Bull. Natl. Mus. Nat. Sci., Ser. B, **36**(2), pp. 39–42, May 22, 2010

Leaf Blade Thickness Differentiation between Coastal and Freshwater Populations of the Coastal Plant *Vitex rotundifolia* (Verbenaceae)

Hiroaki Setoguchi*, Nobuchika Ishibashi, Koichi Fujita, Hisashi Matsubara, Keiko Terada and Haruki Yagi

Graduate School of Human and Environmental Studies, Kyoto University,
Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto, 606–8501 Japan
* E-mail: seto@botany.mbox.media.kyoto-u.ac.jp

(Received 24 November 2009; accepted 24 March 2010)

Abstract *Vitex rotundifolia* (Verbenaceae) is one of the coastal plants that commonly grows on sandy seashores, but also occurs at Lake Biwa, a freshwater inland lake. To assess morphological differentiation between coastal and inland individuals with regard to the leaf thickness, we measured nine populations of *Vitex rotundifolia* from Lake Biwa, Ise Bay in Pacific Ocean side and Wakasa Bay in Sea of Japan side. We measured leaf blade thickness and applied one-way analysis of variance (ANOVA) to test the difference. Post-hoc multiple comparison resulted in four major groups recognizing all inland populations from Lake Biwa as a distinct group while affiliation of coastal populations was assigned to other three groups regardless to localities. Inland individuals tended to have thinner leaves, implying intraspecific differentiation due to adaptation to habitat environments.

Key words: coastal plant, geographical variation, isolation, Lake Biwa, leaf thickness, salinity stress, *Vitex rotundifolia*.

Introduction

Vitex rotundifolia L.fil. (Verbenaceae) is a deciduous creeping tree, which grows on sandy seashores in the temperate-tropic zone including Japan. The seed is covered with spongy pericarp and its dispersal is accomplished by seawater for long distance. *V. rotundifolia* inhabits frontline of coastal vegetation and never grows in inland, implying this plant grows under salinity stress by airborne salt and higher osmotic pressure of soil. Tolerance to airborne saltwater is critical to the survival of the coastal populations; in coastal plant communities, the distribution of species can sometimes be determined according to their tolerance to airborne saltwater (Oosting and Billings, 1942; Oosting, 1945; Boyce, 1954; Barbour, 1978; Barbour *et al*., 1985; Rozema *et al*., 1985; Sykes and Wilson, 1988; Hesp, 1991; Maun, 1994; Greipsson and Davy, 1996; Wilson and Sykes, 1999). However, in Japan, this plant also inhabits the sandy lakeshore in Lake Biwa, a freshwater lake located in central Japan.

Lake Biwa is an ancient lake that was formed about four million years ago (Yokoyama, 1984; Kawabe, 1989, 1994; Meyers *et al*., 1993), and harbors many other coastal plants that commonly inhabit the seashore, including *Calystegia soldanella* (L.) Roem. et Schult. (Convolvulaceae), *Lathyrus japonicus* Willd. (Leguminosae), *Arabis kawasakiana* Makino (Cruciferae), *Raphanus sativus* L. var. *raphanistroides* Makino (Cruciferae), *Dianthus japonicus* Thunb. (Caryophyllaceae), and *Pinus thunbergii* Parl. (Pinaceae) (Kitamura, 1968). These plants are assumed to have migrated to the inland lake from coastal populations during the period when Lake Biwa had been adjacent to the seashore, and inland lake populations might have later become isolated from the coastal populations (Takaya, 1963; Kitamura, 1968).

The landlocked plants at the freshwater lake may have become morphologically differentiated with regard to long-term isolation and/or salt-tolerance as a result of adaptive evolution to the specific habitat over the long term. In particular, leaf succulence is correlated to exposure to salinity stress in coastal plants (Boyce, 1951; Hesp, 1991; Maun, 1994). Noda *et al*. (2009) assessed physiological differentiation between coastal and inland individuals of *Calystegia soldanella* with regard to the response to salinity stress, and found that inland individuals decrease leaf blade thickness than those of coastal ones.

Coastal plants which inhabit both in Lake Biwa and coast are appropriate example to determine the allopatric differentiation in morphology in relation with adaptation to habitat environment. In this study, we aimed to examine morphological differentiation in leaf blade thickness of coastal plant *Vitex rotundifolia* between the inland and coastal habitats. We have measured the leaf blade thickness for 183 individuals from nine populations that located in Lake Biwa and seashore, and discussed ecological factors and genetic background that may affect morphology.

Materials and Methods

Vitex rotundifolia is a deciduous tree and develops new leaves in early May and blooms in late July in Kinki district, Japan. To assess mature leaves, measurements of *V. rotundifolia* individuals were conducted in late July of 2008 and 2009 in natural habitats in Lake Biwa (three populations), Pacific Ocean side (Ise Bay, three populations) and Sea of Japan side (Wakasa Bay, three populations). Localities and their coordination are summarized in Table 1. All voucher specimens were deposited in National Museum of Nature and Science, Japan (TNS). Inland populations in Lake Biwa have been decreased the number of populations, and only the three populations are remained as small patches. We used electric callipers for measurement of leaf blade thickness. The average thickness of each leaf was estimated based on three measurements from different part within a leaf, and then ten fully matured leaves were examined for each individual. Mean value was calculated for each individual, and data set of the ten individuals from each population was subjected to One-way ANOVA to test the difference in the leaf blade thickness among the populations. Post-hoc multiple comparison by Tukey HSD test was used to determine the significant difference between each population. The statistical analyses were performed using SPSS version 10 (SPSS, Chicago).

Results and Discussion

The leaf blade thickness was normally distributed, and values were significantly different among populations (ANOVA, $p<0.001$; Table 2). Leaf blade thickness varied among populations ranging from 0.302 ± 0.002 mm (mean$\pm$standard

Table 1. Sampling localities, coordinates and number of individuals for *Vitex rotundifolia*

Region	No.	Locality	Coordinates		Number of individuals
			latitude	longitude	
Lake Biwa	1	Maiami	35°08′24″N	135°59′47″E	20
	2	Shinkaihama	35°16′56″N	136°01′06″E	10
	3	Sabae	35°08′47″N	136°01′16″E	25
Pacific (Ise Bay)	4	Matsunase	34°36′22″N	136°34′42″E	30
	5	Kawajiri	34°35′55″N	136°37′15″E	29
	6	Yoshizaki	34°55′00″N	136°38′40″E	27
Sea of Japan (Wakasa Bay)	7	Sakajiri	35°37′19″N	135°57′58″E	21
	8	Matsubara	35°36′36″N	135°55′29″E	10
	9	Wada	35°37′00″N	135°56′25″E	11

Table 2. Result of one-way analysis of variance (ANOVA) of leaf thickness among populations

Morpholoical characteristic	Source of variation	SS	dF	MS	*F* value	*p* value
Leaf thickness	Among groups	0.127	8	0.016	27.762	<0.001
	Within groups	0.099	174	0.001		
	Total	0.226	182			

SS, Sum of Squares ; dF, degree of Freedom; MS, Mean Squares; *F, F*-statistics; *p, p* value for the null hypothesis.

error; 1. Maiami, Lake Biwa) to 0.383±0.007 mm (8. Matsubara, Wakasa Bay) (Fig. 1). The leaf blade thickness suggested a geographical trend, where inland populations from Lake Biwa showed a thinner thickness than seashore populations (Fig. 1, Turkey HSD). Post-hoc multiple comparison resulted in four groups recognizing all inland populations (populations 1–3) as a distinct group while affiliation of seashore populations was assigned to other three groups regardless to their localities (Ise Bay on the Pacific side or Wakasa Bay on the Sea of Japan side).

Thus, inland individuals inhabited in Lake Biwa populations have significant thinner leaf blade. This result implies the landlocked coastal plants of *Vitex rotundifolia* in Lake Biwa may have adapted to the habitat environment, i.e., freshwater lakeshore without salinity stress. Leaf succulence in coastal plants is induced by salinity stress (airborne salt loading on the leaves and branches), resulting in salt-induced hypertrophy and the doubling or tripling of leaf thickness (Boyce, 1951). Noda *et al*. (2009) detected physiological differentiation between inland and coastal individuals of *Calystegia soldanella* in terms of their response to salinity stress. Inland individuals at Lake Biwa showed significant decrease of photosynthetic ability as a result of saltwater sprayed onto leaf blades, corresponding to stomatal closure, whereas coastal individuals showed an insignificant decrease in the same parameter. Additionally, coastal individuals tended to have thicker leaf blades than those of inland individuals even after freshwater irrigation. Conversely, inland individuals developed thinner leaf blades than those of coastal ones even after saltwater irrigation and saltwater spray onto leaf

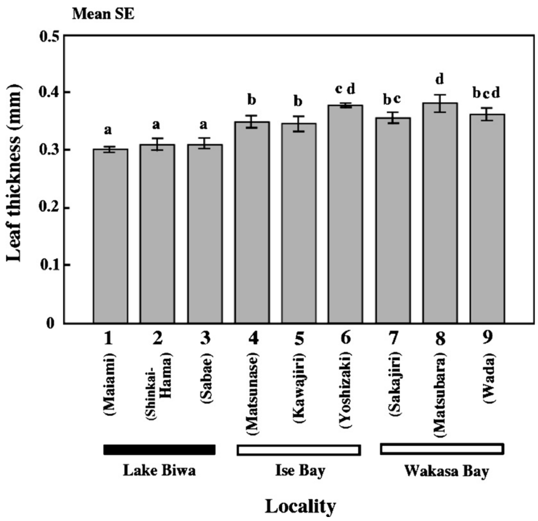

Fig. 1. Means and standard errors of dimensions of the leaf thickness in *Vitex rotundifolia*. Different abbreviation above the vertical bar means significant difference in post-hoc Tukey HSD test.

blades. Thus, the inland plants have adapted to freshwater environment in terms of physiological responses to surrounding environment in terms of genetic background. These findings suggest that the same responses to salinity stress would be also expected in *Vitex rotundifolia*.

Recent molecular phylogeography revealed the heterogeneous genetic structure between the inland and coastal populations, corroborating the long-term isolation of coastal plants at Lake Biwa (Noda *et al*., unpublished). *Vitex rotundifolia* plants at Lake Biwa may have caused a morphological differentiation in leaves in terms of loss of leaf succulence along with loss of salinity tolerance during long-term isolation within inland. Further studies including transplanting experiments and photosynthetic responses to salinity stress should be examined for understanding the physiological background.

Acknowledgements

We thank Hajime Ikeda (Kyoto University) for assisting statistic analyses. This study was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science to HS (13575011).

References

Barbour, M. G. 1978. Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. Oecologia 32: 213–224.

Barbour, M. G, Jong, T. M. and Pavlik, B. M. 1985. Marine beach and dune plant communities. In: Chabot, B. F. and Mooney, H. A. (eds.), Physiological Ecology of North American Plant Communities, pp. 296–322. Chapman & Hall and Methuen, New York.

Boyce, S. G. 1951. Salt hypertrophy in succulent dune plants. Science 114: 544–545.

Boyce, S. G. 1954. The salt spray community. Ecological Monograph 24: 29–67.

Greipsson, S. and Davy, A. J. 1996. Sand accretion and salinity as constraints on the establishment of *Leymus arenarius* for land reclamation in Iceland. Annals of Botany 78: 611–618.

Hesp, P. A. 1991. Ecological processes and plant adaptations on coastal dunes. Journal of Arid Environments 21: 165–191.

Kawabe, T. 1989. Stragigraphy of the lower part of the Kobiwako Group around the Ueno Basin, Kinki District, Japan. Journal of Geoscience of Osaka City University 32: 39–90.

Kawabe, T. 1994. The history of Lake Biwa. In: The Research Group for the Natural History of Lake Biwa (ed.), The Natural History of Lake Biwa, pp. 25–72. Yasaka Shoboo, Tokyo (in Japanese).

Kitamura, S. 1968. Flora of Ohmiensis. Hoikusha, Osaka.

Maun, M. A. 1994. Adaptations enhancing survival and establishment of seedlings on coastal dune systems. Vegetatio 111: 59–70.

Meyers, P. A., Takemura, K. and Horie, S. 1993. Reinterpretation of late Quaternary sediment chronology of Lake Biwa, Japan, from correlation with marine glacial-interglacial cycles. Quaternary Research 39: 154–162.

Noda, A., Nomura, N. and Setoguchi, H. 2009. Chlorophyll fluorescence in response to salt stress in the coastal plant *Calystegia soldanella*: a comparison between coastal and freshwater populations. Bulletin of the National Museum of Nature and Science, Series B 35: 123–129.

Oosting, H. J. and Billings, W. D. 1942. Factors effecting vegetational zonation on coastal dunes. Ecology 23: 131–142.

Oosting H. J. 1945. Tolerance to salt spray of plants of coastal dunes. Ecology 26: 85–89.

Rozema, J., Bijwaard, P., Prast, G. and Bruekman, R. 1985. Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. Vegetatio 62: 499–521.

Sykes, M. T. and Wilson, J. B. 1988. An experimental investigation into the response of some New Zealand sand dune species to salt spray. Annals of Botany 62: 159–166.

Takaya, Y. 1963. Stratigraphy of the Paleo-Biwa group and the paleogeography of Lake Biwa. Memoirs of the College of Science, University of Kyoto, Series B 30: 81–119.

Wilson, J. B. and Sykes, M. T. 1999. Is zonation on coastal sand dunes determined primarily by sand burial or by salt spray? A test in New Zealand dunes. Ecological Letters 2: 233–235.

Yokoyama, T. 1984. Stratigraphy of the Quaternary systems round Lake Biwa and geohistory of the ancient Lake Biwa. In: Horie, S. (ed.), Lake Biwa, Monographiae Biologicae 54, pp. 43–138. Dr W. Junk Publishers, Dordrecht.