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MORPHO-ANATOMICAL DIFFERENTIATION WITHIN POPULATIONS OF *CALLUNA VULGARIS* L. (HULL) ON THE SUBMERIDIONAL TRANSECT MURMANSK – BATUMI

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Abstract

Calluna vulgaris is an ornamental bush widespread from the Kola Peninsula in the north to the Georgian ridges (Batumi) in the south. Today it is in considerable use for pharmacopoeial purposes as it has a lot of bioactive substances in different parts (leaves, roots, shoots, flowers, etc.). Having a wide habitat, the heather demonstrates minor range of morpho-anatomic feature variability that is represented by its systematical characteristics. Heather is a monotype of *Calluna*. This article presents brief results from morphological and anatomical analysis of four *Calluna vulgaris* populations growing on the submeridional transect Murmansk – Batumi. The main aim of the study was to investigate and clarify the mechanism of adaptive variation to the contrast environment conditions. The discriminant analysis was performed using the complex of 22 morpho-anatomic leaf characters. As a result it was found out that *Calluna vulgaris* populations growing at the habitat boundary had some common features that led to growth of plant xeromorphism (decrease of stomata number, trichome number increase, cuticle height rise). *Calluna vulgaris* populations growing in the central part of the habitat on the Russian Plain, despite their sharp isolation, also had one important similar feature – significantly smaller size of chlorenchyma cells which is probably connected with growing of plants beyond the canopy.

Key words: adaptive radiation, isolation, leaf anatomy, xeromorphism.

Introduction

Today the molecular-genetic methods are successfully used to study the microevolutionary processes in the plant populations. On the other hand, the traditional morpho-anatomical methods, which reflect phenotypic selection trend and degree as well as population adaptive radiation under influence of heterogeneous environment, geographical and ecological habitat (Sannikov et al. 2014), are also informative and used for this purpose.

Calluna vulgaris L. (Hull) was chosen as an object to study the processes within the insular marginal populations of the habitat. Following the taxonomists' assumption the populations of this species, which belongs to monotypic genus *Calluna,* do not undergo the morpho-phenotypical adaptive radiation within wide and ecological-geographical heterogeneous habitat – from Transatlantic in Europe to

Trans-Urals in Western Siberia and from the Kola Peninsula (Murmansk) to Colchis (Batumi) (Rayner 1913, Hagerup 1953, Sannikov et al. 2016). However, this hypothesis was not proved with quantitative genetic and phenotypic researches.

At the same time our researches revealed reliable differentiation (on complex of lamina anatomical features) between marginal western (London, England) and eastern (Zavodouspenskoe, Sverdlovsk region) populations (Cherepanova et al. 2015).

The purpose of the article is to perform the comparative analysis of geographic variation and differentiation of some morpho-anatomical features of Central European, southern (Batumi) and northern (Murmansk) marginal populations of *C. vulgaris*.

Materials and Methods

The comparative analysis of seed morphological variation and morpho-anatomical variation of annual sprout of *C. vulgaris* was carried out on the geographical transect going from north to south of the habitat: Murmansk – Luga – Bryansk – Batumi (Fig. 1). The first three populations grew under the canopy of vicariant stands of pine forests and cowberry-heather-moss forests of Russian Plain and the last one – on the south-east coast of the Black Sea.

The structure of *C. vulgaris* annual sprouts were studied using about 11–40 fresh and soaked samples taken from each population. The seed capsules were gathered in August and September. For each sample we measured the length and thickness of 5 leaves taken from the

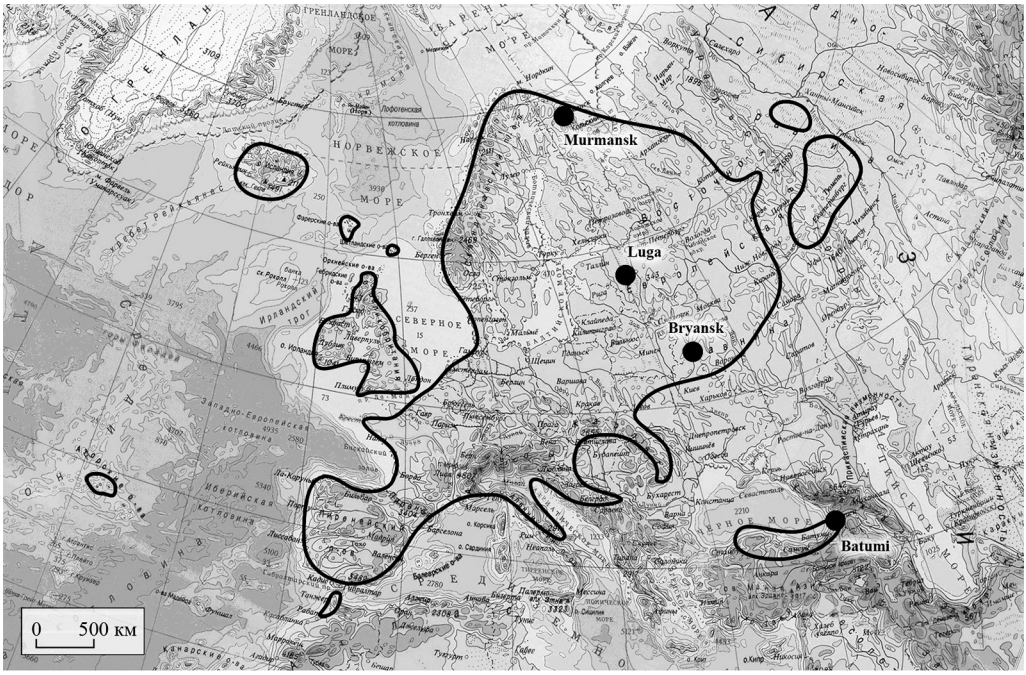

Fig. 1. Schematic map showing the range of *C. vulgaris* and location of population sampling on the submeridional geographic transect Murmansk – Batumi.
Note: The *C. vulgaris* range is marked with the line.

middle part of each of three terminal annual sprouts. The samples of leaves were soaked in a mixture of ethanol and glycerin (3:1). The cross-sections were cut manually with a razor (Fig. 2). Five sections were cut for each leaf and the tissue samples were taken for epidermis maceration, which was performed using the material previously soaked in water solution of NaOH (10 %).

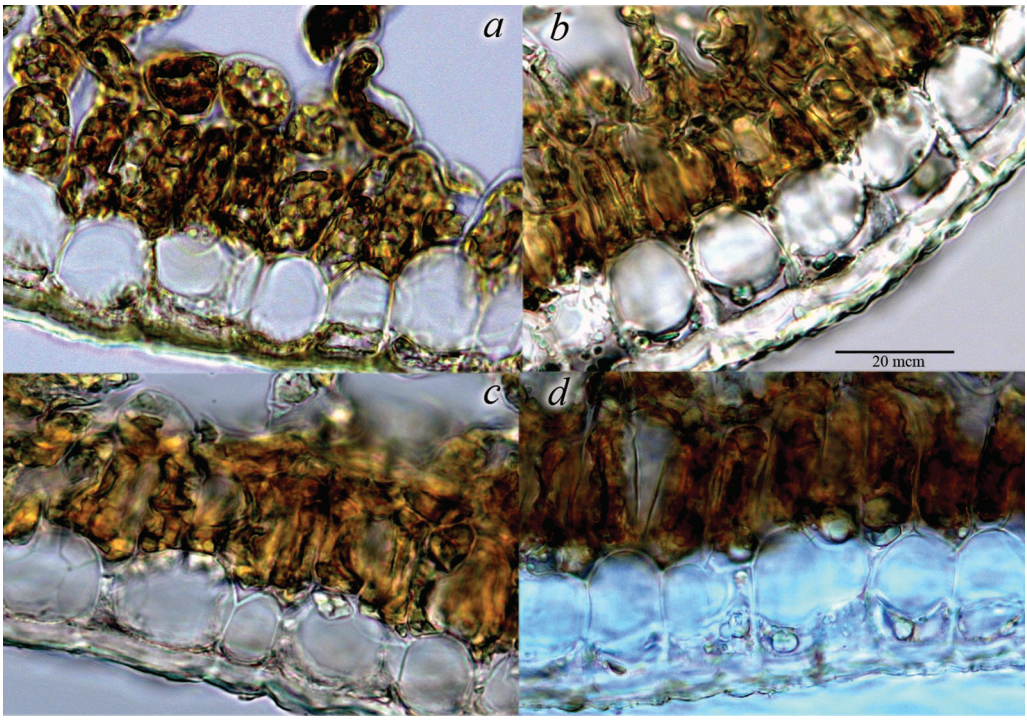

Fig. 2. Sheet of *C. vulgaris* cross-sections: *a* – Murmansk, *b* – Luga, *c* – Bryansk, *d* – Batumi.

Twenty six morpho-anatomical parameters' characters of annual sprout were analyzed (Table 1). The parameters' dimensions of morpho-anatomical structure of leaves were measured with threefold and fivefold replication applying the binocular loupe Carl Zeiss Stemi 2000-C and light microscope Carl Zeiss, as well as software AxioVision Rel. 4.8.

The statistical analysis was carried out on the basis of 26 morpho-anatomical characters of annual sprout (internode length, thickness and length of the leaf, linear parameters of chlorenchyma cells and epidermis cells, number of stomata and trichomes, vascular bundle). For statistical processing (discriminant analysis, ANOVA – Kruskal-Wallis one-way analysis of variance), statistical software package Statistica 8.0 was used.

Results and Discussions

The minimal number of stomata was observed in hot and wet climate in Batumi (12.10±0.57 pcs for 10 μm), and maximal number – in cool and wet climate in Luga

Table 1. Average morpho-anatomical parameters of leaf in *C. vulgaris* populations ($p \leq 0.001$).

Parameters		Murmansk	Luga	Bryansk	Batumi
		N=20	*N*=14	*N*=16	*N*=12
		$M \pm m_M$	$M \pm m_M$	$M \pm m_M$	$M \pm m_M$
Cuticle thickness, μm		3.86±0.18	3.97±0.28	2.9±0.14*	4.36±0.22*
Stomata number, pcs.		18.24±0.39	19.69±1.14	16.95±0.88	12.10±0.57**
Trichome number, pcs.		14.76±0.5	15.65±0.75	14.20±1.17	17.63±0.99
Upper epidermis cells	height, μm	28.68±1.39	26.61±1.44	26.27±1.23	31.76±5.05**
	length, μm	58.89±2.55	58.78±2.4	65.32±1.47	59.74±3.05
	thickness, μm	27.26±1.58	29.39±4.46	25.39±1.09	25.34±1.45
	area, μm^2	1377.65 ±111.87	1195.19 ±56.37	1395.47 ±102.76	1309.33 ±101.38
	perimeter, μm	175.95±8.25	180.48±5.02	200.19±9.25	177.48±9.2
	elongation	0.47±0.02	0.49±0.06	0.40±0.02	0.43±0.03
Palisade tissue cells	length, μm	34.31±1.21	26.19±1.14	31.51±1.07	35.50±2.88
	thickness, μm	14.53±0.26	11.82±0.55**	14.87±0.96	14.53±0.37
	area, μm^2	453.58±22.81	290.85 ±19.87*	353.40±17.60	465.65±20.85
	perimeter, μm	89.19±2.56	67.99±4.98**	81.08±2.38	90.62±2.62
	elongation	0.43±0.02	0.46±0.03	0.47±0.03	0.43±0.03
Spongy parenchyma cells	length, μm	19.80±0.58	16.76±1.06*	19.07±0.47	21.18±1.08
	thickness, μm	20.56±4.68	13.29±1.48*	14.40±0.47	15.52±0.93
	area, μm^2	269.18±15.60	192.74±17.48	228.41±10.18	285.81±28.05
	perimeter, μm	62.56±1.64	54.99±3.25	58.29±0.99	64.86±3.07
	elongation	1.05±0.24	0.86±0.15	0.76±0.02	0.74±0.04
Vascular bundle	length, μm	82.18±2.93	60.33±3.22	59.29±1.75	68.18±2.38
	thickness, μm	79.79±3.14	77.65±3.46	60.35±1.47	79.98±3.79
	elongation	0.98±0.04	1.30±0.04	1.03±0.03	1.18±0.06
Leaf	length, mm	1.23±0.08	1.26±0.03	1.31±0.05	1.22±0.07
	thickness, mm	0.54±0.02	0.37±0.03*	0.44±0.03	0.50±0.03
	elongation	0.42±0.02	0.30±0.02	0.34±0.03	0.43±0.04

Note: $p \leq 0.05$ – *, $p \leq 0.001$ – ** (Kruskal-Wallis one-way analysis of variance), *N* – number of specimens in the analyzed sample, *M* – mean, *mM* – standard error.

(19.69±1.14 pcs for 10 μm). Together with smaller number of stomas the maximal number of trichomes (17.63±0.99 pcs for 10 μm) was found out here (see Table 1).

The number of stomata decrease and trichomes number increase occurring from west to east of heather habitat (Oslo, Norway – Zavodouspenskoe) along with climate dryness increase have been already mentioned before (Fig. 2). The overall trend decrease of stomata number from west to east and from south to north of the habitat was observed. Together with stoma number decrease occurring from west to east, the trichome number increase took place in the same direction. Proba-

bly the heather responded to the climate humidity reduction with the trichome number increase and stoma number decrease to avoid excessive transpiration (Zvereva 2010, Blagushka and Sozinov 2014).

The tendency of general reduction in linear parameters of epidermis cells occurring from the habitat boarders to its central part, previously observed in a row west-east, was preserved. In marginal southern population the epidermis cell height grew sharply (Table 1), while in the central part the values of this parameters were minimal (Bryansk – 26.27±1.23 μm) (Fig. 2).

The cuticular layer thickness grew along with the epidermis cell height from the center to the periphery of the habitat and reached its maximal value in the south 4.36±0.22 mcm. According to several authors, the strong cuticle development prevents the plans from excessive transpiration during daylight hours (Yakovleva and Barmicheva 2005, Dommee 1969, Grant and Hunter 1962, Mohamed and Gimingham 1970). The main function of cuticle, as well as of epidermis, is to avert the moisture loss in conditions of its deficiency (Zvereva 2010). Matching of epidermis height and cuticular layer thickness can demonstrate that the *C. vulgaris* population growing in Batumi was adapted to the dry climate best of all (Fig. 2).

The comparative analysis of sizes of palisade tissue cells and spongy parenchyma cells represented similar tendencies of linear increase of these parameters occurring from the center to the periphery of the habitat. The maximal linear dimensions of palisade tissue cells were found out in Batumi (35.50±2.88 μm – length, 465.65±20.85 μm^2 – area), and minimal – in Luga (26.19±1.14 μm – length, 11.82±0.55 μm – thickness, 290.85±19.87 μm^2 – area). The heather growing near the forest-tundra on the northern border of the habitat (Murmansk) was characterized with almost maximal linear dimensions of palisade tissue cells and spongy parenchyma cells (Table 1).

We did not found out any variability pattern for leaf conduction system linear parameters – vascular bundle – occurring from north to south of the habitat (Table 1).

The result of the step-by-step analysis was the inclusion of 19 parameters in the discriminant analysis model (Table 2).

The parameters characterizing the anatomical structure of the leaf were the most significant (Table 2). Those describing leaf morphology were included in the discriminant analysis model in the minimum number (leaf elongation, leaf thickness, mm).

The most determined population were Murmansk and Bryansk (distance between groups – 8.23, $p \leq 0.001$), Murmansk and Luga (distance between groups – 6.41, $p \leq 0.001$). The closest of leaf parameters are Batumi and Murmansk (distance between groups – 3.92, $p \leq 0.001$), Luga and Bryansk (distance between groups – 4.79, $p \leq 0.001$) populations.

The value of the vectors for the first two factors, describing the discriminant model, amounted to 5.17, 2.50 accordingly. The discriminant analysis (Fig. 3) of *C. vulgaris* populations growing in Luga and Bryansk revealed their differentiation.

Table 2. A summary analysis of the discriminant functions calculated for leaf parameters included in model ($p \leq 0.001$).

Parameters	Wilks' Lambda	Partial Lambda	*F*-remove (3.35)	*p*-level	Tolerance	1-Tolerance
Vascular bundle length, μm	0.023	0.641	6.542	0.001	0.036	0.964
Stomata number, pcs.	0.031	0.478	12.752	0.000	0.472	0.528
Vascular bundle elongation, μm	0.021	0.716	4.621	0.008	0.045	0.955
Palisade tissue cells area, μm^2	0.024	0.631	6.818	0.001	0.253	0.747
Cuticle thickness, μm	0.020	0.747	3.945	0.016	0.637	0.363
Upper epidermis cells elongation	0.020	0.764	3.609	0.023	0.008	0.992
Spongy parenchyma cells elongation	0.019	0.806	2.807	0.054	0.038	0.962
Spongy parenchyma cells thickness, μm	0.018	0.831	2.370	0.087	0.037	0.963
Leaf elongation	0.018	0.823	2.507	0.075	0.209	0.791
Palisade tissue cells thickness, μm	0.019	0.794	3.036	0.042	0.562	0.438
Spongy parenchyma cells area, μm^2	0.016	0.941	0.738	0.537	0.365	0.635
Upper epidermis cells area, μm^2	0.017	0.888	1.472	0.239	0.212	0.788
Upper epidermis cells perimeter, μm	0.023	0.655	6.154	0.002	0.091	0.909
Upper epidermis cells height, μm	0.020	0.750	3.899	0.017	0.023	0.977
Vascular bundle thickness, μm	0.019	0.794	3.029	0.042	0.040	0.960
Upper epidermis cells thickness, μm	0.019	0.783	3.232	0.034	0.008	0.992
Trichome number, pcs.	0.019	0.780	3.296	0.032	0.428	0.572
Palisade tissue cells length, μm	0.018	0.839	2.245	0.100	0.318	0.682
Leaf thickness, mm	0.017	0.895	1.367	0.269	0.194	0.806

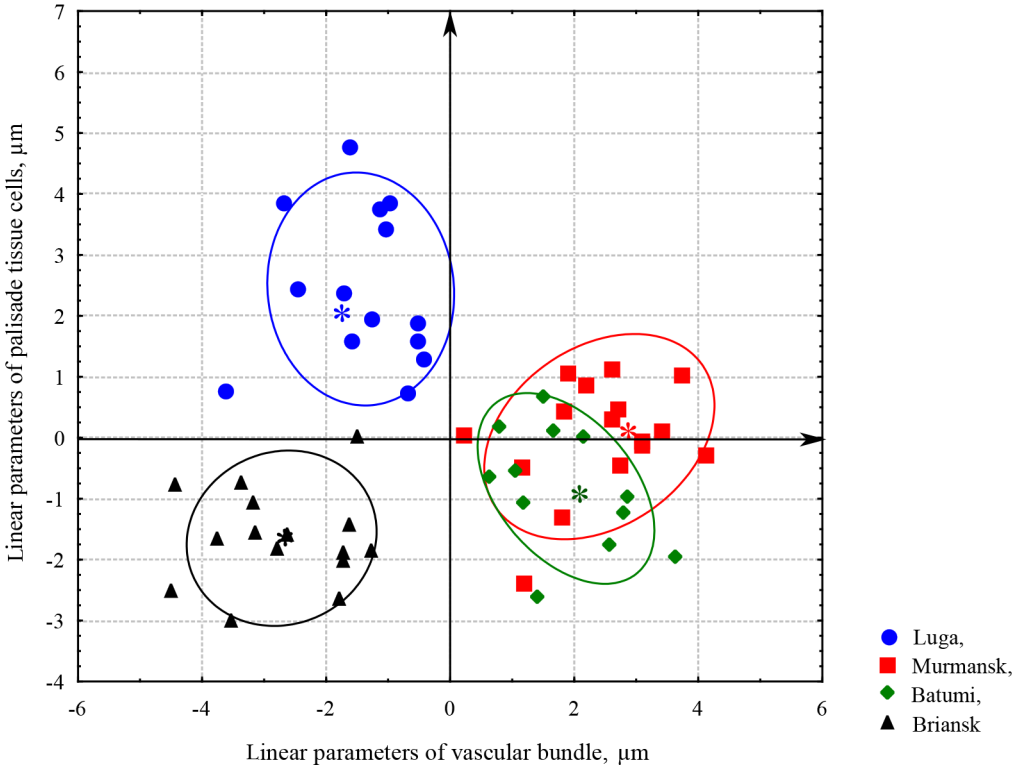

Fig. 3. Graphical representation of discriminant analysis results of morpho-anatomical parameters of leaves.
Note: * – Means of canonical variables.

At the same time the allocation areas populations growing in Murmansk and Batumi overlapped which evidenced for the resemblance of their leaf blade structure. The most significant features for that were the height, thickness of palisade tissue and length vascular bundle (Table 2).

Conclusion

As a result of comparative study of morpho-anatomical characters of leaf in vicariant populations of *C. vulgaris* growing on submeridional transect Murmansk – Batumi, we found out an increase in linear parameters of heather leaf cells (epidermis, chlorenchyma), as well as in xerophytism parameters (stomata number decrease, trichome number increase, growth of cuticle height) along with reduction of leaf blade overall dimensions occurring from periphery to habitat borders, that was previously revealed on the transect going from west to east of the habitat (London – Zavodouspenskoe) (Cherepanova 2015). Along with that the heathers growing in open space were characterized with more xeromorphic structure, which was represented by chlorenchyma cells reduction.

Acknowledgements

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