



# Isozyme analysis and DNA contents estimation of *Melampyrum subalpinum* agg. and *M. nemorosum* populations - preliminary results



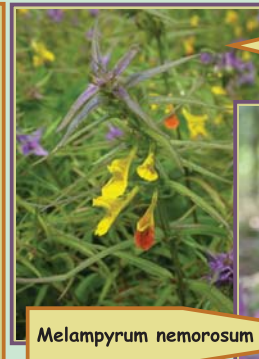
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## Introduction

*Melampyrum subalpinum* group shows the interesting variation pattern in the Central Europe. Morphological variation of populations decreases from the Wienerwald highland towards the Upper Austria/Styria Alps but is still markedly higher there than the variation in the Czech Republic and Slovakia. Efforts to describe this variation pattern formally brought numerous (and often rather confusing) names of taxa. Among them, *M. subalpinum* (Jur.) A. Kern. is today adopted for a conspicuously polymorphic type, which occurs in different (mainly limestone) habitats and is considered to be endemic in the northeastern border of the Alps in Austria (Fischer, Adler, Oswald 2005). On the contrary, *M. bohemicum* A. Kern. is morphologically uniform and occurs mainly on sandy substrates. This type had been considered to be endemic in the Czech Republic and Slovakia (Hadač 1966a, b). Recent morphological studies (Reiner 1994, Štech 2006) described morphological variation of the group in the Central Europe and have formulated a hypothesis about the origin of observed morphological variation pattern. Old hybridization between *M. subalpinum* s. str. and *M. nemorosum* L. has been proposed as an important source of morphological diversity in the Wienerwald region. Another source of variation may be the diversity of habitats and host plants in/on *M. subalpinum* s. str. grows in this region. *M. bohemicum* has been suggested a marginal range of morphological variability of *M. subalpinum* s. str.

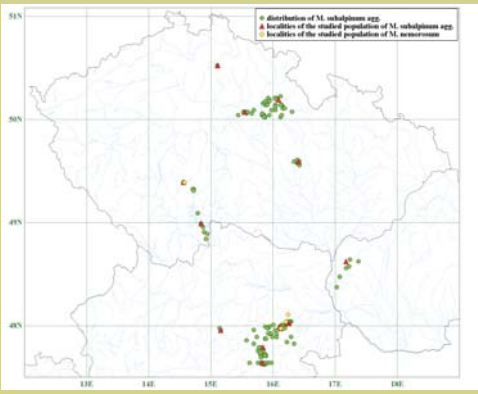


*Melampyrum nemorosum*

*Melampyrum subalpinum* with narrow leaves



## Distribution area of *Melampyrum subalpinum* agg. and position of localities included in the recent study



## Methods

### » Sampling

Populations from different parts of the Austrian distribution area of studied group were chosen for genetic study. Four populations of plants with narrow leaves (1 - Semmering, 2 - Losenheim, 3 - Peutenburg, 4 - Merkenstein) and three populations of plants with broad leaves (5 - Hofstätten, 6 - Einöde, 7 - Gumpoldskirchen) were chosen. Seven populations were sampled from all particular areas in the Czech Republic (8 - Majdalena, 9 - Příběnice, 10 - Kněževs, 11 - Týniště n. O., 12 - Sopčech, 13 - Žehrov) and Slovakia (14 - Šaštín-Stráže). Four populations of *M. nemorosum* especially from Wienerwald region were also sampled (15 - Heidelhof, 16 - Rotes Kreuz, 17 - Gießhübel, 18 - Příběnice).

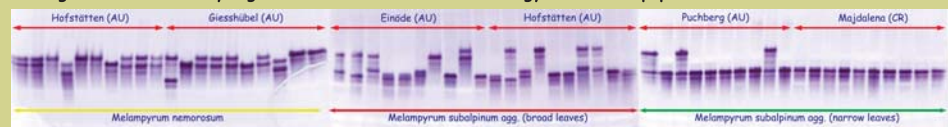
### » DNA content analysis

Absolute DNA content was estimated by FCM. Standard procedure of the absolute DNA content estimation was carried out. Three plants from one population were analysed in three days subsequently. One population from each particular morphological distinct types were included in this analysis. Three populations of "*M. bohemicum*" were analysed because of detected variation. Relative DNA content were analysed in all studied population. Ten plants were collected for this analysis.

### » Isozyme analysis

Isozyme analysis was carried out on ten plants from each population. Four enzymatic systems were tested: Shikimate dehydrogenase (SKDH); leucine aminopeptidase (LAP); aspartate aminotransferase (AAT); alcohol dehydrogenase (ADH).

## Representative SKDH isozyme banding patterns of five Austrian populations and one Czech population. Three groups can be distinguished from the zymogram as well as from the morphology of studied populations.



*Melampyrum subalpinum* agg. (A plant with narrow leaves from CR here known as *Melampyrum bohemicum*)

## Results

» Though all species in the genus *Melampyrum* have eighteen chromosomes, surprisingly high differences in absolute DNA content were found.

- Compared with *M. subalpinum*, *M. nemorosum* has significantly lower DNA content. The difference between *M. subalpinum* with narrow leaves and *M. nemorosum* is about 13%.
- Small (ca 2%), but statistically significant difference occurred also in broad leaved *M. subalpinum* populations, compared to the narrow leaved populations.
- Czech and Austrian populations with narrow leaves showed to have a comparable content of DNA.
- Surprisingly the studied population from Slovakia significantly differs (ca 4%) from all other studied populations.

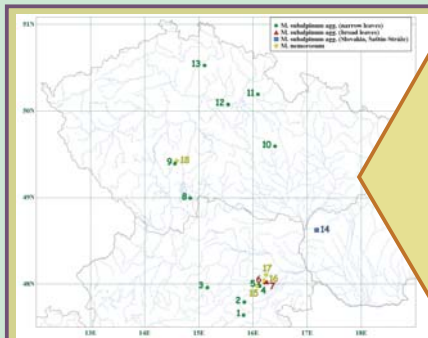
» Till now isozyme analysis was accomplished only on six populations. Preliminary results imply, that there are three groups:

- 1) Czech and Austrian narrow leaved populations appear to be close.
- 2) *M. nemorosum* populations are markedly different from the rest of the populations.
- 3) Austrian populations with broad leaves are rather variable.

In this moment number of analysed populations is insufficient for definite results. Currently other populations from Austria and Czech and Slovak Republic are being analysed.

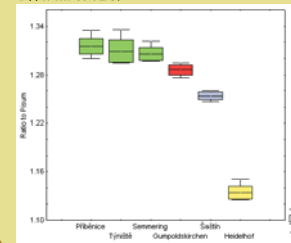
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## DNA content graph

DNA content (ratio to *Pisum*) in selected populations of *M. subalpinum* agg. and *M. nemorosum*. Four significantly different groups are shown by different colours.



## Conclusions

Present studies revealed surprising variation in DNA content among studied groups. The center of the morphological diversity of *M. subalpinum* in the Wienerwald seems to be also the center of the genetic diversity. Preliminary results do not contrast with the hypothesis about identity of Czech populations with majority of Austrian populations. However there is not enough data available for conclusions about potential hybridization between *M. subalpinum* group and *M. nemorosum*. DNA content ratios among parental and potential hybridogenous populations do not support this hypotheses clearly.

Further studies will be aimed on other methods of DNA study (e.g. PCR-RFLP). Causes of differences in the DNA amount between Slovakian population (not different morphologically) and all others will be studied. Studied group will be extended by morphologically similar plants from the Balkan Peninsula.

*Melampyrum subalpinum* agg. with broad leaves



## Acknowledgements

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