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Population Genetics of the Asian Parti-Colored Bat, *Vespertilio sinensis*: Insights Into Seasonal Migration in the Japanese Archipelago

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Understanding bat movement patterns is essential for epidemiology and bat conservation. However, such information is lacking in East Asia, including the Japanese archipelago. It is also unclear whether the straits surrounding the Japanese archipelago affect bat movement. We conducted a population genetic analysis for the Asian parti-colored bat (*Vespertilio sinensis*), distributed in Far East Russia and East Asia, including Japan. Samples were collected from three colonies in Hokkaido and eight in Honshu and sequenced for a mtDNA Cytochrome-b region. The population structure of this species was examined using phylogenetic and molecular variance analyses, which revealed a genetic separation between Hokkaido and Honshu; this suggested that the Tsugaru Strait, located between Hokkaido and Honshu, is a geographic barrier. Our demographic analysis revealed that *V. sinensis* of Hokkaido and Honshu had different population expansion histories. The genetic divergence between Hokkaido and Honshu further suggests that two-way migration across the Tsugaru Strait did not occur with *V. sinensis*. However, in the Mantel test, which was restricted to the Honshu population, the response to isolation by distance differed in summer and winter. This seasonal difference in response may indicate higher fidelity to summer roost or habitat compared to wintering areas. When combined with the results of a previous banding study, which showed that females exhibited two-way movement and males exhibited one-way movement, our findings support the conclusion that *V. sinensis* is migratory. These results provide new insights into the movement patterns of bat species within the Japanese archipelago.

Key words: phylogeography, demographic history, Tsugaru Strait, conservation, mitochondrial DNA

INTRODUCTION

Migratory behavior is observed in broad taxa, such as birds, insects, and mammals (Fleming and Eby, 2003; Newton, 2010; Chapman et al., 2015). Information on migratory activities can provide beneficial countermeasures for epidemiology and conservation. For example, birds provide insights into migration behavior that are used to assess the risk of viruses, such as avian influenza (Reed et al., 2003; Hill et al., 2012). In addition, it is known that the migratory behavior of some bird species causes them to be at high risk of becoming entangled in wind turbines. Knowledge of their migratory behavior patterns could provide conservation benefits for these species (Cryan and Brown, 2007; Masden et al., 2009). Bats are the only mammals that can fly, and there are more than 1400 known species (Wilson and Mittermeier, 2019). Bats are also natural hosts of pathogens, including pathogens that cause zoonoses such as rabies virus, SARS-like coronaviruses, and Nipah virus (Pape et al., 1999; Yob et al., 2001; Wang et al., 2006). In addition, bats sometimes fly into wind turbines, which has a remarkable impact on bat populations (Barclay et al., 2007; Piorkowski and O'Connell, 2010). For example, in Europe, species that migrate long distances at high altitudes are listed as high risk for collisions (Rodrigues et al., 2015). Thus, understanding the movement patterns of bats could assist with disease mitigation (disease risk assessment, modeling disease dynamics, and planning effective emergency responses) and conservation efforts related to threats such as wind turbines (Moussy et al., 2013).

In the field of bat movement ecology, migration and dispersal are generally distinguished from one another. According to Moussy et al. (2013), migration is a regular, seasonal, and two-way movement of populations between regions. Dispersal is a one-way movement from one location to another, typically undertaken by juveniles or immature individuals (natal dispersal). Female bats generally form maternity colonies to breed communally, often gathering in large numbers. These colonies provide benefits such as social thermoregulation and information transfer (Kerth, 2008). Since females have more stringent roosting requirements than males during the summer breeding season (Speakman and Thomas, 2003), sex-biased migration is commonly

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observed in bats (Fleming and Eby, 2003). This results in female bats being more likely to migrate than male bats. The tendency of females toward fidelity to the natal area (natal philopatry), as well as male dispersal, are typically observed in mammals (Clutton-Brock and Lukas, 2012); similar trends have been observed in bats (Moussy et al., 2013). Therefore, understanding bat movement activity is necessary for implementing effective conservation measures (Kunz et al., 2007; Moussy et al., 2013). Understanding how the behavior of bats differs by sex is also essential (Safi et al., 2007). However, information on the ecology of bat migration and related issues in Japan is lacking.

The Japanese archipelago has several known geographical boundaries that significantly influence the spatial distribution of terrestrial organisms. The Tsugaru Strait (Blakiston line), located between Hokkaido and Honshu, is a geographic barrier to terrestrial mammals (Kondo, 1993). The distribution patterns of several bat species also appear to be influenced by this strait. For example, *Miniopterus fuliginosus* is widely distributed in the Japanese archipelago but not in Hokkaido, north of the strait. However, some species, such as *Myotis sibiricus* and *Myotis petax*, are not distributed in Honshu; they are only distributed in Hokkaido. Many other species, such as *Vespertilio sinensis* and *Myotis ikonnikovi*, are distributed both south and north of the Tsugaru Strait; the function of this strait for these bats has not been assessed (Sano et al., 2015).

The Asian parti-colored bat, *V. sinensis*, is widely distributed from East Asia to Far East Russia, a region which includes Japan (Burgin et al., 2015). In the Japanese archipelago, *V. sinensis* is distributed in Hokkaido, Honshu, Shikoku, and Kyushu (Fukui, 2015), all of which are separated by straits. This species forms maternity colonies from spring to summer only in pregnant females, and colony size ranges from tens to several thousand individuals (Fukui, 2015). Some of these colonies have frequently been sighted on man-made structures relatively close to humans, such as on buildings and in slits under elevated bridges (Fukui and Bat Research Group of Centennial Woods Fan Club, 2001; Osawa et al., 2014). In contrast, only a single or several male individuals roost during the maternity period (Kamiyama et al., 2024), suggesting that this species has different roosting strategies for the two sexes. In a banding survey of this species in Japan, the longest recorded movement was 784 km for males (Mukohyama, 2002) and 266 km for females (Sato et al., 2017), suggesting that this species is capable of long-distance migration. Furthermore, banded individuals have been recaptured in hibernacula, away from their maternity colonies (Sato et al., 2017), further supporting the possibility of cross-strait migration. However, it remains unclear whether regular seasonal two-way migration occurs. Because *V. sinensis* lives in close proximity to humans and has a high flight ability, knowledge of its movement potential, biological geography, and mating strategy is important for conservation and public health.

However, the seasonal movements of large populations of this species have not been confirmed, and the possibility of genetic isolation among populations geographically separated by straits or other barriers has not been discussed. In this study, we conducted a population genetic analysis of *V. sinensis* to investigate the possibility of seasonal migration and genetic isolation among populations separated by straits or other geographical barriers within the Japanese archipelago.

MATERIALS AND METHODS

Sampling

Tissue samples for genetic analysis were collected from nine localities across the Japanese archipelago, including six maternity colonies of adult females and five hibernating colonies of females and males (Table 1; Fig. 1; see Supplementary Table S1). The sampling period was from October 2021 to June 2023. Tissue samples were collected from the bat wing membrane using a biopsy punch (Kai Industries, Seki, Japan) and preserved in 99% ethanol. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Tokai University (Approval numbers: 210512, 221024, and 232043). Permission to capture the bats was obtained from the Ministry of the Environment and each prefecture involved.

Table 1. Genetic diversity of *V. sinensis* mtDNA *Cytb*. Populations were treated as distinct if captured in the same location but in different seasons.

Population number	Locality		Season	No. of sequences	Sex (F/M)	No. of haplotypes	Nucleotide diversity (π)	Haplotype diversity (h)
1	Hokkaido	Kutchan	Summer	23	16/7	9	0.00537	0.86957
2			Winter	30	23/7	5	0.00219	0.69195
3		Takikawa	Summer	30	26/4	11	0.00396	0.86897
4	Honshu	Utsunomiya	Summer	30	30/0	13	0.00550	0.90805
5		Shichinohe	Summer	30	29/1	11	0.00419	0.81379
6		Kokonokamachi	Summer	18	18/0	5	0.00447	0.80392
7		Kumagaya	Summer	30	30/0	10	0.00523	0.85287
8			Winter	5	2/3	4	0.00649	0.90000
9		Kyoto	Winter	14	3/11	11	0.00546	0.95604
10		Takahata	Winter	31	9/22	7	0.00382	0.76559
11		Higashimatsushima	Winter	32	14/18	6	0.00284	0.68548
		Total	11	273		42	0.00709	0.89687

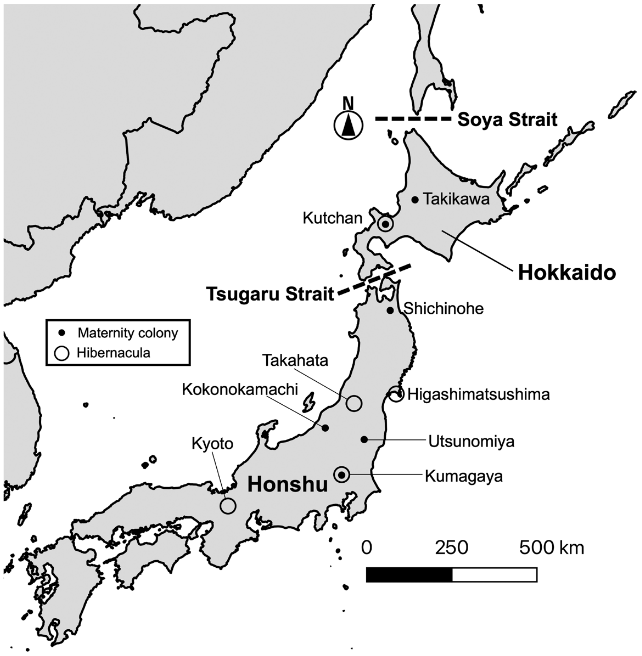

Fig. 1. Collection localities of *V. sinensis*. Circles indicate collected localities and seasons; black circles indicate maternity colonies (summer); white circles indicate hibernating colonies (winter).

DNA extraction and sequencing

Genomic DNA was extracted from the tissues using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The complete sequences of Cytochrome-b (*Cytb*) in mitochondrial DNA (mtDNA) were amplified by polymerase chain reaction (PCR) using primers adjusted to 5 pmol/μL MY-130 and Bat15R (Kawai et al., 2003, 2006). PCR amplifications were performed in a total volume of 25 μL using a KOD FX (Toyobo, Osaka, Japan) with 12.5 μL of 2×PCR Buffer for KOD FX, 5 μL of 2 mM dNTPs, 1.5 μL of each primer, 0.5 μL KOD FX, 3.0 μL distilled water, and 1 μL template DNA containing 5–20 ng genomic DNA. PCR amplification was conducted in a T100 Thermal cycler (Bio-Rad, Hercules, CA) with the conditions: pre-denature at 94°C for 2 min, 30 cycles of denaturing at 98°C for 10 s, annealing at 50°C for 30 s, extension at 68°C for 1 min, and final extension at 68°C for 4 min. PCR products were visualized on a 1.5% agarose gel and purified by isopropanol precipitation. Purified PCR products were sequenced on a SeqStudio Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) using a BigDye Terminator ver. 1.1 Cycle Sequencing Kit (Applied Biosystems) with the same primers as those used for PCR.

Genetic diversity

Nucleotide diversity (π) and haplotype diversity (h) of the mtDNA *Cytb* were calculated in each population using DnaSP Ver. 6.12.03 (Rozas et al., 2017). For the investigation of the differences in these diversities between the summer and winter populations, a Wilcoxon rank-sum test was conducted using R software ver. 4.0.2 (R Core Team, 2020).

Phylogenetic analysis

The Neighbor Joining (NJ) tree was constructed using the distance matrix estimated with Kimura's two-parameter model, incorporating transitions and transversions and a uniform substitution rate. The analysis was performed using MEGA 11 software (Tamura et al., 2021). Sequences of *Vespertilio murinus* (accession numbers: AF376834, JX570911, and LC052293) were used as the outgroup in the phylogenetic analysis. Statistical support for the analyses was estimated using the bootstrap method with 10,000 bootstrap replicates. The median-joining method was used to infer the haplotype network with POPART ver. 1.7 (Leigh and Bryant, 2015).

Population genetic structure

An analysis of molecular variance (AMOVA) and pairwise F_{ST} were performed using Arlequin (Excoffier and Lischer, 2010) to assess the level of genetic differentiation between populations. The deviation of the pairwise F_{ST} from zero was tested using permutations (10,000 replicates). Isolation by distance (IBD) was tested using the Mantel test based on Pearson's product-moment correlation method with permutations (9999 replicates) and performed using the R package vegan (Oksanen et al., 2007). This analysis used a matrix of pairwise standardized F_{ST} values (F_{ST} / [1 – F_{ST}]) and a matrix of geographic distances between colonies.

Population demographic history

Five haplotype groups were grouped based on the NJ tree and haplotype network results to examine their demographic histories. The demographic expansion of the population was tested using neutrality tests and mismatch distribution analysis. The neutrality test, Tajima's D (Tajima, 1989), and Fu's F_S (Fu, 1997) values were calculated using Arlequin with a permutation (10,000 replicates). The mismatch distribution was simulated using the sudden and spatial expansion model, with both simulations implemented in Arlequin. Significant differences from the model of sudden and spatial expansion were assessed using the sum of squared deviations (SSD) and the Harpending raggedness index (*Hri*) with parametric bootstrapping (10,000 replicates).

RESULTS

Genetic diversity

Forty-two haplotypes were identified for 1140 bp of the mtDNA *Cytb* region in 273 individuals of *V. sinensis* and were registered in GenBank (accession numbers: LC788984–LC789025; see Supplementary Table S2). The total nucleotide diversity (π) and haplotype diversity (h) were 0.00709 (min–max: 0.00219–0.00649) and 0.89687 (min–max: 0.668548–0.95604), respectively (Table 1). There was no significant difference in diversity values between the summer and winter (Welch two-sample *t*-test, π: $P = 0.329$, h: $P = 0.537$).

Phylogenetic relationships and population genetic structure

To clarify the phylogenetic relationships among haplotypes, an NJ tree analysis was conducted that included published sequences from East Asia (GenBank accession numbers KX467594, KJ081440, and KM092493). The NJ tree was divided into two major groups: haplotype group A, consisting of individuals captured primarily in Hokkaido, and haplotype group B (B-1–B-4), consisting of individuals captured primarily in Honshu (Fig. 2). Haplotype group A was monophyletic, supported by a high bootstrap value. In contrast, the monophyly of haplotype group B as a whole was not supported by the bootstrap analysis, although subgroups B-2 and B-3 were strongly supported. Thus, the monophyly of haplotype group B remained unclear, and the phylogenetic relationships between haplotype groups could not be fully resolved. AMOVA analysis revealed significant genetic variance between the Hokkaido and Honshu groups (60.9%, $P < 0.005$) but no significant variance within populations of

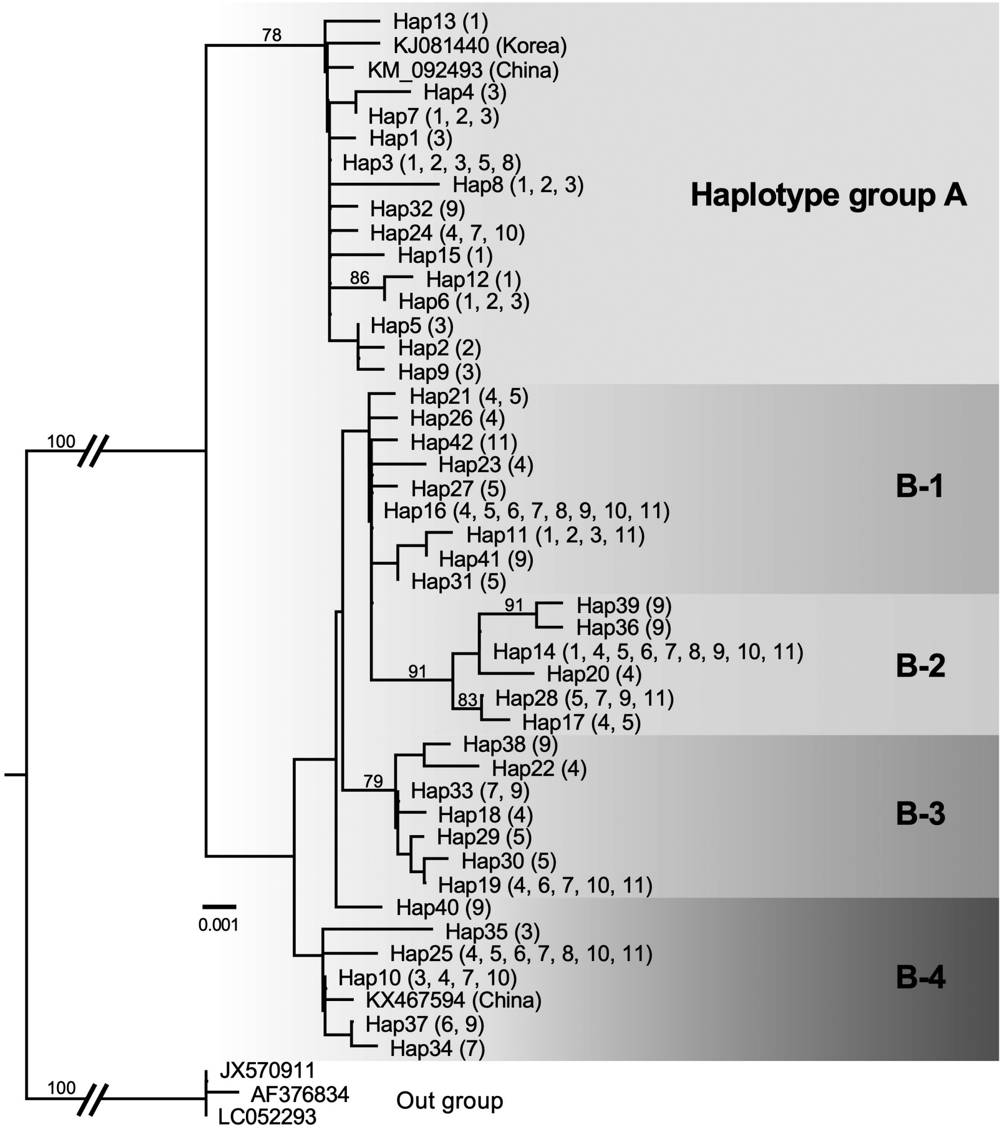

Fig. 2. Neighbor-Joining tree for 42 haplotypes and three continental sequences (GenBank accession numbers KX467594, KJ081440, and KM092493) of *V. sinensis* mtDNA *Cytb*. Sequences of *V. murinus* were used as the outgroup. Bootstrapping values > 70% are shown at branches on the tree. The scale indicates the number of substitutions per site. A figure in parentheses indicates a population in which that haplotype was found.

these groups (0.6%, $P = 0.076$, Table 2). The median-joining network was divided into five distinct groups: haplotype groups A, B-1, B-2, B-3, and B-4 (Fig. 3). Eight mutations were observed between haplotype group A and the other groups. Haplotype group A, which consisted mostly of individuals from Hokkaido, exhibited a star-like phylogeny, irrespective of season. Haplotype group B-1 also displayed a star-like phylogeny dominated by Hap 16. In contrast, haplotype groups B-2, B-3, and B-4 showed more complex phylogenetic structures. Most individuals captured in the hibernacula of Hokkaido and Honshu shared haplotypes with the summer populations from the same areas.

Demographic analyses were performed on the subdivided haplotype groups. All haplotype groups exhibited negative Tajima's *D* values, and only those of B-1 were statistically significant ($P < 0.05$, Table 3). Similarly, negative Fu's F_S values were observed for all haplotype groups, and only those of B-1 were statistically significant ($P < 0.005$). The mismatch distribution analysis revealed that haplotype groups A, B-1, and B-3 were unimodal, whereas haplotype groups B-2, B-4, and the combined group B were multimodal (Fig. 4). Haplotype group A showed no significant results for SSD or *Hri*, suggesting it fits the model of sudden and spatial expansion. Conversely, the combined haplotype group B

Table 2. Analysis of the molecular variance (AMOVA) results for the mtDNA *Cytb* of *V. sinensis* in the Japanese archipelago.

Soruce of variation	Fixiation index	*P*-value	Variation (%)
Hokkaido vs Honshu (FCT)	0.60903	<0.005	60.9
Among populations within groups (FSC)	0.01522	0.076	0.6
Within populations (FST)	0.61498	<0.001	38.5

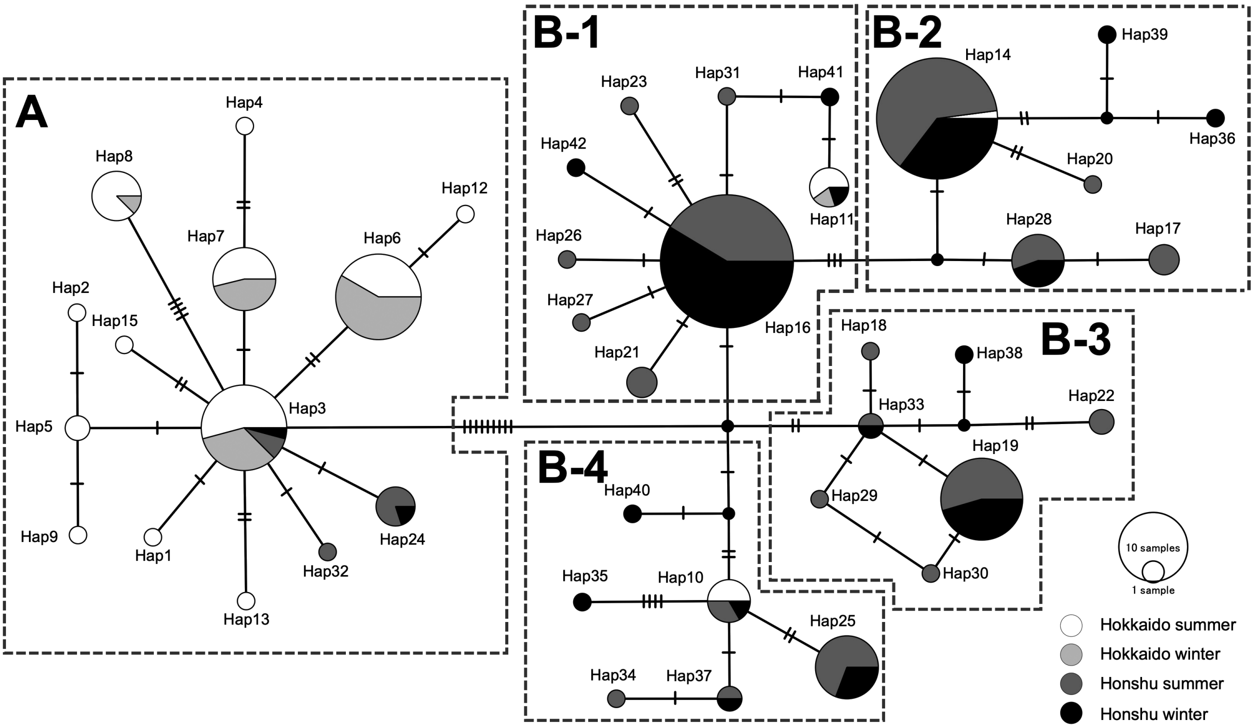

Fig. 3. Median-joining network for 42 haplotypes of *V. sinensis* mtDNA *Cytb*. Circle size indicates the number of individuals sharing the same haplotype. The no caption dots indicate the unsampled haplotypes. Four datasets, which differ by locality and season, are provided for each haplotype and indicated by color.

Table 3. Mismatch distribution analysis and neutrality test results of *V. sinensis* mtDNA *Cytb*.

Haplotype group	Sudden expansion		Spatial expansion		Tajima's *D* (*P*)	Fu's *Fs* (*P*)
	SSD (*P*)	Hri (*P*)	SSD (*P*)	Hri (*P*)		
A	0.004 (0.639)	0.023 (0.848)	0.002 (0.831)	0.023 (0.941)	−1.263 (0.087)	−3.316 (0.103)
B-1	0.013 (0.383)	0.262 (0.523)	0.005 (0585)	0.262 (0.664)	−1.653 (0.024)	−5.385 (0.004)
B-2	0.238 (0.000)	0.424 (0.944)	0.027 (0.478)	0.424 (0.550)	−1.320 (0.085)	−0.889 (0.332)
B-3	0.272 (0.000)	0.056 (0.999)	0.004 (0.738)	0.156 (0.780)	−1.155 (0.139)	−2.480 (0.056)
B-4	0.049 (0.284)	0.188 (0.197)	0.026 (0.537)	0.188 (0.493)	−1.166 (0.118)	−0.059 (0.497)
B (all)	0.062 (0.010)	0.166 (0.000)	0.048 (0.146)	0.166 (0.146)	−1.032 (0.133)	−5.713 (0.080)

exhibited significant deviations from the SSD ($P < 0.05$) and *Hri* ($P < 0.05$) values expected under the sudden expansion model. Haplotype groups B-2 and B-3 exhibited significance only for SSD ($P < 0.05$), suggesting that these groups did not undergo rapid expansion but rather experienced spatial expansion. Furthermore, the mismatch distribution analysis indicated that Hokkaido and Honshu have distinct demographic histories.

The IBD test was performed on the Honshu group, and the data set consisted of summer and winter colonies, each with four facilities (Fig. 5). Among all populations and summer populations, the Mantel test showed a significant correlation (all: $R = 0.521$, $P < 0.05$; summer: $R = 0.955$, $P < 0.05$). However, there was no significant correlation among the winter populations ($R = 0.023$, $P = 0.458$). Furthermore, IBD was not detected when the analysis was restricted to female samples from the winter population ($R = -0.015$, $P = 0.416$).

DISCUSSION

We examined the population genetic structure and demographic history of the Asian particolored bat, *V. sinensis*, in the Japanese archipelago. Our analysis revealed a distinct genetic separation between the populations of Hokkaido and Honshu, represented by Haplotype groups A and B (Table 2; Figs. 2, 3), suggesting that the Hokkaido and Honshu groups have distinct historical genetic structures. There was also an indication that the individuals from the Hokkaido and Honshu genetic groups were mixed in small numbers within several hibernating and maternity colonies. These results suggest that although the two groups have been genetically separated for a long time, a small number of individuals had come into secondary contact, forming the current population. Therefore, many females (maternal genes) were restricted to movements within Hokkaido or Honshu, suggesting fidelity to their natal area of birth, and transboundary movements were considered rare. The

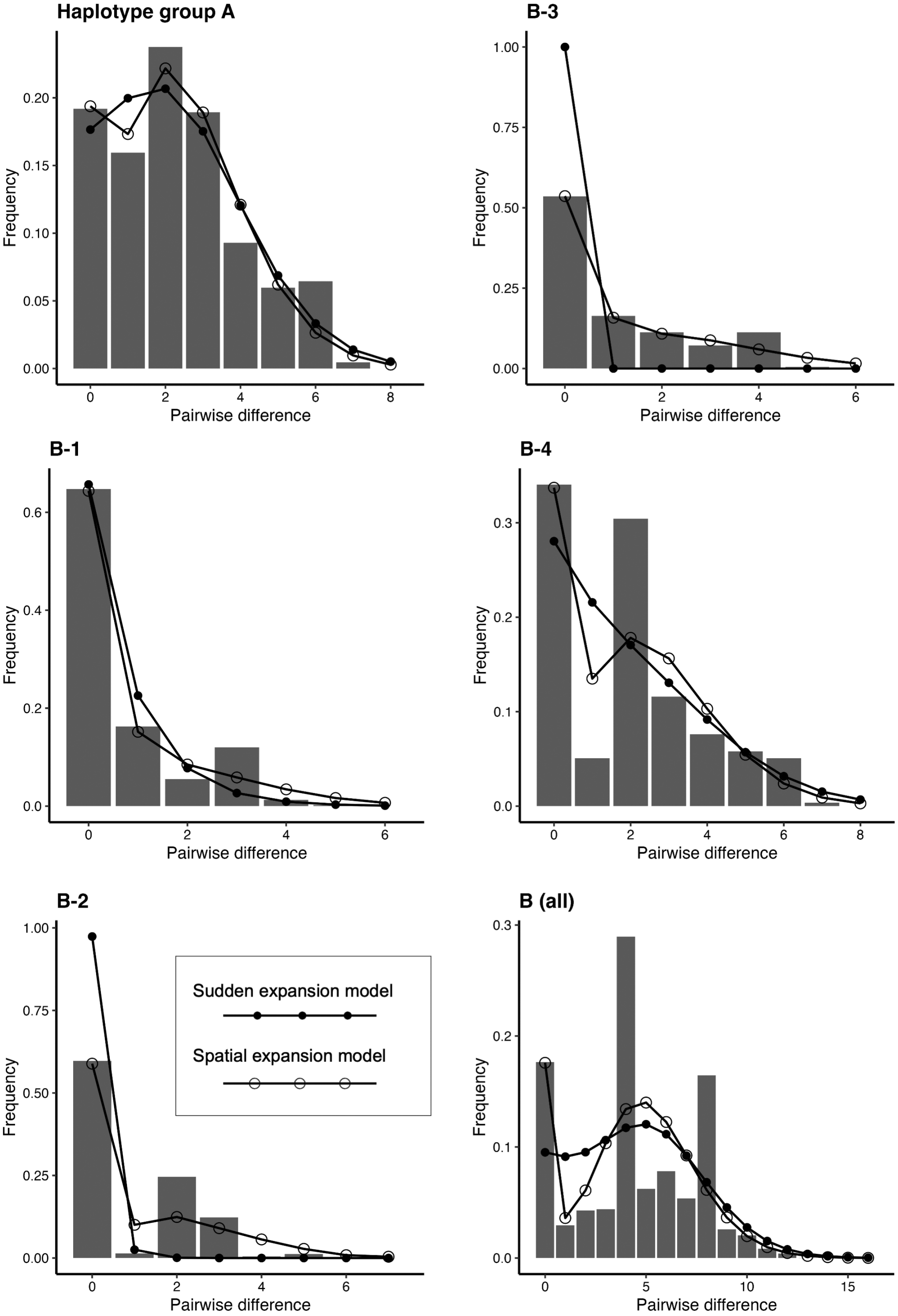

Fig. 4. Mismatch distribution analysis for each haplotype group of *V. sinensis* mtDNA *Cytb*. The bar indicates the frequency of the observed pairwise difference. Dots and lines indicate a model frequency; black circles indicate a sudden expansion model and white circles indicate a spatial expansion model.

genetic separation of Hokkaido and Honshu also provides important insights for two key questions: 1) Does migration across the Tsugaru Strait occur? and 2) How was the present distribution determined?

First, our results suggest that migration across the Tsugaru Strait does not occur in *V. sinensis*, as most individuals from Hokkaido and Honshu (haplotype groups A and B) are not mixed between the populations, regardless of the season. However, the 11 species of Scandinavian bats, including *V. murinus*, the sibling species of *V. sinensis*, have

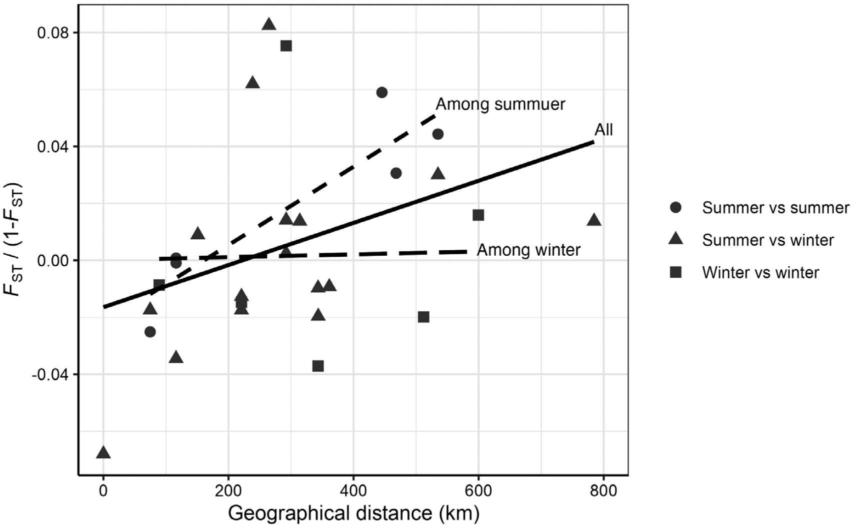

Fig. 5. Mantel test for isolation by distance on two datasets based on *V. sinensis* mtDNA *Cytb* in Honshu: summer and winter populations. Dots and triangles represent genetic [F_{ST} / (1 – F_{ST})] versus geographic distance (km) for the summer and winter populations, respectively.

been observed flying over the ocean (Ahlén et al., 2009). Therefore, it is not unlikely that *V. sinensis* could also fly over the ocean. *Miniopterus schreibersii* (aspect ratio 7.0: Norberg and Rayner, 1987) and *Myotis capaccinii* (aspect ratio 6.18: Mehdizadeh et al., 2018) in the Balearic Islands are seasonal migrant species that have been observed flying approximately 40 km in one flight (Amengual et al., 2007). The morphology of bat wings, especially the aspect ratio, is closely related to flight ecology and migration ecology (Norberg and Rayner, 1987; Fleming and Eby, 2003). *Vespertilio sinensis* has a relatively narrow wing morphology aspect ratio of 6.68 (Fukui et al., 2011). Its wing ratio is within the range of wing ratios (5.9–7.0) of bat species known to be regional migrants of 100–500 km (Norberg and Rayner, 1987; Fleming and Eby, 2003), suggesting that it is capable of long-distance flight and can migrate across the Tsugaru Strait, a distance of 20 km. Nevertheless, the suggested result of a long period of genetic isolation between the Hokkaido and Honshu populations is surprising and raises the question of what mechanisms maintained this isolation. In contrast, Yasui et al. (2022) suggested that the Tsugaru Strait is not a barrier for *M. ikonnikovi*, which is less capable of flight than *V. sinensis* and showed a genetically nested geographical distribution pattern, regardless of geographical barriers such as the strait. This suggests that some bat species may have been influenced by past geographical barriers, demographic history, or other factors such as population isolation and migratory ecology in shaping their distribution patterns in the Japanese archipelago, whereas others have not and are expanding their ranges.

Second, demographic analysis revealed that the *V. sinensis* in Hokkaido has a history of sudden population expansion that occurred more recently, whereas the Honshu population as a whole shows a history of spatial population expansion (Table 3, Fig. 4). These findings indicate that the history of population expansion differs between the Hokkaido and Honshu populations, thus implying a complex colonization history among populations of the intraspecies. The Hokkaido population was likely colonized once by an ancestor from other regions, whereas Honshu harbors at least four temporally and/or spatially isolated groups that are currently mixed (Table 3; Figs. 2, 3, 4). Currently, haplotypes are shared among the populations of Honshu, and there is no evidence of geographic differentiation. These results suggest that genetic exchange has occurred to a considerable extent between the populations of the Honshu group throughout their evolutionary history. According to Suzuki (2015), terrestrial mammals in Hokkaido have shorter divergence times from their closest relatives on the continent than those in Honshu and the Ryukyu Islands. Our findings showed a similar pattern. However, we could not estimate the divergence time and relationships between the Japanese archipelago and the continent because of the unknown optimal evolutionary rate and the need for more mtDNA *Cytb* sequences from the continent. Therefore, the exact population demographics remain unknown.

The Mantel test, restricted to Honshu, revealed IBD among populations, except for the winter populations (Fig. 5). In addition, the Mantel test applied to winter populations, with a sample of females only, did not show IBD. This suggests that maternal genes are unique to each summer population and region, whereas winter populations appear to be more mixed. This seasonal difference may reflect higher fidelity to summer roosts or habitats than to winter sites. Furthermore, these results may suggest female philopatry, as summer populations (i.e., maternity colonies) are composed solely of females. In summer, female bats form maternity colonies, which consist only of pregnant females, and colony size ranges from tens to several thousands of individuals (Fukui, 2015). Male bats roost singly or in small groups, never with females during the maternity and nursing period (Kamiyama et al., 2024), but they hibernate with females from winter to spring. These sex-specific roosting behaviors are a known mechanism driving sex differences in migratory and dispersal patterns (Moussy et al., 2013). Therefore, our results suggest that female-biased migratory behavior may occur. At least in *V. sinensis*, maternity and hibernation colonies disband at the end of each season (Fukui, 2015; Kamiyama et al., unpublished data), and they likely migrate between these sites regardless of geographical range. Previous banding studies of this species have observed movement from maternity colonies to hibernacula or in the opposite direction in females, and only the former has been observed in males (Sato et al., 2017). Based on our results and previous studies, it is reasonable to assume that *V. sinensis* is a seasonal migrant bat with a regional range. The discovery that this bat species migrates seasonally offers valuable insight from a conservation biology perspective. Currently, the migration of bats is not considered in the operation of wind power plants or in environmental impact assessments (Kawai, 2017). Reports indicate an increase in bat fatalities from collisions with wind turbines during the fall season (Kawai, 2017; Kasahi et al., 2018; Sato et al., 2022). This present finding suggests that the distance and spatial proximity to known colonies of seasonally migrating bats should be considered when establishing and operating wind farms.

In conclusion, *V. sinensis* is a regional migratory bat, with the Tsugaru Strait being a geographic barrier for the majority; specifically, the Hokkaido and Honshu populations are genetically and historically distinct. Additionally, a loose connection between the colonies encompassed by Hokkaido and Honshu was shown. These findings provide valuable insights for conservation and epidemiology, and it is worth noting whether they are common to other bat species in the Japanese archipelago. The seasonal migration of the Honshu population within a regional range also strongly indicates the need for a new perspective in various fields, such as conservation and epidemiology. However, realizing effective conservation and disease risk assessments requires further research to deepen the understanding of phylogeography, migration direction, and sex-based differences. In particular, while it has been shown that females exhibit summer habitat fidelity and two-way migration, additional research, including the analysis of nuclear genomes, is needed to determine whether males also disperse. Furthermore, because our sampling area was limited to only part of the Japanese archipelago—specifically, from Hokkaido to Kyoto—it is unknown whether this species migrates seasonally and what effect the Strait has in the western part of the Japanese archipelago. *Vespertilio sinensis* colonies in the west may have a loose connection, given that the northeastern Honshu population has IBD. Further studies focusing on the western part of the Japanese archipelago and Honshu are needed to clarify these issues.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

KK designed the research; SK and KK conducted fieldwork; SK conducted the lab work and analyzed the data; and SK drafted the manuscript, after which both coauthors made important contributions.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs230119)

Supplementary Table S1. Sampling locations and dates of *V. sinensis*.

Supplementary Table S2. Matching of Genbank accession numbers and haplotypes.

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