

Differences in Leaf Morphology between Native and Exotic Dandelion Species in the Southern part of Koshin District, Japan

Teruo ARASE¹, Tetsuo OKANO¹ & Taizo UCHIDA²

¹Faculty of Agriculture, Shinshu University

²Faculty of Engineering, Kyushu Sangyo University

甲信地方南部における在来および帰化タンポポの葉の形態的差異

荒瀬輝夫¹・岡野哲郎¹・内田泰三²

¹信州大学農学部, ²九州産業大学工学部

要旨: フィールド調査において、タンポポの在来種と帰化種を見分ける花以外の基準を得ることを目的として葉の形態を調査した。2010、2011 および 2012 年春季に、甲信地方南部(天竜川, 富士川流域)の 8 地点において、在来種(シナノタンポポ: *Taraxacum hondoense* Nakai) と帰化種(セイヨウタンポポ: *T. officinale* Weber) の葉を採集し、画像解析によって葉のサイズを計測して葉形を解析した。各形質の種間差を比較するとともに、要因(種、地点、個体間)ごとの影響を分散分析に基づく寄与率(ρ)で評価、比較した。その結果、葉形の 2 つの指標(細長度および複雑度)によって、2 種を 2 つのグループとして明確に分けることができ、シナノタンポポはセイヨウタンポポに比べて葉形が細長く、複雑度が低い集団としてグルーピングされた。葉形への寄与率は、種が 23.1~27.3%、個体が 31.7~35.2%と大きかった。地点の寄与率は細長度で 13.0%と低く、複雑度で 28.0%と大きかった。複雑度への地点の寄与は前報(長野県北部)と異なる結果であり、葉形が 2 種を分類する基準となるかについて、データ蓄積に加え他の形質も検討必要と思われた。

キーワード: シナノタンポポ, セイヨウタンポポ, 葉形, 分類, フィールド調査

Key words: *Taraxacum hondoense*, *Taraxacum officinale*, Leaf shape, Taxonomy, Field survey

Introduction

To distinguish between the native dandelion, *Taraxacum hondoense* Nakai, and the exotic dandelion, *Taraxacum officinale* Weber in Japan, the most informative and stable taxonomic character is the morphology of the outer involucre bracts of the capitulum. (e.g. Ohwi, 1992; Shimizu, 1997). However, genetic studies have shown that the native

and exotic dandelion species hybridize with each other and that the outer involucre bracts of the resulting crosses appear most similar to those of the pure native species (Ogawa, 2004; Watanabe *et al.*, 1997). While this confusion between the native and exotic species can be resolved by considering the general habitat preferences of the plants, the existence of hybrids potentially complicates the identification of dandelions using flower morphology



Figure 1 Geographic location of Koshin Region and the sampling sites of *Taraxacum hondoense* and *T. officinale*. The shaded area indicates areas above 1,000 m above the sea.

alone.

The native and exotic dandelion species also have different life history characteristics. For example, the native species only blooms in spring, whereas the exotic species only blooms from spring to autumn and has shorter recovery rates, in terms of leaf area, after episodes of disturbance (Sawada *et al.*, 1982). While these life history traits are ecologically important, they cannot be used to distinguish between the two dandelion species in the field as they need to be observed over extended periods of time.

We therefore focused on dandelion leaf morphology as dandelion leaves can be observed throughout the year and are thus well suited for identification purposes. However, few studies on dandelion leaf morphology have been published to date, and the influence of environmental factors on leaf shape has not yet been clarified (Denawa *et al.*, 1979; Arase *et al.*, 2013). Further, information on the differences in leaf shape between species is limited to descriptions in several illustrated plant guide books (e.g. Ohwi, 1992; Shimizu, 1997). However, we previously demonstrated that leaf shape was a sufficiently robust taxonomic character to distinguish

between native and exotic dandelion species in the Chikuma River Basin in the northern Koshin Region (Arase *et al.*, 2013).

In order to collect data and confirm the validity of leaf morphology as a new taxonomic character for identifying native and exotic dandelion species lacking inflorescences, this study examined the size and shape of dandelion leaves harvested from both species in the field in the southern Koshin Region. The differences between the species, collection sites, and individuals were then analyzed using statistical methods.

Methods

We surveyed areas in the southern Koshin Region (i.e. the Fuji River Basin in Yamanashi Prefecture and the Tenryu River Basin in Nagano Prefecture) in central Japan where the native dandelion, *T. hondoense*, and the exotic dandelion, *T. officinale*, are both common (Shimizu, 1997) (Figure 1).

In the spring of 2010, 2011 and 2012, eight sites where both species grew abundantly were surveyed (Figure 1 and Table 1). Two leaves were collected per

Table 1 Sampling sites of *Taraxacum hondoense* and *T. officinale*.

Site No.	Latitude			Elevation (m)	Habitat type
	deg.	min.	sec.		
1 Hokuto	35	49	49	700	Roadside
2 Minami-Alps	35	38	34	475	Orchard
3 Koufu	35	35	43	255	Unused land
4 Minobu	35	28	54	235	Roadside
5 Nanbu	35	14	47	100	Unused land
6 Minami-minowa	35	51	49	760	Forest road
7 Matsukawa	35	35	16	460	Roadside
8 Shimojo	35	23	18	590	Levee of paddy fields

individual and four individuals were collected per species (8 sites × 2 species × 4 individuals × 2 leaves = 128 samples in total). Images of individual leaves were captured with a scanner at a resolution of 400 dpi (i.e. 0.0635 mm per dot) and the traits of leaf length (L), leaf width (W), leaf margin circumference (C) and leaf area (A) per leaf were measured with image processing software (Motic Images Plus 2.0S, Speed Fair Co., Ltd., Hong Kong).

To further characterize leaf shape, we employed indices for slenderness and intricateness (Arase *et al.*, 2013), which can be expressed as follows:

$$\text{Slenderness} = L/W \quad (1)$$

$$\text{Intricateness} = C^2/A \quad (2)$$

where the minimum value of (2) is 4π (=12.56) if the shape of the leaf approximates a perfect circle.

The percentage variability of each factor relative to the total variability (ρ) was calculated by analysis of variance (ANOVA) and was used as an estimate of

how each factor influences overall data variability (Arase *et al.*, 2013). For example, if the factor is expressed as X , then the ρ percentage can be expressed as follows:

$$\rho_X = (S_X - f_X \times V_e) / S_T \times 100 (\%),$$

where S_X , f_X , V_e , and S_T represent the sum of the squared deviations for Factor X (variability between the groups in factor X), degree of freedom for factor X , mean square for error, and the total sum of the squared deviations (total variability), respectively. Thus, ρ percentage indicates the proportion or contribution of each factor to the total variability, corrected for the degree of freedom.

Results

The leaf characteristics of *T. hondoense* and *T. officinale* at each collection site are shown in Table 2. Except for leaf area (A), the results of the ANOVA showed that all of the factors (sites, species, interaction of sites × species, and individuals) were significantly related to each trait (F-test, $p < 0.02$). In addition, no relationship was observed between species and leaf size (L , W , C and A), which differed among sites. Conversely, for leaf shape (i.e. slenderness and intricateness), the values obtained for slenderness were significantly larger in *T. hondoense* (3.7 to 5.5) than in *T. officinale* (2.8 to 3.8) at five sites (Tukey's Honestly Significant

Table 2 Leaf size and shape of *Taraxacum hondoense* and *T. officinale* at each site.

Species	Site	Leaf size				Leaf shape	
		Leaf length (L) (cm)	Leaf width (W) (cm)	Leaf circumference (C) (cm)	Leaf area (A) (cm ²)	Slenderness (L/W)	Intricateness (C^2/A)
<i>T. hondoense</i>	Hokuto	18.0 ± 3.2 ab	4.0 ± 0.7 cd	69.5 ± 17.7 cd	29.7 ± 7.4 a	4.6 ± 1.2 bcd	165.9 ± 58.3 fgh
	Minami-Alps	16.1 ± 3.9 bc	3.6 ± 0.6 cde	56.2 ± 15.8 de	19.5 ± 9.3 cd	4.5 ± 0.3 bcde	204.0 ± 116.5 rfg
	Koufu	12.0 ± 1.4 d	3.4 ± 0.8 de	41.5 ± 11.0 f	18.8 ± 3.7 de	3.7 ± 0.8 ef	99.8 ± 55.4 h
	Minobu	12.3 ± 3.1 d	2.6 ± 0.7 fg	41.2 ± 14.7 f	13.8 ± 5.5 efgh	4.8 ± 0.6 ab	131.0 ± 62.3 gh
	Nanbu	18.6 ± 3.1 a	3.4 ± 0.6 cde	51.4 ± 9.7 f	24.9 ± 4.9 abc	5.5 ± 0.8 a	110.2 ± 42.2 h
	Minami-minowa	14.8 ± 3.2 c	3.5 ± 0.6 cde	69.3 ± 14.3 cd	17.6 ± 5.1 def	4.2 ± 0.5 bcde	292.1 ± 96.4 cd
	Matsukawa	9.3 ± 2.1 e	2.3 ± 0.4 fg	39.0 ± 8.4 f	8.8 ± 3.0 hi	4.1 ± 0.5 bcde	178.4 ± 35.1 efgh
	Shimojo	10.9 ± 1.9 de	2.8 ± 0.7 efg	45.4 ± 12.4 ef	15.3 ± 5.5 efg	4.0 ± 1.1 cdef	150.6 ± 86.6 gh
<i>T. officinale</i>	Hokuto	16.3 ± 3.0 bc	4.9 ± 1.2 ab	103.5 ± 35.8 b	25.8 ± 9.1 ab	3.4 ± 0.4 fg	420.4 ± 155.7 bc
	Minami-Alps	15.2 ± 3.1 c	4.2 ± 1.1 bc	76.5 ± 28.8 c	18.9 ± 5.8 de	3.7 ± 0.4 ef	320.7 ± 163.2 cd
	Koufu	10.6 ± 2.5 de	3.1 ± 0.6 efg	51.0 ± 11.5 ef	12.9 ± 5.9 fgh	3.4 ± 0.2 fg	212.3 ± 35.6 efg
	Minobu	7.1 ± 2.6 f	2.2 ± 0.9 g	41.5 ± 23.2 f	6.7 ± 4.0 i	3.4 ± 0.3 fg	263.7 ± 152.2 de
	Nanbu	16.0 ± 7.2 bc	4.0 ± 1.0 cd	69.0 ± 25.9 cd	19.1 ± 9.8 de	3.8 ± 1.1 def	261.6 ± 104.8 de
	Minami-minowa	15.2 ± 4.0 c	5.7 ± 2.0 a	125.3 ± 59.5 a	22.9 ± 11.6 bcd	2.8 ± 0.4 g	691.8 ± 318.2 a
	Matsukawa	10.3 ± 2.3 de	3.1 ± 0.5 efg	56.8 ± 16.3 de	9.9 ± 2.8 ghi	3.3 ± 0.5 fg	355.3 ± 168.1 bc
	Shimojo	11.0 ± 1.9 de	3.1 ± 0.9 efg	51.3 ± 14.5 ef	11.1 ± 3.4 ghi	3.8 ± 1.0 def	241.9 ± 74.6 def
s.e.		1.2	0.5	8.2	3.2	0.4	50.8
HSD (5%)		2.1	0.8	14.6	5.6	0.8	89.9

Each record indicates the mean ± standard deviation per leaf (n = 8).

Different letters in columns denote significant difference determined by Tukey's HSD ($p < 0.05$).

Table 3 Percentage variability of each factor relative to the total variability (ρ %) for leaf size and shape of dandelions.

Factor	d.f.	Leaf size				Leaf shape	
		Leaf length	Leaf width	Leaf circumference	Leaf area	Slenderness	Intricateness
Site	7	45.9	36.2	35.4	43.6	13.0	28.1
Species	1	2.0	5.3	9.9	2.1	27.3	23.1
Site \times Species	7	4.0	8.5	6.4	4.3	6.1	6.2
Individual	48	41.4	36.4	41.7	37.0	31.7	35.2
error	64	6.7	13.6	6.6	12.9	21.8	7.4

Difference (HSD) test, $p < 0.05$). At all sites, the values obtained for intricateness in *T. officinale* were significantly larger than those in *T. hondoense* (Tukey's HSD, $p < 0.05$).

Table 3 shows the percentage contribution of each factor to total variability (ρ) for each of the leaf size traits and leaf shape. For leaf size, the differences observed between species, sites, and individuals ranged from 2.0 to 9.9%, 35.4 to 54.9%, and 36.4 to 41.7%, respectively. For leaf shape, the differences between species, sites, and individuals ranged from 23.1 to 27.3%, 13.0 to 28.1%, and 31.7 to 35.2%, respectively. Consequently, only the leaf shape character, slenderness, was relatively independent between sites.

Figure 2 is a scatter plot of average leaf shape (i.e. slenderness vs. intricateness) for both species at the different sites examined in this study. The figure

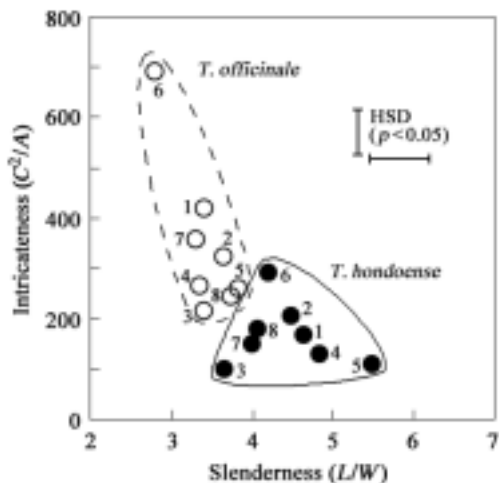


Figure 2 Variations in leaf shape of *Taraxacum hondoense* and *T. officinale* among sites. Numbers adjacent to points correspond to the site numbers shown in Figure 1 and Table 1.

shows that *T. hondoense* and *T. officinale* formed two distinct groups. Despite some overlap between the two groups (i.e., where slenderness = 4 and intricateness = 250), no convergence or reversal was detected between the two species at each of the sites. This difference between the two groups was likely due to the leaves of *T. hondoense* tend to be more slender and simpler than those of *T. officinale*.

Discussion

Our findings showed that slenderness was only slightly affected by geographic location (Table 3) and that the leaf shape parameters of slenderness and intricateness could be used to distinguish between the two species (Figure 2). These findings suggest that, in addition to flower morphology, dandelion leaf shape is a morphological character that can be used to distinguish between dandelion species throughout the year.

However, the dandelion species examined in this study could not be discriminated based on leaf size alone (Tables 2 and 3). The reason for this is because the environmental conditions vary between sites and growth conditions likely have a direct, quantitative effect on leaf size. For example, the growth stage of plants in perennial pastures has been shown to have a marked effect on specific leaf area (Maeda and Yonetani, 1981), and the same may be true for dandelions. Consequently, the effect of leaf size on dandelion species identification needs to be examined in greater detail in the future.

These results generally corroborate those of a previous study conducted in the northern Koshin Region (Arase *et al.*, 2013), with the exception that intricateness varied considerably between sites ($\rho = 28.1\%$). This exception might be caused by the large variation in the intricateness of *T. officinale* leaves

observed among sites in the present study (Figure 2). Regarding the variation observed in the intricateness of dandelion leaves, the influence of environmental factors on compound leaf margins is unclear (Denawa *et al.*, 1979). Consequently, additional surveys of leaf shape, particularly intricateness, need to be conducted in order to more accurately evaluate the robustness of leaf shape for use in taxonomic studies. The potential application of other leaf shape characters that have not yet been investigated also needs to be examined in the future.

In addition, two polyploid microspecies and several formae exist within *T. hondoence*; for example, the forma *yokouchii* has finely lobate leaves (Shimizu, 1997). *T. officinale* has three polyploid microspecies (Shimizu, 1997), and several strains are considered to exist within the species based on the occurrence of specialized dandelion rusts (Harasawa and Yamada, 1976). Any further surveys should therefore be conducted over a larger area in order to confirm whether latent genotypes or ecotypes of dandelions have can be identified based on leaf morphology.

Conclusions

In order to determine whether leaf morphology was sufficiently robust and taxonomically informative to distinguish between native and exotic dandelion species, we examined leaf morphology traits of both species of dandelions from the southern Koshin Region of central Japan. We surveyed eight sites where the native *T. hondoence* and the exotic *T. officinale* were abundant. Two leaves were collected from each individual, and four individuals of each species were sampled in the spring of 2010, 2011 and 2012. Scans of the leaf samples were analyzed and morphometric differences between the two species were compared statistically. Specifically, to estimate how each factor (sites, species, and individuals) influences each leaf character, the percentage variability (ρ percentage) of each factor to the total variability by ANOVA was calculated.

The results showed that two indices of leaf shape (slenderness and intricateness) successfully

discriminated between *T. hondoence* and *T. officinale*, with the leaf shape of *T. hondoence* generally tending to be more slender and simpler than that of *T. officinale*. While the percentage variability by site was low for slenderness ($\rho = 13.0\%$), it was markedly larger for intricateness ($\rho = 28.1\%$). Consequently, additional data should be collected to assess the potential application of leaf shape, especially intricateness, as a taxonomic character for distinguishing between the two dandelion species. Other, as yet undefined, leaf-shape characters should also be examined in the future.

However, the findings of the present study showed that it was not possible to reliably distinguish between the two species based on leaf size alone and more data needs to be collected in order to determine whether leaf size is indeed sufficiently robust to discriminate between species. A larger survey therefore needs to be conducted in order to further clarify the relationships, if any, between the genotypes or ecotypes within these species.

References

- 1) Arase, T., Kumagai, M, Okano, T. and Uchida, T. (2013) Differences in leaf morphology between native and exotic dandelion species in the Chikuma River Basin, Japan. The Annals of Environmental Science Shinshu University 35: 26 - 31
- 2) Denawa, C., Harasawa, I. and Yamada, T. (1979) Ecological studies of genus *Taraxacum* (9) Leaf margin of dandelions. Bulletin of Tokyo Gakugei University, Series VI 31: 1 - 9 (in Japanese with English summary)
- 3) Harasawa, I. and Yamada, T. (1976) Ecological studies of genus *Taraxacum* (4) Specialization of dandelion rusts (*Puccinia taraxaci* Polw.). Bulletin of Tokyo Gakugei University, Series VI 28: 52 - 60 (in Japanese with English summary)
- 4) Maeda, S. and Yonetani, T. (1981) Optimum cutting stage of forage plants V. seasonal changes in the relationships between weight of the whole tops and that of leaf blades, and leaf areas with growth of Italian ryegrass population. Journal of Grassland Science, 27 (2) : 200 - 207 (in Japanese with Japanese summary)
- 5) Ogawa, K. (2004) Problems in field surveys of target plants, dandelions (*Taraxacum*), caused by hybridization between Japanese and introduced species. Bulletin of

Kansai Organization for Nature Conservation 26 (1): 51
- 55 (in Japanese)

- 6) Ohwi, J (Kitagawa, M. ed.) (1992) New flora of Japan, revised edition. pp. Shibundo Co. Ltd. Publishers, Tokyo. pp. 1552 - 1558 (in Japanese)
- 7) Sawada, S., Kasaishi, Y. and Nakamura, Y. (1982) Difference in adaptive strategy of production process between indigenous and naturalized dandelions under artificial disturbance. Japanese Journal of Ecology 32: 347 - 355
- 8) Shimizu, T. ed. (1997) Flora of Nagano Prefecture. The Shinano Mainichi Shinbun, Nagano. pp.1162 - 1168 (in Japanese)
- 9) Watanabe, M., Maruyama, Y. and Serizawa, S. (1997) Hybridization between native and alien dandelions in the western Tokai District (1) Frequency and morphological characters of the hybrid between *Taraxacum platycarpum* and *T. officinale*. The Journal of Japanese Botany 72: 51 - 57 (in Japanese with English summary)

(原稿受付 2014. 3. 10)