

Comparative Analyses of Stomatal Size and Density among Ecotypes of *Aster hispidus* (Asteraceae)

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ABSTRACT

To determine the size and the density of stomata among different environments, we conducted anatomical analyses using *Aster hispidus* var. *hispidus* (open field), *As. hispidus* var. *leptocladus* (serpentine soil), and *As. hispidus* var. *insularis* (coastal). The stomatal size was not significantly different among these ecotypes but the density of stomata in the serpentine and coastal ecotypes was significantly lower than that of *As. hispidus* var. *hispidus*, which suggests that these ecotypes have experienced selection that reduced the density of stomata for adaptation to the dry conditions of serpentine and coastal areas.

Keywords: *Aster hispidus*; Coastal; Ecotype; Density; Guard Cell; Serpentine; Size

1. Introduction

Stomata control the movement of gases in and out of a leaf, make carbon dioxide available for photosynthesis, and control the loss of water from the leaf through transpiration. Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis. The stomatal pores that perforate leaf surfaces are among the best-characterized examples of the fundamental biological relationship between form and function [1]. The density of the stomata determines the stomatal conductance to CO₂ and H₂O [2,3] because gaseous diffusion is regulated through turgor-mediated variation in the aperture of stomatal pores [4,5]. The closure of stomata under dry atmospheric or soil conditions limits CO₂ diffusion from the atmosphere to chloroplasts; therefore, stomatal physiology is inextricably linked with stomatal physiology is inextricably linked to the physiology of photosynthesis [6]. As a result, the patterning of stomata on leaf surfaces is correlated strongly with hydrological conditions [7,8].

Aster hispidus Thunb. (Asteraceae) is widely distributed across China, Korea, and Japan, and is found in lowland open fields and grasslands. This species has intraspecific taxa in Japan, namely *As. hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus* (Makino) Okuyama, *As. hispidus* var. *koidzumianus* (Kitam.) Okuyama, *As.*

hispidus var. *tubulosus* K. Asano ex T. Shimizu, and *As. hispidus* var. *insularis* (Makino) Mot. Ito [9,10]. Of these varieties, *As. hispidus* var. *leptocladus* is found in serpentine areas of central Honshu, Kyushu, and Shikoku, and *As. hispidus* var. *insularis* is restricted to coastal areas along the Pacific Ocean of western Shikoku and eastern Kyushu [9,11]. It is possible that the ecological transitions from open habitats (*As. hispidus* var. *hispidus*) to serpentine (*As. hispidus* var. *leptocladus*) or coastal conditions (*As. hispidus* var. *insularis*) has driven the evolution of stomatal patterning. The stomatal number may provide insights into evolution in terms of leaf anatomy and the response to different ecological changes. However, the extent to which the diversity of stomatal traits is linked to habitat remains unknown. The aim of this study was to characterize the anatomy of *As. hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus*, and *As. hispidus* var. *insularis* with regard to the occurrence of stomata on the abaxial leaf surface. The stomatal number changed (*i.e.*, stomata density was reduced on the surface) as the ecotypes of *As. hispidus* moved into regions with distinct environments.

2. Materials and Methods

The samples used in this study were obtained from open (*Aster hispidus* var. *hispidus*), serpentine (*As. hispidus* var. *leptocladus*), and seashore fields (*As. hispidus* var. *insularis*) (Figure 1, Table 1).

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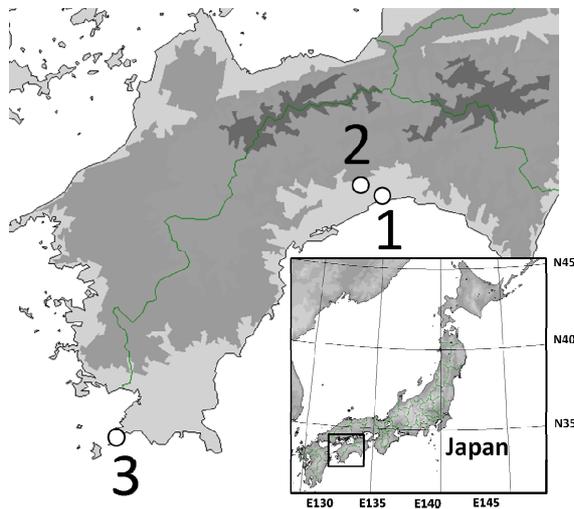


Figure 1. Sampling localities used in this study. For other abbreviations, see Table 1.

Table 1. Sampling localities used in this study.

Species	Sample size	Locality no.	Locality	Latitude and Longitude
<i>Aster hispidus</i> var. <i>hispidus</i>	58	1	Kochi Prefecture, Nankoku City, Monobe	33°33'12"N, 133°40'43"E
<i>Aster hispidus</i> var. <i>leptocladus</i>	22	2	Kochi Prefecture, Kochi City, Ikku	33°35'40"N, 133°35'12"E
<i>Aster hispidus</i> var. <i>insularis</i>	15	3	Kochi Prefecture, Hata-gun, Otsuki-Cho Hirayama	32°47'15"N, 132°40'4"E

Locality no. corresponds to that given in **Figure 1**.

We analysed 58, 22, and 15 leaves from individuals of *As. hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus*, and *As. hispidus* var. *insularis*, respectively. The middle part of a leaf blade along the midrib was examined. To identify the morphology of the guard cells and the density of the stomata, the abaxial leaf surface was replicated using Suzuki's Universal Micro-Printing (SUMP) method. Replicas of each leaf (1 mm²) were prepared to determine the stomatal density and to measure the guard cell size and epidermal cell size (10 cells per leaf) using cellSens standard Imaging software (Olympus Co., Tokyo) (**Figure 2**). Moreover we treated as an individual value the average value of the measured data. The collected data were analysed using Tukey's honestly significant difference (HSD) test to compare the characters. The numerical data were subjected to statistical analysis based on [12].

3. Results and Discussion

The guard cell size of *Aster hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus*, and *As. hispidus* var. *insularis*

was $204.31 \pm 23.55 \mu\text{m}^2$, $227.71 \pm 55.25 \mu\text{m}^2$, and $207.49 \pm 20.24 \mu\text{m}^2$, respectively, and there was no significant differences among them (**Table 2**). Moreover, the density of stomata (N/mm²) in *As. hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus*, and *As. hispidus* var. *insularis* was 70.66 ± 13.29 , 28.52 ± 6.79 , and 33.42 ± 4.09 , respectively (**Table 2**). The stomatal density in *As. hispidus* var. *leptocladus* and *As. hispidus* var. *insularis* was significantly fewer than that of *As. hispidus* var. *hispidus*. The abaxial epidermal cell size of *As. hispidus* var. *hispidus*, *As. hispidus* var. *leptocladus*, and *As. hispidus* var. *insularis* was $883.71 \pm 239.24 \mu\text{m}^2$, $1563.65 \pm 475.82 \mu\text{m}^2$, and $2012.09 \pm 319.85 \mu\text{m}^2$, respectively (**Table 2**). The abaxial epidermal cell size of *As. hispidus* var. *leptocladus* and *As. hispidus* var. *insularis* was significantly smaller than that of *As. hispidus* var. *hispidus*. Thus, *As. hispidus* var. *leptocladus* and *As. hispidus* var. *insularis* showed reduced stomatal density because of increased epidermal cell size and decreased stomatal number relative to *As. hispidus* var. *hispidus*.

Stomatal control of water loss allows plants to occupy habitats with fluctuating environmental conditions. A reduction in stomatal density may have occurred in the *As. hispidus* complex as an adaptation to reduce the evaporation and transpiration of water. In fact, plants growing in high-salinity habitats exposed to more or less constant water stress. *Aster hispidus* var. *insularis* occurs in coastal areas on the Pacific Ocean side. To survive coastal conditions, plants have developed growth strategies such as increased water-use efficiency via C₄- or CAM metabolism [13], succulent growth, and extensive root systems [14]. Therefore, *As. hispidus* var. *insularis* has adapted to coastal areas by developing succulent growth and by reducing stomatal density.

Geologically unique areas are often centres of biodiversity, and thus plant speciation. Serpentine soils present a generally stressful growing environment for plants because of a number of edaphic factors such as a low Ca/Mg ratio, low levels of essential plant nutrients, poor water retention, and high concentrations of several heavy metals [15]. In particular, high levels of nickel in the soil block a plant's ability to take in soil nutrients. In fact, plants growing on serpentine soils were found to have a low Ca/Mg ratio and high concentrations of heavy metals [16]. Moreover, because serpentine soils are shallow and low in organic matter and clay, they also cannot hold water well [15], suggesting that this environment is similar to the dry conditions. Our results indicate that the serpentine-adapted variety *As. hispidus* var. *leptocladus* had a lower density of stomata than *As. hispidus* var. *hispidus* (**Table 2**). This finding suggests that this stomatal type developed for adaptation to dry environments. Similarly, [17] reported that the stomatal size and density of the serpentine ecotype of *Adenophora*

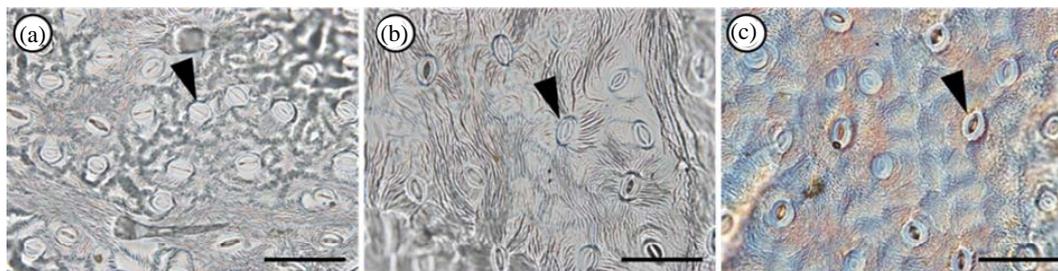


Figure 2. Suzuki's Universal Micro-Printing method (SUMP) replicas. Abaxial epidermal cells of (a) *Aster hispidus* var. *hispidus*; (b) *As. hispidus* var. *leptocladus*; and (c) *As. hispidus* var. *insularis*. Arrowheads indicate stomata. Bar = 100 μm .

Table 2. Anatomical measurement (average \pm standard deviation) of the *Aster hispidus* ecotypes.

Trait	<i>Aster hispidus</i>					
	var. <i>hispidus</i>		var. <i>leptocladus</i>		var. <i>insularis</i>	
Guard cell size (μm^2)	204.31 \pm 23.55	a	227.71 \pm 55.25	b	207.49 \pm 20.24	ab
Stomatal density (N/mm ²)	70.66 \pm 13.29	b	28.52 \pm 6.79	a	33.42 \pm 4.09	a
Epidermal cell size (μm^2)	883.71 \pm 239.24	a	1563.65 \pm 475.82	b	2012.09 \pm 319.85	c

Columns marked by different letters differ significantly according to the Tukey's HSD test ($p < 0.05$).

triphyllo (Thunb.) A. DC. var. *japonica* (Regel) H. Hara (Campanulaceae) were not significantly different from those of the normal type of this species; however, the leaf size of the serpentine ecotype was significantly smaller than that of the normal ecotype of *Ad. triphylla* var. *japonica*. Therefore, the stomatal number per leaf of the serpentine ecotype was lower than that of the normal type of this species [17], which is consistent with our results of decreased stomatal number per leaf in serpentine areas.

We analysed the size and density of stomata in the *As. hispidus* complex using samples adapted to different environments. However, the samples used for our analyses were collected from one population for each taxon. [18] indicated that the leaf morphology of *As. hispidus* var. *leptocladus* was significantly different among populations. Considering this report, it is possible that the size and/or density of the stomata of *As. hispidus* var. *leptocladus* and *As. hispidus* var. *insularis* may vary among populations. Therefore, additional samples from other populations will shed additional light on stomatal differentiation in different environments.

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