Differences in Leaf Morphology between Native and Exotic Dandelion Species in the Chikuma River Basin, Japan

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千曲川流域における在来および帰化タンポポの葉の形態的差異

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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・種子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、長野県千曲川流域の 7 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。統計的に各形質の種間差を検定するとともに、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た。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。葉形に対する各要因の寄与率は、種が 26.1～37.8%、個体が 25.1～32.0%であったのに対し、地点が 6.6～9.0%と著しく小さかった。よって葉形は地点による影響がわずかであり、シナノタンポポとセイヨウタンポポを見わける基準となりうることが示唆された。

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

Since the 1970s, an increase in the number of assessments of changes in the natural environment has resulted in numerous surveys being conducted on the distributions of native and exotic dandelions (genus *Taraxacum*) in Japan (e.g. Hotta, 1977). These dandelion surveys are considered important for reasons related to environmental education, through which we can improve our understanding the surrounding environment by observing the plants familiar to us, and also for assessing environmental developments or disturbances by examining the distributions of native and exotic species (Ogawa 2004).

In addition to pollen morphology and seed size, which are highly seasonal, the most important and stable taxonomic character trait used to distinguish

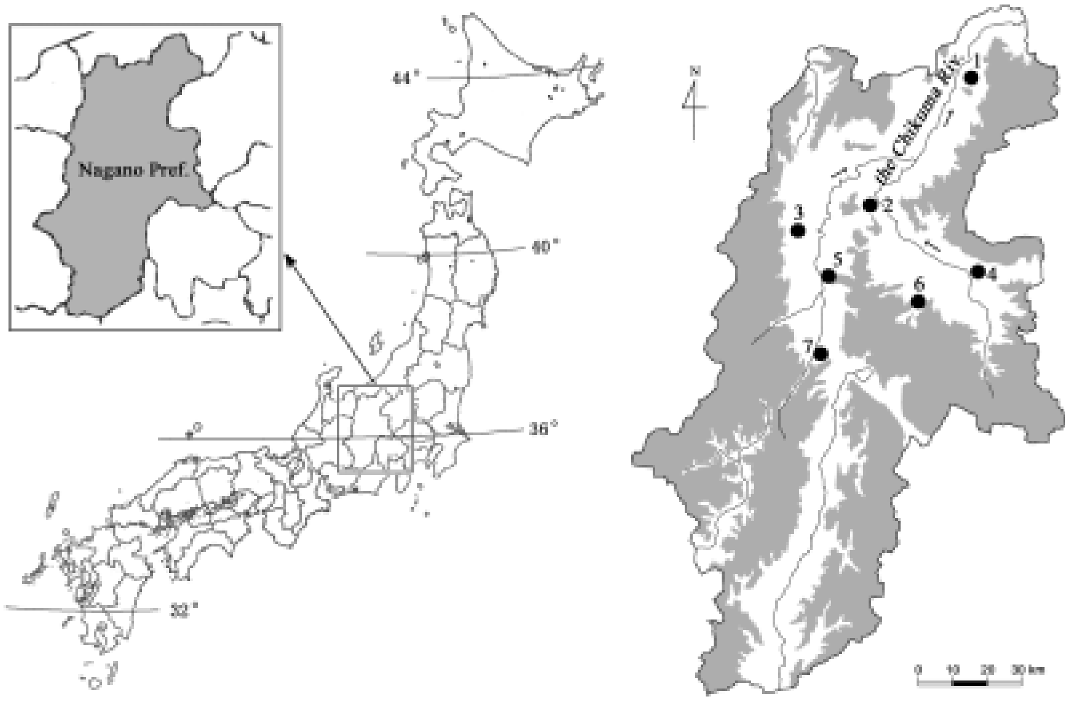

Figure 1　Location of Nagano Prefecture and the sampling sites of *Taraxacum hondoense* and *T. officinale*. The shaded area indicates areas higher than 1,000 m above the sea.

between native and exotic dandelion species in Japan is the morphology of the outer involucral bracts of the capitulum. (e.g. Ohwi, 1992; Shimizu, 1997). However, genetic studies have shown that native and exotic dandelion species hybridize, and that the outer involucral bracts of the resulting crosses appear most similar to those of pure 'native species' (Ogawa, 2004; Watanabe *et al*. 1997). While this confusion between native and exotic species can be resolved by considering the general habitat characteristics of the plants under consideration, the existence of hybrids potentially complicates the identification of dandelions using flower morphology alone.

Native and exotic dandelion species also differ with respect to their life histories. Native species only bloom in spring, whereas exotic species bloom from spring to autumn, and the latter is at an advantage in terms of higher recovery rate of leaf area during periods of disturbance (Sawada, *et al*. 1982). Although these traits are important ecologically, they cannot be used to distinguish between dandelion species in the field as they require extended periods of observation.

We therefore focused on the leaf morphology of dandelions. Since the leaves of dandelions can be observed in any season of the year, leaf morphology is well suited for use as a character for identification. However, few studies on dandelion leaf morphology have been published to date, and the effect of environmental factors on compound leaf margin is currently unclear (Denawa *et al*., 1979). Further, information on the differences in leaf shape among species is limited to descriptions in several illustrated plant guide books (e.g. Ohwi, 1992; Shimizu, 1997).

In order to obtain a new taxonomic character to distinguish between native and exotic dandelion species with no inflorescences, this study examined the size and shape of dandelion leaves harvested from both species in the field. The differences between the species, sites and individuals were then analyzed using a variety of statistical methods.

Methods

We surveyed the Shinano River Basin in Nagano Prefecture, the central district of Japan (Figure 1), where the native dandelion, *Taraxacum hondoence* Nakai, and the exotic dandelion, *T. officinale* Weber,

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

Site No.		Latitude °	′	″	Elevation (m)	Habitat
1	Kijima-daira	36	49	10	580	Bank around a shrine
2	Chikuma	36	30	19	470	Levee of paddy fields
3	Matsukawa	36	25	51	640	Levee of paddy fields
4	Komoro	36	19	46	560	Levee of paddy fields
5	Azumino	36	18	33	580	Bank of a farmland
6	Nagawa	36	13	22	740	Levee of paddy fields
7	Shiojiri	36	7	4	690	Levee of paddy fields

are both commonly distributed (Shimizu, 1997).

In the spring of 2010, 2011 and 2012, seven sites (Figure 1 and Table 1) where both species grew abundantly were surveyed. Two leaves were collected per individual and four individuals were collected per species (7 sites × 2 species × 4 individuals × 2 leaves = 112 samples in total). Images of individual leaves were captured at a resolution of 400 dpi (i.e. 0.0635 mm per dot) with a scanner and the traits of leaf length (L), leaf width (W), leaf 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 characterize leaf shape, we employed indices for 'slenderness' and 'intricateness', which can be expressed as follows:

Slenderness $=L/W$

Intricateness $=C^2/A$

where the latter gives minimum value of 4π (=12.56) if the leaf shape is a perfect circle. Figure 2 shows an example of a leaf image and the datasets of each species.

The percentage variability of each factor relative to the total variability (ρ) was calculated using an analysis of variance (ANOVA), and was used as an estimate of how each factor influences overall data variability. 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 squared deviations for Factor X (variability between the groups in factor X), degree of freedom for factor X, mean square for error, and total sum of squared deviations (total variability), respectively. Thus, the ρ percentage indicates the proportion or contribution of each factor to the total variability, corrected with its degree of freedom.

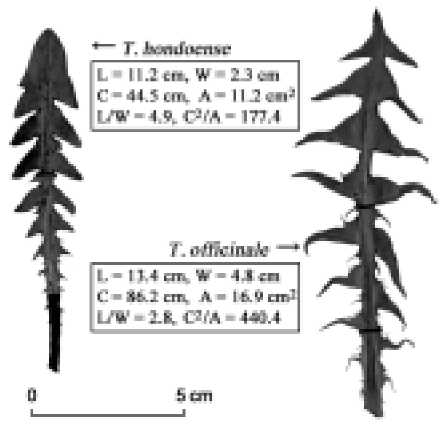

Figure 2 Images and associated data of leaves from *Taraxacum hondoense* and *T. officinale* from the Kijima-daira site. Please refer to the text and Table 2 for L, W, C, A, L/W and C^2/A.

Results

The leaf sizes and shapes of *T. hondoence* and *T. officinale* at each site are shown in Table 2. The ANOVA results showed that all of the factors (sites, species, interaction of sites × species, and individuals) had a significant effect on each trait (F-test, $p<0.01$). However, no relationship was observed between species and leaf size. Which species is larger than the other was found to differ among sites. Conversely, for leaf shape, the values obtained for slenderness were significantly larger in *T. hondoence* (4.3 to 5.9) than in *T. officinale* (3.2 to 3.7) at all sites except sites No. 2 and 4 (Tukey's HSD, $p<0.05$). Similarly, values obtained for intricateness were significantly larger in *T. officinale* than in *T. hondoence* at five sites, except sites No. 4 and 7 (Tukey's HSD, $p<0.05$).

Table 3 shows the percentage variability of each factor to total variability (ρ) for each trait of leaf size and shape. For leaf size, the percentage difference obtained for species, site, and individuals was 2.5 to 14.8%, 19.2 to 45.8%, and 27.9 to 44.7%, respectively. For the trait of leaf shape, the percentage difference obtained for species, sites, and

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)
T. hondoense	Kijima-daira	9.8 ± 1.0 fg	2.2 ± 0.4 e	32.3 ± 6.8 i	10.2 ± 2.2 ef	4.6 ± 0.5 bc	107.2 ± 39.1 e
	Chikuma	14.1 ± 2.8 bcd	3.2 ± 1.0 cd	65.7 ± 18.9 cdef	17.6 ± 6.8 bc	4.5 ± 0.8 bc	257.9 ± 97.6 bc
	Matsukawa	16.6 ± 2.6 a	2.8 ± 0.3 de	56.7 ± 6.5 defg	17.8 ± 3.4 bc	5.9 ± 1.1 a	189.2 ± 59.4 cd
	Komoro	11.6 ± 2.2 defg	2.8 ± 0.6 de	48.1 ± 11.8 gh	12.0 ± 3.1 ef	4.3 ± 0.8 bcde	197.6 ± 61.5 cd
	Azumino	15.7 ± 3.5 b	3.4 ± 0.8 cd	52.6 ± 13.7 fg	22.8 ± 11.9 a	4.7 ± 0.6 b	134.5 ± 49.6 de
	Nagawa	18.5 ± 2.6 a	3.8 ± 0.6 bc	75.2 ± 13.3 bc	22.4 ± 4.6 a	5.0 ± 1.0 ab	258.2 ± 68.6 bc
	Shiojiri	11.8 ± 1.6 defg	3.0 ± 1.0 cd	56.8 ± 15.0 defg	10.9 ± 3.7 ef	4.3 ± 1.6 bcd	318.0 ± 141.7 b
T. officinale	Kijima-daira	12.7 ± 1.3 cde	3.7 ± 0.6 bc	73.4 ± 11.6 bc	12.8 ± 2.8 de	3.4 ± 0.3 def	429.3 ± 87.3 a
	Chikuma	12.3 ± 2.3 cdef	3.3 ± 0.4 cd	67.3 ± 13.7 cde	10.8 ± 2.7 ef	3.7 ± 0.5 cdef	423.4 ± 83.2 a
	Matsukawa	15.2 ± 2.2 b	4.6 ± 1.1 a	82.6 ± 21.4 ab	18.9 ± 6.0 abc	3.4 ± 0.7 def	388.1 ± 139.4 a
	Komoro	9.2 ± 1.4 g	2.8 ± 0.5 de	38.2 ± 9.7 hi	8.5 ± 1.8 f	3.3 ± 0.3 def	185.1 ± 96.6 d
	Azumino	15.4 ± 4.4 b	4.6 ± 1.0 a	93.8 ± 32.4 a	21.1 ± 8.7 ab	3.3 ± 0.5 ef	421.5 ± 126.0 a
	Nagawa	15.0 ± 3.1 bc	4.1 ± 0.9 ab	69.5 ± 18.1 bcd	16.3 ± 5.3 cd	3.7 ± 0.4 cdef	300.7 ± 72.6 b
	Shiojiri	10.3 ± 1.7 efg	3.4 ± 0.8 cd	55.2 ± 12.4 efg	11.5 ± 4.6 ef	3.2 ± 0.8 f	276.1 ± 65.3 b
	s.e.	1.5	0.4	7.5	2.4	0.5	39.4
	HSD (5%)	2.6	0.7	13.2	4.2	1.0	69.7

Each record indicates the mean ± standard deviation per leaf (n =8). Different letters in columns denote significantly different means as determined by Tukey's HSD (p <0.05).

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

Factor	d.f.	Leaf size				Leaf shape	
		Leaf length	Leaf width	Leaf circumference	Leaf area	Slenderness	Intricateness
Site	6	45.8	19.2	22.5	36.8	6.6	9.0
Species	1	2.5	14.8	8.9	1.9	37.8	26.1
Site × Species	6	6.0	11.5	21.2	5.3	4.5	24.2
Individual	42	27.9	40.2	35.8	44.7	25.1	32.0
error	57	17.8	14.3	11.5	11.4	26.0	8.6

individuals ranged from 26.1 to 37.8%, 6.6 to 9.0%, and 25.1 to 32.0%, respectively. Consequently, leaf shape proved relatively independent of differences between sites.

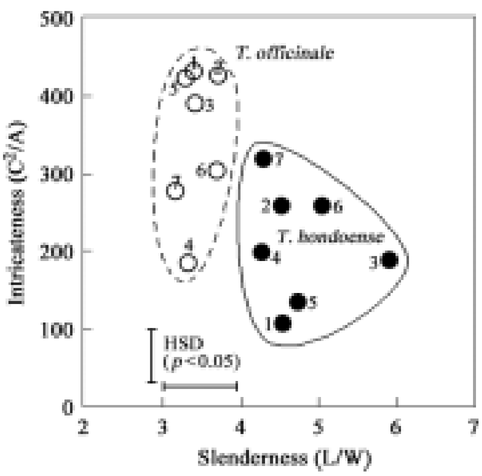

Figure 3 Variations in leaf shape of *Taraxacum hondoense* and *T. officinale* among sites. Numbers adjacent to points correspond to the site numbers shown in Figure 1 and Table 1.

Figure 3 is a scatter plot of average leaf shape (i.e. slenderness vs. intricateness) of both species at different sites in the study. The figure clearly shows that *T. hondoence* and *T. officinale* formed two distinct groups, primarily because the leaf shape of *T. hondoence* tends to be more slender and simpler than *T. officinale*.

Discussion

The present study demonstrated that leaf morphology could be used as new taxonomic character to identify and distinguish between dandelion species. In addition, the influence of different factors on individual leaf traits was evaluated quantitatively by assessing the percentage variability of each factor to the total variability (ρ). The findings showed that leaf shape characteristics (slenderness and intricateness) were only slightly affected by site location (Table 3) and that these parameters could be used to separate the two species into two distinct groups (Figure 3). These findings suggest that, in addition to flower and seed morphology, dandelion leaf shape is a robust morphological character that can be used to distinguish between dandelion species throughout the year.

In general, environmental factors such as light intensity and soil moisture are known to influence leaf shape in plants. It is therefore considered worthwhile to determine whether the weather conditions and the soil environment at of each of the sites in this study had any influence on leaf morphology. Since the ρ percentages of leaf shape appeared to vary between individuals (Table 3), collecting more than one or two individuals at a site is considered important for preventing the misidentification one of species.

Our findings showed that the dandelion species examined in this study could not be discriminated between based on leaf size alone (Tables 2 and 3). The reason for this is because site environment and growth conditions should have a direct quantitative effect on leaf size. In perennial pastures, the growth stage of plants 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 classification needs to be investigated further in the future.

In addition, in *T. hondoence*, two polyploid microspecies and several formae exist within the species; 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). A larger–area survey is therefore considered desirable to confirm whether latent genotypes or ecotypes of dandelions have any relations with leaf morphology.

Conclusions

To obtain a taxonomically informative character for distinguishing between native and exotic dandelion species, we examined the leaf morphologies of dandelions growing in the Shinano River Basin in Nagano Prefecture, the central district of Japan. We surveyed seven sites where the native *T. hondoence* and the exotic *T. officinale* grew abundantly. Two leaves were collected from each individual, and four individuals of each species were sampled. Scans of the leaf samples were analyzed in 2010, 2011 and 2012 and differences between the two species were tested statistically. Specifically, the percentage variability of each factor (sites, species, and individuals) was compared to the total variability by ANOVA.

The results showed that two indices of leaf shape (slenderness and intricateness) successfully discriminated between *T. hondoence* and *T. officinale*; the leaf shape of *T. hondoence* tended to be more slender and simpler than *T. officinale*. In addition, the percentage variability for leaf shape was markedly smaller between sites (6.6 to 9.0%) than it was between species (26.1 to 37.8%) or individuals (25.1 to 32.0%). Consequently, because it is relatively independent of the influence of site, leaf shape was considered to be a sufficiently robust taxonomic character that could be used to distinguish between the two species.

As for leaf size itself, the results of the present study could not clearly distinguish between the two species 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 to clarify the relationships, if any, between the genotypes or ecotypes within these species.

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