




Seasonal infestation patterns of ticks on Hokkaido sika deer (*Cervus nippon yesoensis*)

Kotaro Shimizu¹ , Michito Shimozuru¹, Masami Yamanaka², Genta Ito², Ryo Nakao³  and Toshio Tsubota¹ 

¹Laboratory of Wildlife Biology and Medicine, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; ²Shiretoko Nature Foundation, Shari-gun, Japan and ³Laboratory of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Research Article

Cite this article: Shimizu K, Shimozuru M, Yamanaka M, Ito G, Nakao R, Tsubota T (2024). Seasonal infestation patterns of ticks on Hokkaido sika deer (*Cervus nippon yesoensis*). *Parasitology* **151**, 1317–1325. <https://doi.org/10.1017/S0031182024001227>

Received: 20 May 2024

Revised: 14 September 2024

Accepted: 16 September 2024

First published online: 15 November 2024

Keywords:

attachment site preferences; co-feeding transmission; co-occurrence; *Haemaphysalis* spp; Hokkaido sika deer; interspecies interactions; intraspecies interactions; *Ixodes* spp; tick; tick–host interactions

Corresponding author:

Toshio Tsubota;

Email: tsubota@vetmed.hokudai.ac.jp

Abstract

Ticks prefer specific feeding sites on a host that are influenced by host–tick and tick–tick interactions. This study focused on the spatiotemporal distribution of ticks in Hokkaido sika deer, an important tick host in Hokkaido, Japan. Tick sampling was performed on the sika deer in the Shiretoko National Park between June and October 2022. Ticks were collected from 9 different body parts of the deer to compare their attachment site preferences. Interspecific and intraspecific relationships among ticks were examined using co-occurrence analysis. The collected ticks were nymphal and adult stages of 4 species: *Ixodes ovatus*, *Ixodes persulcatus*, *Haemaphysalis japonica* and *Haemaphysalis megaspina*. Seasonal variations in tick burden were observed, with *I. persulcatus* and *I. ovatus* peaking in June and declining towards October; *H. japonica* showing low numbers in July and August and increasing from September; and *H. megaspina* appearing from September onwards with little variation. Attachment site preferences varied among species, with a significant preference for the pinna in *I. ovatus* and *I. persulcatus*. *Haemaphysalis japonica* was mainly found on the body and legs between June and August, and shifted to the pinna from September. *Haemaphysalis megaspina* showed a general preference for areas other than the legs. Co-occurrence analysis revealed positive, negative and random co-occurrence patterns among the tick species. Ticks of the same genus and species exhibited positive co-occurrence patterns; *I. ovatus* showed negative co-occurrence patterns with *Haemaphysalis* spp. This study revealed the unique attachment site preferences and distinct seasonal distributions of tick species in the Hokkaido sika deer.

Introduction

Most ectoparasites, including ticks, do not infest their hosts randomly but feed preferentially on specific body parts (Andrews *et al.*, 1981; Chilton *et al.*, 1992; Kiffner *et al.*, 2011; Baer-Lehman *et al.*, 2012; Myserud *et al.*, 2014; Lydecker *et al.*, 2019; Tiffin *et al.*, 2021). The distribution patterns depend on the characteristics of both the host and the tick (Kar *et al.*, 2013). For example, it has been reported that many tick species prefer sites where they can effectively feed, such as the hairless parts of rodents (Pilosof *et al.*, 2012). Feeding efficiency is influenced by the anatomical characteristics of the host and the morphology of the ectoparasite, that is, the compatibility of the feeding apparatus of the parasite and the feeding site on the host. Ectoparasites with short proboscises select body parts with relatively thin epidermis and capillaries close to the body surface, whereas ectoparasites with relatively long proboscises select body parts with a thicker epidermis and deeper capillaries (Pilosof *et al.*, 2012). It is also important for ticks that the animal's mouth or claws are out of reach to prevent their removal during feeding. Host defence efforts and efficiency vary by body site (Reiczigel and Rózsa, 1998), and it has been reported that many ticks that feed on birds prefer their heads as feeding sites because they are difficult to be removed by grooming by the host (Fracasso *et al.*, 2019).

Another important factor affecting the spatiotemporal distribution of ticks is interspecific interactions. Such interspecific interactions include cases where 1 parasitic species promotes or inhibits infestation by another species (Fenton *et al.*, 2010; Hellard *et al.*, 2015). For example, some hard ticks (Acari: Ixodidae) that feed on the eastern rock sengis (*Elephantulus myurus*) may facilitate the invasion of other tick species onto the same host (no records of attachment sites) (Lutermann *et al.*, 2015). In contrast, *Ixodes scapularis* and *Dermacentor albipictus* have been reported to compete for preferred attachment sites on white-tailed deer (*Odocoileus virginianus*) (Fellin and Schulte-Hostedde, 2022). In addition to interspecific relationships, facilitative interactions within the same tick species have been reported, possibly due to the decreased immune response of the host as the tick infestation burden increases (Lutermann *et al.*, 2015).

Understanding tick infestation patterns in host animals is important for efficient tick sampling, effective treatment of animals with repellents and acaricides, and, consequently, control of tick-borne diseases (TBDs) (Schmidtman *et al.*, 1998). Hokkaido sika deer (*Cervus nippon yesoensis*), a subspecies of the sika deer (*Cervus nippon*), is one of the most important hosts for

several tick species of the genera *Ixodes* and *Haemaphysalis* (Tsukada *et al.*, 2014; Elbaz *et al.*, 2020; Iijima *et al.*, 2022). The population of sika deer in Hokkaido has increased over the last 20–30 years (approximately 670 000 between 2014 and 2020) (Ikeda and Koizumi, 2024). Because deer are known carriers of several tick-borne pathogens such as Piroplasmida and *Anaplasma* (Lee *et al.*, 2014; Elbaz *et al.*, 2017), it is of critical importance to understand the spatiotemporal distribution of ticks on Hokkaido sika deer.

The aim of this study was to understand tick infestation patterns in Hokkaido sika deer. More specifically, the research focused on seasonal differences in infestation patterns, preferred attachment sites for each tick species and interspecific interactions between different tick species.

Materials and methods

Study area

This study was conducted in the Rusha area of the Shiretoko Peninsula, eastern Hokkaido, Japan (44°11'N, 145°11'E). The area from the centre of the peninsula to Shiretoko Cape, including the research site, is designated Shiretoko National Park and is a UNESCO World Heritage Site where hunting is prohibited. This area is a narrow estuary coast that stretches from south to north for approximately 3 km, and its climate is classified as Subarctic. Climate information for the sampling period in Utoro (44°03'N, 144°59'E), the closest climate observation point to the study area, is shown in Table 1. Based on the definitions provided by the Japan Meteorological Agency, March–May is defined as spring, June–August as summer, September–November as autumn and December–February as winter. Six native mammals, namely the Hokkaido sika deer, Hokkaido brown bear (*Ursus arctos yesoensis*), red squirrel (*Sciurus vulgaris*), red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and sable (*Martes zibellina*), have been identified in the northwestern part of the national park, including the study area (Kawamura *et al.*, 2022).

Sampling

Deer were captured 48 times for tick collection between June and October 2022, totalling 37 deer, 9 of which were captured more than once. Deer capture and sampling were conducted for 3–4 days each month during the study period. The deer were anaesthetized with 0.1 mg kg⁻¹ medetomidine (Dorbene Vet, Kyoritsu Seiyaku, Co., Tokyo, Japan) using a carbon dioxide-powered gun (Dan-Inject ApS, Borkop, Denmark) for intramuscular injection and awakened with 0.1 mg kg⁻¹ atipamezole (Atipame, Kyoritsu Seiyaku, Co., Tokyo, Japan) injected intramuscularly after all procedures were completed. The design of the capture process was based on previous studies (Onuma *et al.*, 2005).

Deer age was estimated based on tooth eruption and replacement (Ohtaishi, 1977). The sample population consisted of the following age and sex structure: two 1-year-old females, one 2-year-old male, eight 2-year-old females and 26 females aged ≥3 years. All captured deer were tagged with earmarks for identification, and individual deer were not captured more than twice in the same month to ensure that recapture did not affect sampling. Ticks were collected from the head, pinna, body (neck: 50 × 70 mm², body side: 100 × 150 mm² and anus tail) and legs (elbow: 50 × 70 mm², forelimb, knee: 50 × 70 mm² and hindlimb) of the sika deer; only the left half of the body was examined (Fig. 1). Ticks found on the host were recorded as either feeding or questing. Owing to the extremely low number of questing ticks (only a few *Ixodes* spp. males), no distinction between the feeding and questing states was made in subsequent data analyses. The sampling sites were chosen based on previous studies (Matthee *et al.*, 1997; Kiffner *et al.*, 2011; Mysterud *et al.*, 2014). The collected ticks were identified by species, sex and life stage based on their morphological characteristics (Yamaguti *et al.*, 1971) using a stereomicroscope. Due to the difficulty in accurately identifying larvae morphologically, they were not included in the analysis.

Statistical analysis

The abundance and prevalence of each tick species attached to each deer per month were determined. Tick abundance was defined as the mean number of each tick species attached per deer per month, whereas tick prevalence was defined as the proportion of infested individuals among the captured deer. The monthly mean number of ticks per body part was calculated to determine the body parts of the deer that were preferred for tick attachment. The number of ticks on different body parts was compared using the Kruskal–Wallis test (Vargha and Delaney, 1998), with 3 degrees of freedom. Subsequent post-hoc analysis was conducted using the Steel–Dwass method for multiple comparison tests (Neuha and Bretz, 2001). The Kruskal–Wallis test and Steel–Dwass post-hoc analysis were performed separately for each tick life stage (males, females and nymphs) as well as for the total number of ticks across all stages, to compare tick distributions among different body parts. Because most of the captured deer were females aged ≥3 years, comparisons of tick infestation patterns by deer sex or age were not made. All statistical analyses were performed using R version 4.1.0 (R Core Team, 2021), and differences were considered statistically significant at *P* < 0.05. The co-occurrence patterns of different tick species and stages across attachment sites (each body part of the deer) were investigated to evaluate the inter- and intraspecific relationships between ticks. The ‘co-occurrence’ package in R (Griffith *et al.*, 2016) was used to conduct a pairwise co-occurrence analysis of the species for this analysis, determining

Table 1. Climate information for the sampling period in Utoro, Japan, the closest climate observation point to the study area

Month	Precipitation (mm)		Temperature (°C)		
	Total	Highest day	Daily average	Daily high	Daily low
June	127.5	29.5	12.6	17.0	9.0
July	80.0	28.5	19.0	24.1	14.8
August	157.5	48.5	19.6	24.5	15.2
September	107.5	48.5	16.9	22.6	11.8
October	130.5	38.0	10.7	15.3	6.5

The data are provided by the Japan Meteorological Agency, Japan.

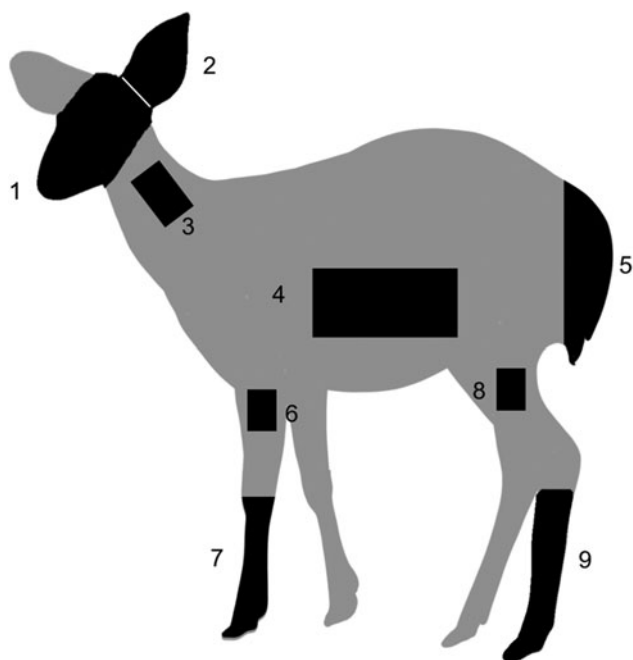


Figure 1. Diagram of the examined sites on the body of the sika deer: (1) head, (2) pinna, body (3: neck $50 \times 70 \text{ mm}^2$, 4: body side $100 \times 150 \text{ mm}^2$ and 5: anus and tail) and legs (6: elbow $50 \times 70 \text{ mm}^2$, 7: forelimb, 8: knee $50 \times 70 \text{ mm}^2$ and 9: hind limb); each part was examined only on the left side.

whether the co-occurrences were positive, negative or random. This package uses an algorithm that calculates the observed and expected frequencies of co-occurrence between each pair of species and stages based on the presence/absence data across a collection on each body part. These associations were interpreted as negative or positive co-occurrences when the co-occurrence frequencies were much lower or higher than expected, respectively. In this study, 4 body parts (head, pinna, body and legs) were examined for each of the 48 host individuals, resulting in a total of 192 observations ($48 \text{ individuals} \times 4 \text{ body parts}$).

Pairs with expected co-occurrence numbers of <1 were excluded from the analysis to minimize the likelihood of spurious results.

Results

All captured deer were infested with at least 1 tick, and 3026 ticks were collected during the study period. The overall composition of the tick population from 48 samplings of sika deer was 21.9% female (663/3026), 52.0% male (1574/3026) and 26.1% nymphs (789/3026). The tick species identified were *Ixodes ovatus* ($n = 441$), *Ixodes persulcatus* ($n = 538$), *Haemaphysalis japonica* ($n = 1793$) and *Haemaphysalis megaspinosus* ($n = 254$) (Table 2). The stage composition of attached ticks varied by species, with *I. ovatus* (males: 51, females: 379 and nymphs: 11), *H. japonica* (males: 1365, females: 135 and nymphs: 293) and *H. megaspinosus* (males: 134, females: 113 and nymphs: 7) being the predominant adults, whereas *I. persulcatus* (males: 24, females: 36 and nymphs: 478) were predominant at the nymphal stage (Table 2).

Monthly variation in tick infestation showed different patterns for each species (Table 2 and Fig. 2). The highest infestation by *I. ovatus* was observed in June ($n = 256$), followed by that in July ($n = 133$), August ($n = 39$), September ($n = 12$) and October ($n = 1$). Infestations of *I. persulcatus* nymph were relatively consistent throughout the sampling period, with a gradual decrease from June to October (June: $n = 156$; July: $n = 131$; August: $n = 151$; September: $n = 68$; October: $n = 32$). In contrast, the number

of *H. japonica* decreased from June to August (June: $n = 201$; July: $n = 93$; August: $n = 65$) but increased rapidly from September ($n = 441$) to October ($n = 993$). *Haemaphysalis megaspinosus* was not observed in June, July or August but appeared in September ($n = 126$) and October ($n = 126$).

The peak prevalence of *I. ovatus* and *H. megaspinosus* was observed in the same month as their peak abundances (Table 2 and Fig. 2). The prevalence of *I. persulcatus*, similar to its abundance, showed little monthly variation and was always higher than 70%. The trend in the prevalence of *H. japonica* was different from that of the other 3 species, with a high prevalence from June to August, even when the abundance was relatively low compared to September to October (June: 90%, July: 80%, August: 100%).

This study revealed that the preference for the body parts to which ticks attach varies significantly depending on the tick species (Fig. 3). In *I. ovatus* adults, significantly higher numbers were observed on the pinna than on other body parts from June to August (June: H -statistic = 36.0; July: H -statistic = 20.8 and August: H -statistic = 18.4). Similarly, *I. persulcatus* nymphs were significantly more abundant in the pinna, and this trend was observed throughout the sampling period, whereas *I. persulcatus* adults showed a slight preference for the head (June: H -statistic = 17.5; July: H -statistic = 29.6; August: H -statistic = 27.9; September: H -statistic = 25.9; October: H -statistic = 20.8). Conversely, *H. japonica* exhibited notable monthly variations in attachment sites. It was primarily found on the body and legs in June, July and August (June: H -statistic = 19.3; July: H -statistic = 18.0; August: H -statistic = 28.4); however, a significant shift was observed in September and October, with a marked aggregation on pinnae across all stages (September: H -statistic = 25.5; October: H -statistic = 17.1). This trend was observed for both nymphs and adults, but only the total number of all stages and the number of males showed statistically significant differences between September and October. In contrast to the other 3 species, which were highly aggregated on specific body parts, *H. megaspinosus* was widely distributed on the head and body, with preference for the pinna (September: H -statistic = 9.2; October: H -statistic = 12.8).

In the analysis of co-occurrence patterns among ticks, 66 pairs of combinations derived from different tick species and stages were considered. Consequently, 13 pairs were removed from the analysis because their expected co-occurrence was <1 , leaving 53 pairs for further analysis. Positive (28%, 15/53), negative (11%, 6/53) and random co-occurrence patterns (60%, 32/53) were observed (Fig. 4). Ticks of the same species and genus tended to exhibit positive co-occurrence patterns. In particular, positive co-occurrence patterns between females and males of the same species were observed in all species and between females and nymphs in *I. persulcatus* and *H. japonica*. Conversely, females of *I. ovatus* showed negative co-occurrence patterns with all stages of *H. japonica* and both males and females of *H. megaspinosus*.

Discussion

This is the first detailed study of tick infestation patterns in the Hokkaido sika deer. Seasonal activity, attachment site preferences and co-occurrence patterns of tick species were investigated. In the current study, 4 tick species, *I. ovatus*, *I. persulcatus*, *H. japonica* and *H. megaspinosus*, were collected from Hokkaido sika deer, and each species showed unique seasonal fluctuations. Specific attachment site preferences were observed for each tick species, with *H. japonica* showing seasonal variation in attachment site preferences. Ticks showed various co-occurrence patterns on the body surfaces of deer, suggesting the presence of inter- and intra-specific interactions between ticks and their attachment sites. These results provided robust evidence that complex host-tick

Table 2. Number of ticks collected from Hokkaido sika deer from June to October 2022

Sampling month		Jun.	Jul.	Aug.	Sep.	Oct.	Total
No. of deer sampled		11	10	10	10	7	48
Species	Stage						
<i>Ixodes ovatus</i>	Male	28	13	8	2	0	51
	Female	227	120	23	9	0	379
	Nymph	1	0	8	1	1	11
	Subtotal	256	133	39	12	1	441
<i>Ixodes persulcatus</i>	Male	12	5	2	2	3	24
	Female	23	4	2	3	4	36
	Nymph	121	122	147	63	25	478
	Subtotal	156	131	151	68	32	538
<i>Haemaphysalis japonica</i>	Male	76	24	0	344	921	1365
	Female	53	30	8	34	10	135
	Nymph	72	39	57	63	62	293
	Subtotal	201	93	65	441	993	1793
<i>Haemaphysalis megaspinoso</i>	Male	0	0	0	49	85	134
	Female	0	0	0	76	37	113
	Nymph	0	0	0	1	6	7
	Subtotal	0	0	0	126	128	254
Total		613	357	255	647	1154	3026

and tick–tick interactions exist during the selection of tick attachment sites, as previously suggested (Andrews *et al.*, 1981; Chilton *et al.*, 1992; Kiffner *et al.*, 2011; Baer-Lehman *et al.*, 2012; Mysterud *et al.*, 2014; Lydecker *et al.*, 2019; Tiffin *et al.*, 2021).

Adults of *I. ovatus* were collected in the highest numbers in June and then declined towards October. Previous studies on the seasonal activity of *I. ovatus* in the natural environment of Honshu, Japan reported that *I. ovatus* was highly active from

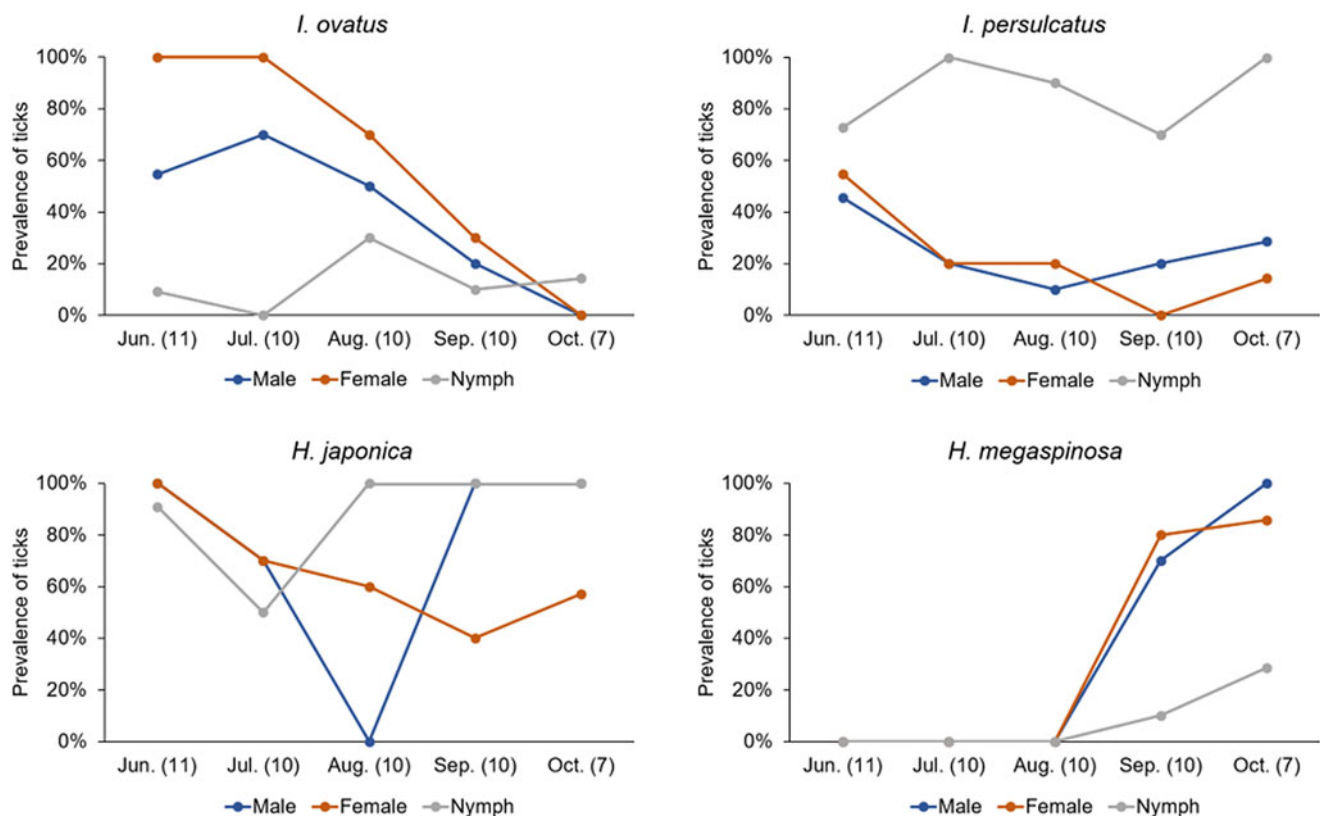


Figure 2. Seasonality of the prevalence of each tick species and their developmental stages parasitizing sika deer from June 2022 to October 2022. The horizontal axis represents the sampling month (number of deer).

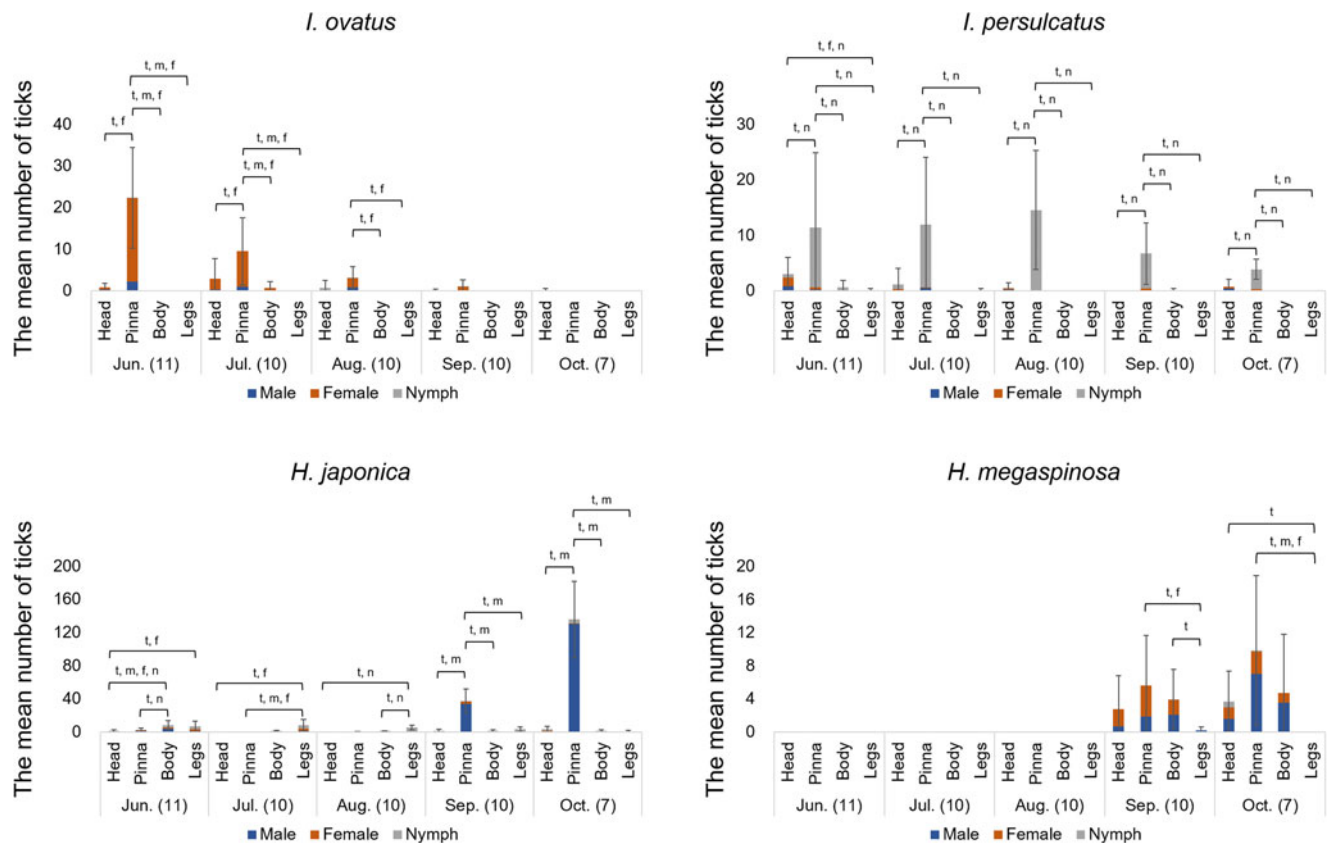


Figure 3. Seasonal occurrence of ticks on the examined attachment sites of infested sika deer from June 2022 to October 2022. Error bars represent standard errors of the total number of ticks attached to each site, and the horizontal axis represents the sampling month (number of deer). (t, m, f, n: $P < 0.05$, based on the Steel–Dwass method for total, male, female and nymph ticks, respectively.).

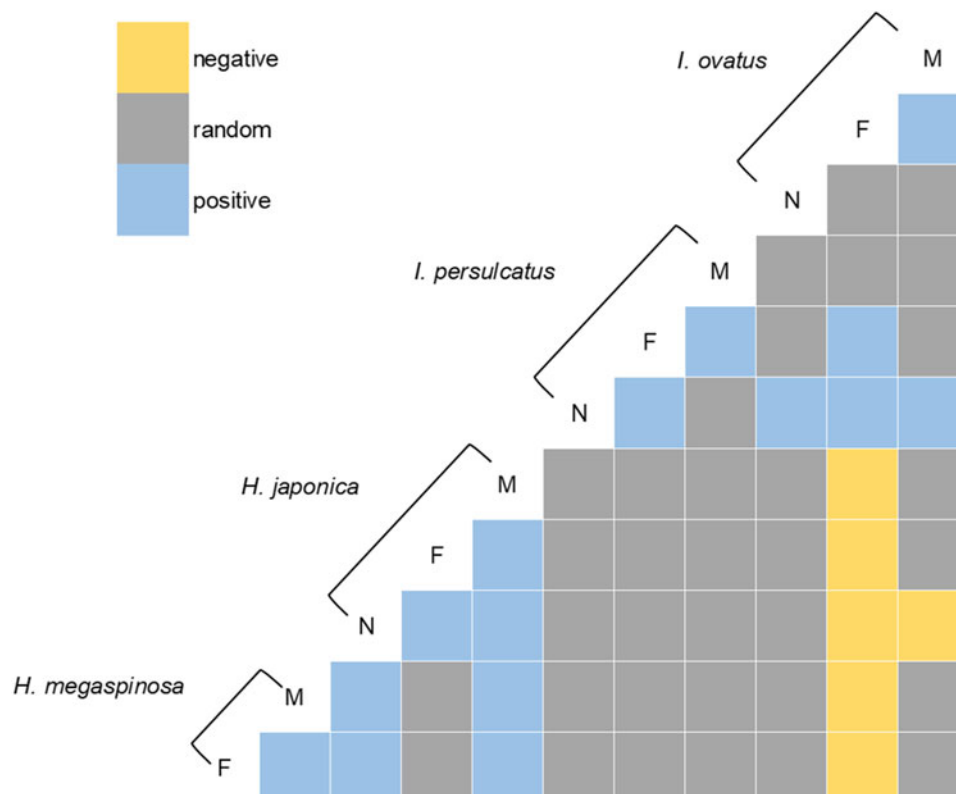


Figure 4. Heat map representing positive, negative and random associations of tick species/stages found in each body part of individual sika deer, as determined by the stochastic co-occurrence model in the ‘co-occurrence’ package in R. Species names and stages are arranged to show columns and rows representing pair-wise relationships with other species/stages (M; male, F; female, N; nymph).

April to mid-July but declined markedly in late-August (Fujimoto, 1996, 1997). Although sampling prior to June was not possible in this study, the activity pattern of *I. ovatus*, with a gradual decline beginning in June, was consistent with the activity pattern of this species reported in previous studies. In contrast, nymphs peaked in abundance and prevalence in August, but the number collected was very small throughout the study period. In general, larvae and nymphs of 2- and 3-host ticks prefer smaller animals, whereas adults prefer larger animals (McCoy *et al.*, 2013). Given that several small- and medium-sized animal species are present in the study area (Kawamura *et al.*, 2022), it is likely that other animal species serve as primary hosts for *I. ovatus* nymphs. This preference for smaller hosts likely contributed to the low number of nymphs collected from the deer in this study.

In contrast to the other 3 species that displayed clear seasonal variation, *I. persulcatus* abundance remained stable throughout the study period. A study in Finland reported that *I. persulcatus* nymphs survived for long periods without exhibiting clear seasonal fluctuations (Pakanen *et al.*, 2020), which is consistent with the results of the current study. Notably, nymphs were predominant throughout the sampling period, and adult ticks were present in very small numbers. As mentioned above, it is well known that nymphal ticks prefer small- to medium-sized mammals as hosts, while adult ticks tend to parasitize larger mammalian species (McCoy *et al.*, 2013). However, the scarcity of *I. persulcatus* adults in this study suggested that there are other suitable hosts for these adults in Hokkaido. The most likely host of *I. persulcatus* adults is the Hokkaido brown bear, another large mammal present in Hokkaido. A previous study examining ticks infesting Hokkaido brown bears reported that most *I. persulcatus* infesting bears were adult ticks (Ozawa and Kadosaki, 1996). However, it should be noted that the previous and current studies have been conducted in different areas of Hokkaido (Ozawa and Kadosaki, 1996). Further investigations involving multiple wild animals at the same location are necessary to gain a better understanding of the host preferences of *I. persulcatus*.

The total number of *H. japonica* was low in summer but increased significantly in autumn. Furthermore, the increase in number from September onwards was primarily due to the presence of males, whereas the numbers of females and nymphs did not show such significant fluctuations. A similar seasonal variation was observed for *H. megaspinosa*, which was collected only from September onwards, with males predominating in October. A previous study on Hokkaido brown bears reported that the abundance of *H. japonica* and *H. megaspinosa* increased from November, which is somewhat different from the results of the current study (Ozawa and Kadosaki, 1996). Although geographic and temporal gaps between this and previous studies should be noted, these differences in results suggested that ticks establish different relationships with different animal species. The male-biased attachment in *Haemaphysalis* spp. observed in the current study has also been previously reported and may be related to reproductive strategies (Ozawa and Kadosaki, 1996; Tsunoda, 2012). Metastriate ticks, including *Haemaphysalis* spp., secrete pheromones that control aggregation, mating and ejaculation after attachment to the host, and they mate only on the body surface of the host, whereas *Ixodes* spp. frequently mate before attachment to the host (Kiszewski *et al.*, 2001). Therefore, it is advantageous for males of *Haemaphysalis* spp. to attach to their hosts earlier than females, wait for mates and remain on the host longer after mating (Tsunoda, 2012). Another possibility is that unmated males attempt to overwinter on the host (Ozawa and Kadosaki, 1996; Doi *et al.*, 2018); however, research on the overwintering strategies of ticks is limited (Kim *et al.*, 2020), and no conclusive statements can be made.

Co-occurrence analysis revealed positive co-occurrence patterns between male and female ticks of the same species, and these patterns were observed in all tick species. These results suggested that the reproductive strategy of ticks promotes male–female aggregation, as previously reported (Sonenshine, 1985, 2004). Additionally, positive co-occurrence patterns between females and nymphs were observed for *I. persulcatus* and *H. japonica*. Differences in the attachment sites at different life stages have been reported in several species (Tiffin *et al.*, 2021). Indeed, *I. persulcatus* nymphs were clustered on the pinna, whereas adults were attached to both the pinna and head, with a slightly higher frequency of attachment to the head. However, these two stages shared the same attachment sites on the head and pinna, possibly resulting in a positive co-occurrence pattern. These results are interesting when considering disease dynamics in nature via co-feeding transmission. Co-feeding transmission occurs when pathogens are transmitted between infected and naïve vectors that feed in close temporal and spatial proximity to hosts that have not yet developed systemic infections (Belli *et al.*, 2017). Co-feeding transmission has been confirmed in several animals and tick species for some pathogens, such as tick-borne encephalitis virus (Labuda *et al.*, 1993, 1996) and *Borrelia burgdorferi* (Gern and Rais, 1996; Ogden *et al.*, 1997; Gern *et al.*, 1998; Randolph *et al.*, 2003). Both pathogens are vectored by *I. persulcatus* in Hokkaido (Hayasaka *et al.*, 1999; Muto *et al.*, 2015; Okado *et al.*, 2021). The results of this study suggested that similarities in attachment site preference between ticks at different stages could potentially facilitate co-feeding transmission. Hence, this study has important implications for understanding the dynamics of TBDs in wildlife and highlights the need for further research to understand how ticks of different species and stages interact with each other. The results of the co-occurrence analysis of different species showed negative co-occurrence patterns between *I. ovatus* and *Haemaphysalis* spp. Three hypotheses may explain the positive and negative co-occurrence patterns of ticks among different species.

The first hypothesis was that similarities and differences in phenology between the same or different genera and species affect co-occurrence patterns. Indeed, previous studies have reported phenological differences between *Ixodes* spp. and *Haemaphysalis* spp. and similarities within the same genus (Ozawa and Kadosaki, 1996; Kanamori *et al.*, 2009). In the current study, *I. ovatus* and *H. megaspinosa* peaked in abundance and infestation rates in summer and autumn, respectively. A previous study also reported clear phenological differences between the two species (Ozawa and Kadosaki, 1996). Based on these results, phenological differences may be the most plausible explanation for the negative co-occurrence patterns observed between the two species.

The second hypothesis was that morphological similarities within the same taxon promote positive co-occurrence among tick species of the same genus. A previous study on similar attachment site preferences within the same subgroups of ectoparasites supports this hypothesis (Pilosof *et al.*, 2012). The most prominent morphological difference between *Ixodes* spp. and *Haemaphysalis* spp. is the length of their mouthparts, which consists of a pair of chelicerae and a single hypostome. *Ixodes* spp. have longer and sharper mouthparts than *Haemaphysalis* spp. and secrete fewer cementing substances to hold the body in place during engorgement (Balashov, 1972). Such morphological differences could influence the availability of host blood vessels and resistance to grooming (Pilosof *et al.*, 2012) and may explain the similarities in attachment site preferences among the same genus groups. The fact that *I. ovatus* adults and *I. persulcatus* nymphs were primarily attached to the pinna during the study period suggests a high degree of compatibility with the pinna. The pinna has been reported to be the preferred feeding site for

certain ticks that feed on ungulates because it is difficult for the host to groom this area with its mouth and legs (Matthee *et al.*, 1997; Handeland *et al.*, 2013; Myserud *et al.*, 2014). In contrast, the distribution of *H. megaspinosa* was wider than that of the other tick species found in the current study and was distributed on the head, pinna and body. Similar attachment patterns have been observed in other tick species (Sundstrom *et al.*, 2021; Tiffin *et al.*, 2021). This suggested that some species may have adapted to a wide range of host body parts. However, the negative co-occurrence patterns of *I. ovatus* and *H. japonica* could not be explained by phenological and morphological differences alone. This is because, although both species had a high prevalence in June and July, the attachment sites were separate, and *H. japonica* showed dynamics such that it moved to the pinna from September onwards when *I. ovatus* was no longer present. This suggested that other factors play a role in the co-occurrence patterns.

The third hypothesis was that negative co-occurrence patterns were formed to avoid interference due to similarities in semiochemical communication, which may explain the patterns of *I. ovatus* and *H. japonica*. Semiochemical-mediated changes in tick behaviour and intra- and interspecific communication are well known (Sonenshine, 2004), and in some cases, some species use the same chemicals. For example, it has been reported that *Ixodes ricinus* and *I. scapularis* exhibit similar arrest behaviours by guanine and xanthine (Sonenshine, 2004). In metastriate ticks, differences in the concentrations of 2,6-dichlorophenol induce different reactions depending on the species (Sonenshine, 1985). Furthermore, when reptile ticks, such as *Bothriocroton hydrosauri*, *Amblyomma albolimbatum* and *Amblyomma limbatum* infest the same host, the movement of males searching for sexually receptive females is constrained, and males display courtship behaviour towards females of different species (Andrews *et al.*, 1982a). It has been suggested that such signal interference may promote spatial separation among tick species (Andrews *et al.*, 1982b; Chilton *et al.*, 1992). Although there have been no previous reports on the interference of semiochemical communication between *I. ovatus* and *H. japonica*, this is the first study to suggest an interspecific interaction between these two species at the field level. Laboratory-based behavioural studies are necessary to investigate the semiochemical-mediated relationship between these two species.

Random co-occurrence was the most frequently observed among all the co-occurrence patterns. It is well known that ticks of different species feed on the same host (Baer-Lehman *et al.*, 2012; Gómez-Rodríguez *et al.*, 2015; Cayol *et al.*, 2018). Therefore, it is not surprising that the neutral interspecific relationships suggested by the random co-occurrence patterns were most frequently observed. Neutral interspecific relationships are beneficial when sufficient resources (hosts) are available for tick survival (Canard *et al.*, 2012; Butler *et al.*, 2021). The tick species collected in this study are generalists that feed on various hosts (Ozawa and Kadosaki, 1996; Sashika *et al.*, 2011; Tsunoda, 2012; Elbaz *et al.*, 2020). Furthermore, sika deer are bigger than other land mammals (the average was 76.2 kg in this study), providing ticks with a large feeding area and abundant blood meals (Iijima *et al.*, 2022), and deer density in the study area has been reported to be very high (Uno *et al.*, 2022). Therefore, it is likely that ticks have sufficient feeding resources in the study area and that a neutral relationship prevailed.

This study suggested that each tick species has unique attachment site preferences on the Hokkaido sika deer body surface and positive, negative or random co-occurrence patterns with ticks of both conspecific and heterospecific groups. However, it is important to note that the observed interactions between tick species

might be the result of various confounding factors. The risk of inferring species interactions from observational data has been previously discussed. Confounding effects may occur at the host individual (host age and sex), population (host sex ratio, age structure and density), parasite (presence of risk factors shared by two or more parasites) or landscape level (geological and climatic factors) (Hellard *et al.*, 2015). In particular, it was not possible to examine how deer host characteristics, such as sex and age affect tick infestation. Nonetheless, this is the first study to suggest the possibility of tick species interactions on wild sika deer, highlighting the need for further experimental studies to elucidate the underlying mechanisms.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements. The authors acknowledge the Shiretoko Nature Foundation staff, Laboratory of Parasitology and Laboratory of Wildlife Biology and Medicine (Hokkaido University, School of Veterinary Medicine) for their technical assistance and sample collection and Editage (www.editage.jp) for English language editing by a native speaker.

Author contributions. K. S., M. S., R. N. and T. T. conceived and planned the project, obtained funding and wrote the manuscript. K. S., M. S., M. Y., G. I. and T. T. conducted the sampling. K. S. identified the ticks and statistical processing.

Financial support. This work was supported by the JST SPRING (grant PH8D230001), Asahi Glass Foundation (grant 2021-94), the Japan Society for the Promotion of Science (grant 21H02350 and 23K23770) and World-leading Innovative and Smart Education (WISE) Program (grant 1801).

Competing interests. None.

Ethical standards. All procedures involving the capture and sampling of live animals were conducted in accordance with the Guidelines for Animal Care and Use, Hokkaido University, and with the approval of the Animal Care and Use Committee of the Graduate School of Veterinary Medicine, Hokkaido University (Permit Number: 22-0016).

References

- Andrews RH, Petney TN and Park B (1981). Competition for sites of attachment to hosts in three parapatric species of reptile tick. *Oecologia* **51**, 227–232.
- Andrews RH, Petney TN and Bull CM (1982a). Reproductive interference between three parapatric species of reptile tick. *Oecologia* **52**, 281–286.
- Andrews RH, Petney TN and Bull CM (1982b). Niche changes between parasite populations: an example from ticks on reptiles. *Oecologia* **55**, 77–80.
- Baer-Lehman ML, Light T, Fuller NW, Barry-Landis KD, Kindlin CM and Stewart RL (2012). Evidence for competition between *Ixodes scapularis* and *Dermacentor albipictus* feeding concurrently on white-tailed deer. *Experimental and Applied Acarology* **58**, 301–314.
- Balashov YS (1972). Bloodsucking ticks (Ixodoidea)-vectors of diseases of man and animals. Translated by O. G. Strekalovski. *Miscellaneous Publications of the Entomological Society of America* **8**, 161–376.
- Belli A, Sarr A, Rais O, Rego ROM and Voordouw MJ (2017). Ticks infected via co-feeding transmission can transmit *Lyme borreliosis* to vertebrate hosts. *Scientific Reports* **7**, 1–13.
- Butler RA, Kennedy ML, Houston AE, Bowers EK, Coons LB, Paulsen D and Trout Fryxell RT (2021). No evidence of competition between the blacklegged tick (Acari: Ixodidae) and American dog tick on the rodent host white-footed deer mouse (Rodentia: Cricetidae) in Southwestern Tennessee. *Journal of Medical Entomology* **58**, 1470–1475.
- Canard E, Mouquet N, Marescot L, Gaston KJ, Gravel D and Mouillot D (2012). Emergence of structural patterns in neutral trophic networks. *PLoS ONE* **7**, 1–8.
- Cayol C, Jääskeläinen A, Koskela E, Kyröläinen S, Mappes T, Siukola A and Kallio ER (2018). Sympatric *Ixodes*-tick species: pattern of distribution and pathogen transmission within wild rodent populations. *Scientific Reports* **8**, 1–10.

- Chilton NB, Bull CM and Andrews RH (1992) Niche segregation in reptile ticks: attachment sites and reproductive success of females. *Oecologia* **90**, 255–259.
- Doi K, Kato T and Hayama SI (2018) Infestation of introduced raccoons (*Procyon lotor*) with indigenous ixodid ticks on the Miura Peninsula, Kanagawa Prefecture, Japan. *International Journal for Parasitology: Parasites and Wildlife* **7**, 355–359.
- Elbaz E, Moustafa MAM, Lee K, Mohamed WMA, Nakao R, Shimozuru M, Sashika M, Younis EEA, El-khodery SA and Tsubota T (2017) Molecular identification and characterization of piroplasm species in Hokkaido sika deer (*Cervus nippon yesoensis*), Japan. *Ticks and Tick-Borne Diseases* **8**, 802–807.
- Elbaz E, Moustafa MAM, Lee K, Ching ALC, Shimozuru M, Sashika M, Nakao R, El-khodery SA and Tsubota T (2020) Utilizing attached hard ticks as pointers to the risk of infection by *Babesia* and *Theileria* species in sika deer (*Cervus nippon yesoensis*), in Japan. *Experimental and Applied Acarology* **82**, 411–429.
- Fellin E and Schulte-Hostedde A (2022) Effects of ticks on community assemblages of ectoparasites in deer mice. *Ticks and Tick-Borne Diseases* **13**, 101846.
- Fenton A, Viney ME and Lello J (2010) Detecting interspecific macroparasite interactions from ecological data: patterns and process. *Ecology Letters* **13**, 606–615.
- Fracasso G, Matthysen E, Dhondt AA and Heylen D (2019) Experimental study of micro-habitat selection by ixodid ticks feeding on avian hosts. *International Journal for Parasitology* **49**, 1005–1014.
- Fujimoto K (1996) Effect of repeated removal in a survey the seasonal activity of *Ixodes ovatus* adults (Acari: Ixodidae) by the flagging method. *Medical Entomology and Zoology* **47**, 375–377.
- Fujimoto K (1997) Disappearance of *Ixodes ovatus* adults (Acari: Ixodidae) in autumn clarified by analysis of field and experimental studies. *Medical Entomology and Zoology* **48**, 275–278.
- Gern L and Rais O (1996) Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). *Journal of Medical Entomology* **33**, 189–192.
- Gern L, Estrada-Peña A, Frandsen F, Gray JS, Jaenson TGT, Jongejan F, Kahl O, Korenberg E, Mehl R and Nuttall PA (1998) European reservoir hosts of *Borrelia burgdorferi* sensu lato. *Zentralblatt für Bakteriologie* **287**, 196–204.
- Gómez-Rodríguez RA, Gutiérrez-Granados G, Montiel-Parra G, Rodríguez-Moreno Á and Sánchez-Cordero V (2015) Diversity and coexistence of ectoparasites in small rodents in a tropical dry forest. *Biotropica* **47**, 484–490.
- Griffith DM, Veech JA and Marsh CJ (2016) Cooccur: probabilistic species co-occurrence analysis in R. *Journal of Statistical Software* **69**, 1–17.
- Handeland K, Qviller L, Vikoren T, Viljugrein H, Lillehaug A and Davidson RK (2013) *Ixodes ricinus* infestation in free-ranging cervids in Norway – a study based upon ear examinations of hunted animals. *Veterinary Parasitology* **195**, 142–149.
- Hayasaka D, Suzuki Y, Kariwa H, Ivanov L, Volkov V, Demenev V, Mizutani T, Gojobori T and Takashima I (1999) Phylogenetic and virulence analysis of tick-borne encephalitis viruses from Japan and far-eastern Russia. *Journal of General Virology* **80**, 3127–3135.
- Hellard E, Fouchet D, Vavre F and Pontier D (2015) Parasite–parasite interactions in the wild: how to detect them? *Trends in Parasitology* **31**, 640–652.
- Iijima H, Watari Y, Furukawa T and Okabe K (2022) Importance of host abundance and microhabitat in tick abundance. *Journal of Medical Entomology* **59**, 2110–2119.
- Ikeda T and Koizumi I (2024) Evaluation of multiple behavioral responses of sika deer to human hunting pressures. *Journal of Wildlife Management* **88**, 1–13.
- Kanamori H, Kawabata H, Arai S, Katayama T, Fujita H, Yano Y and Takada N (2009) Tick fauna associated with sika deer density in the Shimane Peninsula, Honshu, Japan. *Medical Entomology and Zoology* **60**, 297–304.
- Kar S, Dervis E, Akın A, Ergonul O and Gargili A (2013) Preferences of different tick species for human hosts in Turkey. *Experimental and Applied Acarology* **61**, 349–355.
- Kawamura K, Jimbo M, Adachi K, Shirane Y, Nakanishi M, Umemura Y, Ishinazaka T, Uno H, Sashika M, Tsubota T and Shimozuru M (2022) Diel and monthly activity pattern of brown bears and sika deer in the Shiretoko Peninsula, Hokkaido, Japan. *Journal of Veterinary Medical Science* **84**, 1146–1156.
- Kiffner C, Lödige C, Alings M, Vor T and Rühle F (2011) Attachment site selection of ticks on roe deer, *Capreolus capreolus*. *Experimental and Applied Acarology* **53**, 79–94.
- Kim JY, Jung M, Kho JW, Song H, Moon KH, Kim YH and Lee DH (2020) Characterization of overwintering sites of *Haemaphysalis longicornis* (Acari: Ixodidae) and tick infection rate with severe fever with thrombocytopenia syndrome virus from eight provinces in South Korea. *Ticks and Tick-borne Diseases* **11**, 101490.
- Kiszewski AE, Matuschka F and Spielman A (2001) Mating strategies and spermiogenesis in ixodid ticks. *Annual Review of Entomology* **46**, 167–182.
- Labuda M, Nuttall PA, Kožuch O, Elecková E, Williams T, Žuffová E and Sabó A (1993) Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. *Experientia* **49**, 802–805.
- Labuda M, Austyn JM, Zuffova E, Kozuch O, Fuchsberger N, Lysy J and Nuttall PA (1996) Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology* **219**, 357–366.
- Lee K, Takano A, Taylor K, Sashika M, Shimozuru M, Konnai S, Kawabata H and Tsubota T (2014) A relapsing fever group *Borrelia* sp. similar to *Borrelia lonestari* found among wild sika deer (*Cervus nippon yesoensis*) and *Haemaphysalis* spp. ticks in Hokkaido, Japan. *Ticks and Tick-borne Diseases* **5**, 841–847.
- Lutermann H, Fagir DM and Bennett NC (2015) Complex interactions within the ectoparasite community of the eastern rock sengi (*Elephantulus myurus*). *International Journal for Parasitology: Parasites and Wildlife* **4**, 148–158.
- Lydecker HW, Etheridge B, Price C, Banks PB and Hochuli DF (2019) Landscapes within landscapes: a parasite utilizes different ecological niches on the host landscapes of two host species. *Acta Tropica* **193**, 60–65.
- Matthee S, Meltzer DGA and Horak IG (1997) Sites of attachment and density assessment of ixodid ticks (Acari: Ixodidae) on impala (*Aepyceros melampus*). *Experimental and Applied Acarology* **21**, 179–192.
- McCoy KD, Léger E and Dietrich M (2013) Host specialization in ticks and transmission of tick-borne diseases: a review. *Frontiers in Cellular and Infection Microbiology* **4**, 1–12.
- Muto M, Bazartseren B, Tsevel B, Dashzevege E, Yoshii K and Kariwa H (2015) Isolation and characterization of tick-borne encephalitis virus from *Ixodes persulcatus* in Mongolia in 2012. *Ticks and Tick-borne Diseases* **6**, 623–629.
- Mysterud A, Hatlegjerde IL and Sørensen OJ (2014) Attachment site selection of life stages of *Ixodes ricinus* ticks on a main large host in Europe, the red deer (*Cervus elaphus*). *Parasites & Vectors* **7**, 1–5.
- Neuha M and Bretz F (2001) Nonparametric all-pairs multiple comparisons. *Biometrical Journal* **43**, 571–580.
- Ogden NH, Nuttall PA and Randolph SE (1997) Natural Lyme disease cycles maintained via sheep by cofeeding ticks. *Parasitology* **115**, 591–599.
- Ohtaishi N (1977) Age determination of mammals. *Mammalian Science* **17**, 1–17.
- Okado K, Adjou Moumouni PF, Lee SH, Sivakumar T, Yokoyama N, Fujisaki K, Suzuki H, Xuan X and Umemiya-Shirafuji R (2021) Molecular detection of *Borrelia burgdorferi* (sensu lato) and *Rickettsia* spp. in hard ticks distributed in Tokachi District, eastern Hokkaido, Japan. *Current Research in Parasitology and Vector-Borne Diseases* **1**, 100059.
- Onuma M, Takahashi H, Makoto A, Mayumi U, Masatsugu S and Koichi K (2005) The capture and chemical immobilization of wild Hokkaido sika deer (*Cervus nippon yesoensis*). *Japanese Society of Zoo and Wildlife Medicine* **10**, 19–26.
- Ozawa Y and Kadosaki M (1996) Seasonal dynamics of the population of the five species of ticks collected from the brown bear and its habitat in Hokkaido. *Journal of the Japanese Wildlife Research Society* **22**, 29–42.
- Pakanen VM, Sormunen JJ, Sippola E, Blomqvist D and Kallio ER (2020) Questing abundance of adult taiga ticks *Ixodes persulcatus* and their *Borrelia* prevalence at the north-western part of their distribution. *Parasites & Vectors* **13**, 1–9.
- Pilosof S, Lareschi M and Krasnov BR (2012) Host body microcosm and ectoparasite infracommunities: arthropod ectoparasites are not spatially segregated. *Parasitology* **139**, 1739–1748.
- Randolph S, Gern L, Richter D, Allgöwer R and Matuschka FR (2003) Co-feeding transmission and its contribution to the perpetuation of the Lyme disease spirochete *Borrelia afzelii*. *Emerging Infectious Diseases* **9**, 893–896.
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Reiczig J and Rózsa L (1998) Host-mediated site segregation of ectoparasites: an individual-based simulation study. *Journal of Parasitology* **84**, 491–498.

- Sashika M, Abe G, Matsumoto K and Inokuma H (2011) Molecular survey of *Anaplasma* and *Ehrlichia* infections of feral raccoons (*Procyon lotor*) in Hokkaido, Japan. *Vector-Borne and Zoonotic Diseases* **11**, 349–354.
- Schmidtman ET, Carroll JF and Watson DW (1998) Attachment-site patterns of adult blacklegged ticks (Acari: Ixodidae) on white-tailed deer and horses. *Journal of Medical Entomology* **35**, 59–63.
- Sonenshine DE (1985) Pheromones and other semiochemicals of the Acari. *Annual Review of Entomology* **30**, 1–28.
- Sonenshine DE (2004) Pheromones and other semiochemicals of ticks and their use in tick control. *Parasitology* **129**, 405–425.
- Sundstrom KD, Lineberry MW, Grant AN, Duncan KT, Ientile MM and Little SE (2021) Equine attachment site preferences and seasonality of common North American ticks: *Amblyomma americanum*, *Dermacentor albipictus*, and *Ixodes scapularis*. *Parasites & Vectors* **14**, 1–7.
- Tiffin HS, Skvarla MJ and Machtinger ET (2021) Tick abundance and life-stage segregation on the American black bear (*Ursus americanus*). *International Journal for Parasitology: Parasites and Wildlife* **16**, 208–216.
- Tsukada H, Nakamura Y, Kamio T, Inokuma H, Hanafusa Y, Matsuda N, Maruyama T, Ohba T and Nagata K (2014) Higher sika deer density is associated with higher local abundance of *Haemaphysalis longicornis* nymphs and adults but not larvae in central Japan. *Bulletin of Entomological Research* **104**, 19–28.
- Tsunoda T (2012) The tick fauna parasitizing middle- and large-sized mammals in Chiba Prefecture, Central Japan. *Journal of the Natural History Museum and Institute, Chiba* **1**, 33–42.
- Uno H, Inatomi Y, Ueno M and Kaji K (2022) Adaptive management of sika deer populations on Hokkaido Island, Japan. In Kaji K, Uno H and Iijima H (eds), *Sika Deer: Life History Plasticity and Management*. Singapore: Springer Nature Singapore, pp. 553–568.
- Vargha A and Delaney HD (1998) The Kruskal–Wallis test and stochastic homogeneity. *Journal of Educational and Behavioral Statistics* **23**, 170–192.
- Yamaguti N, Tipton VJ, Keegan HL and Toshioka S (1971) Ticks of Japan, Korea, and the Ryukyu Islands. *Brigham Young University Science Bulletin Biological Series* **15**, 1.