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Article

Leaf morphology and anatomy in marginal populations of common heather, *Calluna vulgaris* (L.) Hull from West Siberia and Atlantic Europe

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Abstract

This article deals with the comparative analysis of morphological and anatomical leaf structure in two marginal populations of *Calluna vulgaris* (L.) Hull that are antipodes in terms of their geographical location: one from West Siberia (Tobol River basin) and the other from Atlantic Europe (Bordeaux, Landes, France).

Keywords: Atlantic Europe, *Calluna vulgaris*, leaf anatomy, morphology, population research, Western Siberia.

Introduction

The micro-evolutionary divergence of populations in different parts of a species' distribution range is one of the challenges in contemporary evolutionary plant biology.

Genetic dissimilarities between the most easterly isolated populations of *Calluna vulgaris* (L.) Hull from Tobol River basin in West Siberia and populations in Eastern Europe were previously demonstrated by the authors (Sannikov *et al*., 2013) using analysis of chloroplast DNA. The genus *Calluna* is regarded as monotypic across its entire range from Iceland to Siberia and from Lapland to the Mediterranean. However, over millennia, the adaptive radiation of heather populations in various, at times, contrasting environmental conditions must have affected its phenotypic features.

A number of works describing the morphological and anatomical structures of the above-ground organs and tissues in *C. vulgaris* have been published in Western and Central Europe (Nordhagen, 1937; Beijerinck, 1940; Gimingham, 1960; 1975; Grant and Hunter, 1962; Dommee, 1968; Stevens, 1970; Legg *et al*., 1992; Garcfa-Fayos and Goldarazena, 2008). For Russia and particularly the Tobol River basin, such works are scarcer (Gorchakovsky, 1962; Yakovleva and Barmicheva, 2005; Vasina and Vasin, 2006; Onegin, 2008). Major features of the shoot formation system in heather have been described in detail by Mohamed and Gimingham (1970) and Gimingham (1975) although, when it comes to the structure and

functions of heather leaves and their geographical variability, our knowledge remains incomplete. Data on mechanisms of formation and geographical variation of the internal leaf cavity, abaxial groove, epidermis, stomata system, trichomes and other organs are particularly scarce, contradictory, and hardly comparable. No useful quantitative scales have been developed for comparison between easternmost and European populations concerning important adaptations of these organs and structures.

This article deals with the comparative analysis of morphological and anatomical leaf structure in two marginal populations of *C. vulgaris* that are antipodes in terms of their geographical location: one from West Siberia (Tobol River Basin) and the other from Atlantic Europe (Bordeaux, Landes, France).

Materials and methods

Leafy shoots were collected in the southern forest zone (pre-forest-steppe subzone) of West Siberia, near Zavodouspenskoye in the Tobol River basin, an area of pine forest with mosses, cowberry (*Vaccinium vitis-idaea* L.), and bilberry (*Vaccinium myrtillus* L.), and also from sandy habitats on the Bay of Biscay coast (Bordeaux, Landes, France). The structure of the annotinous shoots was analyzed using fresh and fixed material, each sample set consisting of shoot fragments from 30 individuals, each fragment 5 leaves long, taken from the middle part of the shoot. Fifteen shoots were used for leaf cross-sections (5 for each leaf); the same shoots provided material for epidermis maceration. Leaf samples were fixed in an alcohol/glycerin mixture (3:1). Leaf cross-sections were made manually, with a razor blade. Material used for epidermis maceration was kept in aqueous sodium hydroxide solution. Morphological and anatomical characters were observed with a binocular MC-2-ZOOM, LSM 710 Carl Zeiss. Statistical analyses were carried out using Statistica 8.0 Program. Factor analysis was employed for the study of differences between the populations.

Results and discussion

Detailed descriptions of leaves in a number of representatives of the family Ericaceae are found in Yakovleva and Barmicheva (2005). In many species, leaf margins are involute, so that the abaxial epidermis, containing numerous stomata, is concealed in a groove. Unicellular trichomes are borne around the stomata and at the groove margins. The authors nicknamed the cross-section of the heather leaf shown in the photograph "a butterfly."

As described by Mohamed and Gimingham (1970), in *C. vulgaris*, shoots of the following year are pre-formed at the apex and in distal leaf axils of the annual shoot (Fig. 1–A1). During its first year, the shoot only grows longer; it is in the second year that new vegetative and generative shoots develop in leaf axils.

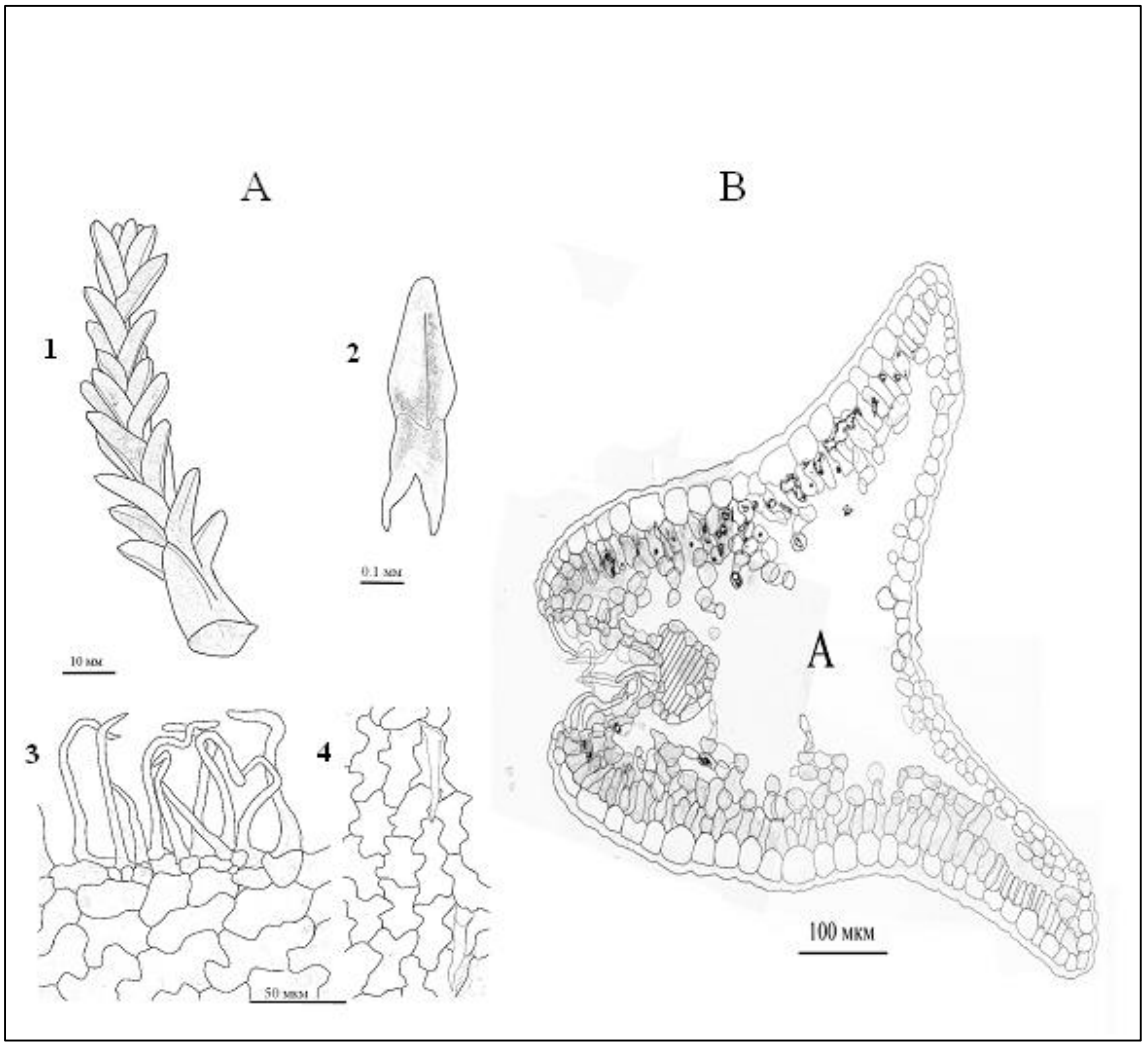

Fig.1. Morpho-anatomical parameters of *C. vulgaris* annotinous shoot.
A: 1 – annotinous shoot fragment; 2 – leaf; 3 – trichomes coating the groove located on the abaxial leaf side; 4 – trichomes located on the adaxial leaf side; B: leaf cross-section (15*20): A: internal leaf cavity

In the environment of pre-forest-steppes in West Siberia, the terminal bud is often killed by the frost when one of the lateral shoots takes over the function of leader. On the Atlantic Coast, the terminal bud and shoot apex usually survive the winter. Generally there are two phases of active growth during the vegetative period. First, the proximal part of the shoot (constituting about two-thirds of its entire length) elongates and then, by the end of the summer, new vegetative buds are formed in proximal leaf axils, while floriferous buds are

formed in distal axils. The distal part of the shoot (one-third of the entire length) starts active growth after flowering. Leaves (Fig. 1–A2) along this part of the shoot are smaller: 1.2±0.01 mm to 2.0±0.04 mm long. Internodes are short, obscured by closely appressed leaves. The largest leaves are those covering proximal parts of annotinous shoots: up to 4.0±0.05 mm. The structure and growth dynamics in European plants are similar (Mohamed and Gimingham, 1970).

Leaves as well as shoots in West Siberian *C. vulgaris* are hairy and three-sided (Yakovleva and Barmicheva, 2005; Onegin, 2008). One face is abaxial, forming a groove which is lined with abaxial epidermis whose central part is curved in and coated with dense unicellular trichomes (Figs. 1–B and 2), a structure similar to that found in plants from the European Atlantic Coast.

Fig.2. Micro-photographs of *C. vulgaris* leaf.
I – abaxial epidermis, II – adaxial epidermis, where 1 – groove;
2 – trichomes; 3 – place of leaf junction with stem, vascular bundle

Cells of the abaxial epidermis are large, with undulating walls. Larger and broader epidermal cells are characteristic for plants from the Atlantic population (Table 1), while cells in the West Siberian plants are thicker. Stomata line both leaf sides along the entire groove forming groups of 3-6 (Figs. 1–A3, 1–B and 2). In West Siberian plants, the average number of stomata per mkm in the groove is 2.5 times as large as that in the plants from the Atlantic Europe (0.952±0.327 and 0.357±0.138, respectively). The area of an epidermis cell in the Bordeaux plants is on average 1.5 times larger than that in the Tobol River basin (1089.03±102.314 and 734.28±73.261 mkm^2, respectively; Table 1.)

Adaxial leaf surfaces are also covered with unicellular trichomes, though they are smaller and sparser (Figs. 1–A4 and 2). Epidermis cells in the conditions of pre-forest-steppe of

West Siberia are smaller, their walls less undulating than in the abaxial epidermis. In the Atlantic Europe population, the adaxial epidermis is often devoid of stomata or with only a few in the central part of the leaf surface (Fig. 1B).

Table 1. Mean values of studied parameters in *Calluna vulgaris* populations (n=15)

	Epidermis cell					Palisade chlorenchyma cell	
	length	width	thickness	elongation	area	area	thickness
Bordeaux, France							
M+m	52.863 ±3.523***	23.881 ±1.284***	34.673 ±1.510***	2.243 ±0.118*	1089.030 ±102.314**	453.081 ±27.929*	33.959 ±1.460**
SD	13.642	4.972	5.846	0.457	396.260	108.167	5.656
Zavodouspenskoye, Sverdlovsk Region, Russia							
M+m	44.954 ±2.763***	17.847 ±0.879***	42.509 ±3.213***	2.519 ±0.101*	734.276 ±73.261**	390.157 ±18.966*	36.296 ±1.173**
SD	10.701	3.406	12.443	0.390	283.736	73.454	4.544

	Palisade chlorenchyma cell		Pneumatic chlorenchyma cell			
	width	elongation	thickness	width	elongation	area
Bordeaux, France						
M+m	12.693 ±0.410*	0.378 ±0.012*	21.049 ±1.040*	15.138 ±0.763*	0.734 ±0.039*	267.189 ±23.545*
SD	1.588	0.045	4.026	2.956	0.152	91.188
Zavodouspenskoye, Sverdlovsk Region, Russia						
M+m	13.148 ±0.393*	0.369 ±0.018*	21.436 ±0.507*	15.514 ±0.376*	0.729 ±0.023*	246.474 ±9.626*
SD	1.521	0.070	1.962	1.457	0.088	37.283

Note: * - p≥0.5: ** - p≥0.05; *** - p≥0.01

On the leaf cross-section (Fig. 1B), one can find features that *C. vulgaris* has in common with some species of *Cassiope* D.Don. More similarities have been noticed before as regards the annual shoot development (Hagerup, 1953). The vascular bundle is at the bottom of the groove (Fig. 1B). Walls are formed by the abaxial epidermis rich with stomata and densely pubescent.

Chlorenchyma in the heather leaf, in most cases, comprises two strata, distinctly differentiated into palisade and pneumatic tissue, both in the Tobol River basin population and in that from Atlantic Europe. Cells of the palisade chlorenchyma are located in the abaxial part of the leaf, their length on average from 29.08±0.29 to 35.35±0.35 mkm. Intercellular spaces are scarce. Strands consisting of pneumatic chlorenchyma cells sized 5.52±0.30 to 8.70±0.33 mkm reach the vascular bundle. In the central part of the leaf, there is a cavity (Fig. 1B), which in an unfavorable situation might allow for contraction of the overall leaf surface, as the walls of the cavity come closer to each other, and thereby reduce of transpiration. Fewer chlorenchyma cells are adjacent to the adaxial epidermis. These are small (6.40–13.70 mkm)

and rounded. Generally, linear parameters (thickness and width) of the palisade and pneumatic chlorenchyma as well as their surface and length are hardly different when one compares the West European and West Siberian population.

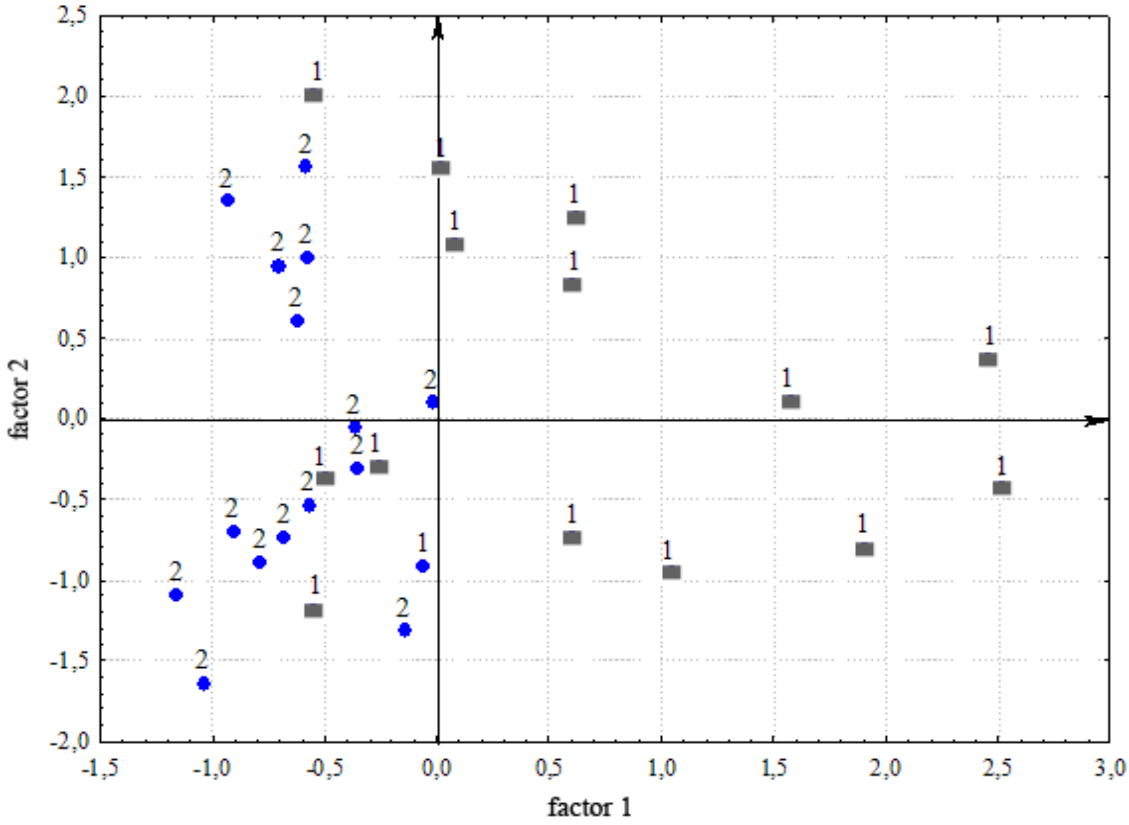

Fig.3. Distribution of individuals in factor environment:
1. Zavodouspenskoye, Russia 2. Bordeaux, France

For the comparison of the Bordeaux and Zavodouspenskoye populations, factor analysis was employed with respect to 15 morphological and anatomical characteristics of the epidermis and chlorenchyma cells. It was found that generally the two populations are hardly differentiated from one another (Fig. 3). Individuals with the largest cells typical for the Atlantic Europe population are concentrated in the first and third quarters of the graph (Fig. 4), while the other two quarters are occupied by individuals with the thickest abaxial epidermis cells characteristic of the Tobol River Basin population (Table 1, Fig. 3).

Conclusions

Literature data on the morphological and anatomical structure of the heather leaf in different parts of the plant's range are rather scanty and controversial. The abaxial epidermis structure has been treated in detail in Yakovleva and Barmicheva (2005). In that work an hypothesis is developed regarding the integrity of the epidermis and its role in the formation of the groove. However, the micro-photographs in the article don't provide enough information for outlining the leaf structure in its entirety. Stevens (1970) provided more detail regarding heather leaf morphology. However, the generalized outline of the leaf cross-section in this work is not precise: no cavity is shown in the central part of the leaf, the vascular bundle is located beneath the abaxial epidermis, and no chlorenchyma cells are depicted. The sketch provided in the article by Onegin (2008) is even less clear. According to the author, the leaf is enclosed, as the margins of the abaxial epidermis touch each other, while the vascular bundle in the leaf center and a multitude of stomata open into the produced cavity.

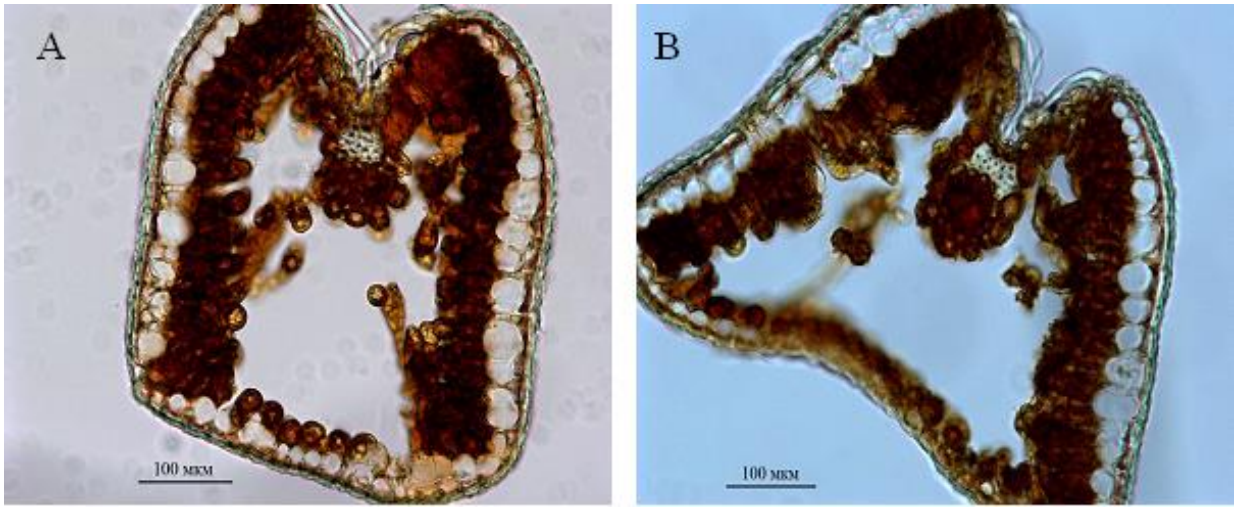

Fig.4. Microphotographs of *C. vulgaris* leaf cross-section: A -- Zavodouspenskoye; B -- Bordeaux

Therefore, the concept of the leaf structure of heather presented in this article is quite different from and only partially in agreement with those proposed by other authors. The results of our research of the morphology and anatomy of heather leaf in contrasting climates of West Siberia and West Europe may be summarized as follows.

1. In both studied populations, the groove is formed by the fold of the abaxial epidermis, which is due to invagination of the central part of the epidermis containing the vascular bundle.

2. Stomata are located on the abaxial leaf surface in the groove and are protected by dense rows of unicellular trichomes.

3. In the central part of the leaf, there is a cavity stretching along the entire leaf length, whose probable function is to facilitate water conservation during the dry period.

4. The groove and adaxial epidermis cells perform a coordinating mechanical function: during the dry period, the adaxial and abaxial surfaces come closer to each other, in the direction toward the vascular bundle, which results in a diminution of the internal cavity and maintenance of the leaf water balance.

Factor analysis (Fig. 3) has demonstrated that in the situation of a comparatively drier climate and soils of pre-forest-steppe of West Siberia, heather leaves are characterized by thicker, though narrower and shorter epidermal cells (Fig. 4). This must have to do with the adaptation of the Tobol River basin population to a drier environment (Petrova *et al*., 2009; Sannikov *et al*., 2013).

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