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Comparative Morphology of Prickles of *Rubus croceacanthus* (Rosaceae) in Kashima Island and Its Neighbouring Areas

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Abstract

Kashima Island (32° 57′ N, 132° 27′ E) is a small island of southern Japan with high population density of sika deer (*Cervus nippon*). To ascertain whether heavy sika deer browsing has resulted in increased plant defences on the island, we conducted a morphological comparison of *Rubus croceacanthus* H. Lév. (Rosaceae) on Kashima Island and in the neighbouring areas on the mainland. We measured the length and density of the leaf and stem prickles. We found that the prickles of *R. croceacanthus* on Kashima Island were significantly longer and at a higher density than those in the neighbouring areas, suggesting that increased prickle length and density is an adaptive defence against sika deer browsing.

Keywords: deer, defence, density, herbivore, island, length, sika

1. Introduction

Many plants without any defense against the browsing by sika deer lead to extinction under high browsing pressure by mammalian herbivores (Takatsuki, 2006). Prickles are common anti-herbivory mechanical defences in thousands of plant species (Grubb, 1992; Gowda, 1996), and they have evolved to avoid browsing by mammalian herbivores in various lineages of angiosperm. For example, African *Acacia* spp. and other woody plants have longer prickles on their lower branches than on their higher branches (Cooper & Owen-Smith, 1986; White, 1988; Gowda & Palo, 2003). In the case of *Urtica thunbergiana* Sieb. et Zucc. (Urticaceae) which had been experienced feeding for many years and had been removed its trichome artificially, the density of them was increased (Pullin & Gilbert, 1989). Some *Citrus* spp. and palms (Arecaceae) have large prickles only on juvenile trees and none or few when mature (Cooper & Owen-Smith, 1986; Cornett, 1986; Clement & Manshardt, 2000). Moreover, as with several other types of induced defence, prickles are known to increase in size and number following herbivory (Milewski et al., 1991; Perevolotsky & Haimov, 1991; Young et al., 2003). Additionally, some number of species decrease under the high browsing pressure by sika deer, others increase such as *Triadica sebifera* (L.) Small (Euphorbiaceae), *Cleyera japonica* Thunb. (Theaceae), *Aralia elata* (Miq.) Seem. (Araliaceae) and *Dryopteris erythrosora* (D. C. Eaton) Kuntze (Dryopteridaceae) (Takatsuki, 1989).

In Japan, numerous studies have examined the effect of deer browsing on plants on small islands. For example, on Akune Island (32° 01′ N, 130° 10′ E), the deer density is as high as 520 per km^2 (Kiyohisa et al., 2005), which results in significant effects on the plant communities of the island (Kido, 2010). Takei et al. (2014) suggested that the long prickles of *Zanthoxylum ailanthoides* Siebold et Zucc. (Rutaceae) act as a defence against heavy browsing by sika deer (*Cervus nippon*) on Akune Island. On Kinkazan Island (32° 57′ N, 132° 27′ E), the density of sika deer is approximately 60 individuals per km^2 (Ito, 1985). The *Fagus crenata* Blume forest, which is the climax forest on Kinkazan, is composed of only large, old trees, suggesting that young trees of this species are subject to heavy browsing by sika deer (Takatsuki & Gorai, 1994). Moreover, *Cirsium amplexifolium* (Nakai) Kitam. var. *muraii* (Kitam.) Kitam. (Asteraceae) has limited distribution on the island and has longer prickles around its leaf edges and stems than those of *C. amplexifolium* (Nakai) Kitam., which occur in a wide area of northern Honshu (Kitamura, 1982). These results suggest that some plants increase the size and/or number of their prickles in response to heavy browsing by sika deer.

Kashima Island (32° 57′ N, 132° 27′ E; 45.1 ha in area) is at the southwest coast of the Shikoku District of Japan and has a relatively high density of sika deer (ca. 38.5 per km^2) (Takatsuki, 1982) (Figure 1). Kashima Island and its opposite shore have approx. 500 m distance and both areas are similarly to abiological environmental conditions (Yagi et al., 1979). Hashigoe (1995) reported that the flora of Kashima Island differs from that of the mainland because of heavy browsing by sika deer. Takei et al. (2013) found that the prickles of *Rubus sieboldii* Blume (Rosaceae) on Kashima were significantly longer and at a higher density than those from ungrazed areas of the mainland (Figure 1B). Although this result suggests that plant species with prickles have increased their physical defences against browsing, the prickles of *Zanthoxylum ailanthoides* are not significantly different from those on plants on the mainland (Takei et al., 2014). Consequently, Takei et al. (2014) suggested that the difference in the high numbers of prickles between *R. sieboldii* and *Z. ailanthoides* was based on the level of browsing pressure on Kashima Island. However, few studies have investigated the comparative morphology of plant physical defences on this island; therefore, further research is required to elucidate whether the length and density of prickles of other plant species have changed in response to heavy browsing.

Rubus croceacanthus H.Lév. (Rosaceae) has prickles on its leaves and stems (Naruhashi, 2001), and occurs on Kashima Island (Hashigoe, 1995) (Figure 1C). Naruhashi (2001) reported that this species also occurs on the mainland close to the island, providing an opportunity to compare prickle morphologies between areas under different browsing pressures on Kashima Island and on the mainland, which has little number of sika deer (Ministry of Environment, 2003). The purpose of this study was to measure the prickle length and density of *R. croceacanthus* on Kashima Island and the mainland, in order to ascertain the effects of browsing by sika deer.

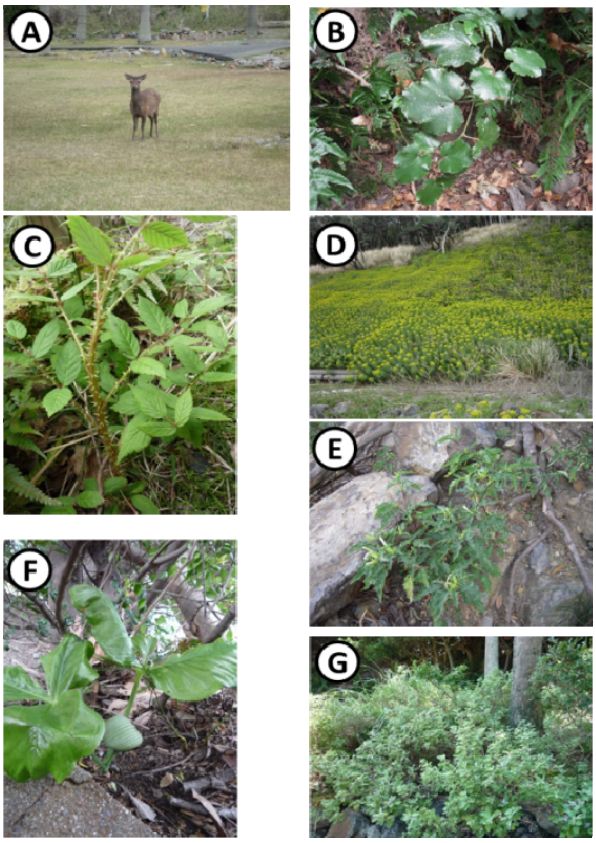

Figure 1. Plants and environments in Kashima Island. (A) sika deer; (B) *Rubus sieboldii* Blume (Rosaceae); (C) *Rubus croceacanthus* H. Lév. (Rosaceae); (D) *Euphorbia jolkinii* Boiss. (Euphorbiaceae); (E) *Datura metel* L. (Solanaceae); (F) *Arisaema ringens* (Thunb.) Schott (Araceae); (G) *Vitex rotundifolia* L. f. (Verbenaceae)

2. Materials and Methods

Samples of *Rubus croceacanthus* were collected from Kashima Island and four neighbouring areas (Ashizuri Cape, Okitsu, Yokonami, and Muroto) in 2012 and 2013 (Figures 2 and 3; Table 1).

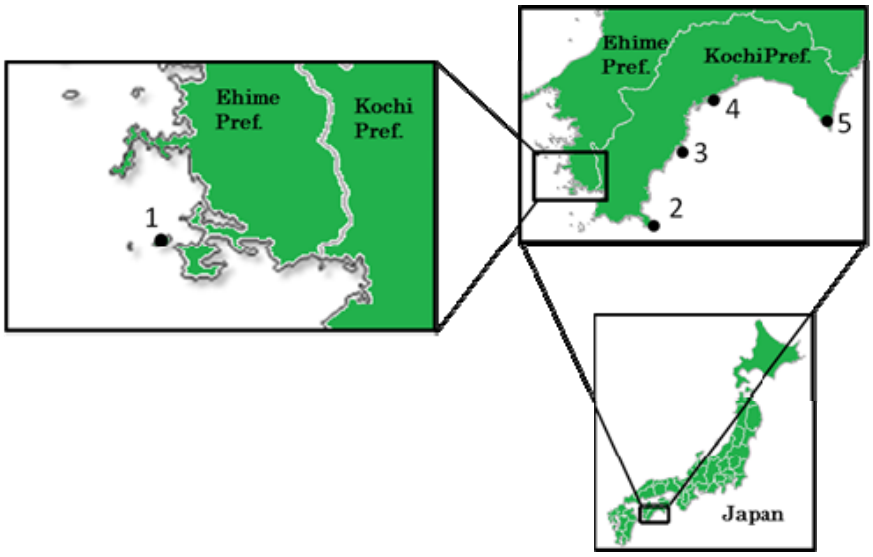

Figure 2. Collecting sites used in the study. 1: Kashima Island, 2: Ashizuri Cape, 3: Okitsu, 4: Yokonami, and 5: Muroto

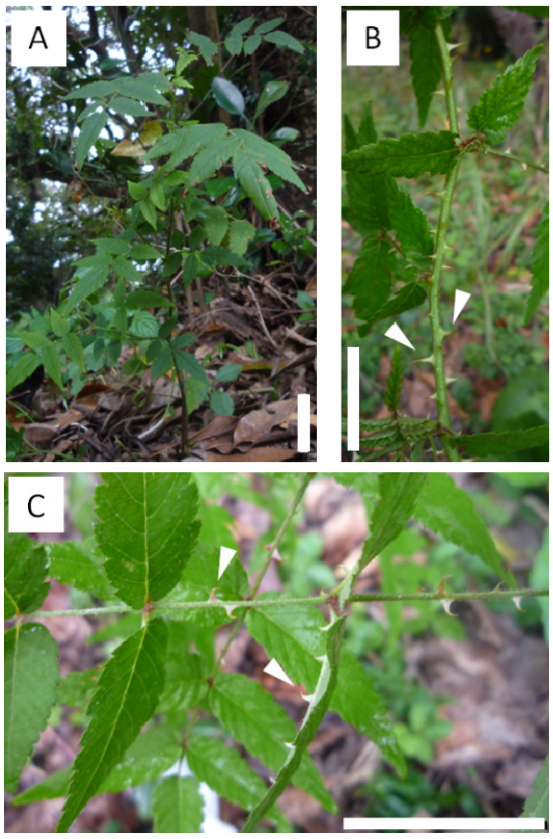

Figure 3. *Rubus croceacanthus* (photographed in Muroto). (A) *Rubus croceacanthus*; (B) The stem; (C) The leaf rachis and midrib. Bar = 5 cm. Arrowheads indicate prickles

Table 1. Sampling locations used in the study

Location name	Location no.	Number of Individuals	Location	Latitude and longitude
Kashima Island	1	30	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho, Kashima	N 32°57′ E 132°27′
Okitsu	3	30	Kochi Prefecture, Takaoka-Gun, Shimanto-Cho, Okitsu	N 33°17′ E 133°19′
Yokonami	4	14	Kochi Prefecture, Susaki City, Uranouchi, Yokonami	N 33°25′ E 133°21′
Muroto	5	30	Kochi Prefecture, Muroto City, Murotomisaki-Cho, Muroto	N 33°15′ E 134°10′

To measure the prickles length and density on the leaf midrib and rachis, 5 fully expanded leaves in the middle of the shoot of each plant were selected. The mean length of leaf prickles was calculated by measuring 5 prickles per leaf under a microscope. Leaf prickle density was calculated by averaging the number of prickles on a square section of 5 leaves per plant. The mean length of stem prickles was calculated as the mean length of five prickles from ten locations halfway up the stem. Stem prickle density was calculated by averaging the number of prickles on a square section of stem surface from ten locations on each plant. All statistical analyses were conducted using SPSS Statistics 19 (IBM SPSS, Chicago, Illinois).

3. Results and Discussion

The morphological measurements are presented in Table 2. The prickles on Kashima Island were significantly longer and at higher densities than those on the mainland (Figure 4; Table 2), suggesting that *Rubus croceacanthus* has adapted to heavy sika deer browsing pressure on Kashima Island. The other traits of *R. croceacanyhus* on Kashima Island had not significant difference with those of neighbouring areas (date not shown). However, the prickles on Muroto were slightly longer and at higher densities than those on Ashizuri Cape, Okitsu and Yokonami, suggesting that the browsing by sika deer effects against plants on Muroto, base on the report of Ministry of Environment (2003).

Table 2. Morphological measurements (mean ± standard deviation) of *Rubus croceacanthus*

Location	Kashima Island	Ashizuri Cape	Okitsu	Yokonami	Muroto
Midrib prickles					
Length (mm)	1.01 ± 0.03a	0.29 ± 0.02d	0.56 ± 0.03c	0.36 ± 0.03d	0.81 ± 0.03b
Density (N/cm²)	1.09 ± 0.06a	0.33 ± 0.03c	0.35 ± 0.03c	0.26 ± 0.02c	0.79 ± 0.04b
Rachis prickles					
Length (mm)	2.35 ± 0.05a	1.51 ± 0.03c	1.92 ± 0.05b	1.39 ± 0.04c	2.05 ± 0.05b
Density (N/cm²)	0.93 ± 0.08a	0.33 ± 0.05c	0.30 ± 0.03c	0.26 ± 0.05c	0.62 ± 0.04b
Stem prickles					
Length (mm)	2.62 ± 0.06a	1.82 ± 0.05c	2.45 ± 0.07ab	1.32 ± 0.14d	2.29 ± 0.06b
Density (N/cm²)	14.19 ± 0.62a	5.54 ± 0.19c	4.11 ± 0.17d	4.64 ± 0.18cd	9.46 ± 0.33b

Values that have the same letter within a column indicate no significant difference at the 5% level (Tukey Honest Significant Difference test).

These results are similar to those obtained by Takei et al. (2013) with *Rubus sieboldii*; both *R. croceacanthus* and *R. sieboldii* have short prickles on their leaves and stems in the absence of browsing. *Rubus* species may be specifically adapted to heavy browsing by sika deer, as shown by Bazely et al. (1991). They specified that the stem prickles of the bramble (*R. fruticosus*) increased in length after its leaves were removed, suggesting the

epigenetic events. We could not find this species on Akune Island, where the density of sika deer is more than thirteen times higher than that on Kashima Island (Ikeda & Iwamoto, 2004; Kiyohisa et al. 2005; Takatsuki, 1980). This shows that *R. croceacanthus* and *R. sieboldii* are adapted to increase the length and density of their prickles at the level of browsing pressure prevalent on Kashima Island, but cannot grow in areas where the browsing pressure is too great. In Kinkazan Island, Takatsuki (2006) reported the similar situation to those of Kashima Island. In contrast to these species, *Zanthoxylum ailanthoides* has relatively long leaf and stem prickles, and prickle length and density in this species is not significantly different between Kashima Island and the mainland, but is increased on Akune Island (Takei et al., 2014), suggesting that *Z. ailanthoides* may be able to resist heavy browsing. The results of the present study, and the results of previous studies by Takei et al. (2013; 2014), suggest that *Z. ailanthoides* can grow in areas where browsing pressure by sika deer is relatively high, whereas *R. croceacanthus* and *R. sieboldii* can not do it. Recently, sika deer have caused serious forest damage in many parts of Japan (Takatsuki & Ito, 2009), including bark stripping in south-west Shikoku (Okumura et al., 2011).

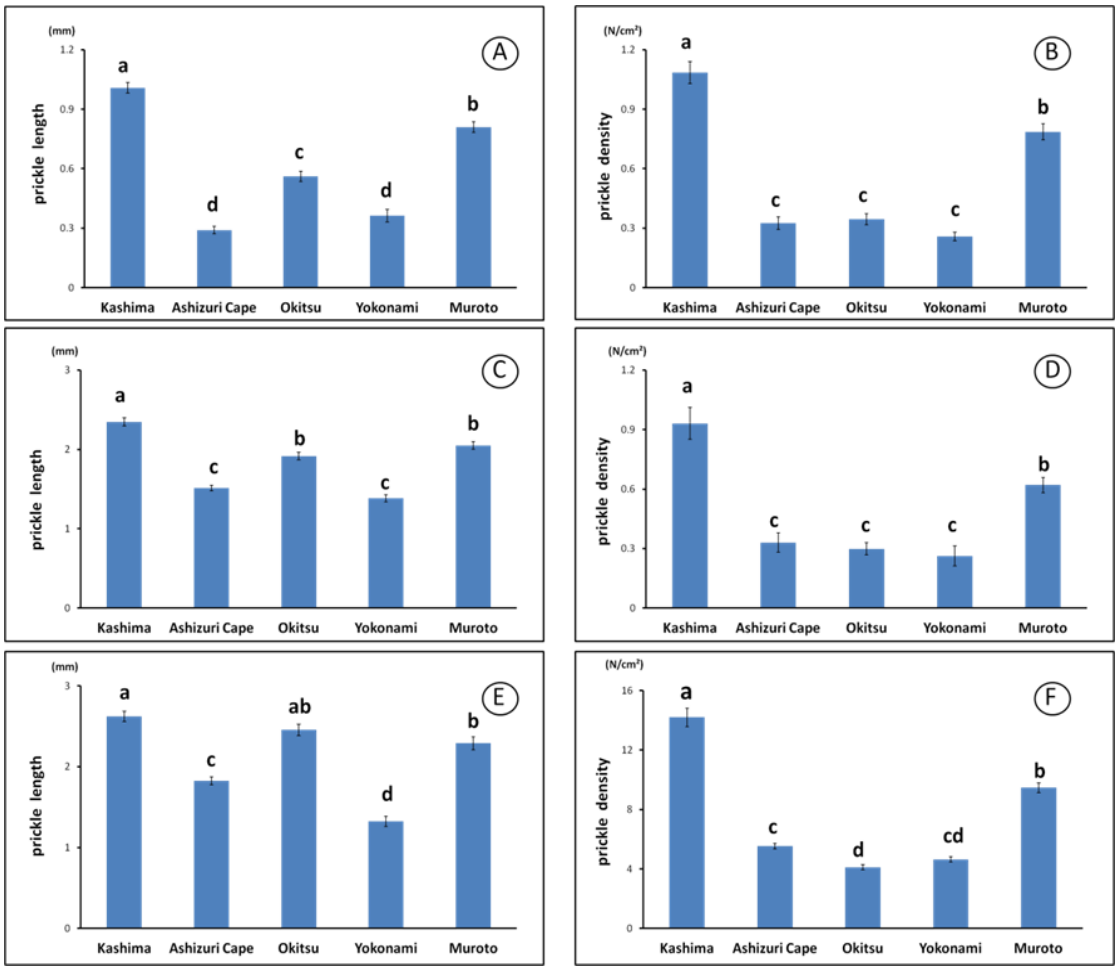

Figure 4. Measurements of the prickles of *Rubus croceacanthus*. Length (A) and density (B) on the midrib; length (C) and density (D) on the leaf rachis; length (E) and density (F) around the stem

Browsing damage to various plants by sika deer is now beginning to occur in areas close to Kashima Island. *R. croceacanthus* and *R. sieboldii* may be able to continue to grow in these areas by increasing their physical defences, until the browsing pressure reaches the same level as on Kashima Island. However, there is a high possibility that they will be lost from these areas when the population size of sika deer increases to such an extent that the browsing pressure exceeds that on Kashima Island. Therefore, future studies should conduct morphological analyses of *R. croceacanthus* and *R. sieboldii* in these areas.

Our results and the results of Takei et al. (2013) suggest that both *R. croceacanthus* and *R. sieboldii* have a similar pattern of increased length and density of prickles on Kashima Island. This suggests that other rosaceous species may also have increased their physical defences against heavy browsing on the island. Kashima Island has three rosaceous species with prickles: *Rosa onoei* Makino var. *onoei*, *Rosa luciae* Rochebr. et Franch. ex Crép., and *Rubus parvifolius* L. (Hashigoe, 1995), and these may also show increased prickle length and density. Hashigoe (1995) reported that there are other plant species with prickles on Kashima Island, such as *Cirsium japonicum* Fisch. ex DC. (Asteraceae) and *Caesalpinia decapetala* (Roth) Alston var. *japonica* (Siebold et Zucc.) H.Ohashi (Fabaceae). We could not find *Cir. japonicum* on Akune Island, but *Cae. decapetala* var. *japonica* does occur on Akune. In fact, Kido (2010) reported that those species have not grown on Akune Island. It is possible that *Cir. japonicum* cannot grow on Akune Island, where the browsing pressure exceeds that on Kashima Island, suggesting that the length and density of the prickles of *Cir. japonicum* has increased on Kashima Island, and the same has occurred in *Cae. decapetala* var. *japonica* on Akune Island. It would be interesting if future studies compared plant morphological characteristics between these islands.

Interestingly, a closely related species *Rubus okinawensis* Koidz. does not have leaf prickles and is mainly found on the Ryukyu archipelago, which includes continental islands without sika deer. Therefore, *Rubus croceacanthus* and *R. okinawensis* may have evolved under differential selection pressures caused by sika deer grazing.

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