Calculated characters of leaves are independent on environmental conditions in *Salix herbacea* (Salicaceae) and *Betula nana* (Betulaceae)

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Abstract

The goal of the study was to check if the shape-describing characters, calculated as ratios of the morphological measured traits are more stable, compared to the latter, and can be treated as independent on environmental conditions. The test was based on the example of leaves of *Salix herbacea* and *Betula nana*. The individuals of the two populations of *S. herbacea* from Tatra Mts. were divided into two groups: with bigger and smaller leaves. The two populations of *B. nana* came from different substrata: the first one, collected from the mire on the lower altitude, had bigger leaves, and the second, collected from the granite plateau and higher altitude, had smaller leaves.

For both species, the measured traits were generally more variable than the ratios calculated on their basis, as expressed by the variation coefficients. The results of Students’ t-test analyses showed statistically significant differences between the two groups of *S. herbacea* and the two populations of *B. nana* with respect to almost all the measured characters, and no such differences for the calculated traits, reflecting the leaf shape.

As the differentiation of the leaf size was probably bound to the environmental factors, the lack of the dependence of the leaf shape on the leaf size could lead to a conclusion of independence of the leaf shape on the environment conditions.

Keywords: morphologic characters, synthetic characters, *Salix herbacea*, *Betula nana*

Introduction

Morphological traits are basic for the description of a plant. Recently, their role has been decreasing in the face of rapid development of molecular biology and modern methods of DNA analysis, treated as more reliable and more sensitive, especially for reflecting the intra- and inter-populational diversity [1, 2]. Still, morphological characters remain important, as they are crucial for the identification of taxa. Various kinds of morphological features, concerning both vegetative and generative organs, are still used in taxonomic studies and analysis of diversity [3–10]. The main criticism of morphological characters is connected with their dependence on changing environmental factors, such as precipitation, temperature, insolation [11–15]. The proposed solution is to use synthetic characters, describing rather the shape of the plant structures, than the size, being regarded as much more stable [5].

The goal of the present study is to test whether the shape-describing characters are more stable and can be treated as independent on environment when compared to measured traits, on the example of leaves. The assumption was that the variability of the measured morphological traits would be greater than in the case of shape describing characters. It was also expected that individuals of various leaf size, sampled in the same geographical region, should not show statistically significant differences with regard to synthetic, shape-describing traits. If the differentiation of the leaf size can be bound with environmental factors, the independence of the leaf shape on the leaf size may lead to a conclusion of independence of the leaf shape of the environmental conditions.

To check the hypothesis, the leaves of two taxonomically unrelated species, interesting as subjects of different biological studies, were examined: *Salix herbacea* L. (Salicaceae) and *Betula nana* L. (Betulaceae).

Material and methods

*Species descriptions*

*Salix herbacea*, an arctic-alpine dwarf shrub, is relatively well known [16–23]. Its genetic diversity and phylogeography has been lately characterized by Alsos et al. [24]. The willow has been also interesting as an important fossil material, but recently it has gained more attention as a possibly endangered species in the perspective of the climate warming. Still, a thorough examination of its morphological variability in the whole range is lacking. In the Tatra Mountains, two morphological
types, differing with regard to the leaf size, have been reported: besides the typical form, *F. macrophylla* Sét. having leaves 23–27 mm long [25].

Betula nana is a small, circum-boreal shrub. In its southernmost localities the birch forms small, relic populations, while in the northern part of the species range it is important as one of the few, if not the only one, woody plants [26–28]. Studies on Betula nana focused on its occurrence in the early Holocene floras [29–31], hybridization with *Betula pubescens* [28,32–36] and importance in forming the mountain birch (*Betula pubescens* var. *tortuosa*) [32–38]. In Scandinavia, *B. nana* builds vast populations and shows low morphological variation. However, the morphological differences between tundra and mire populations, as well as decreasing of the leaf size in severe climatic conditions, were described [28]. The relation of the leaf size to altitude for other birch species was also reported [39].

**Measurements and analysis**

For each taxon, samples from two populations were analyzed, a sample consisted of approximately 30 specimens, for every specimen 5–10 leaves were collected. Individuals growing no closer than 3 m to each other were selected, to avoid sampling the same genet. The location of sampled populations are given in Tab. 1.

Measurements were taken on the dried material. Leaves were scanned and then the scans were measured with diGiShape 1.9.177 (Cortex Nova), teeth were counted from the scans and synthetic traits were calculated (Tab. 2, Tab. 3).

To find possible two morphological types of leaves of *Salix herbacea* in the Tatra Mountains [25], the k-means method of the cluster analysis was used, on the basis of means of individuals of two populations together. For comparisons between two distinguished groups of the willow and between two populations of the dwarf birch, Student’s t-test was performed. The deviation of means of traits was checked on the box and whiskers graphs and the variation coefficients were calculated. For all calculations and analysis STATISTICA 9.0 (StatSoft, Inc.) was used.

**Results**

K-mean clustering allows distinguishing two groups of 60 individuals of *Salix herbacea*: group A with bigger leaves, and group B having smaller leaves, consisting of 23 and 37 individuals, respectively (Fig. 1a, Tab. 2).

For *Salix herbacea*, the values of the variation coefficients, calculated for characters describing the leaf size (Tab. 2) reached more than 15%, while for characters illustrating the leaf shape only up to 10%, with the exception of the character 12 (19.21%). The number of teeth (character 6) and the number of teeth/perimeter (character 13) had variation coefficients about 20%. The coefficient of the petiole length (character 3) was highest (almost 30%; Fig. 2).

Leaves traits of *Betula nana* were generally less variable. For characters 1–5 the coefficient values ranged from 10.53 to 22.27%, for characters 7–11 – from 4.11 to 6.94%, for the character 12, it equaled 14.71% and for character 6 and 13, 14.98% and 14.06%, respectively. Again, the petiole length (character 3) was the most variable trait, with the variation coefficient: 22.27% (Fig. 2).

The analyses of characters’ means and standard deviations values gave analogical findings, as they are shown for the example of *Salix herbacea* only (Fig. 1a–c).

Two groups of *Salix herbacea* individuals were created on the basis of measured characters (1–5), so in the t-test these features differed the groups statistically significantly, as well as the number of teeth (6). All synthetic traits, characterizing the shape of leaves (7–12) and the leaf margin (13), showed no statistically significant differences (Tab. 2).

In the case of the dwarf birch, the t-test showed statistically significant differences for traits: 1–2 and 4–6. From among the measured characters, only the petiole length (3) did not differ the populations significantly. The differences found for the synthetic traits were not significant, with the exception of character 13 (Tab. 3).

**Discussion**

Although within the two studied populations of *Salix herbacea* in the Tatras, the form *macrophylla* was not found, the individuals created two distinct groups. Their values of the leaf length and width were within the limits described in European and local floras [18,26,40]. Still, the differences between the groups with the respect to leaves sizes were great enough to be statistically significant. The morphological variability of *Salix herbacea* was seldom characterized, only the stomatal index was thoroughly studied [19,20,41,42]. Wijk [16,17] reported some correlations between the biomass, the density and the cover of the species in relation to the longevity of the snow cover, as well as the influence of the growing season length on the shoot increment. The material for the present study was not collected in one year (Tab. 1) and the share of the distinguished groups in populations varied. Within the population Sh1, six individuals belonged to the group A (bigger leaves) and 23 to B (smaller leaves), while in the population Sh2, 17 and 14, respectively. This dissimilarity could have been caused by different climatic conditions in particular years. What should be emphasized, the results of Students’ t-test analyses for the two groups fulfilled the assumption that the measured traits differed them significantly, while the synthetic ones did not.

**Tab. 1** Location of analyzed populations of *Salix herbacea* and *Betula nana*.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Taxon</th>
<th>Locality and character of a site</th>
<th>No. of individuals</th>
<th>Alt.</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Year of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh1</td>
<td><em>Salix herbacea</em></td>
<td>Poland, Tatra Mts., Świnicka Przełęcz (granite slopes)</td>
<td>29</td>
<td>2000</td>
<td>20°00.38'</td>
<td>49°13.08'</td>
<td>2005</td>
</tr>
<tr>
<td>Sh2</td>
<td><em>Salix herbacea</em></td>
<td>Poland, Tatra Mts., slopes of Rysy (granite slopes)</td>
<td>31</td>
<td>1997</td>
<td>20°04.86'</td>
<td>49°10.45'</td>
<td>2008</td>
</tr>
<tr>
<td>Bn1</td>
<td><em>Betula nana</em></td>
<td>Norway, Buskerud, Geilo (mire)</td>
<td>33</td>
<td>800</td>
<td>8°12.41'</td>
<td>60°32.08'</td>
<td>2006</td>
</tr>
<tr>
<td>Bn2</td>
<td><em>Betula nana</em></td>
<td>Norway, Buskerud, Geilo, Ustetind (granite plateau)</td>
<td>32</td>
<td>1200</td>
<td>8°00.10'</td>
<td>60°27.38'</td>
<td>2006</td>
</tr>
</tbody>
</table>

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In the case of *Betula nana* the results were not so clear, although similar. It should be underlined that the two compared populations of the birch grew on different substrata: population Bn1 on the mire, while population Bn2 on the granite mountain plateau (Tab. 1). The populations were not much distant but their altitude also differed: the mire (population Bn1) lied 800 m a.s.l., and the plateau (Bn2) ~1200 m a.s.l. The differences between the leaf sizes of these two samples were statistically significant, with the exception of the petiole length. The smaller leaf size on the plateau could be explained by the influence of the substratum, or of the more severe climatic conditions, as reported by de Groot et al. [28]. Białobrzeska and Truchanowicz [39] also found a tendency for the leaf size to decrease with increasing altitude in their studies on the *Betula pubescens/carpatica* in the Stolowe Mountains in Poland. They also indicated the importance of the insolation for the leaf size, but in the present study both populations growth on the plain ground, under similar exposure conditions. On the other hand, Kovačić and Nikolić [43] described poor correlations between ecological factors and *Betula pendula* leaf shape and size in Croatia.

It should be emphasized that the two populations of the dwarf birch, growing in various environmental conditions, but geographically close, differed statistically significantly in respects of the leaf size, but not of the synthetic characters describing leaf shape. This can be regarded as a proof of independence of the leaf shape of the environmental conditions, at least for the species studied.

Other interesting findings of the present study are similar proportions of variability of the same characters between...
Fig. 1  Analyses of *S. herbacea* characters means for two groups: A – bigger leaves; B – smaller leaves.  
a Characters describing the leaf size.  
b Characters describing the leaf shape.  
c Characters describing the leaf blade margin. According to Tab. 2, numbers of characters, are given in left upper corners. Point – arithmetic mean; box – standard deviation; whiskers – 1.96 standard deviation.

Fig. 2  Coefficients of variation for *S. herbacea* and *B. nana*. Character numbers according to Tab. 2: frame – characters describing the leaf size; shade – characters describing the leaf shape; circles – characters describing the leaf blade margin.
the two species (Fig. 2). The most variable character in both shrubs is the petiole length, and this value divided by the sum of the blade and petiole lengths (i.e. character 12) is one of the two most variable synthetic characters. For both species, the measured features are more variable than the calculated ones, with the exceptions of the previously mentioned character 12, and character 13. The latter describes the margin of the leaf blade and is calculated as the number of teeth divided by the perimeter, so the resulting character shows the number of teeth per 1 mm. Both source characters are moderately variable, and the variation coefficients of character 13 calculated on their basis is very close to that of character 6. Thus the variability of the number of teeth and of the number of teeth per 1 mm is almost the same for both species. What is more, the variation coefficients of abovementioned ratios (characters 12 and 13) have almost the same values for both species but this might be accidental.

The higher variability of the petiole length may be just characteristic for this trait, but it also may be a result of measurement technique: some petioles are slightly curved, while measurements are taken along the straight line. Petioles are too short and their curves too weak to be detected. At the same time, the higher variability of the calculated trait 13 (number of teeth per 1 mm) could not be explained by the methods used and so seem to be a typical characteristic of the leaves of both species.

It can be concluded that the study allowed to confirm the hypothesis that calculated ratios are more stable than measured traits and they do not differ significantly across western European mixed oak stands. Ann For Sci. 2002;59(7):777–787. http://dx.doi.org/10.1051/forest:2002065


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