Quaternary Environmental Changes Shaped Mitochondrial DNA Diversity in the Large Japanese Wood Mouse Apodemus speciosus in Hokkaido, Japan

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Original paper

Quaternary environmental changes shaped mitochondrial DNA diversity in the large Japanese wood mouse *Apodemus speciosus* in Hokkaido, Japan

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Abstract. Quaternary environmental change provided opportunities for rapid population expansion; however, the process of building the population spatial structures remains poorly understood. In this study, we determined the mitochondrial cytochrome *b* and control region sequences of 43 individuals of the large Japanese wood mouse (*Apodemus speciosus*) from Hokkaido, northern Japan and analyzed these data along with those from 40 other individuals. Consistent with the findings of our previous study, we found that two rapid expansion events, after the last glacial maximum (LGM) and Marine Isotope Stage (MIS) 4, shaped population genetic pattern of *A. speciosus* in Hokkaido. In northeastern Hokkaido, several ancient lineages that originated during MIS 3 were detected, whereas central Hokkaido was dominated by haplotypes descended from a single lineage that survived the LGM, suggesting that the populations of western part of Hokkaido were newly formed by westward migration from eastern Hokkaido during the post-LGM warm period. Alternatively, as post-LGM vegetation recovery is thought to have occurred gradually from west to east in Hokkaido, population expansion started in the west and moved gradually to the east, resulting in eastward haplotype movement; thus, western and eastern Hokkaido may have served as the haplotype source and sink, respectively.

Key words: cytochrome *b*, ice age impact, population genetic structure, small rodent, sudden population expansion.

The mechanism by which genetic diversity is created and maintained is a fundamental question in evolutionary biology. Phylogeographic studies of terrestrial mammals have shown that Quaternary environmental changes influenced population dynamics and genetic structure (Hewitt 2000, 2004; Oshida et al. 2009; Sakka et al. 2010; Kozyra et al. 2021). Such changes occurred at a 100 000-year cycle, affecting species distribution ranges and facilitating the movement of individuals between islands by lowering the sea level by ~120 m to form land bridges (Hewitt 2000). It has been found that the extent and timing of the effects of Quaternary environmental changes on population dynamics, such as rapid demographic growth, were influenced by local topography, ocean currents, and latitude and also varied among species and populations (Hewitt 2000; Oshida et al. 2009; Burbrink et al. 2016; Honda et al. 2019; Nakamoto et al. 2021). However, it remains to be well documented how the population expansion events have shaped the spatial structure of genetic diversity in response to environmental changes.

The Japanese archipelago is a highly suitable setting for such studies, with multiple segmented spaces including the three central main islands of Honshu, Shikoku, and Kyushu, and many peripheral islands including the Hokkaido, Sado, and Satsunan Islands (Fig. 1A), which are separated from the main central islands by deep water (e.g., –120 m, Tada et al. 1999). These islands are arranged along a north–south axis, and each has a different climate. Numerous species exist in the Japanese archipelago, many of which have colonized the archipelago since millions of years ago (Serizawa et al. 2000; Nunome et al. 2007, 2010; Kirihara et al. 2013; Honda et al. 2019; Nakamoto et al. 2021), allowing comparison of the effects of Quaternary environmental changes within and between species. Several evolutionary studies

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of mammals inhabiting the Japanese archipelago have been conducted using mitochondrial DNA (mtDNA) sequences (e.g., Kinoshita et al. 2012, 2015; Ohdachi et al. 2018; Endo et al. 2020; Sato et al. 2020; Yoshida et al. 2020; Nagata et al. 2021). However, the factors shaping mtDNA diversity have not been sufficiently understood. Only two studies have suggested that in Hokkaido, the northernmost island of Japan, the mtDNA diversity of wood mice and voles were strongly influenced by population dynamics following the last glacial maximum (LGM), while the wood mice were also found to have lineages that diversified prior to the LGM (Suzuki et al. 2015). It is not clear however how two genetic diversification events at different periods affect the outcomes of spatial genetic structure.

The large Japanese wood mouse *Apodemus speciosus* is a common species that is distributed throughout the Japanese archipelago, including Hokkaido, Honshu, Shikoku, Kyushu as well as adjacent islets. The mtDNA diversity of *A. speciosus* has been examined in several studies (Suzuki et al. 2004; Tomozawa and Suzuki 2008; Tomozawa et al. 2010, 2014; Suzuki et al. 2015; Sato et al. 2020). In a previous study, we determined the mitochondrial cytochrome *b* gene (*Cytb*) and control region (CR) of *A. speciosus* and identified two clades, large and small in terms of numbers of nucleotide substitutions, with star-shaped patterns in the phylogenetic network, clade I (central main islands of Honshu, Shikoku, and Kyushu) and clade IIa (northern main island of Hokkaido), indicative of rapid expansion events at different historical times (Suzuki et al. 2015). The Hokkaido clade showed a star-shaped pattern as a whole and additionally included a younger single star-shaped sub-cluster in the interior of the network (Suzuki et al. 2015). Hence three population expansion events were observed in *A. speciosus* in the previous study (Suzuki et al. 2015). The data of pollen fossil records for *Quercus* species, which produce acorns, the main wood mouse food resource, indicate five historical population growths during the late Quaternary (Igarashi et al. 1993; Igarashi and Oba 2006; Igarashi et al. 2011; Igarashi 2013, 2016). Assuming that population growth of *A. speciosus* followed those of the *Quercus* species, we assigned these mtDNA expansion events in *Apodemus* species to possible critical paleoclimatic time points as inferred from population growths of *Quercus* species. The star-shaped patterns of the interior cluster of the clade IIa from Hokkaido and the clade I from Honshu, Shikoku, and Kyushu were suggestive of post-glacial events related to the LGM and penultimate glacial (PGM) maxima, respectively. During these periods, expansions are considered to have started in early Marine Isotope Stage (MIS) 1 (ca. 15 000 years ago) and MIS 5e (ca. 130 000 years ago), respectively, since haplotypes in these clusters are now observed in offshore islands separated by deep water (e.g., ~120 m), which is impossible without land bridge formation (Suzuki et al. 2015). However, when the expansion of the clade IIa began remains to be determined due to the small amount of data analyzed for the clade (Suzuki et al. 2015). Meanwhile, Hanazaki et al. (2017) investigated the local population dynamics of the small Japanese wood mouse *A. argenteus* based on variation among mtDNA sequences and assessed population expansion events. Considering fossil pollen data (Igarashi et al. 1993; Igarashi and Oba 2006; Igarashi et al. 2011; Igarashi 2013, 2016) and fatty acid compositions (Yamamoto et al. 2014), Hanazaki et al. (2017) determined that a critical stage in the onset of population expansion occurred during early MIS 3 (ca. 53 000 years ago). The timing of the early expansion of the clade IIa in *A. speciosus* might be correlated to that estimated in *A. argenteus*.

The objective of the present study was to elucidate and assess the factors driving spatial variation in *A. speciosus* genetic diversity on Hokkaido, where two descendent groups of mtDNA haplotypes derived from ancient and recent population expansion events coexist (Suzuki et al. 2015).

Materials and methods

Sample and data collection

We analyzed the entire *Cytb* coding region (1140 bp) and CR hypervariable region I (552 bp after exclusion of indels) sequences of 106 *A. speciosus* individuals. Sequences were obtained from the DNA Data Bank of Japan (DDBJ), European Nucleotide Archive (ENA), and GenBank nucleotide databases (Suzuki et al. 2015); other sequences were newly determined for 43 individuals collected in Hokkaido in 2017 (Table 1). Field collection was performed with permission from the Hokkaido prefectural government for academic research only, following the Guidelines for the Procedure of Obtaining Mammal Specimens as Approved by the Mammal Society of Japan.

Table 1. List of specimen of *Apodemus speciosus* used in this study

Collection locality	Specimen code	Haplogroup
Hokkaido		
1. Otoineppu	HS5387	A
	HS5388	A
	HS5389	A
	HS5390	A
2. Nayoro	HS3922	A
	HS3923	B
3. Misato, Kitami	HS5118	B
	HS5120	A
	HS5114	B
	HS5115	A
	HS5127	A
4. Misaki, Abashiri	HS5660	B
Notoro, Abashiri	HS5661	B
	HS5662	A
	HS5663	B
	HS5664	A
	HS5665	A
	HS5666	B
	HS5667	A
	HS5668	B
5. Shari	HS5441	B
	HS5442	A
	HS5444	B
	HS5445	B
6. Rausu	HS5439	B
7. Shibecha	HS5263	A
	HS5264	B
	HS5265	A
	HS5266	B
	HS5267	B
	HS5268	A
	HS5269	A
	HS5270	B
8. Fukagawa	HS2218	A
	HS3318	A
	HS3321	A
9. Furano	HS5710	A
	HS5711	B
	HS5713	A
	HS5714	A
	HS5715	A
	HS5717	B
	HS5718	B
10. Obihiro	HS5698	A
	HS5699	B
	HS5700	A
	HS5701	A
	HS5702	B
	HS5703	B
	HS5704	B
	HS5705	A
	HS5706	A
	HS5707	A
11. Otofuke	HS2656	A
11. Otofuke	HS2783	A
12. Toyokoro	HS5709	B
13. Erimo	HS5612	A
14. Tobetsu	HS4147	A
15. Bibai	HS97	A
16. Sapporo	HS3667	A
	HS3672	A
17. Naganuma	HS238	A
	HS240	A
	HS242	A
18. Tomakomai	HS5135	A
	HS5136	A
19. Kimobetsu	HS4906	B
	HS4907	A
20. Date	HS5129	A
	HS5130	B
	HS5131	A
	HS5132	A
	HS5133	A
	HS5134	A
21. Rankoshi	HS3836	B
22. Hokuto	HS5138	A
23. Hakodate	HS4547	A
	HS5472	B
	HS5473	B
	HS5478	B
	HS5543	A
	HS5544	A
	HS5545	A
Honshu/Shikoku/Kyushu		
Yokohama, Kanagawa Pref.	HS22	C
Saga, Kochi Pref.	HS53	C
Hiwa, Hiroshima Pref.	HS73	C
Koriyama, Fukushima Pref.	HS127	C
Mt. Shiragami, Kumamoto Pref.	HS143	C
Dogo, Oki Is.	HS172	C
Dozen (Nishinoshima), Oki Is.	HS173	C
Dozen (Nishinoshima), Oki Is.	HS174	C
Dozen (Nishinoshima), Oki Is.	HS175	C
Dozen (Nishinoshima), Oki Is.	HS176	C
Mt. Amagi, Shizuoka Pref.	HS189	C
Kamikoshikijima Is.	HS277	C
Ashiu, Kyoto Pref.	HS282	C
Mt. Moriyoshi, Akita Pref.	HS301	C
Oze, Gunma Pref.	HS303	C
Mt. Tsurugi, Tokushima Pref.	HS310	C
Mt. Nachi, Wakayama Pref.	HS349	C
Karumai, Iwate Pref.	HS375	C
Katsuura, Chiba Pref.	HS378	C
Aomori, Aomori Pref.	HS389	C
Miyakonojo, Miyazaki Pref.	HS3002	C
Fukuejima Is.	HS3010	C
Akiyoshidai, Yamaguchi Pref.	HS3014	C

DNA sequences of the underlined DNA codes were determined in this study; all other sequences were obtained from the DNA databases DDBL/ENA/GenBank (e.g., Suzuki et al. 2015).
Locality numbers for samples collected from Hokkaido are shown in Fig. 1.

Sequencing analysis

Genomic DNA was extracted from ethanol-preserved tissue samples using a QIAamp DNA Mini Kit (QIAGEN) following the manufacturer's protocol. *Cytb* and CR sequences were determined as reported previously (Suzuki et al. 2015). The sequences were aligned using the Muscle function of the MEGA7 software (Kumar et al. 2016). All sequences obtained in this study were deposited in the International DDBJ/ENA/GenBank DNA database (accession nos. LC655789–LC655874).

Phylogenetic and population genetic analysis

A maximum-likelihood (ML) tree was constructed with concatenated mtDNA sequences of *Cytb* and CR using MEGA7 (Kumar et al. 2016) with the GTR + G + I model. The best fit model was determined using Akaike Information Criterion (AIC) implemented in MEGA7. Node reliability was assessed using 500 bootstrap replicates. Median-joining (MJ) networks were constructed using the concatenated mtDNA sequences with PopART v1.7 (Leigh and Bryant 2015).

Geographical trends in genetic diversity were examined using landscape shape interpolation analysis, implemented in Alleles In Space (AIS; Miller 2005). We converted the latitude and longitude of each sampling locality to XY coordinates on an arbitrary scale, using the plane rectangular coordinate system of Japan (Zone 12, 44°00'N, 142°25'E), following the instruction of AIS. Because sample sizes differed among localities, we used one sequence per haplotype at each location to avoid sampling bias. Using the geographic coordinates and DNA sequences of *Cytb*, we generated a Delaunay triangulation-based connectivity network of sampling localities and their relative genetic distances, which decreased with increasing geographic distance.

We used ARLEQUIN v3.5.1 to calculate nucleotide (π) and haplotype diversity (Hd) using each locus data (Excoffier and Lischer 2010). Mismatch distribution analyses and neutrality tests, Tajima's *D*, and Fu's *F*s, were performed to evaluate rapid population expansion using ARLEQUIN. The significance of neutrality was assessed using 1000 replicates of coalescent simulations. Historical population expansion was assessed through mismatch distribution analysis (Rogers and Harpending 1992). The expected distribution was simulated using the sudden expansion model (Schneider and Excoffier 1999; Excoffier 2004), which was validated using a parametric bootstrap approach with 1000 replicates. For each replicate, we compared the sum of squared deviations (SSD) between the observed and expected distributions with the SSD between the simulated and expected distributions using ARLEQUIN. We applied the raggedness index (*r*) (Harpending 1994) as a test statistic for sudden expansion model estimation. The expansion parameter tau (τ) was estimated in each set of haplotypes (we call it Group) to search for signs of sudden demographic expansion using ARLEQUIN (Excoffier and Lischer 2010). The start times (*t*) of expansion events were calculated using the equation $t = \tau/2\mu k$, where μ is the evolutionary rate in years and *k* is the length of the analyzed fragment (1140 bp) (Suzuki et al. 2015; Hanazaki et al. 2017). There is growing evidence to support that the rate of evolution of mtDNA in animals is not constant and is time-dependent, especially in the early stages of divergence, partly due to the involvement of mild deleterious mutations (e.g., Ho et al. 2005, 2011; Inoue and Suzuki 2022). Hence, here we used the time-dependent evolutionary rate of *Cytb* in small mammals (Honda et al. 2019; Suzuki 2021).

Results

Phylogeny and spatial distribution of mtDNA variation

We determined the *Cytb* (1140 bp) and CR (552 bp) sequences of mtDNA in 43 samples of *A. speciosus* newly collected from Hokkaido. A 2-bp insertion observed in two CR sequences (HS5444, HS378) was removed from subsequent phylogenetic analyses. We combined these sequences with those from databases ($n = 63$) to obtain 106 sequences in total: 83 sequences from 23 localities on Hokkaido and 23 sequences from 20 localities on Honshu, Shikoku, and Kyushu (Table 1, Fig. 1A).

We constructed an ML tree (Fig. 2) and MJ network (Fig. 3) using the concatenated sequences of *Cytb* and CR. In the MJ network, we found star-shaped clusters representing Hokkaido and Honshu/Shikoku/Kyushu, as previously reported (Suzuki et al. 2015). The Hokkaido cluster encompassed a cluster with small numbers of nucleotide substitutions (Group A; Fig. 3). The remaining Hokkaido haplotypes and the Honshu cluster were designated Groups B and C, respectively (Fig. 3). The ML tree showed that neither of monophyly for Group A and B from Hokkaido was supported (Fig. 2). The geographic distribution of Groups A and B is shown in Fig. 1A. Group A was observed throughout Hokkaido, but was more abundant in central Hokkaido, whereas Group B appeared frequently in southern and eastern Hokkaido.

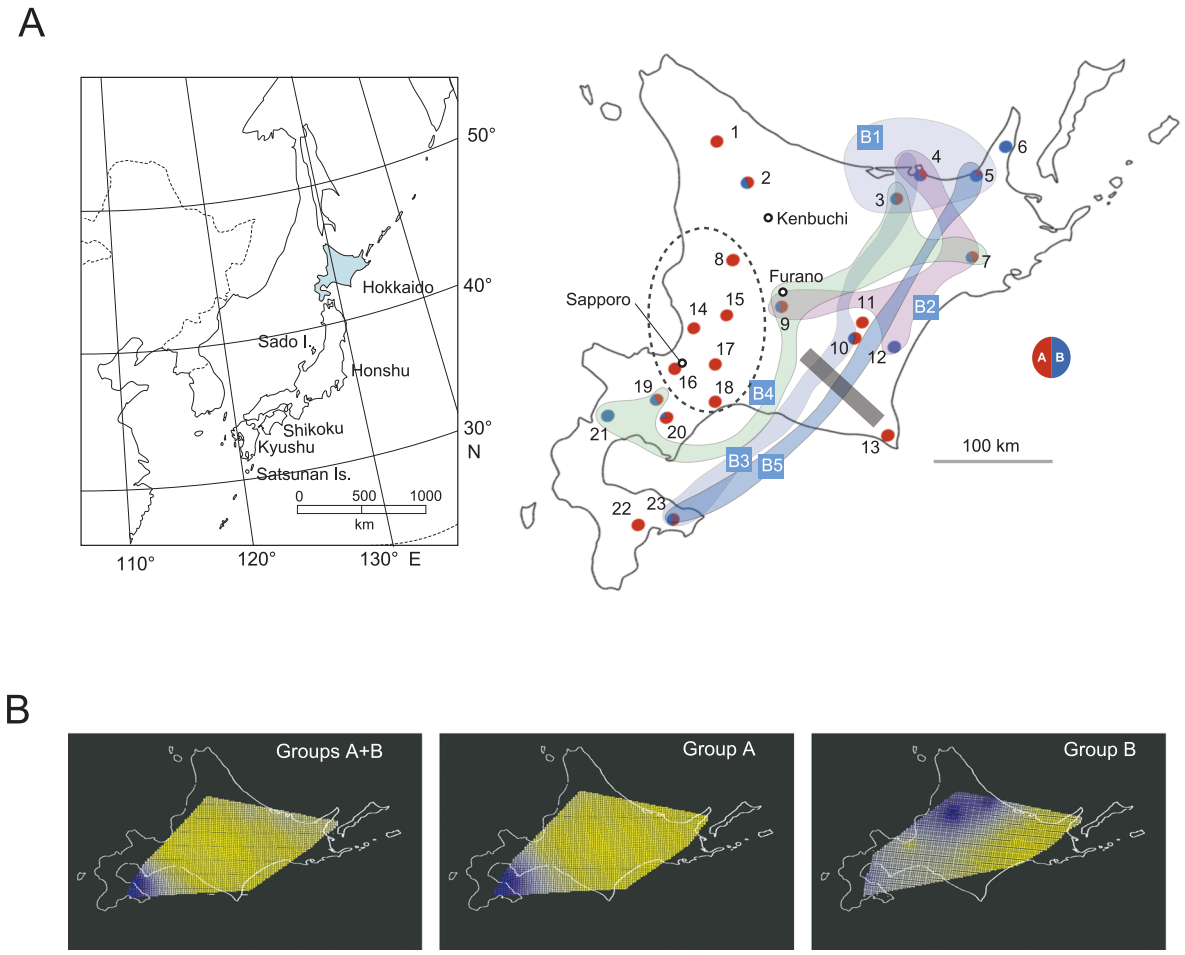

Fig. 1. (A) Sampling localities for the large Japanese wood mouse *Apodemus speciosus* in Hokkaido, the northern island of the Japanese archipelago. Geographic distribution of mitochondrial Groups A and B. Dotted circle indicates the approximate area of central Hokkaido. The geographic ranges of subgroups B1–5 of Group B are shown. Dark gray shading indicates the Hidaka mountain range. (B) Genetic landscape interpolation analysis results for mitochondrial Groups A and B. Light (yellow in online version) and dark (blue in online version) shading indicate high and low genetic diversity, respectively.

The ML tree showed four subgroups (B1–B5) of haplotypes connected by short branches, supported by moderate to high bootstrap values (73–99%). Haplotypes from B1 and B2 tended to be confined to eastern Hokkaido, whereas those from B3, B4, and B5 were distributed in both southern and eastern Hokkaido. Overall, members of Group B, which was derived from an older period than those of Group A, tended to remain within eastern Hokkaido.

We performed landscape shape interpolation analysis of the *Cytb* sequences from Hokkaido (Fig. 1B). In Group A, overall high genetic diversity in Hokkaido was observed, while only the southwestern part was colored dark gray (blue in online version) , showing low genetic diversity. Group B showed relatively high genetic diversity in the eastern part.

Analyses for rapid population expansion

In each gene sequence dataset of *Cytb* and CR, the values of Hd, π, and τ were lower and higher in Groups A and C, respectively (Table 2). The value of τ was lower in Group B than Group C in the *Cytb* sequences, but nearly the same in Groups B and C in the CR sequences. Tajima's *D* and Fu's *F*s neutrality tests were significantly negative in almost all datasets examined, but Tajima's *D* was not significant in the CR dataset of Group B. Mismatch distribution analysis showed that the sudden expansion model was not rejected in all datasets except *Cytb* sequences in Group B in terms of both SSD and RI (Table 2, Fig. 4).

It has been reported that the different evolutionary rate should be applied to time estimations at different ages because of the time dependency of the evolution-

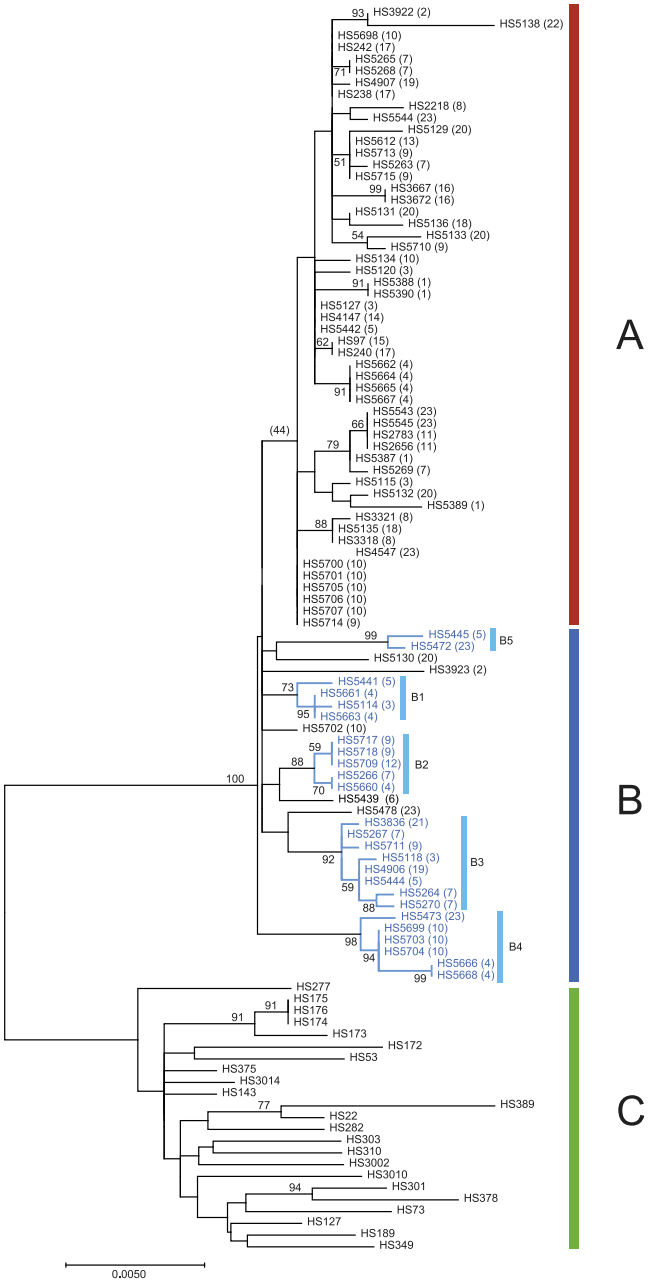

Fig. 2. Maximum-likelihood trees constructed from 106 concatenated sequences (cytochrome *b* and the control region, 1692 bp) of *Apodemus speciosus*. Three clusters represented by haplotypes from Hokkaido (Groups A and B) and Honshu/Shikoku/Kyushu (Group C), and four subgroups of Group B, are indicated. In Groups A and B, numbers in parentheses next to species names indicate the sampling locality (Table 1, Fig. 1A). Node numbers indicate bootstrap values (1000 replicates). Bar indicates substitution numbers per site.

ary rates (Suzuki 2021). Times of the onset of expansion events were therefore estimated using the *Cytb* data, considering the time-dependent evolutionary rate curves as shown in *Cytb* of small mammals, which are similar among different lineages, such as wood mice, voles, and moles (Suzuki 2021). Accounting the degree of the τ values for *Cytb* in Groups A (τ = 2.88), B (τ = 4.99), and C (τ = 7.84) (Table 2), we selected different evolutionary rates of 0.11, 0.047, and 0.028 substitutions/site/million years, respectively, according to the evolutionary rates estimated for each time scale of the τ value (Honda et al. 2019; Suzuki 2021). The start times of the expansion events in Groups A, B, and C were estimated to be 11 500, 46 600, and 122 000 years ago, corresponding to the early stages of MIS 1, MIS 3, and MIS 5, respectively. Furthermore, when the data set (n = 83) of Groups A plus B was examined, signals of rapid population expansion were obtained in the test of Tajima's D and Fu's Fs, and the value of τ obtained (τ = 4.41) was similar to that of Group B (Table 2), suggesting that the expansion started at the early MIS 3.

Discussion

Possible factors shaping rapid expansion events in small terrestrial mammals

In this study, we constructed a phylogenetic tree using the *Cytb* and CR sequences (1692 bp) of *A. speciosus* from the Japanese archipelago, revealing three groups (A–C) as observed in our previous study (Fig. 2; Suzuki et al. 2015). The constructed network of the concatenated (Fig. 3) and *Cytb* (1140 bp; data not shown) sequences exhibited small (Group A: τ = 2.88 in *Cytb*), medium (Group B: τ = 4.99 in *Cytb*), and large (Group C: τ = 7.84 in *Cytb*) star-shaped characteristics in the network. The rapid expansion of Groups A and C was supported by non-significant SSD and RI statistics and significant P values for Tajima's D and Fu's Fs tests in both *Cytb* and CR (Table 2, Fig. 4). The rapid population expansion of Group B was generally supported, but not by SSD or RI analyses of the *Cytb* data or Tajima's D for the CR data (Table 2). This significant divergence between observed and expected mismatch distributions may be explained by a secondary expansion event, as indicated by subgroups B1–5 (Fig. 3).

The formation of these small and large groups was likely caused by rapid expansion events initiated 10 000 and 130 000 years ago, as post-LGM and post-PGM events, respectively (Suzuki et al. 2015). The substantial effects of these glacial maxima on population expansion have been suggested in mtDNA analyses of several Japanese mammals including the small Japanese wood mouse (Suzuki et al. 2015; Hanazaki et al. 2017), western Japanese mole *Mogera wogura* (Nakamoto et al. 2021),

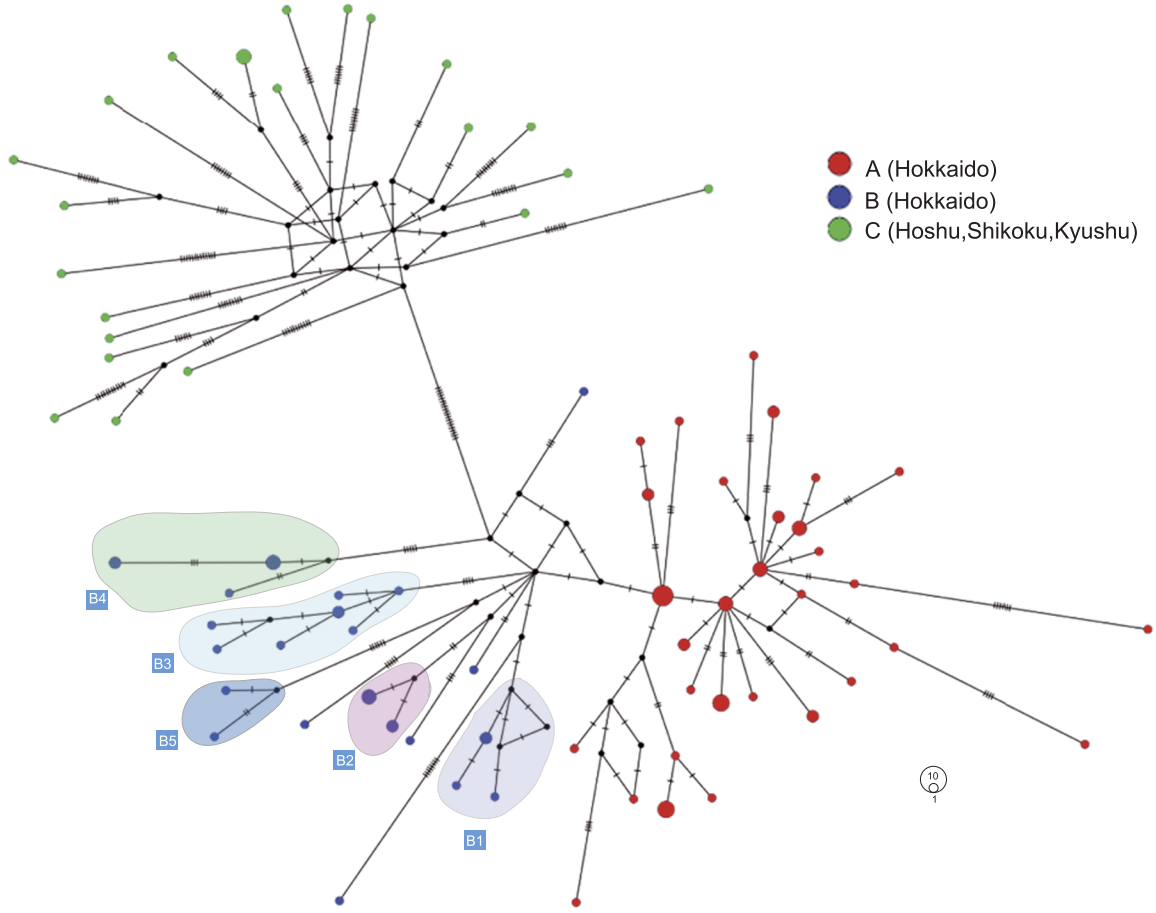

Fig. 3. Median-joining networks constructed from 106 concatenated sequences (cytochrome *b* and the control region, 1692 bp) of *Apodemus speciosus*. Three clusters are represented by haplotypes from Hokkaido (Groups A and B) and Honshu/Shikoku/Kyushu (Group C). Two subgroups (A1, A2) of Group A and five subgroups (B1–5) of Group B are indicated. Mutation numbers between haplotypes are indicated by hatch marks. Circle size is proportional to sample size.

Table 2. Genetic diversity indices and detection of rapid expansion events with the mismatch distribution analysis of two mitochondrial DNA markers (*Cytb* and CR) for *Apodemus speciosus*

Gene	Haplogroup	N	Hd	π	Tajima's *D*	Fu's *Fs*	RI	SSD	τ
Cytb	A	53	0.922	0.003	–1.994**	–12.806**	0.028	0.002	2.88
	B	30	0.956	0.004	–1.613*	–8.823**	0.131**	0.054**	4.99
	C	23	0.988	0.008	–1.767*	–10.995**	0.010	0.003	7.84
CR	A	53	0.899	0.004	–1.916**	–19.733**	0.052	0.002	2.13
	B	30	0.952	0.009	–0.523	–6.460**	0.008	0.001	6.01
	C	23	0.988	0.013	–1.803**	–13.442**	0.018	0.019	5.97

See Figs. 3 and 4 for corresponding phylogroups of *A. speciosus*.
N: sample size, Hd: haplotype diversity, π: nucleotide diversity, SSD: sum of squared deviations, *r*: Harpending's raggedness index, τ: expansion parameter.
$P < 0.05$ (*), $P < 0.001$ (**)

Japanese giant flying squirrel *Petaurista leucogenys* (Oshida et al. 2009), and red-backed vole species (genus *Myodes*, Honda et al. 2019). Therefore, we can conclude that the effects of both glacial maxima had a great impact on the population dynamics of *A. speciosus* and other small mammals in the Japanese archipelago.

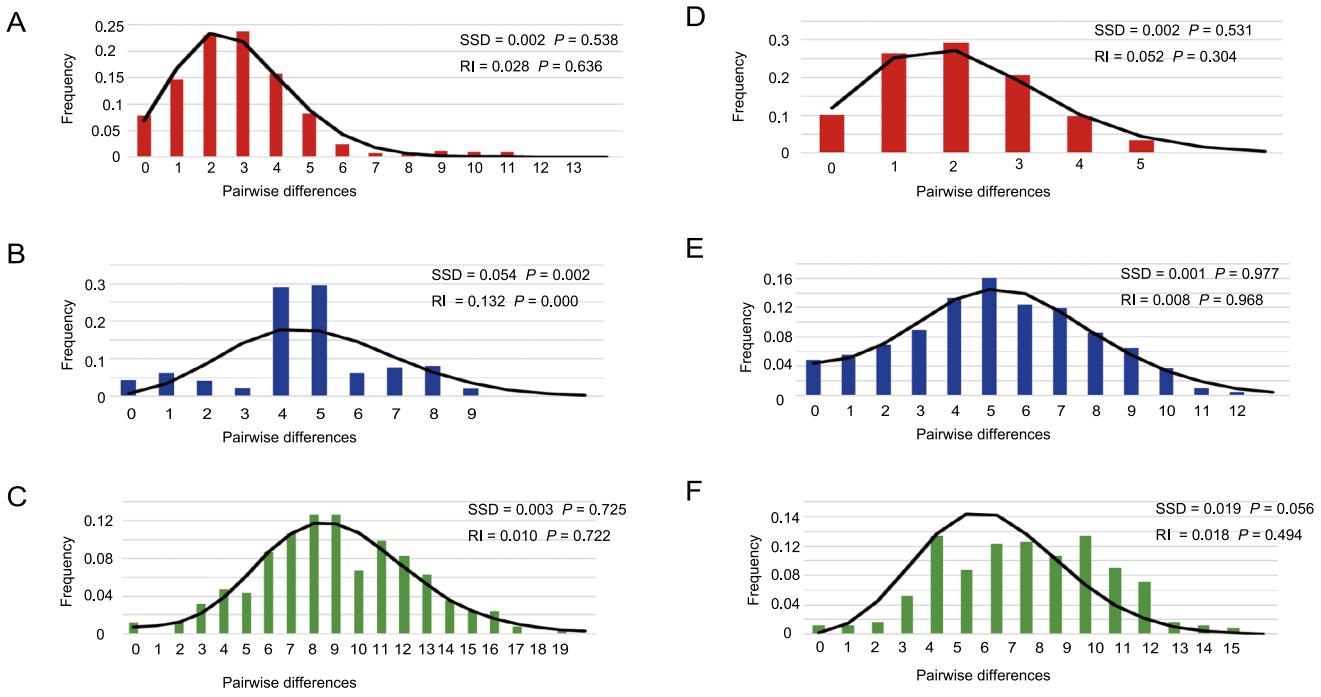

Fig. 4. Mismatch distribution of the mitochondrial gene sequences: cytochrome *b* (*Cytb*; A–C) and the control region (CR; D–F). Each Group of A (A, D), B (B, E), and C (C, F) was analyzed. Bars indicate observed frequency; line indicates the expected frequency under the sudden expansion model. SSD, sum of squared deviations; RI, Harpending's raggedness index.

The τ values of the groups (Table 2) suggest that the medium-sized Group B (*Cytb*, τ = 4.99) is the consequence of an event that occurred between the post-LGM (Group A; *Cytb*, τ = 2.88) and post-PGM (Group C; *Cytb*, τ = 7.84) events. The climate change from MIS 4 to MIS 3 is known to have had a tremendous impact on the global climate (Doughty et al. 2021; Geibert et al. 2021). Rapid population growth of the Holarctic northern red-backed vole (*Myodes rutilus*) in North America is reported to have been associated with an expansion event ca. 50 000 years ago, when large ice sheets shrank in size throughout the continent (Kohli et al. 2015). The grassland-dwelling striped field mouse *A. agrarius* occurring in a broad area of northern Eurasia from Europe to Asia showed a rapid expansion signal during MIS 4-3 in mtDNA sequence data (Kozyra et al. 2021). Interestingly, the forest-dwelling Asian house rat (*Rattus rattus* complex mitochondrial Lineage II, defined by Pagès et al. 2010 and Aplin et al. 2011), which occurs in East and Southeast Asia including Myanmar, also showed rapid expansion signals in mtDNA analysis (Maung Maung Theint et al. 2020), suggesting that the effects of climate change on small rodents associated with the early MIS 3 were widespread and numerous at the global level. In the Japanese archipelago, MIS 4 was characterized by more snowfall and greater glacial development due to the presence of high mountain ranges (e.g., Hidaka Mountains, Hokkaido; Fig. 1) compared to the climate change impacts of the LGM (Sawagaki et al. 2004), and therefore it might be sufficient to trigger a population bottleneck as a prerequisite event for an expansion event to occur (Hanazaki et al. 2017). In an mtDNA study of *A. argenteus*, Hanazaki et al. (2017) concluded that an expansion event was triggered by warming during the early MIS 3. In our previous mtDNA study of grassland *Myodes* voles, which occur in a broad area of Hokkaido (*M. rufocanus*) and in the high elevation grasslands of western Honshu (*M. smithii*), we observed signals of multiple expansions, presumably initiated during early MIS 3 (Honda et al. 2019). These considerations led to the hypothesis that changes in the environment during the late MIS 4 and early MIS 3 caused the rapid expansion of Group B in the Hokkaido population of *A. speciosus*.

Possible scenarios for the formation of the spatial genetic structure of A. speciosus *in Hokkaido*

The distribution patterns of the genetic structure of Groups A and B of *A. speciosus* populations in Hokkaido are shown in Figs. 1 and 5A. The newly generated Group

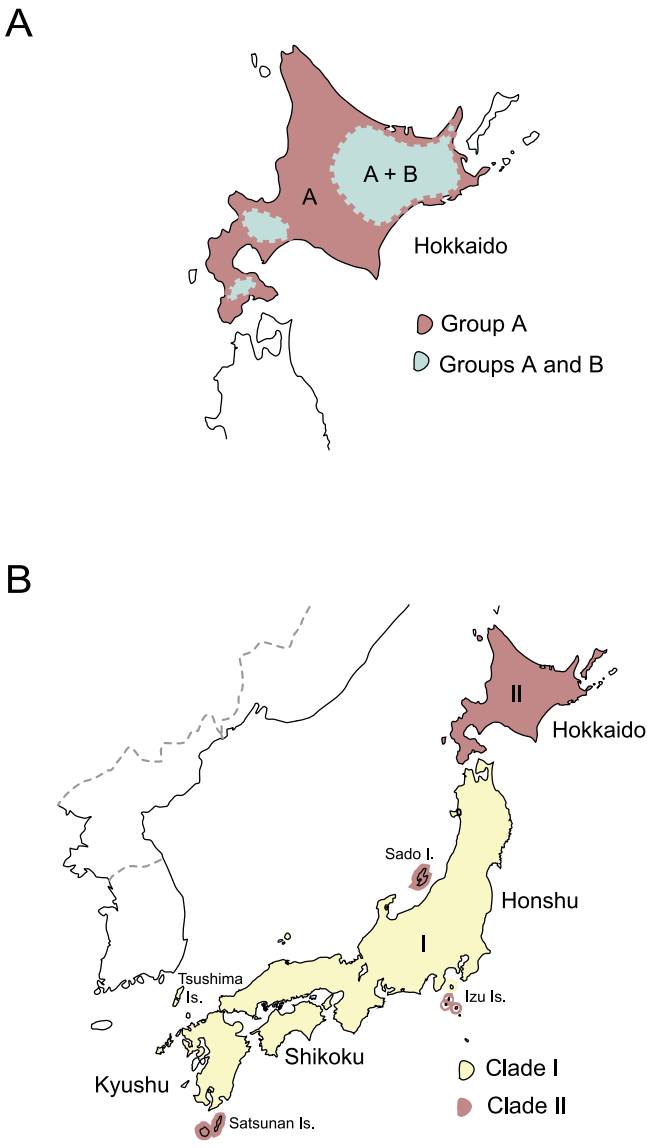

Fig. 5. Geographic distribution of distinct mitochondrial lineages in Japanese wood mice. Schematic representation of Groups A and B in *A. speciosus* from Hokkaido (A, this study), and two distinct clusters of *A. speciosus* from the Japanese archipelago (B, Suzuki et al. 2004; Tomozawa and Suzuki 2008; Tomozawa et al. 2014; Suzuki et al. 2015). This geographic structure is well explained by the concept that sites with low genetic diversity serve as sources of postglacial colonization, while those with high genetic diversity become sinks for migrating haplotypes. In addition, simultaneous mtDNA expansion occurs as one haplotype tends to drive out the others, whereas islands and apical regions tend to evade this effect.

A was found to be dominant in central Hokkaido, but coexisted with the older Group B in peripheral areas. Generally, glacial refugia are assumed to harbor more genetically distant alleles than areas colonized after a glacial period. Thus, in addition to Group A, several distinct lineages belonging to Group B were detected in the eastern part of Hokkaido (Fig. 1A), suggestive of its role as a glacial refugium. In contrast, limited numbers of distinct lineages were detected in central and southern parts of Hokkaido, suggesting that the populations of western part of Hokkaido were newly formed by westward migration from eastern Hokkaido during the post-LGM warm period.

Alternatively, the high genetic diversity indicated by the presence of several distinct lineages of Group B in eastern Hokkaido can also be explained by the migration of haplotypes that survived the LGM in south and central parts of Hokkaido. This can be considered in the context of source-sink dynamics (e.g., Fazio et al. 2004). It assumes an eastward shift of glacial retreat in Hokkaido, which can be supported by a pollen fossil study that showed increasing dominance of *Quercus* (oak trees) in southwest Hokkaido ca. 1000 years earlier than in northern Hokkaido (Igarashi et al. 2011). This indicates that it is important to note that areas of high genetic diversity are not necessarily sources of diversity, but rather may be areas that have characteristics as sinks. In *A. speciosus*, the mtDNA clade (Clade II) from offshore islands of Sado Island, Hokkaido Island, Izu Islands, and Satsunan Islands (Fig. 5B) are thought to have originated by intermittent migrations from the central main islands to the offshore islands during the construction of the land bridge-like structure at the periods of glacial maxima (Suzuki et al. 2004; Tomozawa and Suzuki 2008), resulting in a higher degree of genetic diversity in the offshore islands as a whole. This is explained in the context of source-sink as well.

Conclusion

In this study, we assessed intraspecific mtDNA variations in *A. speciosus* in the Japanese archipelago. We obtained rapid expansion signals in three distinct groups using *Cytb* and CR sequence data and estimated times of the onset of the expansion events with *Cytb* data using the available time-dependent evolutionary rates (Suzuki 2021). In addition to confirming the occurrence of rapid expansion events in the post-LGM and post-PGM of the Hokkaido and Honshu/Shikoku/Kyushu populations, respectively, we considered that severe environmental changes in the early MIS 3 period triggered rapid expansion of the Hokkaido population. Notably, higher genetic diversity was found in eastern Hokkaido and lower genetic diversity was observed in central Hokkaido, which can be explained by westward migration from eastern Hokkaido to central Hokkaido during the post-

LGM warm period. Alternatively, it can be explained by the occurrence of an efficient post-LGM expansion event in central Hokkaido that replaced other existing haplotypes. Several small-scale expansion events (subgroups B1–5, Fig. 3) also occurred in southern Hokkaido; therefore, we suggest that haplotypes were dispersed from these sources to the sink of eastern Hokkaido, resulting in higher diversity in the peripheral area. In summary, the framework of *A. speciosus* mtDNA diversity was formed by rapid population expansion events associated with late-Quaternary environmental changes that further influenced the degree and spatial pattern of mtDNA diversity. These changes, together with various ecological and geographical factors, appear to have influenced *A. speciosus* genetic diversity.

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