渓流沿い植物タニガワコンギク(キク科) の形態学的および解剖学的比較

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Comparative morphology and anatomy of rheophytic *Aster microcephalus* var. *ripensis* (Asteraceae)

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Systematic investigation of various plants has shown that extreme and rapid divergence in morphological characteristics occurs in association with ecological shifts to different environments (Grant 1998). An example of an environment that may undergo ecological shifts is the one alongside streams and rivers, in which plants are called rheophytes. By definition rheophytes are plant species which are in nature confined to the beds of swift-running streams and rivers and grow there up to flood-level, but not beyond the reach of regularly occurring flash floods (van Steenis 1981). Rheophytes also show characteristic morphological features, e.g., stenophylly (Kato 1999). Rheophytes belong to more than 60 families, from bryophytes to angiosperms (van Steenis 1981), and similar adaptive modifications may occur independently within these families. Thus, to clarify the process from closely related land species to rheophytes, anatomical studies have previously been conducted in Japan. These studies on rheophytic species such as *Osmunda lancea* Thunb. (Imaichi and Kato 1992), *Farfugium japonicum* (L.) Kitam. var. *luchuense* (Masam.) Kitam. (Usukura et al. 1994, Nomura et al. 2006), *Dendranthema yoshinaganthum* (Makino ex Kitam.) Kitam. (Tsukaya 2002), and *Rhododendron indicum* (L.) Sweet f. *otakumi* T.Yamaz. (Setoguchi and Kajimura 2004), showed that these species decrease the size and/or number of cells to give rise to narrow leaves. However, most studies were confined to certain regions, owing to the limited distribution of rheophytic species. The strength of selective pressures such as flash floods after heavy rain differs for each river, and thus, morphological characteristics of rheophytic species tend to change between different riverside localities.

Aster microcephalus (Miq.) Franch. et Sav. var. *ripensis* Makino (Asteraceae) is endemic to Japan and is distributed in western regions of Honshu, Shikoku, and north-eastern regions of Kyushu. It is closely related to the land species *A. microcephalus* var. *ovatus* (Franch. et Sav.) Soejima et Mot. Ito, which grows far from rivers and is distributed across the temperate and subtropical regions of Japan (Kitamura 1982). *A. microcephalus* var. *ripensis* is a putative rheophyte because its habitat is limited to mountain riversides of its distribution area and it has narrow lanceolate leaves. The collection of samples from large areas would thus enable the comparison of morphological variations of this rheophytic var. *ripensis* between different riversides.

To clarify the anatomical differentiation of leaves between *A. microcephalus* var. *ripensis* and var. *ovatus*, and also the differences in leaf size among various habitats such as speed of stream in river and frequency of flooding of *A. microcephalus* var. *ripensis*, we characterized the variation of leaves by morphological and anatomical analyses.

Materials and methods

Plant materials

All samples of *A. microcephalus* var. *ripensis* and var. *ovatus* examined in this study were collected from the field. For *A. microcephalus* var. *ripensis*, a total of 370 plants representing 12 populations, from the Shimanto River (2 populations), Nahari River (2 populations), Monobe River (2 populations), Yoshino River (2 populations), and Kushida River (4 populations) were sampled. For *A. microcephalus* var. *ovatus*, a total of 30 plants (1 population) were analysed. The sites of collection are indicated in Fig. 1 and Table 1.

Morphological analysis

For morphological analysis, the plants were analysed for the following characteristics: continuous macromorphological variability of leaves; length and width of the leaf blade; and angle of the leaf base. We calculated the leaf

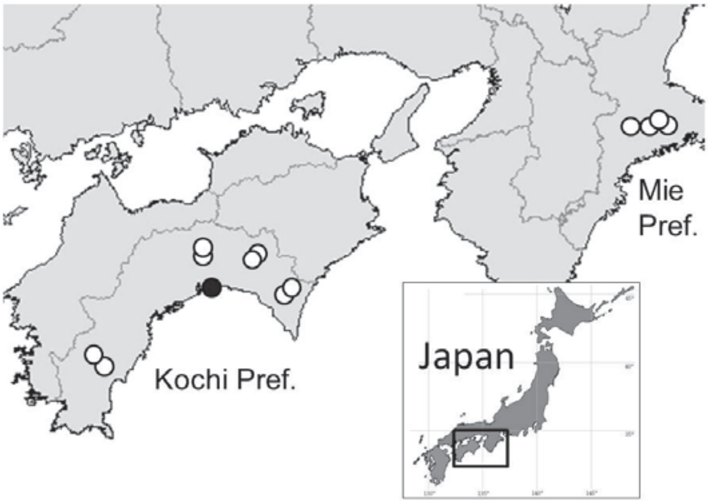

Fig. 1. Sampling localities of plants used in this study. White circles indicate *Aster microcephalus* var. *ripensis*. Black circle indicates *A. microcephalus* var. *ovatus*. For other information, see Table 1.

Table 1. Sampling localities used in this study

Variety	Locality (river) name	No. samples	Locality	Latitude	Longitude
Aster microcephalus var. *ripensis*	Shimanto River	34	Kochi Prefecture, Takaoka-Gun, Shimanto-Cho, Tohwakawaguchi	N 33°13'	E 132°50'
		30	Kochi Prefecture, Takaoka-Gun, Shimanto-Cho, Johguh	N 33°10'	E 133°03'
	Yoshino River	63	Kochi Prefecture, Tosa-Gun, Tosa-Cho, Motose	N 33°45'	E 133°33'
		38	Kochi Prefecture, Nagaoka-Gun, Motoyama-Cho, Sakamoto	N 33°46'	E 133°33'
	Monobe River	44	Kochi Prefecture, Kami City, Monobe-Cho, Minamiike	N 33°45'	E 133°55'
		32	Kochi Prefecture, Kami City, Monobe-Cho, Kubo	N 33°46'	E 133°57'
	Nahari River	48	Kochi Prefecture, Aki-Gun, Umaji-Mura, Yanase	N 33°36'	E 134°06'
		20	Kochi Prefecture, Aki-Gun, Umaji-Mura, Umaji	N 33°33'	E 134°02'
	Kushida River	11	Mie Prefecture, Matsuzaka City, Iitaka-Cho, Awano	N 34°25'	E 136°15'
		16	Mie Prefecture, Matsuzaka City, Iitaka-Cho, Akaoke	N 34°25'	E 136°19'
		30	Mie Prefecture, Matsuzaka City, Iinan-Cho, Fukano	N 34°28'	E 136°24'
		4	Mie Prefecture, Taki-Gun Taki-Cho, Katano	N 34°28'	E 136°24'
A. microcephalus var. *ovatus*		30	Kochi Prefecture, Nankoku City, Monobe (Fac. Agr., Kochi Univ.)	N 33°33'	E 133°40'

index (leaf length per leaf width). Measurements were obtained using a digital caliper. Leaf measurements were taken from five fully expanded stem leaves per plant (Figs. 2 A–C, E).

Anatomical analysis

For anatomical analysis, fully expanded leaves were collected from each individual. To count the number of cells on the blade, the surface of each leaf was peeled off by using Suzuki's Universal Micro-Printing (SUMP) method. We measured the epidermal cell size, guard cell size, and stomatal density among four different sites of the leaf (Fig. 2 (D) I–IV). Then, the central part of the leaf (Fig. 2 (D) I) was analysed to determine the number and size of the epidermal cells and the size of the guard cells. Replicas of each leaf (1 cm^2) were made for measuring the density and size of the stomata. We analysed 10 cells per SUMP image for each leaf by using a light microscope.

Statistical analysis

We compared differences between the two varieties by using a *t*–test. To compare locality differences for *A. microcephalus* var. *ripensis*, we used Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). Because the leaf index in either population was not normally distributed, nonparametric pairwise comparison was conducted (Steel–Dwass test, Dwass 1960; Steel 1960).

Results

Morphological measurements

A. microcephalus var. *ripensis* generally had a shorter leaf length than did *A. microcephalus* var. *ovatus* (33.7 ± 12.2 mm and 47.1 ± 11.5 mm, respectively) (Table 2). However, the size difference was more conspicuous for the leaf width (7.4 ± 3.6 mm in *A. microcephalus* var. *ripensis* and 21.2 ± 5.7 mm in *A. microcephalus* var. *ovatus*) (Fig. 3). The leaf area was estimated as 140 ± 124 mm^2 for *A. microcephalus* var. *ripensis* and 525 ± 249 mm^2 for *A. microcephalus* var. *ovatus*. These traits were signifi-

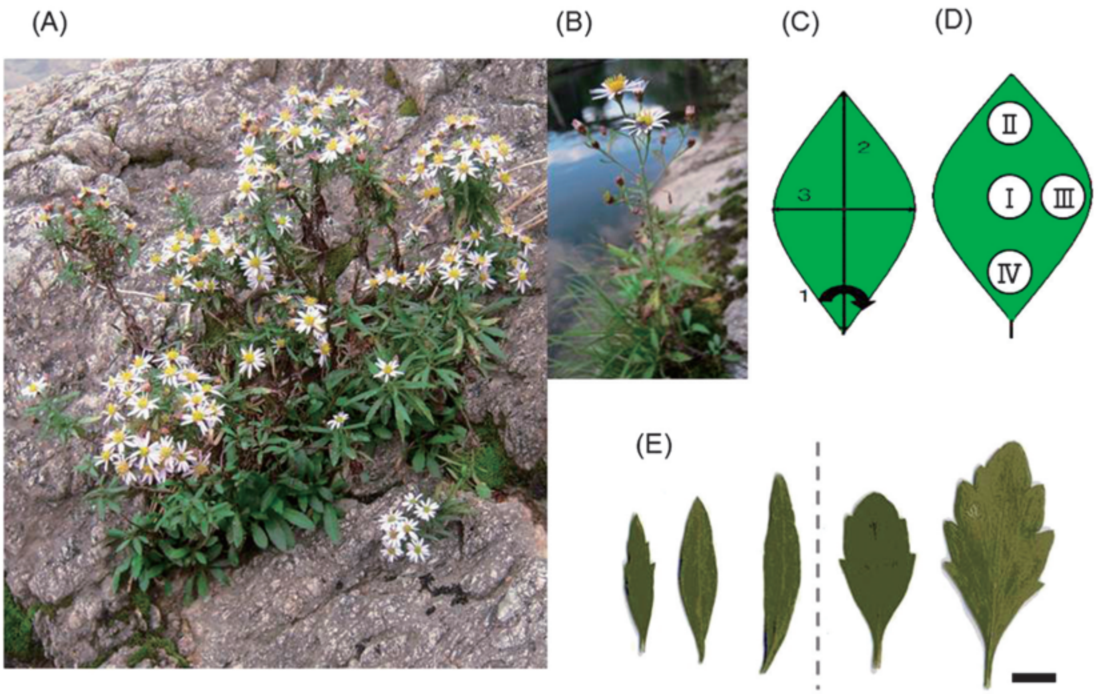

Fig. 2. The plants of *Aster microcephalus* var. *ripensis*. (A) *A. microcephalus* var. *ripensis* in the Kushida River. (B) *A. microcephalus* var. *ripensis* in the Yoshino River. (C) Diagram of leaf measurements. 1: angle of leaf base; 2: leaf length; 3: leaf width. (D) Measured parts of leaf for epidermal cells. I: central part; II: distal part; III: marginal part; IV: proximal part. (E) Leaf shape of *A. microcephalus* var. *ripensis* and var. *ovatus*. Bar = 1 cm.

Table 2. Morphological and anatomical measuerments (average ± standard deviation) of *Aster microcephalus* var. *ripensis* and var. *ovatus*

Trait	*ripensis*		*ovatus*		Significance[1]
Morphological measurements					
Leaf length (mm)	33.7 ± 12.2		47.1 ± 11.5		**
Leaf width (mm)	7.4 ± 3.6		21.2 ± 5.7		**
Leaf size (mm^2)	140 ± 124		525 ± 249		**
Leaf index	5.1 ± 1.9		2.3 ± 0.4		**[2]
Angle of leaf base (°)	24.2 ± 6.7		43.9 ± 8.0		**
Anatomical measurements					
Epidermal cell size (μm^2)					
I. Central	660.6 ± 153.4	a	1117.2 ± 265.8	a	**
II. Distal	628.4 ± 133.7	a	1053.4 ± 239.6	a	**
III. Marginal	629.9 ± 137.3	a	1106.0 ± 293.1	a	**
IV. Proximal	649.4 ± 137.3	a	1130.6 ± 294.8	a	**
Epidermal cell number	225155 ± 109201		576395 ± 159067		**
Guard cell size (μm^2)					
I. Central	119.4 ± 18.5	a	140.3 ± 23.5	a	**
II. Distal	114.8 ± 20.8	a	137.4 ± 20.1	a	**
III. Marginal	117.4 ± 21.7	a	137.7 ± 22.9	a	**
IV. Proximal	117.0 ± 20.0	a	143.3 ± 23.0	a	**
Stomatal density (N/mm^2)					
I. Central	343 ± 103	a	230 ± 51	a	**
II. Distal	340 ± 100	a	242 ± 51	a	**
III. Marginal	346 ± 100	a	237 ± 54	a	**
IV. Proximal	332 ± 94	a	222 ± 51	a	**

[1] ** indicates $p < 0.05$ according to the *t*-test.
[2] Nonparametric pairwise comparison was conducted (Steel-Dwass test).
Numbers marked by the same letter (a) do not differ significantly among four sites in the leaf according to the Tukey's HSD test ($p < 0.05$).

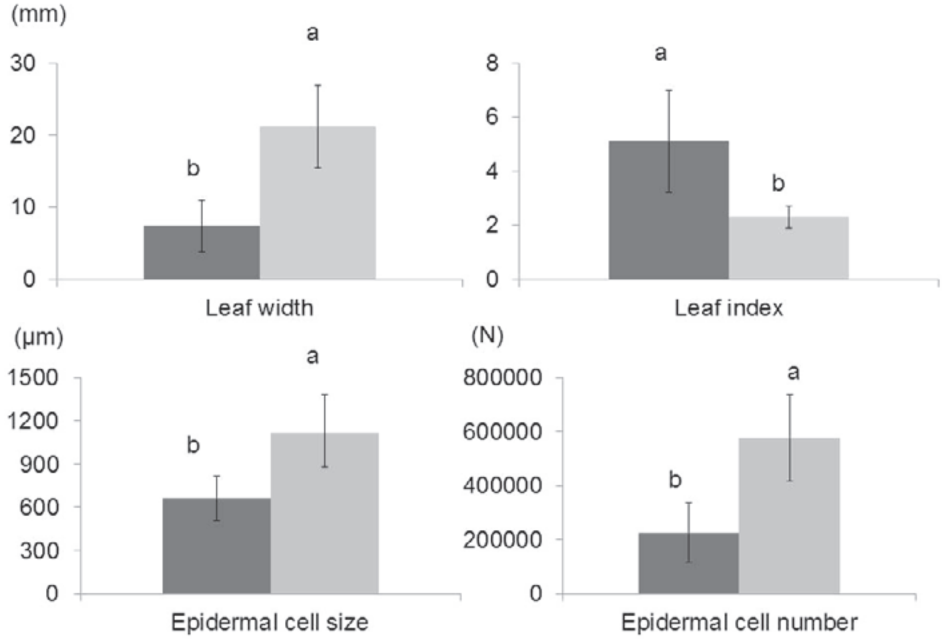

Fig. 3. Morphological and anatomical measurements of *A. microcephalus* var. *ripensis* (dark grey columns) and var. *ovatus* (light grey). Different letters (a, b) indicate significant differences detected by the *t*-test or nonparametric pairwise comparison (Steel-Dwass test) ($p < 0.05$).

cantly different between the two varieties (p < 0.01 in the t-test). We also calculated the leaf index value as the ratio of leaf length to leaf width, as specified by Tsukaya (2002). The leaf index was 5.1 ± 1.9 for *A. microcephalus* var. *ripensis* and 2.3 ± 0.4 for *A. microcephalus* var. *ovatus*. The average angle of the leaf base was 24.2° ± 6.7° for *A. microcephalus* var. *ripensis* and 43.9° ± 8.0° for *A. microcephalus* var. *ovatus*.

Epidermal cells

By using Tsukaya's (2002) method, we measured the epidermal cell size among four different sites of the leaf, with a result that there was no significant difference among the sites (Fig. 2, Table 2). We therefore compared the size and number of epidermal cells in the central part of the leaf between the two varieties (Table 2). The epidermal cell size in the case of *A. microcephalus* var. *ripensis* was significantly smaller (660.6 ± 153.4 μm^2) than that in the case of *A. microcephalus* var. *ovatus* (1117.2 ± 265.8 μm^2) (Fig. 3). The epidermal cell number estimated by dividing mean leaf size by epidermal cell size was approximately 225,000 ± 109,000 for *A. microcephalus* var. *ripensis* and 576,000 ± 159,000 for *A. microcephalus* var. *ovatus*. Thus, the epidermal cell number was lower in *A. microcephalus* var. *ripensis* than in *A. microcephalus* var. *ovatus*. The guard cell size was found to be 119.4 ± 18.5 μm^2 for *A. microcephalus* var. *ripensis* and 140.3 ± 23.5 μm^2 for *A. microcephalus* var. *ovatus* (a significant difference). Additionally, the stomatal density (343 ± 103 N/mm^2) of *A. microcephalus* var. *ripensis* was significantly higher than that of *A. microcephalus* var. *ovatus* (230 ± 51 N/mm^2).

Comparison of *A. microcephalus* var. *ripensis* among rivers

To examine the difference in the leaf size of *A. microcephalus* var. *ripensis* among various habitats, we compared the results for each river. The leaf length differed significantly as follows: Monobe River > Kushida River = Shimanto River > Nahari River = Yoshino River (Table 3). Moreover, the leaf width for the Monobe River (11.1 ± 3.2 mm) was significantly greater than that for the other rivers, whereas that for the Shimanto River (5.3 ± 1.7 mm) and the Kushida River (5.6 ± 4.1 mm) was significantly smaller (Fig. 4). In other words, the order of leaf width was Monobe River > Nahari River = Yoshino River > Kushida River = Shimanto River. Thus, the leaf width for the Monobe River was significantly broader than that for all other rivers. The leaf index also was showed same trend with the leaf width. In other words, the leaf index of the Monobe River (3.9 ± 0.8) and the Nahari River (4.1 ± 1.0) was significantly smaller than that of the other rivers, whereas that for the Shimanto River (6.6 ± 1.8) and the Kushida River (6.7 ± 1.9) was significantly higher. The average angle of the leaf base was 27.6° ± 5.5° for the Monobe River, 26.2° ± 6.5°

Table 3. Morphological and anatomical measurements (average ± standard deviation) of *Aster microcephalus* var. *ripensis*

Trait	Shimanto River		Monobe River		Yoshino River		Nahari River		Kushida River	
Morphological measurements										
Leaf length (mm)	33.2±8.9	b	41.9±10.8	a	29.1±7.7	c	29.7±7.5	c	34.0±18.6	b
Leaf width (mm)	5.3±1.7	c	11.1±3.2	a	6.9±2.6	b	7.5±2.1	b	5.6±4.1	c
Leaf size (mm^2)	92±50	c	245±124	a	107±68	bc	117±57	bc	129±194	b
Leaf index [1]	6.6±1.8	a	3.9±0.8	c	4.5±1.4	b	4.1±1.0	c	6.7±1.9	a
Angle of leaf base (°)	20.6±5.4	c	27.6±5.5	a	26.2±6.5	b	26.2±6.6	ab	19.3±5.4	c
Anatomical measurements										
Epidermal cell size (μm^2)	615.5±102.0	bc	752.1±183.0	a	575.8±113.9	c	628.6±113.9	bc	675±141.9	b
Epidermal cell number	177111±92687	b	192782±59194	a	337942±137214	b	237795±81402	b	217739±116764	b
Guard cell size (μm^2)	126.4±17.6	a	121.9±20.9	ab	112.4±20.6	c	115.9±20.5	bc	110.7±18.0	c
Stomatal density (N/mm^2)	348±80	b	314±101	bc	344±78	b	399±86	a	286±112	c

Columns marked by different letters differ significantly according to the Tukey's HSD test (p < 0.05).
[1] Nonparametric pairwise comparison was conducted (Steel-Dwass test).

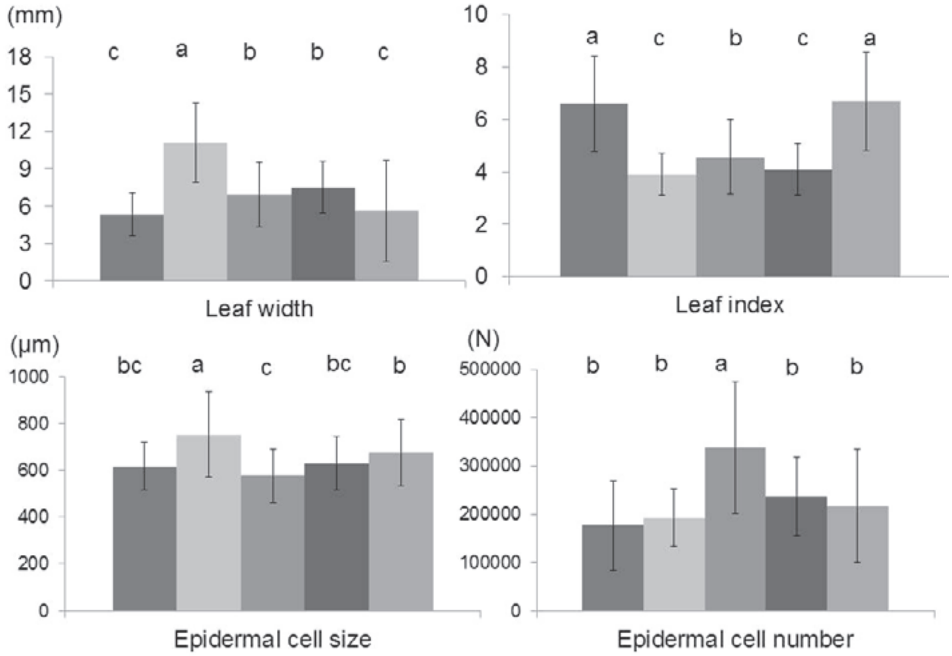

Fig. 4. Morphological and anatomical measurements of *A. microcephalus* var. *ripensis* among rivers. From left to right: Shimanto River, Monobe River, Yoshino River, Nahari River, and Kushida River, in each figure. Different letters (a–c) indicate significant differences detected by the Tukey's HSD test or nonparametric pairwise comparison (Steel–Dwass test) ($p < 0.05$).

for the Yoshino River, 26.2° ± 6.6° for the Nahari River, 20.6° ± 5.4° for the Shimanto River, and 19.3° ± 5.4° for the Kushida River.

With respect to the anatomical analysis of *A. microcephalus* var. *ripensis*, the epidermal cell size for the Monobe River was significantly larger than that for all other rivers (Table 3). In addition, the number of epidermal cells for the Monobe River was significantly higher. The guard cell size for the Shimanto River was larger than that for all other rivers, whereas the stomatal density for the Nahari River was significantly higher.

Discussion

We showed that *A. microcephalus* var. *ripensis* has a significantly narrower leaf than does *A. microcephalus* var. *ovatus* (Table 2). In fact, the leaf shape of *A. microcephalus* var. *ripensis* was streamliner (leaf index is 5.1), which is in consistency with the leaf indices of rheophytic species (>4) (Kato 1999). Moreover, our results suggest that a lower number and lesser size of the epidermal cells contribute to stenophyllization of *A. microcephalus* var. *ripensis*. This finding is similar to that of previous anatomical studies on *Dendranthema yoshinaganthum* (Tsukaya 2002). Our morphological data for *A. microcephalus* var. *ripensis* further indicate that the angle of the leaf base is strongly correlated with the leaf length, and also with the leaf width. Moreover, a decrease in the angle of the leaf base leads to the development of a lanceolate leaf. Thus, our results indicate that stenophyllization of the leaves in *A. microcephalus* var. *ripensis* is accompanied by the transition from ovate to lanceolate leaves.

We also compared the leaf indices of *A. microcephalus* var. *ripensis* among the habitats of the five different rivers. Although there have been no previous studies of comparison among multi-populations of rheophytic species, Imaichi and Kato (1997) claimed that *F. japonicum* var. *luchuense* plants growing closer to streams tended to have greater numbers of lanceolate leaves than those growing further away. It was suggested that this phenomenon was the result of habitat selection pressures along a gradual decrease of flooding frequency. In the present study, the leaf indices of *A. microcephalus* var. *ripensis* populations growing at the Monobe River were lower than those of populations growing at the other rivers studied, suggesting that selection pressure was weak. In addition, the leaf size at the Monobe River populations was the largest implying that the leaf size, which affects photosynthetic ability, also may be a selected trait. It is possible that other rheophytes are also subjected to weaker selection pressures at the Monobe River. *Rhododendron ripense* Makino (Ericaceae) is a semi-evergreen shrub endemic to Japan and distributed in western regions of Honshu, Shikoku, and north-eastern regions of Kyushu (Yamazaki 1993). It is also a putative rheophyte because its habitat is limited to riversides and it has narrow lanceolate leaves. According to Yamanaka and Takezaki (1959) and the Flora of Kochi Prefecture (2009), rheophytic *R. ripense* has not been found at the Monobe River, although the species does grow at other riversides. Taken together, these results suggest that rheophytic species are poorly or not suited to grow along the Monobe River. However, the reasons for this remain unclear. In contrast, it is interesting that leaf indices of *A. microcephalus* var. *ripensis* populations growing at the Shimanto River and the Kushida River were significantly higher. This suggests that strong selection pressure affects the leaf form at these riversides. However, no correlation was found between the degree of stenophyllization and available rainfall data from Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html), due perhaps to the observation points being not close to the rivers studied. Further study is needed to evaluate the relationship between the degree of stenophyllization of leaf and frequency of flooding.

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References

Dwass, M. 1960. Some k-sample rank-order tests. In Olkin, I. (ed), Contributions to Probability and Statistics, pp. 198–202. Stanford university Press, California.

Grant, P. R. 1998. Patterns on islands and microevolution. In: Grant P. R. (ed), Evolution on islands, pp. 1–17. Oxford University Press, NewYork.

Imaichi, R. and Kato, M. 1992. Comparative leaf development of *Osmunda lancea* and *O. japonica* (Osmundaceae): heterochronic origin of rheophytic stenophylly. Bot. Mag. Tokyo **105**: 199–213.

Imaichi, R. and Kato, M. 1997. Speciation and morphological evolution in rheophytes. In: Iwatsuki, K. and Raven, P. H. (eds), Evolution and diversification of landplants, pp. 309–318. Springer-Verlag, Tokyo.

Kato, M. (1999) Adaptation and evolution of rheophytes. In: Kato, M. (ed), Evolutionary morphology of plants, pp. 131–183. University of Tokyo Press, Tokyo. (in Japanese)

Kitamura, S. 1982. Compositae (Asteraceae). In: Satake, Y., J. Ohwi, S., Kitamura, S., Watari, S. and Tominari, T. (eds), Wild flowers of Japan Herbaceous Plants vol. III, pp. 156–235. Heibonsha, Tokyo. (in Japanese)

Nomura, N., Setoguchi, H. and Takaso, T. 2006. Functional consequences of stenophylly for leaf productivity: comparison of the anatomy and physiology of a rheophyte, *Farfugium japonicum* var. *luchuense,* and a related non-rheophyte, *F. japonicum* (Asteraceae). J. Plant Res. **119**: 645–656.

Setoguchi, H. and Kajimura, G. 2004. Leaf morphology of the rheophyte, *Rhododendron indicum* f. *otakumi* (Ericaceae). Acta. Phytotax. Geobot. **55**: 45–54.

Steel, R. D. G. 1960. A rank sum test for comparing all pairs of treatments. Technometrics **2**: 197–207.

Tsukaya, H. 2002. Leaf anatomy of a rheophyte, *Dendranthema yoshinaganthum* (Asteraceae), and of hybrids between *D. yoshinaganthum* and a closely related non-rheophyte,

D. indicum. J. Plant Res. **115**: 329–333.
Usukura, M., Imaichi, R. and Kato, M. 1994. Leaf morphology of a facultative rheophyte, *Farfugium japonicum* var. *luchuense* (Compositae). J. Plant Res. **107**: 263–267.
van Steenis, C. G. G. J. 1981. Rheophytes of the world. Sijthoff and Noordhoff, Alpen aan den Rijn.
Yamanaka, T. and Takezaki, K. 1959. Distribution and ecology of *Rhododendron ripense* Makino, with reference to the vegetation and flora on rocky river-bank. J. Jpn. Bot. **34**: 215–224. (in Japanese)
Yamazaki, T. 1993. Ericaceae. In: Iwatsuki, K., Tamazaki, T., Boufford, D. E. and Ohba, H. (eds), Flora of Japan vol. IIIa, pp. 6–63. Kodansha, Tokyo.

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渓流沿い植物であるタニガワコンギク（*Aster microcephalus* var. *ripensis*）（キク科）とその近縁の陸生種ノコンギク（*A. microcephalus* var. *ovatus*）を用いて形態学的および解剖学的比較を行った。形態学的解析の結果，タニガワコンギクはノコンギクよりも葉が細く，葉形指数は5.1 ± 1.9（タニガワコンギク）と2.3 ± 0.4（ノコンギク）であった。解剖学的解析の結果，タニガワコンギクの狭葉化は細胞の数と大きさの両方が減少していたことに起因していた。さらに，ノコンギクに対してタニガワコンギクは，孔辺細胞も小さく，単位面積あたりの気孔密度も有意に高かった。タニガワコンギクの葉形指数を各河川間で比較した結果，高知県の物部川水系は他の河川よりも有意に低い値となった。したがって，物部川水系では増水頻度などの選択圧が他の河川よりも低い可能性が示唆された。
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