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Comparative Analysis of Prickles on *Rubus sieboldii* (Rosaceae) between Grazed and Ungrazed Areas in Southwestern Shikoku, Japan

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Abstract

Kashima Island (32º57′N, 132º27′E) is a very small island (45.1 ha) in southwestern Shikoku, Japan with a high density (ca. 38.5/km^2) of Sika deer (*Cervus nippon*). To examine induced defences of plants against the Sika deer, we conducted morphological analyses of prickles of *Rubus sieboldii* Blume (Rosaceae) between grazed (Kashima Island) and ungrazed areas (neighbour locations) in southwestern Shikoku. The length and density of prickles on leaves and around stems were measured. The prickles of the plants on Kashima Island were significantly longer and denser than those of the other areas, implying that the increased length and density of prickles were an induced defence of *R. sieboldii* on Kashima Island.

Keywords: island, sika deer, herbivory, density, blackberry, prickle length, defence

1. Introduction

Plant–herbivore interactions are widespread in ecosystems, and the plants have evolved a variety of defences against herbivory, such as chemical substances and structural modifications (Janzen, 1966; Bernays & Janzen, 1988; Yamazaki & Kikuzawa, 2003). The structural modifications of plants such as thorns, spines, and prickles are often assumed as anti-herbivore defences, especially against large herbivores. Therefore, unpalatable plants could be selected for to avoid grazing and browsing under a high density of herbivores (Janzen 1986; Bernays & Janzen, 1988; Brazely et al., 1991; Matsuki et al., 2004). In Japan, some plants had gained characteristic morphologies to avoid grazing from Sika deer (*Cervus nippon*) on a small island. For example, Kinkazan Island is a small land mass situated about one km off the Pacific coast of Honshu, with a high density of Sika deer (ca. 63.2/km^2) that have been protected for a long time as sanctuary animals (Takatsuki, 1983). *Cirsium amplexifolium* (Nakai) Kitam. var. *muraii* (Kitam.) Kitam. (Asteraceae) is endemic to this island and has longer spines around the leaf edges and stems than those in *Cirsium amplexifolium* var. *amplexifolium*, which occurs in wide areas in northern Honshu (Kitamura, 1981). Takatsuki (2006) suggested that, in *Cirsium amplexifolium* var. *muraii,* the long spines might have been selected to avoid heavy grazing by the Sika deer.

Kashima Island (32º57′N, 132º27′E) is a very small land area (45.1 ha) in southwestern Shikoku, Japan (Figure 1). The climate of this island lies in the warm temperate zone; the mean temperature of the coldest month (January) is 7.1ºC and the annual precipitation is 1903 mm (data taken at Misho in Ainan-cho) according to the Japan Meteorological Agency (2011). Evergreen broad-leaved forests are the climax vegetation (Hashigoe, 1995). Some unpalatable plants have become prominent on the island because of the high density (ca. 38.5/km^2) and heavy grazing of Sika deer (Figure 2A) (Takatsuki, 1982a, b; Hashigoe, 1995). Takatsuki (1982a) performed a cafeteria test, offering 63 species of plants to Sika deer, 36 of which the deer avoided eating. One of these, *Rubus sieboldii* Blume, which is distributed from the western Honshu to Ryukyu archipelago (Momiyama, 1989), was probably not consumed because of its prickles which served as anti-herbivore defence (Figure 2B) (Takatsuki, 1982a). If its prickles are defensive structures, it is possible that they could be induced; one may expect long and densely distributed defence structures on plant parts that have been subjected to herbivory. Therefore, it is

possible that the prickles on plants in this island are longer than that on plants of the same species from other areas. The aim of this study was to examine the prickle legethening of this species by comparing individuals on Kashima Island with those of the neighbouring areas.

2. Materials and Methods

The samples of 118 individuals of *Rubus sieboldii* used in this study were collected in 2011 from Kashima Island and the four neighbouring areas (Figure 1, Table 1).

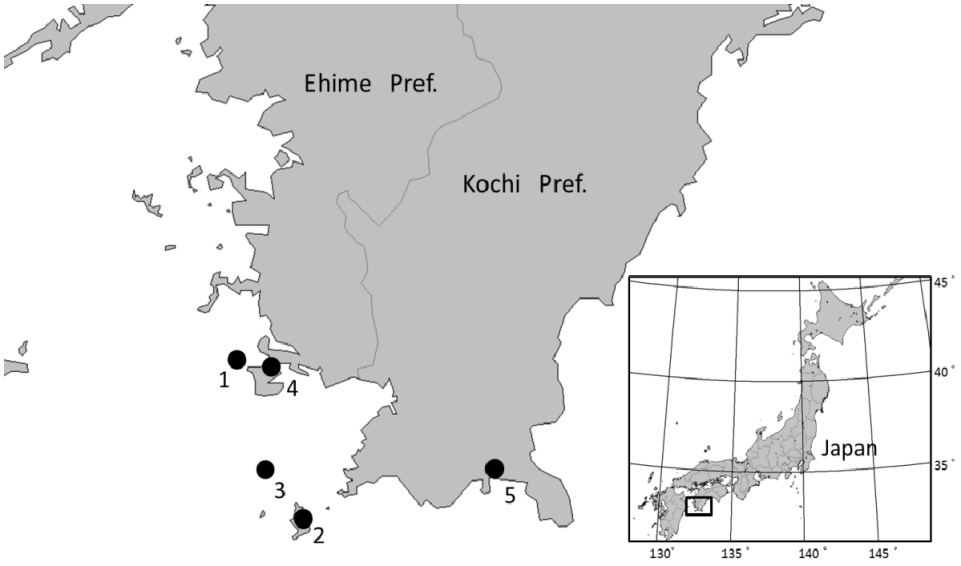

Figure 1. Sampling locations used in this study

1 - Kashima Island, 2- Okinoshima Island, 3- Uguru Island, 4- Uchidomari, and 5- Matsuzaki.

Table 1. Sampling locations in this study

Location name	Location no.	Number of Individuals	Location	Latitude and longitude
Kashima Island	1	35	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho, Kashima Island	N 32°56′ E 132°27′
Okinoshima Island	2	12	Kochi Prefecture, Sukumo City, Okinoshima-Cho, Okinoshima Island	N 32°43′ E 132°32′
Uguru Island	3	23	Kochi Prefecture, Sukumo City, Okinoshima-Cho, Uguru Island	N 32°48′ E 132°29′
Uchidomari	4	20	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho, Uchidomari	N 32°56′ E 132°30′
Matsuzaki	5	28	Kochi Prefecture, Tosashimizu City, Kagumi Matsuzaki	N 32°46′ E 132°55′

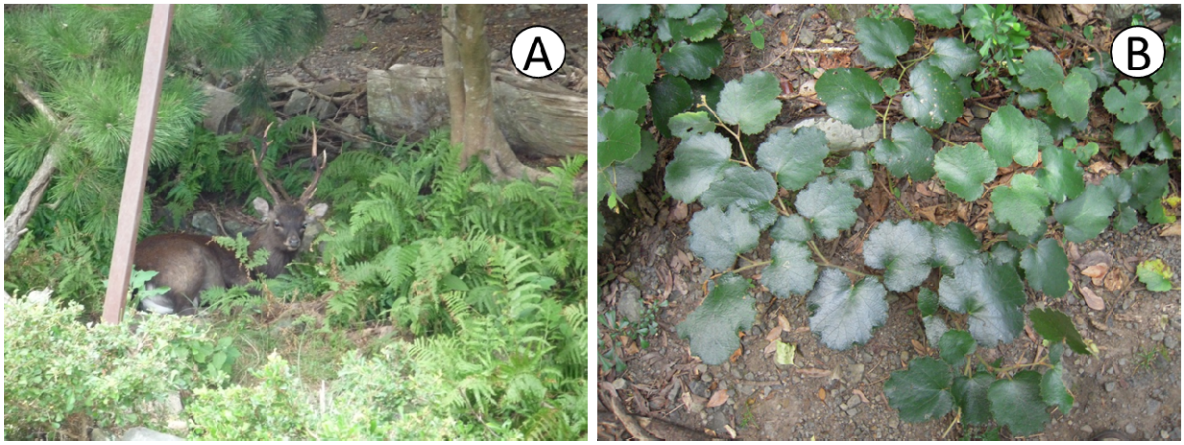

Figure 2. Sika deer (A) and *Rubus sieboldii* (B) in Kashima Island

To measure the length and density of prickles on the adaxial and abaxial sides of the leaves and prickles around stems of *R. sieboldii*, five fully expanded leaves at the middle part of the shoot of each plant were collected per individual. The average length of leaf prickle on a plant was calculated by measuring 10 prickles per leaf under a microscope, on five leaves per plant. The density of prickles was calculated by averaging the number on a square section from those five leaves per individual. The average length of prickles on a stem was calculated as the average from five prickles at 10 locations near the middle part of the height of the stem. The density was measured by counting the number per a square section of surface area around the stem at 10 locations on each individual. All study-specific statistical analyses were performed using SPSS Statistics 19 (IBM SPSS, Chicago, Illinois).

3. Results and Discussion

We analyzed the length and density of prickles on the adaxial and abaxial sides of leaves and around stems of *Rubus sieboldii* on opposite shores of Kashima Island and neighbouring islands (Okinoshima and Uguru Islands) (Figure 1). The results of morphological analysis are shown in Table 2 and Figure 3. *R. sieboldii* on Kashima Island had significantly longer and denser prickles on the stem than plants from the other areas (Table 2), suggesting that the high density and grazing pressure of Sika deer were correlated with the increased length and density of prickles of *R. sieboldii* in Kashima Island. Our results were similar to those mentioned above for *Cirsium amplexifolium* var. *muraii* in Kinkazan Island, Japan, another location with a high density of Sika deer (Takatsuki, 1977; 2006). However, it is questionable whether such ecological response would arise under exposure to a high density of herbivores. Some studies have reported that a high density of herbivores has been correlated with longer thorns. For example, Pullin and Gibert (1989) indicated that grazing pressure on *Urtica dioica* L. (Urticaceae) led to high stinging trichome densities after grazing and mechanical damage between the grazed and ungrazed areas. Moreover, branches of *Acacia drepanolobium* Harms ex Sjöstedt (Fabaceae) within reach of goats had significantly longer thorns than higher branches on the same plants or branches at all heights on nearby plants protected from ungulate herbivory (Young, 1987), and a similar pattern has been reported qualitatively on trees eaten by giraffes (Foster & Dagg, 1972). In addition, Milewski et al. (1991) and Takada et al. (2003) reported that the degree of herbivory was different between prickled and non-prickled *Acacia seyal* Del. and *Damnacanthus indicus* C. F. Gaertn., respectively. Studies on various other species have shown that actual and simulated herbivory correlated with long thorns (Abrahamson, 1979; Myers, 1987; White, 1988). In addition, most of these studies showed that the morphological modifications of plants occurred after a short period under high herbivore density. Takatsuki (2006) also claimed that such modifications on plants on Kinkazan Island in Japan appeared quickly under exposure to a high density of Sika deer. From these results, therefore, we expected that the high grazing pressure in Kashima Island should quickly lead to the increased length and density of prickles on both sides of leaves and shoots of *R. sieboldii*, implying that it is due to induced defence of *R. sieboldii* to sika deer herbivory.

Table 2. Morphological measurements (average ± standard deviation) of *Rubus sieboldii*

Location	Kashima Island	Okinoshima Island	Uguru Island	Uchidomari	Matsuzaki
Leaf prickles					
adaxial side					
length (mm)	1.71±0.55a	1.29±0.51ab	1.19± 0.23b	1.01± 0.40b	1.15± 0.25ab
density (N/cm^2)	0.50± 0.47a	0.15± 0.19ab	0.46± 0.32ab	0.04± 0.06b	0.45± 0.41ab
abaxial side					
length (mm)	1.22±0.30a	0.86±0.33b	0.84± 0.16b	0.84± 0.21b	0.87± 0.12b
density (N/cm^2)	0.72± 0.43a	0.23± 0.30bc	0.46± 0.28abc	0.16± 0.16c	0.65± 0.42ab
Stem prickles					
length (mm)	1.57 ± 0.42a	1.03± 0.30b	1.08 ± 0.13b	1.16 ± 0.24b	1.16± 0.22b
density (N/cm^2)	11.25± 4.61a	5.53± 2.61b	6.81± 2.23b	5.03± 2.88b	7.92± 2.29b

Columns marked by different letters differ significantly according to the Scheffe test ($p < 0.05$).

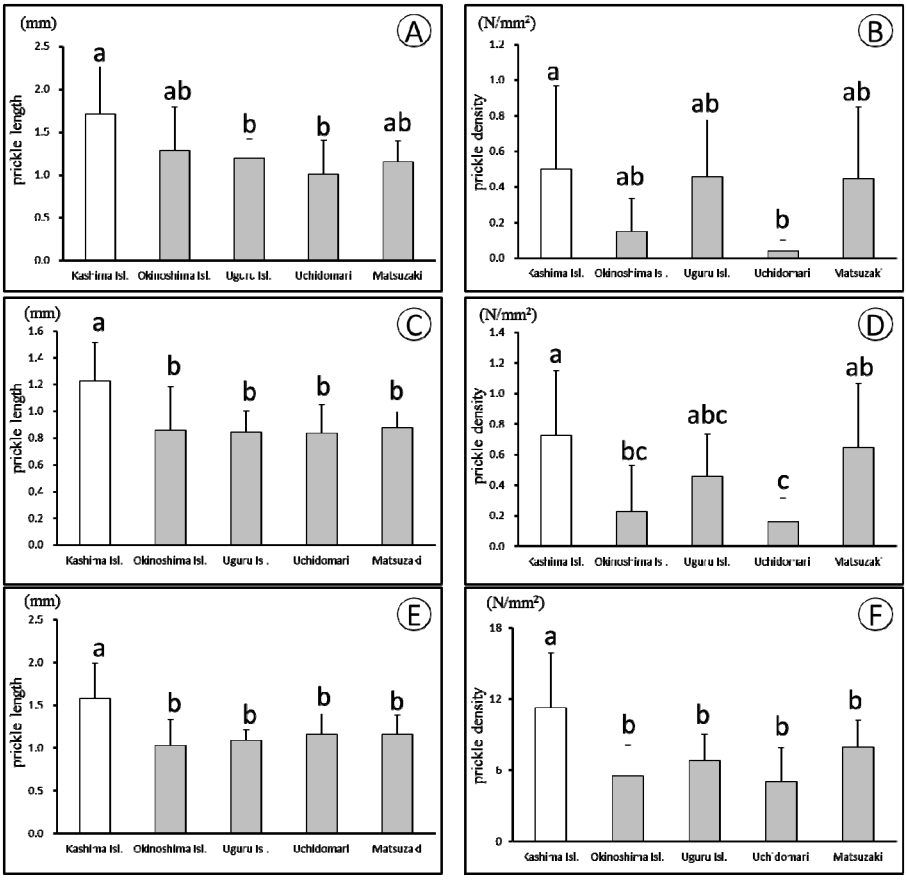

Figure 3. Morphological measurements of prickles of *Rubus sieboldii*. (A) length on adaxial side of leaf; (B) density on adaxial side of leaf; (C) length on the abaxial side of leaf; (D) density on the abaxial side of leaf; (E, F) length and density around the stem

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