

Leaf Adaptation of *Eurya japonica* Thunb. (Pentaphylacaceae) in Coastal Area

Masayuki Shiba¹, Tomoki Tate² & Tatsuya Fukuda¹

¹ Graduate School of Integrative Science and Engineering, Tokyo City University, Tokyo, Japan

² Graduate School of Integrated Arts and Sciences, Kochi University, Nankoku, Kochi, Japan

Correspondence: Tatsuya Fukuda, Graduate School of Integrative Science and Engineering, Tokyo City University, 1-28-1 Tamazutsumi, Setagata, Tokyo, Japan. E-mail: tfukuda@tcu.ac.jp

Received: August 26, 2022

Accepted: September 22, 2022

Online Published: September 24, 2022

doi:10.5539/jps.v11n1p31

URL: <https://doi.org/10.5539/jps.v11n1p31>

Abstract

To clarify the process of plant adaptation to coastal areas, we conducted morphological and anatomical analyses of *Eurya japonica* Thunb. (Pentaphylacaceae). There was no significant difference in leaf shape between the inland and coastal populations, although the leaves in coastal populations tended to be thicker. However, our anatomical analysis revealed significant differences in stomatal size and adaxial and abaxial epidermal cell sizes. The smaller stomata of the coastal population of this species were effective in reducing transpiration during gas exchange. Furthermore, the expansion of epidermal cells could be an adaptive strategy to retain water in leaves in coastal environments.

Keywords: adaptation, anatomy, *Eurya japonica*, morphology, coastal area

1. Introduction

Various adaptation processes frequently result from spatial variations in selective environmental forces acting on phenotypic diversity (Hereford, 2009). As selective forces operate, they may reduce heritable variation within a population, leading to the specialization of individuals (Kawecki & Ebert, 2004). Conversely, in highly stochastic environments, selection can increase the potential of a species for phenotypic plasticity (Albert et al., 2011). Therefore, research on adaptation is essential for understanding the response of a species to environmental changes (Jump & Peñuelas, 2005; Aitken et al., 2008). The environment provided by the land-sea interface supports many plant species that are not found further inland (van der Maarel, 2003). Harsh conditions in many coastal areas limit the range of plant species that can be successfully grown (Rozema et al., 1985). Most coastal plant species have gained characteristics which make them ideally suited to withstand the effects of the coastal environment such as strong persistent on-shore winds carrying sand and salt spray which dry out the leaves and soil (Hesp, 1991; Maun, 1998; Ciccarelli et al., 2009). Therefore, coastal adaptation is often a product of environmental variations in geographical space (Ciccarelli et al., 2010). Coastal plants are known for their rich biodiversity and high level of endemism, including a concentration of rare and threatened taxa and high diversity of endemic plant species (Burgess et al., 1998; Lovett, 1998; Myers et al., 2000; Azeria et al., 2007), and they have implications for biodiversity conservation (van der Meulen & Udo de Haes, 1996; Cori, 1999; Schlacher et al., 2008). Particular attention has been focused on understanding ecophysiological mechanisms involved at the cellular and molecular levels (Elhaak et al., 1997; Migahid & Elhaak, 2001; Tunala et al., 2012; Kumekawa et al., 2013; Ohga et al., 2013; Sunami et al., 2013; Takizawa et al., 2022). It is uncertain whether plant species widely distributed from inland to coastal areas have adapted with or without morphological changes. For example, Tunala et al. (2012) indicated that the epidermal cells of the coastal variety *Aster hispidus* Thunb. var. *insularis* (Makino) Okuyama (Asteraceae) were larger but fewer than those of *As. hispidus* Thunb. var. *hispidus* and had succulent leaves to store water. Additionally, Sunami et al. (2013) reported that leaf hairs on the abaxial side of leaves were correlated with the stomatal density of this variety; the fewer the hairs on the leaf, the lower the stomatal density to avoid transpirational water loss. Ohga et al. (2013) suggested that the coastal population of *Adenophora triphylla* var. *japonica* (Regel) H.Hara (Campanulaceae) has evolved relatively thick leaves via a heterochronic process to store water. Generally, stomata are the major gates for gas exchange in leaves (Raschke, 1975; Schoch et al., 1980; Brent & Ram, 2000). Guard cells surrounding the stomata contain chloroplasts, and they increase stomata sugar concentration, which in turn causes water absorption and swelling of cells (Jarvis,

1976; Ewers et al., 2001). Additionally, stomatal conductance depends on leaf characteristics, such as the size, number, and frequency of stomata and leaves (Cole & Dobrenz, 1970; Teare et al., 1971; Cihá & Brown, 1975). Therefore, plants in wide habitats, including coastal areas, adapt to coastal conditions through morphological and anatomical changes.

Grasses differ from trees due to the spatial separation of rooting niches and the differential utilization of below-ground resources such as water. Trees exclusively access water in deeper soil horizons, whereas grasses use water primarily in the topsoil (Hesla et al., 1985; Knoop & Walker, 1985; Sala et al., 1989). Therefore, water stress on herbs in coastal areas is considered to be much greater than that on trees because of shore winds carrying sand and salt spray. However, Cruz et al. (2019) reported that *Avicennia schaueriana* Stapf & Leechm. ex Moldenke (Acanthaceae), a dominant coastal tree, had small leaves with a low stomatal conductance and transpiration. However, Takizawa et al. (2022) reported that the stomatal size of *Ligustrum japonicum* Thunb. (Oleaceae) was significantly smaller in coastal areas than that in inland areas. Thus, analysis of the adaptations of tree species in coastal areas suggested that tree species also undergo some morphological changes to adapt to coastal areas. Furthermore, these stomatal studies revealed that the adaptation pattern in coastal areas reduced the density of stomata in grass species, while tree species decreased stomata size, suggesting that stomatal adaptation in coastal areas had different modes between grass and tree species. However, only a few studies have reported on the coastal adaptation of tree species.

Eurya japonica is a broad-leaved evergreen woody perennial approximately 1-3.5 m tall that is widely distributed in warm temperate forests in Japan (from Honshu to Ryukyu) and Korea (Tsuyama, 1989). Nagamasu (2006) and Nakajima and Yoshizaki (2018) reported that *E. japonica* comprises a coastal forest with *Eurya emarginata* (Thunb.) Makino, *Machilus thunbergii* Siebold et Zucc. (Lauraceae), and *Ilex integra* Thunb. (Aquifoliaceae). Shiba et al. (2021) indicated that *E. japonica* in riverside populations develops thicker leaves and a higher stomatal density than those in inland populations for adaptation to strong solar radiation, and waterlogged environments. Moreover, serpentine populations of this species have small and thick leaves and reduced stomatal size to minimize water loss via gas exchange (Shiba et al., 2022). Findings on *E. japonica* suggested that this species has adapted to unique environments by changing its morphological and anatomical characteristics. This species is expected to undergo some morphological variations when colonizing coastal areas. Therefore, investigating the coastal adaptation of *E. japonica* is important when considering coastal forests that play a major role as ecosystems with high conservation value. Therefore, this study aimed to clarify the *E. japonica* adaptation leaf traits by comparing individuals between coastal and inland areas using morphological and anatomical approaches (Figure 1).



Figure 1. *Eurya japonica* Thunb. a: coastal (Tei), b: inland (Noichi)

2. Method

The *Eurya japonica* samples examined in this study were collected from a field in Shikoku, Japan. The collection locations are shown in Figure 2 and Table 1. The inland populations near each coastal area were used

as controls. Two hundred and ten individuals (30 individuals per population) representing 7 populations involved in 4 coastal areas and 3 populations were sampled. Among these, Ishima in Tokushima Pref. is a small island in the Kii Channel in the Pacific Ocean between Honshu and Shikoku at the eastern-most tip of Shikoku (Figure 2). Although it is difficult to define coastal forests on this island, individuals sampled from these islands were used as the coastal area samples in this study, because *E. japonica* had been grown together with the following coastal endemic species: *Pittosporum tobira* (Thunb.) W.T.Aiton (Pittosporaceae) and *E. emarginata* in the coastal forests. According to the Japan Meteorological Agency, our coastal sampling areas had stronger winds than inland areas (Figure 3).

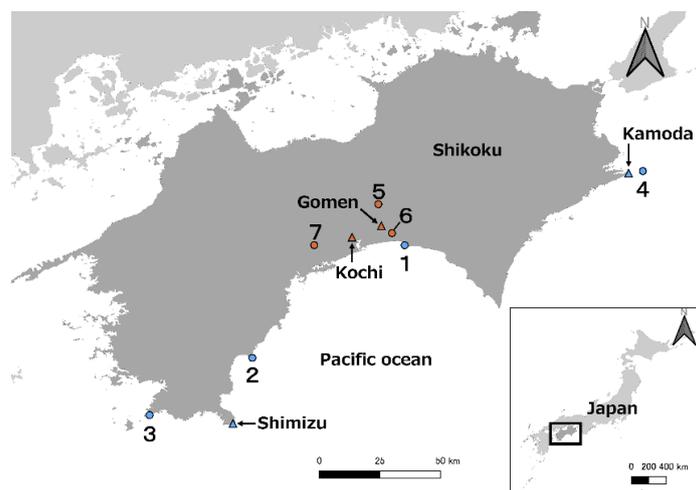


Figure 2. Sampling localities in this study

Number in corresponds to that given in Table 1. Blue and brown circles indicate coastal and inland areas, respectively. Blue and brown triangles indicate anemometer locations near coastal and inland areas, respectively.

Table 1. Sampling localities used in this study

Type	Locality name & number*	Locality	Latitude and longitude	
Coastal				
	Tei	1 Tei, Yasu-cho, Konan City, Kochi Pref.	33 °31'N	133 °45'E
	Inomisaki	2 Ida, Kuroshio-cho, Hata-gun, Kochi Pref.	33 °01'N	133 °04'E
	Kashiwajima	3 Kashiwajima, Otsuki-cho, Hata-gun, Kochi Pref.	32 °77'N	132 °65'E
	Ishima	4 Ishima, Ishima-cho, Anan City, Tokushima Pref.	33 °85'N	134 °81'E
Inland (control)				
	Shigeto	5 Kashinotani, Tosayamada-cho, Kami City, Kochi Pref.	33 °41'N	133 °39'E
	Noichi	6 Ohtani, Noichi-cho, Konan City, Kochi Pref.	33 °34'N	133 °42'E
	Okina	7 Okina, Hidaka-mura, Takaoka-gun, Kochi Pref.	33 °30'N	133 °20'E

*: locality no. corresponds to that given in Figure 2.

For morphological analysis, individuals were measured for leaf blade length and width and leaf thickness using a digimatic caliper (CD-15CXR; Mitutoyo Corporation, Kanagawa, Japan) and digimatic outside micrometer (MDC-SB; Mitutoyo Corporation). Leaf measurements were taken from a fully expanded leaf at the midpoint of plant height. Three leaves were randomly selected from one individual, and the average value was calculated.

Fully expanded leaves were collected from each individual for anatomical analysis. To count the number of stomata on the leaf blade, the abaxial surface of leaves was peeled off using Suzuki's universal micro-printing (SUMP) method (Kijima, 1962). Briefly, the middle part of the blade along the midrib was examined to determine the number and size of stomata. Replicas of each leaf (1 cm²) were prepared to determine the stomatal density (Number/μm²) and size of five leaves per leaf. The stomatal size was calculated using the following formula: stomatal length × stomatal width/2, based on a study by Kumekawa et al. (2013). The copied SUMP images for each individual were examined once using a light microscope (CX41; OLYMPUS, Tokyo, Japan).

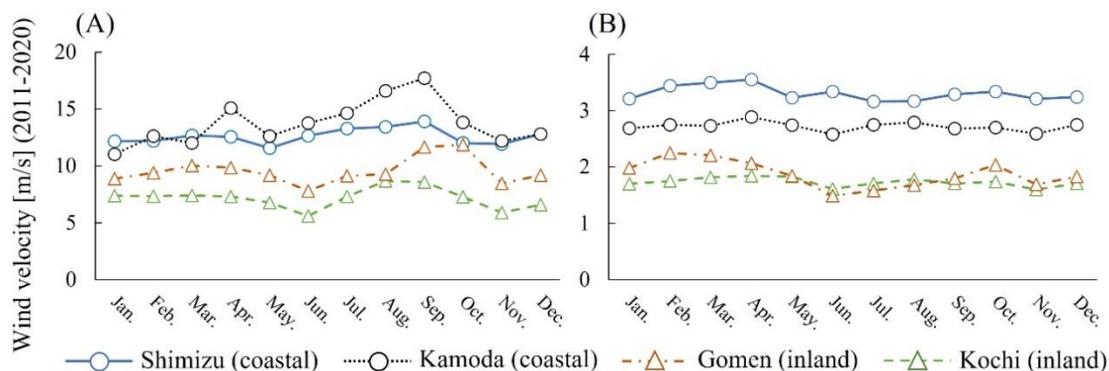


Figure 3. Comparison of wind speeds in the neighborhood of the sampling localities

Localities are shown in Figure 2. (A) Comparisons of average of maximum wind velocity (2011-2020) and (B) comparisons of average of mean wind velocity (2011-2020) between Shimizu (coastal), Kamoda (coastal), Gomen (inland) and Kochi (inland). Open circles are wind velocity data of coastal areas, and open triangles are wind velocity data of inland areas.

3. Results

A summary of *Eurya japonica* leaf morphology measurements is presented in Table 2. In the four coastal areas (Tei, Inomisaki, Kashiwajima, and Ishima), leaf sizes were $1,295.86 \pm 80.81$, $1,221.21 \pm 38.93$, $1,095.85 \pm 54.43$, and $1,401.50 \pm 60.61$ mm²; leaf thicknesses were 285.43 ± 3.71 , 293.20 ± 4.48 , 249.38 ± 14.21 , and 227.73 ± 12.72 μm, respectively. In three inland areas (controls [Shigeto, Noichi, and Okina]), leaf sizes were $1,149.85 \pm 27.62$, $1,252.92 \pm 47.81$, and $1,177.75 \pm 41.92$ mm², and leaf thicknesses were 191.43 ± 8.90 , 173.01 ± 7.90 , and 183.85 ± 6.34 μm, respectively. There were no significant morphological differences in *E. japonica* between the coastal and inland areas. In this study, the leaf index values were calculated as the leaf length to width ratio based on Tsukaya (2002). A significant difference in leaf index values indicates that the population with a large value has narrow leaves. The leaf indexes of the coastal populations Tei, Inomisaki, Kashiwajima, and Ishima were 2.45 ± 0.06 , 2.39 ± 0.04 , 2.58 ± 0.06 , and 2.46 ± 0.05 , and those of inland controls Shigeto, Noichi, and Okina were 2.66 ± 0.05 , 2.37 ± 0.04 , and 2.47 ± 0.05 , respectively. However, there was no significant difference in leaf index between the coastal and inland populations.

Table 2. Leaf morphology (average ± standard error) of *Eurya japonica*

	Coastal			
	Tei	Inomisaki	Kashiwajima	Ishima
size (mm ²)	1,295.86±80.81 ab	1,221.21±38.93 abc	1,095.85±54.43 bcd	1,401.50±60.61 a
thickness (μm)	285.43±3.71 ab	293.20±4.48 abc	249.38±14.21 bc	227.73±12.72 cd
leaf index*	2.45±0.06 ab	2.39±0.04 b	2.58±0.06 ab	2.46±0.05 ab

Table 2. (Continued).

	Inland (control)		
	Shigeto	Noichi	Okina
size (mm ²)	1,149.85±27.62 bc	1,252.92±47.81 abc	1,177.75±41.92 bc
thickness (μm)	191.43±8.90 de	173.01±7.90 e	183.85±6.34 e
leaf index*	2.66±0.05 a	2.37±0.04 b	2.47±0.05 ab

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

*: Tsukaya (2002)

For anatomical analyses, we measured adaxial and abaxial epidermal cell sizes using SUMP samples from coastal and inland areas containing *E. japonica* from all examined locations (Table 3 & Figure 4). The adaxial epidermal cell sizes of the coastal areas Tei, Inomisaki, Kashiwajima, and Ishima were 580.17 ± 19.71 , 619.83 ± 20.25 , 505.38 ± 14.38 , and 557.64 ± 11.25 μm², respectively, and those of abaxial were 503.40 ± 16.08 , 578.79 ± 19.26 , 442.01 ± 4.09 , and 443.57 ± 6.84 μm², respectively. In the inland areas Shigeto, Noichi, and Okina,

Table 3. Anatomical measurements (average \pm standard error) of epidermal cell size (μm^2) in *Eurya japonica*

	Coastal			
	Tei	Inomisaki	Kashiwajima	Ishima
adaxial	580.17 \pm 19.71 ab	619.83 \pm 20.25 a	505.38 \pm 14.38 c	557.64 \pm 11.25 bc
abaxial	503.40 \pm 16.08 b	578.79 \pm 19.26 a	442.01 \pm 4.09 c	443.57 \pm 6.84 c

Table 3. (Continued).

	Inland (control)		
	Shigeto	Noichi	Okina
adaxial	401.69 \pm 7.76 d	400.94 \pm 10.61 d	411.94 \pm 9.59 d
abaxial	354.63 \pm 12.45 d	356.44 \pm 3.66 d	328.79 \pm 3.77 d

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

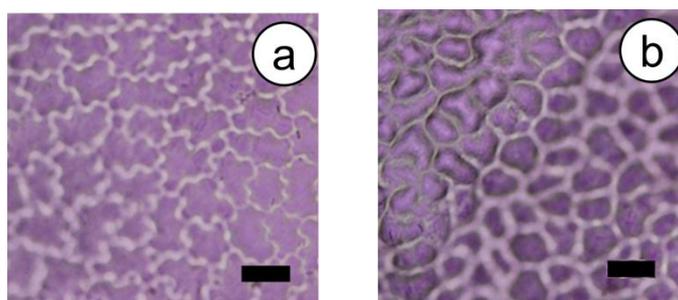


Figure 4. Epidermal cell on adaxial surface

a: coastal (Tei), b: inland (Shigeto). Bar = 30 μm

Table 4. Anatomical measurements (average \pm standard error) of mesophyll tissues using longitudinal section in *Eurya japonica*

	Coastal			
	Tei	Inomisaki	Kashiwajima	Ishima
epidermal cell at adaxial side (μm)	25.90 \pm 0.84 a	26.95 \pm 0.59 a	26.18 \pm 1.09 a	23.68 \pm 1.02 a
palisade tissue (μm)	92.25 \pm 1.91 a	93.70 \pm 1.31 a	75.43 \pm 8.64 abc	82.35 \pm 5.82 ab
spongy tissue (μm)	144.80 \pm 1.94 a	148.25 \pm 3.09 a	127.80 \pm 6.59 a	103.38 \pm 5.85 bc
epidermal cell at abaxial side (μm)	22.48 \pm 0.74 ab	24.30 \pm 0.45 a	20.88 \pm 0.68 b	18.83 \pm 0.60 c

Table 4. (Continued).

	Inland (control)		
	Shigeto	Noichi	Okina
epidermal cell at adaxial side (μm)	13.28 \pm 0.70 c	17.18 \pm 0.58 b	17.65 \pm 0.63 b
palisade tissue (μm)	63.20 \pm 3.90 bc	59.88 \pm 3.36 c	61.58 \pm 3.45 c
spongy tissue (μm)	105.38 \pm 6.33 bc	82.30 \pm 5.26 c	90.43 \pm 3.82 bc
epidermal cell at abaxial side (μm)	9.58 \pm 0.49 e	13.65 \pm 0.34 d	14.20 \pm 0.63 d

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

adaxial epidermal cell sizes were 401.69 \pm 7.76, 400.94 \pm 10.61, and 411.94 \pm 9.59 μm^2 , respectively, and those of abaxial were 354.63 \pm 12.45, 356.44 \pm 3.66, and 328.79 \pm 3.77 μm^2 , respectively. The *E. japonica* cell sizes between the coastal and inland areas were significantly different. Additionally, we measured the heights of the epidermal cells and palisade and spongy tissues that contributed to the thickness of the leaves using the longitudinal section of the leaf, as shown in Table 4 and Figure 5. In the coastal areas Tei, Inomisaki, Kashiwajima, and Ishima, the heights of epidermal cells on the adaxial side, palisade tissue, spongy tissue and epidermal cells on the abaxial side were 25.90 \pm 0.84, 26.95 \pm 0.59, 26.18 \pm 1.09 and 23.68 \pm 1.02, 92.25 \pm 1.91, 93.70 \pm 1.31, 75.43 \pm 8.64 and 82.35 \pm 5.82, 144.80 \pm 1.94, 148.25 \pm 3.09, 127.80 \pm 6.59 and 103.38 \pm 5.85, and 22.48 \pm 0.74, 24.30 \pm 0.45, 20.88 \pm 0.68 and 18.83 \pm 0.60 μm , respectively. In the inland areas, the heights of

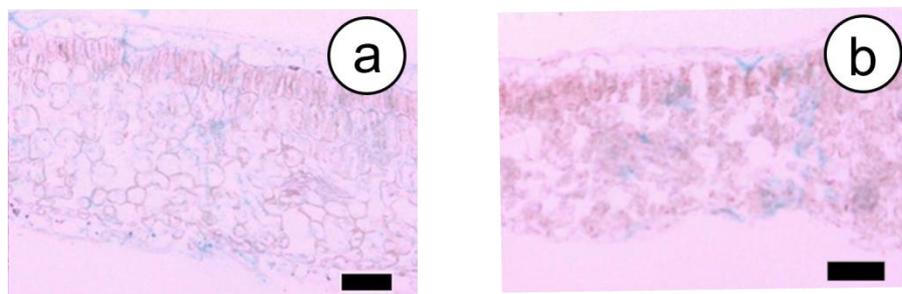


Figure 5. Longitudinal section of leaf

a: coastal (Tei), b: inland (Shigeto). Bar = 50 μm

Table 5. Anatomical measurements (average \pm standard error) of stomata in *Eurya japonica*

	Coastal			
	Tei	Inomisaki	Kashiwajima	Ishima
size (μm^2)	87.03 \pm 1.60 c	88.69 \pm 1.36 c	90.25 \pm 1.19 c	89.27 \pm 1.49 c
density (N/ μm^2)	233.95 \pm 6.49 c	261.78 \pm 3.89 b	288.18 \pm 10.44 a	238.64 \pm 4.28 bc

Table 5. (Continued).

	Inland (control)		
	Shigeto	Noichi	Okina
size (μm^2)	104.75 \pm 1.34 ab	107.02 \pm 1.93 a	100.17 \pm 1.64 b
density (N/ μm^2)	243.33 \pm 6.49 bc	233.26 \pm 5.67 c	259.19 \pm 4.90 bc

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

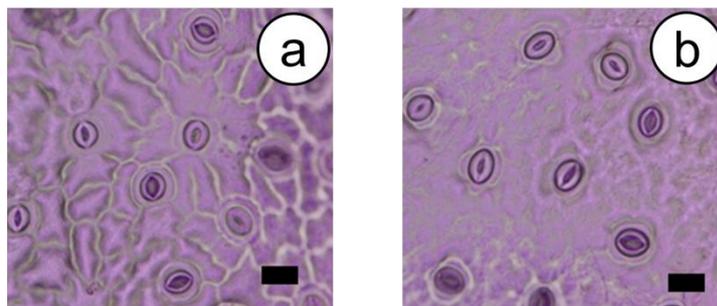


Figure 6. Stomata on abaxial surface

a: coastal (Tei), b: inland (Shigeto). Bar = 20 μm

epidermal cells were 13.28 ± 0.70 , 17.18 ± 0.58 and 17.65 ± 0.63 , 63.20 ± 3.90 , 59.88 ± 3.36 and 61.58 ± 3.45 , 105.38 ± 6.33 , 82.30 ± 5.26 and 90.43 ± 3.82 , and 9.58 ± 0.49 , 13.65 ± 0.34 and 14.20 ± 0.63 μm , respectively. The results showed that the heights of epidermal cells on both sides were significantly different between coastal and inland populations. However, those of palisade and spongy tissues showed no significant differences. Furthermore, we measured the mean stomatal size (guard cell area based on guard cell pair length and width) and density (Table 5 & Figure 6). There were significant differences between the stomatal sizes of the four coastal populations (87.03 ± 1.60 , 88.69 ± 1.36 , 90.25 ± 1.19 , and 89.27 ± 1.49 μm^2 in Tei, Inomisaki, Kashiwajima, and Ishima, respectively) and those of the inland populations (104.75 ± 1.34 , 107.02 ± 1.93 , and 100.17 ± 1.64 μm^2). However, there were no significant differences between the stomatal density (233.95 ± 6.49 , 261.78 ± 3.89 , 288.18 ± 10.44 , and 238.64 ± 4.28 N/ μm^2 in Tei, Inomisaki, Kashiwajima, and Ishima, respectively) of the coastal populations and those of the inland populations (243.33 ± 6.49 , 233.26 ± 5.67 , and 259.19 ± 4.90 N/ μm^2 in Shigeto, Noichi, and Okina, respectively).

4. Discussion

Some coastal adaptive species, such as *Pittosporum tobira*, *Camellia japonica* L. (Theaceae), *Cirsium maritimum* Makino (Asteraceae), and *Dianthus japonicus* Thunb. (Caryophyllaceae) were present in our coastal sampling areas, and the above-mentioned coastal adaptive species *Adenophora triphylla* var. *japonica* was grown in neighboring areas where we collected samples, suggesting that strong coastal selection pressures affect *Eurya japonica* and these species. Changes in the leaf morphology of coastal species are commonly observed in plants subjected to increased drought and salinity stresses. These were caused by increased leaf water content and subsequent accumulation of large amounts of solute without increasing the osmotic pressure of cells (Suárez & Sobrado, 2000). In the present study, we found that coastal *E. japonica* has significantly smaller stomata and larger epidermal cells than inland area species, indicating that the former gained adaptive characteristics in coastal areas. Many studies have reported that coastal adaptation included a decrease in stomatal density (Tunala et al., 2012; Ohga et al., 2013; Kumekawa et al., 2013). Contrastingly, Takizawa et al. (2022) reported that woody *Ligustrum japonicum* had significantly smaller stomatal sizes in coastal areas than those in inland area species and hypothesized that woody plants could not invade coastal areas from inland areas without stomatal changes, although woody plants could adapt to coastal areas by reducing stomatal size. In fact, herbaceous plants have limited capacity for water retention, but woody plants can retain water in the trunk under dry conditions (Takizawa et al., 2022); however, since the results were for only one woody plant (*L. japonicum*), studies on different woody taxa are needed to test this hypothesis. Our findings are consistent with the hypothesis that *L. japonicum* (Oleaceae) has a large phylogenetic distance from *E. japonica* (Pentaphragmalaceae) based on angiosperm phylogeny (Chase et al., 1993; Soltis et al., 2000; Savolainen & Chase, 2003; Soltis & Soltis, 2004). Therefore, smaller stomatal modifications to introduce inland area species to coastal areas suggest that this process is a common trend in the coastal adaptation of woody plants. To gain a deeper understanding of coastal adaptation in woody plants, it would be interesting to clarify whether changes in stomatal size are genetic or plastic. Frankes et al. (2009) indicated that such stomatal changes were likely due to plasticity; therefore, the modification in stomatal size associated with coastal adaptation of these woody plants may be plastic. In the future, it is necessary to examine plasticity through the cultivation experiment of *E. japonica*.

Our anatomical analyses showed that the epidermal cells of coastal *E. japonica* were larger in size but fewer in number than those of inland. Liu et al. (2005) reported larger cell size in drought-tolerant genotypes in *Boehmeria nivea* (L.) Gaud. (Urticaceae). Moreover, Liu et al. (2011) indicated that artificial tetraploid plants of *Dendranthema nankingense* (Nakai) Tzvel. (Asteraceae), which have larger cells than those in normal diploid plants, showed higher levels of abiotic stress tolerance, including drought and salinity stresses. In addition, Kumekawa et al. (2013) showed that the expansion of cells of coastal endemic taxa, *Aster hispidus* var. *insularis*, could be considered an adaptive strategy to coastal environments that allowed the saving of water in plant bodies. These studies suggested that large cell size of leaf could be resistant of dry condition such as coastal areas. However, the size of the leaves is increased when the cell size are enlarged, and therefore the influence of physical stress such as wind and flying sand is increased in coastal areas. From these reasons, we considered that the number of epidermal cell in coastal *E. japonica* was tended to reduce than that in inland area. It will be necessary for further studies to investigate the correlation between cell size and water content in leaves of these species.

Some genetic studies have provided evidence for the coordination of cell proliferation and expansion of the leaf. For example, the reduction in the final leaf area is compensated for by an increase in the size of individual leaf cells when cell proliferation in a leaf is reduced because of certain mutations, which could aid in understanding the regulation of cell proliferation and expansion at the organ level (Tsukaya, 2002; Horiguchi et al., 2005; Tsukaya, 2008). Kim and Kende (2004) reported that the loss-of-function mutation of model plants, which positively regulate cell proliferation in leaf primordia, causes the typical compensation syndrome (Horiguchi et al., 2005). Additionally, several other mutations that affect leaf cell proliferation cause the compensation syndrome (Mizukami & Fischer, 2000; Ullah et al., 2001; Autran et al., 2002; Nelissen et al., 2003; Clay & Nelson, 2005; Ali et al., 2007; Fujikura et al., 2007). These genetic backgrounds of cell proliferation and leaf expansion are useful considering evolutionary developmental studies of leaf differentiation between coastal and inland *E. japonica*. Therefore, it would be interesting to compare the leaf morphologies of transgenic model plants by introducing these candidate homologs isolated from them.

We analyzed the coastal adaptation of *E. japonica* using morphological and anatomical data. These results provide an unbiased interpretation of the coastal adaptation. Our analyses were highly effective in revealing the coastal adaptation process of *E. japonica*. However, these data were least effective in providing definitive answers to the question of their patterns of adaptation along the seaside. It has been shown that *E. japonica*

adapts to the characteristic environment, such as along riversides (Shiba et al, 2021) and serpentine areas (Shiba et al, 2022), and to coastal environment where selective pressure is strong by changing its morphology. For example, in the genus *Eurya*, *E. emarginata* is a coastal dioecious (Tsuyama, 1989), broad-leaved, and woody perennial species distributed in the Southern China and Japan (western region to Chiba Pref. of Honshu, Shikoku, Kyushu, and Ryukyu Islands) and a few locations in the southern coastal area of the Korean Peninsula (Tsuyama, 1989). In Japan, *E. emarginata* is cultivated along roads and hedges far from coastal areas (data not shown). These inland *E. emarginata* individuals may be subject to different selective pressures in coastal areas. These may have gained adaptive morphology from inland to coastal areas, contrary to the findings of our study. Coastal species are scattered across various taxa in angiosperms and have a history of coastal adaptation. Research on coastal adaptation may lead to environmental conservation of coastal areas, where a high rate of environmental change has been observed.

Acknowledgments

We wish to thank Dr. Yoshizaki S for providing much help. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

- Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Ecological Application*, *1*, 95-111. <https://doi.org/10.1111/j.1752-4571.2007.00013.x>
- Ali, F., Horiguchi, G., Yano, S., & Tsukaya, H. (2007). Analysis of leaf development in fugu mutants of *Arabidopsis* reveals three compensation modes that modulate cell expansion in determinate organ. *Plant Physiology*, *144*, 988-999. <https://doi.org/10.1104/pp.107.099325>
- Albert, C. H., Grassein, F., Schurr, F. M., Vieilledent, G., & Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics*, *13*, 217-225. <https://doi.org/10.1016/j.ppees.2011.04.003>
- Autran, D., Jonak, C., Belcram, K., Beemster, G. T. S., Kronenberger, J., Grandjean, O., ... Traas, J. (2002). Cell numbers and leaf development in *Arabidopsis*: a functional analysis of the STRUWWELPETER gene. *EMBO Journal*, *21*, 6036-6049. <https://doi.org/10.1093/emboj/cdf614>
- Azeria, E. T., Sanmartín, I., Stefan, Ås, Carlson, A., & Burgess, N. (2007). Biogeographic patterns of the east African coastal forest vertebrate fauna. *Biodiversity and Conservation*, *16*, 883-912. <https://doi.org/10.1007/s10531-006-9022-0>
- Brent, E. E., & Ram, O. (2000). Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. *Tree Physiology*, *20*, 579-589. <https://doi.org/10.1093/treephys/20.9.579>
- Burgess, N. D., Clarke, G. P., & Rodgers, W. A. (1998). Coastal forests of eastern Africa: status, endemism patterns and their potential causes. *Biological Journal of the Linnean Society*, *64*, 337-367. <https://doi.org/10.1111/j.1095-8312.1998.tb00337.x>
- Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., ... Albert, V. A. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden*, *80*, 528-580. <https://doi.org/10.2307/2399846>
- Ciccarelli, D., Balestri, M., Pagni, A. M., & Forino, L. M. C. (2010). Morpho-functional adaptations in *Cakile maritima* Scop. subsp. *maritima*: comparison of two different morphological types. *Caryologia*, *63*, 411-421. <https://doi.org/10.1080/00087114.2010.10589754>
- Ciccarelli, D., Forino, L. M. C., Balestri, M., & Pagni, A. M. (2009). Leaf anatomical adaptations of *Calystegia soldanella*, *Euphorbia paralias* and *Otanthus maritimus* to the ecological conditions of coastal sand dune systems. *Caryologia*, *62*, 142-151. <https://doi.org/10.1080/00087114.2004.10589679>
- Ciha, A. J., & Brown, W. A. (1975). Stomatal size and frequency in soybean. *Crop Science*, *15*, 309-313. <https://doi.org/10.2135/cropsci1975.0011183X001500030008x>
- Clay, N. K., & Nelson, T. (2005). The recessive epigenetic swellmap mutation affects the expression of two step II splicing factors required for the transcription of the cell proliferation gene STRUWWELPETER and for the timing of cell cycle arrest in the *Arabidopsis* leaf. *The Plant Cell*, *17*, 1994-2008. <https://doi.org/10.1105/tpc.105.032771>
- Cole, D. F., & Dobrenz, A. K. (1970). Stomata density of alfalfa (*Medicago sativa* L.). *Crop Science*, *10*, 61-63.

- <https://doi.org/10.2135/cropsci1970.0011183X001000010024x>
- Cori, B. (1999). Spatial dynamics of Mediterranean coastal regions. *Journal of Coastal Conservation*, 5, 105-112. <https://doi.org/10.1007/BF02802747>
- Cruz, M. V., Mori, G. M., Signori-Müller, C., da Silva, C. C., Oh, D. H., Dassanayake, M., ... de Souza, A. P. (2019). Local adaptation of a dominant coastal tree to freshwater availability and solar radiation suggested by genomic and ecophysiological approaches. *Scientific reports*, 9, 19936. <https://doi.org/10.1038/s41598-019-56469-w>
- Ewers, B. E., Oren, R., Johnsen, K. H., & Landsberg, J. J. (2001). Estimating maximum mean canopy stomatal conductance for use in models. *Canadian Journal of Forest Research*, 31, 198-207. <https://doi.org/10.1139/x00-159>
- Elhaak, M. A., Migahid, M. M., & Wegmann, K. (1997). Ecophysiological study on *Euphorbia paralias* under soil salinity and sea water spray treatments. *Journal of Arid Environments*, 35, 459-471. <https://doi.org/10.1006/jare.1995.0151>
- Fujikura, U., Horiguchi, G., & Tsukaya, H. (2007). Dissection of enhanced cell expansion processes in leaves triggered by a defect in cell proliferation, with reference to roles of endoreduplication. *Plant and Cell Physiology*, 48, 278-286. <https://doi.org/10.1093/pcp/pcm002>
- Frankes, P. J., Drake, P. L., & Beerling, D. J. (2009). Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globules*. *Plant Cell and Environment*, 32, 1737-1748. <https://doi.org/10.1111/j.1365-3040.2009.002031.x>
- Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, 173, 579-588. <https://doi.org/10.1086/597611>
- Hesla, B. I., Tieszen, H. L., & Boutton, T. W. (1985). Seasonal water relations of savanna shrubs and grasses in Kenya, East Africa. *Journal of Arid Environments*, 8, 15-31. [https://doi.org/10.1016/S0140-1963\(18\)31334-X](https://doi.org/10.1016/S0140-1963(18)31334-X)
- Hesp, P. A. (1991). Ecological processes and plant adaptations on coastal dunes. *Journal of Arid Environments*, 21, 165-191. [https://doi.org/10.1016/S0140-1963\(18\)30681-5](https://doi.org/10.1016/S0140-1963(18)30681-5)
- Horiguchi, G., Kim, G. T., & Tsukaya, H. (2005). The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *The Plant Journal*, 43, 68-78. <https://doi.org/10.1111/j.1365-313X.2005.02429.x>
- Jump, A. S., & Peñuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letter*, 8, 1010-1020. <https://doi.org/10.1111/j.1461-0248.2005.00796.x>
- Jarvis, P. G. (1976). The interpretation of variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society of London*, 273, 593-610. <https://doi.org/10.1098/rstb.1976.0035>
- Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letter*, 7, 1225-1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Kijima, M. (1962). Laboratory Manual of Botany. Hirokawa Publishing Corporation, Tokyo (In Japanese).
- Kim, J. H., & Kende, H. (2004). A transcriptional coactivator, AtGIF1, is involved in regulating leaf growth and morphology in *Arabidopsis*. *Proceedings of the National Academy of Sciences of USA*, 101, 13374-13379. <https://doi.org/10.1073/pnas.0405450101>
- Knoop, W. T., & Walker, B. H. (1985). Interactions of woody and herbaceous vegetation in a Southern African savanna. *Journal of Ecology*, 73, 235-253. <https://doi.org/10.2307/2259780>
- Kumekawa, Y., Miyata, H., Ohga, K., Hayakawa, H., Yokoyama, J., Ito, K., ... Fukuda, T. (2013). Comparative analyses of stomatal size and density among ecotypes of *Aster hispidus* (Asteraceae). *American Journal of Plant Sciences*, 4, 524-527. <https://doi.org/10.4236/ajps.2013.43067>
- Liu, F., Liu, Q., Liang, X., Huang, H., & Zhang, S. (2005). Morphological, anatomical, and physiological assesment of ramie (*Boehmeria nivea* (L.) Gaud.) tolerance to soil drought. *Genetic Resources and Crop Evolution*, 52, 497-506. <https://doi.org/10.1007/s10722-004-7071-3>
- Liu, S., Chen, S., Chen, Y., Guan, Z., Yin, D., & Chen, F. (2011). *In vitro* induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulture*, 127,

- 411-419. <https://doi.org/10.1016/j.scienta.2010.10.012>
- Lovett, J. C. (1998), Eastern tropical African centre of endemism: a candidate for world heritage status. *Journal of East African Natural History*, 87, 359-366. [https://doi.org/10.2982/0012-8317\(1998\)87\[359:ETACOE\]2.0.CO;2](https://doi.org/10.2982/0012-8317(1998)87[359:ETACOE]2.0.CO;2)
- Maun, M. A. (1998). Adaptations of plants to burial in coastal sand dunes. *Canadian Journal of Botany*, 76, 713-738. <https://doi.org/10.1139/b98-058>
- Migahid, M. A., Elhaak, M. A. (2001). Ecophysiological studies on some desert plant species native to the Mediterranean area in Egypt. *Journal of Arid Environments*, 48, 191-203. <https://doi.org/10.1006/jare.2000.0750>
- Mizukami, Y., & Fischer, R. L. (2000). Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proceedings of the National Academy of Sciences of USA*, 97, 942-947. <https://doi.org/10.1073/pnas.97.2.942>
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858. <https://doi.org/10.1038/35002501>
- Nagamasu, H. (2006). *Eurya*. In: K. Iwatsuki, D. E. Boufford, H. Ohba, (Eds.), *Flora of Japan Ila* (pp. 405-411). Kodansha Tokyo. <https://doi.org/10.2307/25065724>
- Nakajima, Y., & Yoshizaki, S. (2018). Plant community type and site conditions of coastal forests consisting of evergreen broad-leaved tree species in warm temperate zone in south-west Japan. *Journal of the Japanese Society of Revegetation Technology*, 43, 596-604. <https://doi.org/10.7211/jjsrt.43.596>
- Nelissen, H., Clarke, J. H., De Block, M., De Block, S., Vanderhaeghen, R., Zielinski, R. E., ... Van Lijsebettens, M. (2003). DRL1, a homolog of the yeast TOT4/KTI12 protein, has a function in meristem activity and organ growth in plants. *The Plant Cell*, 15, 639-654. <https://doi.org/10.1105/tpc.007062>
- Ohga, K., Muroi, M., Hayakawa, H., Yokoyama, J., Ito, K., Tebayashi, S., ... Fukuda, T. (2013). Coastal adaptation of *Adenophora triphylla* (Thunb.) A. DC. var. *japonica* (Regel) H. Hara (Campanulaceae). *American Journal of Plant Sciences*, 4, 596-601. <https://doi.org/10.4236/ajps.2013.43078>
- Raschke, K. (1975). Stomatal action. *Annual Review of Plant Physiology*, 26, 309-340. <https://doi.org/10.1146/annurev.pp.26.060175.001521>
- Rozema, J., Bijwaard, P., Prast, G., & Broekman, R. (1985). Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. *Vegetatio*, 62, 499-521. <https://doi.org/10.1007/BF00044777>
- Sala, O. E., Golluscio, R. A., Lauenroth, W. K., & Soriano, A. (1989). Resource partitioning between shrubs and grasses in the Patagonian steppe. *Oecologia*, 81, 501-505. <https://doi.org/10.1007/BF00378959>
- Savolainen, V., & Chase, M. W. (2003). A decade of progress in plant molecular phylogenetics. *Trends in Genetics*, 19, 717-724. <https://doi.org/10.1016/j.tig.2003.10.003>
- Schlacher, T. A., Schoeman, D. S., Dugan, J., Lastra, M., Jones, A., Scapini, F., & McLachlan, A. (2008). Sandy beach ecosystems: key features, management challenges, climate change impacts, and sampling issues. *Marine Ecology*, 29, 70-90. <https://doi.org/10.1111/j.1439-0485.2007.00204.x>
- Schoch, P. G., Zinsou, C., & Sibi, M. (1980). Dependence of the stomatal index on environmental factors differentiation in leaves of *Vigna sinensis* L.: 1. Effect of light intensity. *Journal of Experimental Botany*, 31, 1211-1216. <https://doi.org/10.1093/jxb/31.5.1211>
- Shiba, M., Tate, T., & Fukuda, T. (2021). Rheophytic adaptation of *Eurya japonica* Thunb. (Ternstroemiaceae). *International Journal of Biology*, 13, 65-73. <https://doi.org/10.5539/ijb.v13n2p65>
- Shiba, M., Tate, T., & Fukuda, T. (2022). Adaptive leaf morphology of *Eurya japonica* Thunb. (Ternstroemiaceae) in serpentine areas. *Journal of Plant Studies*, 11, 10-18. <https://doi.org/10.5539/jps.v11n1p10>
- Soltis, D. E., Soltis, P. S., Chase, M. W., Mort, M., Albach, D., Zanis, M., ... Farris, J. S. (2000). Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL* and *atpB* sequences. *Botanical Journal of Linnean Society*, 133, 381-461. <https://doi.org/10.1006/boj.2000.0380>
- Soltis, P. S., & Soltis, D. S. (2004). The origin and diversification of angiosperms. *American Journal of Botany*, 91, 1614-1626. <https://doi.org/10.3732/ajb.91.10.1614>
- Suárez, N., & Sobrado, M. A. (2000). Adjustments in leaf water relations of mangrove (*Avicennia germinans*)

- seedlings grown in a salinity gradient. *Tree Physiology*, *20*, 277-282.
<https://doi.org/10.1093/treephys/20.4.277>
- Sunami, T., Ohga, K., Muroi, M., Hayakawa, H., Yokoyama, J., Ito, K., ... Fukuda, T. (2013). Comparative analyses of hairless-leaf and hairy-leaf type individuals in *Aster hispidus* var. *insularis* (Asteraceae). *Journal of Plant Studies*, *2*, 1-6. <https://doi.org/10.5539/jps.v2n1p1>
- Takizawa, E., Tate, T., Shiba, M., Ishii, C., Yoshizaki, S., & Fukuda, T. (2022). Coastal adaptation of *Ligustrum japonicum* Thunb. (Oleaceae): a case study of stomatal adaptation pattern into coastal forests. *Journal of the Japanese Society of Coastal Forest*, *21*, 1-8.
- Teare, I. D., Peterson, C. J., & Law, A. G. (1971). Size and frequency of leaf stomata in cultivars of *Triticum aestivum* and other *Triticum* species. *Crop Science*, *11*, 496-498.
<https://doi.org/10.2135/cropsci1971.0011183X001100040010x>
- Tsukaya, H. (2002). Organ shape and size: a lesson from studies of leaf morphogenesis. *Current Opinion in Plant Biology*, *6*, 57-62. <https://doi.org/10.1016/S1369526602000055>
- Tsukaya, H. (2008). Controlling size in multicellular organs: focus on the leaf. *PLoS Biology*, *6*, e174.
<https://doi.org/10.1371/journal.pbio.0060174>
- Tsuyama, H. (1989). Theaceae. In Y. Satake, H. Hara, S. Watari, & T. Tominari, (Eds.), *Wild Flowers of Japan (woody plants)* (pp.138-147). Heibonsya Tokyo. (in Japanese)
- Tunala, Hayakawa, H., Minamiya, Y., Gale, S. W., Yokoyama, J., Arakawa, R., & Fukuda, T. (2012). Foliar adaptations in *Aster hispidus* var. *insularis* (Asteraceae). *Journal of Plant Studies*, *1*, 19-25.
<https://doi.org/10.5539/jps.v1n2p19>
- Ullah, H., Chen, J. G., Young, J. C., Im, K. H., Sussman, M. R., & Jones, A. M. (2001). Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. *Science*, *292*, 2066-2069.
<https://doi.org/10.1126/science.1059040>
- van der Maarel, E. (2003). Some remarks on the functions of European coastal ecosystems. *Phytocoenologia*, *33*, 187-202. <https://doi.org/10.1127/0340-269X/2003/0033-0187>
- van der Meulen, F., & Udo de Haes, H. A. (1996). Nature conservation and integrated coastal zone management in Europe: present and future. *Landscape and Urban Planning*, *34*, 401-410.
[https://doi.org/10.1016/0169-2046\(95\)00234-0](https://doi.org/10.1016/0169-2046(95)00234-0)

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).