

Misaki Iwamoto · Chika Horikawa  
Megumi Shikata · Naoko Wasaka  
Teiko Kato · Hiroaki Sato

## Stinging hairs on the Japanese nettle *Urtica thunbergiana* have a defensive function against mammalian but not insect herbivores

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**Abstract** Thorns and hairs of plants can serve as defenses against herbivores, although they may not have evolved under selection by herbivory. Japanese nettles, *Urtica thunbergiana*, in Nara Park, Nara Prefecture, Japan, where sika deer have been protected for 1200 years, bear many more stinging hairs than those in areas with few or no deer. Previous studies suggested that such hairy nettles evolved under natural selection imposed by intense deer browsing, because stinging hairs deterred deer browsing and because among-population variation in hair density was associated with deer abundance. To confirm this hypothesis, we examined (1) whether stinging hairs affected oviposition and feeding preferences of herbivorous insects and (2) the degree to which they deterred deer via laboratory and field experiments with hairy nettles from Nara Park and with almost-hairless nettles from another area. A specialist butterfly, Indian red admiral, showed no oviposition or larval feeding preferences for either hairy or hairless nettles. Insect damage levels did not significantly differ between the two variants. In contrast, deer browsed hairless nettles more heavily than hairy ones. In hairy nettles, however, the level of deer browsing was not proportional to stinging-hair density, presumably because the hairy nettle population had reached a plateau for resistance as a result of long-term strong directional selection for stinging hairs. These results corroborate the hypothesis that hairy nettles in Nara Park evolved through natural selection under intense deer browsing.

**Keywords** Insect damage · Evolution of defensive traits · Indian red admiral · Indirect interactions · Larval feeding and oviposition preferences

### Introduction

Thorns, spines, prickles (here collectively termed thorns), and trichomes (called hairs here) on leaves and along stems can serve as defenses against herbivores (Hanley et al. 2007). For instance, Milewski et al. (1991) examined the effects of *Acacia seyal* thorns on the browsing behavior of giraffes using thorn removal experiments in the field and demonstrated that de-thorned branches suffered more giraffe herbivory than did intact branches on the same plants. Sletvold et al. (2010) investigated the interaction between the perennial herb *Arabidopsis lyrata* and its specialist herbivore, the diamondback moth *Plutella xylostella*; oviposition by moth females and the level of leaf damage by moth larvae were negatively correlated with leaf-hair density. These results indicated that leaf hairs deterred not only larval feeding but also oviposition.

Nevertheless, thorns and hairs are unlikely to be effective defenses against both vertebrates and invertebrates (Walters 2011). Thorns are more effective against vertebrates, while hairs are more deterrent to invertebrates because of the relative sizes of the plants and herbivores (Grubb 1992). Thorns deter mammals from browsing the plant by inflicting painful mouth wounds. They are, however, ineffective against invertebrates, such as lepidopteran larvae and aphids, which can maneuver around them. In contrast, hairs are too small to wound mammals but can obstruct the free movement of insects.

Stinging hairs on stems, petioles, and laminae of the European nettle *Urtica dioica* are composed of a multicellular pedestal surmounted by an elongate, tapering, stinging cell (Thurston 1974). Those hairs are thought to have evolved as a defense against mammalian herbivores but not invertebrates. This supposition is supported by the facts that: (1) stinging hairs are silicified and contain a toxic fluid that causes sharp pain when injected into human skin (Pollard and Briggs 1984b); (2) stinging hairs are much denser on leaves in populations that are

M. Iwamoto · C. Horikawa · M. Shikata · N. Wasaka · T. Kato · H. Sato (✉)  
Department of Biological Sciences, Faculty of Science,  
Nara Women's University, Nara 630-8506, Japan  
E-mail: scarab@cc.nara-wu.ac.jp  
Tel.: +81-742-203937  
Fax: +81-742-203937

subject to high herbivory pressure by large mammals than in populations that have lacked browsing pressure for decades (Pollard 1986; Pollard and Briggs 1982, 1984a; Pullin and Gilbert 1989); (3) mammalian grazers such as rabbits (*Oryctolagus cuniculus*) and sheep (*Ovis aries*) avoid plants with more stinging hairs (Pollard and Briggs 1984b); (4) the level of insect damage is not associated with the density of stinging hairs in the field (Pollard and Briggs 1984b); and (5) there is no evidence that stinging hairs deter or interfere with feeding by invertebrate herbivores such as red admiral butterfly (*Vanessa atalanta*), Japanese beetle (*Popillia japonica*), grasshopper (*Chortophaga viridifasciata*), and snail (*Anguispira alternata*) (Tuberville et al. 1996).

Like *U. dioica*, the Japanese nettle *U. thunbergiana* Siebold et Zucc. also has stinging hairs. Kato et al. (2007) examined stinging-hair densities in nettles of Nara Park, Nara City, central Japan, where many sika deer (*Cervus nippon* Temminck) have been protected for 1200 years in an area of 6.6 km<sup>2</sup>. They found that the park's nettles had 58–630 times higher stinging-hair densities on leaves than did nettles in other areas with few or no sika deer. They also showed that the park's native nettles were less frequently browsed by deer than nettles with few or no stinging hairs that were transplanted from an unbrowsed area into the park. Furthermore, they showed that nettles raised from seeds in a greenhouse retained similar stinging-hair densities of wild populations from which the seeds originated, suggesting that stinging-hair density was controlled genetically. Recently, Shikata et al. (2013) investigated the effects of soil fertility, light intensity, and deer habitat-use frequency on stinging-hair density in 19 wild nettle populations, including those of Nara Park. They revealed that stinging-hair density was positively correlated with deer habitat-use frequency and independent of soil fertility and light intensity. Therefore, we hypothesize that nettles with high stinging-hair densities in Nara Park evolved under natural selection imposed by intense browsing by sika deer.

However, to confirm this hypothesis, we need to solve at least two problems. First, whether the high density of stinging hairs on Nara Park nettles affects insect behaviors such as oviposition and feeding is unclear. Although stinging hairs on *U. dioica* were experimentally shown not to interfere with insect feeding (Tuberville et al. 1996), this experiment used nettles that varied in stinging-hair density by at most threefold. In the weedy shrub *Wigandia urens* (Boraginaceae), contrary to predictions, the level of damage by insects was higher on bristly leaves with stinging hairs than on smooth leaves without stinging hairs (Cano-Santana and Oyama 1992). Thus, dense stinging hairs on Nara Park nettles might have a defensive function against insects or, conversely, permit more insect damage. In either case, deer browsing might induce more stinging hairs, which might indirectly affect the behavior and/or density of insect herbivores. This problem is related to the phenomenon of trait-

mediated indirect interactions in ecological communities (Ohgushi et al. 2012).

Second, the degree to which stinging hairs are effective against sika deer is uncertain. As mentioned above, Kato et al. (2007) compared browsing levels on two extreme variants from different populations, i.e., plants that were virtually devoid of stinging hairs and those that were heavily armed. They showed that hairy nettles suffered much less damage than hairless ones. Unfortunately, they failed to consider within-population variation in stinging-hair density, so whether damage level decreases consistently with an increase in hair density remains uncertain.

The objective of the present study was to determine whether the stinging hairs of *U. thunbergiana* had a defensive function against insect herbivores and the degree to which they contributed to resistance against sika deer. We examined oviposition and larval feeding preferences of a specialist butterfly (Indian red admiral) for hairy and almost-hairless nettles, compared levels of damage by insects in the two variants, and analyzed the relationship between stinging-hair density and the likelihood of deer browsing. Finally, from the viewpoint of trait-mediated indirect interactions among deer, nettle, and herbivorous insects, we considered the possibility that an increase in stinging hairs caused by intense deer browsing would affect herbivorous insects.

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## Materials and methods

### Study organisms

The Japanese stinging nettle *Urtica thunbergiana* is a wind-pollinated perennial plant that grows on forest edges in central and southern Japan (Kitamura and Murata 1961). *Urtica thunbergiana* is nitrophilous, as has been reported for *U. dioica* (Olsen 1921; Molisch and Dobat 1979; Kato 2001).

Nettles used in this study originated in Nara Park (6.6 ha, 34°41'N, 135°51'E, 110 m a.s.l.) and Takatori Castle Site (34°25'N, 135°49'E, 550–580 m a.s.l.), Nara Prefecture, central Japan. In Nara Park, several hundred sika deer have been protected for more than 1200 years, and nettle leaves have much higher densities of stinging hairs than those in other locations with no evidence of sika deer presence (Kato et al. 2007). Takatori Castle Site is 28.5 km south of Nara Park. The area around the site was not considered to be inhabited by sika deer, because there were no signs such as footprints, dung pellets, or browsing marks during the course of the present study.

The Indian red admiral butterfly, *Vanessa indica* Herbst, occurs from India through Indochina and China to Japan. This butterfly is multivoltine, and its larvae feed mainly on *Boehmeria nivea*, *B. sylvestris*, *B. spicata*, *B. japonica*, *U. angustifolia*, and *U. thunbergiana* of the family Urticaceae in Japan (Teshirogi 1990). In Nara Park and Takatori Castle Site, Indian red admirals

overwinter usually as adults and rarely as larvae. Overwintered adult females lay eggs in late March to mid-April. Eggs hatch in April, and larvae grow into pupae through five instars.

#### Oviposition preference in Indian red admiral

Twenty-five well-grown nettles were collected in each of Nara Park and Takatori Castle Site on 20 November, 2006, and transplanted to a corner of the campus of Nara Women's University, which is 500 m from Nara Park. On the campus, nettles with few stinging hairs on leaves grow naturally (Shikata et al. 2013). Nettles from Nara Park and Takatori Castle Site were alternated at intervals of 1 m in two rows that were 4 m apart. One row consisted of 24 plants and the other 26. These nettles were exposed to oviposition by *V. indica* females. The number of butterfly eggs was counted on all leaves of each nettle on 20 July, 2007. These eggs were laid by females of the first generation in the year.

The size of each nettle was approximated by the volume of a cylinder:  $\pi \times \text{maximum length} \times \text{maximum width} \times \text{height}$  on 26 July, 2007. For each plant, two leaves on each of the second, third, and fourth nodes from the tip of major shoots were removed, and their densities of stinging hairs were measured. The upper surface of each leaf was scanned using an image scanner (EPSON GT-X770, Seiko Epson Co., Suwa, Japan) with a resolution of 300 dpi. On the digital image, all stinging hairs on the surface were counted, and leaf area was measured using the freeware LIA32 for Windows95 ver. 0.376 $\beta$ 1 (Yamamoto 1997). Density was calculated by dividing the number of stinging hairs by the leaf area (cm<sup>2</sup>).

#### Larval feeding preference in Indian red admiral

Twenty-eight and 23 eggs of *V. indica* on nettle leaves were sampled in Nara Park and Takatori Castle Site, respectively, on 1 April, 2008. Eggs and leaves were incubated at 24 °C under a photoperiod of 14L:10D to induce hatching. Short-term (24 h) larval preference for nettles of Nara Park and Takatori Castle Site was assessed by a choice-test. Test arenas were plastic transparent cups (10 cm diam. below, 13 cm diam. above, 10 cm in height) containing one nettle leaf from each location on the bottom. The areas of those leaves were nearly equal. A larva within 12 h of hatching was placed at the midpoint between the two leaves and allowed to feed ad libitum at 20 °C under 14L:10D. Twenty-four hours later, we recorded which leaf was consumed. Then, these leaves were removed, and two new leaves were supplied. This procedure was repeated every day until the second day of the second instar. Data from the first days of the first and the second instars were analyzed. When a larva consumed both leaves nearly equally, data from the next day were used. When the larva again showed no preference, that replicate was discarded.

#### Damage by chewing and leafmining insects

Twenty-five nettles were collected in each of Nara Park and Takatori Castle Site on 9 and 10 March, 2010. Each nettle was divided at the rhizome into three clonal parts. They were planted individually in 1.6-L plastic pots that were filled with gardening soil (Takii No Baiyōdo, Takii Co, Japan) containing 300 mg/L N, 470 mg/L PO<sub>3</sub>, and 390 mg/L K. They were raised in a greenhouse under 85 % shading and watered periodically. On 18 March, 2011, 80 surviving nettles (40 from each site) were transplanted individually in 4.3-L plastic pots filled with the same soil as above. Forty pairs of nettles (one from each site) were placed at a distance of more than 3 m from each other on the campus of Nara Women's University; the two pots of each pair were 10 cm apart. For each nettle, the total number of leaves and the number of leaves consumed by chewing and leafmining insects (>20 % of area removed) were counted on 31 May and 28 June, 2011. The percentage of consumed area was estimated by eye. Then, the proportion of consumed to total leaves was calculated. Common chewing insects were green weevils (*Phyllobius* sp.), mother-of-pearls (*Pleuroptya ruralis*), and Indian red admirals, while leafmining insects were agromyzid flies. The two types of insect damage were combined, because they had the same impact on the loss of the photosynthetic organ.

#### The likelihood of deer browsing

Ten of the 80 nettles that were used to assess insect damage (five from each site) were randomly chosen on 5 July, 2011. For each nettle, the number of leaves was counted, and four intact leaves were removed from major shoots. For each leaf, stinging-hair density (cm<sup>-2</sup>) was obtained as described above. Five pairs, each of which consisted of a nettle from Nara Park and one from Takatori Castle Site, were placed at least 20 m apart from each other in Nara Park. Nettles of a pair were set 3 m apart from one another. They were exposed to deer browsing under natural conditions for 24 h. To obtain the likelihood of deer browsing for each nettle, we counted the number of leaves eaten by deer and divided it by the initial number of leaves. This procedure was replicated using other nettles on 12–13, 21–22, and 28–29 July and 3–4 August. In total, 30 pairs were examined. Seven pairs of nettles in which one or both plants were toppled by browsers were excluded from subsequent analysis.

#### Statistical analyses

Before subsequent analyses, stinging-hair density was log-transformed to improve the fit to a normal distribution (Krebs 1998). The proportions of leaves consumed by insects (>20 % of area removed) and leaves

eaten by deer were arcsin-transformed. The data are presented as mean  $\pm$  SD before transformation.

The oviposition preference of Indian red admirals for nettles of Nara Park or Takatori Castle Site was analyzed by single factor analysis of covariance (ANCOVA). Plant size was an independent variable (i.e., the covariate), the number of eggs per plant was a dependent variable, and nettle population was a fixed factor.

Larval preference for nettle leaves of Nara Park or Takatori Castle Site was analyzed for the first and second instars according to Sokal and Rohlf's (2012) replicated goodness-of-fit tests. First, homogeneity of the ratios of larvae choosing leaves of Nara Park and Takatori Castle Site was tested for each larval population using the heterogeneity  $G$  test. Then, goodness of fit to a 1:1 ratio was tested for the pooled data by means of a standard  $G$  test.

Differences in the levels of insect and browsing damage between nettles of Nara Park and Takatori Castle Site were tested using a paired  $t$  test.

We hypothesized that the likelihood of deer browsing a nettle of Nara Park would be related to the stinging-hair density on its leaves and the potential intensity of deer browsing to where and when the nettle was placed. The potential was expressed by the proportion of leaves of the Takatori Castle Site nettles eaten by deer, based on the assumption that nettles of Takatori Castle Site would be readily consumed by deer because they bore few stinging hairs. The proportion of leaves eaten by deer was not significantly associated with stinging-hair density in nettles of Takatori Castle Site ( $r = -0.390$ ,  $df = 21$ ,  $P = 0.065$ ). The hypothesis was tested via multiple linear regression analysis, where the dependent variable was the proportion of leaves eaten by deer in nettles of Nara Park and the predictor variables were stinging-hair density and potential browsing intensity.

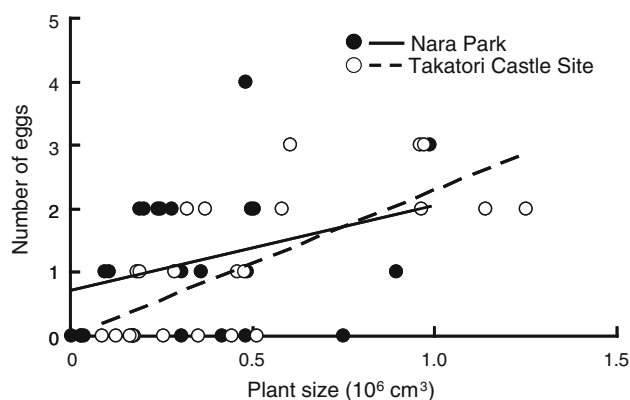
Statistically significant difference in stinging-hair density between nettles of Nara Park and Takatori Castle Site was tested with a mixed model nested analysis of variance (Sokal and Rohlf 2012), where population was a fixed factor, individual was a random factor nested within population, and leaf was a random factor nested within individual.

All analyses were performed with SPSS ver 15.0J (SPSS Inc 2006).

## Results

### Oviposition preference in Indian red admiral

Forty-seven of 50 transplanted nettles (23 for Nara Park, 24 for Takatori Castle Site) survived until the survey date. In total, 27 and 26 eggs of Indian red admiral were found on nettles of Nara Park and Takatori Castle Site, respectively. About one-third of the eggs on nettles of Nara Park were laid at the tips of stinging hairs. ANCOVA revealed no statistical significance in the interaction between nettle population and nettle size



**Fig. 1** Scatterplot and regression lines of the number of *Vanessa indica* eggs per plant versus plant size, which was calculated by  $\pi \times$  maximum length  $\times$  maximum width  $\times$  height, for nettles originating from Nara Park and Takatori Castle Site

(Fig. 1; Table 1), and thus the regression slopes of the number of eggs versus nettle size for the two populations were considered to be equal. The number of eggs was significantly associated with nettle size. We did not find a significant effect of nettle population on the number of eggs. These results indicated that butterfly females laid eggs according to nettle size without discriminating the nettle populations.

Nettles of Nara Park had significantly higher stinging-hair densities than nettles of Takatori Castle Site ( $2.4 \pm 1.38 \text{ cm}^{-2}$  and  $0.04 \pm 0.04 \text{ cm}^{-2}$ , respectively,  $F_{s[1,35]} = 228.9$ ,  $P < 0.001$ ; data for six nettles of Nara Park and four nettles of Takatori Castle Site were accidentally lost).

### Larval feeding preference in Indian red admiral

In the first or second instars, there were no significant differences in the ratios of larvae that chose leaves of Nara Park or Takatori Castle Site between larvae of the respective ( $G$  test for homogeneity; Table 2). The  $G$  test for goodness of fit to a 1:1 ratio was applied for the pooled data. In each instar, the observed distribution did not significantly deviate from an even distribution ( $G$  test for 1:1 ratio; Table 2). These results indicated that larvae chose leaves without discriminating the nettle populations. First and second instar larvae were observed to eat leaf tissues without consuming stinging hairs, which were excised and left in the cup.

### Leaf damage by herbivorous insects and sika deer

There was no significant difference in the proportions of leaves damaged by insects ( $>20\%$  of area removed) between nettles of Nara Park and Takatori Castle Site on 31 May or 28 June (Fig. 2). In contrast, a significant difference was found in the proportions of leaves eaten by deer between the populations (Fig. 3). Deer ate the



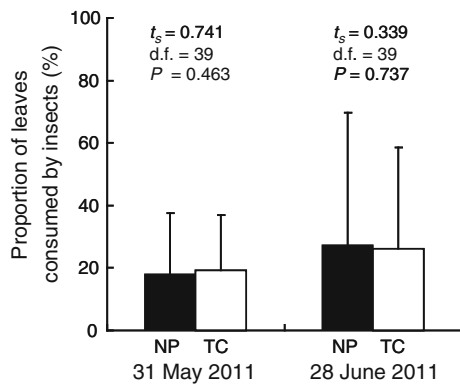
**Table 1** Analysis of covariance table for the data shown in Fig. 1, testing for the effect of nettle size (covariate) and nettle population (fixed factor) on the number of *Vanessa indica* eggs laid on nettles (dependent variable)

Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F<sub>s</sub></i>	<i>P</i>
For homogeneity of regression slopes					
Nettle population × nettle size	1	0.907	0.907	1.034	0.315
Residual	43	37.741	0.878		
For main effect of nettle population					
Nettle size	1	16.489	16.489	18.773	< 0.001
Nettle population	1	1.233	1.233	1.404	0.242
Residual	44	38.648	0.878		

*df* degree of freedom, *SS* sum of squares, *MS* mean square

**Table 2** Contingency tables for nettle leaves from Nara Park and Takatori Castle Site which were chosen by 1st or 2nd instar larvae of *Vanessa indica* hatched from eggs sampled in Nara Park and Takatori Castle Site when two leaves were offered, followed by the results of replicated goodness-of-fit tests consisting of a heterogeneity *G* test and a standard *G* test

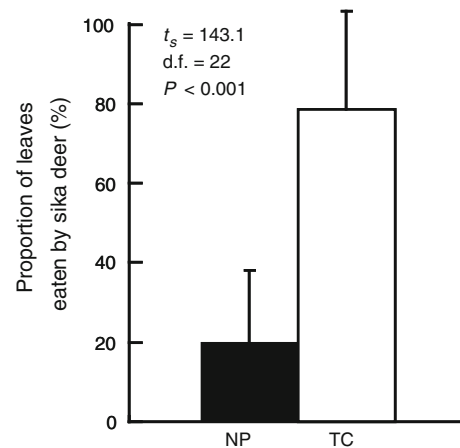
	Source of eggs	Nettle leaves chosen		Sum
		Nara Park	Takatori Castle Site	
Instar I	Nara Park	8	9	17
	Takatori Castle Site	9	8	17
	Sum	17	17	34
	<i>G</i> test for homogeneity	$\chi_s^2 = 0.118, df = 1, P = 0.732$		
	<i>G</i> test for 1:1 ratio	$\chi_s^2 < 0.001, df = 1, P = 0.999$		
Instar II	Nara Park	6	11	17
	Takatori Castle Site	10	8	18
	Sum	16	19	35
	<i>G</i> test for homogeneity	$\chi_s^2 = 1.458, df = 1, P = 0.228$		
	<i>G</i> test for 1:1 ratio	$\chi_s^2 = 0.129, df = 1, P = 0.720$		



**Fig. 2** Leaves consumed by herbivorous insects (> 20 % of area removed) as a percentage of the total number of leaves for each of two nettle populations. *Bar* + *SD*, *NP* Nara Park, *TC* Takatori Castle Site

whole shoots of six nettles of Takatori Castle Site, while they left all or part of shoots of every nettle of Nara Park. Deer were sometimes observed to jump back and shake their heads immediately after their mouths contacted nettles of Nara Park. They did not show such pain responses to nettles of Takatori Castle Site.

Stinging-hair density was significantly higher in nettles of Nara Park than in those of Takatori Castle Site ( $1.1 \pm 0.48$  and  $0.01 \pm 0.01 \text{ cm}^{-2}$ , respectively,  $F_{s[1,44]} = 115.2, P < 0.001$ ).



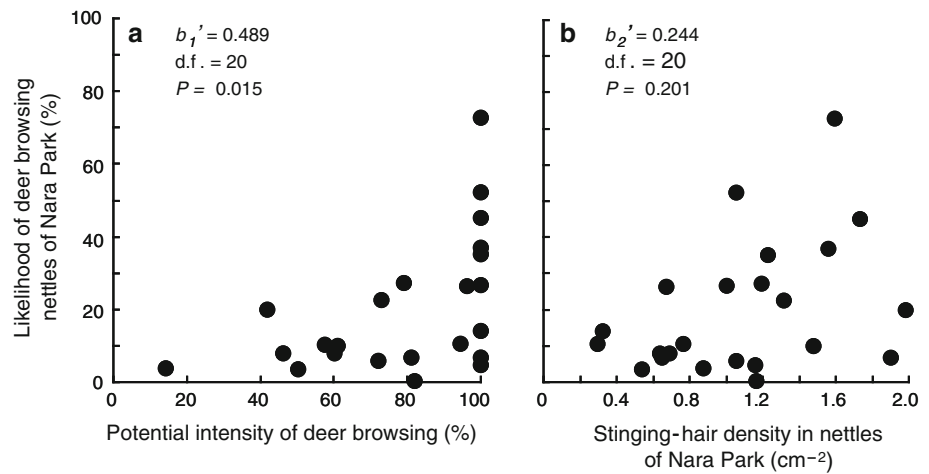
**Fig. 3** Leaves eaten by sika deer as a percentage of the total number of leaves in each of two nettle populations. *Bar* + *SD*, *NP* Nara Park, *TC* Takatori Castle Site

Multiple linear regression analysis provided the following prediction equation:

$$y = 0.310x_1 + 6.294x_2 + 3.429$$

where *y* is the arcsin-transformed proportion of leaves eaten by deer for nettles of Nara Park (i.e., the likelihood of deer browsing), *x*<sub>1</sub> is the arcsin-transformed proportion of leaves eaten by deer for nettles of Takatori

**Fig. 4** Scatterplot of the likelihood of deer browsing nettles of Nara Park (i.e., the proportion of leaves eaten by deer) against the potential intensity of deer browsing (i.e., the proportion of leaves eaten by deer for nettles of Takatori Castle Site) (a) and against the stinging-hair density for nettles of Nara Park (b).  $b_1'$ ,  $b_2'$  standardized partial regression coefficients calculated using transformed variables



Castle Site (i.e., the potential intensity of deer browsing at that place and time), and  $x_2$  is the log-transformed stinging-hair density for nettles of Nara Park. The coefficient of multiple determination was 0.356 and the  $F_{s[2,20]}$  was 5.525 ( $P = 0.015$ ). The standardized partial regression coefficient of  $x_1$  was significantly  $> 0$  (Fig. 4 a), whereas that of  $x_2$  was not significantly different from 0 (Fig. 4b). These results indicated that the likelihood of deer browsing on nettles of Nara Park varied with potential browsing intensity and, noticeably, was independent of stinging-hair density.

## Discussion

Our previous studies supported the hypothesis that a much higher density of stinging hairs on nettles of Nara Park had evolved as a defense against sika deer (Kato et al. 2007; Shikata et al. 2013). To confirm this hypothesis, we needed to address at least two problems: whether stinging hairs serve as defenses against insect herbivores and the degree to which stinging hairs deter sika deer. By comparing (hairy) nettles of Nara Park and (almost hairless) nettles of Takatori Castle Site, we found that (1) stinging hairs affected neither the oviposition preference nor larval feeding preference for nettles of Indian red admiral, (2) stinging hairs did not reduce leaf damage by insect herbivores, and (3) although the likelihood of deer browsing was much lower in hairy nettles than in hairless nettles, it was not correlated with stinging-hair density in hairy nettles. These findings suggest that stinging hairs scarcely serve as defenses against insect herbivores and that higher stinging-hair densities are not necessarily more effective in defending the plants from deer browsing. The former corroborates our hypothesis but the latter raises doubt about it.

No significant associations between insect damage and stinging-hair density were reported in the European nettle *U. dioica* by Pollard and Briggs (1984b) or Tuberville et al. (1996). As those authors suggested, insect herbivores are probably small enough to walk around,

climb over, or eat around stinging hairs. In fact, they observed that larvae of the red admiral *V. atalanta* ate leaf tissues without ingesting stinging hairs. We observed this behavior in first and second instar larvae of the Indian red admiral *V. indica*. Furthermore, females of *V. indica* often attached eggs at the tips of stinging hairs as well as on the leaf epidermis, implying that stinging hairs did not influence egg laying. Thus, stinging hairs, even at high densities, have little effect in reducing insect damage.

One might assume that Indian red admirals at the oviposition preference test site (the campus of Nara Women's University) were adapted to lay eggs on nettles of Nara Park, because the two sites are only 500 m apart. We cannot categorically deny the possibility, but it is unlikely because nettles with almost hairless leaves grow naturally on the campus (Shikata et al. 2013). Thus, the butterflies may not be adapted to the hairy nettles of Nara Park and should therefore prefer to oviposit on the hairless nettles of Takatori Castle Site. However, our results suggested that butterflies laid eggs on both variants equally. Thus, Indian red admirals do not appear to be adapted to either hairy or hairless nettles for oviposition. This conclusion may apply to other herbivorous insects as well.

Stinging hairs on leaves of *U. thunbergiana* effectively deter deer browsing but not insect oviposition or herbivory. Dense stinging hairs were found along the stems as well as on leaves in nettles of Nara Park (data not shown), preventing the whole plant from being consumed by the ungulate. These results confirm the hypothesis that nettles with high stinging-hair densities in Nara Park evolved under natural selection imposed by intense deer browsing, as previous studies suggested (Pollard and Briggs 1982, 1984a, b, Pullin and Gilbert 1989, Tuberville et al. 1996, Kato et al. 2007, Shikata et al. 2013). If the hypothesis is correct, we could predict that nettles with higher stinging-hair densities would suffer less damage by deer. However, the level of deer browsing was not proportional to stinging-hair density (Fig. 4b). This result is inconsistent with that of Pollard

and Briggs (1984b), who demonstrated that browsing by rabbits or sheep was negatively correlated with stinging-hair density in *U. dioica*.

The inconsistency between the two studies could be explained not by the fact that different mammals were involved but by the difference in stinging-hair density. The range of hair densities in the present study (0.29–1.98 cm<sup>-2</sup>) was much narrower than that in Pollard and Briggs (1984b) (0–101.2 cm<sup>-2</sup>). Statistically, the lack of correlation between stinging-hair density and deer browsing level could arise stochastically from the narrow range of stinging-hair densities.

From an evolutionary viewpoint, we can propose a more likely explanation. Pollard and Briggs (1984b) conducted their experiment with plants from several populations to compare the levels of mammalian herbivory across a wide range of hair densities. Thus, they examined the likelihood of mammalian browsing among different populations, while we examined it within a single (Nara Park) population, which has been exposed to intense browsing pressure for 1200 years. Our results showed that nettles of Nara Park suffered much less deer browsing than those of Takatori Castle Site and that the damage was independent of stinging-hair density within the Nara Park population. These findings imply that the resistance of *U. thunbergiana* to deer browsing would increase with stinging-hair density until a plateau was reached. In other words, different densities of stinging hairs would have the same resistance beyond a threshold value.

As a result of long-term strong directional selection for hairy plants, the Nara Park population may not only be at a plateau for resistance but also at a selection limit, where the population fails to respond to selection (Falconer and Mackey 1996). A cessation of response to selection is not necessarily caused by loss of genetic variance. Theoretical and empirical studies have indicated that genetic variance can be maintained at a selection limit (Mousseau and Roff 1987; Falconer and Mackey 1996). Furthermore, even if genetic variance is lost at a selection limit, phenotypic variance may be found; often it increases (Falconer and Mackey 1996). In fact, the range of stinging-hair densities in the Nara Park population was wider than that in the Takatori Castle Site (Fig. 4b). Whether or not the Nara Park population is at a selection limit deserves to be examined.

Our finding that stinging hairs had little effect on insect attack suggested that there was no indirect effect of sika deer on herbivorous insects through an increase in stinging-hair density. However, this conclusion does not mean that there is no indirect relationship between sika deer and herbivorous insects. Intense browsing by sika deer could change not only stinging-hair density but also chemical characteristics, such as water and nitrogen contents (Shoonhoven et al. 2005; Barrett and Stiling 2007). If so, chemical alteration of leaves might affect the growth performance of herbivorous insects. Our finding disagrees with a result of Cano-Santana and Oyama

(1992). They found that *Wigandia urens* plants with stinging hairs suffered more damage by herbivorous insects than plants with no stinging hairs. They explained this result with the fact that bristly plants had higher contents of water, nitrogen, and phosphorus in their leaves. In *U. thunbergiana*, nutritional traits may not affect insect herbivory. To explore these possibilities, we must study the nutritional traits of hairy and hairless nettles and the responses of herbivorous insects to nutritional variation.

In conclusion, we revealed that stinging hairs effectively deter sika deer from browsing *U. thunbergiana* and have little effect in reducing insect attack. These findings support the hypothesis that nettles with high stinging-hair density in Nara Park evolved through natural selection under intense deer browsing. Contrary to our prediction, there was no significant relationship between intra-population variation in stinging-hair density and the likelihood that deer browsed the plants, presumably because the population was at a plateau for resistance due to long-term directional selection for hairy plants.

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