# Genetic Distinctness and Variation in the Tsushima Islands Population of the Japanese Marten, *Martes melampus* (Carnivora: Mustelidae), Revealed by Microsatellite Analysis

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A carnivoran mammal endemic to Japan, the Japanese marten (*Martes melampus*) is native in for-ested regions on Honshu, Shikoku, Kyushu (main islands of Japan), and the Tsushima Islands. The Tsushima population is classified as a different subspecies (*M. m. tsuensis*) from populations on the main islands (*M. m. melampus*). To elucidate the genetic structure of the Tsushima population, we genotyped 101 individuals from the Tsushima Islands and 43 individuals from Honshu and Kyushu using 10 microsatellite loci, and performed population genetic analyses on the genotype data. Genetic diversity was lower in the Tsushima population than in three geographic populations on the main islands: heterozygosity was 0.189–0.364 in the former, compared to 0.457–0.747 in the latter. In addition, high pairwise *F*st values (0.485–0.682) and Nei's standard distance (0.550–1.183) between the Tsushima and main-island populations indicated a high degree of genetic differentia-tion. Finally, a Bayesian clustering analysis showed that the Tsushima population is apparently dif-ferentiated from the main-island populations and comprises two genetic clusters. A factorial correspondence analysis corroborated these results. Our results suggest that restricted gene flow or inbreeding may have reduced genetic diversity in the Tsushima population, which has been geo-graphically isolated from the main-island populations since the formation of Tsushima Strait.

**Key words:** geographic isolation, Japanese marten, *Martes melampus*, microsatellite, population genet-ics, Tsushima Islands

## INTRODUCTION

Populations on smaller, peripheral islands are generally genetically distinct from main-island or continental popula-tions, due to geographic isolation. To better understand these differences, it is necessary to elucidate the genetic charac-teristics and structure of island populations. Comparative studies among island populations can provide valuable insights into the migration and isolation history of the popu-lations.

The Japanese marten, *Martes melampus*, is a carniv-oran mammal endemic to Japan. This medium-sized noctur-nal mustelid is native to forested regions on the main islands (Honshu, Kyushu and Shikoku) and Tsushima Islands, but is not native to Hokkaido (Imaizumi, 1960; Masuda, 2009). The Tsushima Islands population (Tsushima marten) is clas-sified as a different subspecies (*M. m. tsuensis*) from popu-

lations on the main islands (*M. m. melampus*). Japanese martens on the main islands with two different types of win-ter coat color were previously considered distinct subspe-cies (a yellow type, *M. m. melampus*; a brown type, *M. m. bedfordi*), but both are now regarded as *M. m. melampus* (Ellerman and Morrison-Scott, 1966; Anderson, 1970).

The Tsushima marten has a paler coat than do martens on the main islands and has black spots from the throat to the chest (Abe and Ishii, 1987). It is listed as 'near threatened' (NT) on the Red List released by the Japanese Ministry of the Environment in 2007, and was designated a natural monument by the Japanese Agency of Cultural Affairs in 1971.

The origin of the Tsushima marten is closely related to the geological history of the Tsushima Islands. During the Middle Pleistocene, the Japanese archipelago was twice connected (about 0.63–0.64 and 0.43 million years ago, Mya) to the Eurasian Continent by land bridges (Nagaoka, 2001a). In the Late Pleistocene, the Japanese archipelago generally remained separated from the Eurasian Continent by straits, with high mountains on the land bridges remain-ing as islands. The Tsushima Islands between Kyushu

Island and the Korean Peninsula are such islands: they were last connected to Kyushu as late as 20,000 years ago, but have not been connected to the Japanese main islands since Tsushima Strait formed 15,000 years ago (Nagaoka, 2001a). By contrast, Oshima (1990, 1991, 2000) reported that the age of isolation of the Tsushima Islands from Korean Peninsula and the main islands of Japan is much older, about 100,000–150,000 years ago. Thus, the geological history of strait formation remains controversial.

The Tsushima Islands consist of two neighboring islands, North (*Kami*, literally "upper") and South (*Shimo*, literally "lower"), separated broadly by a bay (Fig. 1). Originally connected by two isthmuses at the narrow points, the islands are now completely separated by the artificial Manzeki Channel (40 m wide) and Ofunakoshi Channel (50 m wide) (Figs. 1 and 5); the former was completed in its present form around 1900 (Nagaoka, 2001b), and the latter in 1672 (Tsushima Development Bureau, 2011). Manzeki Bridge and Ofunakoshi Bridge crossing the isthmuses are presently the only two dry connections between the two islands.

There have been several previous genetic studies on the Japanese marten. Kurose et al. (1999) and Hosoda et al. (2000) used complete mitochondrial cytochrome *b* DNA sequences for molecular phylogenetic analyses. Hosoda et al. (1999) used partial cytochrome *b* sequences and restriction fragment length polymorphisms in nuclear ribosomal genes. These studies revealed genetic differentiation between the main-island and Tsushima marten populations, and genetic discontinuity among the main-island popula-

tions, but did not show the Tsushima population as genetically independent, likely due to an insufficient number of molecular markers and small sample sizes. A more comprehensive study by Sato et al. (2009), which analyzed combined sequences from three mitochondrial loci (cytochrome *b*, control region, and NADH dehydrogenase subunit 2) and the nuclear growth hormone receptor, found that the Tsushima marten is genetically homogeneous, with low genetic diversity, and is well differentiated from populations on the main islands.

Microsatellite markers have recently been used for population genetic studies. Microsatellites are DNA regions scattered throughout the genome, and consist of tandem repeats with less than 10 base-pair motifs. The number of repeats is polymorphic due to slippage of the repeats during DNA replication. The polymorphisms can be utilized either for individual identification or population genetic analyses (Frankham et al., 2002). Conservation genetic studies on species of mustelids have used microsatellites to investigate genetic diversity and population genetic structure in native or introduced populations. For example, in a study on the Danish pine marten (*Martes martes*), which has recently declined in number, Pertoldi et al. (2008) found that geographic populations show different levels of loss of genetic diversity. Based on this result, the authors warned against introducing individuals from populations with low genetic diversity into those with high genetic diversity. In another example, Lecis et al. (2008) investigated the phylogeography of the American mink (*Mustela vison*) invasive in Spain for purposes of population management. Geographic populations of American minks in Spain are genetically differentiated due to low gene flow and low migration rates among populations. As described above, microsatellites can be informative molecular markers for understanding population genetic structure in detail.

In the present study, we investigated the genetic structure of the Tsushima marten population by genotyping using 10 microsatellite loci and compared this population with those on the main islands. Based on the results, we discuss the genetic diversity and structure of the Tsushima Islands population.

## MATERIALS AND METHODS

### Sample collection

Sample muscle or liver tissue obtained from 144 road-killed Japanese martens from 1988 to 2011 was stored in 70–100% ethanol at 4°C until use. Samples from the Tsushima population were collected from both the North and South Islands. Samples from the main islands were collected from Chiba Prefecture (*n* = 9), the Chubu District (including Gifu and Nagano Prefectures) (*n* = 11), and northern Kyushu (Kitakyushu) (*n* = 23) (Fig. 1).

### DNA extraction, PCR amplification, and microsatellite scoring

Total DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen). Extracted DNA (concentration about 1–100 ng/μl) was preserved at 4°C in 200 μl of TE buffer until use. PCR amplifications were performed in a TP600 thermal cycler (Takara) using primers for 10 microsatellite loci identified in the European pine marten, *M. martes* (Natali et al., 2010: Table 1). Reactions were conducted in 20 μl volumes containing 2.0 μl of 10x PCR buffer (Takara), 1.6 μl of 2.5 mM dNTP mixture, 0.1 μl of r*Taq* DNA polymerase (5 units/μl, Takara), 0.2 μl of each forward (labeled with FAM, NED, VIC or PET fluorescent dye) and non-labeled reverse primer (25 pmol/μl), and 1.0 μl of DNA extract. PCR conditions were one cycle at 94°C
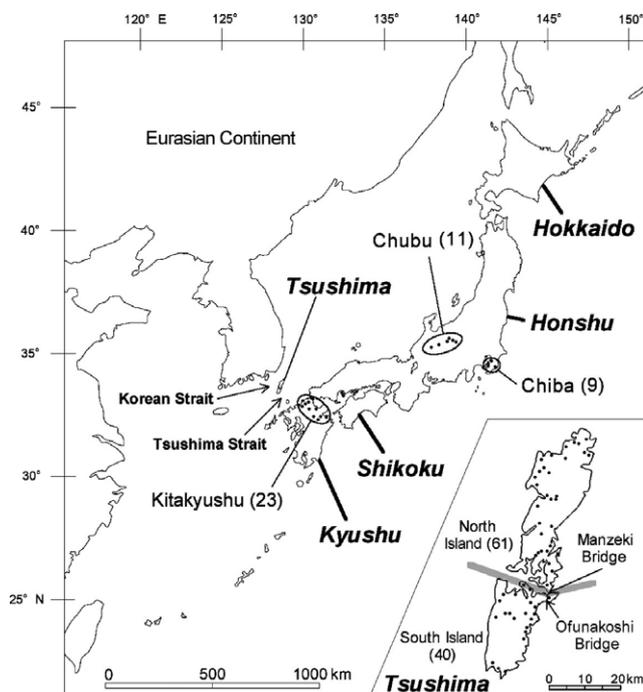


**Fig. 1.** Map of the Japanese archipelago showing sampling locations (dots) and the number of samples from Japanese martens. On the Tsushima Islands (insert, lower right), animals were collected from both the North and South Islands. Samples from populations on the main islands were grouped into three geographic areas (Chiba, Chubu, and Kitakyushu), indicated by circles. Numbers in parentheses are sample sizes.

for 5 min; 35 cycles of 94°C for 40 sec, 55°C for 40 sec, and 72°C for 1 min; and one cycle at 72°C for 5 min. Diluted PCR products were pooled in sets of five loci and analyzed using an Applied Biosystems 3730 DNA analyzer. Allele sizes were determined by co-running a size standard (GS 600 LIZ; ABI), and DNA fragments were scored using GENEMAPPER 4.0 (ABI).

### Data analyses

We calculated observed ($H$o) and expected ($H$e) heterozygosities, deviations from Hardy-Weinberg equilibrium, and linkage disequilibrium for each locus using ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). Microsatellite data were assigned to five geographic populations (North and South Islands of Tsushima, Chiba, Chubu, and Kitakyushu) based on sampling location (Fig. 1), and ARLEQUIN was then used to calculate $H$o and $H$e, deviations from Hardy-Weinberg equilibrium and linkage disequilibrium for each geographic population, and pairwise $F$st values. Allelic richness and a within-population inbreeding estimate ($F$is) were calculated using FSTAT 2.9.3.2 (Goudet, 2001). Nei's standard genetic distances ($D$s; Nei, 1978) were calculated using GENETIX 4.05.2 (Belkhir et al., 2004). From $F$st and $D$s values, neighbor-joining networks were constructed with MEGA 4 (Tamura et al., 2007). The number of private alleles for each locus was counted among the five geographic populations, including the North and South populations of Tsushima.

Recent effective population size reductions were investigated using BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996), which tests for excess heterozygosity as the consequence of a genetic bottleneck. Microsatellite data were tested under a 100% stepwise mutation model (SMM; Kimura and Ohta, 1978) and a two-phase mutation model (TPM with 70% SMM; Di Rienzo et al., 1994), with significance assessed by the sign test and the Wilcoxon signed-rank test, and also by testing allele frequency distributions for shifts from equilibrium L-shape (Luikart et al., 1998). Because of the small sample size, the nine individuals from Chiba were excluded from this analysis.

Bayesian clustering and assignment tests for the two Tsushima populations were implemented in STRUCTURE 2.3 (Pritchard et al., 2000). The STRUCTURE analysis was run with five repetitions of 10,000 iterations of Markov chain Monte Carlo, following a burn-in of 10,000 iterations at $K = 1–5$. To estimate the real number of sub-populations ($K$), log-likelihood ratios [LnP(D)] were calculated. When an individual had a $q$ value (estimated membership in a cluster) $\geq 0.7$, it was assumed to be a member of that particular cluster. GENETIX was used to visualize patterns of differentiation among the five geographic populations, through a factorial correspondence analysis (FCA) (Benzecri, 1973) of individual multilocus genotypes. FCA was performed for the two island populations of the Tsushima marten to further visualize their differentiation pattern.

## RESULTS

### Genetic characteristics of 10 microsatellite loci

In the Tsushima marten, six loci on the North Island (Mar02, Mar08, Mar21, Mar43, Mar56, and Mar64) and three on the South Island (Mar02, Mar08, and Mar43) were monomorphic. By contrast, all loci in the main-island populations were polymorphic, except for Mar21, which was monomorphic in the Kitakyushu population.

All loci analyzed were variable among all Japanese martens, showing 3–15 alleles per locus (Table 1). Table 1 also lists $H$o and $H$e values per locus. All loci deviated from Hardy-Weinberg equilibrium ($P < 0.05$). Significant linkage disequilibrium was observed between two pairs of loci (Mar02 and Mar19; Mar19 and Mar43).

### Genetic diversity within geographic populations and relationships among populations

Among the five geographic populations, multiple private alleles were observed for each locus (Table 2). The largest numbers of private alleles were 11 each from the Chubu and Kitakyushu populations; the other three geographic populations (North and South Islands of Tsushima and Chiba) showed lower numbers (0 or 1) (Table 2). Between the two populations on Tsushima, three private alleles were

**Table 1.** Characteristics of ten microsatellite loci used in this study.

| Locus | Repeat motief | Primer sequence (5'–3')* | Allele size range (bp) | Number of alleles | Heterozygosities | |
|---|---|---|---|---|---|---|
| | | | | | $H$o | $H$e |
| Mar02 | (CA)17 | F: FAM-CCCTCCTTTTCTTTTTCTTTCC R: CCGTTCTCTGAGTGAAATGC | 136–160 | 10 | 0.188 | 0.408 |
| Mar08 | (CA)22 | F: FAM-CCCTTTAGTTGGCACAGTCC R: CTTTGGCATGAGTCATTTGG | 129–153 | 10 | 0.194 | 0.461 |
| Mar15 | (CT)12(CA)11 | F: VIC-TAAGTGGTCCCATCCACACC R: CCAAATGGACATGTAATGTAGGC | 175–193 | 8 | 0.403 | 0.685 |
| Mar19 | (CT)15(CA)8 | F: FAM-GAAGTAGTCCAAGTGTCCATCG R: TTGTCTTTCCCTGACTTATTTGG | 200–216 | 8 | 0.424 | 0.643 |
| Mar21 | (CA)24 | F: NED-ACATGCATACCTCCCAGACC R: TTTGCTTCCTCCATCTCTCC | 147–151 | 3 | 0.028 | 0.041 |
| Mar36 | (CA)24 | F: PET-TGAGTTTGGTGGGAGAGAGG R: TTCACTGCCAATATTATCTTCTCAAG | 215–229 | 8 | 0.340 | 0.510 |
| Mar43 | (CA)26 | F: VIC-CTTGTCACCCCAGGAGAGG R: CCTAAGCCCAAATCTAAGTGC | 142–170 | 15 | 0.236 | 0.471 |
| Mar53 | (CA)18 | F: NED-TCTCCAGCATTTACCTTTACCC R: GAACAGCCAACCCCATACC | 231–249 | 9 | 0.521 | 0.748 |
| Mar56 | (CA)21 | F: VIC-TCTGCACTTAACCCCTCTCC R: AGGGCCATTTGTCTCTTGC | 190–202 | 5 | 0.153 | 0.214 |
| Mar64 | (CA)21 | F: PET-GGCCCCAAAGTCTTACAGTTC R: CGTTTTGAATCATGCTGTGG | 178–192 | 8 | 0.361 | 0.596 |
| Average | | | | 8.4 | 0.285 | 0.478 |

*Cited from Natali et al. (2010). Forward primers were labeled fluorescently with FAM, NED, PET or VIC.

**Table 2.** Frequencies of private alleles in each of five geographic populations of the Japanese marten.

| Locus | Number of alleles per locus among all individuals | Private alleles in each geographic population | | | | | | Total |
| | | Tsushima Islands | | Main islands | | | |
| | | North (n = 61) | South (n = 40) | Chiba (n = 9) | Chubu (n = 11) | Kitakyushu (n = 23) | |
|---|---|---|---|---|---|---|---|
| Mar02 | 10 | 0 | 0 | 0 | 2 | 1 | 3 |
| Mar08 | 10 | 0 | 0 | 0 | 3 | 1 | 4 |
| Mar15 | 8 | 0 | 0 | 0 | 0 | 1 | 1 |
| Mar19 | 8 | 0 | 0 | 1 | 0 | 0 | 1 |
| Mar21 | 3 | 0 | 1 | 0 | 0 | 1 | 2 |
| Mar36 | 8 | 0 | 0 | 0 | 1 | 1 | 2 |
| Mar43 | 15 | 0 | 0 | 0 | 2 | 5 | 7 |
| Mar53 | 9 | 0 | 0 | 0 | 1 | 0 | 1 |
| Mar56 | 5 | 0 | 0 | 0 | 1 | 0 | 1 |
| Mar64 | 8 | 0 | 0 | 0 | 1 | 1 | 2 |
| Total | | 0 | 1 | 1 | 11 | 11 | 24 |

**Table 3.** Frequencies of private alleles between the two populations of the Japanese marten on the Tsushima Islands.

| Allele | Number of alleles par locus among all Tsushima martens | Private alleles | |
| | | North Island | South Island |
|---|---|---|---|
| Mar02 | 1 | 0 | 0 |
| Mar08 | 1 | 0 | 0 |
| Mar15 | 3 | 0 | 0 |
| Mar19 | 2 | 0 | 0 |
| Mar21 | 2 | 0 | 1 |
| Mar36 | 2 | 0 | 0 |
| Mar43 | 1 | 0 | 0 |
| Mar53 | 3 | 0 | 0 |
| Mar56 | 2 | 0 | 1 |
| Mar64 | 3 | 0 | 1 |
| Total | 20 | 0 | 3 |

**Table 4.** Genetic diversity in each of five geographic populations of the Japanese marten. $R$ was based on the minimum sample size, $n$ = 9.

| Geographic population | Sample size | Average value of allelic richness ($R$) | Heterozygosity values | | $F_{is}$ |
| | | | $H_o$ | $H_e$ | |
|---|---|---|---|---|---|
| North Island | 61 | 1.530 | 0.364 | 0.393 | 0.075 |
| South Island | 40 | 1.667 | 0.189 | 0.238 | 0.206 |
| Chiba | 9 | 2.800 | 0.457 | 0.541 | 0.164 |
| Chubu | 11 | 5.738 | 0.747 | 0.780 | 0.043 |
| Kitakyushu | 23 | 5.097 | 0.587 | 0.668 | 0.124 |

observed in the southern subpopulation (Table 3).

Table 4 shows average values of $H_o$, $H_e$, allelic richness ($R$), and $F_{is}$ for the five geographic populations. The two populations (North and South Islands) on Tsushima showed lower values for $H_o$ (0.364 and 0.189, respectively), $H_e$ (0.393 and 0.238), and $R$ (1.530 and 1.667) than did the three populations on the main islands ($H_o$, 0.457–0.747; $H_e$, 0.541–0.780; $R$, 2.800–5.783). The South Island population of Tsushima had a higher $F_{is}$ value (0.206) than the range for the main-island populations (0.043–0.164). All loci were in Hardy-Weinberg equilibrium ($P$ > 0.05) in at least one population, and significant linkage equilibrium was observed in at least one pair of populations. High pairwise $F_{st}$ (0.485–0.682) and $D_s$ (0.550–1.183) values were observed

**Table 5.** $F_{st}$ (lower matrix) and $D_s$ (upper matrix) values between geographic populations. All $F_{st}$ values were statistically significant ($P$ < 0.05).

| | Chiba | Chubu | Kitakyushu | North Island | South Island |
|---|---|---|---|---|---|
| Chiba | | 0.394 | 0.500 | 1.115 | 1.183 |
| Chubu | 0.188 | | 0.145 | 0.550 | 0.593 |
| Kitakyushu | 0.215 | 0.060 | | 0.725 | 0.715 |
| North Island | 0.662 | 0.506 | 0.497 | | 0.050 |
| South Island | 0.682 | 0.518 | 0.485 | 0.177 | |
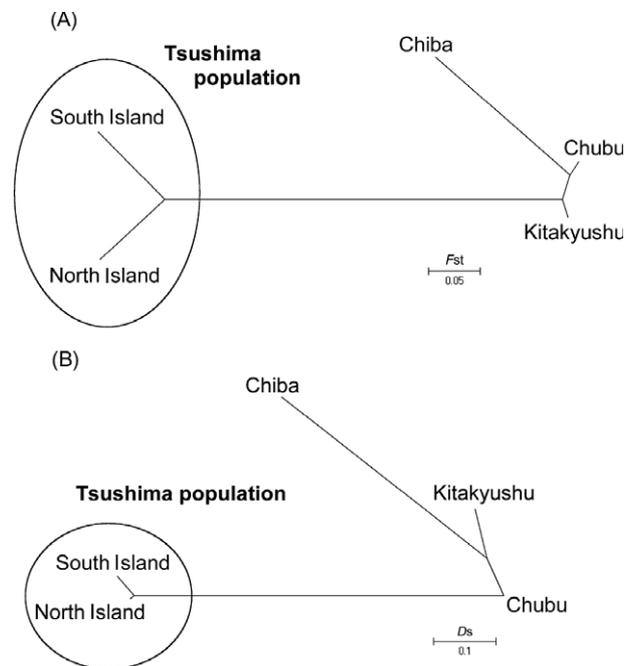


**Fig. 2.** Neighbor-joining networks among geographic populations, constructed using pairwise $F_{st}$ **(A)** and $D_s$ **(B)** values. The two sub-populations on Tsushima are circled. The scales show genetic distances.

between the Tsushima and the main-island populations, whereas the genetic distances between the two populations of Tsushima ($F_{st}$, 0.177; $D_s$, 0.050) and among the three main-island populations ($F_{st}$, 0.060–0.215; $D_s$, 0.145–

0.500) were lower (Table 5). In the neighbor-joining networks based on pairwise $F$st (Fig. 2A) and $D$s values (Fig. 2B), the Tsushima populations were distantly separated from the main-island populations.

The BOTTLENECK analysis indicated no significant excess in heterozygosity in the four populations tested. This means that these four geographic populations have not experienced recent bottlenecks.

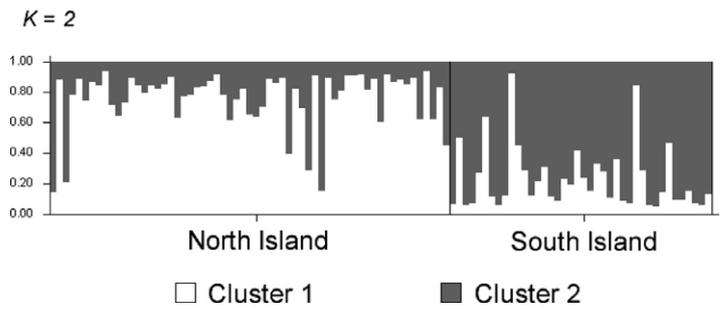### Analyses of population structure

Bayesian clustering and assignment tests using STRUCTURE calculated the value of the log-likelihood ratio [LnP(D)], which was largest at $K = 2$ (Fig. 3). FCA showed a division of the five populations into two groups (the main-island group and the Tsushima group) (Fig. 4A). Individuals from each of the three geographic populations (Chiba, Chubu, and Kitakyushu) in the main-island group formed a single cluster, but were more scattered than those from the Tsushima group. FCA for the Tsushima populations (Fig. 4B) showed separation between most individuals sampled from the North Island and those from the South Island, though there was overlap, with individuals from the South Island having greater spread.

In the STRUCTURE analysis of the Tsushima populations, individuals were assigned to one of two clusters at $q \geq 0.7$. Table 6 indicates the number of individuals in each cluster. Cluster 1 consisted mostly of individuals from the North Island, and Cluster 2 mostly of those from the South Island. Figure 5 shows the geographic distribution of individuals in the two clusters on the Tsushima Islands.

### DISCUSSION

#### Characteristics of the microsatellite loci in the Japanese martens

We genotyped 144 samples using 10 microsatellite loci. When all the genotype data we analyzed were treated as one population, all loci showed deviations from Hardy-Weinberg equilibrium, and many loci indicated significant linkage disequilibrium. Because this result could have resulted from analyzing all data from multiple genetically isolated populations as that from one population (Wahlund effect: Wahlund, 1928), we then analyzed each of five geographic populations independently.



**Fig. 3.** Population structure of the Tsushima marten inferred from a STRUCTURE analysis. Each individual is represented by one thin vertical column partitioned into the $K$ segments, and arranged according to geographic population. Log-likelihood ratios [LnP(D)] were largest at $K = 2$. Two clusters (1 and 2) were detected.
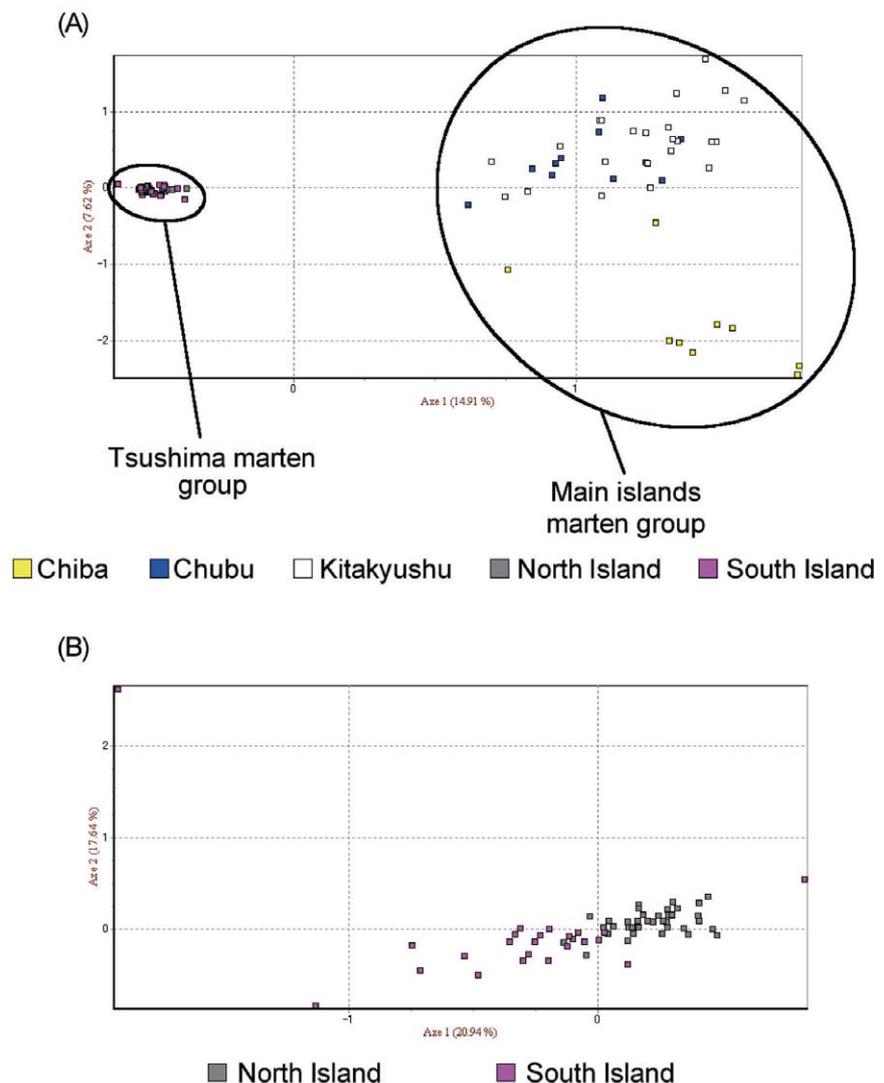


**Fig. 4.** Factorial correspondence analysis (FCA) of all five geographic populations **(A)** and only the two populations on Tsushima **(B)**. Each mark in the plots represents one individual, and different colors represent different geographic populations. **(A)** The main-island martens are genetically well separated from the Tsushima martens. **(B)** Individuals in the North Island population on Tsushima tend to be separated from those in the South Island population, although the two distributions overlap.

## Genetic relationships between the Tsushima and main-island marten populations

Pairwise *F*st and *D*s showed large genetic differentiation between the Tsushima and main-island marten populations (Table 5; Fig. 2A, B). Likewise, FCA (Fig. 4A) indicated a genetic separation between the Tsushima and main-island populations. Similarly to our results, Sato et al. (2009) detected a separation between the Tsushima and main-island populations in a phylogenetic analysis using three mitochondrial loci. Because Tsushima Island has not been connected to the Japanese main islands since the last marine transgression in the Late Pleistocene (Oshima, 1990, 1991, 2000; Nagaoka, 2001a), genetic differentiation between the Tsushima and Japanese main-island populations can be attributed to longstanding geographical isolation.

## Genetic diversity and structure of the Tsushima marten population

Five loci were monomorphic in the North Island, and three in the South Island, populations on Tsushima. In addition, the two populations on Tsushima showed lower *H*o, *H*e, and allelic richness than the three main-island populations, and fewer private alleles (Tables 2 and 4). Sato et al. (2009) similarly reported low genetic diversity in mitochondrial loci in the Tsushima marten. On the other hand, our study indicated that the Tsushima marten has not experienced a recent bottleneck, and also found a high *F*is value for the South Island population (0.206). The low level of genetic diversity in the Tsushima marten may have resulted from founder effect and/or gradual elimination of alleles by genetic drift through long isolation on the island in the absence of intense environmental changes, as consistent with mtDNA data of Sato et al. (2009).

The STRUCTURE analysis (Table 6) detected two clusters (1 and 2) in the Tsushima marten. Cluster 1 was composed mainly of individuals from the North Island, and Cluster 2 mainly of those from the South Island (Table 6; Fig. 5). FCA likewise indicated a division of individuals between two islands of Tsushima (Fig. 4B). In addition, in a comparison between the North and South Islands, three private alleles were detected in the former but none in the latter (Table 3), indicating higher variation on the South Island and genetic isolation between the two islands. Sato et al. (2009) likewise reported two individuals from the South Island with mtDNA haplotypes different from those on the North Island, but also reported homogeneity in nuclear growth hormone receptor gene sequences among all Tsushima martens. This incongruence in the nuclear DNA variation suggests that the higher genetic diversity of the microsatellite loci resulted from a higher mutation rate.

Several factors potentially limit gene flow between the North and South Islands. The average size of the marten's home range throughout the year on the Tsushima Islands is about 0.8 km² for males and about 0.7 km² for females (Tatara, 1994a, b; Hosoda and Tatara, 1996), small areas compared to the size of the Tsushima Islands (696 km²; length 82 km from north to south; width 18 km from east to west). In addition, both the North and South Islands have many bays and mountains (Nagaoka, 2001b), which presumably impede dispersal within Tsushima. Even when the North and South Islands were still connected by two narrow

**Table 6.** Frequencies of individuals assigned (at *q* ≥ 0.7) to two genetic clusters detected on the Tsushima Islands.

| Geographic population | Clusters by STRUCTURE analysis | | Number not assigned (*q* < 0.7) | Total |
|---|---|---|---|---|
| | Cluster 1 | Cluster 2 | | |
| North Island | 46 | 4 | 11 | 61 |
| South Island | 2 | 30 | 8 | 40 |
| Total | 48 | 34 | 19 | 101 |
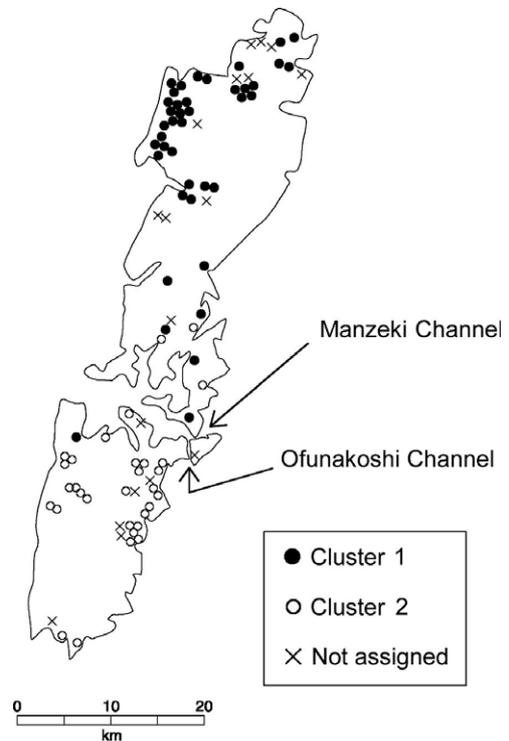


**Fig. 5.** Map of Tsushima Islands showing the distribution of sampling locations for individuals in Cluster 1, Cluster 2, and unassigned by the STRUCTURE analysis; each mark on the map indicates one individual. 'Not assigned (X)' means individuals with *q* < 0.7.

isthmuses, gene flow across the isthmus would have been limited due to the marten's small home-range size. However, completion of Manzeki Channel around 1900 (Nagaoka, 2001b) and Ofunakoshi Channel in 1672 (Tsushima Development Bureau, 2011) undoubtedly curtailed the already limited gene flow between North and South Islands.

Our study revealed a low level of genetic diversity in the Tsushima marten populations. Restricted populations with minor genetic variation (genetic homogeneity) have an increased probability of extinction from sudden environmental changes, infectious diseases, or random demographic events (Soule and Mills, 1998; Johnson et al., 2001). Further research is necessary to elucidate the transition in genetic structure and the process of adaptive evolution in the Tsushima marten.

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