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MORPHOMETRIC AND RAPD STUDY OF THE *MELAMPYRUM SYLVATICUM* GROUP IN THE SUDETEN, THE ALPS AND CARPATHIANS

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Abstract: The *Melampyrum sylvaticum* group is a heterogeneous group at the inter- and infra-specific level. A wide range of morphological characters was examined on specimens collected for this study. The corolla size, the corolla colour and the anther length are considered the most important morphological characters at the interspecific taxonomic level. The morphological and molecular variation of the *Melampyrum sylvaticum* group in the Sudeten, Alps and Carpathians was analyzed. The morphometric study of European populations of *Melampyrum sylvaticum* group partly clarified the pattern of the variation of selected characters in this region. The RAPD analysis manifests a very similar trend of the population grouping. The combined analysis of morphological and molecular data gives the most explicit results. Central European yellow flowering populations of *M. sylvaticum* group form two distinctive groups. The first one is an exclusive type of the Alps and of the Hercynian Mountains (except the Sudeten). The second group has its centre of distribution in the eastern part of the Carpathians. This type was described as *Melampyrum herbichii* from the present Ukrainian Carpathians and was regarded to be endemic to the Eastern and Southern Carpathians. Populations from the Western Carpathians and Sudeten Mts. are polymorphic. They seem to be closer to *M. herbichii* based on the RAPD markers, whereas the morphology of most populations from these regions is closer to *M. sylvaticum* s.str. The bract shape seems to be important for discrimination between both groups of populations along with the flower characters and a modest ecological differentiation. The Eastern Carpathian *Melampyrum saxosum* with white corolla is very close to *M. herbichii* based on the studied morphological characters.

Keywords: Central Europe, Genetic diversity, *Melampyrum herbichii*, *Melampyrum saxosum*

INTRODUCTION

The genus *Melampyrum* L. belongs to the hemiparasitic group of the former family *Scrophulariaceae*. This group was currently added to the *Orobanchaceae* family, based on the molecular studies (OLMSTEAD et al. 2001). The major part of these hemiparasitic genera has a typical infraspecific variation pattern – the seasonal variation. The principle of this phenomenon is the occurrence of two or more variants in a single species. Variants differ by so-called seasonal characters. The most important seasonal characters are number of internodes and that of intercalary internodes (i.e., internodes between the uppermost branches and the lowest flowers of the terminal inflorescence), number and length of branches and flowering branches, leaf and bract width and length, and flowering period (cf. SOÓ 1926–1927, ŠTECH 2000b). The early-flowering types have few internodes and few short branches, the late-flowering types have many internodes, some intercalary internode, many flowering branches, and usually more narrow leaves than early-flowering types. The seasonal variation is very important in many species of the genus *Melampyrum*. Many intraspecific taxa were usually described in different species of the genus *Melampyrum* based on the seasonal characters. However, some species groups of this genus show an important geographical variation in morphological characters influenced only marginally by the seasonal variation. *M. sylvaticum* group is one example. Several more-or-less independent trends in the variation of this group are conspicuous. Several seasonal types are known in this group, but the most important geographical variation covers "non seasonal" characters and is concentrated in the Eastern and Southern Carpathian region. This variation manifests especially in the flower size and flower colour. Three taxa were described from this point of view. Diagnostic characters of these taxa and their distribution are still very confused. *Melampyrum sylvaticum* L. is the commonest species, which is widespread in all regions of the group distribution (north and Central Europe). *Melampyrum herbichii* WOŁ. is often supposed to be endemic in the Eastern and Southern Carpathians (e. g. SOÓ & WEBB 1972), but there are records from the Western Carpathians too (e. g. JASIEWICZ 1958, MANICA 1973, ŠÍPOŠOVÁ 1997) and even some sporadic and equivocal records from the Sudeten Mountains (JASIEWICZ 1958). *M. saxosum* BAUMG. with white flowers is indicated usually for the Eastern and Southern Carpathians only. The variation at the infraspecific level is enormous, leading to a large number of described infraspecific taxa and related nomenclatural problems. RONNIGER (1911) had studied the genus *Melampyrum* in the Alps and he considered plants from the subalpine ecosystems – *M. sylvaticum* subsp. *laricetorum* (A. KERN.) RONNIGER – to be identical with the Carpathian *M. herbichii*. This opinion is usually not accepted. However, *M. carpaticum* SCHULT. – another taxon described from the Western Carpathians – is often synonymized with *M. sylvaticum* subsp. *laricetorum* (SOÓ 1926–1927, JASIEWICZ 1958, HARTL 1974).

In this work (i) morphological variation of *Melampyrum sylvaticum* group in Central Europe was studied, (ii) populations from important geographical regions of *Melampyrum sylvaticum* group were studied by RAPD analysis in attempt to resolve infraspecific variation, and (iii) variation pattern from the taxonomic and phytogeographic point of view was interpreted on the basis of separated and combined morphological and molecular data sets.

METHODS

Plant material and sampling strategy

Almost 420 plants of the *Melampyrum sylvaticum* group were studied in 25 populations from the Sudeten, the Alps and the Carpathians (Fig. 1, Appendix). Between 10 and 20 plants from each population were usually studied from a morphological point of view. A set of 140 *Melampyrum sylvaticum* accessions from the Czech Republic, Slovakia, Austria and Switzerland was selected for RAPD analysis. For later collected plants (in 2003), we have just morphologhical data available, but these populations are very important, because one of them is from a population cited in the original description of *M. herbichii* (Mount Hoverla). The material of *Melampyrum saxosum* was also acquired in 2003.

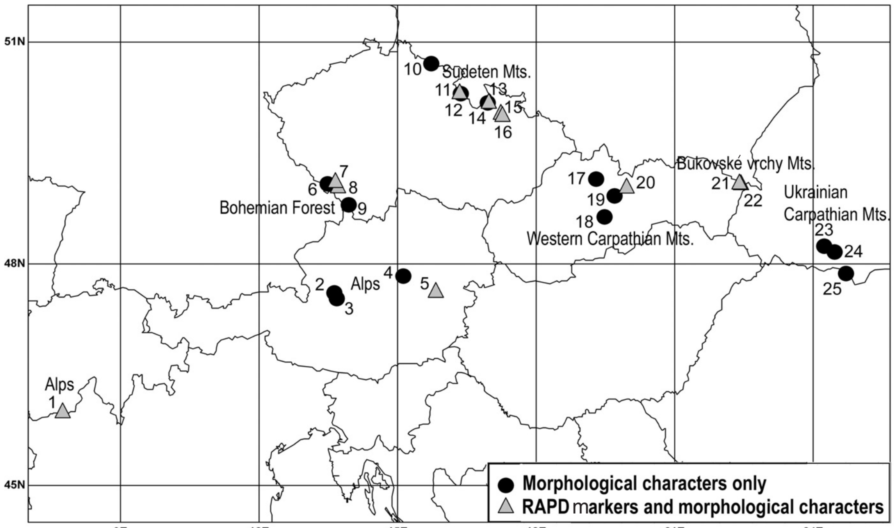

Fig. 1. Map of localities of the studied populations of the *Melampyrum sylvaticum* group.

Morphometric analysis

Morphological characters considered useful in the determination of microspecies and some subspecies, as well as the characters showing high variation in the herbarium material were chosen. Twelve characters were measured on the flower (calyx tube length, upper calyx tooth length, upper calyx tooth width, corolla length, corolla tube length, corolla base length, corolla height, lower lip of corolla length, lower lip of corolla height, upper lip of corolla length, upper lip of corolla height, anther length – Fig. 2) and three characters (bract length, bract width, distance of the widest part of bract from its base) on the first and the fifth bract.

RAPD analysis

We used RAPD as second methodological approach to identify infraspecific variation of the *Melampyrum sylvaticum* group in Central Europe. Genomic DNA was extracted from 0.5 mg of leaves according to the method (modified sorbitol extraction) developed in DNA laboratory of the Institute of Botany ASCR by ŠTORCHOVÁ et al. (2000). Polymerase chain reactions were carried out using forty-nine arbitrary primers. After screening selected decamer primer set OPA from Operon Technology, OPA1 (5´-CAGGCCCTTC-3´), OPA8 (5´-GTGACGTAGG-3´) and UBC40 (5´- TTACCTGGGC-3´) were used subsequently because they revealed a high level of polymorphism between populations (from 13 to 15 polymorphic bands per primer). The amplification conditions were optimized according to COBB & CLARKSON (1994). The 25 µl reaction contained 1.5 mM $MgCl_2$, buffer with NH_4Cl, 0.2 µM primer, 1 units of Taq polymerase (MBI Fermentas), 200 mM each of dATP, dGTP, dCTP and dTTP, and 1µl of template DNA. PCR were done in GeneE termocycler (Techne) and subjected to the following cycle profile: 1 cycle at 95 °C for 5 min followed by

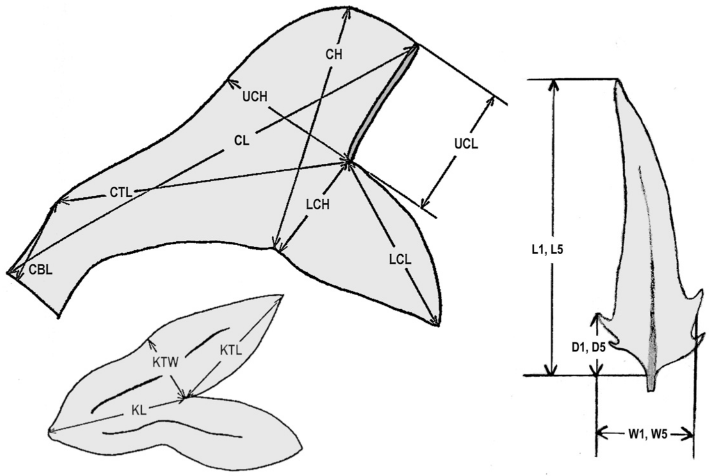

Fig. 2. Studied morphological characters: KL – calyx tube length, KTL – upper calyx tooth length, KTW – upper calyx tooth width, CL – corolla length, CTL – corolla tube length, CBL – corolla base length, CH – corolla height, LCL – lower lip of corolla length, LCH – lower lip of corolla height, UCL – upper lip of corolla length, UCH – upper lip of corolla height; L1, L5 – length of the first respective fifth bract, W1, W5 – width of the first respective fifth bract, D1, D5 – distance of the widest part of the bract from its base.

40 cycles at 95 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min. PCR products were visualized by ethidium bromide with 0.5× TBE buffer in 1.3% agarose gels. Products of amplification from DNA samples from different populations were run on the same gel.

The results were obtained under reproducible conditions (same primer concentration for all analyses, same Taq polymerase source and magnesium ion and template concentration, under the same temperature profile). Each sample was analyzed two times and included in the study only if the phenotypic patterns were identical. Only reliably scored bands with a size from 498–1857 bp and with overall distribution among the plants were included in the analysis. Bands of equal fragment size were interpreted as homologous and the staining intensity of the band was not considered as a difference. Thirty-seven scored markers were polymorphic among populations and each population appears to be characterized by a unique electrophoretic pattern combination. Binary data (scored as presence/absence) matrices were produced from the scored RAPD markers for individual plants.

Statistical analyses of morphological characters and RAPD phenotypes

To evaluate and compare discriminatory power of morphological characters with RAPD data, a dissimilarity measure working well with both quantitative and qualitative variables must be used. We have chosen the commonly used Gower distance, calculated as a 1 − complement of the Gower similarity coefficient (GOWER 1971). The resulting distances were further square-rooted, because such transformation results in Euclidean properties of this metric (LEGENDRE & LEGENDRE 1998). The Gower similarity measure has two variations differing in treatment of “double zeros” (i.e., handling the case when a qualitative

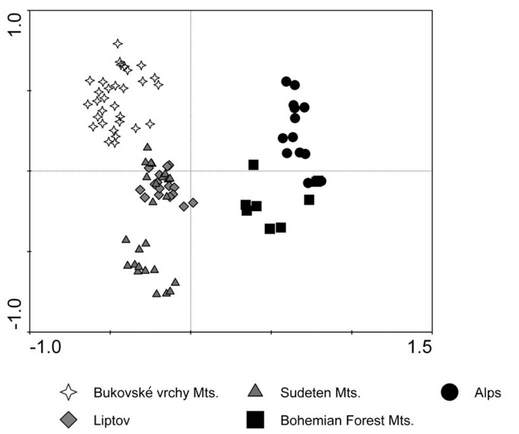

Fig. 3. PCoA ordination diagram based on RAPD markers.

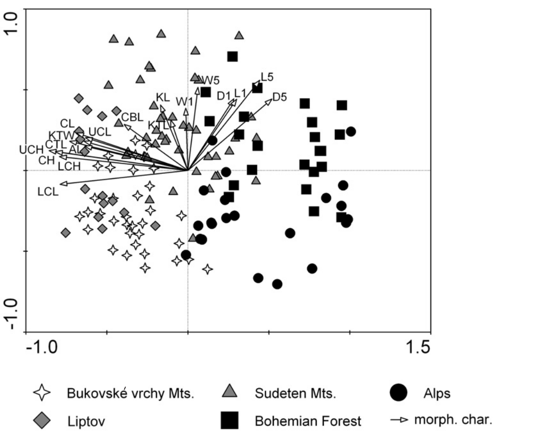

Fig. 4. PCoA ordination diagram of plants (for which both types of data were available) based on the morphological characters. Directions of changes of these characters are projected. For explanation of abbreviations of morphological characters see Fig. 2.

variable has a value of 0 for both compared objects). We selected the form ignoring the double zeros (PODANI 1999).

The distance matrices were calculated based on the morphological characters, on the data obtained from RAPD analysis, or using both datasets combined together. Therefore, only the plants where both types of data were available were used in these comparative analyses. We used the cluster analysis in the program Statistica for Windows (STATSOFT 1999) to classify the plants into distinct groups. We have chosen the unweighted pair-group average (UPGMA; SNEATH & SOKAL 1973) method for the process of cluster fusion. We have also used the principal coordinates analysis (PCoA; LEGENDRE & LEGENDRE 1998) method to visualize more quantitatively the relation between studied plants, using the Gower distance. The PCoA method can be further extended into constrained ordination method, called distance-based redundancy analysis (db-RDA; LEGENDRE & ANDERSON 1999). We have used db-RDA to compare the discrimination between two recognized groups, based on the RAPD data with the one provided by the morphological characters and to test the hypothesis that the morphological characters have their own, additional contribution to this differentiation. This question is important because practical determination of field-collected material must rely on morphological characters and also the RAPD results cannot reflect all the genetic differences between studied plants, not even all those manifested in plant morphology. To compare intra- and inter-population variation for morphological and RAPD datasets, a db-RDA using classification of plants into regional populations as an explanatory variable was provided. The percentage of total variance in these two sets of characters was then estimated using variance component calculation, as described by QUINN & KEOUGH (2002: 189).

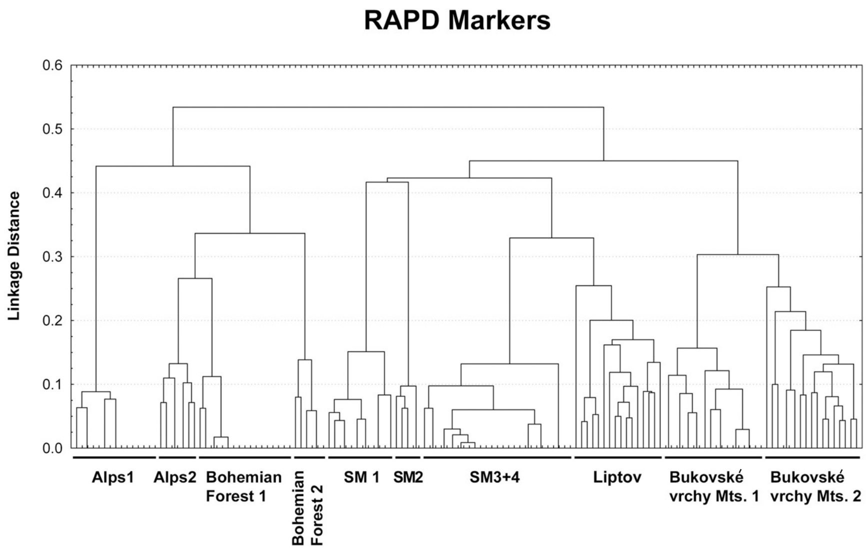

Fig. 5. Dendrogram from a cluster analysis based on a similarity matrix calculated from RAPD marker data (Gower distance). SM – Sudeten Mts.

The plants from the populations clearly representing one of the two distinguished taxa (*M. sylvaticum* and *M. herbichii*) were used as the calibration set for a linear discriminant analysis (LDA; LEGENDRE & LEGENDRE 1998) and the resulting discriminant function was then used to classify the plants from geographically intermediate populations and to estimate the cross-validated classification errors. Discriminating morphological characters were selected using a forward selection procedure with each step supported by a Monte-Carlo permutation test of significance.

The PCoA and db-RDA methods were applied to our data using the CANOCO software version 4.5 (TER BRAAK & ŠMILAUER 2002), and the discriminant analysis was calculated with the R package (ITHAKA & GENTLEMAN 1996), version 1.7.

RESULTS

Diagnostic bands

RAPD fragments were used to identify infraspecific variation of *Melampyrum sylvaticum* group in Central Europe. We originally tested 49 primers, and from these just three RAPD primers were sufficient to detect genetic differences among all populations represented by 140 sampled individuals. Totally, 37 polymorphic bands were scored for the 140 plants representing 10 populations. Four common bands that occurred in all plants of *Melampyrum* taxa were found. Within the Liptov and Bukovské vrchy Mts. more RAPD phenotypes were found than in the populations from the Bohemian Forest and the Alps. We have not found any specific RAPD markers for morphologically intermediate populations (i.e. Western Carpathians and Sudeten).

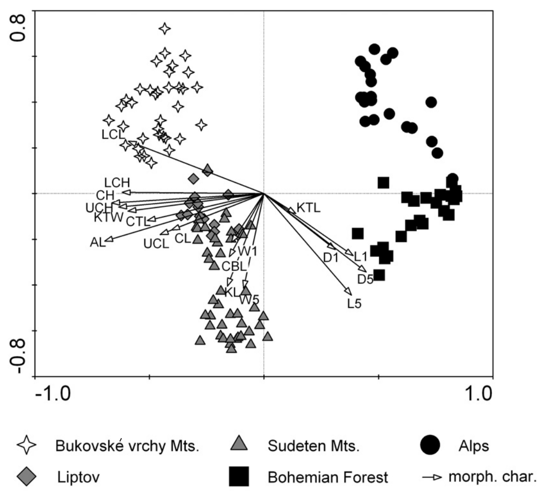

Fig. 6. PCoA ordination diagram of plants (for which both types of data were available) based on both RAPD markers and morphological characters. Directions of changes of these characters are projected. For explanation of abbreviations of morphological characters see Fig. 2.

Genetic structure of populations

The within-population variation is substantially smaller for RAPD data (47.6% of the total variation, based on calculated variance components for db-RDA) than for morphological characters (68.5% of the total variation). This difference between morphological and RAPD data can also be seen by comparing the results of two PCoA ordinations in Fig. 3 and Fig. 4. The two first axes explain 39.5% of total variation of the RAPD dataset and 33.7% of the morphological one. As seen on the dendrogram of cluster analysis of RAPD data (Fig. 5), particularly the intra-population genetic variation in the Alps and Bohemian Forest is very low. Plants from each particular population constitute a discrete cluster in the cluster analysis, based on the similarity matrix calculated from RAPD markers (Gower distance). Only plants from two geographically very close populations from the Jeseníky Mountains are partly mixed (Fig. 5). It seems that the populations of the *Melampyrum sylvaticum* group from different regions of Central Europe are separable by RAPD markers into two geographically restricted groups. Plants from the Alps and from the Bohemian Forest constitute one group (western group) in the cluster analysis results and the second group (eastern) includes all molecular studied populations from the Carpathians and the Sudeten. However, plants from the Sudeten Mountains and the Liptov constitute a cluster separated from another, representing populations of the Bukovské vrchy Mountains, which are often considered to be a part of the Eastern Carpathians. The PCoA of RAPD markers gives a similar result. The first axis separates the plants from the Alps and Bohemian Forest from the plants of the Carpathians and Sudeten Mountains. The distribution along the second axis indicates a larger genetic variation in the eastern group of populations.

The variation pattern of morphological characters is obviously more complex and the cluster analysis does not separate any clearly determinate group. The groups of plants can also not be readily distinguished in the results of PCoA based just on the morphological characters. However, the ordination of plants along the first PCoA axis suggests that the most conspicuous variation trends exist between plants from the western and the eastern part of Central European area of distribution of the *Melampyrum sylvaticum* group (Fig. 4). It is probable that the general trend of morphological variation, which is concordant with the genetic variation pattern, is mixed with other influences (e.g. ecological plasticity, influence of seasonal variation, etc.).

Table 1. Percentage of explained variation in db-RDA. The classification into western and eastern group was used as the explanatory variable. The plant scores from PCoA based on the molecular, morphological or combined data set were used as response variables. Only plants from the Alps, Bohemian Forest and Bukovské vrchy Mts. were included.

Analysis	Variation explained by canonical axis (%)	*F* statistic	*P* value
RAPD markers	39.7	51.429	0.001
Morphological characters	34.1	40.344	0.001
Combined	41.7	55.698	0.001

Table 2. Marginal explanatory effects of individual morphological characters in the discriminant analysis. λ_1 – the eigenvalue of the first discriminant axis; *F* – pseudo-F statistic measuring relative amount of variation explained by the canonical axis (see TER BRAAK & ŠMILAUER 1998, eq. 3.12, p. 51); *P* – type I error estimate based on a Monte Carlo permutation test (TER BRAAK & ŠMILAUER 1998: 44).

Variable	λ_1	*F*	*P*
L1	0.55	261.94	0.001
L5	0.49	206.47	0.001
LCL	0.49	205.02	0.001
LCH	0.46	179.88	0.001
D1	0.39	133.28	0.001
D5	0.36	119.99	0.001
CH	0.35	115.62	0.001
AL	0.34	108.72	0.001
UCH	0.24	67.82	0.001
UCL	0.23	63.12	0.001
CTL	0.19	50.92	0.001
KTL	0.19	49.29	0.001
CBL	0.13	31.14	0.001
CL	0.11	24.91	0.001
KL	0.05	12.13	0.001
W5	0.02	3.91	0.042
KTW	0.01	1.90	0.163
W1	0.00	0.06	0.804

The combined analysis of the RAPD markers and morphological data set confirms the separation of plants from the Alps and Bohemian Forest apart from plants from the Carpathians and Sudeten Mountains. The ordination diagram of PCoA based on the combined data set (Fig. 6) shows two basic groups.

Differentiation among population groups

The db-RDA method was used to compare the discriminatory power of the morphological and RAPD dataset, when discriminating between the two recognized groups. The scores from the PCoA based on the molecular, morphological and combined data set were used as the characteristics of plants. The first 10 PCoA axes derived from each dataset were used. Only plants from the Alps, Bohemian Forest and Bukovské vrchy Mts. were included in these analyses, as the populations from the Sudeten Mountains and the Western Carpathians were demonstrated as intermediate in the previous analyses. The comparison of the percentage of variation explained by the canonical axes in particular analysis is presented with the other statistics in Table 1.

The variation of RAPD markers is explained by the classification into western or eastern group of populations somewhat better than the variation of morphological characters. It is important that the highest percentage of variation is explained in the analysis based on the combined dataset. The partial db-RDA, in which scores on the first 10 axes of PCoA based on the RAPD markers were used as covariates enables us to identify the unique contribution of the morphological characters to the discrimination between the two population groups, absent in the RAPD marker data. This contribution is small (only 1.4 % of the total variation), but

Table 3. Conditional effects from a stepwise selection and canonical coefficients for standardized variables in the LDA. $\lambda \mid A$ – the eigenvalue representing conditional explanatory power that the variable has after the variation explained by the variables preceding this one was already accounted for; *F* – pseudo-F statistic used in the Monte Carlo permutation test of the significance of conditional effect of the particular variable; *P* – type I error estimate based on a Monte Carlo permutation test and refering to a null hypothesis of no conditional effect of the considered variable; Canonical coefficients – canonical coefficient of the variables selected into the final model of the discriminant analysis. Value of the latter coefficient represents strength and direction of differences between the two groups of plants with respect to the particular variable.

Variable	$\lambda \mid A$	*P*	*F*	Canonical coefficients
L1	0.55	0.001	261.94	0.9278
LCL	0.18	0.001	132.58	-1.0216
W1	0.04	0.001	35.01	-0.3320
L5	0.02	0.001	28.26	0.3792
CL	0.02	0.001	12.04	0.6435
KTL	0.01	0.001	12.96	0.5152
LCH	0.01	0.002	10.32	-0.4615
AL	0.00	0.003	10.44	-0.3865
KTW	0.01	0.029	4.68	-0.2680
KL	0.00	0.020	5.45	0.2111
CBL	0.00	0.124	2.38	
W5	0.00	0.339	0.91	

statistically significant (*F* = 2.47, Monte Carlo permutation test *P* = 0.018). The visualization of this analysis says that the anther length and lower lip of corolla length contained the largest independent information for the determination of plants from the Bukovské vrchy Mts. and the distance of the widest part of bracts from their bases are correlated with the plants from the Alps and Bohemian Forest.

Classification of populations

Important differences in morphological characters were investigated using the LDA. All plants with available morphological data from the Alps, Bohemian Forest, Bukovské vrchy Mts. and Ukrainian Carpathians were included in this analysis. The marginal effects, which document the single contribution of characters to the separation of both groups, regardless of correlation with the others characters, are presented in Table 2.

The most important discriminatory characters are evident from the table of conditional effects, which accounts for the correlation between characters (Table 3) and trends in the character values are evident from the sign of their canonical coefficients.

The larger length of the bracts and of the upper calyx tooth seems to be diagnostic for the western group of populations. The significance of the whole corolla length is surprising and can be the result of sampling design. The long and wide ("high" – LCH) lower lips of corolla and long anther are typical for plants from the Bukovské vrchy Mts. and Ukrainian Carpathians. It also seems, that these plants tend to have shorter and broader teeth of calyx.

The LDA was calculated for all available plants, even those not used in the previous analyses, including plants of *Melampyrum saxosum* from the Ukrainian Carpathians. The ten best predictors (L1, LCL, W1, L5, CL, KTL, LCH, AL, KTW, KL – for details see Fig. 2) were used for the calculation of the discriminate function. The position of plants on the discriminant axis is visualized in Fig. 7. Plants were separated into four groups (*M. sylvaticum* s.str., *M. herbichii*, *M. saxosum* and the intermediate populations; see also Discussion for further explanation). The very good difference between plants from the Bukovské vrchy Mountains and Ukraine and plants from the Alps and Bohemian Forest is evident. Within the latter group, there are no differences between studied regions. Very notable is the position of plants of *Melampyrum saxosum*, which practically coincides with the position of plants from

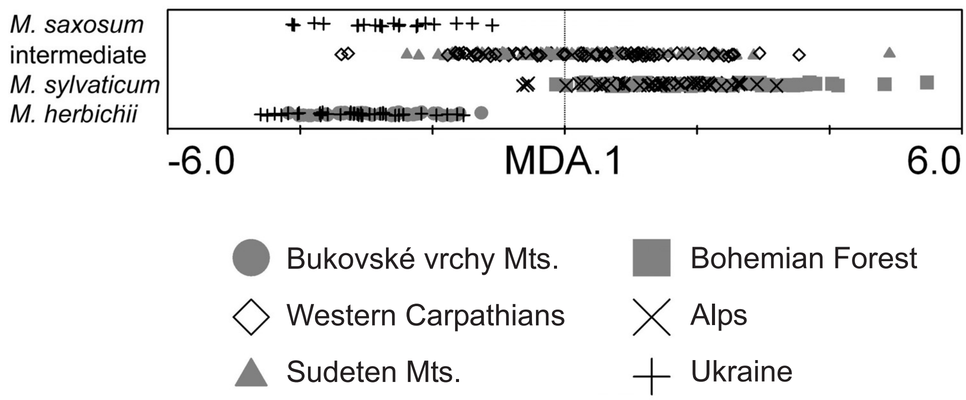

Fig. 7. LDA. Canonical scores of individual plants on the discriminant axis. Plants of "pure" type of *Melampyrum sylvaticum* (Alps, Bohemian Forest) and "pure" type of *M. herbichii* (Bukovské vrchy Mts., Ukrainian Carpathians) were used as the calibration set.

the Bukovské vrchy Mts. and the Ukrainian Carpathians. Plants from the Sudeten Mountains and Western Carpathians are very polymorphic and overlap with positions of Alps and Bohemian Forest plants as well as the plants from Ukraine and Bukovské vrchy Mts.

The intermediate state of morphological characters in populations from Sudeten Mountains and Western Carpathians is obvious from Table 4, which lists for individual populations the "probability" (on percentage scale) of membership in the two classes.

DISCUSSION

Morphological variation and distribution of *Melampyrum sylvaticum* group

The existing knowledge about relation of morphological properties with the distribution of microspecies of the *Melampyrum sylvaticum* s.str. is not very accurate. The discovered patterns of morphological and molecular variation can be used for important taxonomic and phytogeographic conclusions. *Melampyrum sylvaticum* is obviously the most widespread type of the group. The white flowering *M. saxosum* described from Transylvania was sporadically indicated from the regions other than the Eastern and Southern Carpathians (e.g. MEUSEL et al. 1978, HARTL 1974). All records outside the Eastern Carpathian region are probably misidentifications. In the Czech Republic some reports originate from the Krušné hory Mts., Krkonoše Mts., Mt. Králický Sněžník, and Jeseníky Mts. All these records are partly confusions with *Melampyrum pratense* L. but mostly nomenclatural confusions (with *Melampyrum sylvaticum* subsp. *dentatum* ČELAK. and *M. saxosum* – cf. ČELAKOVSKÝ 1871, ŠTECH 2000a). *M. herbichii* was initially regarded as the endemic species of the Eastern and Southern Carpathians too. Firstly, JASIEWICZ (1958) published this taxon from the Western Carpathians and the Sudeten Mountains. On the other hand, HARTL (1974) did not mention this taxon at all. The treatment of genus *Melampyrum* in the Flora of Czech Republic (ŠTECH 2000a) included *M. herbichii* based on the preliminary analyses of morphological data presented also here.

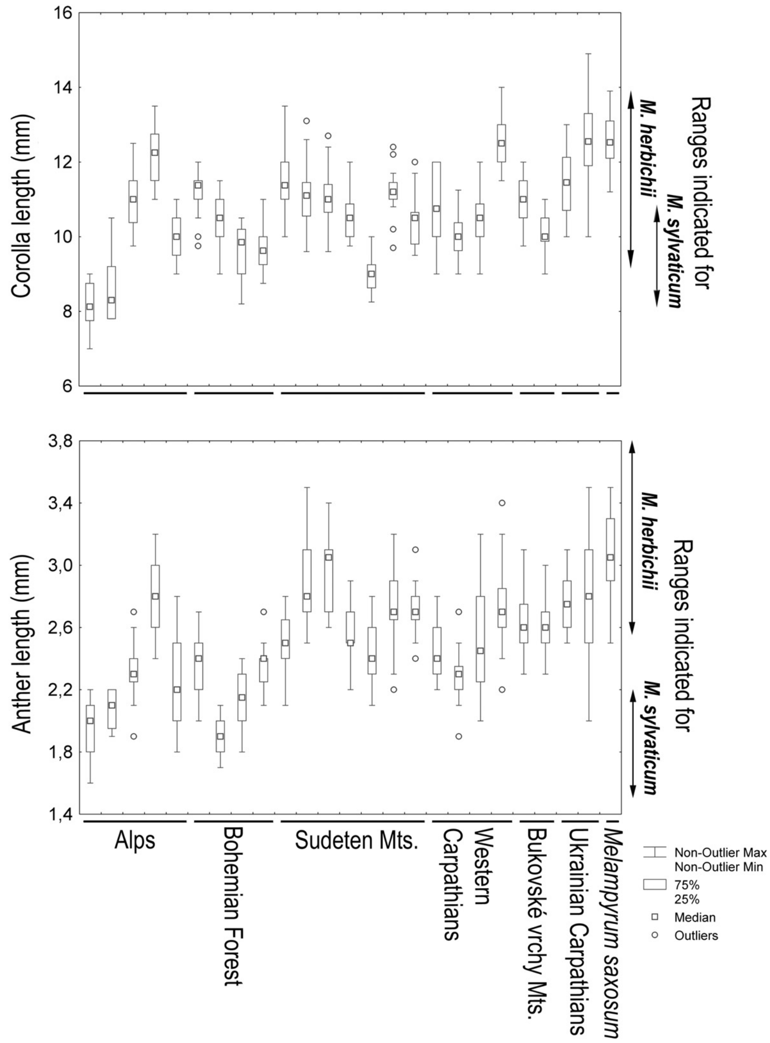

Fig. 8. Comparison of character values of the studied populations with the published ranges (SOÓ & WEBB 1972).

The corolla length and anther length are usually indicated to be different between *M. sylvaticum* s.str. and *M. herbichii*. The indicated ranges vary according to various authors (Table 5).

According to these published diagnostic characters, the majority of the studied populations would be assigned to the *M. herbichii* or they would be seen as intermediate (Fig. 8). But the occurrence of *M. herbichii* in the Alps and in the Bohemian Forest is not very probable. Hence, the elementary application of these morphological characters is not possible. The variation in flower size is more complex. It is very probable that some other factors, such as seasonal type of a population influence flower size. The surrounding plant communities, host

Table 4. Cross-validated estimate of class membership for individual populations, based on LDA.

Region	Locality number (see Appendix)	Class W (*M. sylvaticum*)	Class E (*M. herbichii*)	Number of classified plants
Alps	1	100%	0%	6
Alps	5	100%	0%	13
Alps	3	100%	0%	4
Alps	4	87%	13%	15
Alps	2	100%	0%	20
Bohemian Forest	7	100%	0%	14
Bohemian Forest	8	100%	0%	7
Bohemian Forest	9	100%	0%	20
Bohemian Forest	6	100%	0%	20
Eastern Carpathians, Bukovské vrchy Mts.	21	0%	100%	20
Eastern Carpathians, Bukovské vrchy Mts.	22	0%	100%	20
Ukrainian Carpathians	24	0%	100%	20
Ukrainian Carpathians	23	0%	100%	19
Sudeten Mts., Krkonoše Mts.	10	75%	25%	20
Sudeten Mts., Rychlebské hory Mts.	13	44%	56%	18
Sudeten Mts., Rychlebské hory Mts.	14	94%	6%	16
Sudeten Mts., Orlické hory Mts.	12	91%	9%	33
Sudeten Mts., Orlické hory Mts.	11	71%	29%	7
Sudeten Mts., Jeseníky Mts.	16	80%	20%	10
Sudeten Mts., Jeseníky Mts.	15	80%	20%	20
Western Carpathians, Chočské vrchy Mts.	17	93%	7%	15
Western Carpathians., Poľana Mts.	18	100%	0%	20
Western Carpathians, Liptov	20	50%	50%	20
Western Carpathians, Nízke Tatry Mts.	19	40%	60%	20
Ukrainian Carpathians – *M. saxosum*	25	0%	100%	20
Grand Total		64%	36%	417

plant and other ecological factors, such as altitude, also have an important influence. However, the tendency of plants from the Carpathians and Sudeten Mountains to have larger flowers (especially the dimensions of corolla lips and anther) is still obvious. Plants from the western part of Central European distribution area of *Melampyrum sylvaticum* group can be labelled as the *Melampyrum sylvaticum* s.str. According to our results, the plants from the Bukovské vrchy Mountains and Ukrainian Carpathians should be classified as *Melampyrum herbichii*. The populations from the Western Carpathians and Sudeten Mountains are in many morphological characters intermediate between the above two types. The plants from the eastern group of *Melampyrum sylvaticum* group populations usually have shorter and wider bracts. The tooth presence at bract sides was not included in the analyses, but it was observed. The plants from the eastern group of populations have very often one or two teeth on each side of the lower bract. ČELAKOVSKÝ (1871) described the *M. sylvaticum* var. *dentatum* based on this character from the Sudeten Mountains (Krkonoše Mts. and Mt. Králický Sněžník). This character, however, should also be typical for *M. sylvaticum* subsp. *laricetorum* (A. KERN.) RONNIGER from the Alps and can be a response to the alpine conditions above the timberline.

Table 5. Comparison of differences between taxa of the *Melampyrum sylvaticum* group indicated by SOÓ & WEBB (1972), HARTL (1974) and JASIEWICZ (1958).

Taxon	Authors			
	SOÓ & WEBB	HARTL	JASIEWICZ	
	range	range	range	average
Corolla length (mm)				
M. sylvaticum	(6–)8–10(–11)	(5–)8–10	(5–)7–9.5(–11)	8.3
M. herbichii	(7–)9–11(–14)	-	-	-
M. herbichii (Tatry Mts.)	-	-	(7–)8–11.5(–13)	9.5
M. herbichii (Eastern Carp.)	-	-	(8–)9–12.5(–14)	10.6
M. saxosum	8–13	(9–)10–12(–13)	(7.5–)9–12.5(–14)	11.0
Anther length (mm)				
M. sylvaticum	1.5–2.2	1–1.5	(1.4–)1.6–2.1(2.3)	1.84
M. herbichii	2.5–4	-	-	-
M. herbichii (Tatry Mts.)	-	-	(2.0–)2.4–3.2(–3.5)	2.68
M. herbichii (Eastern Carp.)	-	-	(2.0–)2.5–3.6(–4.2)	3.03
M. saxosum	2.5–4	2.5	(2.2–)2.8–3.6(–4.2)	3.24

Distribution of genetic variation

The relative genetic similarity of the plants from the Alps and Bohemian Forest among the greater variation range in region of Carpathians and Sudeten Mts. suggest some ideas about the potential origin of this variation pattern. The intra-population differentiation is generally very low (Fig. 5). The highest variation within populations was revealed in the Carpathians (see also Fig. 5). This may indicate a possibility of influence of genes from Eastern Carpathian plants to populations of *M. sylvaticum* s.str., which were more widespread in Central Europe in the past. Although the hybridization is not confirmed in the genus *Melampyrum* (ŠTECH 2005), there are some indications of such possibility in the other groups of the genus. The similarity of morphological characters and of ecological requirements (mainly the alpine and subalpine plant communities) between *M. herbichii* and *M. saxosum* suggests the possibility of the origin of *Melampyrum herbichii* by hybridization of *Melampyrum saxosum* and *M. sylvaticum* s.str. A possibility of hybrid origin of *M. herbichii* was mentioned already by JASIEWICZ (1958). This hypothesis can be tested in future by appropriate molecular methods.

All these questions concerning morphological variability and genetic pattern at intra- and inter-population levels of *Melampyrum sylvaticum* group must in the future be investigated in detail in the entire distribution area of the studied group. A lot of infraspecific taxa still cannot be interpreted. For instance, the relation between *M. herbichii* and *M. carpaticum*, which is often seen as a seasonal type of *M. sylvaticum* s.str., is unclear.

CONCLUSIONS

(1) The genetic intra-population differentiation in the studied taxa is very low.

(2) Morphological variation is complex. The principal geographical trend seems to be concordant with the genetic pattern, but other factors probably distort this trend.

(3) Two groups of populations of the *Melampyrum sylvaticum* group with yellow corolla can be distinguished in the Central Europe. The western group, widespread in the Alps and Hercynian Mountains (except the Sudeten), belongs to *M. sylvaticum* s.str. The studied populations from the Bukovské vrchy Mountains and Ukrainian Carpathians can be clearly classified as *M. herbichii*. Populations from the Western Carpathians and Sudeten Mountains are more polymorphic on both intra- and inter-population levels. All studied populations from this region seem to be closer to *M. herbichii* based on the RAPD markers. However, most populations are more similar to the *M. sylvaticum* s.str. from the morphological point of view. The white flowering *Melampyrum saxosum* is notably similar to *M. herbichii* in the studied morphological characters.

(4) This pattern of variation could imply old hybridization, introgression and migration on the Carpathian and Sudeten ridge in the westward direction. Further detailed genetic study of the group in its entire distribution could address these issues and help to clarify taxonomic status of *Melampyrum herbichii* and of other taxa.

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APPENDIX

List of localities of population samples

***Melampyrum sylvaticum* (incl. *M. herbichii*)**

The Alps: **Switzerland**

(1) Wallis, Zermatt: forest in the north-eastern foothills of the Matterhorn massif SW of the village, 46°01′00″ N, 7°45′00″ E, 1900 m a.s.l., 25.7.1996 (Alps 2 in figures).

The Alps: **Austria**

(2) Upper Austria, Sankt Agatha: spruce forest around the path ca. 2 km SW of the village, N 47°36′26″ E 13°38′00″, 550 m a.s.l., 21.7.1997.
(3) Upper Austria, Ober Traun: path margin in the north-eastern slope of the Dachstein massif ca. 2.5 km (S)SW of the lower railway station at the south-eastern margin of the Dachteinhof settlement, N 47°31′52″ E 13°41′05″, 1600 m a.s.l., 20.8.1995.
(4) Lower Austria, Lackenhof: forest edge near road at the east-south-eastern foothills of the Scheblingstein mountain ca. 4.5 km SSW of the village, N 47°50′05″ E 15°07′37″, 700 m a.s.l., 13.8.1997.
(5) Lower Austria, Semmering: forest edge in the north-eastern hillside of the Hirschen Kugel mountain ca. 800 m NNW of the summit, N 47°38′49″ E 15°49′48″, 1100 m a.s.l., 23.6.1996 (Alps 1).

Bohemian Forest: Czech Republic

(6) SW Bohemia, Bohemian Forest, Srní: heath eastwards of the road at the southern margin of the village, N 49°04′47″ E 13°29′05″, 850 m a.s.l., 5.7.1996.
(7) SW Bohemia, Bohemian Forest, Javorník: meadow edge ca. 1 km SSW of the village, N 49°07′52″ E 13°39′22″, 900 m a.s.l., 30.7.1996 (Bohemian Forest 1).
(8) SW Bohemia, Bohemian Forest, Vimperk: spruce forest southwards of settlement Kamenná hora ca. 5 km W of the town, N 49°03′33″ E 13°42′37″, 950 m a.s.l., 5.8.1995 (Bohemian Forest 2).
(9) SW Bohemia, Bohemian Forest, Nová Pec: birch scrubs along road ca. 1 km NNW of railway station Nová Pec, N 48°47′43″ E 13°56′37″, 730 m a.s.l., 29.6.1997.

Sudeten Mountains: Czech Republic

(10) NE Bohemia, Krkonoše Mts., Pec pod Sněžkou: heath south-west of the bridge across the Úpa River ca. 1.5 km NNW of the central crossroads in the village, N 50°42′28″ E 15°43′46″, 860 m a.s.l., 1.8.1997.
(11) NE Bohemia, Orlické hory Mts., Sedloňov: roadside ca. 2.5 km E(S)E of the church in the village, N 50°20′01″ E 16°20′50″, 870 m a.s.l., 9.7.1995 (SM1).
(12) NE Bohemia, Orlické hory Mts., Deštné v Orlických horách: forest in the southern slope ca. 1.6 km E(S)E of the church in the village, N 50°18′02″ E 16°22′09″, 750 m a.s.l., 9.7.1995.
(13) N Moravia, Rychlebské hory Mts., Velké Vrbno: heath around the path ca. 1.25 km SSW of the Paprsek Mountains Hotel, N 50°12′14″ E 16°58′42″, 920 m a.s.l., 31.7.1996 (SM2).
(14) N Moravia, Rychlebské hory Mts., Staré Město pod Sněžníkem: spruce forest near road ca. 1.75 km NN(E) of the church in the town, N 50°10′36″ E 16°57′10″, 580 m a.s.l., 30.7.1997.
(15) N Moravia, Jeseníky Mts., Karlov: scrubs in the lower part of the Velká Kotlina cirque ca. 3 km SS(E) of the Praděd mountain, N 50°03′23″ E 17°14′14″, 1150 m a.s.l., 11.7.1995 (SM3).
(16) N Moravia, Jeseníky Mts., Karlov: spruce forest ca. 1 km SS(W) of the Kopřivná hill NW of the village, N 50°01′35″ E 17°16′25″, 700 m a.s.l., 11.7.1995 (SM4).

Western Carpathians: Slovakia

(17) Central Slovakia, Chočské vrchy Mts., Valašská Dubová: spruce forest around the path ca. 2.25 km ENE of the church in the village, N 50°01′35″ E 17°16′25″, 800 m a.s.l., 14.7.1997.

(18) Central Slovakia, Poľana Mts., Detva: summit region of Mt. Poľana, N 48°38′00″ E 19°29′00″, 1300 m a.s.l., 7.7.1997 (leg. J. CHRTEK et Z. KAPLAN).

(19) Central Slovakia, Nízke Tatry Mts., Čertovica: alpine meadows around the path in the Kumštové sedlo saddle ca. 3.5 km NW of the chalet in the Čertovica saddle, N 48°55′00″ E 19°42′00″, 1540 m a.s.l., 6.7.1997.

(20) Central Slovakia, Liptovská kotlina valley, Važec: barrens south-west of the village ca. 400 m WNW of the Važecká jaskyňa cave, N 49°03′20″ E 19°57′40″, 810 m a.s.l., 15.6.1996 (Liptov).

Eastern Carpathians: Slovakia

(21) E Slovakia, Bukovské vrchy Mts., Runina: alpine meadows at the Pľaša hill ca. 4.5 km N of the village, N 49°06′40″ E 22°24′20″, 1150 m a.s.l., 18.6.1996 (Bukovské vrchy Mts. 1).

(22) E Slovakia, Bukovské vrchy Mts., Runina: alpine meadows in the saddle westwards of the Riaba skala hill ca. 4 km N(NE) of the village, N 49°06′10″ E 22°26′20″, 1160 m a.s.l., 17.6.1996 (Bukovské vrchy Mts. 2).

Eastern Carpathians: Ukraine

(23) Ukrainian Carpathians, Svydovets Mts., alpine pastures at north-eastern slopes of the Mount Blyznitsa ca. 1.75 km N of the Blyznitsa summit, N 48°14′25″ E 24°14′24″, 1410 m a.s.l., 17.6.2003.

(24) Ukrainian Carpathians, Chernogora Mts.: alpine pastures between Mount Hoverla and Mount Pietros ca. 2.75 km W of the Hoverla summit, N 48°09′37″ E 24°27′50″,1580 m a.s.l., 11.7.2003.

Melampyrum saxosum

Eastern Carpathians: Ukraine

(25) Ukrainian Carpathians, Chivchin Mts., Burkut: alpine meadows at Mt. Chivchin, ca. 0.5 km northwards of the summit, ca. 8.5 km S of the village, N 47°52′09″ E 24°42′38″, 1640 m a.s.l., 9.7.2003.