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**Dispersal, distribution and genetic diversity of
Melampyrum subalpinum group**

Ph.D. Thesis

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Annotation

This thesis is focused on the dispersal, distribution, and genetic diversity of the taxonomically highly diverse *Melampyrum subalpinum* group. A complete revision of the localities of *M. subalpinum* in the Czech Republic and Slovakia is presented. The genetic variation and population structure of the *M. subalpinum* group across its distribution range is described based on allozymes, nuclear and chloroplast gene sequencing, and genome size. Signs of historical hybridization with *M. nemorosum* were found in some populations. A comparison of seed dispersal by ants between the co-occurring *M. subalpinum* and *M. pratense* and the influence of differences in this process are presented. A new mean of seed dispersal (endozoochory) is introduced for *Melampyrum*. Myrmecochorous dispersal distances are tested and Holocene migration possibilities are discussed taking into account endozoochory.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejněpřístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejich internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Jan Chlumský

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To my parents and grandma

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List of papers and authors contribution

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Jan Chlumský initiated the study, was responsible for the design of experiments, collected a major part of the data in the field and wrote the manuscript.

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Jan Chlumský participated in the specimen collection, tested for applicable enzyme systems, performed major part of allozyme analyses, participated in zymograms interpretation and statistical analyses and wrote the manuscript.

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Peer-reviewed bulletin of the Czech Botanical Society without IF.

*Jan Chlumský revised in field all recently known and historically reported localities of *M. subalpinum* (*M. bohemicum*) in CZ and SK, collected data about the populations including phytosociological relevés and wrote the manuscript.*

Štech, M, **Chlumský, J**, Herbstová, M, Košnar, Jan, Košnar, Jiří, Koutecký, P, Fér, T, Trávníček, P and Suda, J (2016): Disentangling the evolutionary history of *Melampyrum subalpinum* agg. (Orobanchaceae) in Central Europe. *Manuscript*.

Jan Chlumský participated in the field sampling, laboratory work (pre-sequencing purification, flow-cytometry) and the revision of the manuscript. This participation is hereby confirmed by the signature of the first author:

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Ing. Milan Štech Ph.D.

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Chapter I

General introduction

Photo on previous page (M. Štech): *Melampyrum subalpinum*,
Losenheim, Austria

General introduction

Knowing the genetic variation, present distribution, reproduction strategy, and means of dispersal of plants can tell us a lot about their history and consequently about the history of the area they occur in.

The present distribution of plants in the northern hemisphere was substantially shaped by climatic oscillations during the Pleistocene and mainly during the last glacial maximum. Plants were forced to migrate south and to lower altitudes to find optimal conditions. The impact of these oscillations on plant diversity was more significant in the areas with east to west oriented mountain ranges (Europe), which represent considerable barriers for plant migration, compared to areas with north to south oriented ranges (North America, eastern Asia) where plants can migrate more easily. In Europe, these extensive climatic changes caused significant migration, fragmentation, and even the extinction of populations (Comes and Kadereit, 1998; Hewitt, 1999). Taxa with broad distribution were forced to withdraw and survive in geographically restricted, favourable refugia. Early phylogeographic studies focused mainly on trees and their migration within the wider scale of the whole continent (e.g. Sigurgeirsson and Szmidt, 1993; Demesure et al., 1996; Dumolin et al., 1997; King and Ferris, 1998; Sinclair et al., 1999). A similar pattern of Holocene recolonization was found for different taxa and hence three main southern refugia were defined – the Iberian, Apennine, and Balkan peninsulas (Taberlet et al., 1998; Hewitt, 1999).

Based on early works the paradigm has been postulated, that trees were present only in these southern refugia and that the rest of non-glaciated Europe was covered by steppe-tundra. It was unclear how trees and herbs could have recolonized northern latitudes so fast after the last glacial period. This problem was called *Reid's paradox of rapid plant migration* (Clark et al., 1998). The explanation suggested by Clark et al. (1998) presumes that occasional long-distance dispersal events helped such species to expand their distribution range and reach further/more distant, suitable habitats during the recolonization. Although this theory might be partly valid, later palaeoecological works based on the presence of wood fossils at intermediate latitudes and modern molecular phylogeographic studies have repeatedly indicated trees persisting far to the north during the last glacial maximum from the original supposed refugia (Carcaillet and Vernet, 2001; Stewart and

Lister, 2001; Palmé et al., 2003; Lascoux et al., 2004; McLachlan and Clark, 2004; Willis and van Andel, 2004; Magri et al., 2006; Maliouchenko et al., 2007). These small northern microrefugia (Rull, 2009; 2010) were probably pockets of vegetation with higher humidity and unusual microclimatic conditions which allowed the persistence of trees during the last glacial maximum (e.g. parts of river floodplains) and enabled the rapid recolonization of the continent. Tzedakis (2013) questions the microrefugia concept and points out that the fossil, pollen, and genetic evidence for northern tree microrefugia is often equivocal and is not able to locate the exact position of particular microrefugia. Tzedakis (2013) also questions the credibility of Late Pleniglacial refugia in northernmost Europe and suggests that temperate trees didn't extend much further than 45°N and that the boreal trees limit was 46°N in Western Europe and 49°N in Eastern Europe. However according to Rull (2014), even in the case of the eventual absence of glacial microrefugia in northernmost Europe there is still sufficiently robust evidence for tree microrefugia in the rest of the continent outside of the traditional southern macrorefugia.

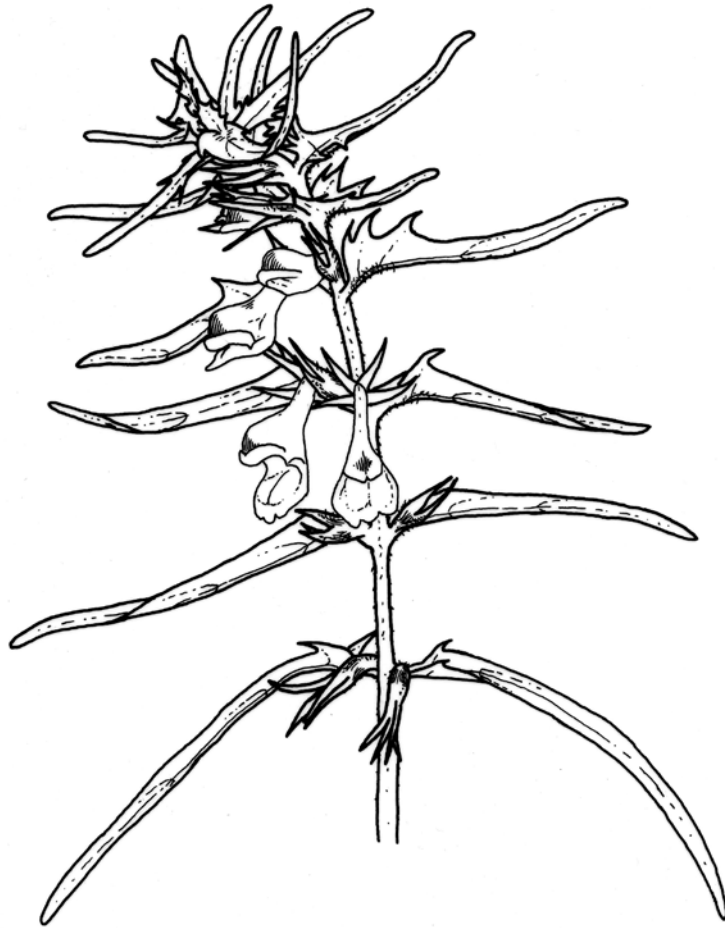
The holocene phylogeography of trees became well explored over time and detailed studies of other groups started to emerge (e.g. Grivet and Petit, 2002; Fér et al., 2007; Daneck et al., 2011), however studies focused on herbs are still rather rare (e.g. Rejzková et al., 2008; Wróblewska, 2008; Pfeiffer et al., 2009). A very interesting group for phylogeographic studies are annual plants, however only a few studies have been conducted so far (e.g. Sharbel et al., 2000; Koch and Bernhardt, 2004; Beck et al., 2008; Vrancken et al., 2009). Their specific life strategy with a fast change of generations may show different levels and structuring of genetic variation. Vrancken et al. (2009) observed an absence of geographic structure in the cpDNA variation of the annual hemiparasite *Rhinanthus angustifolius* (parasiting on herbs). Authors assign this lack of geographic structure either to the possibility that the central haplotypes of the species survived the ice ages in several shared refugia across Europe, and subsequent recolonization took place with the ancestral haplotypes, or the indistinct geographic structuring is due to hybridization with closely related species *R. minor* which blurred the possibly structured original haplotype pattern.

Many plant groups possess very high morphological variability which leads to ambiguous description of numerous taxa with uncertain

relationships. In the case of annual plants, rapid selection, population oscillations with subsequent genetic drift, or hybridization, play an important part in speciation events, which may occasionally result in a gradient of morphological changes ranging from the obvious to the very subtle, inexplicit, and hard to classify. These processes can result in a very fine differentiation of various morphotypes and microspecies. A good example of such groups are annual hemiparasites which are often taxonomically complicated aggregates of several variable microspecies which are difficult to identify (Koutecký et al., 2012; Yeo, 1978).

A very important and sometimes underestimated factor that can influence the phylogeographic pattern of the species is plant dispersal. Plants deal with their immobility by dispersing pollen and seeds which consequently affects their total distribution, reproduction, evolution, population dynamics, and persistence (Primack and Miao, 1992). If we focus on seed dispersal, we can highlight several direct benefits resulting from this important process. Via dispersal, seeds can reach sites favourable for germination and growth and escape from sibling and parent-sibling competition (Howe and Smallwood, 1982; Reichenberger and Pyke, 1990; Wenny, 2001). Seeds dispersed away from other seedlings have a higher probability of survival as they avoid infection with the pathogens and parasites possibly carried by other individuals. Further away there is also a higher chance of avoiding predators bound to the area inhabited by parental plants (Wenny, 2000) and a lower chance of inbreeding with closely related individuals (Keller and Waller, 2002). The means of dispersal of the species can also inform us of its migration rate and consequently about the speed and possibilities of recolonization of suitable habitats after the last glacial period.

An interesting group for studying postglacial migration would be tree parasites. The fact that these plants are dependent on the host tree presence may represent a useful limitation for the gene flow which can result in a more distinctive geographic pattern of Holocene migration. If we focus on the genus *Melampyrum*, another gene-flow limitations that can help trace the migration routes and population relations are entomogamy and the fact that heavy *Melampyrum* seeds are dispersed by ants, which is rather slow and highly limited means of dispersal.



Chapter II

Introduction to the studied group
Melampyrum subalpinum

Picture on previous page: *Melampyrum subalpinum*, the main inflorescence

Introduction to the studied group *Melampyrum subalpinum*

Variation in Melampyrum subalpinum group

The *Melampyrum nemorosum* group is a morphologically well-defined group of hemiparasitic annuals which covers almost the whole of Europe. The delimitation of species inside this group is rather complicated. In the current conception the group consists of 15 species (Soó, 1927; Soó and Webb, 1972) with intraspecific taxa based mainly on seasonal variation, however their real taxonomic status wasn't analyzed sufficiently yet. Among these species *M. subalpinum* is a morphologically well-delimited group containing highly diverse taxa with a specific distribution area restricted to Central Europe (Fig. 1).

An important variation in this group can be found in leaf and bract width, bract coloration, or calyx indumentum. With the exception of taxa from the Balkan peninsula with uncertain relationships to Central-European plants, the center of morphological variation of the group lies in Austria in the Vienna Forest (Wienerwald) on the north-eastern edge of the Alps (Štech, 2006). Austrian populations were originally described as *M. nemorosum* var. *subalpinum* (Juratzka, 1857). However, due to high morphological variability, several other taxa with different delimitation have been described since. Plants from the Czech Republic and Slovakia were described as *M. bohemicum* A. Kern. (Kerner, 1881). One year later a detailed study of the group was published by Beck (1882). Broad-leaved plants were described as *M. subalpinum* s. str. and narrow-leaved plants as *M. angustissimum* (with early-flowering type *M. subalpinum* var. *grandiflorum* (Kerner) Beck). Czech populations were classified as close to *M. angustissimum*. Due to the high variability of broad leaved populations Wiesbaur (1883) described relatively narrow-leaved populations with closed corolla as *M. stenotaton* and assigned them to *M. subalpinum* s. str. as its marginal morphological form. Beck's (1882) concept of *M. subalpinum* s. str. and *M. angustissimum* was accepted by Austrian botanists until recently whereas *M. bohemicum* was considered as a separate taxon. Flora Europaea (Soó and Webb, 1972) distinguishes Austrian and Czech populations as *M. subalpinum* and *M. bohemicum*. However, more recent morphometric studies have not revealed any morphological differences between Czech, Slovak, and Austrian narrow-leaved populations (Štech, 2000; 2006). The problematics of Austrian population variability was studied by Reiner

(1994). Based on morphometrics and seasonal traits he divided *M. subalpinum* into two subspecies. *M. s.* subsp. *grandiflorum* as an early morphotype of narrow-leaved plants which blooms in May and June, and *M. s.* subsp. *subalpinum* as a late morphotype blooming from July to the start of September. The latter morphotype is further divided into two varieties; a narrow-leaved variety, *M. s.* subsp. *subalpinum* var. *subalpinum*, and a wide-leaved variety, *M. s.* subsp. *subalpinum* var. *thermale*, occurring only in the area of the Vienna Forest. This different delimitation of *M. subalpinum* s. str. is caused by an absence of typification of the name *M. nemorosum* var. *subalpinum* Juratzka. In his diploma thesis, Reiner suggested typifying this name by narrow-leaved plant, however this typification has yet to be validly published. Reiner's solution was used in the second and third edition of the Austrian botanical key (Fischer et al., 2005; 2008).

Štech (2006) considers *M. bohemicum* to be an identical taxon to the Austrian narrow-leaved populations traditionally designated as *M. angustissimum*. Wide-leaved populations of *M. subalpinum* s. str. originated according to Štech (2006) from hybridization between narrow-leaved populations and *M. nemorosum*.

The taxonomy of the *M. subalpinum* group has so far been based solely on morphological, ecological, and geographical characteristics. For a disentangling of the relationships between different morphotypes and regions it is necessary to combine morphological characteristics with appropriate molecular markers. Modern molecular methods such as the sequencing of single copy nuclear gene and chloroplast DNA can also shed some light on the assumed hybridization of *M. subalpinum* with *M. nemorosum*, which possibly resulted in the formation of wide-leaved *M. subalpinum* populations in the area of the Vienna Forest.

Distribution range

The distribution of Austrian *M. subalpinum* populations was most recently discussed by Štech (2006) and Reiner (1994). It occurs in an area extending from the Vienna forest to the Upper Styria Alps. An overview of Czech and Slovak localities was published by Hadač (1966), who analysed the environmental conditions in this part of the distribution area. In the Czech Republic, *M. subalpinum* can be found in southern Bohemia in the vicinity of the Lužnice river, in north-eastern Bohemia in the area of the Labe and Orlice floodplains, and in eastern Bohemia in

the Hornosvratecká vrchovina hills. A small northernmost locality discovered by Holub (1996) near Žehrov in Český ráj is considered to be recently introduced according to its isolation and small size. Slovak *M. subalpinum* populations are located in the western part of the country in the sandy area of Záhorie.

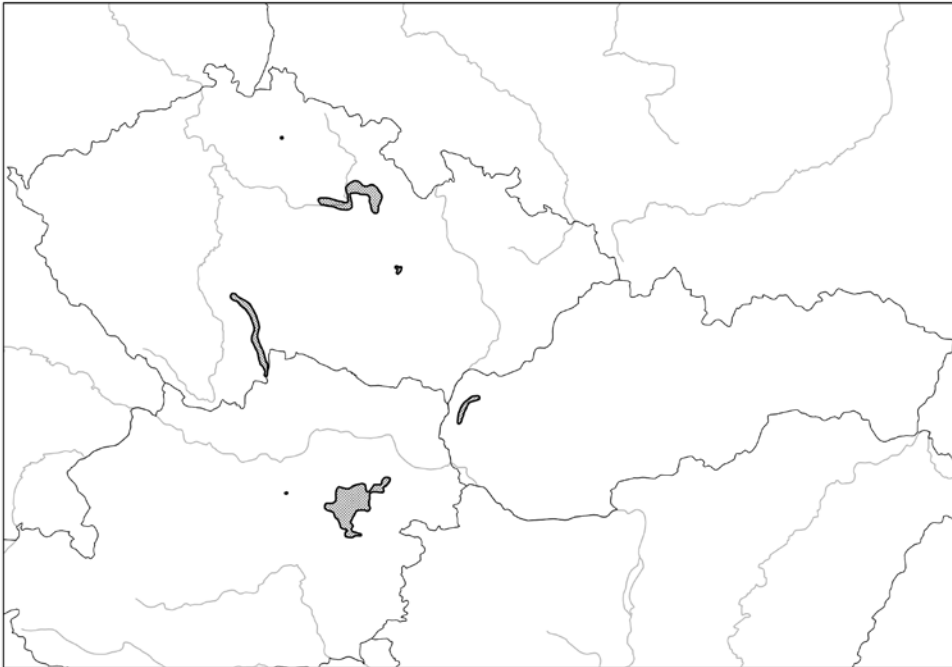


Figure 1. Distribution area of *M. subalpinum* agg.

Holocene history

The discontinuous, patchy distribution of *M. subalpinum* group raises questions about the migration history of the species during and after the last glaciation period. Particularly fragmented are mostly the areas outside of the presumed evolutionary center in the Alps (Štech, 2006). Hadač (1966) speculates that the distribution range of a *M. subalpinum* ancestor during the last glaciation reached from the Austrian Alps to the Záhorie area in Slovakia. He supports this idea with a hypothesis claiming, that during the last ice age pine forests survived in this area and thus it was suitable for the survival of the plant. On the contrary Hadač (1966) claims that concurrently the Czech Republic was covered with tundra and hence *M. subalpinum* was not able to survive there.

Hadač (1966) assumes that in the Preboreal period light birch-pine forests also spread out as far as Central Europe followed by Boreal expansion of pine forests with hazel shrubs to the mountains, which enabled *M. subalpinum* to spread from Slovak localities through Moravia and Eastern Bohemia, and further to recent localities. Current knowledge however assumes that trees were much more widespread during the last glaciation period than previously believed (Wills and van Andel, 2004; Svenning, et al., 2008) and hence *M. subalpinum* could spread to recent marginal localities much earlier and from other directions than solely from Slovak Záhorie. The present distribution of species in the Alps is matches the hypothetical last glacial refugium in the North Eastern Calcareous Alps (Tribsch and Schönswetter, 2003).

Dispersal in Melampyrum

Seed dispersal by ants can be found in at least 11 000 species in 334 genera and in 77 families (Lengyel et al., 2010). It was first described by Sernander (1906). The plant adaptation for myrmecochory is a lipid-rich seed appendage known as an elaiosome whose anatomical origin varies depending on the species. Ants are attracted to this appendage and transport the seed to the nest where they consume the elaiosome or feed it to larvae. Afterwards, the seeds are discarded either in the nest or outside it. This way the seed is transported away from the parental plant, receives protection from predators, and it is often left at a site suitable for germination and growth (Beattie and Hughes, 2002).

The longest observed seed dispersal by ants is 180 m (Whitney, 2002), however this value refers to *Acacia ligulata* from the arid zones of Australia, which are extreme in terms of ant-mediated dispersal distances. For temperate forest herbs the highest observed myrmecochorous dispersal is 70 m (Sernander, 1906). This value is, compared to latter studies, still exceptionally high and Heinken (2004) questions its credibility as an average distance for temperate forest herbs is only a few meters per year (Cain et al., 1998; Gómez and Espalader, 1998). Likewise *M. pratense* (which inhabits similar biotopes to *M. subalpinum*) generally travels less than one meter per year (Heinken, 2004). Myrmecochory is important for local migration and population preservation, however for long-distance migration occasional accidental transfers are necessary. If we compare myrmecochorous dispersal speed with the total distribution of some *Melampyrum* species

(and other myrmecochorous plants), it is highly insufficient, and, at this rate, ants would not be able to repopulate formerly glaciated areas during the Holocene up to the present state. Long-distance dispersal is also biologically very important and can not be considered as missing. It affects colonization probabilities, population persistence probabilities in a fragmented habitat, and metapopulation structure (Ouborg, et al. 1999).

So far, no other means of seed dispersal have been described for *Melampyrum*. The most likely means of long-distance dispersal is endozoochory, and even though the probability of survival of *Melampyrum* seeds in the animal gut is unknown (Cain et al., 1998) it could be one of the neglected means of dispersal for many plants that are presently considered as solely myrmecochorous.

Often, more than one *Melampyrum* species grow together, and also other myrmecochorous species can occur in the same habitat. It is unknown how this co-occurrence affects population dynamics and the dispersal success of involved myrmecochores in the case of preference for some species by ants over others.

Genetic variation

Variation is present, in some form, in the natural populations of all organisms (Lowe, et al., 2004). If we focus on the genetic variation of a species we can uncover the historical events connected with postglacial colonization, historical and present population size changes, the impact of environmental factors and anthropogenic disturbances, as well as the species breeding system, hybridization, and polyploidization (e. g. Aparicio et al., 2002; Phillipp and Siegismund, 2003; Rosenbaumová et al., 2004; Leimu and Mutikainen, 2005; Leimu et al., 2006; Wróblewska, 2008; Chung et al., 2013).

Genetic variation within the whole distribution area of the *M. subalpinum* group remains unknown. According to the central-marginal concept (Eckert et al., 2008) within-population genetic diversity should decline and among-population differentiation should increase from the center of the species geographical range to the periphery. The morphological variability of the group is consistent with this theory as populations from the centre of distribution area in the Vienna Forest are highly diverse and marginal populations from the Czech Republic and

Slovakia are uniform (Štech, 2006). Yet the question remains of whether the genetic variation of populations follows this gradient in a similar way.

Some studies state that small and isolated peripheral populations can face the negative effects of inbreeding, genetic drift, and the lowered genetic variation caused by bottleneck and founder effects (Lynch et al., 1995; Young et al., 1996; Tomimatsu and Ohara, 2003; Leimu et al., 2006; Chung et al., 2013). The recent decline of some marginal populations may be, apart from other environmental factors, also caused by a loss of genetic diversity.

The main objectives and hypotheses of the thesis

The unique delimited distribution range of the *M. subalpinum* group, which can be labelled as endemic in Central Europe, raises questions about the migrational history of the group in the Holocene, its means of dispersal to achieve recent distribution, the possible relictiness of some populations, and recent population dynamics in connection to myrmecochory.

- 1) We hypothesize that the center of genetic variation is congruent with the center of morphological variation of the group and that Czech and Slovak populations are taxonomically identical with Austrian narrow-leaved populations.
 - What is the extent and pattern of population variation among different morphotypes and geographical areas?
 - Is the level of genetic variation of the *M. subalpinum* group correlated with its morphological variation?
 - What is the size of marginal populations and what are the characteristics of the stands they grow on?

- 2) We hypothesize that broad-leaved populations of *M. subalpinum* are the result of an old hybridization event between narrow-leaved populations and *M. nemorosum*.
 - Does any genetic marker indicate a past hybridization event between *M. subalpinum* agg. and *M. nemorosum* L.

- 3) We hypothesize that *M. subalpinum* populations survived the last glacial maximum in microrefugia located in Central Europe.
 - Is it possible to appraise any colonization history events of the *M. subalpinum* group in Central Europe?

- 4) We hypothesize that small size of populations of *M. subalpinum* may be influenced by the seed preference for the seeds of the co-occurring *M. pratense* by ants.
 - Are the seeds of one of the species (*M. pratense* or *M. subalpinum*) dispersed by ants preferentially?

- 5) We hypothesize that myrmecochory in *Melampyrum* is important for dispersal strictly on a local scale, whereas long distance dispersal happens by different means – probably endozoochory.
 - What are the myrmecochorous dispersal distances in the area with high ant density?
 - Is endozoochorous dispersal in the genus *Melampyrum* possible?

Contents of the thesis

Chapter 1 reviews the general knowledge about the phylogeography, genetic variation and dispersal in plants with the focus on annuals.

Chapter 2 introduces the *Melampyrum subalpinum* group and reviews the problematics of the group from various points of view.

Chapter 3 contains a complete revision of localities of *M. subalpinum* (*M. bohemicum*) in the Czech Republic and Slovakia. The estimation of size for all localities is presented for future comparison and dangers for the *M. subalpinum* populations are discussed.

Chapter 4 provides a comparison of seed dispersal by ants between co-occurring *M. subalpinum* and *M. pratense* and comments on the influence of differences in this process. A new mean of seed dispersal (endozoochory) is introduced for *Melampyrum*. Myrmecochorous dispersal distances are tested and Holocene migration possibilities are discussed taking into account endozoochory.

Chapter 5 describes genetic variation and population structure across the distribution range of the *M. subalpinum* group based on allozyme analysis. Genetic variation is compared to the known morphological variation of the group and to the central-marginal concept. Allelic enrichment due to assumed former hybridization with *M. nemorosum* is discussed.

Chapter 6 provides a further description of the genetic diversity of the *M. subalpinum* group based on genome size analysis, and nrDNA and cpDNA sequencing. The phylogeographic history of the species is commented on, taking into account tree microrefugia in Central Europe. Further evidence of an old hybridization with *M. nemorosum* is introduced.

Chapter 7 summarizes the main results of this thesis.

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Chapter III

**Rozšíření černýše českého (*Melampyrum bohemicum*) v
České republice a na Slovensku – revize po čtyřiceti
letech**

**Distribution of *Melampyrum bohemicum* in the Czech
Republic and Slovakia – revision after forty years**

Chlumský, J and Štech, M (2011): *Zprávy České Botanické Společnosti* 46:
1–16 (in Czech).

Photo on previous page (M. Štech): *Melampyrum subalpinum*, Doubí u
Tábora, Czech Republic

Rozšíření černýše českého (*Melampyrum bohemicum*) v České republice a na Slovensku – revize po čtyřiceti letech
Distribution of *Melampyrum bohemicum* in the Czech Republic and Slovakia – revision after forty years

Abstract

Because of uncertainty in present extent of *Melampyrum bohemicum* A. Kern. populations in the Czech and Slovak Republic and alarming announcements about withdrawal of populations from their localities, complete revision of all historically mentioned localities was carried out. The aim of the survey was to determine range and size of all recent populations for future comparison. The revision was based mainly on the paper by Professor Emil Hadač (Hadač, 1966) which gathered old herbarium data. New data from recent botanists were added. During the revision *M. bohemicum* was found in 50 localities consisting of 76 populations. The species wasn't found on twenty historical localities. Some recent knowledge about biology and taxonomy of *M. bohemicum* is compared to Hadač (1966).

Keywords: *Orobanchaceae*, *Melampyrum*, flora of Central Europe, geographical distribution, revision of localities

Nomenklatura: Kubát et al. (2002), zástupci rodu *Melampyrum* jsou při první zmínce v textu uvedeni s autorskými zkratkami.

Úvod

Melampyrum bohemicum A. Kern. je poloparazitická jednoletá rostlina zařazená v Červeném seznamu ČR do kategorie C3 – ohrožený taxon (Procházka, 2001) a také chráněná podle Přílohy 2 vyhlášky MŽP ČR č. 395/1992 Sb. v kategorii ohrožených druhů. Stejně tak na Slovensku je druh zařazen do červeného seznamu v kategorii ohrožený druh (Feráková et al., 2001) a chráněn podle Přílohy 5 Vyhlášky MŽP SR č. 24/2003 Z. z. Jeho rozšíření je vázáno výhradně na území střední Evropy a v minulosti byla tato rostlina považována dokonce za československého endemita (Hadač, 1966, 1977; Hendrych, 1981). Dle současného taxonomického pojetí, podpořeného výzkumy genetické variability, však můžeme najít populace náležející k tomuto druhu i v Rakousku. Taxonomicky náleží *M. bohemicum* v rámci série *Eunemorosa* (Soó, 1927)

do morfologicky značně proměnlivé skupiny *Melampyrum subalpinum* (Jur.) A. Kern., jejíž problematika je v současnosti řešena pomocí molekulárních metod. *Melampyrum bohemicum* se na našem území vyskytuje vzácně a jeho populace jsou většinou malé, z čehož plyne i potenciální značné ohrožení tohoto fyto geograficky mimořádně významného taxonu. Vzhledem k velmi malému celkovému areálu jsou znepokojující i sdělení některých floristů o ústupu *M. bohemicum* na známých lokalitách.

Hadač (1966) shromáždil z herbářových sbírek veškeré dostupné údaje o výskytu *M. bohemicum* a sám navštívil všechny základní oblasti výskytu. Přesto byla velká část uváděných lokalit naposledy revidována staršími autory mnohdy i před více než sto lety.

Tyto důvody spolu s probíhajícím podrobným studiem taxonomie skupiny *M. subalpinum* vedly k rozhodnutí provést úplnou revizi všech uváděných lokalit tohoto druhu.

Metodika

V červenci a srpnu roku 2006 byla provedena revize všech recentně i historicky uváděných lokalit *Melampyrum bohemicum* v České republice a na Slovensku. Revize navazuje na práci Hadače (1966), který se detailně zabýval rozšířením *M. bohemicum* a excerpoval dostupné herbářové údaje o výskytu taxonu v bývalém Československu. Nové floristické údaje o výskytu *M. bohemicum* byly získány především z prací Holub (1996), Tichý (1996), Kaplan (2005) a Štech (2005). Další údaje byly získány od Agentury ochrany přírody a krajiny ČR a z České národní fytoocenologické databáze (Chytrý and Rafajová, 2003), která je na požádání dostupná na internetové adrese (<http://www.sci.muni.cz/botany/vegsci/dbase.php?lang=cz>). Řada údajů o recentním výskytu *M. bohemicum* byla získána z rukopisných materiálů R. Kurky a M. Štecha. I přes intenzivní snahu se nepodařilo získat diplomovou práci B. Michkové vypracovanou v roce 1982 na Pedagogické fakultě Univerzity Hradec Králové, která se zabývala tímto druhem. Práce bohužel není k dispozici v tamní knihovně ani u autorky samotné.

Smyslem mapování bylo kromě zjištění aktuálního rozšíření *M. bohemicum* položit výchozí bod pro následný monitoring změn v populacích. Z tohoto důvodu byly některé větší populace rozděleny na subpopulace. Jako subpopulace byly označovány jednotlivé výskyty

rostlin, které náležely do stejného lesního celku, případně rostly podél jednoho úseku silnice a byly od sebe vzdálené maximálně do 1 km.

Na revidovaných lokalitách byly zaznamenány následující charakteristiky: Přesná lokalizace pomocí přístroje GPS (souřadný systém WGS 84), nadmořská výška a charakteristika populace s poznámkami o lokalitě a potenciálním ohrožení. Dle přesné lokalizace byl zjištěn geologický podklad lokality na mapovém serveru České geologické služby (<http://www.geology.cz/extranet/geodata/mapserver>). Na většině lokalit byl zaznamenán fytoocenologický snímek, který byl uložen v České národní fytoocenologické databázi (Chytrý and Rafajová, 2003). Velikost populace byla vzhledem k meziročním oscilacím u tohoto jednoletého druhu odhadnuta do hrubé ordinační škály (desítky, stovky, tisíce a desetitisíce rostlin).

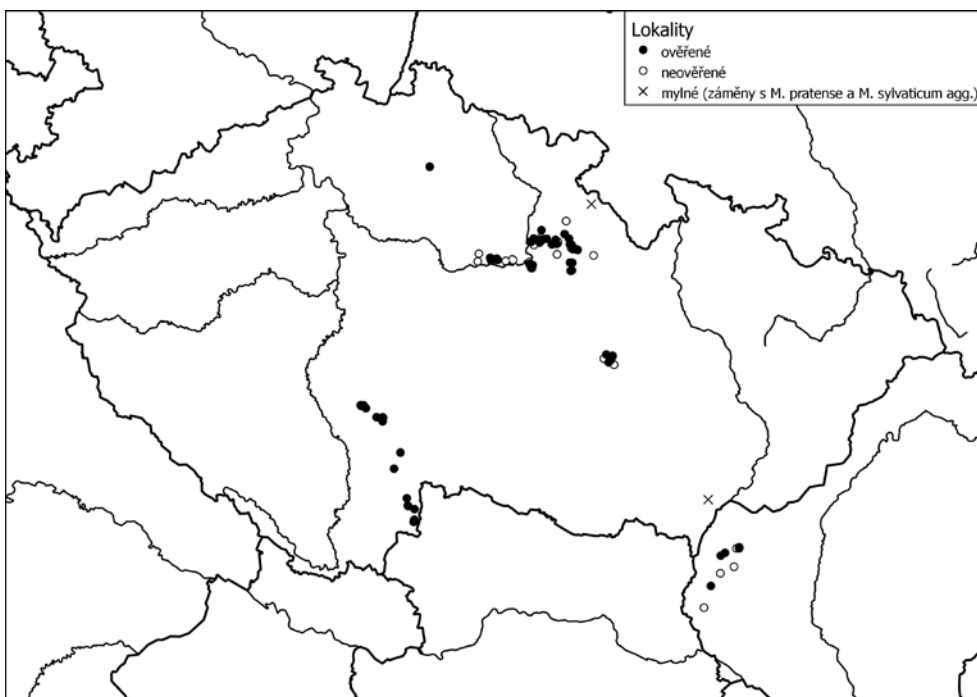
V průběhu revize nebyl zpravidla sbírán dokladový materiál. Většina lokalit je doložena sběry z jiných návštěv lokalit a tyto jsou uloženy v herbáři katedry botaniky Přírodovědecké fakulty JU (CBFS).

Stručná taxonomická problematika skupiny *Melampyrum subalpinum*

V příbuzenstvu *Melampyrum nemorosum*, kam skupina *M. subalpinum* patří, je tradičně za nejvýznamnější taxonomický znak považováno odění kalicha. Skupina *Melampyrum subalpinum*, jak je chápána v této práci, je vymezena oděním, které je omezeno výhradně na žilky kalicha a okraje kališních cípů a je tvořeno středně dlouhými (do 1,5 mm) a dopředu směřujícími chlupy. Pomineme-li balkánské taxony s nejistými vztahy k rostlinám střeoevropským, je centrum diverzity této skupiny v oblasti Vídeňského lesa, kde roste většina morfologicky odlišitelných typů. Rostliny z území Rakouska byly původně popsány Juratzkou na úrovni variety jako *M. nemorosum* var. *subalpinum* (Juratzka, 1857). Vzhledem k velké mezipopulační variabilitě byly postupně popisovány další taxony, které byly různými autory různě chápány a jsou příčinou řady taxonomických i nomenklatorických obtíží. Rostliny objevené v Čechách byly nejdříve uváděny pod různými jmény vztahujícími se na rakouské rostliny (přehled viz Holub, 1996) až nakonec byly popsány v roce 1881 jako *Melampyrum bohemicum* A. Kern. (Kerner, 1881) a zároveň také jako *M. nemorosum* subsp. *fallax* Čelak. (Čelakovský, 1881). Validní publikace jména v druhovém ranku (*M. fallax* Čelak.) je sporná (Holub, 1996). V roce 1882 publikoval detailní studii skupiny Beck (1882). Rostliny se širokými listy chápal jako *M. subalpinum* s. str. a úzkolisté

rostliny popsal jako *M. angustissimum* Beck. Později Beck(1893) sloučil *Melampyrum bohemicum* a *M. angustissimum* do jednoho druhu (se jménem *M. fallax*) a rozlišoval je pouze jako variety tohoto druhu spolu s časným sezónním typem *M. fallax* var. *grandiflorum* (A. Kern.) Beck. Výskyt všech těchto variet udával z území Rakouska a stále je na druhové úrovni odlišoval od *M. subalpinum* s. str. (Beck, 1893). Taxony *M. subalpinum* s. str. a *M. angustissimum* byly akceptovány rakouskými autory do nedávné doby, a také *M. bohemicum* pozdější čeští i rakouští autoři považovali obvykle za samostatný druh (Ehrendorfer and Gutermann, 1973). Rovněž Flora Europaea (Soó and Webb, 1972) rozlišuje rakouské a české populace jako *M. subalpinum* a *M. bohemicum*. Pro *M. bohemicum* jsou jako jeden z rozlišovacích znaků uváděny zelené horní listeny přesto, že *M. bohemicum* má často listeny fialové nebo alespoň fialově naběhlé (Holub, 1996; Štech, 2000). Se skutečností také nekoresponduje udávaná šířka listů. Flora Europaea uvádí šířku listů pouze 2 mm, kdežto ve skutečnosti může mít *M. bohemicum* listy široké 2–9 mm (Štech, 2000, 2006). Naopak v případě *M. subalpinum* Flora Europaea bere v úvahu hlavně širokolisté populace, pro které uvádí šířku listů (2–)14–18 mm a o rakouských úzkolistých populacích se zvláště nezmiňuje.

Detailně se problematikou variability rakouských populací zabýval Reiner (1994). Jeho řešení bylo přejato také do druhého a s úpravou i třetího vydání rakouského klíče (Fischer et al., 2005, 2008). V tomto pojetí je *M. subalpinum* s. l. rozděleno na dva poddruhy odlišované na základě sezónních znaků (např. doba květu, počet lodyžních a interkalárních článků, počet větví a kvetoucích větví). *Melampyrum subalpinum* subsp. *grandiflorum* (A.Kern) Soó je časný morfotyp úzkolistých rostlin údajně kvetoucí v květnu až červnu a *M. subalpinum* subsp. *subalpinum* zahrnuje pozdní morfotypy kvetoucí od července do začátku září. Tento poddruh je dále členěn na dvě variety. Varieta *subalpinum* zahrnuje úzkolisté rostliny a var. *thermale* Reiner ined. širokolisté populace z oblasti Vídeňského lesa v Rakousku. Zcela opačné pojetí *M. subalpinum* s. str. je způsobeno tím, že dosud nebyla platně provedena typifikace jména *M. nemorosum* var. *subalpinum* Juratzka. Reiner ve své rukopisné diplomové práci navrhnul typifikaci úzkolistými rostlinami (Reiner, 1994). Pokud by však toto jméno bylo typifikováno širokolistými rostlinami, pak by zřejmě nejstarším jménem pro úzkolisté rostliny bylo *M. bohemicum* A. Kern.



Obr. 1. – Mapa současného a historicky uváděného rozšíření *Melampyrum bohemicum* v České republice a na Slovensku.

Fig. 1. – Distribution map of recent and historical localities of *Melampyrum bohemicum* in the Czech Republic and Slovakia.

Štech (2006) považuje *M. bohemicum* za taxon totožný s rakouskými populacemi *M. angustissimum*. Přesto, že jsou populace *M. angustissimum* morfologicky variabilnější, dá se *M. bohemicum* chápat z pohledu morfologického jako výsek variability rakouských úzkolistých rostlin. Širokolisté populace *M. subalpinum* s. str. vznikly dle Štecha (2006) starou hybridizací populací *M. angustissimum* a *M. nemorosum*. Testování těchto hypotéz a analýza genetické variability celého komplexu jsou hlavní náplní projektu řešeného v současné době.

Současné rozšíření a charakteristika stanovišť *M. bohemicum* v České republice a na Slovensku – srovnání s historickým stavem

Melampyrum bohemicum se na našem území nejhojněji vyskytuje ve východní části širšího Polabí, kde je vázán především na terasy Labe a Orlice, roztroušeně roste i v jižních Čechách, a to zejména na terasách Lužnice od jižního Třebońska až po Příběnice a na jedné lokalitě nedaleko

Kardašovy Řečice. Malá arela je v širším okolí Olešnice u Kunštátu, kde se často vyskytuje i na bázemi bohatých až vápnitých nepísčitých stanovištích. Byl také objeven na jediné lokalitě v Českém ráji (viz obr. 1). Vyskytuje se od planárního do submontánního stupně (viz ,obr. 2) s výškovým maximem na Českomoravské vrchovině na vrchu Fouska u Trpína (ca 660 m) (Štech, 2000). Na Slovensku *M. bohemicum* roste pouze na několika lokalitách v písčitých světlých borech v oblasti Záhorí (Hadač, 1966; Šípošová and Štech, 1997).

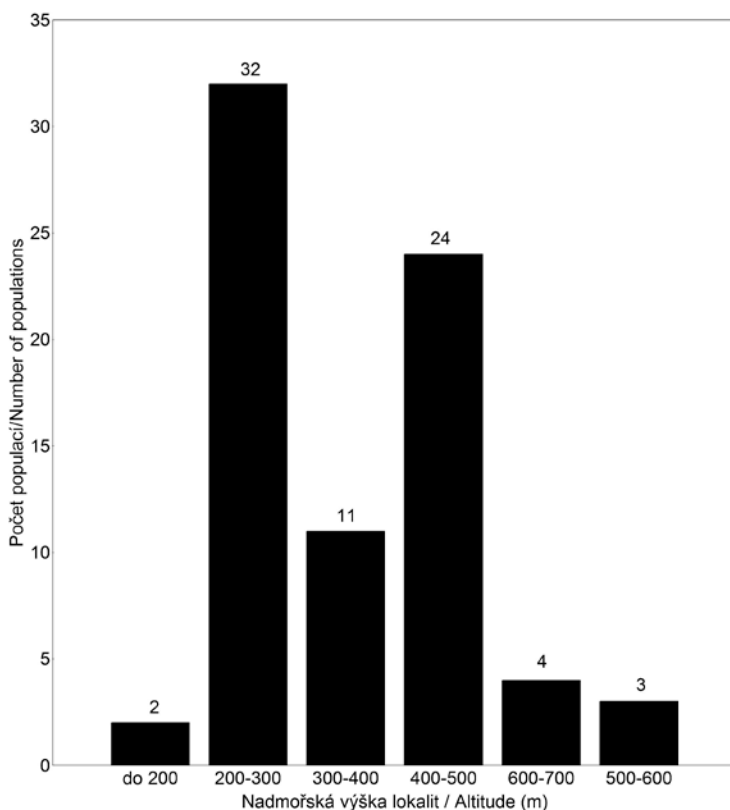
V současné době byl druh ověřen na 50 lokalitách s celkem 76 populacemi (viz příloha 1). Část lokalit (19) uváděných Hadačem (1966) se nepodařilo ověřit (viz příloha 2). Vzhledem k tomu, že některé lokality jsou naposledy udávány na konci 19. století, je pravděpodobné, že řada z nich již neexistuje. Významnou roli však také hraje fakt, že mnohé starší údaje jsou pojaty velmi široce a nelze vyloučit, že některé lokality se ještě v budoucnu podaří ověřit. Dvě Hadačem (1966) uváděné populace náleží k jiným druhům, jak bylo zjištěno revizí citovaných herbářových dokladů (Štech, 2000). Jedná se o lokalitu u Dobřan v Orlických horách, kde došlo k záměně s *M. sylvaticum* agg. a o lokalitu na Hodonínsku, kde došlo k záměně s *M. pratense*.

Přestože je černýš český na mnoha lokalitách již neznámý, přibyla od doby Hadačovy revize (1966) řada lokalit nových. V jižních Čechách byly objeveny nové populace v okolí Majdaleny, Kardašovy Řečice, Doubí u Tábora, Plané nad Lužnicí a několik nových lokalit v širším okolí zříceniny hradu Příběnice. Mimo jižní Čechy je kromě několika drobných lokalit v blízkosti Pardubic a na slovenském Záhorie významný nález izolované nejsevernější lokality nedaleko Žehrova v Českém ráji.

Během aktuální revize bylo nalezeno několik nových populací, které se nacházejí v sousedství již známých lokalit a dvě nové lokality v blízkosti Ústrašic na Táborsku a nedaleko Kardašovy Řečice (viz příloha 1). Údaje poskytnuté AOPK a údaje z národní fytoocenologické databáze (Chytrý and Rafajová, 2003) se vesměs kryly s údaji Hadače (1966) a vlastními nálezy či excerpcí M. Štecha.

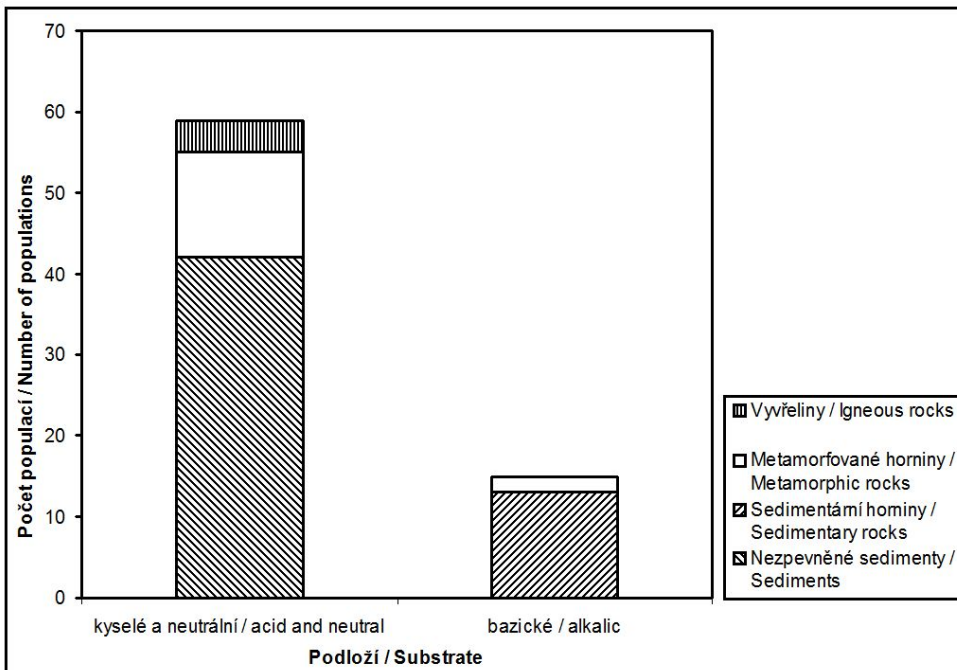
Hadač (1966) ve své práci uvádí, že *M. bohemicum* roste v borech nebo světlých doubravách v asociacích *Pino-Quercetum* nebo *Luzulo-Quercetum*. Při revizi lokalit v roce 2006 se všechny lokality *M. bohemicum* nalézaly v sekundárních kulturních lesích, které lze zařadit do syntaxonomického systému jen velmi obtížně. Uvažovat by se dalo jen o lokalitách ze Záhorí, které Hadač (1966) řadí do asociací *Pino-Quercetum*

zahoricum a *Dicrano-Pinetum zahoricum*. Tyto světlé bory na pískách mají specifickou flóru, která je zcela odlišná od podrostu českých borů. Na těchto lokalitách byly populace *M. bohemicum* nejpočetnější, což může souviset se světlostí borových lesů a také s řídkce zapojeným bylinným patrem na pískách. Fytcenologické snímky, které byly pořízeny na všech lokalitách druhu, je možné vyhledat v České národní fytcenologické databázi (Chytrý and Rafajová, 2003) pod čísly uvedenými u jednotlivých lokalit v příloze 1.



Obr. 2. – Rozdělení populací *Melampyrum bohemicum* dle nadmořských výšek.

Fig. 2. – *Melampyrum bohemicum* populations categorized on the basis of the altitude.



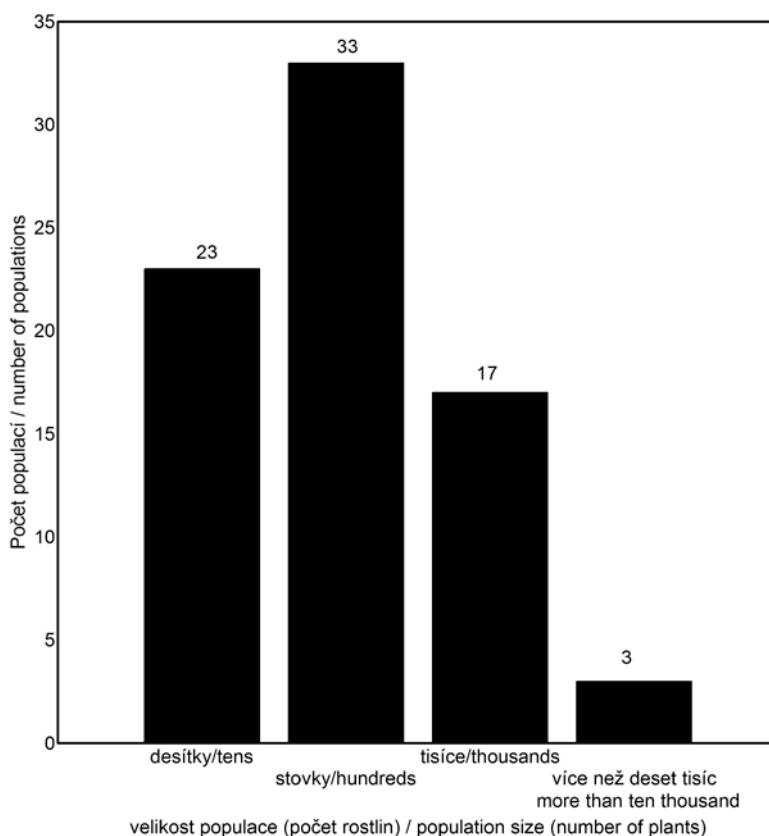
Obr. 3. – Rozdělení populací *Melampyrum bohemicum* dle geologických charakteristik.

Fig. 3. – *Melampyrum bohemicum* populations categorized on the basis of geological characteristics.

Hadač (1966) uvádí, že převážná část lokalit má písčité podklad. Při současné revizi však bylo zjištěno, že populace často rostly i na štěrkopískovém podkladu či na hlinito-písčitých půdách. Stejně tak matečným substrátem nemusí být pouze písky. Z geologických map bylo zjištěno, že podklad lokalit mnohdy tvoří přeměněné horniny, výjimečně též vyvřeliny a lokality jsou často v blízkosti výchozů hornin s vyšším obsahem bazických iontů (viz obr. 3). Hadač (1966) se o vápencovém podkladu na lokalitách *M. bohemicum* nezmiňuje. Naopak pro rakouské populace *M. subalpinum* agg. uvádí jako jeden z rozlišovacích znaků, že se proti *M. bohemicum* vyskytují na vápenci. Teprve Tichý (1996) uvádí populace rostoucí na vápencovém podloží z lokalit v blízkosti Olešnice u Kunštátu. Při aktuální revizi bylo zjištěno, že *M. bohemicum* se na vápnitých podkladech (vápnitých jílovcích, slínovcích a prachovcích) vyskytuje i ve východním Polabí. *Melampyrum bohemicum* tedy roste na vápencovém podkladu častěji, než se předpokládalo (viz obr. 3), a přibližuje se tak stanovištními nároky druhu *M. angustissimum*.

Melampyrum bohemicum vyhledává především světlejší lesy, případně světlá místa s narušeným stromovým patrem. Snižující se světlost způsobená kompaktním stromovým zápojem a expanzí křovin, která je obecným trendem současného vývoje lesů (Hofmeister et al., 2004, 2009), se dá považovat za jednu z hlavních příčin ústupu populací tohoto druhu. Ze všech lokalit bylo patrné, že *M. bohemicum* velice často roste pouze v okolí silnic, cest a pěšin, a to i v územích, ze kterých je v minulosti udáván jako hojný druh. Největší populace jsou v současnosti soustředěny především do světlých borů na pískách v oblasti Záhorie. Velmi často *M. bohemicum* roste na terasách řek nebo v jejich blízkosti (např. nad nivou řeky) a v okolí terénních zlomů, což zřejmě rovněž souvisí s příznivějšími světelnými podmínkami, a to nejen v současnosti, ale i v minulosti. Také Hadač (1966) uvádí, že smíšené háje s duby, lípou a javory jsou pro *M. bohemicum* příliš stinné a vlhké. Druh přesto může prosperovat v podobných společenstvech, pokud jsou dostatečně světlá. Například u Vysoké nad Labem roste velká prosperující populace v lipovém háji (s příměsí trnovníku akátu), který je velmi světlý a bez podrostu křovin.

Hlavními faktory recentně ohrožujícími tento druh v České republice jsou tedy zejména postupující zastínění a ruderalizace lokalit (zarůstání druhy *Calamagrostis epigejos*, *C. arundinacea*, *Impatiens parviflora*, *Carex brizoides*, *Pteridium aquilinum*), nevhodné lesnické zásahy (velkoplošné mýcení, hustá výsadba smrku na lesní světliny), vysoká fragmentace populací a jejich malá velikost. Z obr. 4 je patrné, že jen tři populace *M. bohemicum* dosahují velikosti o desítkách tisíců jedinců. Ve dvou případech se jedná o populace ze světlých borových lesů na slovenském Záhorie a třetí populace je z řídkého a světlého lesa v blízkosti Černé za Bory u Pardubic. Na menším rozsahu dalších populací se již často projevuje limitace světelnými podmínkami. Pouze třináct populací je tisícových a zbylých padesátšest sestává z desítek až stovek jedinců, což může být pro jednoletou rostlinu nepříznivý počet. Na druhou stranu se výskyt na řadě lokalit jeví dlouhodobě stabilní. Některé informace o poklesu velikosti populací lze připsat na vrub přirozeným fluktuacím početnosti, které jsou u jednoletých rostlin běžné. Takovéto změny početnosti byly např. sledovány od roku 2003 na lokalitách v Doubí u Tábora.



Obr. 4. – Rozdělení populací *Melampyrum bohemicum* dle jejich velikostí (odhad počtu jedinců).

Fig. 4. – *Melampyrum bohemicum* populations categorized on the basis of their size (number of individuals estimated).

Současný pohled na historii druhu

Nový pohled na taxonomii celé skupiny *M. subalpinum* a prohlubující se znalosti o vegetaci Holocénu mění i pohled na historii skupiny v poslední době poledové. Nad migrační historií skupiny v těchto oblastech se zamýšlel Hadač (1966), který předpokládal, že společný předek *M. bohemicum* a *M. subalpinum* sahal svým areálem od rakouských Alp po Záhorie na Slovensku. Jako argument pro toto rozšíření Hadač (1966) uvádí, že se v této oblasti v poslední době ledové udržely borové lesy vhodné pro výskyt *M. subalpinum*, zatímco Čechy pokrývala tundra. Později byla podle Hadače (1966) záhorská arela oddělena a dále se

vyvíjela samostatně. V preboreálu se na naše území rozšířily světlé březoborové lesy a v boreálu se bory s lískou rozšířily i vysoko do hor (Ložek, 2007). Tato období tedy byla vhodná pro šíření *M. subalpinum*. Dle Hadačovy teorie (Hadač, 1966) se pak již ustálený druh *M. bohemicum* šířil přes Moravu a východní Čechy dále. Dnes však již převládá názor, že bezlesí v poslední době ledové zdaleka nebylo tak rozšířené, jak se dříve předpokládalo (Wills and van Andel, 2004), a je tedy pravděpodobné, že měl předek *M. subalpinum* agg. daleko větší četnost výskytu a možnosti šíření.

Heinken (2004) uvádí, že *M. pratense*, které obývá podobné biotopy jako *M. subalpinum*, urazí za rok od centra zavlečení vzdálenost převážně menší než jeden metr, což koresponduje s rychlostí šíření jiných myrmekochorních rostlin. Takováto rychlost šíření by byla pro dosažení dnešního areálu *M. subalpinum* zcela nedostačující. Myrmekochorie má zásadní význam pro udržení a rozšíření populace v lokálním měřítku, avšak pro migraci na větší vzdálenosti jsou nutné ojedinělé dálkové přesuny diaspor. Hadač (1966) uvádí možnost přenosu semen větrem, což je ale vzhledem k velikosti a hmotnosti semen téměř nemožné. Jedním z pravděpodobnějších přirozených způsobů je endozoochorie. Soudě dle častých nálezů rostlin s ukousnutým hlavním květenstvím či viditelně spasených populací jsou černýše zvěří s oblibou vyhledávány. Cain et al. (1998) předpokládá, že schopnost přežití semene po průchodu trávicím traktem je relativně nízká. Výsledky v současnosti probíhajícího projektu však naznačují, že semena rodu *Melampyrum* jsou běžně schopna projít trávicím traktem přežvýkavců v neporušeném stavu. Tato forma přenosu je tedy pravděpodobně jedním z významných způsobů šíření rostlin v Holocénu (Cain et al., 1998; Pakeman, 2001), černýše nevyjímaje.

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Příloha 1. – Přehled revidovaných lokalit s ověřeným výskytem *M. bohemicum*. Sublokality náležející pod jedinou lokalitu jsou odděleny středníkem. Lokality jsou odděleny tečkou a pomlčkou. V popisu lokalit je uvedena řádová velikost populací, nadmořská výška lokality, souřadnice středu populace (WGS 84), číslo fytoocenologického snímku uloženého v České národní fytoocenologické databázi (Chytrý and Rafajová, 2003), poznámka k lokalitě a ohrožení (pokud je k dispozici) a v závorce datum poslední revize a autor. Pokud je to nutné pro upřesnění polohy obce, je v hranatých závorkách uvedeno nejbližší větší sídlo.

Appendix 1. – Summary of successfully revised localities of *M. bohemicum*. Sublocalities belonging to one locality are divided by semi-colon. Localities are divided by coma and dash. For each locality, rough size of the population, altitude, GPS coordinates of the center of the population (WGS 84), number of phytosociological relevé from the Czech National Phytosociological Database (Chytrý and Rafajová, 2003), notes about the site and its threats (if available) and in brackets date and author of the last revision are included. In case it's necessary to specify a location of a village, nearest larger village or town is stated in brackets.

Česká republika

Termofytikum:

15c Pardubické Polabí: Sopřeč, u křižovatky Sopřeč – Strašov – Břehy, ca 2 km JJZ od obce, stovky až tisíce rostlin v borovém lese s *Quercus robur*, 240 m n. m., 50,07890°N, 15,54553°E (23. VIII. 2006 J. Chlumský et J. Svobodová); Sopřeč, ca 980 m J od křižovatky Sopřeč – Strašov – Břehy v boru u silnice, stovky rostlin v úseku ca 100 m, 220 m n. m., 50,06899°N, 15,54539°E, Turboveg č. 551395 (23. VIII. 2006 J. Chlumský et J. Svobodová). – Přelovice: ca 2 km ZSZ od kostela v Přelovicích, lesní světlina SV od rybníka Černý Nadýmač, desítky až stovky rostlin roztroušeně na světlině v lese, 240 m n. m., 50,07627°N, 15,58875°E, Turboveg č. 551394 (22. VIII. 2006 J. Chlumský et J. Svobodová); Přelovice, 1,5 km ZJZ od kostela v obci, doubrava u silnice na Vlčí

Habřinu, stovky až tisíce rostlin v doubravě s borovicí na okraji silnice v úseku ca 30 m, 230 m n. m., 50,07073°N, 15,59530°E, Turboveg č. 551393 (22. VIII. 2006 J. Chlumský et J. Svobodová). – (Pardubice) Bělobranská dubina, Spojil, 590 m SV – SSV od křižovatky v obci na okraji lesa u lesní pěšiny, desítky rostlin v doubravě na ploše ca 30m², ohrožení: velmi malá populace, 230 m n. m., 50,04638°N, 15,82786°E, Turboveg č. 551392 (22. VIII. 2006 J. Chlumský et J. Svobodová). – Bělobranská dubina, Sezemice, 2,7 km JJZ od kostela v obci, okraj silnice mezi Černou za Bory a Sezemicemi, několik desítek rostlin u silnice v příkopu a v jeho těsné blízkosti, les s duby a smrky, ohrožení: místy hustý porost semenáčů *Quercus rubra*, 230 m n. m., 50,04154°E, 15,84028°E, Turboveg č. 551391 (22. VIII. 2006 J. Chlumský et J. Svobodová); Bělobranská dubina, Sezemice, 2,75 km JJZ od kostela v obci, les u silnice mezi Černou za Bory a Sezemicemi, desítky rostlin, ohrožení: malá populace, 230 m n. m., 50,04111°N, 15,83910°E (22. VIII. 2006 J. Chlumský et J. Svobodová). – Bělobranská dubina, Veska, 680 m ZSZ od kostela v obci, okraj lesa za rodinnými domy, stovky rostlin na ploše ca 1500 m² v lese s borovicí, dubem a břízou, ohrožení: zarůstá *Melica nutans*, 230 m n. m., 50,04098°N, 15,85254°E, Turboveg č. 551390 (22. VIII. 2006 J. Chlumský et J. Svobodová). – Černá za Bory, 650 m V od vlakové zastávky v obci, JZ část lesa S od silnice mezi obcemi Zminný a Černá za Bory, desetitisíce rostlin roztroušeně v celé JZ části borového lesa a po jeho okrajích, ohrožení: místy zarůstá *Calamagrostis epigejos*, 240 m n. m., 50,02766°N, 15,84306°E, Turboveg č. 551389 (22. VIII. 2006 J. Chlumský et J. Svobodová).

Mezofytikum:

39 Třeboňská pánev: Planá nad Lužnicí, okraj lesa ca 50 m J od rybníka Hejtman, stovky až tisíce rostlin v doubravě navazující na bor, 410 m n. m., 49,33250°N, 14,72173°E, Turboveg č. 551363 (5. VIII. 2006 J. Chlumský et J. Svobodová); Planá nad Lužnicí, ca 200 m JJV od rybníka Hejtman na SV okraji borového lesa u lesní cesty, stovky rostlin, ohrožení: výrazně stínící hustá výsadba mladých borovic, 410 m n. m.,

49,32243°N, 14,72463°E (5. VIII. 2006 J. Chlumský et J. Svobodová). – Doubí u Tábora, ca 950 m SSZ od vlakové zastávky v obci v pásu mezi železnicí a silnicí E55, stovky rostlin rozptýleně v porostu, 420 m n. m., 49,33000°N, 14,71456°E, Turboveg č. 551360 (5. VIII. 2006 J. Chlumský); Doubí u Tábora, ca 450 m SSZ od vlakové zastávky v obci v prostoru mezi železnicí a silnicí E55, stovky rostlin, 420 m n. m., 49,32562°N, 14,71579°E (5. VIII. 2006 J. Chlumský). – Doubí u Tábora, ca 150 m Z od vlakové zastávky v obci, borový les u staré silnice, velká populace tisíců rostlin na ploše 4000 m², 425 m n. m., 49,32243°N, 14,71519°E, Turboveg č. 551355 (1. VIII. 2006 J. Chlumský). – Doubí u Tábora, ca 460 m JJV od vlakové zastávky v obci, stovky až tisíce rostlin roztroušeně v borovém lese po obou stranách trati (i v bezprostřední blízkosti na okraji náspu), ostrůvkovitě hojněji, ohrožení: silná ruderalizace, zvláště kolem trati, 420 m n. m., 49,31799°N, 14,71956°E, Turboveg č. 51358 (5. VIII. 2006 J. Chlumský et J. Svobodová). – Doubí u Tábora, 1 km JJV od vlakové zastávky v obci, smrkový les nad zářezem trati, populace tisíců rostlin na ploše ca 700 m², 425 m n. m., 49,31334°N, 14,72134°E, Turboveg č. 551359 (5. VIII. 2006 J. Chlumský et J. Svobodová). – Kardašova Řečice, ca 2,5 km JJZ od železniční stanice v obci na okraji borového lesa u pěšiny s turistickou značkou, desítky rostlin na čerstvé mýtině a dále v borovém porostu kolem pěšiny v úseku ca 240 m, 445 m n. m., 49,16297°N, 14,84647°E, Turboveg č. 551382 (16. VIII. 2006 J. Chlumský et J. Svobodová). – Kolence, ca 900 m JV od centra obce, les při silnici do Novosedel, velká populace mnoha tisíc rostlin v lese s dubem a borovicí podél obou stran silnice, ohrožení: lokálně postupuje ruderalizace, 445 m n. m., 49,08643°N, 14,79793°E, Turboveg č. 551383 (18. VIII. 2006 M. Štech et J. Chlumský). – Majdalena, ca 2,5 km SSZ od nádraží v obci u lesní cesty s turistickou značkou k rozvodí Lužnice a Nové řeky ca 200 m od hlavní silnice, stovky rostlin v úseku asi 150 m kolem cesty, okraj druhotného borového lesa s přimíšenými duby letními na JV orientované hraně terasy nad nivou Lužnice, 440 m n. m., 48,98466°N, 14,84436°E, Turboveg č. 551350 (16. VII. 2006 J. Chlumský et Z.

Chlumská). –Kosky [Majdalena], ca 720 m JJZ od mostu přes Kostěnický potok v obci nad pravým břehem Lužnice v lese za chatami, malá populace desítek rostlin v smrkovém lese s dubem, ohrožení: velikost populace a zarůstání *Carex brizoides* a *Pteridium aquilinum*, 450 m n. m., 48,94421°N, 14,88637°E, Turboveg č. 551384 (19. VIII. 2006 J. Chlumský et Z. Chlumská). – Suchdol nad Lužnicí, ca 540 m SSV od mostu přes Lužnici v obci, při soutoku Lužnice s Dračicí (terasa na pravém břehu Lužnice), stovky rostlin na světlinách kolem lesní cesty směřující k Lužnici a ve smrkovém lese u řeky, 450 m n. m., 48,90893°N, 14,89216°E, Turboveg č. 551385 (19. VIII. 2006 J. Chlumský et Z. Chlumská). – Klikov, ca 800 m Z od mostu přes Dračici v obci, okraj lesní cesty vedoucí z J k soutoku Lužnice a Dračice asi 150 m od silnice mezi Klikovem a Suchdolem nad Lužnicí, velmi malá populace několika desítek rostlin na ploše ca 2 × 3 m, ohrožení: v případě dalšího zhoustnutí stromového patra by populace měla nedostatek světla, 464 m n. m., 48,90650°N, 14,89470°E, Turboveg č. 551351 (16. VII. 2006 J. Chlumský et Z. Chlumská). – Františkov, ca 300 m JV od mostu silnice na Rapšach na terase nad levým břehem Dračice, populace podél hrany vyznívá na Z, stovky rostlin, ohrožení: místy okraj populace ohrožen ruderalizací, zarůstání *Impatiens parviflora*, juvenilní rostliny *Symphoricarpos albus*, 465 m n. m., 48,89154°N, 14,94009°E, Turboveg č. 551354 (20. VII. 2006 M. Štech et J. Chlumský). – Halámky, 800 m JV od hájenky v obci po obou stranách hlavní silnice Halámky – Neunagelberg v místě křížení s lesní cestou, V od silnice tisíce rostlin v boru na ploše ca 200 m², Z od silnice tisíce rostlin na ploše ca 500 m², 467 m n. m., 48,84177°N, 14,93738°E, Turboveg č. 551353 (20. VII. 2006 M. Štech et J. Chlumský). – Halámky, 2,8 km J od křižovatky v obci, pravý břeh Lužnice asi 1 km V od Lesních Chalup, poslední terasa nad nivou Lužnice, ústí úvozové cesty do nivy, asi 15 rostlin na ploše 2 × 4 m v mladém borovém lese, ohrožení: vzhledem k počtu rostlin vysoké, 460 m n. m., 48,83049°N, 14,93284°E, Turboveg č. 551352 (20. VII. 2006 M. Štech et J. Chlumský). **41 Střední Povltaví:** Příběnice, J až JZ svah nad silnicí kolem zříceniny Příběnice ca 150 m JJZ

od zbytků věže, roztroušeně desítky až stovky rostlin, 420 m n. m., 49,39162°N, 14,56269°E, Turboveg č. 551361 (5. VIII. 2006 J. Chlumský); Příběnice, po okrajích obezděného otevřeného prostoru v centru zříceniny, 30 m S od zbytků věže, desítky až stovky rostlin, ohrožení: populace zarůstá okolní vegetací, S od tohoto místa roste odděleně populace *M. nemorosum*, 425 m n. m., 49,39142°N, 14,56421°E (5. VIII. 2006 J. Chlumský); Příběnice, 100 m SZ od věže v areálu zříceniny v lískovém křoví, desítky rostlin, 425 m n. m., 49,39245°N, 14,56244°E (5. VIII. 2006 J. Chlumský); Příběnice, asi 580 m JV od zříceniny na svahu nad Lužnicí při okraji lesní cesty, 7 rostlin ve velmi husté vegetaci s *Calamagrostis epigejos* a *Rubus idaeus*, ohrožení: vysoké, populace vytlačována expanzními druhy, 460 m n. m., 49,38838°N, 14,56655°E (5. VIII. 2006 J. Chlumský). – Bečice u Tábora, světlá dubohabřina na svahu nad Lužnicí asi 650 m VSV od kostela v Bečicích v blízkosti křižovatky lesních cest, stovky rostlin, 450 m n. m., 49,384333°N, 14,562388°E (27. VI. 2010 J. Chlumský et V. Jílková). – Malšice u Tábora, asi 1,5 km V od zříceniny Příběnice na mohylovém pohřebišti, smrkový les s tisíci rostlin, 480 m n. m., 49,39191°N, 14,58351°E (5. VIII. 2006 J. Chlumský); Malšice u Tábora, asi 1,5 km VJV od zříceniny hradu Příběnice, 300 m JJZ od mohylového pohřebiště, stovky rostlin roztroušeně na svahu u lesní cesty v úseku ca 200 m, ohrožení: značné, lokalita zarůstá *Calamagrostis arundinacea*, 460 m n. m., 49,38966°N, 14,58216°E (5. VIII. 2006 J. Chlumský). **42b Tábořsko–vlašimská pahorkatina:** Slapy, les u silnice ca 1,5 km JZ od obce, pěšina na Z okraji lesa, roztroušená populace stovek rostlin na okraji a uvnitř lesa, ohrožení: silná ruderalizace, mýcení, 520 m n. m., 49,37760°N, 14,60035°E, Turboveg č. 551356 (1. VIII. 2006 J. Chlumský). – Ústrašice, 750 m JZ od křižovatky v obci po obou stranách silnice z Ústrašic do Želeče, ca 200 m před okrajem lesa v okolí křižující lesní cesty, tisíce rostlin na ploše ca 460 m², ohrožení: mýcení, populace těsně sousedí s lesnickou školkou, 440 m n. m., 49,33371°N, 14,67733°E, Turboveg č. 551357 (1. VIII. 2006 J. Chlumský). **55c Rovenská pahorkatina:** Žehrov, ca 980 m JJV od křižovatky v obci v bývalém lomu u

silnice, roztroušeně stovky rostlin v lomu a jeho blízkosti, 280 m n. m., 50,52346°N, 15,10729°E, Turboveg č. 551396 (23. VIII. 2006 J. Chlumský et J. Svobodová); Žehrov, při silnici u býv. lomu ca 970 m JJV od křižovatky v obci, stovky rostlin v příkopu u silnice v úseku ca 200 m, 280 m n. m., 50,52301°N, 15,10653°E (23. VIII. 2006 J. Chlumský et J. Svobodová). **61a Křivina:** Rašovice [Týniště nad Orlicí], ca 970 m SSZ od křižovatky v obci u silnice z Rašovic do Křivic, pod kopcem Hlava (Hlaváč), stovky až tisíce rostlin po obou stranách cesty v boru s přimíšenými buky, 300 m n. m., 50,16135°N, 16,12835°E, Turboveg č. 551369 (11. VIII. 2006 J. Chlumský et J. Svobodová). **61b Týnišťský úval:** Běleč nad Orlicí, 1,3 km ZSZ od křižovatky v obci při silnici mezi Bělčí a Svinary na hraně terasy nad nivou Orlice, přes silnici proti hájovně Škvárovka v chatařské osadě, tisíce rostlin pod borovicemi a na mýtině s výsadbou mladých smrků, 245 m n. m., 50,20584°N, 15,92646°E, Turboveg č. 551381 (13. VIII. 2006 J. Chlumský et J. Svobodová). – Bělečko, ca 1,6 km SSV od křižovatky v obci při silnici z Bělečka do Třebechovic p. O., malá populace desítek rostlin u odbočky na lesní cestu, ohrožení: zarůstá *Carex brizoides*, 270 m n. m., 50,16388°N, 15,96336°E, Turboveg č. 551380 (13. VIII. 2006 J. Chlumský et J. Svobodová). – Křivice, ca 1,25 km SZ od kostela v obci na okraji lesa poblíž jedné z lesních cest vedoucích k vojenskému prostoru, tisíce rostlin na ploše ca 1000 m² v boru na okraji s duby, 280 m n. m., 50,18350°N, 16,09675°E, Turboveg č. 551370 (11. VIII. 2006 J. Chlumský et J. Svobodová). – Čestice, ca 1 km ZSZ od vlakové zastávky v obci u lesní cesty, stovky rostlin roztroušeně po stranách lesní cesty v úseku ca 200 m, 280 m n. m., 50,13400°N, 16,13324°E, Turboveg č. 551368 (11. VIII. 2006 J. Chlumský et J. Svobodová); Čestice, asi 270 m SZ od nádraží v obci na malé stránce pod lesem na úpatí vrchu Malý Chlum za rodinným domkem, asi 20 rostlin, ohrožení: zarůstá *Calamagrostis epigejos*, malá populace, 300 m n. m., 50,13202°N, 16,14481°E (11. VIII. 2006 J. Chlumský et J. Svobodová). – Kostelec nad Orlicí, při lesní cestě ca 620 m JJZ od hájovny u Kostelce n. O. v blízkosti býv. pískovny, desítky rostlin rozptýleně na okraji mladé smrkové výsadby a v listnatém lese,

315 m n. m., 50,10688°N, 16,18907°E, Turboveg č. 551366 (10. VIII. 2006 J. Chlumský et J. Svobodová); Kostelec nad Orlicí, ca 760 m JJZ od hájovny u Kostelce n. O. po obou stranách hlavní silnice mezi Kostelcem n. O. a Čermnou n. O., stovky rostlin ve smrkovém lese s duby, 320 m n. m., 50,10536°N, 16,18889°E (10. VIII. 2006 J. Chlumský et J. Svobodová). – Zdelov, ca 1,8 km SV od hájovny ve Zdelově u silnice ze Zdelova do Kostelce n. O., 1,2 km V od rybníka v Častolovických Horkách, několik desítek rostlin v úseku ca 100 m, 280 m n. m., 50,11335°N, 16,16443°E, Turboveg č. 551367 (10. VIII. 2006 J. Chlumský et J. Svobodová); Zdelov, ca 1 km VSV od hájovny ve Zdelově u silnice ze Zdelova do Kostelce n. O., 760 m JV od rybníka v Častolovických Horkách, stovky rostlin, 280 m n. m., 50,10999°N, 16,15475°E (10. VIII. 2006 J. Chlumský et J. Svobodová).

61c Chvojenská plošina: Vysoká nad Labem, 150 m JJV od hájovny v obci v lipovém háji na vyvýšenině nad silnicí, háj se stromovým patrem tvořeným *Tilia cordata* (a několika stromy *Robinia pseudacacia*), tisíce rostlin na ploše 1300 m², zcela jistě zde *M. bohemicum* parazituje na lípě, 260 m n. m., 50,15285°N, 15,84125°E, Turboveg č. 551378 (12. VIII. 2006 J. Chlumský et J. Svobodová); Vysoká nad Labem, 520 m JV od hájovny v obci při lesní cestě, porost tisíců rostlin po stranách lesní cesty na okraji křovinaté paseky, 260 m n. m., 50,15118°N, 15,84681°E, Turboveg č. 551377 (12. VIII. 2006 J. Chlumský et J. Svobodová). – Nový Hradec Králové, ca 500 m JV od rybníka Biřička u hlavní silnice z Nového Hradce Králové do Býště, stovky až tisíce rostlin na Z straně silnice na ploše ca 1000 m², 280 m n. m., 50,16741°N, 15,86620°E, Turboveg č. 551379 (13. VIII. 2006 J. Chlumský et J. Svobodová); Nový Hradec Králové, ca 700 m JV od rybníka Biřička u hlavní silnice z Nového Hradce Králové do Býště, roztroušeně desítky až stovky rostlin kolem hlavní silnice, ohrožení: ruderalizace, 280 m n. m., 50,16612°N, 15,86817°E (13. VIII. 2006 J. Chlumský et J. Svobodová). – Hoděšovice, ca 40 m V od hájovny Bažantnice, stovky rostlin v úseku ca 80 m, 280 m n. m., 50,15725°N, 15,392501°E, Turboveg č. 551375 (12. VIII. 2006 J. Chlumský et J. Svobodová); Hoděšovice, ca 130 m SV od hájovny Bažantnice,

smrčina u křižovatky lesních cest, tisíce jedinců, dále populace pokračuje po 150 m S, 280 m n. m., 50,15798°N, 15,92571°E (12. VIII. 2006 J. Chlumský et J. Svobodová); Hoděšovice, ca 100 m SZ od dřevěny v bývalé osadě Mazurovy chalupy, stovky rostlin, dříve možná větší populace, ohrožení: mýcení (12. VIII. 2006 J. Chlumský et J. Svobodová). – Hoděšovice, u silnice ca 480 m Z od obce, ca 120 m od V okraje lesa, stovky rostlin, 300 m n. m., 50,14710°N, 15,90948°E, Turboveg č. 551376 (12. VIII. 2006 J. Chlumský et J. Svobodová). – Vysoké Chvojno, Buky u Vysokého Chvojna, 1,8 km Z od hájovny v Jezovinách po straně hlavní silnice u plotu a za plotem lesnických školek, stovky rostlin v úseku ca 150 m, 300 m n. m., 50,13634°N, 16,00045°E (11. VIII. 2006 J. Chlumský et J. Svobodová). – Suté břehy [Týniště nad Orlicí], rozcestí Suté břehy u zlomu terasy nad nivou Orlice, stovky rostlin kolem celého rozcestí, 250 m n. m., 50,15732°N, 16,02848°E, Turboveg č. 551371 (11. VIII. 2006 J. Chlumský et J. Svobodová). – Nová Ves [Týniště nad Orlicí], při silnici 680 m V od hájovny v Jezovinách, několik desítek rostlin v příkopu a na okraji lesa u silnice, 300 m n. m., 50,13796°N, 16,03532°E, Turboveg č. 551372 (11. VIII. 2006 J. Chlumský et J. Svobodová). – Dolní Jelení, ca 500 m VJV od osady Rousínov na okrajích silnice, tisíce rostlin roztroušeně kolem cesty, ostrůvkovitě větší porosty, ohrožení: místy zarůstá *Pteridium aquilinum*, 285 m n. m., 50,04659°N, 16,12945°E, Turboveg č. 551373 (12. VIII. 2006 J. Chlumský et J. Svobodová); Dolní Jelení, 3,8 km V(J)V od kostela v Horním Jelení a 1,4 km SZ od nádraží v Plchůvkách, při lesní cestě na Plchůvky, několik desítek rostlin roztroušených v borůvčí, 300 m n. m., 50,04517°N, 16,14651°E (12. VIII. 2006 J. Chlumský et J. Svobodová); Dolní Jelení: 2,4 km VJV od křižovatky v Dolním Jelení, u silnice z Dolního Jelení do osady Prochody, asi 20 rostlin rozptýlených v borůvčí, 300 m n. m., 50,04507°N, 16,14081°E (12. VIII. 2006 J. Chlumský et J. Svobodová); Dolní Jelení, ca 1 km VJV od osady Rousínov na okraji silnice, stovky rostlin, 290 m n. m., 50,04603°N, 16,13269°E (12. VIII. 2006 J. Chlumský et J. Svobodová). – Dobříkov [Vysoké Mýto], na 900 m SSV od kostela v obci na okraji lesní cesty, stovky rostlin po obou

stranách lesní cesty ve smrkovém lese, 320 m n. m., 50,00888°N, 16,14135°E (12. VIII. 2006 J. Chlumský et J. Svobodová); Dobříkov [Vysoké Mýto], ca 0,5 km SSZ od kostela v obci, SZ roh nesekané stráně na okraji lesa, několik desítek rostlin, 310 m n. m., 50,00751°N, 16,13196°E, Turboveg č. 551374 (12. VIII. 2006 J. Chlumský et J. Svobodová). **67 Českomoravská vrchovina:** Nyklovice, u lesní cesty ca 1,8 km VJV od křižovatky v obci, svah nad levým břehem Hodonínky (Nyklovického potoka), stovky rostlin na okraji smrkového lesa v úseku ca 35 m, 620 m n. m., 49,60185°N, 16,37135°E, Turboveg č. 551386 (21. VIII. 2006 J. Chlumský et J. Svobodová). – Kněževes [Olešnice u Kunštátu], ca 1 km SSZ od kaple v obci na SZ svahu Kavinského potoka při lesní cestě se žlutou tur. značkou, desítky až stovky rostlin na ploše ca 600 m², 530 m n. m., 49,59475°N, 16,41799°E, Turboveg č. 551364 (7. VIII. 2006 M. Štech, Jan Košnar, J. Svobodová, J. Chlumský et T. Peterka); Kněževes [Olešnice u Kunštátu], asi 760 m SZ od kaple v obci, při lesní cestě na mýtině s výsadbou mladých modřínů a na okrajích lesní cesty, tisíce rostlin na ploše ca 450 m², 530 m n. m., 49,59178°N, 16,41661°E (7. VIII. 2006 M. Štech, Jan Košnar, J. Svobodová, J. Chlumský et T. Peterka). – Trpín [Olešnice u Kunštátu], 900 m JJZ od kostela v obci, JZ okraj lesa na vrchu Fouska, tisíce rostlin v březovém háji s přimíšeným smrkem a místy v lemu mezi lesem a polem, ohrožení: subpopulace na okraji pole zarůstají *Calamagrostis epigejos*, 640 m n. m., 49,58455°N, 16,39945°E, Turboveg č. 551365 (7. VIII. 2006 J. Chlumský et J. Svobodová). – Olešnice u Kunštátu, 1,7 km J od kostela v obci Trpín nad levým břehem Hodonínky, u lesní cesty nad býv. lomem u silnice Olešnice – Nyklovice, stovky rostlin na ploše ca 200 m² a další populace o 100 m J, několik desítek rostlin, 600 m n. m., 49,57538°N, 16,40224°E, Turboveg č. 551388 (21. VIII. 2006 J. Chlumský et J. Svobodová). – Velké Tresné, ca 800 m JJV od křižovatky v obci, v údolí Tresného potoka na terase nad potokem na kraji lesní cesty, stovky rostlin na okraji smrkového lesa na ploše ca 60 m², ohrožení: zarůstání mladými smrkem, 540 m n. m.,

49,56416°N, 16,38700°E, Turboveg č. 551387 (21. VIII. 2006 J. Chlumský et J. Svobodová).

Slovensko

Obvod eupanónskej xerothermnej flóry (Eupannonicum):

4 Záhorská nížina: Šaštín – Stráže, ca 2 km JJZ od žel. zastávky v obci, doubrava u silnice v blízkosti hájovny, niekoľik desítek rastlin v mladé doubravě s borovicí, 200 m n. m., 48,61874°N, 17,13850°E, Turboveg č. 551397 (25. VIII. 2006 J. Chlumský). – Borský Mikuláš, ca 3 km Z od obce, bor J od silnice do Šaštína, desiatitisíce rastlin v řídkém boru na jemném písku na ploše ca 4 ha, 180 m n. m., 48,63115°N, 17,17033°E, Turboveg č. 551398 (25. VIII. 2006 J. Chlumský). – Šajdíkové Humence, ca 180 m V od kostela v obci ve světlém boru s duby a na jeho okraji, plošně v lese mezi obcí a pískovnou desiatitisíce až statisíce rastlin, 200 m n. m., 48,65257°N, 17,27207°E, Turboveg č. 551399 (25. VIII. 2006 J. Chlumský); Šajdíkové Humence, ca 400 m SV od kostela v obci, okraj boru podél panelové lesní cesty, tisíce až desiatitisíce rastlin, ohrožení: zarůstá *Calamagrostis epigejos* a výmladky *Robinia pseudacacia*, 200 m n. m., 48,65409°N, 17,27477°E (25. VIII. 2006 J. Chlumský); Šajdíkové Humence, bor 450 m JJV od kostela mezi obcí a pískovnou, tisíce až desiatitisíce rastlin, 200 m n. m., 48,64954°N, 17,27324°E (25. VIII. 2006 J. Chlumský). – Malacky, u lesní cesty 170 m SV od býv. hájovny Červený Kríž (momentálně vojenský objekt), desítky rastlin roztroušeně kolem lesní cesty v borovém lese v úseku ca 250 m, ohrožení: populace značně zarůstá *Calamagrostis epigejos*, 200 m n. m., 48,47697°N, 17,05714°E, Turboveg č. 551400 (25. VIII. 2006 J. Chlumský). – Malacky, světlý borový les JV od silnice ca 0,7 km JZ od býv. hájovny Červený Kríž (momentálně vojenský objekt), stovky rastlin roztroušeně v borovém lese, 185 m n. m., 48,472071°N, 17,053008°E, (10. VII. 2009 M. Štech et T. Štechová).

Příloha 2. – Přehled historicky uváděných lokalit *M. bohemicum*, na kterých se výskyt nepodařilo ověřit. Popis lokalit je přejat doslovně z článku Hadače (1966).

Appendix 2. – Summary of unsuccessfully revised historical localities of *M. bohemicum*. Description of localities follows the paper by Hadač (1966).

Česká republika

Termofytikum:

13a Rožďalovická tabule: V lese ohrada u Vchynic, ca 213 m (Košťál in Čelakovský 1890, MP) **15b Hradecké Polabí:** Ad marginem silvae Horka supra Přepychy, ca 320 m (Rohlena 28. 8. 1927 PR, BRNU). **15c Pardubické Polabí:** Mezi Kladruby a Týncem n. L. v borobřezovém lese na písčité půdě ca 250 m n. m. (Čelakovský 1873). – Bor u Živanic (E. Hadač, MP). – Lesy u Bohdanče (Jahn, BRNU); les Horka u Bohdanče, ca 230 m (J. et E. Hadač 1948, E. Hadač 1966). – U hájovny jv. od Strášova 18. 6. 66 (E. Hadač). – Bělobranská dubina (při silnici Pardubice – Sezemice), 220 m, s *Melampyrum vulgatum*. (J. et E. Hadač 1948, MP). – Štěpánovsko, ca 255 m (Rohlena 1923). – Les nad Starými Holicemi, 320 m (Košťál in Rosůlek 1903); pravděp. totožné s lokalitou: „Holice, v obecním lese“, 29. 7. 1899 Tocl, PR, BRNU, resp. „Holice, při lesní cestě k Borohrádku“ (30. 8. 1897 Tocl PR).

Mezofytikum:

61b Týnišťský úval: Horecká stráž jihovýchodně od Čestic (Mikyška apud Hadač 1966). **61c Chvojenská plošina:** Kóta 284 m mezi háj. Dva Šraňky a Bělečkem (Mikyška apud Hadač 1966). – Les u os. Borek (J. Hadač MP). – Albrechtice, ca 260 m (Rohlena 1928, J. Hadač MP), les Lípava u A. (Mikyška apud Hadač 1966). **60 Orlické opuky:** U Potštýna, ca 300 m, 8. 1896 (Čelakovský fil. PR). **67 Českomoravská vrchovina:** V borech u Rovečného („Rovečín“), ca 600 m, 8. 1878 (Fleischer PR). – Olešnice: u lomu při silnici k Crhovu, 550 m, druhotná smrčina (E. Hadač).

Slovensko

Obvod eupanónskej xerothermnej flóry (Eupannonicum):

4 Záhorská nížina: Studienka („Hasprunka“), v písčitém boru 17. 8. 1946 (Holzknecht, BRNU). – Pod Bozajvrškom juhových. Mikulášova, 225 m, 16. 8. 1965 (Hadač). – Šajdíkové Humence: poľesí Hrušov odd. 71d (Růžička 1964) 185–206 m n. m. – Plavecký Štvrtok (Holuby sec. Soó 1927), ca 170 m n. m.



Chapter IV

Roles of species-preferential seed dispersal by ants and endozoochory in *Melampyrum* (Orobanchaceae)

Chlumský J, Koutecký P, Jílková V and Štech M (2013): *Journal of Plant Ecology* 6: 232–239

Photo on previous page: One plot from dispersal experiment with *Formica polyctena* ants and *Melampyrum* seeds

Roles of species-preferential seed dispersal by ants and endozoochory in *Melampyrum* (Orobanchaceae)

Abstract

Aims

Melampyrum pratense and *M. subalpinum* are two myrmecochorous species, which possess similar habitat requirements and frequently occur together. Despite this, their population sizes differ markedly. *Melampyrum pratense* populations are usually very large, whereas *M. subalpinum* has rather small and isolated populations. We suggest that such an imbalance might be partially influenced by the difference in ant-mediated seed-removal rates.

Genus *Melampyrum* is considered to be exclusively myrmecochorous, though to achieve the recent distribution of some *Melampyrum* species during the Holocene myrmecochory would be highly insufficient. We suggest that endozoochory takes place in the long-distance migration, whereas myrmecochory is important for the removal of seeds on a local scale.

Methods

For seed-preference analysis, *M. pratense* and *M. subalpinum* mixed seed samples were placed around *Formica polyctena* anthills. After a period of time, the remaining seeds of both species were counted for each sample. The results were analysed by analysis of variance and generalized linear mixed-effect model. To test myrmecochorous removal distances, *M. pratense* seeds were covered with fluorescent dactyloscopic powder and placed in the vicinity of a large ant trail. The area around the starting plot was searched in the dark using UV LED torchlight 7 h after the beginning. The distance from the starting plot was measured for each seed found. Birds, rodents, leporine and a ruminant were fed with *M. pratense* seeds and fresh plants to test the possibility of endozoochorous dispersal of the species. Animal droppings were searched for intact seeds.

Important Findings

Our field studies show that from mixed seed samples, containing both species, ants significantly preferred the seeds of *M. pratense*. This may be one of factors that has positive influence on *M. pratense* success in seed dispersal on mixed stands and consequently in the colonization of favourable sites. Experiments focusing on ant-mediated dispersal

distance revealed that *F. polyctena* ants are able to move seeds over a distance of 36 m in only 7 h. This distance is among the furthest known myrmecochorous removals of forest plant seeds. A new *Melampyrum* seed disperser *Oligolophus tridens* (Opiliones) was observed repeatedly. Our pilot study documented that *Melampyrum* seeds are able to pass through the digestive tract of a cow intact. This suggests that large ruminants such as deer, bison or forest-grazing livestock may function as important longdistance dispersers of *Melampyrum* species.

Keywords: endozoochory, *Melampyrum*, myrmecochory, Orobanchaceae, seed dispersal

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Chapter V

Is genetic diversity congruent with morphological diversity across the distributional range of the *Melampyrum subalpinum* group (Orobanchaceae)?

Chlumský, J, Koutecký, P, Plačková, I and Štech, M (2016): *Flora* 220: 74–83.

Photo on previous page (M. Štech): *Melampyrum subalpinum*

Is genetic diversity congruent with morphological diversity across the distributional range of the *Melampyrum subalpinum* group (Orobanchaceae)?

Abstract

The *Melampyrum subalpinum* group consists of morphologically diverse populations traditionally treated as closely related taxa with patchy distribution limited to Central Europe. The centre of the morphological variability and geographical distribution of the group lies on the north-eastern edge of the Alps in the Vienna Forest, while marginal, morphologically uniform populations occur in the Czech Republic and Slovakia. Genetic variation and population structure within the distribution range of the group remains unknown; we hypothesise that the marginal populations are genetically depauperate. Allozymes were used to assess the genetic structure of 27 populations present throughout the distribution area; four *Melampyrum nemorosum* populations from the Vienna Forest were also analysed because of the presumed hybridization. An artificial pollination experiment was carried out to examine the possibility of autogamy. Four enzyme systems were clearly resolved and scored for one polymorphic locus each with a total of 20 alleles.

Seven out of 49 flowers with preserved stamens developed seeds after self-pollination. Genetic variation was generally congruent with the known pattern of the morphological variation of the group. The allelic richness was higher in the Austrian populations than in marginal Czech and Slovak populations. Some wide-leaved populations from the Vienna Forest had a rather high number of alleles which may be caused by allelic enrichment due to former hybridization with *M. nemorosum*. Czech and Slovak populations are genetically derived from Austrian populations. The high differentiation among populations suggests that the current gene flow between populations is limited. The high inbreeding coefficient in some populations indicates that there is a certain level of selfing within the populations. The pollination experiment does not contradict the possibility of autogamy. In general, our data are congruent with the central-marginal model with more variable Austrian populations and less variable isolated and probably partly inbreeding Czech and Slovak populations.

Keywords: allozymes, population genetics, hemiparasites, marginal populations

Introduction

Genetic variation of a species can reveal historical events connected with postglacial colonization (Wróblewska, 2008; Chung et al., 2013), historical and present population size changes (Leimu et al., 2006), the impact of environmental factors and anthropogenic disturbances, as well as the species breeding system (Aparicio et al., 2002; Leimu and Mutikainen, 2005), hybridization (Phillipp and Siegismund, 2003), and polyploidization (Rosenbaumová et al. 2004). High levels of variability are seen as healthy, conferring the ability to respond to threats such as disease, parasites and predators, and environmental changes (Amos and Hardwood, 1998). A number of studies has stated that small and scattered peripheral populations tend to be less variable than populations from the centre of distribution of the particular species. The peripheral populations can face the negative effects of inbreeding, genetic drift and the lowered genetic variation caused by bottleneck and founder effects (Lynch et al., 1995; Young et al., 1996; Tomimatsu and Ohara, 2003; Leimu et al., 2006; Chung et al., 2013). However, in some cases small limited populations do not seem to be influenced by these negative effects (Gitzendanner and Soltis, 2000; Mandák et al., 2005; Wróblewska, 2008).

The *Melampyrum subalpinum* group consists of morphologically diverse populations of annual hemiparasitic plants traditionally treated as closely related taxa with a distribution area reaching from the Eastern Alpine foothills to the north of the Czech Republic and the south-western part of Slovakia (cf. Fig. 1). Following the conventional taxonomic concept, at least four taxa are recognized. The centre of morphological diversity lies in the Vienna Forest (Wienerwald), Austria (Štech, 2006). Plants from this region, traditionally designated as *M. subalpinum* (Jur.) A. Kern. s. str. (e.g., Beck, 1882, 1893), have rather wide leaves and bracts, intensively blue-coloured bracts, and relatively dense indumentum on calyx and bracts. These morphotypes are labelled as *M. subalpinum* var. *thermale* in the current Austrian literature (Fischer et al., 2008; Staudinger, 2009). Less variable populations with narrow leaves and bracts and sparse indumentum occur in an area reaching from the Vienna Forest towards the Lower Austria/Styria Alps. Traditionally they are recognized as *M.*

angustissimum Beck but in recent Austrian literature the name *M. subalpinum* var. *subalpinum* is used (Fischer et al., 2008; Staudinger, 2009). Morphologically very similar and very uniform marginal populations known from the Czech Republic and Slovakia are traditionally labelled as *M. bohemicum* A. Kern. (Hadač, 1966). However, based on a comprehensive investigation of morphological variation, Štech (2006) considers these populations conspecific with *M. angustissimum*. The early flowering morphotypes of *M. angustissimum* restricted to the higher altitudes of the Alps are described as *M. grandiflorum* A. Kern. Seasonality is a common phenomenon in the genus *Melampyrum* as well as in related hemiparasitic species and genera (Soó, 1926 Soó, 1926-1927; Zopfi, 1993a,b, 1997) and it does not seem to be systematically important because the early flowering plants are quite rare and it is not possible to analyse them separately. Therefore, in this study one early flowering *M. grandiflorum* population (MarsA) is included in the narrow-leaved group together with *M. angustissimum*.

Genetic variation and population structure within the whole distribution range of the group remains unknown. According to the group's morphological variability and geographical distribution, Štech (2006) presumes that the centre of genetic diversity of the group lies on the north-eastern edge of the Austrian Alps in the Vienna Forest. The marginal morphologically uniform populations from the Czech Republic and Slovakia are expected to be genetically less variable. Some of the marginal populations are at present declining; whether this is due to a loss of genetic diversity in these small isolated populations remains unknown.

Allozymes are reliable and often used markers for the study of population genetic structure (e.g., Hamrick et al., 1981; Phillipp and Siegismund, 2003; Chrtek and Plačková, 2005; Chung et al., 2013). They are easily detected codominant markers which allow (compared to dominant markers, such as AFLP) to calculate for example allelic frequencies or standard population genetic parameters based on heterozygosity (F-statistics, deviations from the Hardy-Weinberg equilibrium). No previous genetic knowledge of the species is required, large numbers of individuals can be analysed at one time for multiple enzymes, and methods of data interpretation and analysis are well developed (Lowe et al., 2004).

In the study we addressed the following questions

(1) What is the extent and pattern of allozyme variation in the *M. subalpinum* group? (2) Is the level of genetic variation of the *M. subalpinum* group correlated with its morphological variation across the whole distributional range? (3) Are marginal isolated populations in the Czech Republic threatened by a loss of genetic variation? (4) Is it possible to appraise any colonization history events of the *M. subalpinum* group in Central Europe?

Methods

Sampling

Sampled populations of the *M. subalpinum* group were chosen to cover the whole distributional range and all morphotypes. Sampling was thorough especially in the Vienna Forest (esp. broader surroundings of Baden) with morphologically diverse populations (Fig. 1, Table 1). Outside of the Alps and Vienna Forest, all regions in which *M. subalpinum* occurred are represented by at least one population. Because of the assumed former hybridization of *M. subalpinum* with *M. nemorosum* in the area of Vienna Forest, 4 local populations of *M. nemorosum* were also included. From each population 10 randomly selected plants were collected and in total 31 populations were sampled. A sample from each plant contained approximately 8 fresh leaves or bracts. Upon collection leaves were wrapped in wet tissues and stored in plastic bags on ice until extraction. Voucher specimens from all sampled populations are deposited in CBFS herbarium in České Budějovice (Czech Republic).

Extraction

Extraction was carried out within 24 h of collection. Approximately 80 mg of leaf tissue was ground in ice-cold Tris–HCl extraction buffer with the addition of a small amount of DOWEX 1 × 8–100 (Cl) and quartz sand. The extraction buffer contained 0.1 M TRIS–HCl pH 8.3, 1% (w/v) l-glutathione reduced, 10 mM MgCl₂·6H₂O, 5% (w/v) sucrose and 0.1% (v/v) 2-mercaptoethanol. Crude homogenates were centrifuged for 10 min at 15,000 rpm. Clear supernatant was stored in deep freeze at –75°C.

Table 1: Sampled populations with description of locality, altitude, geographical coordinates, date of sampling and approximate number of plants per population.

Population code	Locality	Altitude (m a. s. l.)	Coordinates (WGS84)	Date of collection	Size of the population (order)
Czech and Slovak populations					
DoubB	Doubí u Tábora: forest near the road 230 m SSW of the railway station	410	49°19'8.8"N 14°43'1.7"E	26.8.2008	10 ² - 10 ³
KnezB	Kněževés: forest along the path ca 0.7 km NW of the village	550	49°35'30.4"N 16°24'59.8"E	7.8.2006	10 ³
MajdB	Majdaléna: forest edge along the path 2.5 km NNW of the village	445	48°59'4.8"N 14°50'39.7"E	30.8.2005	10 ²
PribB	Příběnice: forest around of the castle ruin of Příběnice	420	49°23'32.8"N 14°33'44.8"E	5.9.2006	10 - 10 ²
SastB	Šaštín: pine forest near the road to Borský Mikuláš 1.4 km ESE of the village	185	48°37'52.3"N 17°10'12.8"E	29.8.2006	10 ² - 10 ³
SoprB	Sopreč: forest near the crossroad 1.8 km SSE of the village	230	50°4'42.9"N 15°32'46.3"E	5.9.2006	10 ³
TyniB	Křivice: forest 1.2 km WNW of the village	280	50°11'1.7"N 16°5'48.4"E	5.9.2006	10 ³
ZehrB	Žehrov: forest near the road 0.9 km SE of the village centre	280	50°31'22.8"N 15°6'23.5"E	5.9.2006	10 ²
Austrian narrow-leaved populations					
HernA	Hernstein: forest margin along the road to Neusiedel 1.6 km NNW of the village	390	47°54'26.6"N 16°5'41.2"E	24.6.2008	10 ³
HirsA	Hirschwang an der Rax: forest above the Höllental road 1.9 km NNW of the village	525	47°43'20.9"N 15°48'15.9"E	21.8.2008	10 ²
HollA	Höllental: forest margin and shrubs near the mouth of the G. Kesselgraben valley	580	47°45'9.6"N 15°44'55.5"E	23.6.2008	10 ³
KaltA	Baden, Kaltenberger Forst: pine forest along the path on the northeast slope of the Soosser Lindkogel 5 km W of the town	590	47°59'46.2"N 16°9'48.0"E	21.8.2008	10 ²
KranA	Kranichberg: hazel shrubs SE of the castle of Kranichberg	660	47°38'38.6"N 15°58'31.4"E	23.6.2008	10 ³

Population code	Locality	Altitude (m a. s. l.)	Coordinates (WGS84)	Date of collection	Size of the population (order)
LangA	Sooß: forest along the path on the ridge southward of the Langer Graben valley	430	47°59'1.5"N 16°11'19.7"E	21.8.2008	10 ²
LoseA	Losenheim: forest along the path 200 m N of the castle ruin of Losenheim	760	47°47'26.8"N 15°50'40.6"E	30.8.2005	10 ³
MarsA	Maria Schutz: spruce forest margin along the road 0.5 km ESE of the Mariaschutz church	730	47°38'25.0"N 15°52'32.9"E	23.6.2008	10 ³
MiesA	Miesenbach: forest margin along the road 2.5 km N of the village	440	47°51'45.7"N 15°59'22.0"E	21.8.2008	10 ² - 10 ³
PeutA	Peutenberg: pine forest above the path 0.3 km WNW of the Peutenberg railway station	430	47°57'20.5"N 15°9'15.1"E	29.8.2006	10 ²
SemmA	Semmering: forest along the road 250 m N of the Hotel Panhans	1030	47°38'24.4"N 15°49'37.3"E	29.8.2006	10 ⁴
SlinA	Sooß: forest along the path 0.3 km S of the Soosser Lindkogel hilltop	640	47°59'19.2"N 16°9'42.0"E	21.8.2008	10 ²
Austrian wide-leaved populations					
Ein2S	Einöde: forest and shrubs along the road to Gaaden 750 m NW of the village centre	320	48°01'29.7"N 16°13'41.8"E	21.8.2008	10 ³
EinoS	Einöde: forest and shrubs along the road to Gaaden 600 m NW of the village centre	320	48°01'25.6"N 16°13'47.0"E	30.8.2005	10 ³
GumpS	Gumpoldskirchen: open forest on the rock outcrops ca 500 m WNW of the village	420	48°2'45.4"N 16°15'40.9"E	29.8.2006	10 ²
HofsS	Rohrbach, Hofstätten: shrubs (Corylus, Carpinus) along the road to the hermitage Hofstätten	420	47°59'34.4"N 16°6'17.5"E	30.8.2005	10 ³
MerkS	Merkenstein: forest above the castle ruin of Merkenstein	450	47°58'58.6"N 16°8'0.3"E	29.8.2006	10 ² - 10 ³
RichS	Gumpoldskirchen, Richardhof: shrubs and forest edge along the road 300 m NNE of the Hotel Richardhof	360	48°3'28.2"N 16°16'34.9"E	21.8.2008	10 ³

Population code	Locality	Altitude (m a. s. l.)	Coordinates (WGS84)	Date of collection	Size of the population (order)
SoosS	Soosß: shrubs along the road 1.4 km W of the centre of the village centre	310	47°59'15.0"N 16°11'53.7"E	21.8.2008	10 ³
<i>M. nemorosum</i> populations					
GaadN	Rosental: shrubs along the road ca 1.7 km SSE of the village	260	48°1'4.0"N 16°11'28.2"E	24.6.2008	10 ³
GiesN	Gießhübl, Tirolerhof - Siedlung: forest on the western edge of the settlement	420	48°6'12.6"N 16°14'2.6"E	30.8.2005	10 ⁴
HofsN	Bad Vöslau, Großau: forest along the road ca 4.3 km WNW of the village Großau	380	47°58'48.0"N 16°7'20.0"E	30.8.2005	10 ²
RotkN	Rotes Kreuz: forest margin ca 2 km SSE of the village Gaaden near Rotes Kreuz crossroad	400	48°2'8.3"N 16°12'34.8"E	29.8.2006	10 ³

Electrophoresis

The extracts (30 µl per sample) were subjected to electrophoresis in vertical polyacrylamide gel slabs (Hoefler SE 600 vertical unit) using separating gel (8.16%) with 1.82 M Tris–HCl buffer, pH 8.9, and stacking gel (4%) with 0.069 M Tris–H₃PO₄ buffer, pH 6.9. Electrode buffer was 0.02 M Tris, 0.24 M glycine, pH 8.3. Ice-refrigerated electrophoresis was carried out by applying a pulsed current at 80 mA for ca. one hour (until the front of samples left the stacking gel) and subsequently at 100 mA for 3 h and 15 min. The following 18 enzymes were tested with a focus on well stained and clearly interpretable zymograms exhibiting some variability: SKDH (1.1.1.25), ADH (1.1.1.1), SOD (1.15.1.1), AAT (2.6.1.1), ENP (3.4.23.6), PRX (1.11.1.7), MDH (1.1.1.37), IDH (1.1.1.42), GDH (1.4.1.2), ACP (3.1.3.2), HEX (2.7.1.1), 6-PGDH (1.1.1.44), PGM (2.7.5.1), PGI (5.3.1.9), DIA (1.6.-.-), LAP (3.4.11.-), EST (3.1.1.-), G-6-PDH (1.1.1.49). From these enzymes 4 were chosen for further analysis (shikimic acid dehydrogenase SKDH, alcohol dehydrogenase ADH, superoxide dismutase SOD and endopeptidase ENP). The other tested enzymes were either invariable or did not provide zymograms of the required quality.

Staining

The staining procedures followed Vallejos (1983) to visualize ADH and Wendel and Weeden (1989) for ENP, SOD and SKDH with the following

modifications. The SOD staining ingredients were 50 ml of 0.05 M Tris-HCl (pH 8.2), 5 mg of NBT, 4.5 mg of EDTA and 1.5 mg of riboflavin. The gel was incubated in the dark at 37°C for 20 min and then placed under lamp light until bands appeared on the dark background. The SKDH ingredients were 30 ml of 0.1 M Tris-HCl (pH 8.4), 30 mg of shikimic acid, 5 mg of NADP, 6 mg of MTT and 1 mg of PMS. The gel was incubated in the dark at 32°C until bands appeared. Ingredients for ENP for solution A were 50 ml 0.2 M Tris-maleic acid (pH 5.5) and 50 ml for rinsing of the gel, 20 mg of Fast Black K salt and 50 mg of MgCl₂·6H₂O. Ingredients for solution B were 2 ml *N,N*-dimethylformamide and 25 mg BANA. Solution A was poured into solution B in the dark. The gel was rinsed in chilled Tris-maleic acid and then incubated in a mixture of solutions A and B at 37°C until bands appeared. ADH staining ingredients for solution A were 40 ml 0.1 M Tris-HCl (pH 7.5), 15 mg NAD, 10 mg MTT and 1 mg PMS. Solution B was 10 ml of chilled ethanol. Solution A was poured over the gel and left to incubate at 32°C. After 3 min solution B was added. If the gel was not sufficiently stained, more ethanol was added after 1 h of incubation. All gels were rinsed in distilled water and wrapped in two cellophane sheets and dried.

Data analysis

Zymograms were scored according to Soltis and Soltis (1989). One variable and easily interpretable locus was chosen in each enzyme system. Within this locus in monomeric SKDH and ENP zymograms alleles were numbered with increasing migration distance from the origin. In dimeric SOD and ADH zymograms heterozygotes possess 3 bands. From these only outer bands were scored as alleles whereas the middle (heterodimeric) band was not considered as an allele. Occasionally occurring secondary bands with notably lower intensity were not scored as alleles. Allele frequencies, percentage of polymorphic loci (P), mean number of alleles (A), mean effective number of alleles (A_e), observed (H_o) and expected (H_e) heterozygosity and fixation index (F_{st}) were calculated using POPGENE version 1.31 (Yeh et al., 1999). Coefficients of inbreeding (F_{is}) were calculated using FSTAT version 2.9.3.2 (Goudet, 1995); their significance (assuming the null hypothesis of $F_{is} = 0$) was tested by a permutation test with 1000 replicates. A dendrogram based on Nei's genetic distances between populations (Nei, 1972) was generated using POPGENE. Nei's genetic distances were used as a metric

in a Principal Coordinate Analysis, which was calculated using Canoco version 5.01 (ter Braak and Šmilauer, 2012).

To determine if genetic distances were correlated with geographic distances, a Mantel test (Mantel, 1967) was performed for different subsets of the studied populations (all populations of *M. subalpinum* agg.; Czech, Slovak, and Austrian narrow-leaved; all Austrian; Austrian narrow-leaved; Austrian wide-leaved; only Czech and Slovak populations). Correlation coefficients between matrices of Nei's genetic distances of populations and their geographical distances were calculated using zt software (Bonnet and van de Peer, 2002); significance of the correlation coefficients was tested by a permutation test with 10,000 replicates.

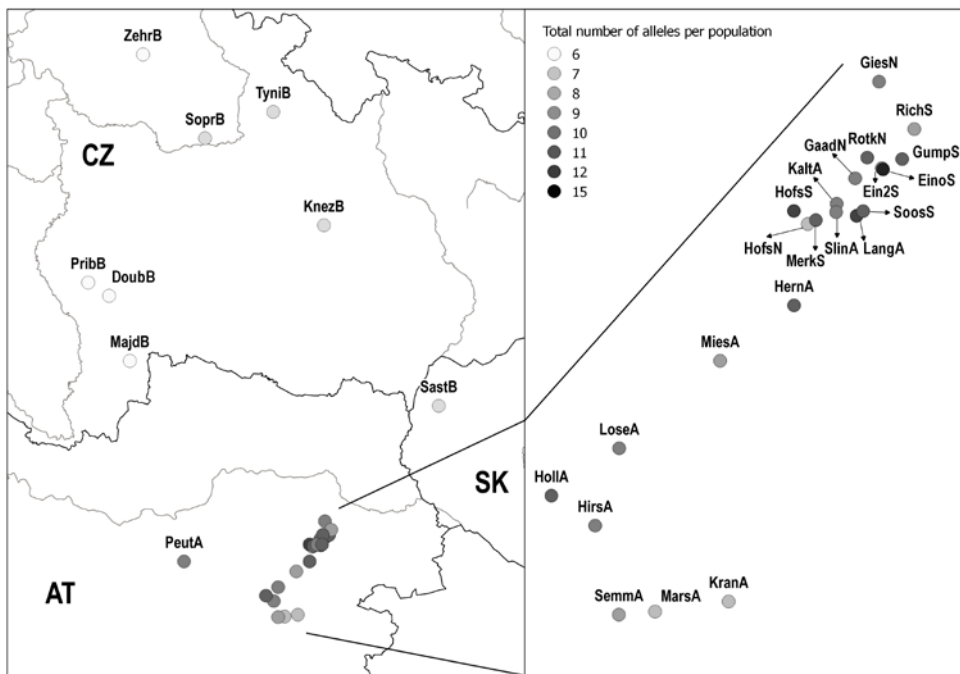


Fig.1. Sampled populations of the *M. subalpinum* group with the total number of alleles per population represented by different shades of grey.

Reproduction experiment

A pollination test was carried out on the Czech DoubB population. Very young unopened flowers on several plants were used for the test. Flowers were alternately either castrated and pollinated a few days later with pollen from another plant (allogamy), not castrated and left to self-pollinate (autogamy) or only castrated (control). After the treatment, the

experimental plants were covered with monofilament sacks to prevent access by pollinators. The flowers were visually checked for the formation of capsules and seeds (well formed × aborted/defective × absent).

Results

Allozymes

For SOD two loci were detected, but only the faster locus provided clear and variable pattern (dimeric, 2 alleles). For ADH two loci were detected as well and only the faster locus was variable and clear enough for scoring (dimeric, 7 alleles). SKDH yielded one monomeric locus with 7 alleles and ENP had one monomeric locus with 4 alleles.

The total number of alleles per population ranged from 6 to 7 in Czech and Slovak populations, 8 to 12 in narrow-leaved Austrian populations and 9 to 15 in wide-leaved populations from the Vienna Forest (Fig. 1). The highest number of alleles (15) was detected in the EinoS population which is morphologically close to *M. nemorosum*. The number of alleles in *M. nemorosum* populations ranged from 8 to 11.

The rarest alleles were ADH 7 with a frequency of 0.002 (LoseA population), SKDH 7 with a frequency of 0.005 (HirsA population), ADH 1 with a frequency of 0.006 (EinoS and HollA populations) and ADH 4 with a frequency of 0.032 (EinoS and SastB populations). The most frequent alleles were ENP 2 with an overall frequency of 0.640 and SOD 2 and ADH 6 with a frequency of 0.613. These alleles were present in most or all of the populations including some *M. nemorosum* populations. Apart from rare alleles that occurred only in one or two populations, we found a unique allele (ADH 5) that occurred only in *M. nemorosum* populations and one wide-leaved *M. subalpinum* population (EinoS) that is morphologically close to *M. nemorosum*. The SKDH 6 allele was found only in 3 wide-leaved *M. subalpinum* populations morphologically close to *M. nemorosum*, but not in any *M. nemorosum* population. Allele ADH 2 occurred only in Austrian populations, both wide- and narrow-leaved, from the Vienna Forest region and in one *M. nemorosum* population, and was absent in narrow-leaved populations from the Alps and Czech and Slovak populations. The ADH 3 allele was very common in both Austrian *M. subalpinum* morphotypes, however it also occurred in one *M. nemorosum* population and in one isolated Czech *M. subalpinum* ZehrB population. The ENP 4 and SKDH 5 alleles occurred in wide- and narrow-leaved populations, both Austrian and Czech, but did not occur in *M.*

Table 2: Allelic frequencies at four polymorphic loci for each population. Alleles are numbered for each enzyme system/locus.

Population	Locus/allele																			
	SOD		SKDH				ENP					ADH								
	1	2	1	2	3	4	5	6	7	1	2	3	4	1	2	3	4	5	6	7
HofsN	0.850	0.150	0.000	0.050	0.400	0.550	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.444	0.556	0.000
GiesN	1.000	0.000	0.050	0.050	0.450	0.450	0.000	0.000	0.000	0.500	0.300	0.200	0.000	0.000	0.000	0.000	0.000	0.350	0.650	0.000
GaadN	0.950	0.050	0.150	0.000	0.200	0.650	0.000	0.000	0.000	0.150	0.650	0.200	0.000	0.000	0.000	0.000	0.000	0.750	0.250	0.000
RotkN	0.950	0.050	0.100	0.000	0.100	0.800	0.000	0.000	0.000	0.000	0.950	0.050	0.000	0.000	0.050	0.200	0.000	0.250	0.500	0.000
EinoS	0.900	0.100	0.150	0.150	0.000	0.300	0.050	0.350	0.000	0.000	0.650	0.000	0.350	0.050	0.550	0.150	0.100	0.100	0.050	0.000
Ein2S	0.850	0.150	0.300	0.000	0.000	0.250	0.000	0.450	0.000	0.000	0.500	0.200	0.300	0.000	0.389	0.000	0.000	0.000	0.611	0.000
HofsS	0.550	0.450	0.150	0.150	0.000	0.000	0.250	0.450	0.000	0.000	0.900	0.050	0.050	0.000	0.389	0.167	0.000	0.000	0.444	0.000
GumpS	0.250	0.750	0.800	0.000	0.100	0.000	0.100	0.000	0.000	0.056	0.611	0.333	0.000	0.000	0.050	0.600	0.000	0.000	0.350	0.000
MerkS	0.650	0.350	0.150	0.000	0.350	0.100	0.400	0.000	0.000	0.000	0.750	0.050	0.200	0.000	0.000	0.550	0.000	0.000	0.450	0.000
RichS	0.200	0.800	0.000	0.000	0.650	0.000	0.350	0.000	0.000	0.000	0.800	0.000	0.200	0.000	0.050	0.400	0.000	0.000	0.550	0.000
SoosS	0.250	0.750	0.250	0.100	0.200	0.000	0.450	0.000	0.000	0.000	0.550	0.250	0.200	0.000	0.111	0.000	0.000	0.000	0.889	0.000
LoseA	0.050	0.950	0.850	0.000	0.000	0.150	0.000	0.000	0.000	0.000	0.450	0.400	0.150	0.000	0.000	0.400	0.000	0.000	0.550	0.050
PeutA	0.050	0.950	0.050	0.950	0.000	0.000	0.000	0.000	0.000	0.050	0.050	0.400	0.500	0.000	0.000	0.750	0.000	0.000	0.250	0.000
SemmA	0.000	1.000	0.600	0.100	0.300	0.000	0.000	0.000	0.000	0.000	0.550	0.300	0.150	0.000	0.000	0.250	0.000	0.000	0.750	0.000
HernA	0.400	0.600	0.450	0.100	0.000	0.000	0.450	0.000	0.000	0.000	0.600	0.350	0.050	0.000	0.050	0.250	0.000	0.000	0.700	0.000
Holla	0.050	0.950	0.750	0.000	0.050	0.000	0.200	0.000	0.000	0.000	0.700	0.200	0.100	0.150	0.000	0.150	0.000	0.000	0.700	0.000
HirsA	0.200	0.800	0.600	0.250	0.000	0.000	0.000	0.000	0.150	0.000	0.600	0.150	0.250	0.000	0.000	0.350	0.000	0.000	0.650	0.000
KaltA	0.200	0.800	0.000	0.000	0.800	0.000	0.200	0.000	0.000	0.000	0.550	0.350	0.100	0.000	0.050	0.300	0.000	0.000	0.650	0.000
KranA	0.200	0.800	0.900	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.600	0.000	0.400	0.000	0.000	0.100	0.000	0.000	0.900	0.000
LangA	0.300	0.700	0.400	0.250	0.300	0.000	0.050	0.000	0.000	0.000	0.650	0.300	0.050	0.000	0.286	0.143	0.000	0.000	0.571	0.000
MarsA	0.400	0.600	0.250	0.750	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.500	0.000	0.000	0.111	0.000	0.000	0.889	0.000
MiesA	0.150	0.850	0.950	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.800	0.150	0.050	0.000	0.000	0.450	0.000	0.000	0.550	0.000
SlinA	0.400	0.600	0.450	0.000	0.250	0.000	0.300	0.000	0.000	0.000	0.450	0.550	0.000	0.000	0.150	0.600	0.000	0.000	0.250	0.000
MajdB	0.500	0.500	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.700	0.300	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
SastB	0.300	0.700	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.950	0.000	0.050	0.000	0.000	0.000	0.900	0.000	0.100	0.000
PribB	0.150	0.850	0.950	0.000	0.000	0.000	0.050	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
SoprB	0.300	0.700	0.900	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.950	0.050	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
ZehrB	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.700	0.000	0.300	0.000	0.000	0.800	0.000	0.000	0.200	0.000
TyniB	0.200	0.800	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.667	0.111	0.222	0.000	0.000	0.000	0.000	0.000	1.000	0.000
KnezB	0.750	0.250	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.300	0.550	0.000	0.000	0.000	0.000	0.000	1.000	0.000
DoubB	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.550	0.000	0.050	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Mean	0.387	0.613	0.490	0.098	0.134	0.105	0.127	0.040	0.005	0.037	0.640	0.169	0.154	0.006	0.069	0.217	0.032	0.061	0.613	0.002

nemorosum. The SKDH 2 and 3 alleles did not occur in the Czech populations, but were present in *M. nemorosum* and wide- and narrow-leaved Austrian populations. The SKDH 4 allele occurred in all *M. nemorosum* populations and 1 wide-leaved population (MerkS) and 1 narrow-leaved Austrian population (LoseA); hence, although it is not unique for any group, it is clearly much more common in *M. nemorosum* populations than in the *M. subalpinum* agg. The allelic frequencies for each locus and each population are given in Table 2.

The effective number of alleles (A_e) per population ranged from 1.112 to 2.940 (Table 3). Among different groups (Table 4), the wide-leaved *M. subalpinum* group had the highest value ($A_e = 2.160$) and the Czech narrow-leaved group had the lowest value ($A_e = 1.340$).

Observed heterozygosity (H_o) ranged from 0.050 to 0.593 (mean 0.340), and mean expected heterozygosity (H_e) ranged from 0.092 to 0.572 (mean 0.373). The Czech and Slovak populations had the lowest H_e suggesting the lowest genetic variation. The inbreeding coefficient (F_{is}) of Czech and Slovak populations ranged from high (0.481) to very low (-0.416), which was also the lowest from all studied populations. The highest value of F_{is} was 0.570 for the Austrian LoseA population. Population characteristics are given in Table 3.

The total fixation index (F_{st}) for all populations was 0.378 which indicates a very high genetic differentiation between populations. In the case of groups of populations, the highest F_{st} was found for the Czech-Slovak group (0.514) and the lowest, but still a considerably high F_{st} was found for *M. subalpinum* group (0.224). A summary of population characteristics for the groups is given in Table 4.

The UPGMA dendrogram (Fig. 2) and the PCoA based on Nei's genetic distances showed similar population relationships. The most isolated population in UPGMA is the Austrian narrow-leaved PeutA population. The remainder of the populations clustered into two major groups. The first group contains all *M. nemorosum* populations and three *M. subalpinum* populations (EinoS, Ein2S, HofS), which are morphologically closest to *M. nemorosum*. The structure of the second major group is less clear. The remaining Austrian wide-leaved populations are dispersed between narrow-leaved populations. Most of the Czech populations are grouped together, yet the KnezB population, the Slovak SastB population, and especially ZehrB are distant from the rest of the Czech populations. These populations have a rather isolated position as seen in the PCoA analysis

(outlying *M. nemorosum* populations were excluded for better resolution). *M. subalpinum* populations morphologically close to *M. nemorosum* (EinoS, Ein2S, HofS) lie remote from the main patch of the Austrian populations with HofS being the closest (Fig. 3).

The results of the Mantel test show a significant positive correlation ($r = 0.698$, $p < 0.001$) between Nei's genetic distances and the geographic distances of the Austrian narrow-leaved populations. Correlations in the most remaining subsets of populations (all populations of *M. subalpinum* agg., Czech, Slovak, and Austrian narrow-leaved, all of the Austrian populations, and Czech and Slovak populations) are positive as well, but markedly lower and they are not, or are only weakly, significant. Weakly significant but negative correlation was revealed in Austrian wide-leaved population (Table 5).

Reproduction experiment

From 35 flowers without stamens pollinated by pollen from different plants (allogamy), 31 flowers developed into a ripe capsule, 1 developed into an aborted capsule and 3 flowers did not produce a capsule. From 49 non-pollinated flowers with preserved stamens (autogamy), 42 did not develop into a capsule and 7 flowers self-pollinated and developed capsules. From 58 non-pollinated control flowers with removed stamens 56 did not develop, 1 developed into the aborted capsule and 1 developed into a ripe capsule.

Discussion

The central-marginal concept claims that within-population genetic diversity declines and among-population differentiation increases from the centre of the species' geographical range to the periphery (Eckert et al., 2008). Although there are some studies questioning the central-marginal concept in the genetic variation of populations (e.g., Mandák et al., 2005; Wróblewska, 2008), our results are consistent with this theory.

The highest genetic variation of *M. subalpinum* agg. among Austrian wide-leaved populations ($H_e = 0.615$) and the highest effective number of alleles per locus ($A_e = 2.160$) is in accordance with the assumptions based on morphology about the diversity centre of the group in the Vienna Forest (Štech, 2006). The adjacent narrow-leaved Austrian populations occurring from the Vienna Forest towards the Lower Austria/Styria Alps display a lower level of variation ($H_e = 0.523$, $A_e = 2.120$) and the marginal Czech and

Table 3: Summary of genetic population characteristics for all studied populations based on allozymes. P (%) = percent of polymorphic locus; A = average number of alleles per locus; A_e = effective allele number; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{is} = inbreeding coefficient (values above 0.25 in bold; values marked with * are significant at 5% level).

Population	P (%)	A	A_e	H_o	H_e	F_{is}	No. of alleles
HofsN	75	2.000	1.710	0.342	0.339	-0.010	8
GiesN	75	2.500	1.640	0.525	0.438	-0.212	10
GaadN	100	2.500	1.720	0.350	0.395	0.119	10
RotkN	100	2.750	1.550	0.250	0.309	0.200	11
EinoS	100	3.750	2.940	0.533	0.506	-0.034	15
Ein2S	100	2.500	2.174	0.569	0.526	-0.089	10
HofsS	100	3.000	2.220	0.547	0.526	-0.044	12
GumpS	100	2.750	1.690	0.431	0.459	0.067	11
MerkS	100	2.750	2.161	0.400	0.534	0.262*	11
RichS	100	2.250	1.160	0.350	0.429	0.192	9
SoosS	100	2.750	2.123	0.350	0.488	0.294*	11
LoseA	100	2.500	1.880	0.175	0.395	0.570*	10
PeutA	100	2.500	1.490	0.275	0.303	0.096	10
Semma	75	2.250	1.590	0.350	0.395	0.119	9
HernA	100	2.750	2.049	0.375	0.533	0.308*	11
Holla	100	2.750	1.970	0.325	0.372	0.133	11
HirsA	100	2.500	1.950	0.425	0.496	0.150	10
KaltA	100	2.500	1.540	0.475	0.445	-0.072	10
KranA	100	2.000	1.820	0.350	0.305	-0.156	8
LangA	100	3.000	2.350	0.593	0.572	-0.038	12
MarsA	100	2.000	1.230	0.364	0.411	0.120	8
MiesA	100	2.250	1.280	0.23	0.311	0.286	9
SlinA	100	2.500	2.180	0.500	0.572	0.133	10
MajdB	50	1.500	1.431	0.300	0.242	-0.256	6
SastB	75	1.750	1.220	0.125	0.183	0.328	7
PribB	50	1.500	1.112	0.050	0.092	0.471	6
SoprB	75	1.750	1.220	0.175	0.183	0.045	7
ZehrB	50	1.500	1.870	0.200	0.195	-0.029	6
TyniB	50	1.750	1.150	0.183	0.215	0.155	7
KnezB	50	1.750	1.240	0.350	0.253	-0.416	7
DoubB	25	1.500	1.760	0.075	0.141	0.481	6

Table 4: Summary of genetic characteristics for groups of populations. P (%) = percent of polymorphic locus; A = average number of alleles per locus; A_e = effective allele number; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{st} = fixation index.

Group	P(%)	A	A_e	H_o	H_e	F_{st}
CZ + SK	100	2.750	1.340	0.182	0.369	0.514
AU narrow	100	4.250	2.120	0.368	0.523	0.226
AU wide	100	4.500	2.160	0.457	0.615	0.224
<i>M. nemorosum</i>	100	3.250	1.740	0.368	0.419	0.146

Table 5: Results of the Mantel test. r = correlation coefficient, p = significance by probability test with 10,000 replicates (value below 0.01 in bold).

Group	r	p
All populations of <i>M. subalpinum</i> agg.	0.090	0.224
CZ +SK + AU narrow	0.158	0.113
AU narrow + AU wide	0.396	0.015
AU narrow	0.698	<0.001
AU wide	-0.350	0.027
CZ + SK	0.213	0.205

Slovak narrow-leaved populations have the lowest variation ($H_e = 0.369$, $A_e = 1.340$) and lowest number of alleles per population as well as a lower percentage of polymorphic loci. The highest genetic variation of the wide-leaved populations may also be partly influenced by an assumed hybridization with *M. nemorosum* populations (Štech, 2006).

This assumption is partly supported by the Mantel test results which showed the highest significant correlation of genetic and geographic distance among Austrian narrow-leaved populations. For wide-leaved populations the correlation was weaker (yet still significant) and negative. The lower correlation is probably caused by the low number of wide-leaved populations which are also restricted to a relatively small area. Another reason might be the possible hybridogenous origin of wide-leaved populations. Hybridogenous populations were observed to possess no significant geographic component in genetic variation, whereas obvious correlations between genetic and geographic distances were detected in non-hybrid populations of the genus *Lotus* (Kramina, 2013).

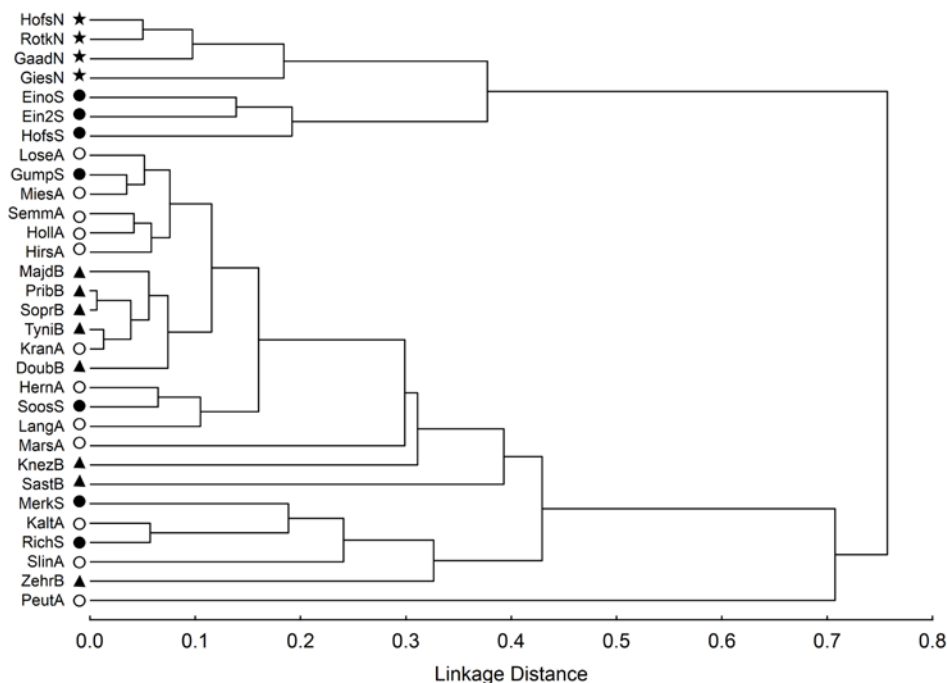


Figure 2: The UPGMA dendrogram based on Nei's genetic distances (Nei, 1972). Symbols explanation: * *M. nemorosum*; ● wide-leaved *M. subalpinum*; ○ Austrian narrow-leaved *M. subalpinum*; ▲ Czech or Slovak *M. subalpinum*

The highest average fixation index ($F_{st} = 0.514$) for the Czech and Slovak populations supports the marginal status of this group and clearly shows that the disjunction of these populations is not a recent event. It is possible that the ancestral taxon was spread in the early Holocene across large areas which included today's localities and started to withdraw when the tree canopy became more dense, and later due to substantial changes to suitable habitats made by man. Recent localities are often found in sparse woods on well-illuminated sites such as slopes and terrace edges of river floodplains, forest margins, or the surroundings of forest roads and clearings (Chlumský and Štech, 2011). Another possibility is that *M. subalpinum* was never widespread in today's marginal areas, but was restricted to small regions with appropriate environmental conditions, and recent localities are the remains of scattered historical distribution.

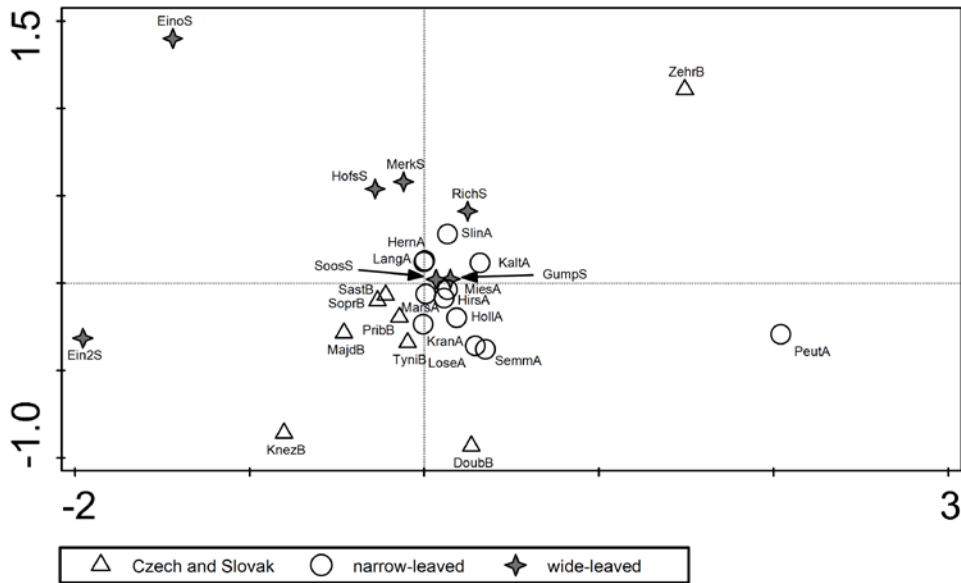


Figure 3: Principal Coordinates Analysis for all studied populations of *M. subalpinum*. For better resolution *M. nemorosum* populations were removed.

Nevertheless, genetic drift (Amos and Hardwood, 1998) as a result of either persistent isolation, bottleneck, or founder effect has had enough time to substantially enhance the genetic differentiation between populations.

The average inbreeding coefficient (F_{is}) was close to 0 in all groups suggesting that there is not a high risk of inbreeding depression. However, a closer look at single populations shows a more complicated pattern. There were 8 populations that had a rather high value for the inbreeding coefficient ($F_{is} > 0.25$, Table 3; note that in four of them it is not significantly different from 0 in a permutation test, which is, however, caused by their low overall variation or even invariability of some loci, which are then omitted from the computation). One of the reasons could be the isolation of populations and therefore higher inbreeding due to crossing with close relatives. The pollination test also showed the partial ability to self-fertilise, which may as well add to the amount of inbreeding. Adaptations for self-fertility are known to increase the probability of establishment following dispersal (Larson and Barrett, 1998) and self-fertility may also be selected in

peripheral populations (Lipow and Wyatt, 2000). However, allogamy is obviously still the preferred reproduction mode in *M. subalpinum* agg.

A common feature of populations with high inbreeding coefficient is that they occupy secondary stands which were presumably colonised by rapid radiation from a small number of plants originating from vanished primary stands. The density and regular distribution of these populations is often very high. It has been observed that land use changes may lead to a decreased genetic diversity within populations shortly after the colonization of secondary stands (Vellend, 2004; Jacquemyn et al., 2004, 2009).

On the contrary, the populations with the lowest inbreeding coefficients are often spatially structured and comprised of a system of patches. Although their sites are usually secondary, they often occur close to their putative primary habitats such as the edges of terraces above watercourses. Disturbance dynamics realized by the river ensured proper light conditions for the survival of the species during the Holocene. The continual occurrence of appropriate conditions can reduce a bottleneck effect and such populations may act as allelic refugia and present higher genetic variability (Comps et al., 2001). In case of sufficient genetic diversity in the population and gradual expansion of population size there is still sufficient opportunity for non-relative allogamy and F_{is} thus may reach even moderate negative values.

In concordance with Honnay and Jacquemyn (2007) there was no significant relationship between population size and the inbreeding coefficient.

Despite the low number of studied loci (but high total number of alleles), the dendrogram (Fig. 2) and principal coordinates analysis (Fig. 3) based on Nei's genetic distances were easily interpretable. The dendrogram separated a cluster containing *M. nemorosum* populations together with some wide-leaved Austrian populations that are morphologically closest to *M. nemorosum*. This pattern supports a hypothesis supposing old hybridization between *M. subalpinum* and *M. nemorosum* assumed on the basis of morphological characteristics (Štech, 2006).

The EinoS population had also the allele ADH 5 which was otherwise specific for *M. nemorosum* populations. Another interesting allele, SKDH 4, common for *M. nemorosum* populations, was present in 3 wide-leaved populations. However, it is also present in the population LoseA, which morphologically belongs to the narrow-leaved populations and occurs in a region without the presence of any *M. nemorosum* population. It is hard to

say if SKDH 4 is an ancestral allele or evidence for an old hybridization event with an inconspicuous morphological manifestation.

As expected, *M. grandiflorum* MarsA population did not differ in any way from the rest of the narrow-leaved Austrian populations. The allelic pool of Czech and Slovak populations is obviously pauperized compared to the Austrian populations and there are no unique alleles present for this area. An interesting fact is that the ADH 4 allele is shared by the Slovak SastB population and Austrian wide-leaved EinoS population, which are morphologically different, but geographically relatively close.

The ZehrB population was, due to its geographic isolation and rather late year of discovery, considered to be introduced (Holub, 1996). However, within the Czech populations unique ADH 3 allele discovered in the ZehrB population (common for Austrian *M. subalpinum* agg. populations and one Austrian *M. nemorosum* population) suggests that the ZehrB population might be considered relic.

Conclusions

Genetic variation estimated by isozyme analyses is congruent with the known pattern of morphological variation of *M. subalpinum* agg.

The allelic richness is higher in the Austrian populations than in the marginal Czech and Slovak populations.

Some wide-leaved populations from the Vienna Forest have a rather high number of alleles. This allelic abundance may be caused by allelic enrichment due to an old hybridization with *M. nemorosum*. The Czech and Slovak populations traditionally designated as *M. bohemicum* are, according to their allozymes, genetically derived from Austrian narrow-leaved populations.

The high differentiation among populations suggests that the current gene flow between populations is not common and populations do not interbreed often. The highest between-population differentiation (F_{st}) in the group of Czech and Slovak populations suggests that they have been isolated long enough for the genetic drift to divide these populations.

The high inbreeding coefficient (F_{is}) in some populations together with the pollination experiment indicates that there might be selfing within the populations.

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Chapter VI

Disentangling the evolutionary history of *Melampyrum subalpinum* agg. (Orobanchaceae) in Central Europe

Štech, M, Chlumský, J, Herbstová, M, Košnar, Jan, Košnar, Jiří, Koutecký, P, Fér, T, Trávníček, P and Suda, J (2016): *Manuscript*.

Photo on previous page (M. Štech): *Melampyrum subalpinum*,
Schneeberg, Austria

Disentangling the evolutionary history of *Melampyrum subalpinum* agg. (Orobanchaceae) in Central Europe

Abstract

The *Melampyrum subalpinum* group represents a taxonomically intricate complex with a unique fragmented distribution area restricted to Central Europe. Morphological variation in the region of the north-eastern edge of the Alps contrasts with a seemingly uniform populations in the fragmented rest of the distribution area. Genetic diversity across the whole distribution area was studied by analysis of genome size, three noncoding regions of cpDNA ($trnT^{UGU}$ - $trnL^{UAA}$, $psbA$ - $trnH^{GUG}$, $rpl32$ - $trnL^{UAG}$), and one single copy gene of nrDNA (*Agt1*). The revealed pattern of genetic variation proves an old, limited hybridization with *M. nemorosum* as one of the sources of the morphological diversity. The occurrence of introgressive populations with the presence of *Agt1* alleles shared with *M. nemorosum* is restricted to the narrow contact zone of the Vienna Forest and Vienna Basin. A surprisingly high diversity outside of the Alps, including genome size differentiation and unique cpDNA haplotypes in some particular parts of the distribution area, supports the long history of the species in the whole distribution area. The restriction of occurrence outside of the Alps to alluvial edges, quaternary terraces, and aeolic sand regions suggests that the basic forming of the distribution area took place during glacial conditions. Hemiparasitic species dependent on host trees were probably restricted to alluvial areas with higher humidity and the continual presence of trees during the last glacial period.

Keywords: genome size, phylogeography, cpDNA, nrDNA, hybridization

This part is the subject of unpublished manuscript. The full version of this part of Chapter VI is archived by the Faculty of Science, University of South Bohemia in the printed version of the Ph.D. Thesis.



Chapter VII

Conclusions

Photo on previous page (M. Štech): Wide-leaved *Melampyrum subalpinum*, Gumpoldskirchen, Austria

Conclusions

The thesis extends current knowledge on the distribution, population structure, phylogeography, and dispersal strategies of the *M. subalpinum* group.

The genetic variation of *M. subalpinum* across its distribution area is congruent with the known morphological variation within the species. The highest genetic variation, in the center of morphological variability, the Vienna forest, is supported by allozyme allelic richness as well as by nuclear and chloroplast haplotype diversity. Within this region, supposedly hybridogenous wide-leaved populations are present, which can further increase the genetic variability.

Allozyme allelic richness across the distribution area is in accordance with the central-marginal concept (Eckert et al., 2008) - it is decreasing outside of the Wienerwald region and it is lowest in the marginal Czech and Slovak populations. (Chapter 5)

Chloroplast and nuclear DNA variability is considerably high in the whole distribution area including morphologically uniform areas and the pauperization of genetic diversity is not as pronounced as in allozymes, which suggests that the distribution is not the result of recent expansion. (Chapter 6)

High allozyme and chloroplast sequence differentiation between isolated populations suggests that gene flow between populations is minimal and that they have been isolated long enough for genetic drift to divide them. (Chapter 5 and 6)

The studied nuclear DNA sequences exhibit rather uniform variability between particular areas. However, there are rare unique alleles in some populations, which suggests their probable relictness. *M. subalpinum* ancestors probably survived the last glaciation period in the same or larger distribution range as present, depending on the tree microrefugia which were concentrated at protected moist sites such as river floodplains (Svenning, et al., 2008). This historical distribution may be reflected by the present common occurrence of *M. subalpinum* localities on slopes and the terrace edges of river floodplains. (Chapter 3, 5 and 6)

A high inbreeding coefficient together with pollination experiments suggest that selfing or crossing with closely related individuals is common in some populations. (Chapter 5)

Both morphological and molecular data support conspecificity between populations from the Czech Republic and Slovakia and Austrian populations. The oldest valid name for this species is *M. subalpinum* (A. Kern) Jur. (Chapter 5 and 6)

Allozymes and nuclear markers show clearly that the Wienerwald populations with the widest leaves are the result of hybridization of *M. subalpinum* with *M. nemorosum*. Chloroplast DNA shows that the hybridization took place mostly on *M. subalpinum* as a maternal plant being pollinated by *M. nemorosum* pollen. There was only one population (and five plants) that carried chloroplast genes of *M. nemorosum*. The rest of the wide-leaved populations exhibited no clear introgression marker in the nuclear DNA. (Chapter 5 and 6)

In the Czech Republic and Slovakia, most *M. subalpinum* populations occur predominantly in secondary cultural spruce or pine forests (however, in the area, or in the vicinity, of original relic sites). The ability to act partly as synantropic species probably enabled *M. subalpinum* to survive the extensive changes of forest composition caused by man. Recent populations in the Czech Republic and Slovakia are mostly very small, consisting sometimes only of a few tens of plants. For an annual plant this is a rather critical state and many populations are at a risk of vanishing from their sites in the case of deforestation or excessive shading of the site by dense tree canopy. (Chapter 3)

On mixed stands with *M. subalpinum* and *M. pratense* ants preferentially remove *M. pratense* seeds and *M. subalpinum* seeds are removed less likely. This may be one of factors that has a positive influence on *M. pratense* success in the seed dispersal at mixed stands and consequently in the colonization of favourable sites. *M. subalpinum* seeds are dispersed less willingly by ants, which negatively affects population size, density, dispersal speed, and the colonization possibilities of this species. This may be one of the reasons for the limited size of most of the *M. subalpinum* populations outside of the Alps. (Chapter 4)

Formica polyctena ants are able to move *Melampyrum* seeds over a distance of 36 m in only 7 hours. This distance is among the furthest known myrmecochorous removals of forest plant seeds. However myrmecochory is still important mainly on a local, intrapopulation scale and it is not sufficient to explain the extent of distribution of some *Melampyrum* species in the areas covered with glacier in the last glacial maximum. For long-distance removal *Melampyrum* seeds are able to be

dispersed by endozoochory. *M. pratense* seeds were able to pass through the digestive tract of a cow in large numbers and mostly unharmed. Endozoochory thus can be a rather common event in *Melampyrum* and can offer an elegant explanation for the long-distance migration of *Melampyrum* and other myrmecochorous plants during the Holocene period or in the event of the loss or formation of new favourable habitats. (Chapter 4)

We have made 16 observations of a new arthropod disperser of *Melampyrum* seeds — the harvestman *Oligolophus tridens* (Opiliones). (Chapter 4)

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Poster: Chlumský J., Jílková V., Koutecký P. et Štech M. (2011): Seed–dispersal possibilities in myrmecochorous genus *Melampyrum* – Is there an interspecific competition for dispersers? - Programme and abstracts: 8.

2008: 2nd Symposium on Biology of Non-Weedy Hemiparasitic Orobanchaceae, České Budějovice

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