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Spatially heterogeneous distribution of mtDNA haplotypes in a sika deer (*Cervus nippon*) population on the Boso Peninsula, central Japan

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Abstract. We used variation in the mitochondrial DNA (mtDNA) control region (*D-loop*) to examine the genetic structure of the sika deer (*Cervus nippon*) population on the Boso Peninsula, central Japan. A total of four haplotypes was found. In order to examine whether or not artificial barriers such as roads, dams, and golf courses affect the spatial heterogeneity of mtDNA haplotypes, we implemented two exclusive spatial analyses (SAMOVA and network analysis based on Monmonier's algorithm) for searching genetic discontinuities between artificial barriers. Prior to the analyses, the whole distribution area was divided into meaningful eight blocks. Analysis of molecular variance (AMOVA) detected significant spatial heterogeneity in the constitution of the haplotypes among the blocks. The subsequent spatial analyses detected some significant spatial discontinuities on borders of the blocks. In particular, the largest discontinuity was observed in the area including motorway Line 81, but the traffic density of Line 81 is generally not very heavy compared to other major roads. These findings suggest that roads could be one of major barriers to hamper migration of sika deer to some extent, but other potential factors such as the location of food resources and/or the history of bottleneck event are also likely to more or less contribute to configure the present patterns of haplotype distribution.

Key words: artificial structure, *Cervus nippon*, gene flow, mitochondrial DNA, population.

Estimation of the patterns of gene flow is one of the central problems in the field of population genetics, because gene flow determines the extent to which local populations of a species form independent evolutionary units (Slatkin 1985). Two following approaches are possible to estimate the amount of gene flow. One of them directly estimates dispersal distances of individuals, for instance, with the aid of the Global Positioning System (GPS) or by means of traditional mark-and-recapture methods. The other is founded on a mathematical model of the interaction of gene flow and other forces (e.g. genetic drift) to predict how much gene flow must have been occurring in order for the patterns observed in the data to be present (Slatkin 1994). Although these two approaches have different drawbacks for estimating gene flow, the latter is greatly advantageous because it incorporates the effects of various kinds of dispersal and averages variations in dispersal over time. Our principal aims are to research genetic population structure in a wild animal using the latter approach.

Movement of individuals is often restricted by physical barriers in wild animals (Avise et al. 1987). Geographic gaps such as rivers, valleys, and mountains have been known to contribute to form genetic structure within or between populations (Avise et al. 1987). Besides these natural barriers, potential artificial barriers to gene flow are roads (Forman and Alexander 1998; Aars and Ims 1999; Mech and Hallet 2001; Riley et al. 2006), dams (Hansen and Loeschcke 1996; Yamamoto et al. 2004), and urbanized areas (Wang and Schreiber

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2001). Thus, environmental modification mediated by human activities is likely to result in habitat fragmentation in wildlife (Ceballos and Ehrlich 2002) with a consequent reduction of genetic diversity and of the levels of gene flow (Young et al. 1996). Understanding the practical effects of such artificial structures on gene flow of wildlife is important in point of detecting of spatial genetic structure in landscape genetics (Manel et al. 2003).

The population density of sika deer (*Cervus nippon*) on the Boso Peninsula in Chiba Prefecture, central Japan, is known to have been relatively high in the latter half of the 19th century, but the prompt population shrinkage occurred in about the 1940s probably because of human settlement of the foothills and hunting (Chiba Prefecture and Deer Research Group on Boso 2004). To restore population size, culling of deer was suspended in 1961. By 1973–74, when the density was at its lowest, a relict deer population was distributed in a local area of the southern peninsula (Koganezawa et al. 1976; Chiba Prefecture and Deer Research Group on Boso 2004) (Fig. 1a). For reasons not entirely known, a rapid resurgence of numbers began in the late 1980s. The present distribution is consequently much wider than ever previously recorded, and its density is also conspicuously high especially in the southern part of its distribution (Chiba Prefecture and Deer Research Group on Boso 2004, Fig. 1). The southwestern limit of the current distribution of sika deer on the Boso Peninsula corresponds well to the motorway Line 34 (see Fig. 1), implying that this road acts as an effective barrier against distribution expansion to the southward. Geographical barriers are of importance as well. A very high mountainous region and deep gorge are absent, but some roads pass along the brink, which could affect dispersion of sika deer. Furthermore, the amount of food resources could cause non-random dispersal in wild animals (Dwyer and Morris 2006), and thus could be another potential factor for weakening random dispersal. However, the precise estimate of the area covered with suitable food plants is generally awkward and the survey of plant species over the area is beyond our scope in the present study. Therefore, here we focused on the relationship between artificial structures and population genetic structure, together with geographical features where artificial structures exist.

In the present study, we assessed the genetic variation and spatial genetic structure in the Boso population using mtDNA *D-loop* sequences which have been frequently used to assess such purposes (e.g. Nagata et al. 1998, 1999; Nabata et al. 2004, 2007; Yamada et al. 2006; Yuasa et al. 2007). Based on the result, we compared the amount of variation in *D-loop* region in other populations (Yuasa et al. 2007) and discussed effects of staple artificial structures and other potential factors on gene flow among subdivided populations.

Materials and methods

Specimens and DNA extraction

To date, local governments have started programs to control the population density of sika deer. The present study was conducted under the operation of the programs. Whole blood, tail or ear tissue, or hair was obtained from 259 individuals of sika deer culled by hunters at Kimitsu, Ohtaki, Ichihara, Kamogawa, and Katsu-ura, in Chiba Prefecture, central Japan, between January and October, 2005 (Fig. 1 and Table 1). Details including geographic information (latitude and longitude) and date of collection of the deer were individually recorded for each site. Whole-blood samples were stored on FTA Mini Cards (Whatman, Middlesex, UK), and other tissues were stored in a freezer until extraction of total DNA. DNA was extracted from the FTA Mini Cards in accordance with the manufacturer's specifications, and from the tissues by using a DNeasy Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol.

Amplification and sequencing

To amplify the mitochondrial *D-loop* region by polymerase chain reaction (PCR), we used the primer set L15926 and H597 (Nagata et al. 1998). Amplification of the DNA extracted from the FTA Mini Card was carried out in reaction solution (50 μL in a tube) containing 5 μL of 10× PCR Buffer II (Applied Biosystems, CA, USA), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μM each primer, and 1.5 units of AmpliTaq DNA polymerase (Applied Biosystems). Amplification from each tissue extract was done in 25 μL of the above solution. PCR was performed under the following conditions: 2 min at 94°C; 40 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 6 min. We also used previously developed primers HD2, HD6 (Nagata et al. 1998), and original primers, HD2R, HD6R, and HDN for sequencing. HD2R (5'-CATCTGGTTCTTTTTTCAGG-3') and HD6R (5'-GCAGTCAATGGTCACAGGAC-3') are complementary to HD2 and

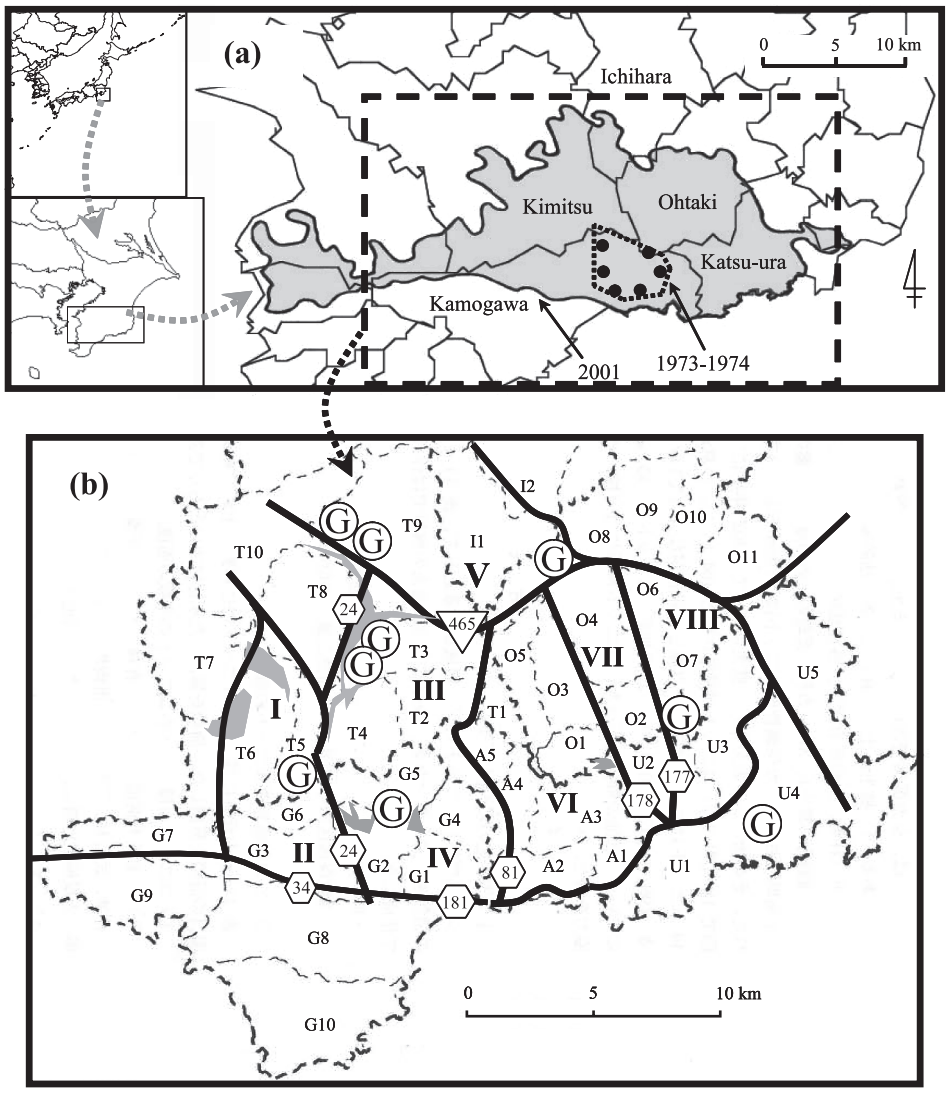

Fig. 1. (a) Changes in the distribution of sika deer, *Cervus nippon*, on the Boso Peninsula, central Japan. Dots show locations of observed field signs of sika deer (e.g. footprints, food remains, droppings, wallows) observed in 1973–1974 (Koganezawa et al. 1976). The distributions in 1973–1974 (dashed line) and in 2001 (shaded area) followed the study of the Chiba Prefecture and Deer Research Group on Boso (2004). (b) Map of the study area on the Boso Peninsula and schematics of boundaries of municipals (bold dashed line) and management units (dashed line), artificial structures such as main roads (solid line), dams or artificial lakes (shaded area), and golf courses (letter "G" in circle). Numbers in reverse-triangle and in hexagon indicate route (National motorway) and line (Prefecture mortorway), respectively. Eight areas enclosed by these structures (Block I–VIII) were determined prior to the analysis of spatial genetic structure. Management units were overlaid in this figure (see also Tables 1 and 3).

HD6, respectively. The nucleotide sequence of HDN is 5'-CATAATGTATCTTACGCACCGG-3'. Before the sequencing reaction, the PCR product was purified with a QIAquick PCR Purification Kit (Qiagen). The purified PCR product was directly sequenced with a BigDye-Terminator Cycle Sequencing Kit (ver. 3.1, Applied Biosystems), and analyzed on an automated DNA sequencer, model 3730 (Applied Biosystems).

Nucleotide and haplotype diversity

All sequences were edited and aligned by CodonCode Aligner (CodonCode Corp). Definition and alignment of tandem repeat units conformed to the methods of Nagata et al. (1999). Haplotype diversity (h) and nucleotide diversity (π) were calculated using DnaSP ver. 4.10.4 (Rozas et al. 2003), excluding the gap region and seventh tandem repeat unit which cause interference in the calculations of the indices (Table 2).

Table 1. Collecting sites, number of sika deers collected from January to October, 2005 (N), and haplotype frequency at each collecting site

Location		N	Haplotype**			
Municipal	Management Unit*		1a	1b	2a	2b
Kamogoawa	A2	51	38	0	11	2
	A3	9	8	0	1	0
	A4	13	12	0	1	0
	A5	5	4	0	1	0
	G1	13	2	0	10	1
	G2	24	17	0	7	0
	G3	18	6	0	12	0
	G4	1	0	0	1	0
	G7	4	3	0	1	0
Kimitsu	T1	5	4	0	1	0
	T2	5	4	0	1	0
	T3	19	14	0	5	0
	T4	4	3	0	1	0
	T5	4	3	0	1	0
	T6	6	6	0	0	0
	T8	6	3	0	3	0
	T9	5	5	0	0	0
Otaki	O2	13	13	0	0	0
	O3	11	8	0	3	0
	O4	7	6	1	0	0
	O5	2	2	0	0	0
	O6	4	4	0	0	0
	O7	12	12	0	0	0
Ichihara	I1	15	14	0	1	0
Katsu-ura	U3	3	2	0	1	0

* Names followed Chiba Prefecture and Deer Research Group on Boso (2004).
** Definition of each haplotype is shown in Table 2.

Table 2. Comparison of nucleotide sequences (variable positions are shown) and numbers of individuals sharing each haplotype (N) in the Boso population of *Cervus nippon*

Haplotype	N	Nucleotide position														
		1 9 7	2 6 3	2 9 2	3 0 2	3 0 7	3 5 9	–	3 9 7	4 8 1	7 5 9	8 1 0	8 2 4	8 5 7	9 5 8	9 7 1
1a	193	A	C	C	T	C		–		A	A	T	T	T	C	–
1b	1	G	·	·	·	·		–		·	·	C	·	C	·	·
2a	62	·	T	T	C	T		*		G	G	·	C	·	·	T
2b	3	·	T	T	C	T		*		G	G	·	C	·	–	T

Dots indicate nucleotide identity with haplotype 1a, and bars indicate lack of the tandem repeat unit (359–397 bp) or single nucleotide. Asterisks between 359 and 397 bp show the tandem repeat unit named d8 by Nagata et al. (1999).

Spatial genetic structure of the Boso population

For the purpose of assessing the spatial patterns of mtDNA haplotypes, we first conducted local interpolation using inverse distance weighting (IDW: Fortin and Dale 2005) in order to estimate the intensity of aggregation of haplotypes. A total of four haplotypes was found in consequence of DNA sequencing, but they consisted of two major haplotypes that differ in the number of tandem repeat unit (see details in Results). Therefore, the interpolation was performed for the major two haplotypes. The common form of the inverse distance weight is

$$\hat{z}(x_0) = \frac{\sum_{j=1}^{m} z(x_j) d_{ij}^{-p}}{\sum_{j=1}^{m} d_{ij}^{-p}}$$

where $\hat{z}(x_0)$ is an interpolated value of variable z for a given point x_0, $z(x_j)$ is the value at the sampled location j, m is the number of neighboring sampling locations based on some definition such as being within a search radius, p is a real value, and d_{ij} is the distance between unsampled location i and sampled location j. In the present study, the IDW interpolation was conducted with $p = 2$ and the number of sampled location for interpolation is 12.

To find an area in which the constitution of the major two haplotypes was changed large, local regression model (Simonoff 1996) was used for fitting data given by dummy variables (i.e., one major haplotype = 0, another haplotype = 1). We also estimated Ripley's *K*-function (Ripley 1981) that was estimated using a Spatstat package (Baddeley and Turner 2005) in statistical program R, version 2.5. The statistical significance of $K(r)$ values for given radii, r, was estimated by 95% confidence intervals generated by 1000 times Monte Carlo simulations of complete spatial randomness (CSR) which is implemented as a homogeneous Poisson process (Diggle 1983). Circle radii for the $K(r)$ analysis increased by 50-m increments up to the maximum distance of 40 km.

Chiba Prefecture Government divided 13 municipalities that were located in and around the distribution of deer into 71 units for the deer management (Chiba Prefecture and Deer Research Group on Boso 2004). We adopted those management units for determining population subdivision (Table 1, Fig. 1b). To assess whether or not staple artificial structures play an important role to restrict gene flow, we divided the population into eight blocks (Block I–VIII) enclosed by main roads, dams or lakes, and golf courses, covered with all sampling points (Fig. 1b, Table 3). Two management units, G7 and T8, which contained only four and six samples, respectively, were excluded from subsequent analysis because they were not included into any of the eight blocks. Based on these blocks, we examined the relationship between the existence of artificial structures enumerated above and spatial genetic structure of the sika population. For comparison of the amount of traffic on major roads in study area, mean number of cars that have passed through each road for several years was calculated (Chiba Prefectural Government 1989, 2000).

Wright's *F* statistics, in which the inbreeding coefficient in a subdivided population (F_T) can be decomposed into components due to nonrandom mating within local populations (F_{IS}) and due to population subdivision (F_{ST}), is frequently used (Wright 1921) for assessing the degree of population substructure and the level of gene flow among (sub)populations. We calculated θ (Weir and Cockerham 1984) as F_{ST} estimator, in which the numbers of samples and locations were entered explicitly, between different blocks described above. θ with unequal sample size was calculated by Arlequin (ver. 3.01, Excoffier et al. 2005).

Two exclusive approaches were used to assess the spatial population structure. First, we conducted Spatial Analysis of Molecular Variance (SAMOVA) which allows us to detect groups of (sub)populations that are genetically and geographically homogeneous using simulated annealing procedure (Dupanloup et al. 2002). In

Table 3. Eight blocks defined by artificial structures, management units containing in each block, the numbers of samples (N), haplotype frequency, and geographic coordinates of the center of the block

Block	Block composition (Management unit)*	N	Haplotype frequency				Geographic coordinates (longitude (E), latitude (N))
			1a	1b	2a	2b	
I	T5, T6	10	9	0	1	0	(140°01'57", 35°11'36")
II	G3, G6	18	6	0	12	0	(140°02'47", 35°08'21")
III	T2, T3, T4, G5	28	21	0	7	0	(140°06'12", 35°12'04")
IV	G1, G2, G4	38	19	0	18	1	(140°06'23", 35°08'42")
V	T9, I1	20	19	0	1	0	(140°06'59", 35°15'53")
VI	T1, O1, O3, O5, A2, A3, A4, A5	96	76	0	18	2	(140°09'50", 35°10'50")
VII	O2, O4, U2	20	19	1	0	0	(140°11'26", 35°13'22")
VIII	O6, O7, U3	19	18	0	1	0	(140°14'14", 35°12'14")

* Names of management unit follow Chiba Prefecture and Deer Research Group on Boso (2004).

hierarchical AMOVA, F_{CT} (proportion of genetic variation among groups of populations to the total variation) shows the extent of differentiation between groups (Excoffier et al. 1992). SAMOVA calculates the largest F_{CT} value corresponding to arbitrary number of groups (K) (Dupanloup et al. 2002). We ran SAMOVA program developed by Dupanloup et al. (2002) with 100-times simulated annealing process for various number of groups (K = 2 to 5) and compared simulated F_{CT} values for determining the best grouping of blocks. Another approach for assessing population structure was based on Monmonier's algorithm which is used to identify the discontinuity. Delaunay triangulation networks between blocks were constructed using geographic coordinates, and pairwise genetic distance was calculated between adjacent blocks. Monmonier's algorithm firstly selected the edge with the largest distance on the triangulation as the starting edge to find the discontinuity and then extended it across the adjacent edge associated with the largest distance. This procedure was continued until the barrier reached the limit of the triangulation or formed a loop by itself or met another barrier (Manni et al. 2004). Pairwise θ between blocks was used as genetic distance, and genetic discontinuities were detected using program BARRIER 2.2 (Manni et al. 2004). Both methods require geographic data, namely latitude and longitude, of blocks and sometimes result in somewhat similar results, while algorithms for finding discontinuities are different (Dupanloup et al. 2002). To determine geographic coordinates of each block, both latitude and longitude of north, south, east, and west extremity of the block were found on a 1:25000 scale map, and line connecting north and south extremities and line connecting east and west ones were each drawn. The point where north-south and east-west line intersect was defined as the center of the block in this study (Table 3).

Results

The mitochondrial *D-loop* region was successfully amplified from all samples, and nucleotide sequences were determined. The length of the compared region varied from 1051 to 1091 bp. Comparison of all the sequences obtained revealed a total of four haplotypes in the Boso population (Table 2). The sequences of these haplotypes have been deposited in EMBL/GenBank/DDBJ (Accession Nos. AB247654–AB247657). Transitions were found at 10 nucleotide sites and gaps at two sites. According to Nagata et al. (1999), the *D-loop* region of sika deer in Japan possesses tandem repeat units of 37 to 40 bp, and samples from Chiba contain either six or seven units. Our haplotypes 1a and 1b contained six units, whereas haplotypes 2a and 2b contained seven (Table 2). Comparison of our results with those of Nagata et al. (1999), in which three haplotypes from Chiba Prefecture, Ama1, Kmo1, and Kmo2, were described, revealed that haplotype Ama1 was identical to haplotype 1a, but we did not find haplotype Kmo1. It was unclear whether haplotype Kmo2 corresponded to either haplotype 2a or 2b, because the partial sequences consisting of 169 bp in the left domain of the control region near tRNA-Phe were not recorded by Nagata et al. (1999). Haplotype 1b was newly observed in our study. Haplotype 1a was mostly dominant throughout the Boso population (74.5% of the total individuals) (Table 1). The percentage nucleotide difference was 0.29% between haplotypes 1a and 1b (3/1050 bp), 0.67% between 1a and 2a/2b (7/1050 bp), and 0.95% between 1b and 2a/2b (10/1050 bp). The haplotype diversity (h) and nucleotide diversity (π) were 0.38 and 0.0025, respectively.

The distribution of each haplotype demonstrated that the constitution of the two dominant haplotypes, 1a and 2a, varied among subpopulations: haplotype 1a was widely observed throughout the localities, whereas the majority of haplotype 2a was localized in the south and southwest (Fig. 2). Haplotype 1b was observed only in a management unit "O4", where the sika deer population had not been confirmed to be distributed in the 1970s, and haplotype 2b was found only in "A2" and "G1" (Table 1). The null hypothesis of complete spatial randomness (CSR) was evaluated for the two major groups of haplotypes consisting of haplotype 1 (haplotype 1a + 1b, see Fig. 3) and haplotype 2 (haplotype 2a + 2b) using the Ripley's K-function. CSR was statistically rejected in the both groups ($P < 0.05$). Interpolation by local regression method revealed that major two haplotype constitution (haplotype 1 and 2 described above) changed largely in an area surrounding motorway Line 81 (indicated by bold arrow in Fig. 3) on which a boundary of block IV and VI exists (Figs. 1b and 3).

Analysis of molecular variance (AMOVA, Excoffier et al. 1992) demonstrated that the global F_{ST} value over all blocks (θ = 0.184) was significantly greater than zero ($P < 0.0001$, based on 10 000 permutations), implying that distinctive heterogeneity of the haplotype distributions (i.e. population structure) exists among blocks.

SAMOVA demonstrated that F_{CT} value was larger for

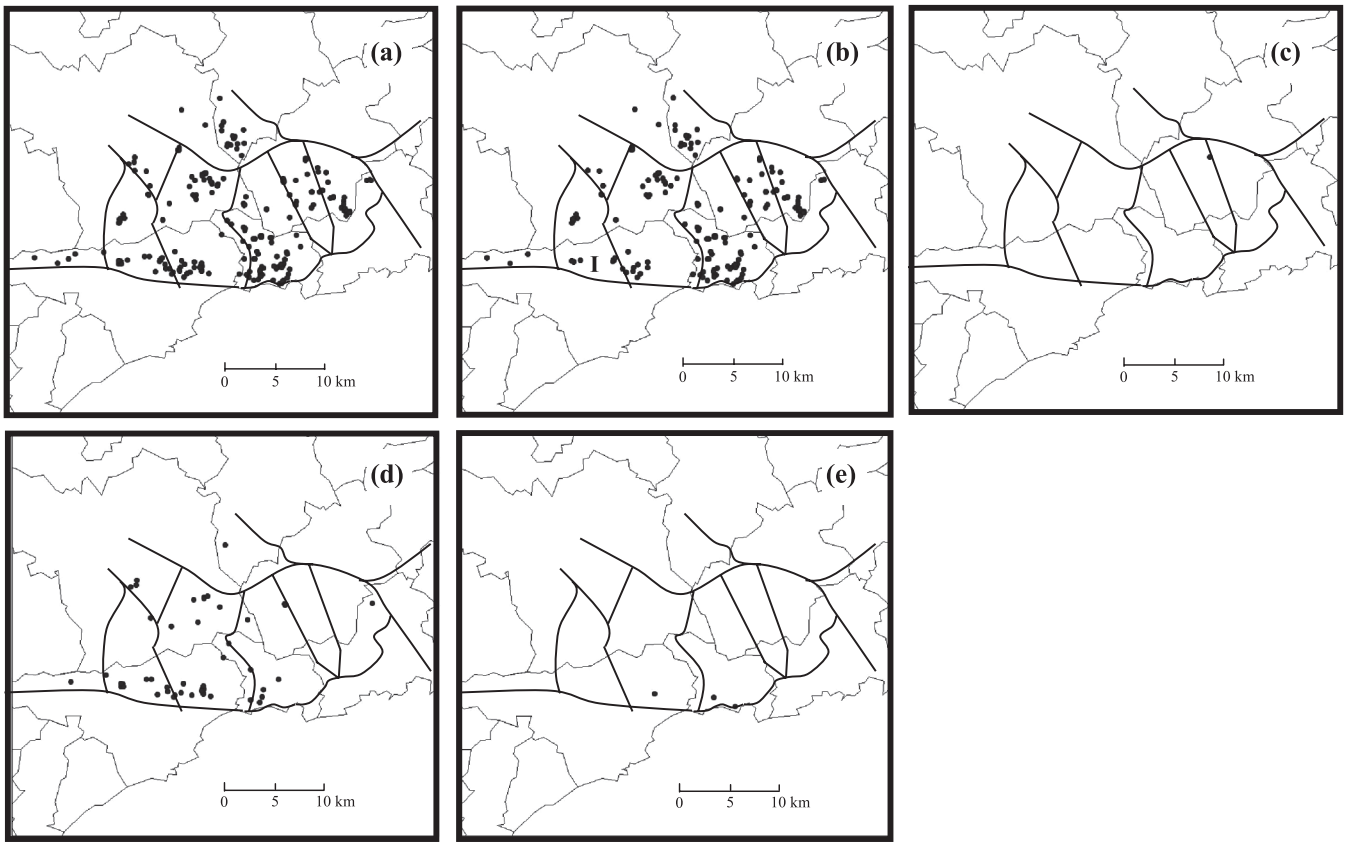

Fig. 2. Distributions of haplotypes on the Boso Peninsula. (a) All pooled haplotypes; (b) haplotype 1a; (c) haplotype 1b; (d) haplotype 2a; (e) haplotype 2b. Dots indicate sampling points where individuals were collected. Some dots overlap because many deer were collected at the same geographic points. Solid lines show the network of main roads at study area (see text for details).

smaller number of groups (K) and that it was the highest when all samples were divided into two groups, block II and the others (F_{CT} = 0.320, Table 4). A significant F_{CT} was also calculated when blocks II and IV were separeted from the others (K = 3, F_{CT} = 0.299, Table 4). This can be interpreted that based on a locus on mtDNA, subdivided populations existing in southwestern part of the whole distribution were genetically distinct to the others in the Boso population. Genetic discontinuities among blocks connected by Delaunay triangulation were delineated using Monmonier's algorithm (Fig. 4), a result showed similar tendency to the consequence obtained by SAMOVA: a main discontinuity was recognized between a pair of block II and IV and the others, and third discontinuity was drawn between block II and IV, while the model of three groups was marginally significant (Table 4). Note that results of these methods were not completely congruous: the second discontinuity obtained by latter analytical method did not correspond with the result of SAMOVA. Such an incongruity would be attributed to the difference in searching algorithm for genetically heterogeneous groups (see Dupanloup et al. 2002 for detailed explanation). In conclusion, these results support the view that some of artificial structures considered in the present study have tended to constrict movement of sika deer and also suggest that primary factors that restrain migration exist between block II, IV and the others.

Discussion

Nucleotide and haplotype diversity

One of the main purposes we intended to analyze was to show the level of genetic variation in the mitochondrial DNA in the Boso population. The percentage difference in nucleotide sequences between haplotypes in the *D-loop* region varied from 0.29% to 0.95%. This gives 0.13–0.43 Myr have passed since haplotypes have diverged, based on the knowledge that a substitution rate in the mitochondrial *D-loop* is 1.11–1.13% per Myr in Cervinae (Randi et al. 2001). Two diversity indices, haplotype diversity (h = 0.38) and nucleotide diversity

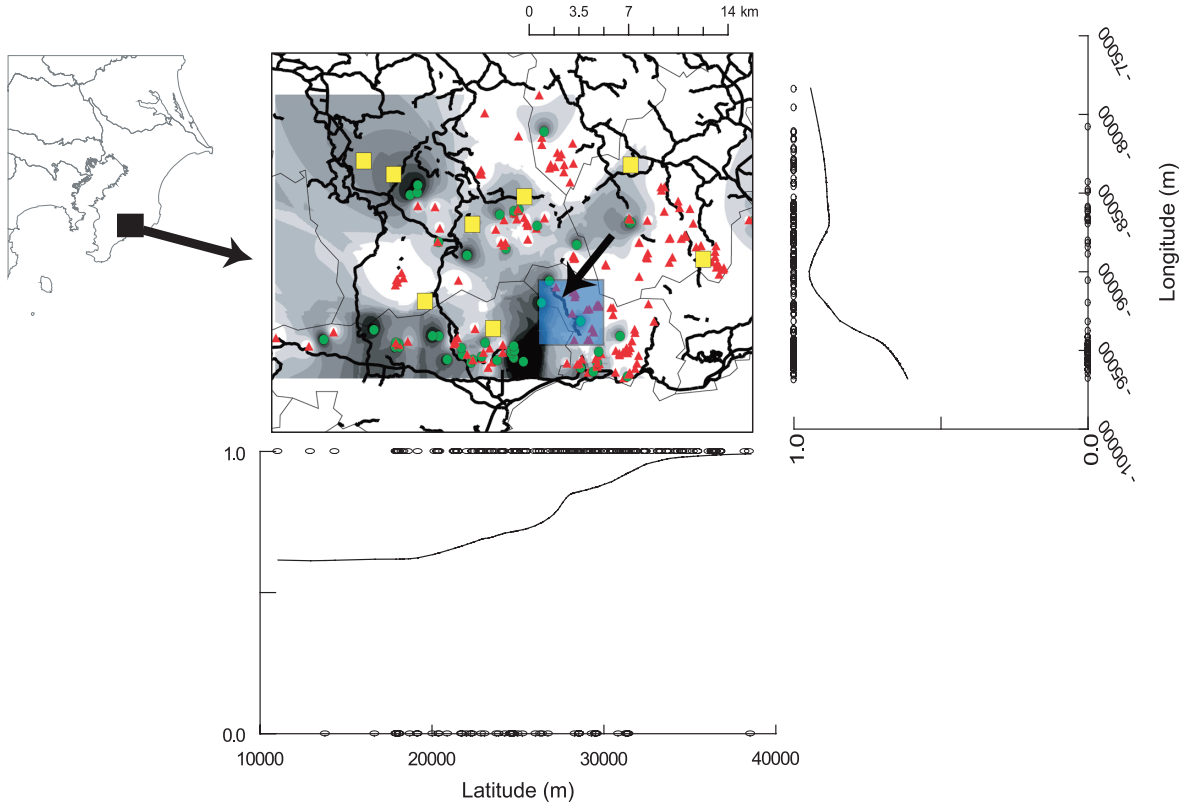

Fig. 3. Distribution of pooled haplotypes (1a + 1b: green circles; 2a + 2b, red triangles). Boundaries between cities (solid black lines), roads that are larger than 5 m width (bold black lines), golf course (yellow squares) are overlaid, respectively. Large bold arrow indicates Line 81 (see Materials and methods and Discussion for details). Local interpolation based on inverse distance weighting (IDW, see Materials and Methods for details) is shown in the area of study (probability of existence of haplotype 2a + 2b is shown by black contours). Bottom and right figures indicate scatterplots of the distribution of pooled haplotypes represented by dummy scores (the score of haplotype 1a and 1b is set as "1" and that of haplotype 2a and 2b as "0"). The unit of latitude and longitude represents projected coordinate (distance unit: meters) from center (i.e. projected distance from the location of N 36°0'0" E 139°50'0"). These plots were interpolated by local regression model (see Materials and methods). The regression lines are overlaid on the scatterplots and the area where a large increment of regression coefficients was observed is shown by blue square.

(π = 0.0025) of the Boso population were included in the range of both diversities in other neighboring population of southern Kanto-Izu district, Honshu (0.063–0.533 in haplotype diversity, 0.0001–0.0063 in nucleotide diversity, see Yuasa et al. 2007). Grant and Bowen (1998) proposed to infer the population history using these diversity indices in marine fishes. According to Grant and Bowen (1998) criteria, populations with both low haplotype diversity ($h < 0.5$) and nucleotide diversity ($\pi < 0.5$) can be interpreted that they recently experienced severe bottlenecks. Since many Japanese populations of sika deer including the Boso one have experienced recovery from severe bottleneck, our estimations could be interpreted as a consequence of such a history. This inference is not discrepant to previous reports on the Boso population (Koganezawa et al. 1976; Chiba Prefecture and Deer Research Group on Boso 2004).

Possible effects of artificial structures and other factors on population genetic structure

While only four haplotypes were detected and the distribution area of sika deer is not very wide, marked heterogeneity of haplotype distribution was detected in the Boso population. In particular, frequency of haplotype 2 (2a + 2b) was relatively high in the southern and the southwestern areas of the whole distribution, whereas haplotype 1 (1a + 1b) was found over the entire area (Figs. 2, 3). From the knowledge of substitution rate in mitochondrial *D-loop* region (Randi et al. 2001), the possibility that haplotype 2 occurred in the last two or three decades and has not still spread over the area is not promising. Therefore, based on the current data, it is most likely that both major haplotypes existed in the last bottleneck event and the distribution patterns of these haplotypes have gradually varied since the population expansion has started. The mechanism inducing

Table 4. Fixation indices corrsponding to the different number of groups (*K*) of blocks inferred by SAMOVA for the sika deer population on the Boso Peninsula

Number of groups (*K*)	Group composition	F_{SC}	F_{ST}	F_{CT}
Two groups	1. Blocks I + III + IV +V +VI +VII + VIII	0.119***	0.401***	0.320***
	2. Block II			
Three groups	1. Blocks I + III +V +VI + VII + VIII	0.021***	0.314***	0.299*
	2. Block II			
	3. Block IV			

*P<0.05, **P<0.01, ***P<0.001 (based on 1000 permutations).

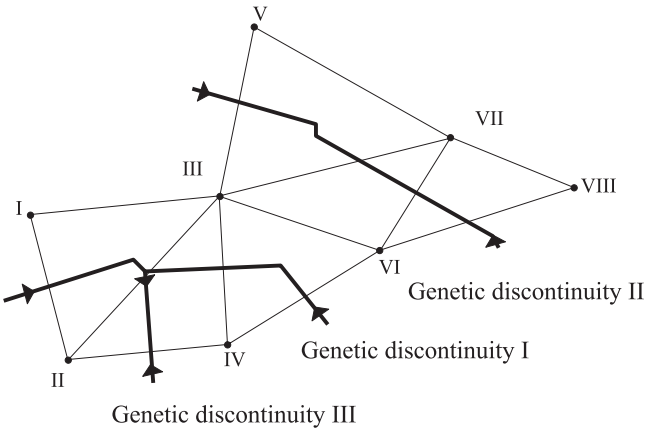

Fig. 4. Delaunay triangulation (solid lines) based on geographic coordinates of the center of the blocks (plots) and three main barriers (bold lines) deduced by pairwise θ between blocks based on Monmonier's algorithm.

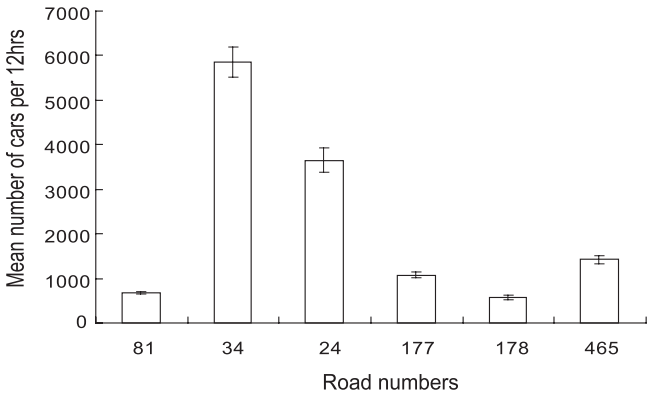

Fig. 5. Mean number of cars that have passed through each road per half a day (12 h: 7:00–19:00) with ±1 standard errors (*SE*) (data from reports of Chiba Prefectural Government 1989, 2000). Data are based on the survey for 35 years (20 years for Load 178 and 465).

heterogeneity of the frequency constitution of haplotypes among subpopulations is a very intriguing issue.

Since artificial structures including roads and dams are known to hamper animal movement across habitats and alter landscape and spatial patterns, the network of these structures is considered to be important to know the patterns of spatial genetic structure of wildlife. In the Boso Peninsula, the motorway Line 34 seems to act as an effective barrier against distribution expansion to the southward (see Fig. 1). Indeed, the average number of cars that have passed through the Line 34 was extremely large among main roads in the study area (Fig. 5). In this study, eight blocks surrounded by roads, dams including artificial lakes, and golf courses, were treated units to be analyzed to assess the effect of artificial structures on gene flow (Table 3, Fig. 1b). AMOVA and spatial analyses detected distinctive genetic structures of sika deer population on the Boso Peninsula. In particular, spatial analyses detected the greatest heterogeneity in the constitution of haplotype frequencies between a pair of block II and IV and the others (Table 4, Fig. 4). Moreover, interpolation and local regression approach revealed the area in which haplotype constitution was conspicuously changed existed in an area surrounding Line 81 (Fig. 3). These concordant results suggest that the Line 81 is a potential candidate that has left sika deer in the block II and IV apart from other area. Road width and vehicle traffic levels could be major determinants of the barrier effect (Forman and Alexander 1998), but width of the Line 81 is relatively narrow (i.e., the minimum width of road is shortest among compared roads) and traffic is generally not so heavy compared with other roads (Fig. 5). A reasonable explanation for the discontinuity is that the Line 81 is running through a steep-walled ravine that may practically function as a geographic barrier. It is worth studying whether or not a similar genetic discontinuity is also found in the area in other animal species.

Our results also suggest that existence of golf courses on management units G2 and T5 has promoted heteroge-

neity of spatial distribution of mtDNA. The construction of these two golf courses has been started in early 1970s, approximately corresponding to the beginning of range expansion of deer population on the Boso Peninsula (see Fig. 1). Another important factor that could affect dispersal patterns in animals is the abundance of resources. When resources are abundant, consumers tend to disperse shorter distances, whereas the dispersal distance would be longer when local resource availability is low (Kareiva and Odell 1987; Dwyer and Morris 2006). These forecast that deer that have migrated from an area in which food resources are relatively poor would stay longer in areas where the food resources are relatively abundant, leading to heterogeneous dispersal. These resource-dependent movements could consequently affect estimations of migration rate between local habitats. Indeed, in the southern parts of the blocks II and IV, there exists a wide area of agricultural fields, which is known to provide high-quality food to deer in this region (Takada et al. 2002; Miyashita et al. 2007). Another detailed study on the distribution of food resource is of course necessary, but the evidence of the agricultural fields supports the theoretical prediction that individuals tend to stay in area where food resource is constantly supplied and as a result, heterogeneous spatial structure of haplotypes arises due to the promotion of local dispersal. Inversely, genetic differentiation will not be facilitated in such area as the amount of food resource is poor, which would induce a high frequency of migration. Not only artificial structures but food resource in block II and IV may have been enhanced the difference in genetic constitution in these from other area.

History of population shrinkage in the studied area cannot also be ignored. It is very likely that present genetic structure is partly derived from the pattern of refugia during 1970s. In fact, previous study on a relict sika population did not take account of the number of refugia nor genetic structure (Koganezawa et al. 1976). The mitochondria are maternally inherited except some evidence (e.g. Ujvari et al. 2007) and therefore, the present distributions of haplotypes can be viewed as a pattern of past dispersal in females. For the detailed study of inferring population structure during bottleneck event, coalescent simulation studies using information of nuclear markers such as microsatellites are inevitable. It is important to understand what the extent of various factors listed above have affected the formation of population genetic structure of sika deer in the Boso peninsula in the subsequent studies.

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References

Aars, J. and Ims, A. R. 1999. The effect of habitat corridors on rates of transfer and interbreeding between vole demes. Ecology 80: 1648–1655.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. and Saunders, C. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489–522.

Baddeley, A. and Turner, R. 2005. Spatstat: an R package for analyzing spatial point patterns. Journal of Statistical Software 12: 1–42.

Ceballos, G. and Ehrlich, P. R. 2002. Mammal population losses and the extinction crisis. Science 296: 904–907.

Chiba Prefecture and Deer Research Group on Boso 2004. Integrated Science Reports on the Management of Sika Deer on Boso Peninsula, Chiba Prefecture. Chiba Prefecture, Chiba, 134 pp. (in Japanese).

Chiba Prefectural Government 1989. Report of Road Traffic Census. Chiba Prefecture, Chiba (in Japanese).

Chiba Prefectural Government 2000. A New Report of Road Traffic Census. Chiba Prefecture, Chiba, 1692 pp. (in Japanese).

Diggle, P. J. 1983. Statistical Analysis of Spatial Point Patterns. Academic Press, New York, 288 pp.

Dupanloup, I., Schneider, S. and Excoffier, L. 2002. A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11: 2571–2581.

Dwyer, G. and Morris, W. F. 2006. Resource-dependent dispersal and the speed of biological invasions. American Naturalist 167: 165–176.

Excoffier, L., Smouse, P. and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.

Forman, R. T. and Alexander, L. E. 1998. Roads and their major ecological effects. Annual Review of Ecology and Systematics 29: 207–231.

Fortin, M-J. and Dale, M. 2005. Spatial Analysis: A Guide for Ecologist. Cambridge University Press, Cambridge, 365 pp.

Grant, W. S. and Bowen, B. W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insighs from sardines and anchoviesand lessons for concervation. The Journal of

Heredity 89: 415–426.

Hansen, M. M. and Loeschcke, V. 1996. Genetic differentiation among Danish brown trout populations, as detected by RFLP analysis of PCR amplified mitochondrial DNA segments. Journal of Fish Biology 48: 422–436.

Kareiva, P. M. and Odell, G. 1987. Swarms of predators exhibit "prey taxis" if individual predators use area-restricted search. American Naturalist 130: 233–270.

Koganezawa, M., Katai, N. and Maruyama, N. 1976. Distribution of sika (*Cervus nippon centralis* Kishida) in the eastern part of the Boso Mountains, Chiba Prefecture. The Nihonzaru 2: 115–121 (in Japanese).

Manel, S., Schwartz, M. K., Luikart, G. and Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18: 189–197.

Manni, F., Guérard, E. and Heyer, E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Human Biology 76: 173–190.

Mech, S. G. and Hallet, J. G. 2001. Evaluating the effectiveness of corridors: a genetic approach. Conservation Biology 15: 467–474.

Miyashita, T., Suzuki, M., Takada, M., Fujita, G., Ochiai, K. and Asada, M. 2007. Landscape structure affects food quality of sika deer (*Cervus nippon*) evidenced by fecal nitrogen levels. Population Ecology 49: 185–190.

Nabata, D., Masuda, R., Takahashi, O. and Nagata, J. 2004. Bottleneck effects on sika deer *Cervus nippon* population in Hokkaido, revealed by ancient DNA analysis. Zoological Science 21: 473–481.

Nabata, D., Kaji, K., Nagata, J. and Masuda, R. 2007. Genetic structure changes of expanding sika deer (*Cervus nippon*) populations in central and western Hokkaido, revealed by mitochondrial DNA analysis. Mammal Study 32: 17–22.

Nagata, J., Masuda, R., Kaji, K., Kaneko, M. and Yoshida, M. C. 1998. Genetic variation and population structure of the Japanese sika deer (*Cervus nippon*) in Hokkaido Island, based on mitochondrial D-loop sequences. Molecular Ecology 7: 871–877.

Nagata, J., Masuda, R., Tamate, H. B., Hamasaki, S., Ochiai, K., Asada, M., Tatsuzawa, S., Suda, K., Tado, H. and Yoshida, M. C. 1999. Two genetically distinct lineages of sika deer, *Cervus nippon*, in Japanese Islands: comparison of mitochondrial D-loop region sequences. Molecular Phylogenetics and Evolution 13: 511–519.

Randi, E., Mucci, N., Claro-Hergueta, F., Bonnet, A. and Douzery, E. J. P. 2001. A mitochondrial DNA control region phylogeny of the Cervinae: speciation in *Cervus* and implications for conservation. Animal Conservation 4: 1–11.

Riley, S. P. D., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K. and Wayne, R. K. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Molecular Ecology 15: 1733–1741.

Ripley, B. D. 1981. Spatial Statistics. John Wiley and Sons Inc, 272 pp.

Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X. and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497.

Simonoff, J. S. 1996. Smoothing Methods in Statistics. Springer, New York, 356 pp.

Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16: 393–430.

Slatkin, M. 1994. Gene flow and population structure. In (L. A. Real, ed.) Ecological Genetics, pp. 3–17. Princeton University Press, Princeton.

Takada, M., Asada, M. and Miyashita, T. 2002. Cross-habitat foraging by sika deer influences plant community structure in a forest-grassland landscape. Oecologia 133: 389–394.

Ujvari, B., Dowton, M. and Madsen, T. 2007. Mitochondrial DNA recombination in a free-ranging Australian lizard. Biology Letters 3: 189–192.

Wang, M. and Schreiber, A. 2001. The impact of habitat fragmentation and social structure on the population genetics of roe deer (*Capreolus capreolus* L.) in Central Europe. Heredity 86: 703–715.

Weir, B. S. and Cockerham, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wright, S. 1921. Systems of mating. Genetics 6: 111–178.

Yamada, M., Hosoi, E., Tamate, H. B., Nagata, L., Tatsuzawa, S., Tado, H. and Ozawa, S. 2006. Distribution of two distinct lineages of sika deer (*Cervus nippon*) on Shikoku Island revealed by mitochondrial DNA analysis. Mammal Study 31: 23–28.

Yamamoto, S., Morita, K., Koizumi, I. and Maekawa, K. 2004. Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. Conservation Genetics 5: 529–538.

Young, A., Boyle, T. and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11: 413–418.

Yuasa, T., Nagata, J., Hamasaki, S., Tsuruga, H. and Furubayashi, K. 2007. The impact of habitat fragmentation on genetic structure of the Japanese sika deer (*Cervus nippon*) in southern Kantoh, revealed by mitochondrial *D-loop* sequences. Ecological Research 22: 97–106.

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