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

**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,
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±standard

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

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

<table>
<thead>
<tr>
<th>Morphological characteristic</th>
<th>Source of variation</th>
<th>SS</th>
<th>dF</th>
<th>MS</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Among groups</td>
<td>0.127</td>
<td>8</td>
<td>0.016</td>
<td>27.762</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>0.099</td>
<td>174</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.226</td>
<td>182</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

SS, Sum of Squares; dF, degree of Freedom; MS, Mean Squares; F, F-statistics; p, p value for the null hypothesis.
experiments and photosynthetic responses to salinity stress should be examined for understanding the physiological background.

Acknowledgements

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References


