

# Temporal change in the spatial genetic structure of a sika deer population with an expanding distribution range over a 15-year period

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**Abstract** Since the 1980s, the sika deer (*Cervus nippon* Temminck, 1838) population of Hokkaido, Japan, has grown, resulting in range expansion. To assess the effects of this range expansion on the spatial genetic structure of the population, we compared subpopulation structures during 2 different periods (168 samples for 1991–1996, and 169 samples for 2008–2010), using mitochondrial DNA (mtDNA; D-loop) and microsatellites (9 loci). The number of gene-based subpopulations decreased across the 15-year period; specifically from four to three subpopulations based on mtDNA, and from two to one subpopulation based on microsatellite DNA. The fusion of the two northern subpopulations caused the change to the mtDNA-based structure, which might be explained by the dispersal of

females from higher to lower density subpopulations. In comparison, the reason for the change in the microsatellite DNA-based structure was unclear, because no significant genetic differentiation was observed between the two study periods. A stable mtDNA-based structure was maintained in the north and central population separated by a west-to-east boundary, while a north-to-south boundary in eastern Hokkaido maintained stability in the eastern subpopulation versus all other subpopulations. The findings of this study demonstrate the importance of understanding gene flow within a structured population to implement effective management efforts; for instance, the culling of one subpopulation might not affect an adjacent subpopulation, because deer movement is limited between the subpopulations.

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## Introduction

Human-based disturbance has caused a major decline in the size of many wildlife populations. Such disturbance includes habitat loss (through agriculture, logging, mining, tourism, and development), over-exploitation, and pest control. Large reductions in population size have caused the fragmentation of many wildlife populations (Rueness et al. 2003; Manel et al. 2004; Kaji et al. 2010), which increases the risk of further reduction (i.e., through higher demographic stochasticity and lower genetic diversity). Recent genetic bottlenecks have been evidenced in various species because of this phenomenon (see Peery et al. 2012 for a review). Fragmented populations become genetically

differentiated from each other, leading to the formation of genetically distinct units, as is the case of restored populations [e.g., white-tailed deer (DeYoung et al. 2003); roe deer (Coulon et al. 2006); red deer (Pérez-Espona et al. 2008; Frantz et al. 2012); black bears (Saitoh et al. 2001; Ishibashi and Saitoh 2004); brown bears (Manel et al. 2004); lynx (Rueness et al. 2003)]. Fortunately, some wildlife populations have recovered, but only due to focused conservation effort (Rueness et al. 2003; Manel et al. 2004; Coulon et al. 2006). As a result, the distribution ranges of such populations have expanded. This phenomenon raises the question of whether the spatial genetic structure also alters during population expansion. If so, what factors elicit such alterations to population structure? Through answering these questions, we might identify the management unit of a wildlife population. Therefore, studies on spatial genetic structure potentially provide valuable data to inform conservation and management policies (Crandall et al. 2000; Moritz 2002).

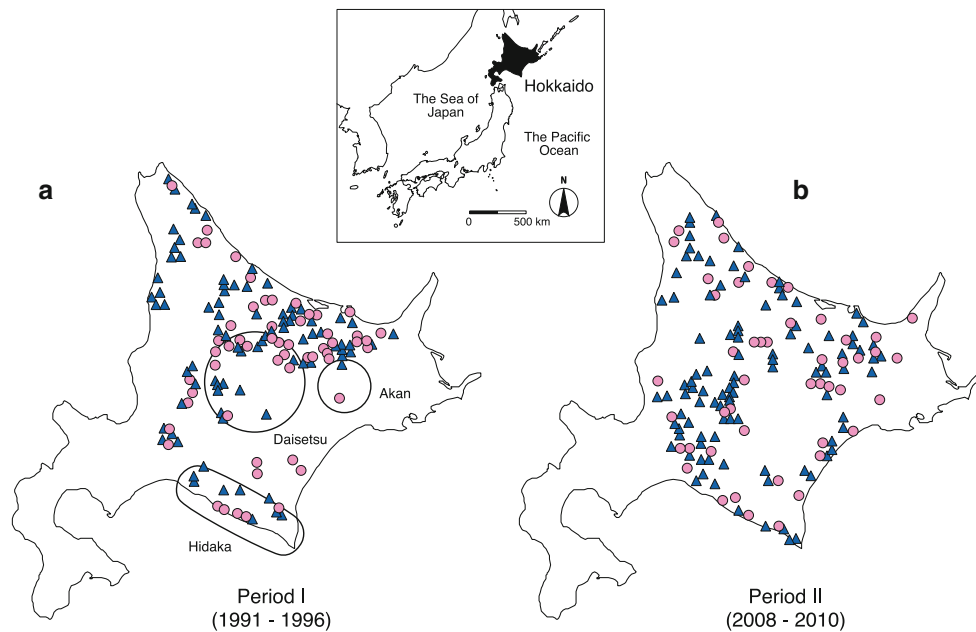
Deer are a typical example of wildlife that has been subject to large reductions and subsequent recovery in population size. Over-exploitation during the last half of the 19th century caused the number and distribution range of deer to decline drastically worldwide (Staines 1977; DeYoung et al. 2003; Côté et al. 2004; Nielsen et al. 2008). However, effective protection measures facilitated the recovery of some deer populations to the point where some became overabundant, particularly in North America and Europe (McShea et al. 1997; Brown et al. 2000; Mysterud et al. 2000; Fuller and Gill 2001; Ward et al. 2008; Warren 2011; Pérez-González et al. 2012). Some studies have attempted to use the spatial genetic structure of populations to identify the management units of such recovered abundant populations (Coulon et al. 2006; Zannèse et al. 2006; Pérez-Espona et al. 2008; Frantz et al. 2012), and have suggested that the landscape features serve as gene flow barriers. For red deer in the Scottish Highlands, the most important barriers to gene flow were identified as sea lochs, followed by mountain slopes, roads, and forests (Pérez-Espona et al. 2008). Motorways have also been suggested to serve as an effective barrier for a population of red deer in Belgium (Frantz et al. 2012). In contrast, Coulon et al. (2006) found no absolute barriers to roe deer populations in France; however, the combination of several landscape features was suggested to cause genetic differentiation.

While such studies provide valuable information about the spatial genetic structure of wildlife populations, the sampling in these studies was only conducted over short timeframes, and thus could not be used to answer the question about the stability of the population structure. Some subpopulations might merge into one, while others might remain distinct. In particular, the spatial genetic

structure of a population that is rapidly recovering from a bottleneck is expected to change.

The sika deer (*Cervus nippon* Temminck, 1838) population in Hokkaido, Japan, presents an ideal model for studying temporal changes in spatial genetic structure during population recovery, taking the effect of density variation on population structure into consideration. The sika deer was abundant throughout Hokkaido prior to the colonization of the island by the Japanese in the late 19th century. Over-exploitation, habitat loss (due to agricultural development and timber extraction in lowland forests), and heavy snowfall contributed to a rapid decline in deer numbers around 1900 (Inukai 1952). To conserve the sika deer population, the Hokkaido Government established hunting regulations (i.e., the prohibition of hunting and/or bucks-only hunting; Kaji et al. 2010). Three major subpopulations survived the bottleneck, which were located in the Akan, Hidaka, and Daisetsu mountain regions (Fig. 1; Nagata et al. 1998; Kaji et al. 2000). Following the implementation of deer protection policies, an increase in grassland area, the extinction of wolves, and a decrease in the number of hunters, the sika deer population recovered, and their distribution range expanded (Kaji et al. 2010). By the mid-1970s, the sika deer population occupied most available habitats in the eastern part of Hokkaido, and had spread to all potential habitats by the 1990s (Kaji et al. 2000). Conversely, the success of the protection measures caused the sika deer to become overabundant, which led to the damage of agricultural land, forests, and natural vegetation, in addition to an increase in the number of traffic collisions. Consequently, hunting regulations were gradually relaxed to place some control on sika deer population numbers (Kaji et al. 2010). In 1998, the Conservation and Management Plan for Sika deer (CMPS), which was operated by the Hokkaido Government, initiated adaptive management practices in eastern Hokkaido, which included intensive female harvesting. In 2000, the target areas were extended to include central Hokkaido, encompassing most parts of the deer distribution range in Hokkaido (Kaji et al. 2010). During this latter period, Nagata et al. (1998) carried out intensive sampling surveys of sika deer between 1991 and 1996 in Hokkaido, and reported that three major mitochondrial DNA (mtDNA) haplotypes were distributed in the three core ranges (Akan, Hidaka, and Daisetsu) that remained after the historical bottleneck. Therefore, by integrating samples recently collected by our research group with those of Nagata et al. (1998), it might be possible to identify temporal changes in the spatial genetic structure during the expansion of this population.

In this study, we aimed to infer the subpopulation structure of a sika deer population inhabiting Hokkaido Island during two distinct periods (Period I: 1991–1996; Period II: 2008–2010), which spanned a 15-year



**Fig. 1** Study area and sampling locations of sika deer in Hokkaido, Japan, during **a** Period I: 1991–1996, and **b** Period II: 2008–2010. Circles indicate female samples; triangles indicate male samples

timeframe. We analyzed the mtDNA (D-loop) and microsatellite DNA (nine loci) from samples collected by Nagata et al. (1998) combined with samples recently collected by our research group. Since mtDNA molecules tend to be inherited maternally in animals (Avisé 2004), the spatial genetic structure revealed by mtDNA sequence variation is a combined product of maternal lineage structure that has accumulated for multiple generations and individual dispersal during a single generation. In comparison, microsatellite DNA molecules are biparentally inherited; thus, the microsatellite DNA-based structure reflects the accumulated dispersal of both sexes for multiple generations. Therefore, if dispersal behavior differs between sexes, different spatial structures would be detected by mtDNA and microsatellite DNA-based analyses. Since deer tend to exhibit male-biased dispersal (Nelson 1993; Clutton-Brock et al. 2002), microsatellite DNA-based structures are expected to show more homogenous patterns in comparison to mtDNA-based structures. Based on these analyses, we discuss the overall population management unit and relevance of the study results for conservation actions.

## Materials and methods

### Study area and sika deer

The study area, Hokkaido Island, is the northernmost island of Japan, which is mountainous and extensively forested (61 % of the total area). The island covers an area of about

77,984 km<sup>2</sup>, and is located at latitudes of 41°24′–45°31′N and longitudes of 139°46′–145°49′E. The island has four distinct seasons, with cool humid summers and cold snowy winters. From 2002 to 2011, the annual average temperatures of Sapporo ranged between 8.8 and 9.8 °C, while the annual precipitations ranged between 843 and 1325 mm (Japan Meteorological Agency, <http://www.data.jma.go.jp/obd/stats/etrn/index.php>). It is warm in the southwestern part of the island and cool in the northeastern part (Stenseth et al. 1998). Less snow accumulates in the eastern part of the island compared to the western part (Kaji et al. 2000).

The sika deer is widely distributed throughout eastern and northeastern Asia, from the Ussuri region of Siberia to northern Vietnam, Taiwan, and Japan (Ohtaishi 1986; Whitehead 1993). It is likely that the wide distribution range of the sika deer, extending from subarctic to tropical zones, has resulted in the species exhibiting considerable morphological variation among populations (Ohtaishi 1986; Terada et al. 2012). The sika deer of Hokkaido is classified as a subspecies, *C. n. yesoensis*, based on its large body size and antlers, in addition to the yellowish-red color of the summer pelage (Imaizumi 1949; Whitehead 1993).

### Sample collection

We used 168 sika deer samples (muscle or liver tissue) that had been collected by Nagata et al. (1998) throughout Hokkaido from 1991 to 1996 (Period I). A further 648 samples were collected from 2008 to 2010 (Period II) by the authors of the current study in cooperation with the

Hokkaido Government and the Hokkaido Hunters Association. Frozen muscle tissues (ca.  $10 \times 10 \times 3$  cm) that had been stored at  $-15$  °C was supplied by hunters, in addition to information about the gender, age (adult:  $\geq 1$  year old; juvenile:  $< 1$  year old), and geographic location from which each sample was obtained. Since the number of samples differed between the two periods, 169 samples (110 males and 59 females) from Period II were selected from the 648 samples, based on sample size, sex ratio, and sample locations from Period I (112 males and 56 females; Fig. 1). Samples from the southern area were not used, because experimental reintroductions were conducted at this location during 1980 and 1981 (Kaji et al. 2000, 2010), with the possible unofficial introduction of deer from outside of Hokkaido (Terada et al. 2013).

In addition to the above datasets, 359 samples were selected from the 648 samples collected during the second period based on the following two steps: (1) juvenile and young individuals were removed, and (2) when several individuals were sampled at the same location, one individual was randomly selected for that location. These 359 samples were used for resampling analyses (see “Resampling analyses”).

#### Molecular analysis

Total genomic DNA was extracted from muscle tissue using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer’s protocol. The D-loop region (606 base-pairs) was amplified using primer L15926 (5'-CTAATACACCAGTCTTGTAACC-3') (Kocher et al. 1989) and primer H597 (5'-AGGCATTTT-CAGTGCCTTGCTTTG-3') (Nagata et al. 1998). PCR amplification was carried out using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) in a 25- $\mu$ l reaction mixture containing 1  $\mu$ l of the DNA extract, 2.5  $\mu$ l of 10 $\times$  PCR Buffer, 2.5  $\mu$ l dNTP, 0.1  $\mu$ l AmpliTaq Gold DNA Polymerase (Applied Biosystems), 1  $\mu$ l of each primer (12.5  $\mu$ M), and 16.9  $\mu$ l UltraPure DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, CA, USA). After incubation at 95 °C for 10 min, cycling was performed for 35 cycles of 1 min at 94 °C, 1 min at 53 °C, and 1 min at 72 °C, with a postcycling extension at 75 °C for 10 min. After removing excess primers and dNTP, the PCR products were labeled using the L15926 primer and a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequences were then determined using an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems). Sequences were analyzed using Sequence Scanner 1.0 (Applied Biosystems), and aligned using MEGA 4.0 (Tamura et al. 2007).

We used nine microsatellite loci: OarFCB193, BM203, BM888 (Talbot et al. 1996); Cervid14 (Dewoody et al.

1995); IDVGA55 (Mezzelani et al. 1995); INRA040 (Vaiman et al. 1994); TGLA127 (Slate et al. 1998); MM12 (Beja-Pereira et al. 2004); and BM4107 (Bishop et al. 1994). PCR amplification was performed in a 25- $\mu$ l mixture containing 1  $\mu$ l DNA Template, 12.5  $\mu$ l AmpliTaq Gold 360 Master Mix (Applied Biosystems), 0.25–1  $\mu$ l Reverse primer (12.5  $\mu$ M), 0.25–1  $\mu$ l Forward primer (12.5  $\mu$ M) end-labeled with a fluorescent dye (NED, PET, VIC or 6-FAM), and 9.5–11  $\mu$ l UltraPure DNase/RNase-Free Distilled Water. After incubation at 95 °C for 10 min, cycling was performed for 30–35 cycles of amplification (30 s at 94 °C, 30 s at melting temperature [ $T_m$ ], 30 s at 72 °C), with a postcycling extension at 72 °C for 7 min. The PCR products were resolved in an ABI PRISM 3100-Avant Genetic Analyzer, and allele sizes were determined for each locus using Gene Mapper 4.0 (Applied Biosystems). To maximize the quality of the genotype data, the presence of null alleles and genotyping errors (such as large allele dropout and stuttering) was examined using Micro-Checker 2.2.3 (van Oosterhout et al. 2004).

#### Mitochondrial DNA data analysis

##### *Standard population genetic analyses*

The number of haplotypes, nucleotide diversity ( $\pi$ , Nei and Tajima 1987), and haplotype diversity ( $h$ , Nei and Tajima 1987) was estimated using DnaSP 5.10.01 (Librado and Rozas 2009).

##### *Inferring subpopulations*

To infer the spatial genetic structure of the population, we used the software GENELAND 4.0.3 (The Geneland development group 2012). GENELAND assigns an individual to a subpopulation based on the genetic information and spatial coordinates, implementing a Bayesian MCMC approach (Falush et al. 2003; Guillot et al. 2005). To infer subpopulation structure and to estimate other parameters, the MCMC was run 10 times, allowing the number of subpopulations ( $K$ ) to vary between 1 and 10, and using the correlated allele frequency model with the following parameters: 100000 MCMC iterations, maximum rate of Poisson process being set to 100, maximum number of nuclei being set to 300, and the uncertainty of spatial coordinates being set to 5 km, according to a grid on a map of Hokkaido used by hunters (Kaji et al. 2000). We calculated the average logarithm posterior probability for each of the 10 runs. To verify the consistency of the inferred subpopulation structures, we compared the subpopulation patterns of the 10 runs. When more than 90 % of runs showed the same subpopulation pattern, we assumed that the consistency of the population was supported, and

selected the run that had the highest average logarithm posterior probability for subsequent analyses. The posterior probability of population membership for pixels was computed with a burn-in of 10,000 iterations, and the number of pixels was set to 200 along both the  $x$ - and  $y$ -axes. Finally, the posterior probability of population membership was computed for each pixel of the spatial domain, and the population membership of individuals to the modal population was inferred.

The frequency and number of haplotypes, nucleotide diversity ( $\pi$ ), and haplotype diversity ( $h$ ) was estimated for each subpopulation in the two periods using DnaSP in the same way as it was used for the whole samples. Genetic differentiation ( $F_{ST}$ ; Weir and Cockerham 1984) between inferred subpopulations for each period was assessed based on haplotypes frequency differences using ARLEQUIN 3.5 (Excoffier and Lischer 2010). Differences in the frequency of haplotypes among subpopulations for each period were examined using Fisher's exact test. Significance was assumed when  $P < 0.05$ .

#### Microsatellite data analysis

##### *Standard population genetic analyses of the whole population*

Standard population genetic analyses were performed with the whole samples collected during each period. The mean number of alleles (MNA) per locus, expected heterozygosity ( $H_E$ ), and observed heterozygosity ( $H_O$ ) were calculated using the Excel Microsatellite Toolkit (Park 2001). The Hardy–Weinberg equilibrium for each locus was tested for each locus using heterozygote deficiency, and was tested globally using the Markov chain method implemented in GENEPOP 4.0 (Rousset 2008; parameter values for the test at the locus level: dememorization number = 10,000, number of batches = 200, number of iterations per batch = 2,000; parameter values for the global test: dememorization number = 1,000, number of batches = 100, number of iterations per batch = 1,000). Linkage disequilibrium was also examined using GENEPOP 4.0 (parameter values: dememorization number = 10,000, number of batches = 800, number of iterations per batch = 8,000).

##### *Inferring subpopulations*

The subpopulation structures were inferred by GENELAND 4.0.3 based on microsatellite DNA data using the uncorrelated frequency model in the same way as it was inferred for mtDNA, except for the parameter set and the number of runs. Parameters were set as follows: 500,000 MCMC iterations, maximum rate of the Poisson process was fixed to 100,

maximum number of nuclei was fixed to 300, and the uncertainty of spatial coordinates was set at 5 km. The MCMC was run 30 times, allowing  $K$  to vary between 1 and 10, to verify the consistency of the inferred subpopulation structures. The posterior probability of population membership was computed for each pixel of the spatial domain ( $200 \times 200$  pixels), and the population membership of individuals to the modal population was inferred. When more than 90 % of runs showed the same subpopulation pattern, we assumed that the consistency of the subpopulation was supported, and selected the run that had the highest average logarithm posterior probability for further analyses.

Pairwise  $F_{ST}$  values were estimated among subpopulations for each periods using ARLEQUIN 3.5.

#### Resampling analyses

To test the null hypothesis that the datasets for Period I and Period II were sampled from the same mother population and to validate the robustness of the original results on spatial genetic structure, we randomly resampled 169 samples from the 359 samples collected for Period II. One-hundred datasets were obtained through this resampling procedure. Spatial genetic analyses using GENELAND 4.0.3 were performed for each of the 100 resampled datasets using mtDNA and microsatellite DNA features, and the results for the original two datasets from Period I and Period II were compared with those produced by the 100 datasets. In addition mtDNA-based and microsatellite DNA-based structures were also inferred by using the 359 samples.

#### Temporal comparison of the subpopulations

To examine genetic differentiation between the two study periods,  $F_{ST}$  was calculated for each subpopulation between the two periods using ARLEQUIN 3.5. Differences in the frequency of haplotypes were also examined for each subpopulation between the two periods using Fisher's exact test.

#### Sightings per unit effort (SPUE)

To test our hypothesis that the spatial genetic structure of a population is altered due to gene flow from high to low density subpopulations, a density index of deer was analyzed in relation to the observed subpopulation structures. The Hokkaido Government requested that hunters report the date and location of hunting, the number and sex of harvested deer, and the total number of live deer that were observed. SPUE (i.e., the number of deer sighted per hunter-day) were converted to a density index based on the reported number of observed deer (Uno et al. 2006). We organized the SPUE for each of 3,599 blocks (approximately  $5 \text{ km} \times 4.6 \text{ km}$ )

**Table 1** Diversity of mtDNA for each inferred sika deer subpopulation during Period I (1991–1996) and Period II (2008–2010)

	<i>N</i>	<i>h</i>	$\pi$	<i>H</i>	Type-a	Type-b	Type-c	Type-d	Type-f
Period I									
1990mtE	27	0.359	0.001	2	21 77.8 %	6 22.2 %	0 0.0 %	0 0.0 %	0 0.0 %
1990mtC	21	0.710	0.002	4	6 28.6 %	1 4.8 %	9 42.9 %	5 23.8 %	0 0.0 %
1990mtN1	80	0.359	0.001	3	17 21.3 %	62 77.5 %	0 0.0 %	1 1.2 %	0 0.0 %
1990mtN2	40	0.703	0.002	5	6 15.0 %	17 42.5 %	1 2.5 %	13 32.5 %	3 7.5 %
Total	168	0.637	0.002	5	50 29.8 %	86 51.2 %	10 5.9 %	19 11.3 %	3 1.8 %
Period II									
2000mtE	47	0.457	0.001	3	32 67.5 %	14 30.3 %	0 0.0 %	1 2.2 %	0 0.0 %
2000mtC	37	0.670	0.002	3	11 29.7 %	0 0.0 %	10 27.0 %	16 43.2 %	0 0.0 %
2000mtN	85	0.517	0.002	4	21 24.7 %	55 64.7 %	0 0.0 %	8 9.4 %	1 1.2 %
Total	169	0.669	0.002	5	64 37.9 %	69 40.8 %	10 5.9 %	25 14.8 %	1 0.6 %

*N* the number of samples, *H* the number of haplotypes, *h* haplotype diversity,  $\pi$  nucleotide diversity, *type-a*, *type-b*, *type-c*, *type-d*, and *type-f* the frequency of haplotype a, b, c, d, and f

placed as a grid on a map of Hokkaido from 2000 to 2010 according to Kaji et al. (2000). This information was used to determine the spatial variation of deer density. Of note, CMPS targeted management activity on a deer subpopulation in eastern Hokkaido in 1998, after which the target areas were extended to include the eastern and central parts of Hokkaido in 2000, covering most parts of the deer distribution range, except for the south-western peninsula (Kaji et al. 2010). Since SPUE could only be obtained from the areas targeted by the CMPS, we focused on the SPUE from of 2,448 blocks between 2000 and 2010. SPUE was not reported for certain blocks that were not entered by hunters. Since hunters did not anticipate successful hunting in these blocks, the SPUE of these blocks was treated as zero. The averaged SPUE was obtained for the subpopulations, and used as a density index. The proportion of blocks where SPUE was higher than zero was also used to determine the proportion of deer presence.

## Results

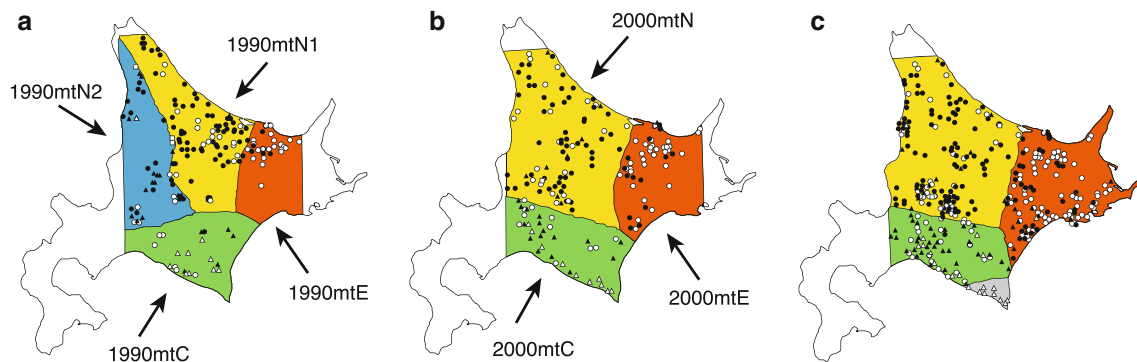
### Mitochondrial DNA analyses

Six hundred and two base pairs of the mtDNA D-loop region were sequenced for all samples ( $n = 168$  for Period I

and  $n = 169$  for Period II). Five haplotypes based on four variable nucleotide sites (base substitution between A and G at nt 178, 251, and 499; base substitution between C and T at nt 457) were found, and were identical to those reported by Nagata et al. (1998): type-a, type-b, type-c, type-d, and type-f. These haplotype sequences were deposited in the databases of GenBank, EMBL, and DDBJ by Nagata et al. (1998) under the following accession numbers: D50128 (type-a), D50129 (type-b), AB004297 (type-c), AB00428 (type-d), and AB004300 (type-f). Haplotype frequencies were similar between Period I (1991–1996) and Period II (2008–2010; Table 1; Fisher's exact test = 0.251). The main haplotypes were type-a, type-b, and type-d, which constituted 92.3 % of all samples in Period I and 93.5 % of all samples in Period II. The geographic distributions of the haplotypes are presented in Fig. 2. In both periods, type-a was the most widespread haplotype, while type-b was primarily distributed in the northern and central regions. The type-d haplotype was primarily distributed in the southern area. Haplotype and nucleotide diversity were low and similar for both periods (Table 1).

### Subpopulation structure

Four subpopulations (*K*) were estimated in nine out of 10 runs for Period I (Fig. 2a). In Period II, three clusters were



**Fig. 2** Map of the subpopulations based on mtDNA haplotypes (D-loop). Memberships of individual sika deer were deduced by GENELAND 4.0.3 for **a** Period I (1991–1996,  $n = 168$ ), **b** Period II (2008–2010,  $n = 169$ ), and **c** Period II (2008–2010,  $n = 359$ ). Samples with haplotype-a are represented by *open circles*, type-b by

*solid circles*, type-c by *open triangles*, type-d by *solid triangles*, and type-f by *open squares*. Four subpopulations were named 1990mtN1, 1990mtN2 (northern Hokkaido), 1990mtE (eastern Hokkaido), and 1990mtC (central Hokkaido) during Period I, and three subpopulations were named 2000mtN, 2000mtE, and 2000mtC during Period II

supported by all 10 runs (Fig. 2b). According to the sampling locations, we named the four subpopulations from Period I and the three subpopulations from Period II as follows: 1990mtN1 ( $n = 80$ ), 1990mtN2 ( $n = 40$ ; northern Hokkaido), 1990mtE ( $n = 27$ ; eastern Hokkaido), and 1990mtC ( $n = 21$ ; central Hokkaido); 2000mtN ( $n = 85$ ), 2000mtE ( $n = 47$ ), and 2000mtC ( $n = 37$ ), respectively. The location of the boundary between eastern and northern Hokkaido remained stable for both periods, and it ran through Abashiri city, Kitami city, Ashoro town, and Urahoro town (Fig. 2). The boundary that divided the northern subpopulation into two subpopulations (1990mtN1 and 1990mtN2) was only observed during Period I. The boundary that separated the central subpopulation from all other subpopulations shifted slightly northwards between the two periods.

The type-a haplotype dominated all other haplotypes in the eastern subpopulation during both periods (1990mtE and 2000mtE; Table 1). The type-b haplotype was dominant in the northern subpopulations during both periods (1990mtN1, 1990mtN2, and 2000mtN), although type-d was also common in 1990mtN2. Type-c was the most common haplotype in the central subpopulation during Period I, followed by type-a and type-d (1990mtC), while type-c was replaced as the most common haplotype by type-d during Period II (2000mtC). Haplotype frequencies differed significantly among subpopulations during both Period I (Fisher's exact test,  $P < 0.0001$ ) and Period II ( $P < 0.0001$ ).

Genetic differentiation among subpopulations was large and highly significant (Table 2). The pairwise  $F_{ST}$  estimated for Period I (0.206–0.461) was generally higher compared to that estimated for Period II (0.175–0.377).

Eighty-five of the 100 resampled datasets showed consistent patterns for subpopulation structure, with two, 34,

**Table 2** Genetic differentiation among subpopulations estimated by pairwise  $F_{ST}$  ( $P$  value) and Fisher's exact test between Period I (1991–1996) and Period II (2008–2010) based on mtDNA features

Subpopulation combination	$F_{ST}$ ( $P$ value)	Fisher's exact test ( $P$ value)
Period I		
1990mtC–1990mtE	0.214 (<0.0001)	<0.0001
1990mtN1–1990mtE	0.461 (<0.0001)	<0.0001
1990mtN1–1990mtC	0.457 (<0.0001)	<0.0001
1990mtN2–1990mtE	0.409 (<0.0001)	<0.0001
1990mtN2–1990mtC	0.289 (<0.0001)	<0.0001
1990mtN2–1990mtN1	0.206 (<0.0001)	<0.0001
Period II		
2000mtC–2000mtE	0.209 (<0.0001)	<0.0001
2000mtN–2000mtE	0.175 (<0.0001)	<0.0001
2000mtN–2000mtC	0.377 (<0.0001)	<0.0001

$P$  values are the probability of  $F_{ST}$  values being at least as extreme as the observed values, assuming that the null hypothesis ( $F_{ST} = 0$ ) is true

and 49 datasets supporting  $K = 2$ ,  $K = 3$ , and  $K = 4$ , respectively. The subpopulation structure of the 34 datasets supporting three subpopulation resembled the one of the original dataset for Period II. The basic subpopulation structure of the 49 datasets supporting four subpopulations was similar to the one of the original dataset for Period II. However, the subpopulation corresponding to 2000mtC was divided into two subpopulations resembling the structure based on the 359 sample-analyses (Fig. 2c; see the next paragraph). The subpopulation structure of the two datasets supporting two subpopulations lacked the subpopulation corresponding to 2000mtE. When focusing on the northern area (Period I: 1990mtN1, 1990mtN2, and 1990mtE; Period II: 2000mtN and 2000mtE), all of the resampled datasets showed the different subpopulation

**Table 3** Temporal comparison of genetic differentiation among the sika deer subpopulations using  $F_{ST}$  ( $P$  value) and Fisher's exact test between Period I (1991–1996) and Period II (2008–2010) for the subpopulations based on the mtDNA features

Subpopulation combination	$F_{ST}$ ( $P$ value)	Fisher's exact test ( $P$ value)
1990mtE–2000mtE	–0.015 (0.550)	0.740
1990mtN1+1990mtN2–2000mtN	–0.008 (0.676)	0.802
1990mtC–2000mtC	0.048 (0.198)	0.219
1990mtN1–2000mtN1'	0.017 (0.162)	0.091
1990mtN2–2000mtN2'	0.039 (0.081)	0.035

structure from that of Period I, indicating that 1990mtN1 and 1990mtN2 were merged together in Period II. Therefore, the null hypothesis that the datasets for Period I and Period II were sampled from the same mother population was rejected.

All of the 10 runs using the 359 samples showed that the number of subpopulations was four, and the subpopulation structure was consistent across the 10 runs (Fig. 2c). The subpopulation structures from the 169 samples and the 359 samples for Period II resembled each other, except for the central subpopulation being separated into two in the analysis using the 359 samples. Based on the 359 samples, the structure of the northern subpopulations evidently differed from those collected during Period I. The area of 1990mtN2 was coupled with that of 1990mtN1 in the 359 sample-based structure. Both boundaries that were observed in the analysis using the 169 samples were confirmed in the analysis using the 359 samples.

#### *Differences between the two periods*

Genetic differentiation between the two periods was examined using the pairwise  $F_{ST}$  and Fisher's exact test for each subpopulation. For the eastern and central subpopulations, no significant genetic differentiation was detected between the two periods (Table 3). For the northern subpopulations, we combined the two subpopulations from Period I (1990mtN1 and 1990mtN2) into one subpopulation, and examined the genetic differentiation with the equivalent subpopulation in Period II (2000mtN). These subpopulations also showed no significant genetic differentiation (Table 3). We then divided the 2000mtN into two units (2000mtN1' and 2000mtN2') based on the boundary separating 1990mtN1 and 1990mtN2, and again examined the genetic differentiation. Genetic differentiation was found between 1990mtN2 and 2000mtN2', whereas 1990mtN1 and 2000mtN1' did not show any significant genetic differentiation.

#### Microsatellite DNA analyses

The MNA and heterozygosity are presented in Table 4. No significant deviation from the Hardy–Weinberg equilibrium expectation was detected for any of the loci when the significance values for multiple comparisons were adjusted using the Bonferroni correction. The analysis of each pair of loci showed no significant linkage disequilibrium after the Bonferroni correction for multiple tests. The number of alleles was the same between Period I and Period II for all loci, except BM4107 (Table 4). Observed frequencies of alleles were not significantly different between the periods for any loci (Fisher's exact test,  $P > 0.090$ ).

#### *Subpopulation structure*

The inferred number of subpopulations was two, and the subpopulation structure was consistent across the 30 runs for Period I. The subpopulation structure with the highest average logarithm posterior probability is presented in Fig. 3a. The subpopulations were named as 1990msN ( $n = 151$ ) and 1990msC ( $n = 17$ ) according to the sampling locations. The modal number of populations was one in all 30 runs for Period II (Fig. 3b).

Genetic differentiation among subpopulations was estimated using  $F_{ST}$ . The pairwise  $F_{ST}$  was significantly large among the subpopulations (1990msN–1990msC) in Period I (Table 5). The samples from Period II were grouped into two units according to the boundaries of the two subpopulations in Period I, with the two units being named as 2000msN' and 2000msC'. Although pairwise  $F_{ST}$  for Period II (2000msN'–2000msC') was lower compared to that for Period I, significant genetic differentiation was obtained (Table 5).

Eighty-nine of the 100 resampled datasets showed consistent patterns for subpopulation structure, with 34, 41, and 14 datasets supporting  $K = 1$ ,  $K = 2$ , and  $K = 3$ , respectively. The population structure of the 34 datasets supporting one subpopulation was the same as the one of the original dataset for Period II, of course. In the 41 datasets supporting two subpopulation three patterns of the subpopulations structure were shown; (1) the subpopulation structure of 16 datasets resembled the one of the original dataset for Period I, (2) in 24 datasets, one subpopulation occupied most of Hokkaido together with another small subpopulation being located on eastern part, and (3) in the remaining one datasets the population was almost evenly divided into eastern and western subpopulations. The 14 datasets supporting three subpopulations showed a combined structure of the above patterns (1) and (2). Because the considerable number of resampled datasets showed the subpopulation structure resembling the one of the original dataset for Period I or Period II, the

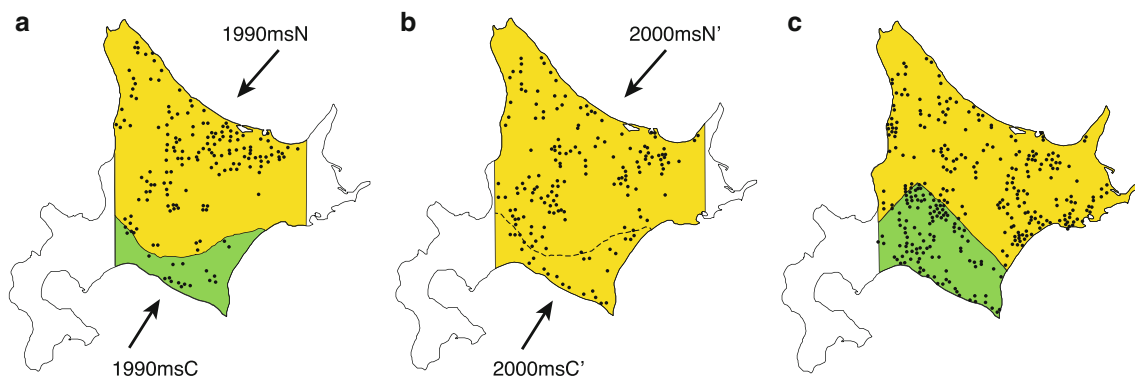


**Table 4** Genetic variation per locus based on the microsatellite DNA features of the whole sika deer population in Hokkaido for Period I (1991–1996) and Period II (2008–2010)

Locus name	Period I				Period II				Fisher's exact test ( <i>P</i> value)
	<i>A</i>	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>P</i> value	<i>A</i>	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>P</i> value	
OarFCB193	2	0.327	0.313	0.800	2	0.260	0.277	0.292	0.367
BM203	2	0.387	0.373	0.751	2	0.284	0.322	0.095	0.166
BM888	3	0.512	0.517	0.214	3	0.503	0.518	0.423	0.559
INRA040	3	0.589	0.655	0.027	3	0.592	0.655	0.023	0.995
Cervid14	2	0.476	0.501	0.312	2	0.462	0.501	0.190	0.818
IDVGA55	6	0.768	0.781	0.273	6	0.751	0.807	0.076	0.090
MM12	2	0.464	0.486	0.341	2	0.503	0.479	0.795	0.695
TGLA127	2	0.208	0.187	1.000	2	0.189	0.209	0.184	0.625
BM4107	3	0.417	0.486	0.022	5	0.527	0.522	0.519	0.603

Fisher's exact tests were performed to test the differences in allele frequencies between the two periods

*A* the number of alleles per locus, *H<sub>O</sub>* observed heterozygosity, *H<sub>E</sub>* expected heterozygosity



**Fig. 3** Map of the subpopulations based on microsatellite DNA features. Memberships of individual sika deer were deduced by GENELAND 4.0.3 for **a** Period I (1991–1996,  $n = 168$ ), **b** Period II (2008–2010,  $n = 169$ ), and **c** Period II (2008–2010,  $n = 359$ ). Subpopulations were named as 1990msN (northern Hokkaido) and 1990msC (central Hokkaido) during Period I. Dots indicate sampling

locations. Although the subpopulation structure was unclear for Period II, samples from Period II were grouped into two units according to the boundary of the two subpopulations in Period I (a broken line), with the two units being named as 2000msN' and 2000msC'

**Table 5** Spatial and temporal comparisons of genetic differentiation for the sika deer subpopulations based on the microsatellite DNA features

Subpopulation combination	$F_{ST}$	<i>P</i> value
1990msN–1990msC	0.048	<0.0001
2000msN'–2000msC'	0.017	<0.0001
1990msN–2000msN'	0.000	0.812
1990msC–2000msC'	0.004	0.342

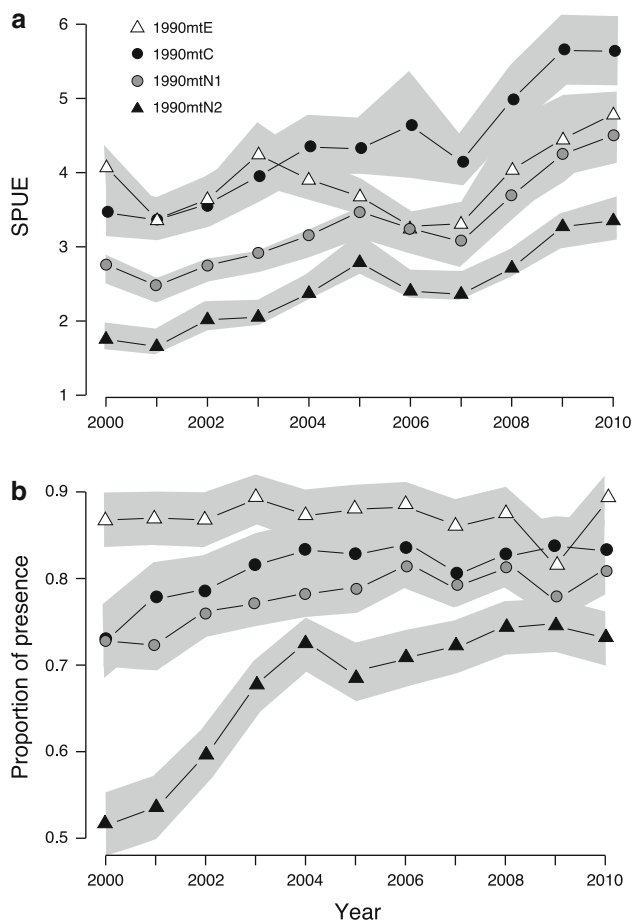
The individuals in Period II were divided into 2000msN' and 2000msC' by the boundary of two subpopulations in Period I. Genetic differentiation was estimated by pairwise  $F_{ST}$ . *P* values are the probability of  $F_{ST}$  values being at least as extreme as the observed values, assuming that the null hypothesis ( $F_{ST} = 0$ ) is true

hypothesis that the datasets for Period I and Period II were sampled from the same mother population was not rejected.

Two subpopulations were consistently detected across the 30 runs in the analysis using the 359 samples (Fig. 3c). The inferred subpopulation structure resembled that from the 168-sample analysis for Period I, although the boundary separating the northern subpopulation from the central one shifted northwards in the 359-sample analysis for Period II.

#### Differences between the two periods

Genetic differentiation between the two periods was examined using the pairwise  $F_{ST}$  for each subpopulation



**Fig. 4** **a** Temporal changes in sika deer sightings per unit effort (SPUE, i.e., the number of deer sighted per hunter-day) reported by hunters. SPUE was averaged for four subpopulations based on the mtDNA-based structure. *Gray zones* denote the 95 % confidence interval. **b** Temporal changes in the proportion of sika deer presence. The proportions represent the number of blocks where SPUE was higher than zero, and were organized for the four mtDNA-based subpopulations. *Gray zones* denote 95 % confidence intervals

(Table 5). No significant differentiation was observed in the comparison between 1990msN ( $n = 151$ )–2000msN' ( $n = 143$ ) and between 1990msC ( $n = 17$ )–2000msC' ( $n = 26$ ).

#### Sightings per unit effort

The density index represented by the averaged SPUE was compared among subpopulations and years. Subpopulations were defined using the mtDNA-based structure for Period I, because the subpopulation structure for Period I might represent the basis of changes in the spatial genetic structure between the two periods. SPUE was not analyzed using on the microsatellite DNA-based subpopulations, because the microsatellite DNA-based structure using the 169 samples for Period II is inconclusive (see “Discussion”).

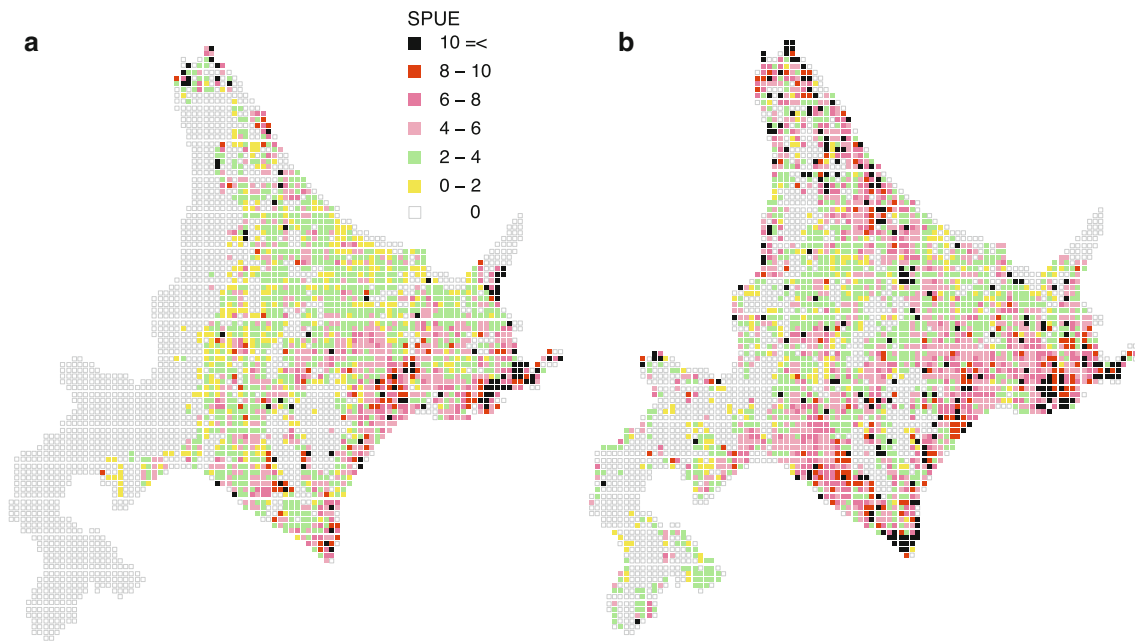
The number of blocks for the four subpopulations detected by the mtDNA-based analysis (1990mtC, 1990mtE, 1990mtN1, and 1990mtN2) was 413, 418, 882, and 735, respectively. The highest average density ( $\pm$ SD) in 2000 was in block 1990mtE ( $4.06 \pm 3.20$ ), followed by 1990mtC ( $3.48 \pm 3.55$ ), 1990mtN1 ( $2.69 \pm 2.89$ ), and 1990mtN2 ( $1.76 \pm 2.49$ ), with no substantial overlap in the 95 % CIs among the subpopulations (Fig. 4). The density of all subpopulations gradually increased between 2000 and 2010, with the highest values being obtained in 2010; 1990mtE:  $4.77 \pm 3.24$ ; 1990mtC:  $5.68 \pm 4.91$ ; 1990mtN1:  $4.39 \pm 4.42$ ; and 1990mtN2:  $3.36 \pm 4.08$ . The subpopulations of 1990mtN1 and 1990mtE showed similar densities during 2005–2010, while the densities of 1990mtC and 1990mtN2 substantially differed to all other densities obtained during this period. The subpopulation with the highest density shifted from 1990mtE to 1990mtC in 2004, while 1990mtN2 remained the lowest subpopulation throughout 2000–2010. The highest rate of increase for density was recorded in 1990mtN2 (1.91), followed by 1990mtC (1.63), 1990mtN1 (1.63), and 1990mtE (1.17).

In contrast to the subpopulation densities, there was a clear variation in the temporal change in the proportion of presence (i.e., the proportion of blocks where SPUE was higher than zero) among subpopulations (Figs. 4, 5). In 2000, the proportion of presence was highest in 1990mtE (0.87), followed by 1990mtN1 (0.73), 1990mtC (0.73), and 1990mtN2 (0.52). The proportion of presence was stable in 1990mtE, with the value for 2010 (0.89) remaining similar to that obtained in 2000. The proportion of presence for 1990mtC and 1990mtN1 was comparative in most years (Fig. 4), showing a gradual increase until 2004, after which the proportion became saturated at around 0.8. There was a noticeable change in the proportion of presence for 1990mtN2, which showed a major increase from 0.52 in 2000 to 0.73 in 2004, and continued to increase at a lower rate until 2009.

Throughout the study period, the proportion of presence for 1990mtN2 remained substantially lower compared to all other subpopulations. While the proportion of presence for 1990mtE was higher compared to all other subpopulations until 2003, it exhibited only a minor difference to 1990mtC and/or 1990mtN1 in all subsequent years.

#### Discussion

The results of this study partly support our prediction that the spatial genetic structure of a rapidly recovering population changes. The mtDNA-based structures changed between the two study periods, whereas the microsatellite DNA-based structures did not change. Here, we discuss the processes of structural change when considering the effects



**Fig. 5** Sightings of sika deer per unit effort (SPUE, i.e., the number of deer sighted per hunter-day) reported by hunters in Hokkaido for **a** 2000 and **b** 2010. The sightings were averaged for 3,599 blocks (about 5 km  $\times$  4.6 km), and placed as a grid on a map of Hokkaido. SPUE was not reported for blocks not entered by hunters. Since

hunters did not anticipate successful hunting in these blocks, the SPUEs of these blocks were treated as zero, and presented as *open blocks*. SPUEs were classified into seven ranks at intervals of two from zero to more than 10, and were represented by a *gray gradation* (this is presented in color in the online version)

of population density and inter-sexual differences in dispersal pattern. The management implications are also presented in relation to the observed differences in the mtDNA- and microsatellite DNA-based subpopulation structures.

#### Temporal changes in subpopulation structure

Between Period I (1991–1996) and Period II (2008–2010), the number of subpopulations decreased from four to three based on the mtDNA analyses. One major change was the fusion of subpopulations 1990mtN1 and 1990mtN2 into subpopulation 2000mtN. In contrast, the changes in subpopulation structure observed by microsatellite DNA analysis were unclear.

Genetic structuring is primarily determined by the dispersal capability and habitat requirements of a given population (Slatkin 1987). Since sika deer were distributed throughout Hokkaido Island in the past, and the sika deer population has recently spread again to most areas of Hokkaido, most areas of Hokkaido are likely to meet the habitat requirements of sika deer. Furthermore, major changes in agricultural pasture and forest area, which are considered to have contributed to the recovery of deer distribution and abundance (Kaji et al. 2010), were not reported during the two study periods ([http://www.e-stat.](http://www.e-stat.go.jp/)

[go.jp/](http://www.e-stat.go.jp/); Department of Fishery and Forestry, Hokkaido 1990–2010), while pasture areas increased and many natural forests were converted into artificial forests during the 1960s and 1970s. Therefore, habitat features might not represent a major factor explaining the change in the spatial genetic structure during the study periods. Alternatively, dispersal behavior and its associated factors (such as population density and geographic structure) might explain the documented changes in the spatial genetic structure of this population. 1990mtN1 and 1990mtN2 were genetically distinct in Period I, but had merged into one subpopulation in Period II (Fig. 2). Since no significant genetic differentiation was observed between 1990mtN1 + 1990mtN2 and 2000mtN, mtDNA features that were different between 1990mtN1 and 1990mtN2 for Period I might have been homogenized by the dispersal of deer by Period II. However, the change in the structure between 1990mtN1 and 1990mtN2 was different. Genetic differentiation was found between 1990mtN2 and 2000mtN2', whereas 1990mtN1 and 2000mtN1' did not show significant genetic differentiation (Table 3). This result indicates that 1990mtN2 might have received more immigrants compared to 1990mtN1. For instance, there might have been more frequent dispersal from 1990mtN1 to 1990mtN2 compared to dispersal in the reverse direction. Spatial variations in changes in SPUE, as a density index, were consistent with

this interpretation. The density of 1990mtN1 was substantially higher compared to 1990mtN2 across the periods. In addition, many vacant habitat blocks in the region of 1990mtN2 became occupied between the two study periods, while the other subpopulations occupied most blocks by 2000 (Figs. 4, 5).

The microsatellite DNA-based subpopulations for Period I appeared to have become considerably mixed by Period II (Fig. 3a, b). However, pairwise  $F_{ST}$  values do not support this change in subpopulations, because genetic differentiation was significant between the hypothetical subpopulations (2000msN'–2000msC') during Period II as well as between the subpopulations (1990msN–1990msC) during Period I. Furthermore, no significant differentiations were observed in the comparison between 1990msN–2000msN' and between 1990msC–2000msC' (Table 5). In addition, the hypothesis that the datasets for Period I and Period II were sampled from the same mother population was not rejected by resampling analyses. Similar subpopulation structures were observed both in the 168-sample analysis for Period I and the 359-sample analysis for Period II (Fig. 3). Therefore, the microsatellite DNA-based subpopulation structure may not have changed across the two study periods; hence, the result indicating that no spatial genetic structure was detected during Period II might have been caused by a sampling bias.

#### Sex-specific movement

Spatial genetic structures were more heterogeneous in the mtDNA-based structure compared to the microsatellite DNA-based structure (Figs. 2, 3). These results indicate that gene flow among subpopulations is asymmetric between mtDNA and microsatellite DNA. This phenomenon might be explained by assuming the presence of male-biased dispersal and female philopatry. Male-biased dispersal occurs in most mammalian species (Greenwood 1980), including deer (Nelson 1993; Clutton-Brock et al. 2002). Male movement contributes towards homogenizing the microsatellite DNA-based structure; however, its contribution is limited for the mtDNA-based structure, because the inheritance system differs between mtDNA and microsatellite DNA; specifically, mtDNA is maternally inherited, while microsatellite DNA is bi-parentally inherited. The result demonstrating that the subpopulation structure of 1990mtE (2000mtE) was maintained within a larger microsatellite DNA-based structure indicates that female movement was limited in these areas, whereas males moved between the mtDNA-based subpopulations. Hence, the phenomenon might be explained by male-biased dispersal and female philopatry.

We have suggested that the fusion of the 1990mtN1 and 1990mtN2 into 2000mtN is explained by the dispersal from

1990mtN1 to 1990mtN2 along a density gradient. The mtDNA features of 1990mtN2 for Period I were significantly altered by Period II. Females are expected to contribute to this alteration. Male immigrants might also alter mtDNA features; however, their contribution is expected to be minor because only females pass mtDNA features onto the next generation.

Although we explained the change in the mtDNA-based subpopulation structure based on the dispersal of females along the density gradient, a density gradient does not always generate mtDNA gene flow. Although SPUE and the proportion of presence were higher in 1990mtE compared to 1990mtN1 in the early 2000s, mtDNA gene flow from 1990mtE to 1990mtN1 was not observed. These results indicate that density effects have a limited influence on dispersal. Other factors might also contribute to the structuring of deer subpopulations.

#### Factors influencing the formation of subpopulations

Two boundaries remained stable throughout the two study periods with respect to the mtDNA-based structure: (1) the west-to-east boundary, separating the northern subpopulations from the central subpopulations traversed the same locations, and (2) the north-to-south boundary in eastern Hokkaido, separating the eastern subpopulation from other subpopulations (Fig. 2). The first boundary was also observed in the microsatellite DNA-based structures from the 168-sample analysis of Period I and the 359-sample analysis of Period II. The first boundary might limit the movement of both sexes, while the second boundary might have had a larger effect on female dispersal, because this boundary was only valid for the mtDNA-based structure.

Igota et al. (2004) captured 57 female sika deer at Shiranuka, which is located 25–30 km east of the boundary separating the eastern subpopulation from the other subpopulations. The authors subsequently released the deer after attaching radio-telemetry units, and tracked their movements patterns during 1997–2001. Long-distance seasonal migration was recorded for 39 females ( $35.1 \pm 3.6$  km; mean  $\pm$  SD), whereas the remaining females were non-migrants or the tracking units failed. The migratory direction was north or east in the majority of cases, with none of the females crossing the east boundary. This tracking study supports the presence of this boundary for the female component of the population, although there was a lack of information about males.

The sampling resolution of the current study might not be sufficiently high enough to obtain a detailed picture about boundaries. Further studies that use a greater number of samples collected by spatially uniform and intensive sampling effort are required to identify the exact landscape barriers to gene flow.

## Management implications

CMPS has been focusing on reducing the size of the subpopulation in eastern Hokkaido, because increased deer abundance in recent years has caused severe agricultural damage. When the target areas for population control were extended to central Hokkaido, the management units (MUs) were defined as western and eastern subpopulations, which roughly correspond to 1990mtN2 + C and 1990mtN1 + E, respectively. However, these management units should be revised based on the subpopulation structure identified in this study.

The current study raises the question of which subdivisions of a population should be used when mtDNA and nuclear DNA analyses present different results. Since mtDNA is maternally inherited, the mtDNA-based subdivisions indicate maternally structured groups. In comparison, nuclear DNA-based subdivisions indicate the effect of gene flow by both males and females; thus, reflecting groups that have the potential for future local adaptation. Therefore, from an evolutionary perspective, a nuclear DNA-based analysis of the subpopulation structure should be adopted. However, from a demographic perspective, an mtDNA-based structure should be adopted. For example, the CMPS has been encouraging hunters to target female sika deer in areas of high population density, because the removal of adult females with high reproductive value represents the most efficient strategy of controlling abundant populations. In other words, the survival rate of adult females strongly influences the population growth rate of wildlife populations (Escos et al. 1994; Walsh et al. 1995; Uno 2006). This interpretation raises the question of whether female-biased hunting effort in 2000mtE would impact 2000mtN. Our study results indicate that this would not be the case, because the dispersal of females between 2000mtE and 2000mtN was limited in both study periods at least. Therefore, the 2000mtN population size would continue to increase, despite the successful control of the 2000mtE subpopulation. Through understanding the genetic structuring and gene flow among the subpopulations, we are able to demonstrate that these two subpopulations should be managed separately, which differs to the interpretation based on the microsatellite DNA-based management unit.

Genetic monitoring presents a promising tool for conservation and management (Schwartz et al. 2007). Our study shows the power of spatial genetic analyses, and the importance of periodical monitoring, for the management of wildlife. In conclusion, the combination of spatial genetic analyses with behavioral, geographic, and demographic datasets would increase the power of these different, yet related, wildlife population analysis techniques.

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