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Development of paternally-inherited Y chromosome simple sequence repeats of sika deer and their application in genetic structure, artificial introduction, and interspecific hybridization analyses.

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Abstract (185/150-250 words)

The effect of sex-biased dispersal in mammalian ecology and evolution can be elucidated by focusing on maternally or paternally inherited DNA polymorphisms. In sika deer, the genetic structure of the maternal lineage has been clarified by studies based on mitochondrial DNA (mtDNA) variations. However, the genetic structure of the paternal lineage has not been well analyzed due to the limited number of point mutations in Y chromosome sequences. In this study, we focused on mutations of highly polymorphic simple sequence repeats (SSR) in the Y chromosome and developed 16 Y chromosome

SSR markers to evaluate male-biased dispersal in sika deer. In total, 55 alleles and 31 multi-locus haplotypes were detected from these 16 loci, revealing clear genetic differentiation among populations (F'_{ST} = 0.783). In particular, the native individuals in Tanegashima and Yakushima Islands, and introduced exotic individuals from Taiwan showed unique alleles. These markers are highly useful for evaluating not only historical male-mediated dispersal, genetic structure and demography of the native populations in Japan, but also the impact of artificial introductions on hybridization, especially the introgression of alleles from escaped farmed individuals to native populations.

Keywords

Cervidae, genetic structure, microsatellite DNA, red deer, short tandem repeat.

Introduction

The sika deer (*Cervus nippon*) is a large mammal with a widespread distribution in East Asia. Although sika deer is considered an iconic species in its native distributional range, its taxonomy and genetic phylogeny are still unclear, especially in Japan. Ohtaishi (1986) classified sika deer into 14 subspecies based on their morphological characteristics, six of which are found in Japan: *Cervus nippon yesoensis* (Hokkaido Island), *C. n. centralis* (Honshu Island and Tsushima Island), *C. n. nippon* (Kyushu, Shikoku and Goto Islands), *C. n. mageshimae* (Mageshima and Tanegashima Islands), *C. n. yakushimae* (Yakushima and Kuchinoerabu Islands) and *C. n. keramae* (Ryukyu Islands). However, examination of mitochondrial DNA (mtDNA) variation has suggested the presence of two genetic groups that do not correspond to the six Japanese subspecies (Tamate et al. 1998; Nagata et al. 1999; Yamada et al. 2006; Nagata 2009; Takiguchi et al. 2012; Liu et al.

2021). The two genetic groups, called the northern and southern mtDNA groups, have their boundary on western Honshu Island and Shikoku Island, and morphological subspecies do not correspond exclusively to each cluster (Fig. 1, Tamate et al. 1998; Nagata et al. 1999; Yamada et al. 2006; Nagata 2009; Takiguchi et al. 2012; Liu et al. 2021). In addition to the discrepancy between morphological classification and mtDNA phylogeny, nuclear DNA analysis also suggests different genetic groups. A lack of spatial genetic structure in the populations of two large islands, Honshu Island and Kyushu Island, and two genetic subgroups on Hokkaido Island and the Ohsumi Islands (Tanegashima and Yakushima Islands) were proposed following a population genetic analysis using nuclear simple sequence repeat (SSR) markers (Goodman et al. 2001; Tamate 2009). This taxonomic confusion and lack of consensus surrounding the genetic structure of the species, makes it difficult to propose conservation units and provide recommendations for genetic management of sika deer in Japan. Although the genetic structure of sika deer in Japan is understudied, recent artificial introductions are known to have disturbed the original structure (Yuasa et al. 2007; Terada et al. 2013; Yamazaki 2018; Eva & Yamazaki 2018; 2019; Matsumoto et al. 2015, 2019; Takagi et al. 2020). For example, the introduced crossbreeds among red deer (*C. elaphus*), Formosan sika deer (*C. n. taiouanus*), and Formosan sambar (*C. unicolor swinhoei*) escaped from captivity and putative F1 hybrids between these exotic deer and native sika deer have been reported from Tomogashima Island, Wakayama Prefecture (Matsumoto et al. 2015, 2019; Takagi et al. 2020). Artificial transfers between different mtDNA groups of Japanese sika deer have also been reported. The two mtDNA haplotypes of the southern group have been observed in Toyama Prefecture, which is the distribution area of the northern mtDNA group (Yamazaki 2018). These southern mtDNA haplotypes are likely to have originated

97 from artificial introductions. Similarly, non-native mtDNA haplotypes have been widely
98 detected across Japan (Nagata et al. unpublished data). These reports suggest that
99 Japanese sika deer are at risk of on-going genetic disturbance through hybridization and
100 introgression with introduced deer. In particular, due to the expansion of sika deer
101 populations in many areas of Japan (Ministry of the Environment 2016), the alleles of
102 non-native deer may be rapidly spreading via genetic surfing (Peischl et al. 2016) and/or
103 genetic drift.

104 In the previous studies, nuclear SSR markers and mtDNA sequences have been
105 used for phylogeographic analysis and detection of introgression from non-native
106 lineages. In contrast to nuclear SSR markers and mtDNA sequences, analysis of Y
107 chromosome variation has the potential to provide insight into the diversity, dispersal,
108 and structure of male lineages. The Y chromosome is paternally inherited without
109 recombination, similar to the maternally inherited mtDNA. Because of this characteristic,
110 Y chromosome markers can describe the genetic structure generated by male-biased
111 dispersal. Recently, Y chromosome-derived markers have been used in several animal
112 species, such as chimpanzee (Hughes et al. 2005), Felidae species (Luo et al. 2007),
113 Japanese macaque (Kawamoto et al. 2008), and brown bears (Hirata et al. 2017). In
114 addition, Y chromosome markers have also been used to evaluate hybridization and the
115 direction of introgression among species, including between wild and domestic species,
116 such as between wild wolves (*Canis lupus*) and domestic dogs in Italy (Iacolina et al.
117 2010) and wild boars (*Sus scrofa*) and domestic pigs in Europe (Iacolina et al. 2016).
118 Despite their potential applications, Y chromosome genetic markers have rarely been used
119 in studies of sika deer. As an exception, Tanaka et al. (2020) sequenced 16 kb of the Y
120 chromosome of sika deer in Japan and identified 10 different haplotypes based on 9

121 substitution sites. However, only one of the ten haplotypes was common and widely
122 distributed geographically, suggesting a limitation for genetic structure analysis. Low
123 levels of substitution variation in the Y chromosome have been observed not only in sika
124 deer but also in five other mammals: lynx, wolf, reindeer, cattle, and field vole (Hellborg
125 & Ellegren 2004), suggesting it might be a common phenomenon in mammal species.
126 Despite this, it is possible that Y chromosome SSR (YSSR) loci may show a
127 higher degree of variation. The average mutation rate of 17 SSR loci in the Y chromosome
128 of humans was estimated as 1.998×10^{-3} per generation per locus (95% CI, 1.501×10^{-3}
129 to 2.606×10^{-3}), a rate similar to autosomal SSR loci (Gusmão et al. 2005). Therefore,
130 YSSR markers in sika deer may facilitate the evaluation of the genetic structure of
131 paternal lineages in more detail. The aims of this study were: 1) to develop YSSR markers
132 for sika deer in Japan; and 2) to evaluate their utility for genetic structure analysis and
133 detection of genetic disturbance due to hybridization and/or introgression.
134
135 **Materials & Methods**
136 The genomic sequences of the red deer (*C. elaphus*), including the sequence
137 information of the Y chromosome (4,026,935 bp) in the genome assembly CerEla1.0
138 (Bana et al. 2018) were obtained from NCBI, GeneBank (Accession No,
139 MKHE01000035). The isolation of the SSR region in the red deer genome sequence and
140 design of PCR primers were performed by Krait (Du et al. 2018). The minimum number
141 of repeats required for the SSR region was 10 and 8, for di- and tri-repeats, respectively.
142 The length of the flanking sequence at both ends was 100 bp. The design parameters for
143 the primers were 18 bp to 27 bp in length with 20 bp being optimal, annealing temperature
144 was 54°C to 65°C with 57°C being optimal, GC content was 30% to 80%, and the PCR

product size was 100 to 300 bp.

In the primary primer screening, DNA samples of 16 sika deer (15 males and 1 female), selected based on sampling location and sex, were used for amplification confirmation. A secondary screening, to assess the amplification stability and diversity of the amplified loci, was performed using DNA from 107 individuals (93 males and 14 females) from nine populations across Japan (Fig. 1). All samples were collected according to the guidelines of the Mammalogical Society of Japan. Sex determination of sika deer DNA samples followed the method of Yamauchi et al. (2000) using the amelogenin gene on the X and Y chromosomes.

The secondary primer screening was performed to evaluate whether the newly developed markers were appropriate for the following two purposes: 1) comparative analyses between Y chromosome and mtDNA genetic structure, i.e., detecting sex-biased genetic structure; and 2) distinguishing between non-native and native deer species. Four populations from the northern group (Hokkaido, Miyagi, Nara, and Hyogo) and four populations from the southern group were selected (Shimane, Miyazaki, Tanegashima Island, and Yakushima Island) (Tamate et al. 1998; Nagata et al. 1999; Yamada et al. 2006; Nagata 2009; Takiguchi et al. 2012; Liu et al. 2021; Fig. 1). To confirm the genotype of exotic deer, samples of exotic deer artificially introduced from Taiwan to Tomogashima Island, in Wakayama Prefecture, Japan were used (Fig. 1, Matsumoto et al. 2015, 2019, Takagi et al. 2020).

The PCR conditions for both primary and secondary screening were performed in 5μl reactions using the QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany) and a protocol for fluorescent dye-labeled primer Tail D (Blacket et al. 2012). Reactions contained 2.5 μl of Multiplex PCR Master Mix, 0.01 μM forward primer, 0.5 μM reverse

primer, and 0.5 μM fluorescently labeled primer. Reactions were then amplified using the following settings: 95 °C for 15 min; 32 cycles of 94 °C for 30 s, 57 °C for 1.5 min and 72 °C for 1 min; and a final extension at 60 °C for 30 min. Product sizes were determined using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, California, USA). For each locus, the number of alleles (*N*a) and gene diversity (*h*) were calculated using GenAlEx 6.5 (hereafter, GenAlEx, Peakall and Smouse, 2006, 2012). Multi-locus haplotypes were determined, then the number of haplotypes (N_{hap}) and the haplotype diversity (*H*) were calculated for each population using GenAlEx. The genetic relationships among multi-locus haplotypes were evaluated by generating a Neighbor-joining (NJ) tree based on the D_A genetic distances (Nei et al. 1983), using Populations 1.2.30beta software (Langella 2007). The levels of genetic differentiation among populations were evaluated by F'_{ST} (Meirmans and Herdrick 2011). Overall and pairwise F'_{ST} values were calculated and their significance was tested by 999 permutations. Moreover, a principal coordinates analysis (PCoA) was conducted based on pairwise F'_{ST} values to evaluate genetic relationships among populations. These genetic structure analyses were conducted using GenAlEx.

Results

In the primary and secondary primer screenings, clear peak patterns of PCR fragments (alleles) were obtained for all 16 loci (Table 1). Fifteen loci contained di-tandem repeats and one locus contained a tri-tandem repeat. None of the loci amplified in female samples. The alleles of 10 loci were similar with just 3 bp differences between the reference red deer sequence (Bana et al. 2018) and the sika deer samples (Table 2). The other six loci had clear differences in their allele sizes with at least 4 bp between sika and

193 red deer. Of particular note was locus AY32, which showed the largest difference with 24
194 bp between the two species (Table 2). A total of 55 alleles were detected across the 16
195 loci. Allelic polymorphism was observed in 14 loci, while two loci, AY04 and AY19, were
196 monomorphic. Moreover, the exotic Tomogashima samples showed unique alleles in nine
197 out of the 16 loci. Of the eight native populations, the Ohsumi Islands (Tanegashima and
198 Yakushima Islands) samples showed unique alleles in four of the 16 loci. The number of
199 alleles (*N*a) ranged from 1 to 7 with an average of 3.4, and the haplotype diversity (*h*)
200 ranged from 0 to 0.685 with an average 0.28. (Table 2).

201 A total of 31 multi-locus haplotypes (Y01-31) were identified in 93 individuals
202 based on the alleles at 14 polymorphic loci (Table 3). All multi-locus haplotypes were
203 unique to each population (Table 3) and the fixation indices (F_{ST}) among populations
204 showed complete differentiation. The number of haplotypes (N_{hap}) in each population
205 ranged from 1 to 11 and the haplotype diversity (*H*) ranged from 0 to 0.964 (Table 3).
206 When genetic differentiation and structure were evaluated across the 14 loci, the overall
207 F'_{ST} value was 0.783 and the pairwise F'_{ST} values ranged from 0.258 to 1.047 (Table 4).
208 The exotic Tomogashima population showed a clear genetic difference from the eight
209 native populations, indicated by F'_{ST} values of more than 0.91 (Table 4, Fig. 2). Among
210 the eight native populations, the Ohsumi Islands showed a relatively large degree of
211 differentiation from the other six populations (Table 4). The NJ-tree showed clear genetic
212 differentiation of the Y31 haplotype that was detected on Tomogashima Island (Fig. 3).
213 Although the four haplotypes from the Ohsumi Islands were genetically closely related,
214 there was no clear phylogeographic relationship for the other haplotypes (Fig. 3).
215

216 **Discussion**

In this study, 16 YSSR markers for sika deer were developed using red deer genomic information. Frank et al. (2020) developed four YSSRs for European red deer (*C. elaphus*), but these loci did not amplify well in sika deer from Japan (Takagi et al. unpublish data). In contrast, the markers developed in this study amplified well in sika deer and will be useful for various analyses focusing on paternal lineages in sika deer (*C. nippon*), red deer (*C. elaphus*), and related species. Recent genome wide phylogenetic analyses have shown that three species in the genus *Cervus*, sika deer (*C. nippon*), red deer (*C. elaphus*), and elk (*C. canadensis*), are part of a monophyletic clade (Hu et al. 2019). Therefore, some of the present markers may be useful for many subspecies of these three species, such as was the case for the Tomogashima Island samples in this study. Sika deer have been introduced into many countries around the world, causing introgression with native deer populations, which is not only a conservation problem, but also has a significant impact on the deer farming industry (Swanson and Putman 2009). Thus, the present YSSR markers provide a new cost-effective tool for genetic management through hybrid analysis among various *Cervus* species.

The present results (Fig. 2, 3. Table 4) showed the clear genetic differentiation of Tanegashima and Yakushima Island populations from the other Japanese populations, a pattern similar to previous studies based on mtDNA (Nagata et al. 1999; Yamada et al. 2007) and nuclear SSR analysis (Goodman et al. 2001; Terada & Saitoh 2018). These two populations are separated from the other Japanese populations by Ohsumi Strait, which formed during the interglacial period of the late Pleistocene (100,000 years ago, Ohshima 1990). Thus, genetic divergence between the two groups (Ohsumi Islands and the rest of Japan) could be due to isolation without gene flow caused by Ohsumi Strait and thus, site-specific mutations might have accumulated. This genetic difference is important not only

241 for understanding the genetic structure of sika deer in Japan, but also for understanding
242 the disturbance of genetic structure by artificial introduction. The mtDNA haplotypes
243 putatively originating from Yakushima Island have been found on Honshu Island,
244 probably due to artificial introductions (Yuasa et al. 2007; Yamazaki 2018). Therefore,
245 the present YSSR markers could be useful for assessing the status of introgression from
246 the Ohsumi Islands lineages into others in Japan. In the past, mtDNA and nuclear DNA
247 markers have been used to detect introduced individuals and their relatives (e.g. Yuasa et
248 al. 2007; Eva & Yamazaki 2018, 2019; Matsumoto et al. 2019; Takagi et al. 2020, Senn
249 and Pemberton 2009; Smith et al. 2018; McFarlane et al. 2020). However, the offspring
250 of crosses between non-native male deer and native female deer are not detected in
251 mtDNA analysis. In nuclear SSR analyses, the admixture of native and non-native alleles
252 can make it difficult to detect the non-native ones, especially in later generations. For
253 example, if the nuclear SSR alleles revert to the native deer ones by repeated backcrossing,
254 depending on the amount of gene flow within species, it can be impossible to find the low
255 frequency alleles derived from non-native deer (Petit & Excoffier 2009). However, even
256 in this case non-native Y chromosome information would be retained in the population
257 through the male descendants.

258 There is a possibility that unique variation has accumulated in each local
259 population of the native Japanese sika deer. All haplotypes in this study were unique to
260 the six populations from Hokkaido, Honshu, and Kyushu (Table 3) and thus, high genetic
261 differentiation among populations was detected (Table 4). The allele differences of the
262 examined markers in this study, probably due to restricted gene flow among populations
263 and the high mutation rate of these loci, suggested that there is enough resolution for the
264 estimation of historical and current population fragmentation and gene flow involving

male migration. In the mtDNA analyses, Miyazaki and Shimane are located in the southern group, and Hyogo, Nara, Miyagi, and Hokkaido are located in the northern group (Fig. 1, Nagata et al.1999; Yamada 2006; Takiguchi et al. 2012; Liu et al. 2021). However, we found no clear relationship between YSSR haplotypes and geographical distribution for these populations (Fig. 3). Thus, the pattern of genetic structure detected by Y chromosome markers was in contrast to that reported in previous mtDNA phylogeographic analyses (Tamate et al. 1998; Nagata et al. 1999; Yamada et al. 2006; Nagata 2009; Liu et al. 2021). The differences in genetic structure between mtDNA and YSSR may be due to historical sex biased dispersal in Japanese sika deer. Therefore, comprehensive analysis using the YSSR markers developed in this study together with nuclear SSRs and mtDNA will greatly contribute to our understanding of the evolutionary history of sika deer.

Conclusion

The YSSR markers developed in this study were shown to be robust markers capable of revealing variation between inter- and intra-species lineages of sika deer. Population genetic analysis using these markers can provide important data about the role of historical male-mediated dispersal in genetic structure and demography as well as the detection of male lineages in hybrids. These markers are likely to be applicable in red deer and due to the low sequence variation of the Y chromosome some of the markers may also be useful for other *Cervus* species. The management of genetic introgression and disturbance of natural genetic structure by non-native introduced individuals requires rapid and early detection as well as long-term genetic monitoring post-disturbance. The

YSSR markers developed in the present study provide a new cost-effective option for the genetic monitoring of deer species.

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Figure

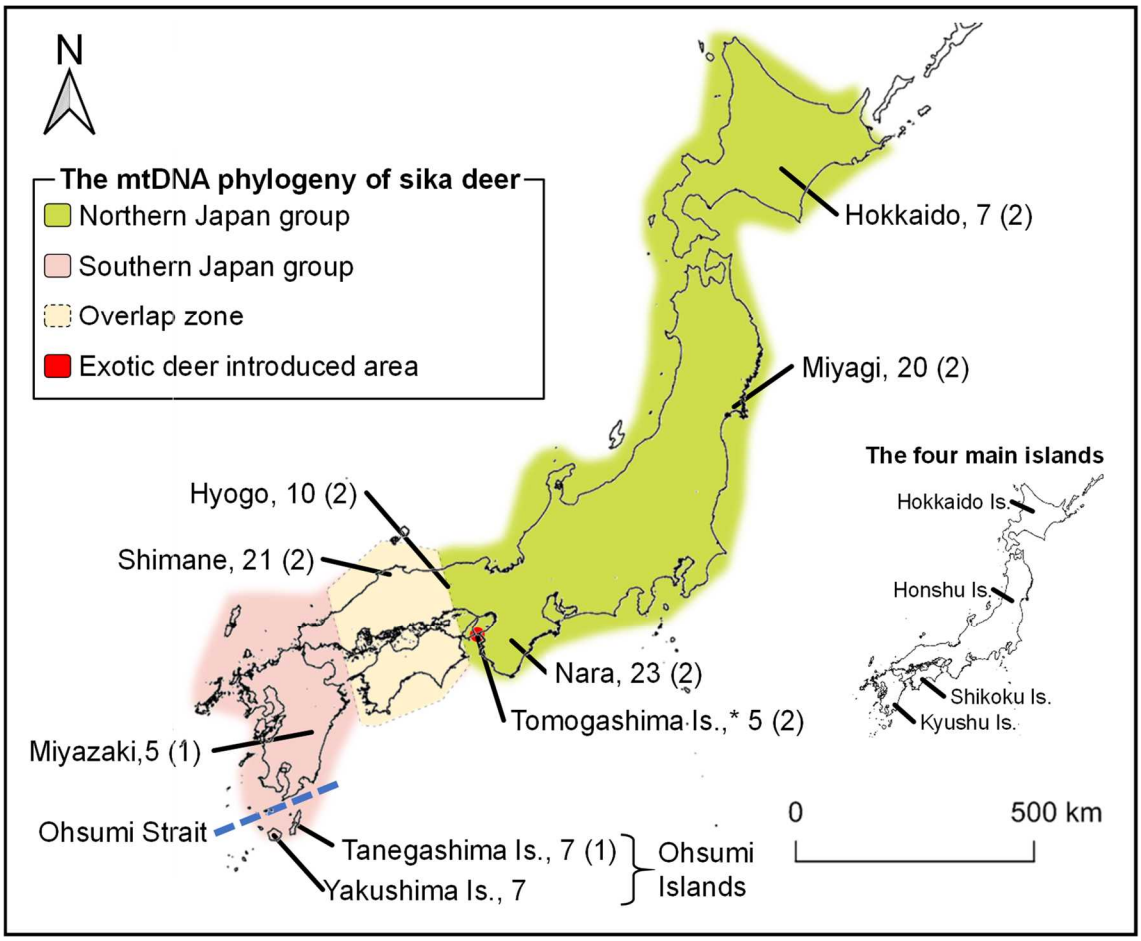
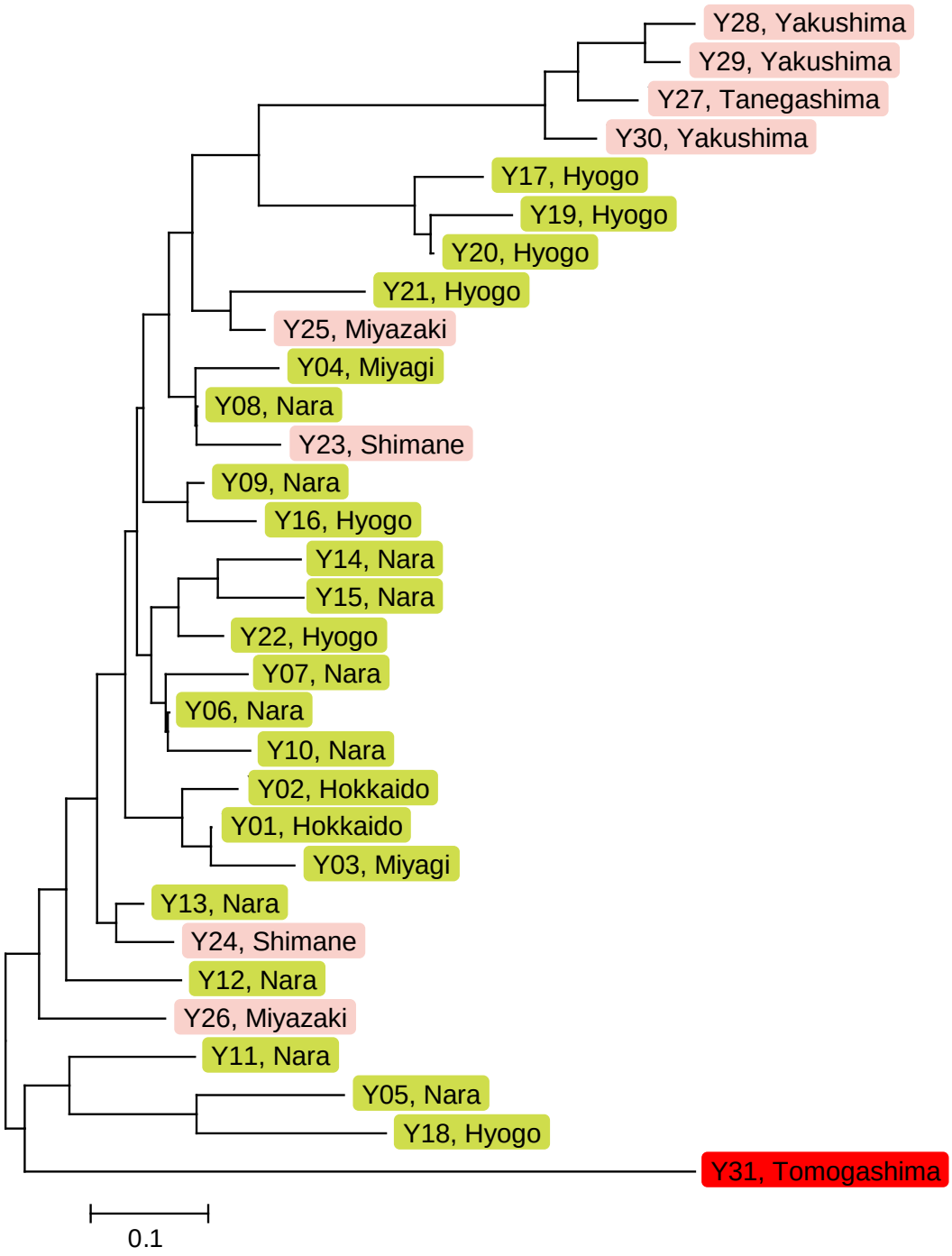


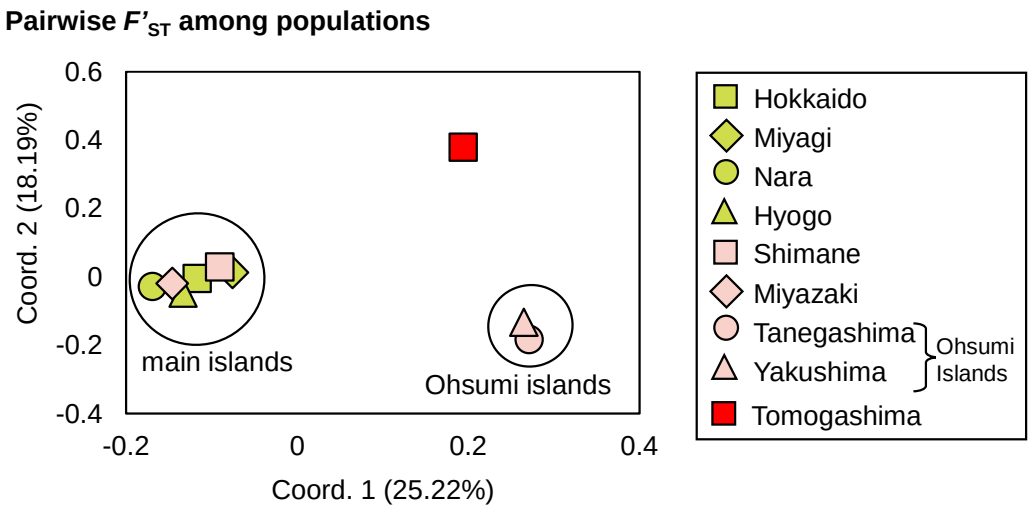
Fig. 1. Sampling sites for the sika deer samples used in the secondary screening of this study. The number after the population name is the number of samples, of which the number of females is shown in parentheses. The mtDNA of sika deer groupings were based on Tamate et al. (1998), Nagata et al. (1999), Yamada et al. (2006), Nagata (2009), Takiguchi et al. (2012), Liu et al. (2021). *The Tomogashima Island population is not pure Formosan sika deer (*C. n. taiouanus*), but a hybrid species between Formosan sambar (*C. unicolor swinhoei*) and red deer (*C. elaphus*). For more information see Matsumoto et al. (2015).



491

492 Fig. 2. The principal coordinate analysis (PCoA) based on pairwise F'_{ST} among nine

493 populations.



494

495 Fig. 3.

496 The neighbor-joining (NJ) tree based on the D_A genetic distances (Nei et al. 1983) among

497 YSSR haplotypes.

498

499

500 **Table**

Table 1. Characteristics of the 16 SSR primers developed based on the genomic information of red deer.

Locus	Primer sequence (5'-3')	Repeat motif	Position
AY02	F: TCTAATGAAGTAGACTGGACCC R: GCATCTCTTTTGCTGTCTCG	$(AC)_{12}$	275760-275783
AY03	F: CTCTTATTTGTTTCAGCGCG R: TTGAACATTGGTCCTTAAACC	$(AC)_{13}$	493373-493398
AY04	F: TGGGAAACGGCTAAATTTAGG R: AACTAGAAAAAGCCCAAGCG	$(TTG)_{9}$	1009611-1009637
AY05	F: GAGGGAGCTGAAGAGAAAGG R: AGCCAATGCAGTATTTGTGC	$(GT)_{16}$	1144665-1144696
AY12	F: ATCATGCAGAAAATGGGTGC R: AAGAGCACACGTGTCTACC	$(GT)_{12}A(TG)_{14}$	2134032-2134084
AY14	F: AGCTTGTATATCCACTCAGC R: TTATGCCTCAGATAGTTCACC	$(TG)_{15}$	2293789-2293818
AY18	F: TGTCCATTCTTCCAACCACC R: CTGGGACAAAAAGAGAGAGC	$(AC)_{16}$	2408497-2408528
AY19	F: TCCAATGTTGTGTTAATTTCTGC R: CATTTCTACTTCATGGGGTGC	$(GT)_{15}$	2441301-2441330
AY20	F: AGTTTGTTGTCATTTATGTCAGG R: CATAAGCACAAAAACTGCAGC	$(AT)_{14}$	2507979-2508006
AY22	F: CGTCATTTCTGGTTTAGGGC R: AACACTTCTGGTTCAGTTGG	$(AC)_{14}$	2535930-2535957
AY26	F: GACTCTATGTCTGCCCTGG R: TCATATCGATTGTTTACAGTAAGAGG	$(TG)_{24}$	2792813-2792860
AY29	F: CTCTTGCAGACAGAAAAGCC R: TCATGGGTTGTCTTTTTGTGG	$(TG)_{16}$	3084769-3084800
AY32	F: GCCTATTTGAACATATCAGTGTAGG R: AAGGATGGGTTAACAGGAGG	$(TA)_{13}$	3202803-3202828
AY33	F: CAAAGTATCATGGTCAAGGCC R: TGGTCTAAAAGTGTGGGAGG	$(TG)_{24}$	3363103-3363150
AY57	F: GAGACCTTTGAAGTGGATGC R: TGTTGAATTGTCTTCCCACG	$(AC)_{14}$	3935255-3935282
AY60	F: TTAGCATCTGAGCTAGCTGG R: AACCCCATGGACAGAATAGC	$(TG)_{19}$	4003045-4003082

501

Table 2. Genetic diversity of sika deer based on Y chromosome markers.

Locus	*n*	N_a	*h*	Size of alleles (red deer*) / sika deer
AY02	93	2	0.062	(175) / 170,172
AY03	93	4	0.083	(174) / 159,169,171,194
AY04	93	1	0	(187) / 186
AY05	93	2	0.285	(188) / 184,186
AY12	93	3	0.294	(173) / 181,183,185
AY14	93	4	0.377	(196) / 208,212,214,216
AY18	93	3	0.161	(214) / 214,216,222
AY19	93	1	0	(222) / 222
AY20	93	4	0.377	(188) / 192,194,196,206
AY22	93	4	0.402	(132) / 133,139,141,143
AY26	93	3	0.483	(176) / 168,170,172
AY29	93	5	0.444	(214) / 215,223,225,227,231
AY32	93	7	0.562	(192) / 159,165,167,169,171,173,175
AY33	93	6	0.685	(167) / 156,158,160,162,164,166
AY57	93	3	0.179	(120) / 120,122,132
AY60	93	3	0.083	(193) / 187,189,214

n , number of sampled individuals; N_a, number of alleles; *h* , haplotype diversity. *The value in parentheses is the corrected value by adding 17bp of "CGGAGAGCCGAGAGGTG" to the value detected in the red deer genome at the time of primer design.

502

Table 3. Genetic diversity of sika deer based on Y chromosome markers for nine sites in the Japanese archipelag

		Population								
		Hokkaido	Miyagi	Nara	Hyogo	Shimane	Miyazaki	Tanegashima	Yakushima	Tomogashima
n		5	20	21	8	19	4	6	7	3
N_{hap}		2	2	11	7	2	2	1	3	1
H		0.6	0.189	0.895	0.964	0.199	0.5	0	0.667	0
Haplotype of YSSR	Y01	2								
	Y02	3								
	Y03		2							
	Y04		18							
	Y05			2						
	Y06			5						
	Y07			1						
	Y08			2						
	Y09			1						
	Y10			1						
	Y11			1						
	Y12			1						
	Y13			5						
	Y14			1						
	Y15			1						
	Y16				2					
	Y17				1					
	Y18				1					
	Y19				1					
	Y20				1					
	Y21				1					
	Y22				1					
	Y23					17				
	Y24					2				
	Y25						3			
	Y26						1			
	Y27							6		
	Y28								4	
	Y29								2	
	Y30								1	
	Y31									3

n , number of samples; N_{hap}, the number of haplotypes in each population; *H*, haplotype diversity in each population.

503

Table 4. Pairwise *F*'st Values based on YSSR genotype.

	Hokkaido	Miyagi	Nara	Hyogo	Shimane	Miyazaki	Tanegashima	Yakushima
Miyagi	0.79							
Nara	0.39	0.53						
Hyogo	0.47	0.58	0.26					
Shimane	0.84	0.74	0.48	0.57				
Miyazaki	0.75	0.71	0.38	0.32	0.74			
Tanegashima	0.98	0.96	0.85	0.79	0.97	0.96		
Yakushima	0.93	0.93	0.84	0.78	0.96	0.92	0.70	
Tomogashima	0.99	0.99	0.93	0.91	0.99	0.98	1.05	0.99

Supplement

Supplement 1. The allele frequencies by locus for each population in the Japanese archipelago. The number in brackets under the Locus name is the corrected allele size of the red deer. The number in brackets below the population name is the sample size. No polymorphisms were found in AY04 and AY19, and therefore not listed.

		Population								
locus	Allele	Hokkaido	Miyagi	Nara	Hyogo	Shimane	Miyazaki	Tanegashima Is.	Yakushima Is.	Tomogashima Is.
		(5)	(20)	(21)	(8)	(19)	(4)	(6)	(7)	(3)
AY02	170	0	0	0	0	0	0	0	0	1
(175)	172	1	1	1	1	1	1	1	1	0
AY03	159	0	0	0	0.13	0	0	0	0	0
(174)	169	1	0.9	1	0.75	1	1	1	1	1
	171	0	0	0	0.13	0	0	0	0	0
	194	0	0.1	0	0	0	0	0	0	0
AY05	184	1	1	1	0.63	1	1	0	0	1
(188)	186	0	0	0	0.38	0	0	1	1	0
AY12	181	1	1	1	1	1	1	0	0	0
(173)	183	0	0	0	0	0	0	1	1	0
	185	0	0	0	0	0	0	0	0	1
AY14	208	0	0	0	0	0	0	0	0	1
(196)	212	0	0	0	0	0	0	1	1	0
	214	1	1	0.95	0.5	1	1	0	0	0
	216	0	0	0.05	0.5	0	0	0	0	0
AY18	214	1	1	0.81	0.88	1	1	1	1	0
(214)	216	0	0	0.19	0.13	0	0	0	0	0
	222	0	0	0	0	0	0	0	0	1
AY20	192	0	0	0.05	0	0	0	0	0.57	0
(188)	194	0	0.9	0.86	0.88	1	0.75	1	0.14	0
	196	1	0.1	0.1	0.13	0	0.25	0	0.29	0
	206	0	0	0	0	0	0	0	0	1
AY22	133	0	0	0	0	0	0	0	0	1
(132)	139	1	1	0.86	0.88	0.11	1	1	1	0
	141	0	0	0.14	0	0.89	0	0	0	0
	143	0	0	0	0.13	0	0	0	0	0
AY26	168	1	0.1	1	1	1	1	0	0	1
(176)	170	0	0.9	0	0	0	0	0	0.86	0
	172	0	0	0	0	0	0	1	0.14	0

Supplement 1 continued

locus	Allele	Population								
		Hokkaido	Miyagi	Nara	Hyogo	Shimane	Miyazaki	Tanegashima Is.	Yakushima Is.	Tomogashima Is.
		(5)	(20)	(21)	(8)	(19)	(4)	(6)	(7)	(3)
AY29	215	0	0	0	0	0	0.25	0	0	1
(214)	223	0	0	0.14	0.25	0	0	1	0.86	0
	225	1	1	0.81	0.63	1	0	0	0.14	0
	227	0	0	0	0.13	0	0.75	0	0	0
	231	0	0	0.05	0	0	0	0	0	0
AY32	159	0	0	0	0	0	0	1	1	0
(192)	165	0	0	0.1	0.25	0	0	0	0	0
	167	1	1	0.48	0.5	0.89	0.75	0	0	0
	169	0	0	0.33	0	0.11	0.25	0	0	0
	171	0	0	0.1	0.13	0	0	0	0	0
	173	0	0	0	0.13	0	0	0	0	0
	175	0	0	0	0	0	0	0	0	1
AY33	156	0	0	0	0	0	0	1	1	0
(167)	158	0	0	0.14	0	0	0	0	0	1
	160	0.4	0.1	0.67	0.13	0	0	0	0	0
	162	0	0.9	0.14	0.5	0.89	1	0	0	0
	164	0	0	0.05	0.38	0	0	0	0	0
	166	0.6	0	0	0	0.11	0	0	0	0
AY57	120	1	1	0.9	0.5	1	1	1	1	0
(120)	122	0	0	0.1	0.5	0	0	0	0	0
	132	0	0	0	0	0	0	0	0	1
AY60	187	1	1	0.95	1	1	1	1	1	0
(193)	189	0	0	0.05	0	0	0	0	0	0
	214	0	0	0	0	0	0	0	0	1

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