

Population Structures of the Red Fox (*Vulpes vulpes*) on the Hokkaido Island, Japan, Revealed by Microsatellite Analysis

TAKUYA OISHI, KOHJI URAGUCHI, KENICHI TAKAHASHI, AND RYUICHI MASUDA

From the Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan (Oishi and Masuda); and the Hokkaido Institute of Public Health, Sapporo, Japan (Uraguchi and Takahashi).

Address correspondence to Dr R. Masuda at the address above, or e-mail: masudary@ees.hokudai.ac.jp.

Abstract

In order to examine the population structures of the red fox (*Vulpes vulpes*) on the Hokkaido Island in Japan, we conducted analysis on 250 foxes from all over the island for 12 microsatellite loci. Assignment tests using the genotype data set showed that they were divided into 6 subpopulations. Of the 6, one was geographically isolated in the southern region and considered definitive subpopulation, whereas the other 5 were not. The slight differences among the latter 5 subpopulations were explained by the high adaptability and long dispersal of the red fox on the Hokkaido Island. Although there are few ecological data to explain the genetic differentiation of the southern population, we have proposed some hypotheses from the present ecological and geohistorical viewpoints. One convincing reason from the ecological viewpoint is the restriction of gene flow to southern Hokkaido from other areas due to geographical isolation resulting from the land shape. The other explanation is the geohistorical division of southern Hokkaido from other regions on the island during the last interglacial age, resulting in the isolation of the fox population.

Key words: clustering, Hokkaido, microsatellite, population structure, red fox, *Vulpes vulpes*

The fauna of the Japanese islands is geographically separated between the Hokkaido and Honshu Islands by the Tsugaru strait, which is a biogeographical demarcation called “Blakiston’s line” (Blakiston and Pryer 1880, Figure 1). There are many endemic mammalian species of mammals in the main islands located south of Blakiston’s line, and the endemic species reach about 40% of all Japanese mammalian species (Abe et al. 2005). On the other hand, the mammalian fauna on the Hokkaido Island, located north of Blakiston’s line, exceptionally does not show such endemism of the other Japanese main islands (Abe et al. 2005), and the terrestrial fauna occurring in Hokkaido is rather similar to southern Siberian fauna (Fujimaki 1994). This is because that the Tsugaru strait (Blakiston’s line) is considerable and separated Hokkaido from the other islands in the last interglacial age, whereas the Hokkaido Island was considered to be connected intermittently to the Eurasian continent via Sakhalin (Ohshima 1990).

Meanwhile, recent molecular genetic studies have revealed that the Hokkaido populations of mammals have particular and distinct features of the phylogeography and

migration history. For example, the distribution of the 3 mitochondrial DNA (mtDNA) lineages of the brown bear (*Ursus arctos*) did not overlap, and each mtDNA lineage was considered to have immigrated separately to the Hokkaido Island (Matsuhashi et al. 1999). The genetic population structures of the sika deer (*Cervus nippon*) in Hokkaido reflected bottleneck effects, resulting from past heavy snows and hunting pressure (Nagata et al. 1998). The molecular phylogeography of other mammals on the Hokkaido Island, such as the least weasel (*Mustela nivalis*) (Kurose et al. 2005) and *Sorex* species (Ohdachi et al. 2001), also showed endemism according to the biogeographical history of the Hokkaido Island.

The red fox (*Vulpes vulpes*) is also widely distributed in Hokkaido, but its population structures have not yet been clarified. Inoue et al. (2007) examined the variations of the mtDNA cytochrome *b* gene and control region of the red fox on Hokkaido. Although they found some haplotype lineages in Hokkaido, their distributions were admixed throughout the entire island, and no clear genetic subdivisions were defined. This could have been caused by the high

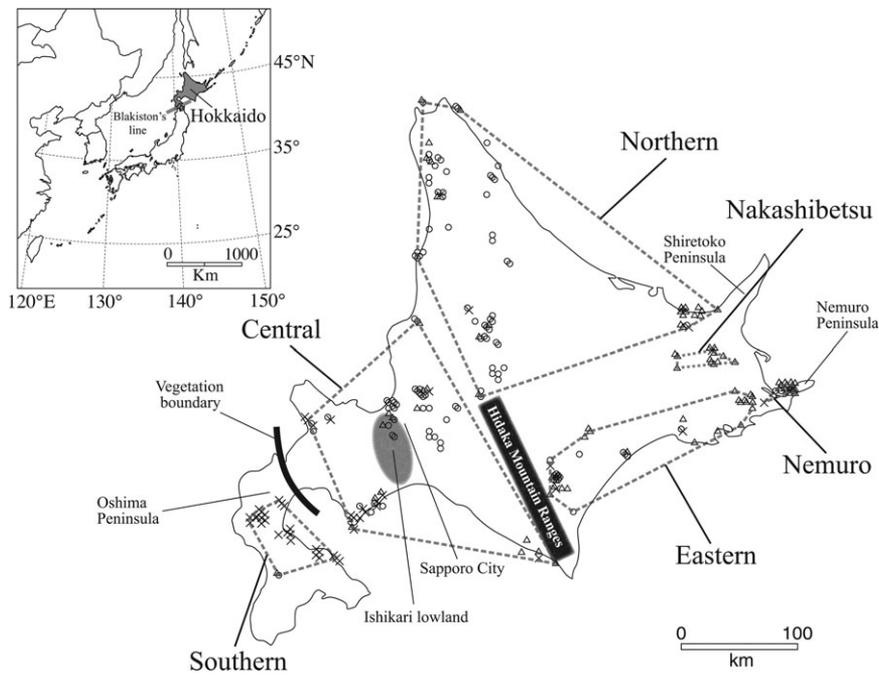


Figure 1. Locations of the red fox subpopulations on the Hokkaido Island, based on genetic clustering analyses of GENELAND and STRUCTURE at $K = 3$. Circles, triangles, and crosses show fox individuals, which were partitioned to segments of yellow, blue, and red, respectively, by the STRUCTURE results at $K = 3$ (see Figure 3). The individuals surrounding a polygon belong to the same subpopulation inferred by the GENELAND analysis. The vegetation boundary on the map was cited from Tatewaki (1958).

adaptability and dispersal ability of the red fox. This species is omnivorous (Yoneda 1981; Doncaster et al. 1990; Lucherini and Crèma 1994; Molsher et al. 2000), and its adaptability is as high as it is distributed widely in the northern hemisphere (Voigt and Macdonald 1984), including some urban areas (Teagle 1967). Behavioral studies revealed that the red fox can disperse over long distance (Storm et al. 1976; Hokkaido 1988).

However, elucidation of some unknown population structures on the island could also help to understand some ecological phenomena of the red fox in Hokkaido. For example, sarcoptic mange, which is a serious skin disease of red foxes caused by the mite (*Sarcoptes scabiei*) and spread by physical contact, has expanded from eastern to western Hokkaido but not to the Oshima Peninsula of southern Hokkaido (Takahashi and Uruguchi 2001). This may indicate a low level of migration (i.e., gene flow) between the southern and other populations of the red fox. Moreover, the Hokkaido Island has a complex geographic shape, such as narrow peninsulas and high mountain ranges, and climatic and vegetative variations between southern and other parts of the island (see Figure 1). These island conditions may also have been related to the formation of the fox population structures.

In addition, it is epidemiologically important to understand the fox population structures in Hokkaido to plan an effective program against zoonoses. This animal plays an important role as the host of a tapeworm (*Echinococcus*

multilocularis) causing a serious zoonosis alveolar echinococcosis in Hokkaido (Oku and Kamiya 2003). In addition, although rabies has been exterminated in Japan, it remains possible that the red fox, which is the main rabies-infected animal in Europe, could spread the infection again in Hokkaido (Uruguchi 2008).

In order to further examine fox population structures in Hokkaido, we conducted biparentally inherited microsatellite analysis on 250 foxes collected throughout Hokkaido and then discuss gene flow among subpopulations and the process of population structuring of the red fox on the island.

Materials and Methods

Sample Collection

We examined muscle tissues of 250 foxes collected widely from Hokkaido for epidemiological survey on *E. multilocularis* infection conducted by the Hokkaido government. Most of the foxes were hunted in 2006, except for Nemuro and Nakashibetsu in 2003. Samples from Sapporo were obtained from road kills in 2001. The tissues were treated at 70 °C for 3 days for inactivation of parasites and then preserved in 99% ethanol at 4 °C.

DNA Extraction, PCR Amplification, and Data Analysis

Total DNA was extracted by a DNeasy Tissue Kit (QIAGEN) and stored in TE buffer at 4 °C. We amplified

Table 1 Basic information on the Hokkaido red fox subpopulations inferred by the GENELAND analysis

Subpopulation	Sample size (<i>n</i>)	Heterozygosities		Mean no. of alleles per locus (<i>A</i>)
		Observed (<i>H_O</i>)	Expected (<i>H_E</i>)	
Southern	28	0.59	0.61	4.78
Central	70	0.63	0.67	7.11
Northern	78	0.67	0.68	8.22
Eastern	41	0.67	0.67	6.33
Nakashibetsu	13	0.61	0.63	5.22
Nemuro	20	0.65	0.63	5.56

12 microsatellite loci for each sample, with the following primers of polymerase chain reaction (PCR): DB1, DB3, DB4, and DB6 (Holmes et al. 1993); C213 (Ostrander et al. 1993); V142, V374, V402, V468, V502, V602, and V622 (Wandeler and Funk 2006). The PCR amplification was carried out in 10 μ l of the reaction mixture containing 1 \times PCR buffer, dNTP mixture (0.2 mM), 0.3 μ l of each primer (0.15 pmol/ μ l), *rTaq* DNA polymerase (0.05 units/ μ l, TAKARA), and 1.0 μ l of each DNA extract. The following PCR programs were performed using a DNA thermal cycler, TAKARA TP600: one cycle of denaturing at 94 $^{\circ}$ C for 3 min; 30–40 cycles of denaturing at 94 $^{\circ}$ C for 1 min, annealing at 52–60 $^{\circ}$ C for 1 min, extension at 72 $^{\circ}$ C for 1 min; and the reaction completion at 72 $^{\circ}$ C for 10 min.

Before sizing microsatellite alleles, 2.5 μ l of the bromophenol blue loading solution (PROMEGA) were added to 2.5 μ l of each PCR product, denatured at 95 $^{\circ}$ C for 2 min, and cooled down immediately on ice for 3 min. Then, we applied the products to an autosequencer HITACHI SQ-5500L and determined the molecular sizes of microsatellite alleles using computer software FRAGLYS 3 (HITACHI). Because no clear PCR results of 3 loci (C213, V374, and V502) were obtained, these loci were excluded from subsequent analysis.

Statistical Analysis

In order to infer the geographic boundaries and the number of subpopulations, we used GENELAND 3.1.4. program (Falush et al. 2003; Guillot, Mortier, and Estoup 2005). This was programmed on the basis of a Bayesian clustering model, assigning individuals to subpopulations from its genetic information (Guillot, Estoup, et al. 2005). GENELAND does not incorporate only genetic information but also

spatial coordinates of sampling points at an earlier stage of simulation, making it possible to optimize the delineation of subpopulations (Guillot, Estoup, et al. 2005). We varied the number of subpopulations (*K*) from 1 to 12 in order to infer the possible number of *K*, and in this step, we set the Markov Chain Monte Carlo (MCMC) parameters as 50 000 iterations and 50 thinning. Correlated and null allele model options were activated, and we used the default setting for other parameters and estimated *K* of our data set from the highest average posterior probability. The assignment of individuals to subpopulations was performed in a separate run, as suggested by Guillot, Estoup, et al. (2005). For these runs, *K* was set to the inferred number of subpopulations, the MCMC parameters were set to 100 000 iteration and 100 thinning, and other parameters were the same as the first step. Ten runs with fixed *K* were performed. For one of the 10 runs with the highest average posterior probability, the posterior probability of subpopulation membership was computed for each pixel of the spatial domain (400 \times 400 pixels).

For each of the inferred subpopulations based on GENELAND results, deviations from Hardy–Weinberg and linkage equilibrium were examined by GENEPOP 3.4 (Raymond and Rousset 1995) (dememorization number = 1000; batch number = 100; number of iterations per batch = 10 000). The observed (*H_O*) and expected (*H_E*) heterozygosities were estimated by ARLEQUIN 3.1.1 (Excoffier et al. 2005). In addition, pairwise *F_{ST}* (Weir and Cockerham 1984) was estimated by ARLEQUIN, and we tested the significance of the observed *F_{ST}* values by 10 000 permutations of individuals among populations. We also estimated Nei's standard genetic distance (*D_S*; Nei 1978) among subpopulations by SPAGeDi 1.2 (Hardy and Vekemans 2002). Using the pairwise *F_{ST}* and *D_S* values, we constructed phylogenetic trees by the neighbor joining method (Saitou and Nei 1987) in MEGA 4 (Tamura et al. 2007).

After dividing populations into subpopulations by GENELAND, we also attempted to infer subpopulations and geographic boundaries by STRUCTURE 2.3.1 program (Pritchard et al. 2000; Falush et al. 2003). The STRUCTURE is also formed by the Bayesian clustering technique, similar to GENELAND, but spatial coordinates are not considered in its simulation process and used only as a method of visualizing the subpopulation membership. In STRUCTURE, it is sometimes difficult to estimate clear geographical boundaries among subpopulations if the subpopulations have

Table 2 *F_{ST}* (lower matrix) and *D_S* (upper matrix) among inferred red fox subpopulations

Subpopulation	Southern	Central	Northern	Eastern	Nakasibetsu	Nemuro
Southern		0.22	0.19	0.21	0.34	0.39
Central	0.10		0.04	0.05	0.18	0.17
Northern	0.09	0.02		0.03	0.11	0.16
Eastern	0.10	0.03	0.02		0.10	0.08
Nakashibetsu	0.14	0.07	0.04	0.04		0.08
Nemuro	0.17	0.08	0.07	0.04	0.03	

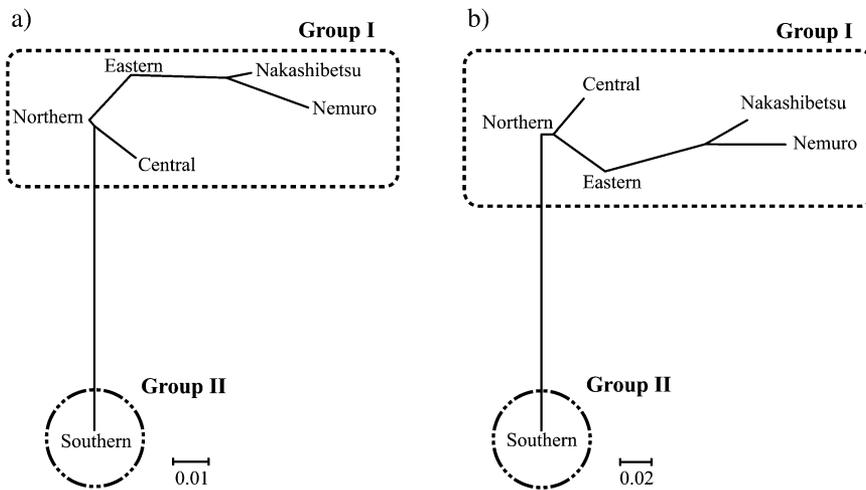


Figure 2. Neighbor joining relationships among the 6 red fox subpopulations, constructed by values of pairwise F_{ST} (a) and D_S (b).

shallow genetic structures. This clustering is, however, purely based on genetic information. In the present study, STRUCTURE was employed to examine the characteristics of the boundaries found by GENELAND. We then performed 5 runs at each value of the fixed parameter K (1–10: number of subpopulations). Each run consisted of 10 000 replicates of the MCMC after a burn-in of 10 000 replicates to examine the most likely number of subpopulations in the present data set by the highest average likelihood. The admixture model and correlated allele frequencies model were used because this combination is thought to provide the highest resolution in the case of a shallow population structure (Falush et al. 2003). We input the subpopulation information obtained from GENELAND analysis for later comparison. All other parameters were set to default values. From the highest average likelihood value, $K = 3$ was estimated as the most likely number of K , but if it would be difficult to detect clear population structure at $K = 3$, we performed additive runs (30 000 burn-in and 100 000 replicates) not only at $K = 3$ but from $K = 2$ to 6 to examine certain boundaries by the same method as Rosenberg et al. (2002).

In order to estimate the proportion of genetic variance explained by the subpopulations inferred by GENELAND and to assess the strength of each GENELAND boundary, we also conducted analysis of molecular variance (AMOVA) by ARLEQUIN. The AMOVA analysis examined the hierarchical proportion of total genetic variance in “among groups,” “among populations within groups,” “among individuals within populations,” and “within individuals.” We input the 6 subpopulations by GENELAND into “populations” and divided the subpopulations into 2 geographically connected “groups.” Then, we considered the strength of boundaries by comparing the variance proportion values of “among groups.”

In addition, we estimated resistance distances among subpopulations by CIRCUITSCAPE 3.5.1 based on circuit and random walk theory (McRae 2006). The resistance

distances were then compared with F_{ST} values and geographic distances in order to consider the effects of the island shape on gene flow. We used a simple binary land/sea map created by DIVA GIS (LizardTech 2005) because of the limited information on the red fox habitat in Hokkaido. The resolution of the grid map of Hokkaido was 730×500 . Each subpopulation was condensed into one node and located on the map, according to the average x, y coordinate of all samples belonging to each subpopulation. We chose the 8-neighbor connection option because it seemed to express the more natural dispersal of animals, and connections were calculated using the average resistance option. Geographic distances were also calculated from the average subpopulation locations described above using Excel X (Microsoft 2002). The resistance, geographic, and genetic distances were compared by the Mantel test and partial Mantel test on FSTAT 2.9.3.2 (Goudet 2001). P values were calculated after 10 000 randomizations.

Results

The GENELAND analysis indicated that the most likely value of K was 7 as the number of subpopulations in the total data set; however, there were only 6 subpopulations to which individuals were assigned. One of the inferred subpopulations was considered “ghost” subpopulation with no individuals assigned. This phenomenon has been previously reported particularly when subpopulations are slightly differentiated, and it is recommended that such subpopulations should be ignored (Guillot, Estoup, et al. 2005; Guillot, Mortier, and Estoup 2005). Therefore, we ignored the ghost subpopulation and named the other 6 subpopulations as follows: Southern ($n = 28$), Central ($n = 70$), Northern ($n = 78$), Eastern ($n = 41$), Nakashibetsu ($n = 13$), and Nemuro ($n = 20$) for sampling locations (Figure 1).

Although, in most subpopulations, no deviations from Hardy–Weinberg equilibrium were detected ($P > 0.05$), only the Central subpopulation ($P \leq 0.05$) had an H_O value

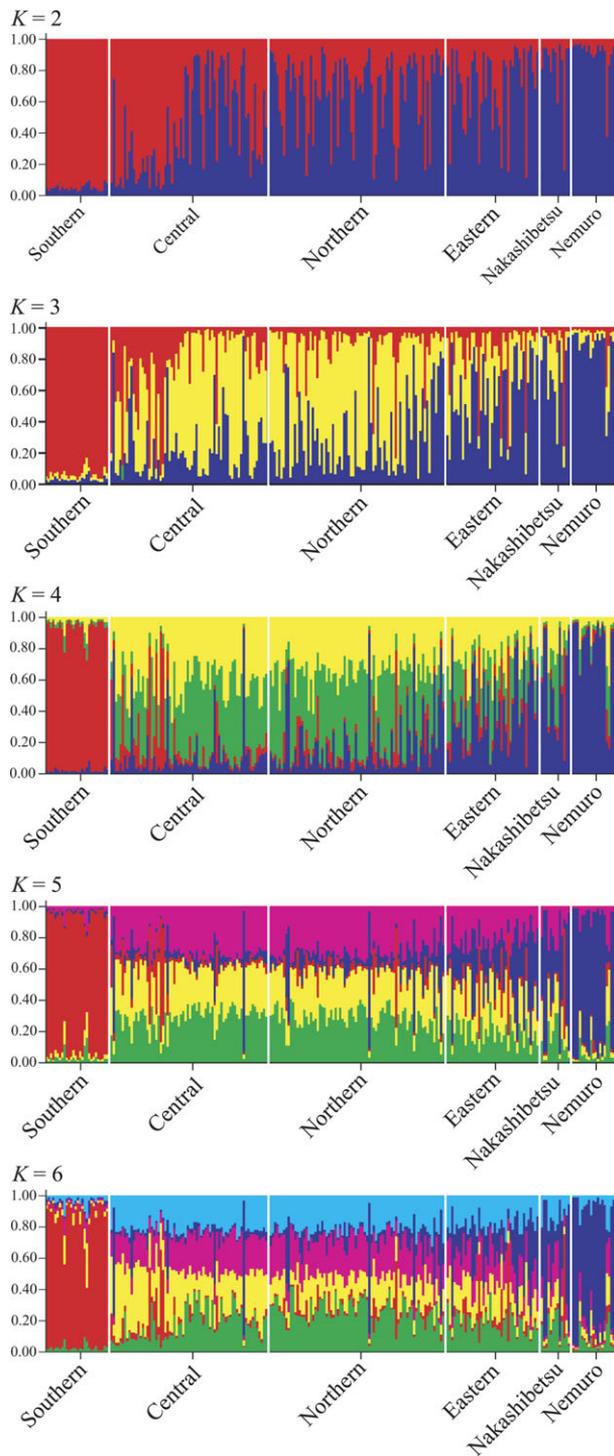


Figure 3. Assignments of individuals due to the STRUCTURE analysis on microsatellite allele data. One thin vertical line for each individual is partitioned into K colored segments that represent the individual's estimated assignment probabilities to each cluster. Six subpopulations were inferred as described below.

lower than H_E (Table 1). This indicates that random mating has not occurred in the Central subpopulation and that there

would be additional unknown subdivisions that could not be identified. Although the GENELAND analysis did not define the entire subdivision of the Central subpopulation, we treated the Central subpopulation in the manners same as the other subpopulations in subsequent analyses. No linkage disequilibrium in the subpopulations was found by the ARLEQUIN analysis.

Pairwise F_{ST} values between the Southern and other subpopulations (0.09–0.17) were higher than other F_{ST} values in all pairs of subpopulations (0.02–0.08 with $P < 0.01$) (Table 2). The D_S values between the Southern and other subpopulations (0.19–0.39) were higher than those of other combinations of subpopulations (0.03–0.18) (Table 2). The phylogenetic trees constructed using F_{ST} and D_S values (Figure 2) showed that the Southern subpopulation was genetically most differentiated from the other subpopulations.

The STRUCTURE analysis showed the existence of explicit boundaries between the Southern and other subpopulations at $K = 3, 4, 5,$ and 6 as summarized in Figure 3. The results indicated that the most likely number of subpopulations was 3 with the highest average likelihood value. At $K = 3$, the geographical boundaries among the 3 clusters were unclear. With higher K numbers (4, 5, and 6) than 3, most individuals were not assigned to any subpopulations with more than 0.70 posterior probability of the population membership, indicating no clear population structures among them. At any number of K , however, the Southern subpopulation was always recognized as a distinctive group.

The result of the AMOVA analysis was similar to that of the STRUCTURE analysis. In all analyses, the proportions of variance in “individuals” reached more than 90% (Table 3), indicating that population structuring was weak throughout the Hokkaido Island. However, the highest percentage of variance among groups (6.9%, Table 3) was obtained when the whole population was classified into 2 groups: the Southern subpopulation versus the others. By contrast, the percentages of variance of “among groups” were less than 3.5% in the other combinations of subpopulations. This indicates that the boundary between Southern and Central subpopulation was clearer than the other boundaries.

Based on all results of genetic analyses, it was concluded that the GENELAND analysis divided the genotype data set into 6 subpopulations. Of the 6 subpopulations, the Southern subpopulation was genetically most differentiated.

The Mantel and partial Mantel test revealed that both the resistance (R) and geographic distances (S) significantly correlated to the genetic distances (G) ($P < 0.05$) (Figure 4). In both tests, the correlation between genetic and geographic distances were stronger than that between genetic and resistance distances (Mantel test: $R^2 = 0.30$ between G and R , $R^2 = 0.41$ between G and S) (partial Mantel test: $r_{RG|S} = 0.55$, $r_{SG|R} = 0.61$; Figure 4). Because the resistance distances were higher when either or both subpopulations were in peninsulas (Table 4), the land shape of the Oshima and Nemura Peninsulas could be related to the gene flow prevention.

Table 3 Differences of variance proportion depending on possible red fox subpopulation groupings based on AMOVA analysis

Grouping	Proportions of variance			
	Within individuals	Among individuals within populations	Among populations within groups	Among groups
Southern/others	90.4	-0.6	3.3	6.9
Northern/others	96.8	-0.6	7.4	-3.5
Eastern/others	97.2	-0.6	6.5	-3.1
Nakashibetsu/others	94.7	-0.6	5.2	0.8
Nemuro/others	92.7	-0.6	4.5	3.4
Southern, Central/others	95.5	-0.6	5.6	-0.4
Northern, Nakashibetsu/others	96.2	-0.6	6.8	-2.5
Eastern, Nemuro/others	95.2	-0.6	5.2	0.2
Nakashibetsu, Nemuro/others	93.0	-0.6	4.2	3.5
Southern, Central, Northern/others	94.8	-0.6	4.7	1.1
Southern, Central, Eastern/others	95.8	-0.6	6.3	-1.5

Discussion

Weak Structuring of the Red Fox Populations Due to Gene Flow on the Hokkaido Island

The present study revealed that the population structure of the red fox is not strongly differentiated throughout most parts of the Hokkaido Island, except for the Southern subpopulation. This is not inconsistent with the previous genetic study (Inoue et al. 2007) reporting no visible genetic structuring within Hokkaido, based on the distribution patterns of maternally inherited mtDNA haplotypes. Some red foxes on Hokkaido were reported to have dispersed more than 30 km (Hokkaido 1988; Uruguchi 2008). Such a shallow population structure could be explained by the high adaptability and far dispersal habit of this species. For example, although the Hidaka mountain range (Figure 1), which is the highest (more than 2000 m) in Hokkaido, could be a geographic barrier between the Central and Eastern subpopulations, the lower values of both F_{ST} (0.03) and D_S (0.05) between them in the present study indicate that geographic isolation by the mountain range is not so significant, leading to gene flow.

Genetic Differentiations of the Southern Subpopulation

The genetic differentiations between the Southern and other subpopulations were relatively stronger. Such population differentiation in southern Hokkaido has been reported in other species. For example, Matsubashi et al. (1999) reported that the brown bear population in Hokkaido is clearly divided by the Ishikari lowland (Figure 1) based on mtDNA data and that the subpopulation in southern Hokkaido is genetically differentiated from other subpopulations. We here propose 2 hypotheses for the differentiation of the Southern population of the red fox from geohistorical and present ecological viewpoints.

From the geohistorical viewpoint, the geographical isolation between the Southern and other regions within Hokkaido in the past might have caused the genetic division. In the last interglacial age (130 000–60 000 years ago), the Ishikari lowland (Figure 1) was reported to have been under the sea, and the Oshima Peninsula was isolated from other areas of the Hokkaido Island (Japan Association for Quaternary Research 1987). Red foxes in Hokkaido might have been divided into 2 or more populations and been genetically differentiated during the last interglacial age.

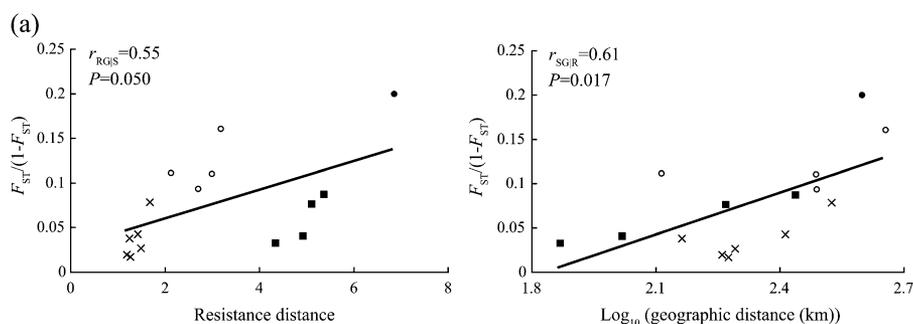


Figure 4. Plots of genetic, geographic, and resistance distances and the results of the partial Mantel test on all subpopulations. Partial correlations and P values are shown, where G , S , R indicate genetic, geographic, and resistance distances, respectively. Closed circle, plots between Southern and Nemuro subpopulations; open circles, those between Southern and other subpopulations; closed squares, those between Nemuro and other subpopulations; and crosses, those between inland subpopulations.

Table 4 Resistance (lower matrix) and geographic distances (km) (upper matrix) among red fox subpopulations

Subpopulation	Southern	Central	Northern	Eastern	Nakasibetsu	Nemuro
Southern		130	308	307	452	397
Central	2.12		183	196	336	274
Northern	2.70	1.21		189	260	186
Eastern	2.99	1.50	1.28		146	104
Nakashibetsu	3.18	1.69	1.43	1.26		74
Nemuro	6.86	5.37	5.11	4.92	4.34	

On the other hand, as an explanation for the subdivision from the present ecological viewpoint, we considered 2 possibilities. The first is that the genetic differentiation of the Southern population may have caused simply by the geographic shape of the distribution areas. The Oshima Peninsula, which covers a wide range of the Southern subpopulation (Figure 1), is the biggest peninsula in Hokkaido, and the narrow and complicated landform could have play the role of a dead end in gene flow. The resistance distance values were higher within a subpopulation in a peninsula (Table 4). The result suggests the idea of gene flow prevention due to the landform; however, resistance distances related to the Southern subpopulations were much lower than those related to the Nemuro subpopulation, although the genetic distances were opposite. This could be because of overestimation of the resistance distances of the Nemuro subpopulation due to the closed entrance to the Nemuro Peninsula. Because the brackish lake Onneto, which is located at the bottom of the peninsula, is part of the sea, we treated it as nonland data in the geographic input file for CIRCUITSCAPE; however, because it is dry at low tide and frozen in winter, the gene flow of foxes could occur more easily between the Nemuro and other inland subpopulations. Because it has not yet been considered that the genetic differentiation of the Southern subpopulation could be due to only resistance distance, we should discuss other possibilities to explain of the differentiation, as follows.

The other possibility from the present ecological viewpoint is that genetic differentiation might have resulted from natal-specific dispersal. Field studies of some vertebrates, such as the prairie deer mouse (*Peromyscus maniculatus*) (Wecker 1963), Townsend's ground squirrel (*Spermophilus townsendii*) (Olson and Van Horne 1998), and cuckoo (*Cuculus canorus*) (Vogl et al. 2002) indicated that individuals tend to disperse preferentially to habitats similar to their natal home ranges. Sacks et al. (2004) also reported that the population structure of the California coyote (*Canis latrans*) was a result of the intraspecific variability in habitat affinities. In Hokkaido, there are some differences in vegetation between the Oshima Peninsula including the Southern area and the other parts. The forests of Hokkaido, except for southern areas, are biogeographically regarded as the transition between temperate and subarctic zones and generally resemble those of the neighboring northern Asian mainland more than those of the Honshu Island in Japan (Tatewaki 1958; Kondo 1993). On the other hand, however, the vegetation in the Oshima Peninsula is relatively similar to that of the Honshu Island, covered by

temperate and cool-temperate deciduous forests, and their border (Tatewaki 1958) almost corresponds with the boundary between Southern and Central subpopulations (Figure 1). Although there are no available reports on the differences in the red fox food habitat or behavior between the southern region and other parts of Hokkaido, there may be some behavioral differences related to these habitats. Natal habitat-biased dispersal could have formed a distinct boundary between the Southern and other subpopulations, as identified in the present study.

There is evidence supporting the present ecological hypothesis above. Recently, sarcoptic mange, which is a serious skin disease of red foxes caused by the mite *S. scabiei*, broke out on the Hokkaido Island. Fox mange was first found in the Shiretoko Peninsula in 1994 then appeared in the Nemuro Peninsula in 1998 (Figure 1). The 2 peninsulas are located on the eastern edges of Hokkaido. After finding mange in these areas, a survey was conducted throughout Hokkaido in 1999. Of the 458 foxes examined, 76 were infected with the mite. Although fox mange was found in 11 of the 14 administrative districts into which Hokkaido is divided, no infected foxes were found in the 3 districts of southern Hokkaido (Takahashi and Uraguchi 2001). This disease is spread by physical contact, such as grooming, mating, and fighting between foxes; therefore, the distribution of infected foxes suggests a low level of fox migration and subsequent gene flow between the Southern subpopulation and the others.

This is the first report examining the red fox population by microsatellite analysis using samples systematically collected on one island. It is interesting to know that the Southern subpopulation of Hokkaido has differentiated from the other subpopulations in spite of their high adaptability and wide dispersal. If the present fox ecological characteristics are responsible for the genetic differentiations, the results of the present study also could give a new insight into the issues of controlling alveolar echinococcosis and preventing of rabies reintroduction to Hokkaido. In order to clarify the reasons why the Southern subpopulation was genetically differentiated, further studies are needed not only from genetic analyses but also from ecological approaches.

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