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Genetic Analysis Reveals Dispersal Patterns of Japanese Serow in Two Different Habitats of a Mountainous Region

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Dispersal increases the costs of feeding and predation risk in the new environment and is reported to be biased toward habitats similar to the natal region in some mammals. The benefits and costs of dispersal often differ between sexes, and most mammals show male-biased dispersal in relation to a polygamous mating system. Japanese serow is generally a solitary and monogamous species. However, recent studies have shown that the sociality of serows on Mt. Asama differs between habitat types. In the mountain forests with low forage availability, solitary habits and social monogamy were observed, while, in alpine grasslands, female grouping and social polygyny were observed, which is probably due to abundant forage availability. We investigated the effects of habitat characteristics and sociality on the dispersal of serows using fecal and tissue samples from two different habitats on Mt. Asama. The *F_{st}* value between the two areas was significantly positive, and the mean relatedness within areas was significantly higher than that between areas, which suggests limited gene flow and natal habitat-biased dispersal. Bayesian clustering analysis showed unidirectional gene flow from forest to grassland, which was probably due to the high forage availability of the grassland. Analyses of the assignment index and mean relatedness did not show male-biased dispersal, even in the grassland, where serows were polygynous. Thus, polygyny in the grassland is not linked to male-biased dispersal. In summary, our study suggests that dispersal patterns in Japanese serows are affected by habitat rather than social differences.

Key words: *Capricornis crispus*, sex biased-dispersal, natal habitat-biased dispersal, gene flow, solitary ungulate, polygyny

INTRODUCTION

Dispersal from natal habitats is one of the important events in an animal's life history that can affect local colonization and population dynamics (Bowler and Benton, 2005). Due to the lack of knowledge of the new environment, feeding efficiency is low and predation risk is high for dispersed individuals, and survival rate for dispersers is reported to be lower than that for non-dispersers in some animals (Johnson and Gaines, 1990; Clutton-Brock and Lukas, 2012). Individuals that disperse into a habitat similar to the natal habitat, which is called natal habitat-biased dispersal, are expected to have a low risk for dispersal; this has been reported in several species of insects and birds (Davis and Stamps, 2004), and also in mammals (McRae et al., 2005; Sacks et al., 2008; Merrick and Koprowski, 2016; Sanz-Pérez et al., 2018).

The ultimate causes of dispersal are thought to be kin interactions, inbreeding avoidance, and habitat variability (Bowler and Benton, 2005). The benefits and costs of dispersal and the dispersal pattern often differ between sexes (Greenwood, 1980; Bowler and Benton, 2005; Lawson Handley and Perrin, 2007). Although most birds show female-biased dispersal, most mammals show male-biased dispersal, and this difference has been suggested to be linked to the mating system; most mammals are polygamous, whereas most birds are monogamous (Greenwood, 1980). Dobson (1982) suggested that polygamous or promiscuous mammals show male-biased dispersal patterns because the cost of mating competition between males is higher than that between females, whereas both sexes disperse in monogamous mammals because the strength of mating competition does not differ between the sexes. Trochet et al. (2016) conducted a phylogenetic analysis using data from various animals and highlighted that the evolution of sex-biased dispersal was more closely linked to parental care and sexual dimorphism than to the mating sys-

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tem. However, studies of dispersal patterns in mammals have mainly been conducted in polygamous mammals, and thus, the data on monogamous mammals are limited. The accumulation of information on the dispersal patterns of monogamous mammals will elucidate the factors that are important for the evolution of sex-biased dispersal.

Japanese serow (*Capricornis crispus*) is a solitary ungulate that inhabits the cool and warm temperate zone, from lowland to the alpine zone in Japan (Ochiai, 2015; Yamashiro et al., 2019; Takada, 2023). Japanese serows have little sexual dimorphism (Miura, 1986), are mainly monogamous, and have an exclusive territory for adults of the same sex (Kishimoto and Kawamichi, 1996; Ochiai and Susaki, 2002). Contrary to other polygamous ungulates which have male-biased dispersal, such as red deer (*Cervus elaphus*; Clutton-Brock et al., 1981), long-term observational data in the Japanese serow indicates that differences in dispersal patterns are not observed between the sexes (Ochiai and Susaki, 2007). All dispersal events by both sexes occurred when a parent or territorial resident of same-sex serow was present in their natal home ranges; thus, dispersal was assumed to occur because of competition for mates and/or resources (Ochiai and Susaki, 2007).

Recent studies on Japanese serow based on behavioral observations have revealed that their social systems vary according to their habitat characteristics even among close habitats (Takada et al., 2020a; Takada and Minami, 2021). In the mountain forests of Mt. Asama, where there are closed habitats with low forage availability, Japanese serows are mainly solitary and monogamous (Takada et al., 2020a). In contrast, in the alpine grasslands of Mt. Asama, where there are open habitats with high forage availability, they occasionally form female groups of two to three individuals (Takada and Minami, 2019a, 2021) and are mainly polygynous (one male with two to five females; Takada et al., 2023). If mating systems affect dispersal patterns, as previously reported

(Greenwood, 1980; Dobson, 1982; Clutton-Brock, 1989), sex dispersal patterns may differ between close habitats in the same mountain. Furthermore, the diet of serows was different between the forest and alpine grassland because of the difference in vegetation between these areas based on elevation differences (Takada and Minami, 2019a; Takada et al., 2021). These dietary and environmental differences may have promoted natal habitat-biased dispersal.

To evaluate the effect of habitat characteristics and mating systems on dispersal patterns, we conducted genetic analysis using samples collected from two areas where mating systems and vegetation are different (Takada and Minami, 2019a, 2021; Takada et al., 2023). First, we investigated gene flow between the two areas using analyses of genetic divergence, genetic structure, and mean relatedness and discussed natal habitat-biased dispersal. We hypothesized that natal habitat-biased dispersal limits gene flow between the two areas because of environmental differences (Hypothesis 1). Next, we investigated the differences in dispersal patterns between the sexes using mean relatedness and assignment test (Favre et al., 1997; Mossman and Waser, 1999), and discussed the effects of mating systems on sex-biased dispersal. We hypothesized that dispersal in forest area is not sexually biased due to monogamy, while dispersal in grassland area is male-biased due to polygyny (Hypothesis 2). The dispersal patterns of Japanese serows are discussed based on these hypotheses.

MATERIALS AND METHODS

Sampling areas

Fecal and tissue samples were collected in two different habitats, a montane forest (hereafter “forest”) and an alpine grassland (hereafter “grassland”), on a south-facing slope of Mount Asama, central Japan (Fig. 1). The forest (36°38'N, 138°47'E) ranges from 1200–1600 m above sea level and covers approximately 200

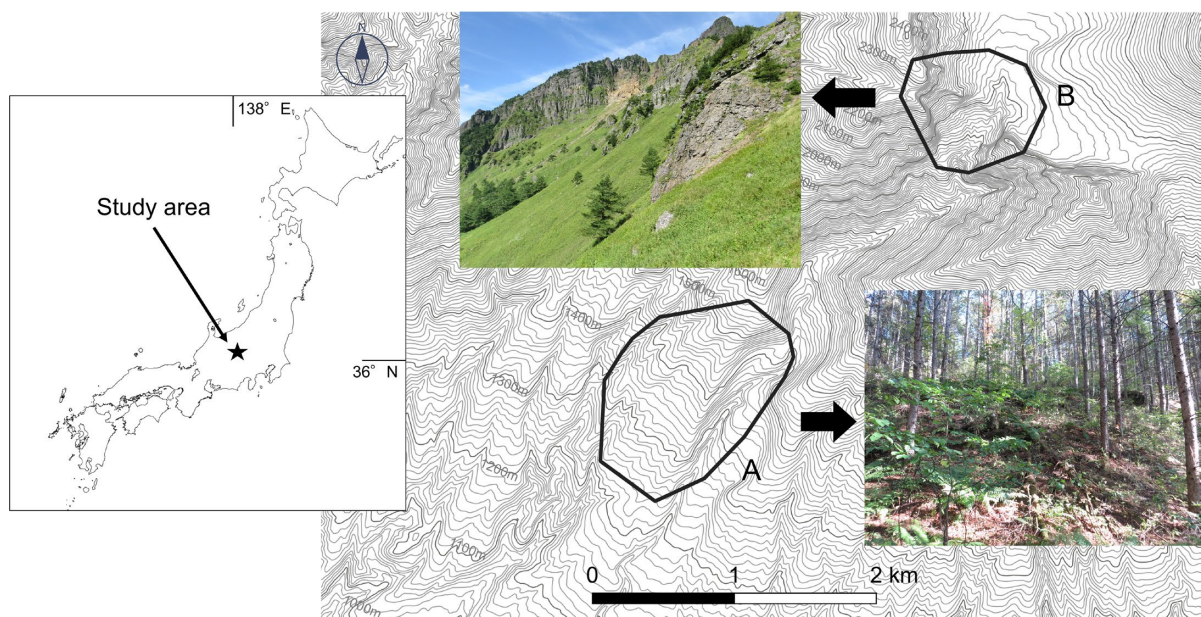


Fig. 1. Locations and views of the two study sites (black frame), the montane forest (A) and the alpine grassland (B), of Mt. Asama, central Japan.

ha of montane zones. The overstory is dominated by coniferous trees of Japanese larch (*Larix kaempferi*) and Japanese red pine (*Pinus densiflora*) (88.3%), with secondary deciduous forests of Mongolian oak (*Quercus crispula*) and white birch (*Betula platyphylla*) (11.7%). The understory is dominated by dwarf bamboo (*Sasa nipponica*) and the thickets are dominated by various broad-leaf trees. Snow covers this site (up to 50–100 cm deep) between late December and March. The grassland is located in an approximately 2.7 km straight line from the forest. The grassland (36°40'N, 138°50'E) ranges from 1900 to 2400 m above sea level and spreads from the subalpine to alpine zones, covering approximately 80 ha. The vegetation consists of grasslands (62.1% of the site, dominated by various grasses and dicotyledonous forb species) and subalpine coniferous forests (37.9% of the area, dominated by Veitch's fir, *Abies veitchii*; northern Japanese hemlock, *Tsuga diversifolia*; and Japanese larch). Snow covers this site (up to 150–250 cm deep) between late December and March. The climatic conditions and seasonal changes are milder in the forest than in the grassland. The forage availability in the grasslands during the warm season is up to six times higher than that in the forests (Takada et al., 2021).

Based on the differences in habitat characteristics, the food habits, social systems, and population characteristics of Japanese serows are quite different. In the forest, Japanese serows mainly feed on deciduous, broad-leaved trees throughout the year (spring to autumn: leaves, winter: twigs, Takada et al., 2021), whereas in the grassland, they feed mainly on grasses and dicotyledonous forb species in warm seasons and on various plants, such as dwarf bamboos and coniferous trees, in winter (Takada and Minami, 2019a). Japanese serows are mainly solitary in both habitats; however, female groups are observed only in the grassland (Takada and Minami, 2019b). A female in the forest holds a solitary territory (Takada et al., 2020a), whereas females in the grassland form group territories (Takada and Minami, 2021). Males in the forest are socially monogamous (Takada et al., 2020a), whereas those in the grassland are socially polygynous (Takada et al., 2023). The population density of the Japanese serows was reported to be higher in the grassland (27.1 ind./km²) than in the forest (4.5 ind./km²) (Takada and Minami, 2019b).

DNA samples

Of the 164 samples, 97 fecal samples and three tissue samples were collected from the grassland, and 60 fecal samples and four tissue samples were collected from the forests. Fecal samples and tissue samples were collected between 2016 and 2019, and 2012 and 2016, respectively. Each fecal pellet group was collected by rubbing the surface of two–three pellets with a cotton swab, rinsing with solution, and storing in lysis buffer (0.5% SDS, 100 mM EDTA, 100 mM Tris-HCl, 10 mM NaCl; Longmire et al., 1997). We collected only fresh or slightly fresh fecal samples that were not collapsed, and avoided collecting old feces, which were collapsed. When multiple fecal pellet groups were found in the same spot (i.e., forming a latrine site), we distinguished each fecal sample based on the color, size, and freshness of fecal pellet groups. Fecal DNA extraction was performed using the QIAamp DNA Stool Mini Kit (QIAGEN, Tokyo, Japan), and DNA was finally eluted with 200 µl of water. Tissue samples were collected from the ears of individuals who had died under natural circumstances or by roadkill, and were stored in ethanol before DNA extraction. Tissue DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Tokyo, Japan), and DNA was eluted with 200 µl of water.

Microsatellite and sex identification marker

A total of 23 microsatellite markers were used for genetic analysis (Table 1). Of these, seven loci developed in other ungulates were reported to be highly polymorphic in Japanese serows

(Nishimura et al., 2010; Yamashiro et al., 2017), seven loci were developed in the long-tailed goral (*Naemorhedus caudatus*; An et al., 2005, 2010), and three loci were developed in the Taiwan serow (*Capricornis swinhoei*; Chang et al., 2012). The primers for the seven loci were modified for multiplex PCR (Table 1). In addition, we developed new microsatellite markers specific to Japanese serow. One of the DNA samples extracted from tissue was quantified using a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and 4.7 µg template DNA was sequenced with 300 bp paired-end MiSeq protocols by Hokkaido System Sciences Co., Ltd. (Hokkaido, Japan). We obtained 3675 sequences including microsatellite regions, and selected candidate markers from 122 sequences that included more than 16 dinucleotide repeats. The primers were designed using Primer 3 (Koressaar and Remm, 2007; Untergasser et al., 2012). We referred to the sequences of three species closely related to the Japanese serow: sheep (*Ovis aries*; accession no. NC_056054–80), goat (*Capra hircus*; accession no. NC_030808–36) and Tibetan antelope (*Pantholops hodgsonii*; accession no. GCA_000400835), whose genomes were registered with NCBI, and selected the primers that showed high similarity to the sequences of the other three species. We identified six new loci that showed high diversity in the samples and used these loci for the analyses.

Sex identification was conducted using the primer set SE47/48, which amplified a part of the amelogenin region and was reported to identify the sex of the Japanese serow (Nishimura et al., 2010). Amelogenin primers have been reported to amplify fragments of 262 bp for the X-chromosome and 217 bp for the Y-chromosome in the Japanese serow (Nishimura et al., 2010).

PCR amplification

Using Multiplex Manager (Holleley and Geerts, 2009), we developed four multiplex PCR primer sets including 23 microsatellite loci and an amelogenin region (Table 1). The PCR amplification was performed in a 5 µl reaction solution containing 2.5 µl of QIAGEN Multiplex PCR Master Mix (QIAGEN), 0.2–0.8 µM of primers (Table 1), and 2 µl of fecal DNA or 1 µl of tissue-derived DNA. After initial incubation at 95°C for 15 min, PCR amplification was performed for 45 cycles consisting of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 60°C for 30 min. Fragment analysis was conducted using a 3730xl DNA analyzer (Thermo Fisher Scientific, New York, USA) by FASMAC, Inc. (Kanagawa, Japan). Peak Scanner Software v1.0 (Thermo Fisher Scientific) was used to determine the genotype of each locus.

Fecal DNA may be degraded; thus, several PCR replicates per sample were required (Taberlet et al., 1996). For fecal DNA, heterozygous and homozygous genotypes were determined if at least two and three independent PCRs yielded the same genotype, respectively (Lampa et al., 2013).

Sex and individual identification

We identified the individuals and sex of the fecal samples using the genotype of primer set 1, which included seven microsatellite loci and the amelogenin locus (Table 1). PCR using this primer set did not amplify the allele on the X-chromosome in any of the samples, including tissue-derived DNA, probably due to multiplex PCR and amplified only the allele on the Y-chromosome in male samples, although PCR using only the amelogenin locus amplified alleles on both the X-chromosome and Y-chromosome. Thus, samples for which the Y-chromosome allele was observed were identified as male, and samples for which the Y-chromosome allele was not observed were identified as female. We confirmed that this multiplex PCR correctly identified sex using DNA extracted from tissues.

Samples that did not yield genotypes at more than five loci were excluded from subsequent analysis. Individual identification

Table 1. Primer sequences and genetic characteristics of 23 microsatellite loci.

Set	Locus	Primer sequence	<i>n</i>	Concentration (μM)	Range	Dye	<i>Na</i>	<i>Ho</i>	<i>He</i>	Reference
1	OarFCB193	F:5'-TTCATCTCAGACTGGGATTGAGAAAGGC-3'	30	0.2	120–122	FAM	2	0.600	0.480	Buchanan and Crawford 1993
		R:5'-GTTCTTGCTTGGAAATAACCCCTCCTGCATCCC-3'								
	SY50	F:5'-GGCTGTGGTGTGGTTTTCT-3' ¹	30	0.3	159–169	FAM	6	0.733	0.738	An et al. 2005
		R:5'-TTCCCAATCTTTCTCTCTCTGTC-3'								
	ILSTS58	F:5'-GCCTTACTACCATTTCAGC-3'	30	0.3	145–153	HEX	4	0.667	0.663	Kemp et al. 1995
		R:5'-CATCCTGACTTTGGCTGTGG-3'								
	FS50	F:5'-AAACCCTGAACCTGTGGTCA-3'	30	0.2	239–253	HEX	3	0.400	0.407	Chang et al. 2012
		R:5'-CAAAAATGGTTCTATGAAACTTCC-3' ¹								
SY76	F:5'-AGGGTTTGCTTTTCAGGAC-3'	30	0.2	120–130	NED	3	0.467	0.545	An et al. 2010	
	R:5'-CATCCATTACAGGAAGACTGC-3'									
SPS115	F:5'-AACGATGCCACACAAAACAAA-3' ¹	30	0.2	191–197	NED	4	0.700	0.674	Moore and Byrne 1994	
	R:5'-AAAGGCAAAAGACACTAGAGGA-3' ¹									
ETH10	F:5'-CAACCCCTCTGTTGTGCTCT-3' ¹	30	0.2	236–244	NED	3	0.467	0.496	Toldo et al. 1993	
	R:5'-ACCTGGGTAGTCGGAGAAGC-3' ¹									
2	BM4107	F:5'-AGCCCCTGCTATTGTGTGAG-3'	30	0.2	151–153	FAM	2	0.500	0.495	Bishop et al. 1994
		R:5'-GTTCTTATAGGCTTTGCATTGTTTCAGG-3'								
	SY141 ²	F:5'-CATAGCCTTGACTAAACGGACC-3'	30	0.6	260–262	FAM	2	0.133	0.124	An et al. 2010
		R:5'-CACCTGCCACATTCGGG-3'								
	SY58	F:5'-CTATTGAACCTGTATCTCCCC-3'	30	0.2	204–230	HEX	5	0.633	0.574	An et al. 2005
		R:5'-GCATTCTGGCTCTGGCAA-3'								
SY12B	F:5'-TGACCCTTTGCTGTATCCTG-3'	30	0.2	109–115	NED	3	0.667	0.604	An et al. 2005	
	R:5'-GGTGAGCCCAGAGAATCTTC-3'									
FS90	F:5'-AAAAGAGGGGTGAGTAGGC-3'	30	0.2	224–252	NED	4	0.600	0.568	Chang et al. 2012	
	R:5'-CGAAGGAAGATGAATCATGG-3'									
3	FS152	F:5'-TTCATGTCTGGGTTTGACCA-3'	30	0.2	120–124	FAM	3	0.633	0.509	Chang et al. 2012
		R:5'-AGAAGCTTGAGCCTCCTCCT-3' ¹								
	SY84	F:5'-GAACTGAACTTGTAGTATGTTGGG-3'	30	0.2	170–176	FAM	2	0.600	0.420	An et al. 2005
		R:5'-TTGTTATGCTTGATGTTATTTGTTAC-3'								
	SY259	F:5'-AGAAAAAGCCTGCACACCAG-3' ¹	30	0.4	117–121	HEX	3	0.833	0.651	An et al. 2005
		R:5'-AGACATAAGGGCGAACAGGA-3' ¹								
ETH225	F:5'-GATCACCTTGCCACTATTTCT-3' ¹	30	0.2	164–174	HEX	4	0.533	0.523	Steffen et al. 1993	
	R:5'-GTTCTTACATGACAGCCAGCTGCTACT-3' ¹									
BM3628 ²	F:5'-CTGAGATGGACTCAGGGAGG-3'	30	0.4	244–248	NED	3	0.200	0.209	Bishop et al. 1994	
	R:5'-GTTCTTGTGGATTGGAAAGGTTAGGC-3'									
4	CC0280 ³	F:5'-TGGAGTTTAGCCAGGTAGGC-3'	30	0.2	210–228	NED	4	0.667	0.636	
		R:5'-GTGGGGGAAAATGGTAATC-3'								
	CC0452 ³	F:5'-GGTCACAAAGAGTGGGCTGT-3'	29	0.2	279–287	HEX	4	0.621	0.706	
		R:5'-TCCTAGCAGGGGAACTAAAGC-3'								
	CC0480 ³	F:5'-CGCACACAAACACACATTCA-3'	29	0.2	113–131	FAM	5	0.828	0.691	
		R:5'-GAAAGTCCGAAGCGTCTGTG-3'								
	CC0580 ³	F:5'-CACCAGGGAAGCCATATTT-3'	29	0.2	190–196	FAM	4	0.586	0.674	
		R:5'-GTGGCAGCTACACTCCTGGT-3'								
	CC0508 ^{2,3}	F:5'-CAGGATTCTTGCTGGAGAA-3'	28	0.2	295–303	FAM	4	0.393	0.586	
		R:5'-TGCTTGGTGTAGTTGGTGTGA-3'								
CC1374 ³	F:5'-GCCTTTTCACTACTGTTGGGTTTC-3'	30	0.2	107–117	HEX	4	0.733	0.747		
	R:5'-CAGAGAGCATGCTTTTCTTAG-3'									

Set: multiplex PCR primer set, *n*: number of genotyped individuals, *Na*: number of alleles, *Ho*: observed heterozygosity, *He*: expected heterozygosity. Multiplex PCR primer set 1 included amelogenin region, SE47/48, labeling by FAM. This region was excluded from this list because it is not a microsatellite locus.

¹The primers were modified in this study.

²The primers were excepted from the analyses due to low genetic diversity and/or null allele existence.

³The primers were developed in this study.

was performed by identity analysis using CERVUS (Marshall et al., 1998; Kalinowski et al., 2007). To confirm the results of individual identification using primer set 1, we performed PCR using primer

set 2 for all individually identified samples at least once. After individual identification, we selected one sample per individual and determined the genotypes of all individuals at 23 microsatellite loci

using four primer sets.

Genetic analysis

We calculated the number of alleles (N_a), observed and expected heterozygosity (H_o and H_e), and the probability of having the same genotype among full siblings (P_{sib}), and confirmed deviations from the Hardy-Weinberg equilibrium (HWE) using GenAEx 6.3 (Peakall and Smouse, 2006, 2012). The presence of null alleles was checked using MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004).

Several genetic analyses were conducted to test hypotheses 1 and 2. If Hypothesis 1 is true, the gene flow between two areas can be limited: the fixation index (F_{st}) value could be significantly positive, individuals in the grassland could be genetically different from individuals in the forest, and relatedness within areas could be expected to be higher than that between areas. If Hypothesis 2 is true, female relatedness could be higher than that of male in the grassland, where the mating system is polygynous, whereas relatedness values could be similar in the forest, where the mating system is monogamous. In addition, analysis of the assignment index showed that females were more philopatric than males only in the grassland.

F_{st} value, which is an index of genetic divergence, was determined using AMOVA in GenAEx 6.3. We conducted Bayesian clustering analysis with Markov chain Monte Carlo methods (MCMC) using STRUCTURE 2.3 (Pritchard et al., 2010). In the admixture model, 1,000,000 MCMC replicates after burn-in of 200,000 replicates were conducted independently 10 times. The number of putative populations, K , was set to range from 1 to 10, and the best value of K , which is the most probable number of clusters, was inferred based on ΔK calculated using Structure Harvester (see Supplementary Figure S1; Earl and von Holdt, 2012). The mean fractional membership of each animal in each cluster (Q) was calculated from 10 iterations using CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and the Q value of each animal was illustrated using DISTRUCT 1.1 (Rosenberg, 2004). Individuals were considered to belong to a single cluster when the highest Q value was > 0.7 , and individuals were considered to be admixed when the highest Q value was < 0.7 (Hirose et al., 2021).

We calculated the QuellerGt (Queller and Goodnight, 1989, "QGM") relatedness estimator using Coancestry (Wang, 2011). We compared QGM relatedness between areas to that within areas in each sex. We also compared QGM relatedness between sexes. Permutation test was conducted using the package 'exactRankTests' (Hothorn and Hornik, 2019) in R 3.6.2 (R Core Team, 2019).

The corrected assignment index (Aic), which is the probability of an individual's assignment to a population as negative values identify migrants and positive values identify residents, was used for the analysis of sex-biased dispersal (Favre et al., 1997; Mossman and Waser, 1999; Colson et al., 2013). We calculated Aic values using GenAEx, and a permutation test was performed to analyze the difference in Aic between sexes and an F-test for equality of two

variances was conducted to compare variances of Aic between sexes in R 3.6.2 (R Core Team, 2019). We compared the number of individuals with negative or positive Aic values between the sexes using Fisher's exact test in R 3.6.2.

RESULTS

Sex and individual identification and genetic diversity

Of seven loci, genotypes at six or more loci, including primer set 1, were successfully identified in 41 of the 60 (68%) forest fecal samples and 61 of the 97 (63%) grassland fecal samples. Of the 102 samples, eight individuals (four males and four females) and 17 individuals (five males and 12 females) were identified from fecal samples in the forest and grassland areas, respectively. Among the seven tissue samples, one sample from each area yielded the genotypes that had already been determined using fecal samples. In total, 11 individuals (six males, five females) in the forest area and 19 individuals (five males, 14 females) in the grassland area were identified. The number of samples for each individual ranged from one to 14. The genetically identified sex was the same among multiple samples from the same individuals.

The genetic characteristics of the 30 individuals are shown in Table 1. The average H_o was 0.583 and for H_e was 0.527 (Table 1). Two loci (SY141 and BM3628) had low diversity, and one locus (CC0508) was estimated to have a null allele; thus, these loci were excluded from further analyses. The total P_{sib} value of the microsatellite loci in primer set 1 was 1.1×10^{-2} . The number of mismatched loci between different individuals was more than one, and none of the results using primer set 2 contradicted the results of individual identification using primer set 1.

Genetic differentiation between areas

The F_{st} value between the two areas was 0.081, and the value was significantly different from zero ($P < 0.01$). The mean relatedness among males within areas (0.126) was significantly higher than that among males between areas (-0.173 , $P < 0.001$; see Supplementary Figure S2), and the mean relatedness among females within areas (0.060) was also significantly higher than that among females between areas (-0.145 , $P < 0.001$; see Supplementary Figure S2).

In the Bayesian clustering analysis, the value of ΔK was highest at $K = 2$ (see Supplementary Figure S1). For the bar plot figures at $K = 2$, all individuals in the forest area belonged to cluster 1, whereas 16 individuals belonged to cluster 2, one individual belong to the cluster 1, and the other



Fig. 2. Bar plot representing the proportion of clusters at $K = 2$ by STRUCTURE. Each serow is represented as a vertical bar, and each color bar length is proportional to the estimated membership of the cluster. The color of cluster 1 is light gray and that of cluster 2 is dark gray at $K = 2$.

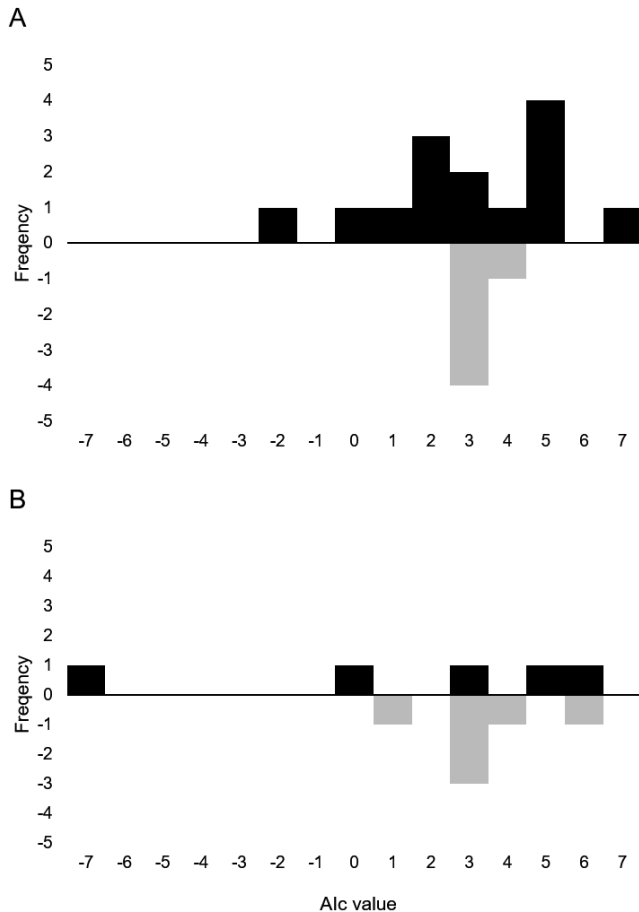


Fig. 3. Distribution of corrected assignment index (Alc) in two areas. A higher or lower Alc value indicates that the individual is from the natal area or from another area, respectively. Females are shown as black bars and males are shown as light gray bars: **(A)** grassland, **(B)** forest.

two were admixed individuals in the grassland area (Fig. 2). The individual that belonged to cluster1 and two admixed individuals were female.

Sex-biased dispersal

The mean Alc value of males (2.665) in the grassland area were similar to those of females (2.649), and the difference was not statistically significant (permutation test, $P = 1$). Although the mean Alc values of males (2.743) in the forest area were higher than those of females in forest area (0.895), the difference was not statistically significant (permutation test, $P = 0.468$). The variance of Alc values was significantly higher in females than in males, in both the grassland area ($P < 0.01$) and the forest area ($P < 0.05$). Two of the 14 females and none of the five males in the grassland area and two of five females and none of six males in the forest area had negative Alc values (Fig. 3). The differences of the ratio were not significant between the sexes in the grassland (Fisher's exact test, $P = 1$) and the forest area (Fisher's exact test, $P = 0.181$).

The mean relatedness among males within the areas (0.126) was not significantly higher than that among females within the areas (0.060, $P = 0.326$; see Supplementary Fig-

ure S2). We independently investigated the mean relatedness in the grassland and forest area. In the grassland area, the mean relatedness among males (0.175) was not significantly higher than that among females (0.048, $P = 0.107$; see Supplementary Figure S2). In the forest area, the mean relatedness among males (0.044) was not significantly lower than that among females (0.162, $P = 0.493$; see Supplementary Figure S2).

DISCUSSION

Individual identification and genotyping success rate of fecal samples

Individual identification is essential for genetic studies of fecal samples. We used primer set 1, which included seven microsatellite loci and the amelogenin region for individual and sex identification. *Plsib* values of primers in set 1 were 1.1×10^{-2} in total. Waits et al. (2001) reported that a *Plsib* value below approximately 0.01 is considered reasonable for individual identification, and thus the *Plsib* value in this study was low enough for individual identification. In addition, the genetically identified sex was the same among the samples that yielded the same genotype. This indicates that this primer set is useful for efficient sex and individual identification in Japanese serows.

Genetic differentiation between two areas and natal habitat-biased dispersal

The F_{st} value between the two areas was significantly different from zero, and Bayesian clustering analysis showed that different clusters dominated in different areas at $K = 2$. These results support Hypothesis 1 that gene flow between the two areas is limited due to habitat differences. This result might be surprising when we consider that the straight distance between the areas was less than 3 km, and most Japanese serows dispersed more than 1 km from their natal birth places (Ochiai and Susaki, 2007). The slope between the two areas is very steep (Fig. 1), and thus topography may be one of the candidate reasons for limited gene flow, as reported in other ungulates (Pérez-Espona et al., 2008; Akomo-Okoue et al., 2022). However, Japanese serows are adapted to steep slopes and rocky cliffs (Takada et al., 2019, 2020b) and are continuously distributed between the two areas, and thus the impact of topography on gene flow may not be significant for serows. Genetic differentiation in this study may be due to the difference in vegetation between the two areas according to the elevation difference (Takada and Minami, 2019a). Natal habitat-biased dispersal has been reported in several insect, bird, and mammalian species (Davis and Stamps, 2004; McRae et al., 2005; Sacks et al., 2008; Merrick and Koprowski, 2016; Sanz-Pérez et al., 2018). Our findings in serows at Mt. Asama were consistent with a report that female mountain gorillas dispersed into areas where the elevation was similar to that of the natal area (Guschanski et al., 2008). Thus, habitat difference might affect genetic difference between two areas at Mt. Asama. Takada et al. (2021) reported that serows in the forest area at Mt. Asama fed mainly on dicotyledonous leaves, whereas those in the grassland fed mainly on graminoids. Such dietary differences may result in natal habitat-biased dispersal of Japanese serows.

Bayesian clustering analysis suggested unidirectional

gene flow between the two areas on Mt. Asama. All individuals in the forest area belonged to Cluster 1, while in grassland areas some individuals were admixed or belonged to Cluster 1, although most individuals belonged to Cluster 2. This suggests that gene flow occurred from forest to grassland area, but not vice versa. One reason for this gene flow may be the difference in food abundance between the areas. Takada et al. (2021) reported that the grassland area on Mt. Asama has abundant forage resources, such as graminoids and forbs, and the forage biomass in the grassland area is higher than that in the forest area. Gene flow from low to high food abundance areas is expected to occur easily; thus, unidirectional gene flow from forest to grassland occurs on Mt. Asama. Social factors, such as exclusive territory for adults of the same sex, might also influence gene flow. Further studies are needed to identify the cause of unidirectional gene flow.

Sex-biased dispersal

The *Alc* values and the mean relatedness were not significantly different between the sexes in either forest or grassland areas. The ratio of individuals with negative *Alc* values was not statistically different between sexes both in forest and grassland areas, although the number of individuals may not have been large enough for statistical power. These results suggest that the dispersal patterns of Japanese serows did not differ between the sexes in the two areas of Mt. Asama. In contrast, the variances of *Alc* were significantly higher in females than in males, both in forest and grassland areas, which suggested female-biased dispersal. Thus, it is difficult to draw conclusions about dispersal patterns in Japanese serows, but our results did not indicate male-biased dispersal. This is not consistent with Hypothesis 2: dispersal in the forest area is not sexually biased owing to monogamy, whereas dispersal in the grassland is male-biased owing to polygyny.

Although the Japanese serow is a primarily monogamous mammal, and the serows in the forest at Mt. Asama showed a monogamous mating system, serows in the grassland area at Mt. Asama show a polygynous mating system (Takada et al., 2023). Male-biased dispersal has been reported in polygamous species such as red deer (*Cervus elaphus*; Clutton-Brock and Albon, 1979), white-tailed deer (*Odocoileus virginianus*; Miller et al., 2010), southern chamois (*Rupicapra pyrenaica*; Loison et al., 1999), and bighorn sheep (*Ovis canadensis*; Corlatti et al., 2011). However, male-biased dispersal was not observed in Japanese serows in Mt. Asama, even in grassland, where serows were mainly polygynous. Ochiai and Susaki (2007) suggested that Japanese serows disperse due to resource competition for both sexes and mating competition for males. Males in grassland areas may benefit more from philopatric dispersal due to higher female density in grassland areas than in forest areas (Takada and Minami, 2019b). Furthermore, as previously discussed, natal habitat-biased dispersal may lead to philopatric dispersal of males in grassland. Recent genetic analyses suggested variability in sex-biased dispersal patterns in some ungulates (Pérez-Espona et al., 2010; Deakin et al., 2021). More detailed genetic studies of ungulates in various environments will elucidate the effects of the social system and environment on dispersal patterns in detail.

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COMPETING INTERESTS

The authors have no competing interest to declare.

AUTHOR CONTRIBUTIONS

This study was designed by EI, HT, and MM. HT led the sampling at Mt. Asama. Experiments and genetic analyses were conducted by MH, YN, and EI. MH and EI conducted statistical analyses. MH prepared the first draft, and all authors reviewed the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: <https://doi.org/10.2108/zs230055>)

Supplementary Figure S1. Inferred ΔK values in the number of clusters by STRUCTURE harvester.

Supplementary Figure S2. Mean relatedness of Japanese serow among sexes within areas and between areas in grassland and forest areas at Mt. Asama.

REFERENCES

- Akomo-Okoue EF, Inoue E, Atteke C, Nakashima Y, Hongo S, Inoue-Murayama M, et al. (2022) Effect of landscape features on the genetic structure of forest duikers (Cephalophinae) in Moukalaba forest, Gabon. *Mamm Biol* 102: 1879–1888
- An J, Min MS, Sommer J, Louis E, Brenneman R, Kwon SW, et al. (2005) Isolation and characterization of 15 microsatellite loci in the Korean goral (*Nemorhaedus caudatus*). *Mol Ecol Resour* 5: 421–423
- An J, Choi SK, Sommer J, Louis E, Brenneman R, Zemanová B, et al. (2010) A core set of microsatellite markers for conservation genetics studies of Korean goral (*Naemorhedus caudatus*) and its cross-species amplification in Caprinae species. *J V Sci* 11: 351–353
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, et al. (1994) A genetic linkage map for cattle. *Genetics* 136: 619–639
- Bowler DE, Benton TG (2005) Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. *Biol Rev Camb Philos Soc* 80: 205–225
- Buchanan FC, Crawford AM (1993) Ovine microsatellites at the OARFCB11, OARFCB128, OARFCB193, OARFCB266 and OARFCB304 loci. *Anim Genet* 24: 145
- Chang YY, Chao MC, Ding ST, Lin EC, Tsao HS, Yuan HW, et al. (2012) Development of microsatellite markers in an ungulate mammal, the Formosan serow (*Capricornis swinhoei*). *Conserv Genet Resour* 4: 755–757
- Clutton-Brock TH (1989) Mammalian mating systems. *Proc R Soc B* 236: 339–372
- Clutton-Brock TH, Albon SD (1979) The roaring of red deer and the evolution of honest advertisement. *Behaviour* 69: 145–170
- Colson KE, Brinkman TJ, Person DK, Hundertmark KJ (2013) Fine-scale social and spatial genetic structure in Sitka black-tailed deer. *Conserv Genet* 14: 439–449

- Corlatti L, Lorenzini R, Lovari S (2011) The conservation of the chamois *Rupicapra* spp. *Mamm Rev* 41: 163–174
- Davis JM, Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends Ecol Evol* 19: 411–416
- Deakin JE, Guglielmo CG, Morbey YE (2021) Effect of the social environment on spring migration timing of a songbird. *Front Ecol Evol* 9: 715418
- Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Anim Behav* 30: 1183–1192
- Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4: 359–361
- Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal, *Crocidura russula*: Evidence from field data and microsatellite patterns. *Proc R Soc B* 264: 127–132
- Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Anim Behav* 28: 1140–1162
- Guschanski K, Caillaud D, Robbins MM, Vigilant L (2008) Females shape the genetic structure of a gorilla population. *Curr Biol* 18: 1809–1814
- Hirose M, Yoshida K, Inoue E, Hasegawa M (2021) Population genetic structure of raccoons as a consequence of multiple introductions and range expansion in the Boso Peninsula, Japan. *Sci Rep* 11: 19294
- Holleley CE, Geerts PG (2009) Multiplex Manager 1.0: A cross-platform computer program that plans and optimizes multiplex PCR. *BioTechniques* 46: 511–517
- Hothorn T, Hornik K (2019) exactRankTests: Exact distributions for rank and permutation tests. version 0.8–30. <https://CRAN.R-project.org/package=exactRankTests/> Accessed 1 July 2019
- Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801–1806
- Johnson ML, Gaines MS (1990) Evolution of dispersal: Theoretical models and empirical tests using birds and mammals. *Annu Rev Ecol Syst* 21: 449–480
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16: 1099–1106
- Kemp SJ, Hishida O, Wambugu J, Rink A, Longeri MLL, Ma RZ, et al. (1995) A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Anim Genet* 26: 299–306
- Kishimoto R, Kawamichi T (1996) Territoriality and monogamous pairs in a solitary ungulate, the Japanese serow, *Capricornis crispus*. *Anim Behav* 52: 673–682
- Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23: 1289–1291
- Lampa S, Henle K, Klenke R, Hoehn M, Gruber B (2013) How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation—A review of available methods illustrated by a case study. *Jour Wild Mgmt* 77: 1490–1511
- Lawson Handley LJ, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Mol Ecol* 16: 1559–1578
- Loison A, Jullien J-M, Menaut P (1999) Subpopulation structure and dispersal in two populations of chamois. *J Mammal* 80: 620–632
- Longmire JL, Maltbie M, Baker RJ (1997) Use of “lysis buffer” in DNA isolation and its implication for museum collections. Lubbock, TX, Museum of Texas Tech University, 1997
- Marshall TC, Slate JBKE, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7: 639–655
- McRae BH, Beier P, Dewald LE, Huynh LY, Keim P (2005) Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Mol Ecol* 14: 1965–1977
- Merrick MJ, Koprowski JL (2016) Evidence of natal habitat preference induction within one habitat type. *Proc R Soc B* 283: 20162106
- Miller BF, De Young RW, Campbell TA, Laseter BR, Ford WM, Miller KV (2010) Fine-scale genetic and social structuring in a central Appalachian white-tailed deer herd. *J Mammal* 91: 681–689
- Miura S (1986) Body and horn growth patterns in the Japanese serow, *Capricornis crispus*. *J Mammal Soc Jpn* 11: 1–13
- Moore SS, Byrne K (1994) Characterization of 65 bovine microsatellites. *Mamm Genome* 5: 84–90
- Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. *Mol Ecol* 8: 1063–1067
- Nishimura T, Yamauchi K, Saitoh Y, Deguchi Y, Aoi T, Tsujimoto T, et al. (2010) Sex determination of the Japanese Serow (*Capricornis crispus*) by fecal DNA analysis. *Jpn J Zoo Wildl Med* 15: 73–78
- Ochiai K (2015) *Capricornis crispus* (Temminck, 1836). In “The Wild Mammals of Japan. 2nd ed” Ed by DS Ohdachi, Y Ishibashi, MA Iwasa, D Fukui, T Saitoh, Shoukadoh Book Sellers and the Mammalogical Society of Japan, Kyoto, pp 314–317
- Ochiai K, Susaki K (2002) Effects of territoriality on population density in the Japanese serow (*Capricornis crispus*). *J Mammal* 83: 964–972
- Ochiai K, Susaki K (2007) Causes of natal dispersal in a monogamous ungulate, the Japanese serow, *Capricornis crispus*. *Anim Behav* 73: 125–131
- Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537–2539
- Peakall ROD, Smouse PE (2006) GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6: 288–295
- Pérez-Espona S, Pérez-Barbería FJ, McLeod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Mol Ecol* 17: 981–996
- Pérez-Espona S, Pérez-Barbería FJ, Jiggins CD, Gordon IJ, Pemberton JM (2010) Variable extent of sex-biased dispersal in a strongly polygynous mammal. *Mol Ecol* 19: 3101–3113
- Pritchard JK, Wen X, Falush D (2010) Documentation for structure software: Version 2.3. University of Chicago, Chicago, pp IL 1–37
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43: 258–275
- R Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/> Accessed 25 December 2017
- Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. *Mol Ecol Notes* 4: 137–138
- Sacks BN, Bannasch DL, Chomel BB, Ernest HB (2008) Coyotes demonstrate how habitat specialization by individuals of a generalist species can diversify populations in a heterogeneous ecoregion. *Mol Biol Evol* 25: 1384–1394
- Sanz-Pérez A, Ordiz A, Sand H, Swenson JE, Wabakken P, Wikenros C, et al. (2018) No place like home? A test of the natal habitat-biased dispersal hypothesis in Scandinavian wolves. *R Soc Open Sci* 5: 181379
- Steffen P, Eggen A, Dietz AB, Womack JE, Stranzinger G, Fries R (1993) Isolation and mapping of polymorphic microsatellites in cattle. *Anim Genet* 24: 121–124

- Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, et al. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res* 24: 3189–3194
- Takada H (2023) Unique spatial behavior of the Japanese serow (*Capricornis crispus*) in the open mountains of Mt. Fuji. *Acta Ethol* 26: 127–132
- Takada H, Minami M (2019a) Food habits of the Japanese serow (*Capricornis crispus*) in an alpine habitat on Mount Asama, central Japan. *Mammalia* 83: 455–460
- Takada H, Minami M (2019b) Do differences in ecological conditions influence grouping behaviour in a solitary ungulate, the Japanese serow? *Behaviour* 156: 245–264
- Takada H, Minami M (2021) Open habitats promote female group formation in a solitary ungulate: The Japanese serow. *Behav Ecol Sociobiol* 75: 1–12
- Takada H, Nakamura K, Watanabe H, Minami M (2019) Effects of the physical and social environment on flight response and habitat use in a solitary ungulate, the Japanese serow (*Capricornis crispus*). *Behav Processes* 158: 228–233
- Takada H, Nakamura K, Minami M (2020a) Spatial organization and mating behavior of the Japanese serow under a low population density. *Mammalia* 84: 219–226
- Takada H, Ohuchi R, Watanabe H, Yano R, Miyaoka R, Nakagawa T, et al. (2020b) Habitat use and the coexistence of the sika deer and the Japanese serow, sympatric ungulates from Mt. Asama, central Japan. *Mammalia* 84: 503–511
- Takada H, Washida A, Yano R, Tezuka N, Minami M (2023) Evolution from monogamy to polygyny: Insights from the solitary Japanese serow. *Behav Ecol Sociobiol* 77: 1–11
- Toldo SS, Fries R, Steffen P, Neiberg HL, Barendse W, Womack JE, et al. (1993) Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mamm Genome* 4: 720–727
- Trochet A, Courtois EA, Stevens VM, Baguette M, Chaine A, Schmeller DS, et al. (2016) Evolution of sex-biased dispersal. *Q Rev Biol* 91: 297–230
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. (2012) Primer3—new capabilities and interfaces. *Nucleic Acids Res* 40 (15): e115
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4: 535–538
- Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Mol Ecol* 10: 249–256
- Wang J (2011) COANCESTRY: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol Ecol Resour* 11: 141–145
- Yamashiro A, Kaneshiro Y, Kawaguchi Y, Yamashiro T (2017) Species, sex, and individual identification of Japanese serow (*Capricornis crispus*) and sika deer (*Cervus nippon*) in sympatric region based on the fecal DNA samples. *Conserv Genet Resour* 9: 333–338
- Yamashiro A, Kaneshiro Y, Kawaguchi Y, Yamashiro T (2019) Dietary overlap but spatial gap between sympatric Japanese serow (*Capricornis crispus*) and sika deer (*Cervus nippon*) on Eastern Shikoku, Japan. *Mammal Study* 44: 261–267

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