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Comparative Analyses of Hairless-Leaf and Hairy-Leaf Type Individuals in *Aster hispidus* var. *insularis* (Asteraceae)

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Abstract

The distribution of *Aster hispidus* Thunb. var. *insularis* (Makino) Okuyama is limited to the coastal areas of southwestern Shikoku. This species has been described as having fatter stems, thicker leaves, and glabrous stems and leaves ciliate on margin. We have found many individuals that ranged from having no hair to many hairs on the abaxial side of the leaves in this variety. We measured the number of leaf hairs, degree of herbivory, and stomatal density of leaves of *A. hispidus* var. *insularis*. Two types of *A. hispidus* var. *insularis* for the presence or absence of leaf hairs were obsereved. The degree of herbivory did not differ significantly between these two types, however, the stomatal density was lower in the hairy-leaf-type individuals than in the hairless-leaf-type individuals.

Keywords: *Aster hispidus* var. *insularis*, leaf, hair, stomata

1. Introduction

Aster hispidus Thunb. (Asteraceae) is distributed from China, Korea, and Japan, inhabits open fields and grasslands in lowland areas, and comprises five infraspecific taxa; *A. hispidus* var. *hispidus*, *A. hispidus* var. *insularis* (Makino) Okuyama, *A. hispidus* var. *tubulosus* K. Asano, *A. hispidus* var. *leptocladus* (Makino) Okuyama, and *A. hispidus* var. *koidzumianus* (Kitam.) Okuyama (Yonekura & Kajita, 2003; Igari, 2007). Among them, *A. hispidus* var. *insularis* is limited to along the seashore of southwestern Shikoku with fatter stems and thicker leaves (Kitamura, 1981, as *Heteropappus hispidus* (Thunb.) Less. subsp. *insularis* (Makino) Kitam.). Tunala et al. (2012) conducted a morphological and anatomical analyses and reported that *A. hispidus* var. *insularis* had larger and thicker stem leaves than that of *A. hispidus* var. *hispidus*, and it was caused by increasing cell size of stem leaves. In addition to these traits, *A. hisipidus* var. *insularis* is distinguished from *A. hispidus* var. *hispidus* in having glabrous stems and leaves ciliate on the margin (Kitamura, 1981; Ito & Soejima, 1995). Many studies recognized that *A. hispidus* var. *insularis* had no hair excluding leaves ciliate on the margin (Makino, 1898; Kitamura, 1981; Ito & Soejima, 1995; Igari, 2007). However, we found many different individuals of *A. hispidus* var. *hispidus* ranging from no hair to many hairs on the stems and on the abaxial side of leaves, although it had fatter stems and thicker leaves, which were characteristics to *A. hispidus* var. *hispidus*. These individuals show sympatry in various areas of the seashore of southwestern Shikoku. However, the roles of the leaf-hair variation among the individuals of *A. hispidus* var. *insularis* is not known.

In general, leaf hairs were widespread in all angiosperms, occurring on the aerial surfaces of most species (Johnson, 1975). In many studies, the leaf hairs in many species were thought to serve as a mechanical defense against attack by herbivores (Hoffman & McEvoy, 1985; Gross & Price, 1988; Tuberville et al., 1996; Mauricio & Rausher, 1997; Elle et al., 1999; Hare & Elle, 2002; Agrawal, 2004; Handley et al., 2005; Valkama et al., 2005). Leaf hairs may also function to protect plants from abiotic stressors such as drought (Ehleringer, 1982; Grammatikopoulos & Manetas, 1994; Espigares & Peco, 1995; Perez-Estrada et al., 2000), temperature (Ehleringer, 1982; Perez-Estrada et al., 2000), and heavy metal toxins (Salt et al., 1995; Gutierrez-Alcala et al., 2000). The accumulation of salts owing to drought in the soil in coastal areas, where *A. hispidus* var. *insularis* is distributed, decreases the soil's osmotic potential and plants suffer from dehydration; therefore, the leaf hairs of *A. hispidus* var. *insularis* could have a function against drought. In addition, the stomatal density was correlated to the transpiration rate (Ouedraogo & Hubac 1982), suggesting that it would be interesting to measure the stomatal density of the hairless- and hairy-leaf types of *A. hispidus* var. *insularis*, because it might reveal a part in determining susceptibility to drought. By studying the functions of the leaf hairs, one can examine the interacting abiotic and biotic factors in *A. hispidus* var. *insularis.* Therefore, we measured morphologies and functions of the hairs on the stem leaves and the stomatal density in *A. hispidus* var. *insularis* individuals with and without leaf hairs.

2. Materials and Methods

The samples of *A. hispidus* var. *insularis* collected in this study were used from the seashore field in 2011. All samples had typical morphologies of *A. hispidus* var. *insularis* in the characters of the fatter stems and thicker leaves. The leaf hairs were counted for all samples of *A. hispidus* var. *insularis.* Then, *A. hispidus* var. *insularis* was categorized into two types, hairless and hair types, by puberulous or glabrous leaves. For the degree of herbivory, we analyzed 30 individuals (Kashiwajima) and 32 individuals (Komomisaki) of hairless type and 30 individuals (Kashiwajima) and 31 individuals (Komomisaki) of hair type of *A. hispidus* var. *insularis* (Figure 1, Table 1). To calculate the degree of herbivory, we observed the percentage of herbivore damage and calculated the size of all the stem leaves (summarized leaf length x leaf width) on each individual. With these measurements, we calculated the percentage values of the degree of herbivory on all individuals (herbivore-damaged size divided by entire leaf size).

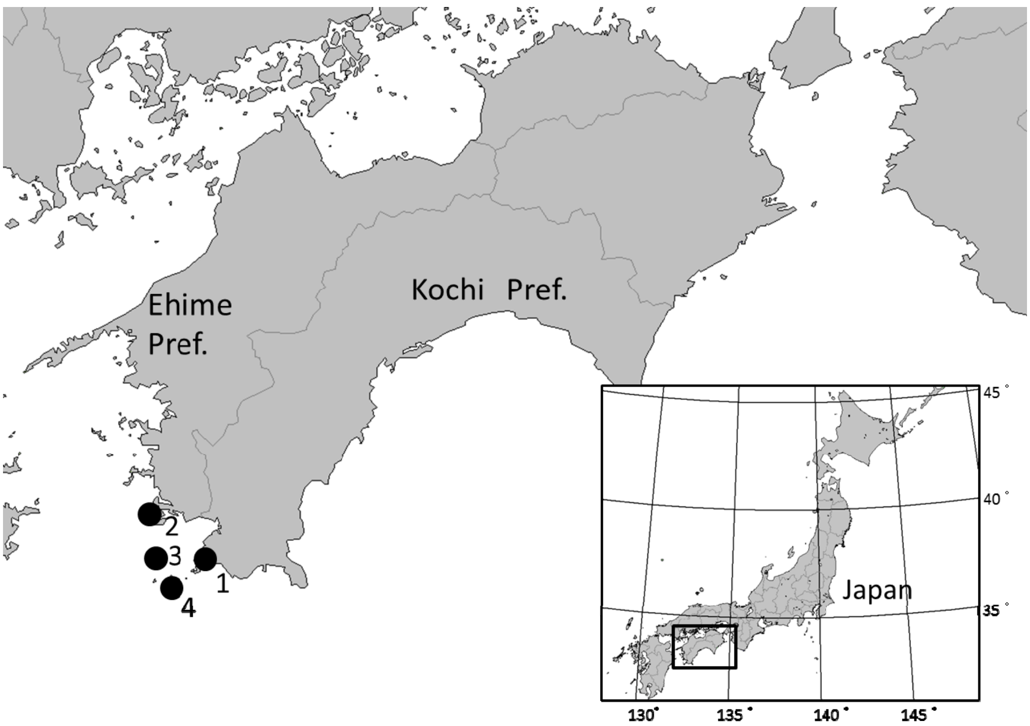

Figure 1. Sampling localities (No. 1-4) observed in this study

Locality names of No. 1 to 4 are Kashiwajima, Komomisaki, Ugurujima, and Okinoshima, respectively. See Table 1 for additional information.

Table 1. Sampling localities used in this study

Locality Name	Locality No.	Locality	Number of individuals		Latitude and longitude
			hairless-leaf	hairy-leaf	
Kashiwajima	1	Kochi Prefecture, Hata-Gun, Otsuki-Cho, Kashiwajima	65	58	N 32°78' E 132°64'
Komomisaki	2	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho, Komomisaki	32	31	N 32°91' E 132°48'
Ugurujima	3	Kochi Prefecture, Sukumo City, Okinoshima-Cho, Ugurujima	6	0	N 32°80' E 132°49'
Okinoshima	4	Kochi Prefecture, Sukumo City, Okinoshima-Cho, Okinoshima	17	7	N 32°72' E 132°55'

Locality No. Corresponds to that given in Figure 1.

To measure stomatal density, we collected 58 and 35 samples of *A. hispidus* var. *insularis* with and without hairs, respectively, from Kashiwajima, Okinoshima, and Ugurujima in southwestern Shikoku (Figure 1, Table 1). Five fully-expanded stem leaves at the middle part of plant height were analyzed from each individual. We used the average value of five leaves for each individual. We measured the leaf size and counted the leaf hairs on the abaxial side of stem leaves. To measure the stomatal density of a leaf, the leaf surface was replicated using Suzuki's Universal Micro-Printing (SUMP) method. The middle part of the blade along the midrib was analyzed to determine the stomatal density. Replicas of each leaf (1 cm^2) were prepared in order to count the stomatal density. The SUMP images were examined twice for each leaf using a light microscope. The collected data were analyzed using *t*-test and *U*-test to compare the characteristics.

3. Results and Discussion

The number of leaf hairs varied from zero to over 300 (Figure 2), suggesting that *A. hispidus* var. *insularis* has not only hairless-leaf type, but also hairy-leaf type. From this result, we divided *A. hispidus* var. *insularis* into two types by of presence or absence of leaf hairs. The leaf size values of hairless-leaf type and hairy-leaf type individuals were 222.0 ± 158.0 mm^2 and 219.2 ± 149.9 mm^2, respectively with no significant difference between them (Table 2). The relationship between the leaf size and leaf hair number was not significantly correlated in each leaf (r^2 = 0.1387), suggesting that the number of leaf hairs was not determined allometrically. The degree of herbivory of hairless-leaf type and hairy-leaf type individuals were 4.6 ± 6.8% and 8.0 ± 9.3%, respectively, in the Kashiwajima population and 3.9% ± 7.2% and 3.6% ± 8.5%, respectively, in the Komomisaki population (Figure 3). There was no significant difference between these traits, suggesting that the leaf hairs play a minor role in mechanical defense against herbivore attack. On the contrary, the stomatal density of hairless-leaf type and hairy-leaf type individuals of *A. hispidus* var. *insularis* were 108.2 ± 17.7 and 96.0 ± 13.0, respectively, with a significant difference between the values (Table 2). Thus, there were fewer stomata in hairy-leaf-type individuals than in the hairless-leaf-type individuals.

Table 2. Morphological and anatomical measurements (average ± standard deviation) of *Aster hispidus* var. *insularis*

Trait	hairless-leaf (n=58)	hairy-leaf (n=35)	Significance
Morphological measurements			
Leaf length (mm)	30.3 ± 9.7	30.2 ± 9.5	n.s
Leaf width (mm)	6.5 ± 2.2	6.4 ± 2.2	n.s
Leaf size (mm^2)	222.0 ± 158.0	219.2 ± 149.9	n.s
Number of leaf hair (N)	----------	153.4 ± 97.3	----
Anatomical measurements			
Stomatal density (N/mm^2)	108.2 ± 17.7	96.0 ± 13.0	**

**: $P < 0.05$. n.s. : $P > 0.05$

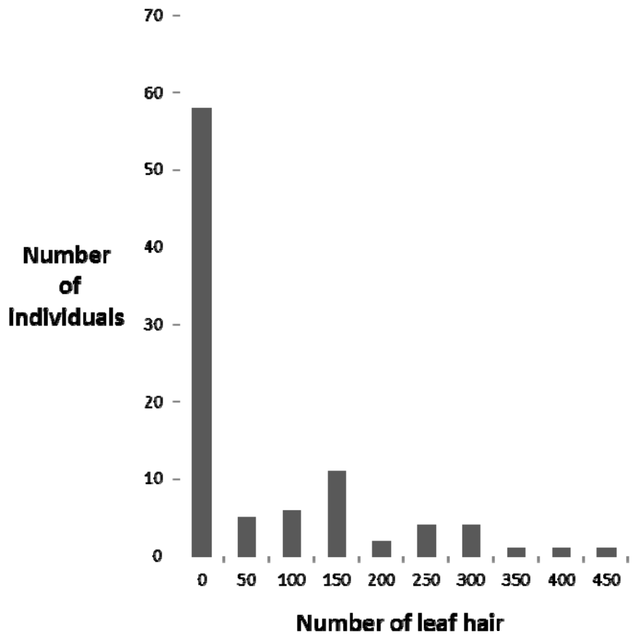

Figure 2. Histogram of hair number on the abaxial side of leaf

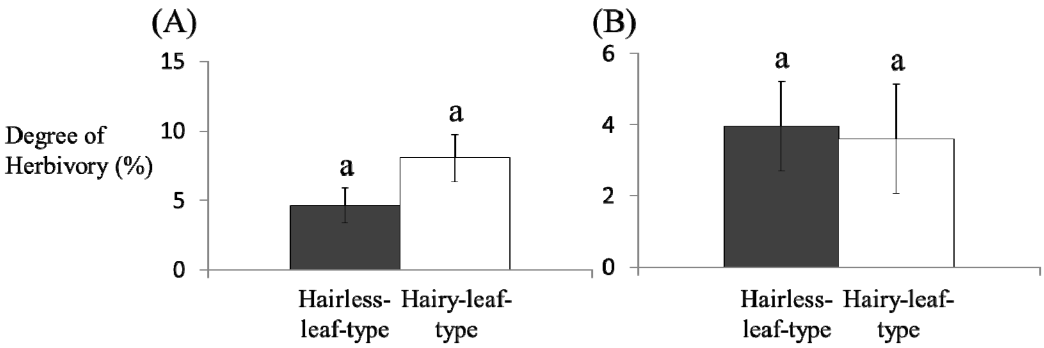

Figure 3. Comparison of the degree of herbivory between the hairless-leaf- and hairy-leaf-type individuals in *Aster hispidus* var. *insularis*

White boxes indicate hairless-leaf-type and black boxes indicate hairy-leaf-type *A. hispidus* var. *insularis* individuals. (A) Kashiwajima; (B) Komomisaki. Same alphabetica (a) indicates no significant difference on *U*-test ($p < 0.05$).

It was unclear whether reduction in the stomatal density of *A. hispidus* var. *insularis* had any adaptive significance. In general, the leaves of the coastal plants were more succulent than those of the inland plants because they can store water and were associated with environments subjected to conditions of drought (Uno et al. 2001). In fact, we found various coastal plants with succulent leaves such as *Cirsium maritimum* Makino (Asteraceae), *Dianthus japonicus* Thunb. (Caryophyllaceae), and *Canavalia lineata* (Thunb.) DC. (Fabaceae) in our study areas. Considering the drought conditions of coastal areas, a decrease in the stomatal density of a leaf was another adaptation that helped prevent excess water loss, because the fewer the stomata, the lesser transpirational water loss (Campbell et al., 1999). Our results indicated that the stomatal density was significantly less in the hairy-leaf-type individuals than in the hairless-leaf-type individuals in *A. hispidus* var. *insularis*.

Additionally, it is very interesting to note that the population of hairy-leaf type individuals of *A. hispidus* var. *insularis* was sympatric to that of hairless-leaf type individuals growing in various areas (Table 1). Kitamura (1981) and Igari (2007) recognized *A. hispidus* var. *insularis* had no hairs on leaves and stems in their reports.

Thus, we considered that the effects from *A. hispidus* var. *hispidus* through hybridization between them would be small for the variation of leaf hairs. Fujikawa et al. (2009) reported the presence of hairy-leaf type individuals using voucher specimens at the MBK herbarium. Because *A. hispidus* var. *insularis* and *A. hispidus* var. *hispidus* cannot be distinguished on the basis of puberulous and glabrous stem leaves, further studies are necessary to investigate more detailed morphological analysis for the two varieties, including the characters of leaf hair and stomatal density. From these results, we propose the following four hypotheses for the leaf hair variation in *A. hispidus* var. *insularis* individuals: (1) the individuals have gained the divergence of leaf hair variation, after speciation of *A. hispidus* var. *insularis* and *A. hispidus* var. *hispidus*, (2) *A. hispidus* var. *insularis* contained the plesiomorphous polymorphic characters for leaf hair variation at speciation from *A. hispidus* var. *hispidus*, (3) *A. hispidus* var. *insularis* have gained the divergence of leaf hair variation through hybridization between glabrous *A. hispidus* var. *insularis* and puberulous *A. hispidus* var. *hispidus*, and (4) *A. hispidus* var. *insularis* evolved fewer times with or without leaf hair variations from *A. hispidus* var. *hispidus*. Yokoo et al. (2009) revealed the phylogenetic relationships among *A. hispidus* varieties that the edaphic varieties, *A. hispidus* var. *insularis,* may have derived allopatrically. Thus, among our hypotheses, hypothesis four could be the most candidate hypothesis. Interestingly, *Chrysanthemum japonense* (Makino) Nakai var. *ashizuriense* Kitam is limited to the distribution of this area (Fujikawa et al., 2009), which species has more hairs on abaxial side of the leaves than those of closely related taxa, *C. japonense* var. *japonense* (Kitamura, 1981). These results imply that it is likely to select the hairly-leaf type individuals of *A. hispidus* var. *insularis* and *C. japonense* in this area. Our results of the presence of individuals with leaf hair and reduction of stomatal density might be one of effective adaptation mechanisms and increasing divergence for coastal areas. Therefore, ecological investigations of the leaf-hair type of *A. hispidus* var. *insularis* individuals will be necessary in the future.

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