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Mitochondrial phylogeography and population history of the large Japanese wood mouse (*Apodemus speciosus*) on Sado Island, Japan

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Abstract. A phylogeographic study of the large Japanese wood mouse, *Apodemus speciosus*, on Sado Island, Japan, was performed based on sequences of the mitochondrial cytochrome *b* gene (1,140 bp). Our previous study covered the entire species range and suggested that the mice on Sado Island are monophyletic, exhibiting two well diverged lineages throughout the island. The present data also supported two lineages (the average number of nucleotide difference was 11.4), showing a weak phylogeographic structure. Given the high sequence divergence observed, we assumed historically subdivided populations within the island. Bayesian coalescent analysis supported a dual-population model rather than that of one large population. The times to most recent common ancestor of all sequences were 293,000 years ago [ka; 95% highest probability density (HPD) 85–634 ka] and 292 ka (HPD 102–605 ka) for the one- and dual-population models, respectively. These results suggest that the populations have undergone repeated separations and reconnections, rather than being subdivided completely through time. Our results are in accordance with other paleogeographic and phylogeographic evidence from the island. The present study highlighted a unique system of producing and maintaining genetic diversity and suggested prehistoric colonization of the *A. speciosus* population on Sado Island, thus supporting the ancient origin of the mammalian fauna of Sado Island.

Key words: *Apodemus speciosus*, Bayesian coalescent analysis, phylogeography, Pleistocene sea-level change, Sado Island.

Sado Island, located in the middle of the Japanese archipelago, represents an enigmatic and interesting field for mammalian biogeography because of its rugged topography and independent geological history. A relatively deep sea strait (>200 m) separates the island from the adjacent island (Honshu), and no geological evidence suggests a past connection of these islands (Japan Association for Quaternary Research 1987). However, some phylogeographic evidence implies the prehistoric colonization of terrestrial mammals on the island (shrews, Ohdachi et al. 2001; wild boars, Watanobe et al. 2004). Thus, the accumulation of phylogeographic information for various taxa on Sado Island is necessary to clarify the origin of the mammalian fauna on the island.

The large Japanese wood mouse, *Apodemus speciosus*, is a useful model species for assessing the possibility of past interisland connections in the Japanese archipelago. Phylogeographic analyses covering the entire species range showed that some groups of small island populations (including Sado Island) harbor a reciprocally monophyletic mitochondrial lineage (Suzuki et al. 2004). Our previous study based on a nuclear gene in combination with mitochondrial genes revealed a strong association between the phylogeographic structure and the connection–separation events among the islands as well as demographic fluctuation during the Quaternary glacial cycles (Tomozawa and Suzuki 2008). Therefore, a more intensive phylogeographic analysis with emphasis on Sado Island will help evaluate the possibility of a past connection between Sado Island and Honshu Island.

The population of *A. speciosus* within Sado Island harbors peculiar genetic variations. Our previous study

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covering the entire species range suggested monophyly of the Sado population of *A. speciosus* and two well diverged mitochondrial lineages within the island [cytochrome *b* (*Cyt b*), 1,140 bp; Tomozawa and Suzuki unpublished data]. This was quite unexpected because the island is small (855 km^2) and located on the periphery of the species range, and thus the population should have had fewer immigrants and experienced a severe bottleneck upon colonization (*i.e.*, founder effect). Indeed, other peripheral populations of similarly sized islands exhibit less sequence divergence within an individual island (Tomozawa and Suzuki 2008). Why such high sequence diversity is maintained in this small island remains unknown. However, we can frame a model to address this question based on the topography of this island. The island consists of two mountain masses, Osado (1,172 m at the highest point) and Kosado (645 m at the highest point), which comprise the northwest and southeast parts of the island, respectively, which are connected by a low flat land (Kuninaka Plain, <10 m above sea level; Fig. 1). Therefore, if the island is currently uplifting, it would have been separated into two landmasses by a sea strait in the past. Bayesian coalescent analysis (Drummond and Rambaut 2007) would be a helpful tool to evaluate such a model of population history.

Materials and methods

Samples

We trapped 23 animals in March 2008 and extracted total genomic DNA from liver tissues using a standard phenol–chloroform protocol. Together with five DNA samples stored in our laboratory, we analyzed 28 samples from 10 localities covering almost the entire island (Table 1, Fig. 1).

Table 1. Samples used in this study

	Location No.	Location name	No. of individuals	Sample code
Osado	1	Washizaki	1	ST23
	2	Kitaujima	2	ST21, 22
	3	Umezu	1	ST20
	4	Himezu	4	ST16-19
Kosado	5	Ryotsu	2	HS101, 104
	6	Sumiyoshi	3	HS2803-2805
	7	Niibo	3	ST1-3
	8	Oguragawa	1	ST4
	9	Mano	5	ST11-15
	10	Shukunegi	6	ST5-10

The location numbers correspond to Fig. 1.

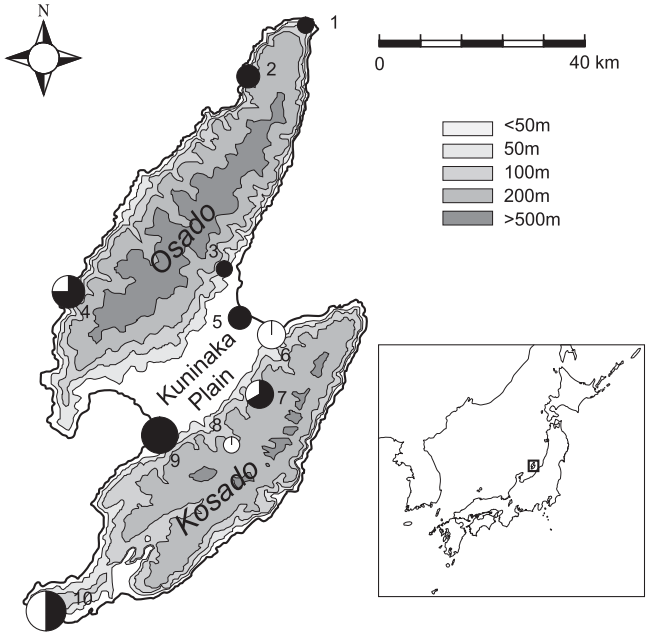

Fig. 1. Geographical origins of the sampling localities given in Table 1. Sampling localities are indicated by pie charts. Each pie chart displays the proportion of S1 (black) and S2 (white) mitochondrial lineages with the size corresponding to the number of samples.

Data analyses

We determined the *Cyt b* gene sequences (1,140 bp) using the method described by Suzuki et al. (2004). Sequences were resolved on a 3100 Genetic Analyzer (Applied Biosystems) and assembled into a single consensus sequence with the aid of PROSEQ software (Filatov 2002).

Nucleotide diversity (π) and the average number of nucleotide differences among all samples and between the two lineages were calculated using MEGA ver.4 (Tamura et al. 2007). The standard errors around these values were obtained by bootstrapping with 1,000 permutations implemented in MEGA.

The phylogenetic trees of the *Cyt b* sequences were constructed using the neighbor-joining (NJ) method with PAUP* version 4.0b10 (Swofford 1993) and Bayesian inference (BI) implemented in MrBAYES version 3.1.2 (Ronquist and Huelsenbeck 2003). We selected the best-fit nucleotide substitution model to our data set using the Akaike information criterion (AIC; Akaike 1974) with Modeltest version 3.7 (Posada and Crandall 1998). The parameter estimates obtained under the best-fit model were used in all phylogenetic analyses. Branch support

was evaluated using 1,000 bootstrap replicates for the NJ analyses. Bayesian searches were run for 10,000,000 generations using four chains, and trees were sampled once every 100 generations. The first 25,000 trees within the burn-in period were discarded. The remaining 75,000 trees with a stable likelihood score were used for the construction of a 50% majority-rule consensus tree. The convergence of Markov chain Monte Carlo (MCMC) chains was confirmed using the program Tracer version 1.3 (Drummond and Rambaut 2007).

The population history was estimated based on an alignment of complete *Cyt b* sequences using the relaxed clock (fixed mean at 2.8% per million years; Michaux et al. 2004) model in BEAST version 1.4 (Drummond and Rambaut 2007). A constant population size was assumed for all runs. Depending on the run, MCMC samples chains were run for 10,000,000 generations, sampling every 1,000 generations under the generalized time reversible (GTR) nucleotide substitution model. The burn-in was set at 10% of the posterior sample. The convergence of MCMC chains was confirmed using the program Tracer. We framed two demographic models: one assumed two subpopulations on the island by specifying taxon groups based on the phylogenetic tree (dual-population model), whereas the other assumed one population throughout history (one-population model). The models were compared by "posterior" values and calculating a $\log_{10}$ Bayes Factor ($\log_{10}$ BF: 0.5–1; Substantial, 1–2; Strong, >2; Decisive) with the aid of Tracer. These parameter setting files (.xml files) were produced using the program BEUti (Drummond and Rambaut 2007) and are available as supplemental data upon request.

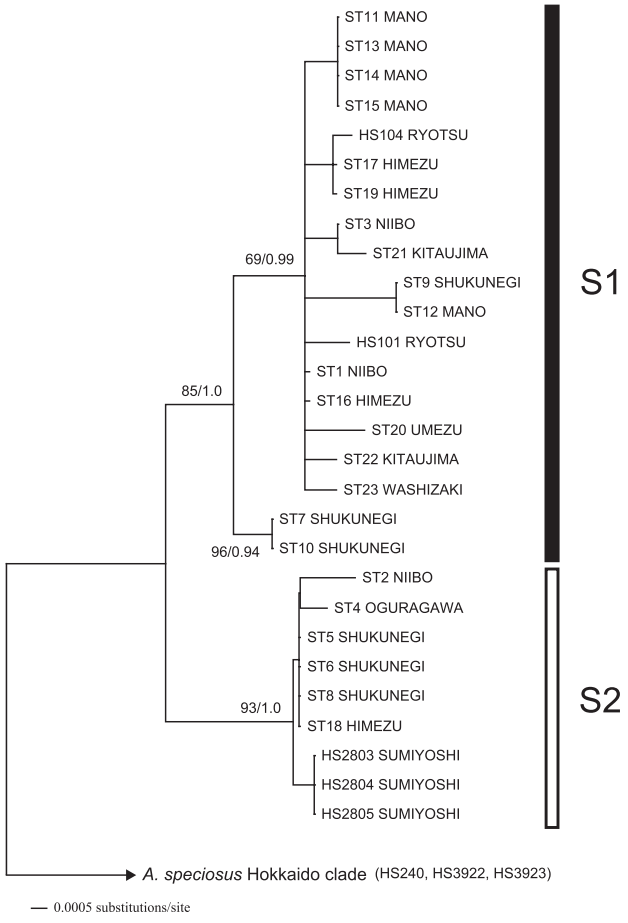

Fig. 2. Neighbor-joining (NJ) tree of 28 sequences of the *Cyt b* gene from *Apodemus speciosus* with bootstrap values (1,000 replicates). The names of the clades are given to the right of the taxa names. Numbers along the branches indicate the percentage of bootstrap support obtained in the NJ analysis and posterior probabilities of Bayesian inference.

Results

The complete *Cyt b* gene sequences (1,140 bp) were determined for all *A. speciosus* samples. In total, 35 sites were variable, which resulted in 16 haplotypes. The average numbers of nucleotide differences and nucleotide diversities (π) were 6.78 (S.E. 1.64) and 0.059% (S.E. 0.014%), respectively. The newly identified haplotypes were deposited in GenBank (AB503218–AB503240).

The best-fit mutation model for the data set (1,140 bp) was a Transversion + I (TVM + I) model. The phylogenetic trees produced using the NJ and BI methods showed very similar topologies (Fig. 2). We found two lineages based on the trees (S1 and S2; Fig. 2). The average nucleotide difference between the two clades was 11.4 (±3.1) per gene. The monophyly of S1 clade was supported by moderate bootstrap values in both analyses.

The Bayesian coalescent analysis stabilized before 100,000 steps of the MCMC runs. The marginal likelihood of a run assuming a dual population was higher than that of the one-population model ($\log_{10}$ BF = 2.15). The time to most recent common ancestry (MRCA) for all samples was estimated to be 293,000 years ago [ka; 95% highest probability density (HPD) 85–634 ka] and 292 ka (HPD 102–605 ka) for the one- and dual-population models, respectively. The S1 clade was estimated to have MRCA between 53 (HPD 10–120 ka) and 55 ka (HPD 9.3–130 ka) for the one- and dual-population models, respectively. The time to MRCA of the S2 clade was estimated to be 154 (HPD 52–300 ka) and 155 ka (HPD 53–294 ka) under the one- and dual population

Table 2. Results of Bayesian coalescent analyses assuming the one-population and dual-population models

		One population			Dual population		
		lower	mean	upper	lower	mean	upper
TMRCA	All clades	85490	293400	634300	102000	292100	605400
	S1 clade	8723	53440	123558	9390	55670	130900
	S2 clade	52580	154900	301330	53930	155600	294200
Mean in (posterior)			–1638.6			–1633.7	

TMRCA indicates the time to most recent common ancestor.

model, respectively. The parameters of each run are presented in Table 2.

Discussion

Colonization of terrestrial mammals on Sado Island

Our previous study covering the entire species range showed monophyly of the Sado Island population, although with a low support value, suggesting one past colonization event. The present study suggested that the time to MRCA of the *Cyt b* sequences was 292 ka (HPD 102–605 ka). The phylogeographical data of some other mammal species suggest prehistoric colonizations of terrestrial animals such as shrews (Ohdachi et al. 2001), wild boars (Watanobe et al. 2004), and moles (500 ka; Kirihara et al. unpublished data) onto Sado Island. However, the estimated timings of these colonizations are debatable and vary among taxa. Conversely, some mammals such as *A. argenteus* (Suzuki et al. 2004), hares (<20 ka; Nunome et al. unpublished data), and voles (*Microtus montebelli*; Tomida personal communication) showed less mitochondrial sequence divergence. These observations suggest that several opportunities to colonize the island took place, or that artificial introductions were made of some mammal species now on Sado Island. Nevertheless, our data on *A. speciosus* as well as other phylogeographic data all strongly suggest that at least a chance to colonize the island presented itself before the middle and late Pleistocene.

Mechanism of maintaining intraisland variation

The results of the Bayesian coalescent inference of population history suggest that the dual-population model is more appropriate than the one-population model. The weak phylogeographic structuring also supports the model of subdivided populations. The S2 clade was mostly sampled from the Kosado area except for one individual, while the Oosado area harbored both clades (Figs. 1 and 2). These data suggest that some (at least two) subdivided populations within the island were not completely isolated.

This situation is realistic given the landscape of the island and the paleogeographic evidence therein. Several marine terraces were reported at different altitudes on the island, suggesting rapid uplifting of the island (Ota 1964; Ota et al. 1992). A marine terrace (~50 m above sea level) in the edge of the Kuninaka Plain is indicative of sea transgression during the last interglacial period (isotope stage 5e: 110–130 ka; Ota et al. 1992). Therefore, if the plain area uplifted through the Pleistocene at the same rate as the last 130,000 years (~0.4 m/kyr), the two mountain masses were likely separated as two isolated islands during the early to middle Pleistocene as well as during the last interglacial period. Such population subdivisions would be periodical because the subsequent sea-level drop during the glacial period would have enabled populations to intermingle. Thus, the period of separation might not have been long enough to produce reciprocally monophyletic lineages in each population. Instead, such a sequence of dividing and mixing events during the glacial cycles would have kept the effective population size high and functioned to maintain the high genetic variability within the small island.

Here we revealed that the population of *A. speciosus* on Sado Island was founded at least prior to human colonization, presumably in the penultimate glacial cycle or the one preceding it. This is supported by the phylogeographic data of other species, which suggest ancient origins of the mammalian fauna on Sado Island. Moreover, the high genetic diversity seen on Sado Island was likely maintained through past population subdivision and contact events associated with Pleistocene sea-level changes. The mechanism for producing and maintaining genetic diversity is analogous to the population subdivision associated with glacial extension in mountainous

areas (Trewick et al. 2000) and thus can be applied to other areas and taxa.

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