	5.0	2.0	5.0	3.0	5.0

Table S4-4 Observed summary statistics. The population number is 1: Northern Koshiki, 2: Southern Koshiki, 3: Goto + Hirado.

mean_K	0
sd_K	0
tot_K	0
mean_H	0
sd_H	0
tot_H	0
prS_1	0
prS_2	0

prS_3	0
mean_S	0
sd_S	0
tot_S	35128
mean_D	0
sd_D	0
mean_FS	0
sd_FS	0

Pi_1	45.8609
Pi_2	170.083
Pi_3	206.84
mean_Pi	140.928
sd_Pi	84.3568
FST_2_1	0.154214
FST_3_1	0.509845
FST_3_2	0.403296

Table S4-5 Posterior distribution for each parameter simulated by ABC toolbox. The median, standard deviation, and 95% BCI (bottom 2.5% and top 2.5%) are summarized.

Parameters		**MAF**	**Northern Koshiki**	**Southern Koshiki**	**Goto + Hirado**	**t 1 (earlier divergence)**	**t 2 (older divergence)**
M1 (rejected)	**Median**	0.255277	652894	1171	704	382	7095.5
	sd	0.143681	2457130	88122.3	2180.29	2141.15	21111.9
	0.025 BCI	0.013185	12776	114	110	26	263
	0.975 BCI	0.486393	8783308	187729	8340.3	8149.05	80896
M2 (rejected)	**Median**	0.2514465	20553	30360	1558.5	643	34262.5
	sd	0.142534	23005	229453	2466.08	2366.68	28087
	0.025 BCI	0.0145283	10321	167	119	13	2899.6
	0.975 BCI	0.4877614	91364.5	840166	8863.3	8509.12	95786.2
M3 (adopted)	**Median**	0.25021	296978.5	826.5	828.5	40	7617.5
	sd	0.143654	2287025	8677.43	2372.27	754.784	24588.2
	0.025 BCI	0.0126952	11590.8	114	111	346	394.975
	0.975 BCI	0.4873665	8573961.8	10610.5	8641.12	2776.02	87708.6

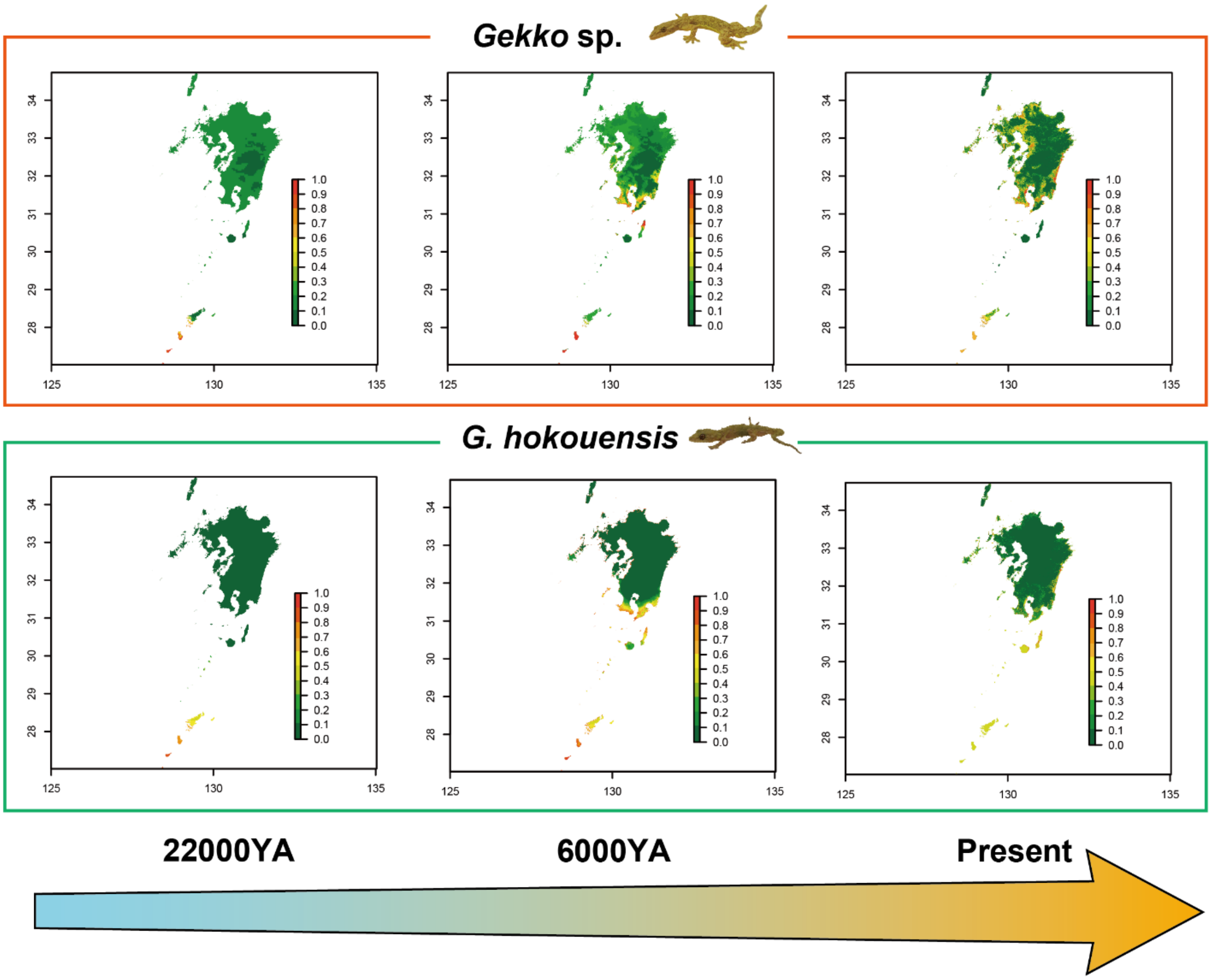

Fig S4-1 Time over changes in suitable habitats of *Gekko* sp. and *G. hokouensis*. Left: in the Last Glacial Period (22000 years ago). Central: after the Last Glacial Period. Right: Present.

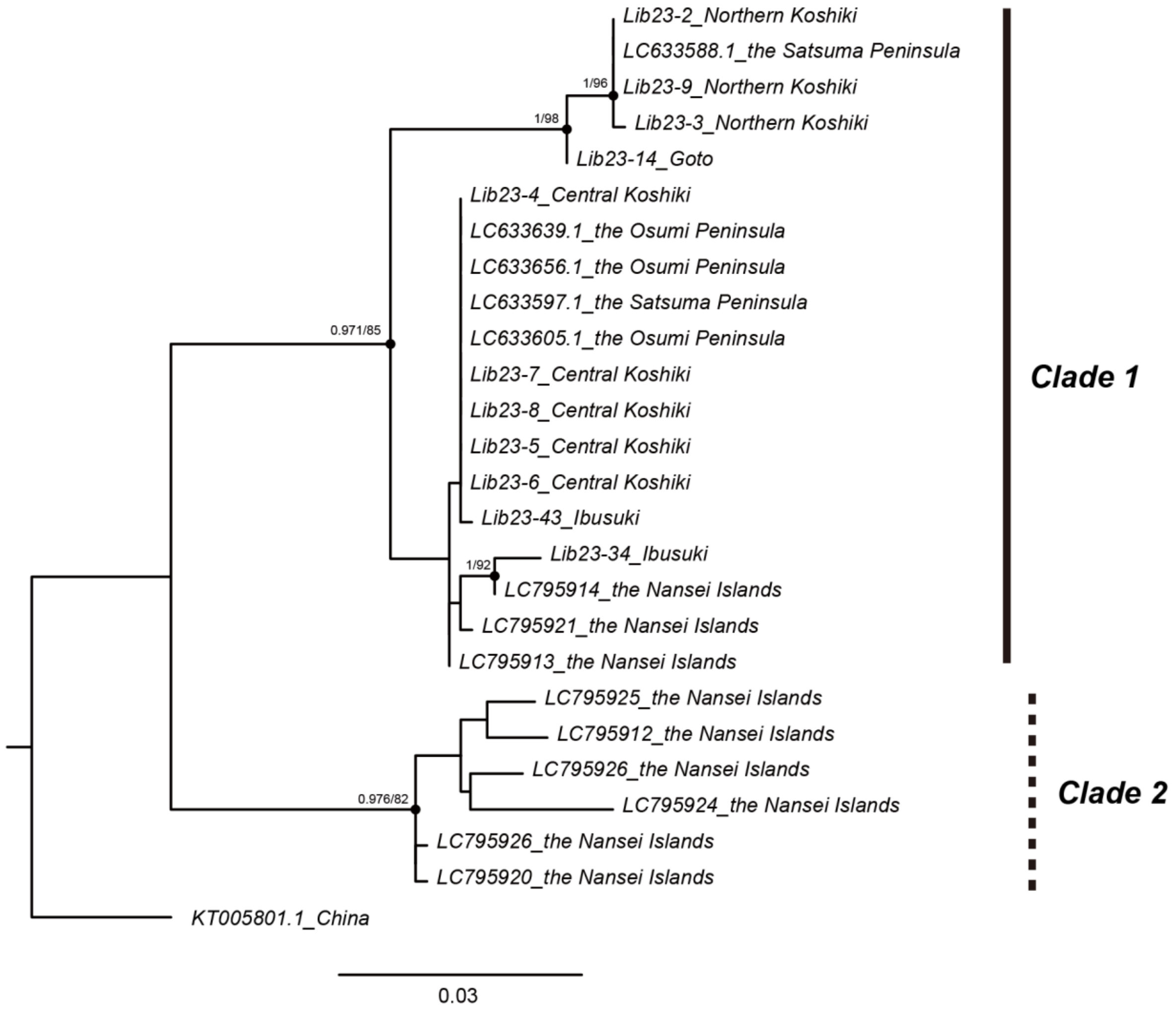

Fig S4-2 A molecular phylogenetic tree based on the ML method and the approximate Bayesian method. It wascreated based on concatenated mtDNA region (12S, rRNA V, 16S). Nodes with a closed circle represent Bayes posterior probabilities higher than 0.99 and UFboot higher than 80%. Labels show the Accession number of GenBank (or Sample No. in my study) and the location.

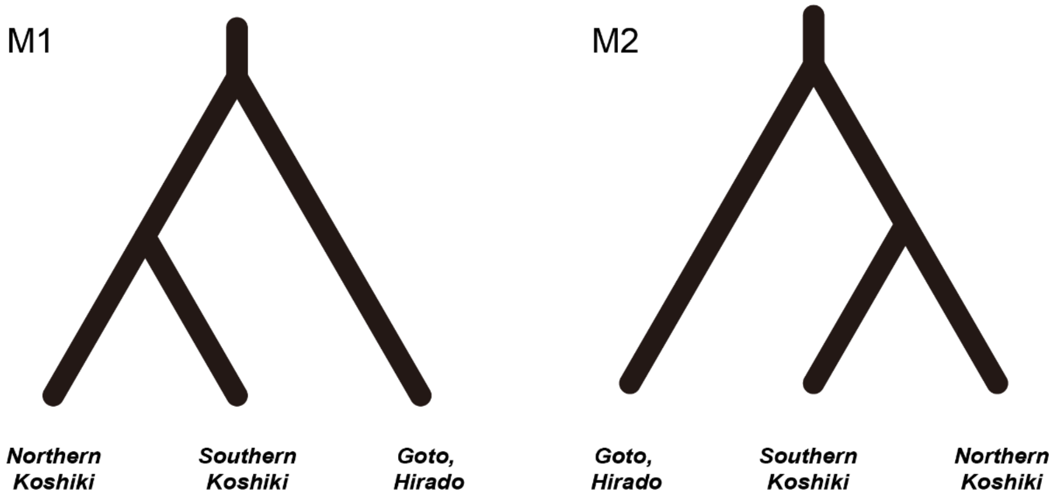

Fig S4-3 The rejected scenarios of the coalescent simulation. M1: First, the population of the Southern Koshiki coalesces into the Northern Koshiki. Next, the population of the Koshiki coalesces into the Goto + Hirado. M2: First, the population of the Southern Koshiki coalesces into the Northern Koshiki. Next, the population of the Goto + Hirado coalesces into the Koshiki.