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**Identification of critical life history stages in the life
cycle of endangered species,
Dracocephalum austriacum L.**

Diploma thesis

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I declare I wrote this study independently using only the cited literature.

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1. Introduction

In the integration process of Europe and approach of the Czech Republic to European legislative claims it is necessary to be also interested in coming together of methods in nature conservation. European concept NATURA 2000 includes integrated program for conservation of habitats and individual endangered species (web 1, web 2, web 3). In the Czech Republic 36 species of vascular plants and 4 bryophytes have been selected for this program. In 2002 basal monitoring of these species was started. It includes counting numbers of fertile and sterile individuals on the localities and monitoring of basic habitat characteristics (Rybka 2002).

This information allows identification of trends in population development, however it does not say anything about factors responsible for these changes. In case of significant decline of population size it is thus not clear which part of life cycle was most affected and should be the target of conservation action.

To gain detailed view on mechanisms responsible for changes in population sizes of rare species a few studies were published. Their authors try to obtain detailed information on population dynamics of the species (e.g. Buchele et al. 1991, Baskin et Baskin 1998, van Buren et Harper 2003). The results of these studies allow to identify critical life history stages and to decide which life history stage should be preferentially supported in case of population decline. Frequent disadvantage of these studies is that they deal only with one restricted region or even one population of the species. That is why the information about variability of population dynamics on larger area is rather limited (but see Willems et Ellers 1995, Nantel et Gagnon 1999, Jongejans et de Kroon 2005). It is thus not clear whether it is possible to apply the conclusion of such study to other populations or even other regions. This would be very useful to know since many of the species are endangered in different countries and understanding dynamics of the species in one area could help to understand dynamics of the species in another area.

Knowledge of demography of the species does not however provide complete information needed for its effective conservation. An important aspect in rare species conservation is not only maintaining sufficient number of individuals on the locality but also their genetic diversity (Lopez-Pujol et al. 2003, Oostermeijer et al. 2003). Although this concept is generally accepted, the information about genetic diversity of rare species in central Europe is very limited (but see Gaudeul et al. 2000, Brzosko et al. 2002, Brzosko et Wróblewska 2003).

The importance of studying genetic diversity in rare species is given by the fact that populations of rare and endangered species are often small and isolated. Genetic diversity in such populations is subjected to strong random changes in allele frequencies called genetic drift. At its most extreme case, genetic drift can lead to loss of alleles from the population and thus loss of polymorphism such that a locus becomes fixed for a single allele (Lowe et al. 2004). Low genetic diversity can lead to reduction of fitness due to expression of deleterious alleles in homozygous state following breeding with close relatives (Lowe et al. 2004) called inbreeding depression (DeMauro 1993, Anderson et Waldmann 2002, Ishihama et al. 2005). By estimating genetic diversity of the populations and linking it to performance of the plants it is possible to test whether the genetic diversity of the population may be limiting its fitness.

In spite of the potential importance of genetic diversity for plant fitness Oostermeijer et al. (2003) showed in a review of papers published between years 1979 and 2000 on the conservation biology of wild plants that there were only a few studies interested in interactions of demography and genetics. Demographic data can provide us essential information on the most critical stages in the life cycle but we also need to understand the importance of genetic diversity for these demographic process. Combination of studies on demography and genetics can thus give us much more reliable information about population dynamics than when studied separately (Colas et al. 1997, Luijten et al. 2002). There are several ways how to link genetics and demography. To get an idea on the effects of inbreeding on demographic transitions, we should perform (simultaneous) field experiments in which we monitor the relative performance of inbred, outcrossed and naturally produced offspring from large and small population (Ouborg et van Treuen 1994, Oostermeijer 1996, Richards 2000, Luijten 2001). Information with respect to Allee-effects on reproductive success can be obtained by studying seed:ovule ratio in a series of small to large populations (Kunin 1997, Lammi et al. 1999, Molano-Flores et al. 1999, Morgan 1999, Luijten et al. 2000). Experiments manipulating population size can be also used to obtain strong evidence of Allee-effects, although this is time-consuming and not always possible (Hackney et McGraw 2001). In all these cases we obtain information on the effect of genetic diversity on part of the life cycle, but almost never direct influence of genetics on population growth rate is studied.

In my study I want to compare population dynamics of an endangered species in two distant regions. I also want to estimate genetic diversity in this species and assess the importance of genetic diversity for population dynamics. As a model species, I chose *Dracocephalum austriacum* L. It is one of critically endangered species in the Czech Republic

and effective conservation strategies are very needed nowadays. Recently there are only 9 localities (Čeřovský et al. 1999) in the Czech Republic and some of them have only a few individuals and are in the risk of extinction (personal observation 2005). Very similar situation is also in neighbouring Slovak Republic (Karasová in verb.) and in other surrounding countries (web 3). That is why this species is included in 36 species of vascular plants in the Czech Republic selected for the European program Natura 2000. Information about ecology, population biology and genetic diversity of populations are almost lacking even though these are needed for creating an effective conservation plan.

In this study I want to fill in this gap by answering the following questions:

- What are the critical life history stages in endangered species *Dracocephalum austriacum*?
- Are there any differences in population dynamics between Czech and Slovak populations?
- What is the genetic diversity of its populations?
- What is the importance of genetic diversity for population dynamics of the species?
- What is the genetic relationship between populations in two distant regions, the Czech and Slovak Republic?
- What are the habitat requirements of this species? What are the differences in habitat conditions between localities?

To do this I studied full population dynamics in 3 Czech and 3 Slovak populations for 3 years (2003-2005). Further I used allozyme analysis to estimate genetic diversity of all Czech and 3 Slovak populations of *Dracocephalum austriacum*. I also performed pollination experiments with isolation of inflorescences and measured habitat characteristics (depth, soil analysis, vegetation composition, aspect, slope etc.) in all Czech (even one already extinct) and most of Slovak populations.

2. Methods

2.1 Study species

Dracocephalum austriacum L. (*Lamiaceae*), is a perennial herb or dwarf shrub with erect or ascending stems up to 60 cm, which are densely leafy and velutinous. Cauline leaves are 3- to 5(-7)-pinnatipartite with segments $20-30 \times 1-2.5$ mm, linear to linear-lanceolate, entire, more or less velutinous with revolute margins. Verticillasters are 2- to 4(-6)-flowered, forming a more or less dense, ovoid to oblong spike. Bracts are 3-fod and aristate. Corolla is 35-50 mm long, blue-violet. The species is diploid ($2n = 14$) (Heywood 1972). It flowers from the second half of May to the first half of June. It grows on rocky steppes and rocky sunny slopes (Hroudá 2002). In the Czech and Slovak Republic this species belongs to C1 species, which means critically endangered species (Čeřovský 1999). For manipulation with this species I needed permission. I also needed permission to enter its localities. I obtained it from Ministries of Environment of the Czech and Slovak Republic – Appendix 7.

The whole distribution range of this taxon is discontinuous and ranges from eastern Pyrenees across France, Italy, Switzerland, Austria, the Czech Republic (northern edge of *Dracocephalum austriacum* distribution range) and the Slovak Republic, Hungary and Romania to the Ukraine (Meusel et al. 1978) – see Figure 1.

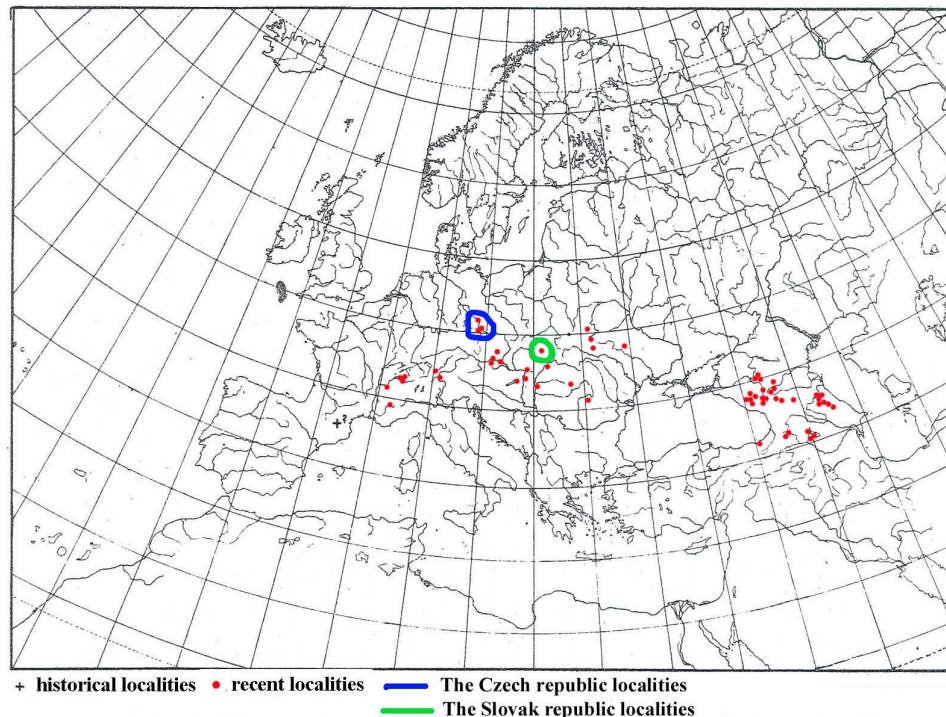


Figure 1: Distribution range of *Dracocephalum austriacum* L. with labeled studied Czech and Slovak regions of localities (according to Meusel et al. 1978).

2.2 Localities in the Czech Republic

Haknovec is the biggest locality in the Czech Republic with cca 500 flowering plants (personal observation 2005). It is situated on southern and southeastern slope of Haknová mountain on the northeast edge of town Karlštejn. It belongs to NPR (National Nature Reserve) Karlštejn. Plants grow here on six rocky outbursts. The locality is overgrowing with shrubs and trees in some places (mainly *Cerasus fruticosa*, *Fraxinus excelsior*, *Berberis vulgaris*, *Rosa* sp.). At this locality a few ping-flowering plants were seen.

Kodská stěna is another locality with cca 55 flowering and 100 non-flowering plants (personal observation 2005). It is situated on the upper edge of Kodská stěna in NPR Koda, about 1 km west of village Srbsko. This population is divided in two microlocalities (cca 15 plants and the rest). This locality is almost free of shrubs and trees. The sunny upper edge seems to be a good place for generative reproduction (more seedlings than elsewhere).

In **Císařská rokle** there are about 90 flowering and 80 non-flowering plants (personal observation 2005). This locality is on the rocky ridge on the left slope of the ravine in NPR Koda, 1 km southwest of village Srbsko. This locality is strongly overgrown with shrubs and trees (*Swida sanguinea*, *Crataegus* sp., *Ligustrum vulgare*, *Fraxinus excelsior*, *Rosa* sp., *Acer campestre*, *Juniperus communis*, *Pyrus pyraeaster*, *Carpinus betulus*, *Cornus mas*, *Cotoneaster integerrimus*).

Kozelská rokle is the smallest locality in the Czech Karst only with 3 flowering and 1 non-flowering plants (personal observation 2005). It is on the top of a rock wall on the right bank of creek Kačák in NPR Karlštejn, about 1 km south of village Hostim. The rock is being overgrown with trees and shrubs (*Swida sanguinea*, *Pyrus pyraeaster*, *Rosa* sp., *Fraxinus excelsior*, *Acer campestre*, *Crataegus* sp., *Prunus spinosa*, *Sorbus aria*).

Velká hora is the second largest locality in Czech Karst with about 315 flowering plants (personal observation 2005). It is situated on rocky edges and ridges on southern and southeastern slope of Velká hora mountain above Kubrychtova bouda in NPR Karlštejn northeast of village Srbsko. It is divided in three microlocalities on rocky edges with about 70, 65 and 180 flowering plant respectively. In this locality a few ping-flowered plants were seen. This locality is a bit endangered with shrubs and trees only from its lower part.

Karlické údolí is a small locality with 6 flowering and 1 non-flowering plants (personal observation 2005) on the top of rocky promontory on the left bank of creek Karlický potok in PR (Nature Reserve) Karlické údolí about 2 km north of village Karlík. Population is endangered with tourist visits and overgrowing with shrubs and trees

(*Cotoneaster integerrimus*, *Tilia platyphyllos*, *Sorbus torminalis*, *Juniperus communis*, *Rosa* sp., *Prunus spinosa*, *Berberis vulgaris*).

Radotínské údolí is locality with 26 flowering and 26 non-flowering plants (personal observation 2005) on rocky ridge on the right bank of Radotínský potok creek about 300 m downstream from Rutický mlýn in PR Radotínské údolí. This locality is also divided into three microlocalities. On the lowest one there are 6 plants, in the middle one there are 12 plants and in the highest one 34 plants. Locality is endangered by overgrowing with shrubs and trees especially from the creek side (*Cotoneaster integerrimus*, *Pinus sylvestris*, *Sorbus aria*, *Quercus robur*, *Berberis vulgaris*, *Corylus avellana*, *Juniperus communis*). The highest microlocality is overgrown mainly with *Dictamnus albus*. Kubíková (1993) recorded on this locality 37 plants (23 flowering and 14 non-flowering) in 1986.

Vanovice, locality with about 33 flowering and 20 non-flowering plants (personal observation 2005), is on the rocky ridge on the right bank of Berounka river above railway, about 1 km northwest of village Krupná. From east, population is overgrowing with *Swida sanguinea*, *Cotoneaster integerrimus*, *Corylus avellana*, *Carpinus betulus*, *Rosa* sp. and *Berberis vulgaris*.

Zázmoníky is the only recent locality in the Czech Republic outside Czech Karst but in 2004 I found only 1 non-flowering plant there. The locality is in a pine forest about 2 km north of village Bořetice in PR Zázmoníky in Hustopečská pahorkatina in southern Moravia. *Dracocephalum* is not here in typical habitat, shaded in the pine forest in quite dense vegetation of *Carex humilis*, *Inula ensifolia* and *Polygonatum odoratum*.

Deblík is the only locality in České středohoří mountains. The population on this locality is nowadays extinct (last plant was observed in 1996 – Hamerský 2000). This locality is on rocky ridge of Deblík mountain about 1 km west of village Círvice. This locality is strongly overgrown with shrubs, mainly *Cotoneaster integerrimus*, and *Prunus spinosa*, *Rosa* sp., *Quercus robur*, *Sorbus aria* and *Ulmus minor*. In 1929 Mittelbach recorded 60 plants (see in Machová et Kubát 2004), in 1984 Kubát found only two plants (Machová et Kubát 2004).

All species names are according to Kubát et al. (2002).

Detailed information about numbers on plants and conditions of populations in Czech Karst from 1983 are deposited in Czech Karst Protected Landscape Area Administration (Moucha 1983, Ložek et al. 1983-2003). Jatiová (2001), Machová et Kubát (2004) and Špryňar (2001) provide information about other localities of *Dracocephalum austriacum*. Moucha (1986) was interested also in species conservation and possibilities of its cultivation.

2.3 Localities in the Slovak Republic

There are 8 localities (3 in Zádielská planina, 2 in Plešivská planina, 2 in Koniarská planina and 1 in Domické škrapy) in NP (National Park) Slovak Karst and 1 locality in Slovak Paradise (in NPR Dreveník).

I was interested in my study especially in three biggest populations:

Zádielský kameň on Zádielská planina is locality with 42 flowering and 44 non-flowering plants (personal observation 2005) on sunny slope of the highest rock in Zádielská planina in NPR Zádielská tiesnava. This locality is overgrowing with shrubs such as *Spirea media* and herbs such as *Polygonatum odoratum*.

Domické škrapy is recently the biggest population in the Slovak Republic with about 100 flowering and 80 non-flowering plants (personal observation 2005). It is situated on a meadow with limestone rocks in NPR Domické škrapy. This locality is also endangered with overgrowing mainly with *Prunus spinosa* and grasses.

Železná vrata is the biggest population on Plešivská planina with 66 flowering and 47 non-flowering plants (personal observation 2005). This locality is on sunny west rocky edge of Plešivská planina. *Juniperus communis* and other shrubs and grasses gradually overgrow this locality.

Most of the other Slovak populations have less than 10 plants (all data by Karasová in verb).

Photos of some localities are available in Appendix 8.

2.4 Characteristics of the localities

2.4.1 Vegetation composition

At each locality vegetation composition was recorded in 4m² quadrates with *Dracocephalum austriacum*. Aspect, slope, altitude and cover of tree, shrub and herb layer were recorded in each plot. These data are available in Appendix 1. Names of species are according Kubát et al. (2002). Using program Canoco (ter Braak et Šmilauer 1998) I performed DCA analysis and constructed graph showing differences in vegetation composition among localities. Using CCA analysis I tested differences in vegetation composition between Czech and Slovak Karst before and after excluding species occurring only in flora of one republic so that I could remove the effect of taxa specific for each region.

2.4.2 Soil analyses

I also took samples of soil for analyses of $\text{pH}_{(\text{H}_2\text{O})}$, C_{ox} , N_t , Ca, Mg, K and P. I took 1–6 samples per population in transects according to locality size. Transects were along longest diameter of the population and I took samples each 5 metres. Soil analyses were made by Bulová in February 2005 in laboratory of AOPK of the Czech republic in Brno. Analyses of $\text{pH}_{(\text{H}_2\text{O})}$ and C_{ox} were done according to ČSN ISO 10390, N_t according to Kjeldahl and Ca, Mg, K and P – according to Mehlich III. For protocols of soil analyses see Protocols of soil analysis on enclosed CD.

I tested differences in soil contents among localities within regions (the Czech and Slovak Republic) using ANOVA with fixed effects. I tested differences between regions using ANOVA with fixed effects on mean values of each parameter per locality. Both tests I did in program S-Plus (MathSoft 1999). For some localities phosphorus contents were under level of detectability, lower than 10 mg/kg. These values I substitute with 5 mg/kg as mean value between no phosphorus content and the detectability level.

2.4.3 Other abiotic characteristics

Depth of soil in transects (puncture every 0.5 m with 30 cm long wire across the locality), aspect in main slope in the locality and GPS coordinates were recorded in all Czech and most of Slovak localities. (GPS coordinates of Slovak localities are not shown here because the populations are endangered by people digging up plants.)

From geological maps 1:50 000 available on www.cgu.cz I obtained information on geological substrate of the localities in the Czech Republic.

2.5 Genetic analysis

Genetic diversity of the populations was examined using allozyme analysis. Allozymes, when compared to other markers, have several main advantages. Markers are co dominant and so allele scoring is possible, they are easy to apply and costs are quite low.

2.5.1 Sampling and extraction

Ten randomly chosen individuals (if possible) were sampled for each population of *Dracocephalum austriacum* in Czech Karst (all 8 populations). Twenty randomly chosen

individuals for 3 populations used for demography study in Slovak Karst (Zádielský kameň, Domické škrapy, Železná vrata) were sampled for genetic analysis. The higher sample size from Slovak populations was due to long distance to laboratory and thus higher probability of degrading enzymes.

Samples of leaves (about 70 mg) were taken at Czech Karst populations at the end of March and in April 2004 just when the leaves sprouted. In Slovak Karst populations the leaves were taken at the beginning of July 2004 and at the beginning of May 2005. They were carried in icebox, kept over night in the fridge, and the next day extracted in allozyme laboratory of Botanical Institute of Academy of Science in Průhonice (see extraction protocols on enclosed CD).

2.5.2 Allozyme analysis

Standard methods of polyacrylamid gel (usually done on starch gel) electrophoresis were followed (Soltis et Soltis 1989). For exact procedure see protocols of electrophoresis and composition of gels, buffers and detect solutions on enclosed CD.

Ten enzyme systems (leucine aminopeptidase LAP, superoxid dismutase SOD, aspartat aminotransferase AAT, glucose-6-phosphate dehydrogenase G6PDH, alcohol dehydrogenase ADH, shikimic acid dehydrogenase SHDH, phosphoglucomutase PGM, malic enzym ME, esterase EST, isocitrate dehydrogenase IDH) were initially tested. Only 4 of them were however selected as based on variability and possibility to score the alleles. Analysed enzyme systems were leucine aminopeptidase (LAP, tree loci), superoxid dismutase (SOD, three loci), glucose-6-phosphate dehydrogenase (G6PDH, two loci), and aspartat aminotransferase (AAT, two loci). All gels are on enclosed CD.

The slowest locus in each system was designated 1, followed 2, 3, etc. and the fastest allele in each locus was designated “a”, followed “b”, “c”, etc.

2.5.3 Analysis of genetic data

Even though I took samples from 8 Czech localities and 3 Slovak localities, for most analysis I used only localities with at least 9 samples so that I could have comparable numbers of samples. I thus exluded Kozelská rokle (2 samples) and Karlické údolí (4 samples).

For each population I computed number of alleles per each locus and population, number of unique alleles for each population and for each region (Czech and Slovak Karst) and proportion of polymorfic loci in program Microsoft Excel. Further Shannon diversity

index, effective number of alleles for each population, H_i (mean observed heterozygosity per individual), H_s (mean expected heterozygosity within populations) for each population and H_t (total expected heterozygosity in the total population) first separately for each region ($H_{t(\text{Czech Karst})}$, $H_{t(\text{Slovak Karst})}$) and then for the two regions together (H_{t1}) was computed using program POPGENE32 (Yeh et Boyle 1997). Using program FSTAT (Goudet 2001) I computed and tested F_{is} for each population. According Wright (1951) I computed F_{is} , F_{st} and F_{it} for all populations together. F_{is} is inbreeding coefficient, which describes the divergence of observed heterozygosity from the expected heterozygosity within populations assuming panmixia. F_{st} is fixation index, which describes the reduction in heterozygosity within populations when compared to the total population due to selection or drift. F_{it} is the overall inbreeding coefficient, which describes the reduction of heterozygosity within individuals relative to the total population due to non-random mating within subpopulation (F_{is}) and population division (F_{st}) (Lowe et al. 2004). F_{st} was computed also for each region separately (Czech and Slovak Karst) as

$$F_{st(\text{Czech Karst})} = [H_{t(\text{Czech Karst})} - H_{s(\text{Czech Karst})}] / H_{t(\text{Czech Karst})}$$

and

$$F_{st(\text{Slovak Karst})} = [H_{t(\text{Slovak Karst})} - H_{s(\text{Slovak Karst})}] / H_{t(\text{Slovak Karst})},$$

where $F_{st(\text{Czech Karst})}$ and $F_{st(\text{Slovak Karst})}$ are proportions of variability that exists between populations, out of total variability in the region of Czech and Slovak Karst, respectively, H_t is total expected heterozygosity in the total population, H_s is the mean expected heterozygosity within populations.

Then $F_{st(\text{Czech and Slovak Karst})}$, proportion of variability between regions, out of total variability, was computed as:

$$F_{st(\text{Czech and Slovak Karst})} = (H_{t1} - H_{t2}) / H_{t1},$$

where H_{t1} is total expected heterozygosity in Czech and Slovak Karst, H_{t2} is mean of total expected heterozygosity for Czech Karst ($H_{t(\text{Czech Karst})}$) and total expected heterozygosity for Slovak Karst ($H_{t(\text{Slovak Karst})}$).

F-statistics were tested (if not written else) using chi-square formula provided by Workman et Niswander (1970):

$$\text{Chi} = 2NF_{st}(k-1) \text{ with } (k-1)(s-1) \text{ degrees of freedom,}$$

where N is the total sample size, k is the number of alleles at the locus, and s is the number of populations.

2.6 Demography

2.6.1 Selection of localities

For study of demographic parameters I selected 3 populations in CHKO (Landscape Protected Area) Czech Karst (Haknovec, Kodska stěna and Císařská rokle) and 3 biggest populations in NP (National Park) Slovak Karst (Zádielský kameň, Domické škrapy and Železná vrata) – Table 1. I selected populations with at least 100 plants so that I could gain good estimates of demography parameters.

Localities in CHKO Czech Karst	No. of marked plants	Localities in NP Slovak Karst	No. of marked plants
Haknovec	200	Zádielský kameň	80
Císařská rokle	150	Domické škrapy	150
Koda	150	Železná vrata	150

Table 1: Localities selected for studying population dynamics in CHKO Czech Karst and in NP Slovak Karst (numbers of marked plants for demography at the localities).

In each population 100–200 individuals (mostly all plant in the locality except individuals on steep rocks, where any movement is very dangerous) were marked. Plants were marked with plastic labels (2×10 cm) and metal plates (2×2 cm, for finding with a metal detector). For three years (2003-2005) number of sterile and fertile stems of each plant was recorded in June or July after flowering.

2.6.2 Demographic analysis

The demographic data were examined by transition matrix models. The projection matrix is isomorphic to the life cycle graph and allows the quantitative demographic data that describe the life cycle of a population with stage structure to be represented in a standard format. The theoretical background of transition matrices and their application for studies of population dynamics are treated in detail by Caswell (1989a). A matrix population model is of the general form

$$\mathbf{A} * \mathbf{x} (t) = \mathbf{x} (t+1),$$

and describes the dynamics of a population comprised by $i (=j)$ stages. \mathbf{A} is a transition matrix with i rows and j columns, containing matrix elements, a_{ij} , which define transitions from population stage j to stage i in a predefined time interval (t to $t+1$), $\mathbf{x}(t)$ is a column vector containing the number of individuals in each stage at time t (Ehrlén 1994).

Analysis of a projection matrix yields the stable stage distribution and the finite rate of increase, λ , of the population. λ may be used as a measure of fitness for organisms possessing a particular set of traits in a particular environment (Lande 1982, Caswell 1989a). Analyses of projection matrices also generate information on the change in population growth rate, $\delta\lambda$, following a small change in a_{ij} (δa_{ij}). This is called sensitivity, s_{ij} , of λ to changes in a_{ij} (Caswell 1989a). In order to compensate for differences in absolute values of a_{ij} , elasticity is often used. It is defined as a proportional change in λ as a result of a proportional change of matrix element (de Kroon et al. 1986). Elasticity is also a measure of an element's contribution to fitness (de Kroon et al. 1986). Elasticities may be summed across selected regions of a matrix in order to compare the relative importance of these regions (Silvertown et al. 1993).

In stochastic models, several matrices are incorporated. A stochastic population process is simulated by sampling, at each time step, one of several possible matrices. This implies that the process does not yield just one stable stage structure, but instead a stationary distribution of population structures, to which populations converge (Ehrlén 1994).

2.6.3 Classification to stages

Plants were classified into 3 stages: seedlings, small plants and large plants. Seedlings were plants with only one thin sterile stem. These were plants that germinated in year they were recorded or one year before. So no seedling could be older than 2 years. It was not allowed for small or large plant to come back to seedling stage even though it had only one sterile stem. Small plants were plants with 2-5 stems and large plants have 6 and more stems. There was no relationship between number of stems and flower probability and so small and large plants stages were classified regardless of flowering stems only according to stem number. The division into small and large plants was based on the attempt to have sufficient number of individuals in each of the categories.

2.6.4 Seed production

Seed production was estimated at each studied locality at 20 randomly selected flowering plants (see data on enclosed CD). Seeds were counted in late June or in July in the time of fruiting. At each selected plant number of black or dark brown hard seeds was counted, length of inflorescence and length of stem was measured. In years 2004 and 2005

also number of calices in inflorescence was counted. Obtained seeds were, after counting, sown at the localities into germination plots (see below).

In 2005 also parameters that could potentially explain seed production per plant were recorded. These were: soil depth, proportion of rock in plant surroundings, shading by trees, number of flowering and non-flowering plants within 50 cm and within 100 cm. Soil depth was measured as average of three measurements in the vicinity of the plant in different directions. Proportion of rock in plant surroundings was estimated as proportion of rock or stones in 20x20 cm square with the target plant in the middle. Shading by trees was recorded using five categories – 0 (no trees and shrubs), 1 (within 20 cm there is a small shrub or tree), 2 (plant is lightly shaded with trees or shrubs), 3 (plant is strongly shaded trees or shrubs), 4 (plant is totally overgrown and strongly shaded with shrubs or trees).

2.6.5 Germination and matrices

Due to low germination rate and high dormancy of the seeds it was quite difficult to measure germination rate of this species. Therefore I used two types of approaches to include germination rate into the matrices:

1) Matrices contain seeds as separate stage (see Figure 3). In years 2003 and 2004 20 x 20 cm plots were set up and seeds were sown into them. Seedling number was recorded in the plots and in the neighbouring plots without seed addition in the following year (see Figure 2). I used data on

seed germination obtained from this experiment and on average seed production of small and large plants in each population to compute

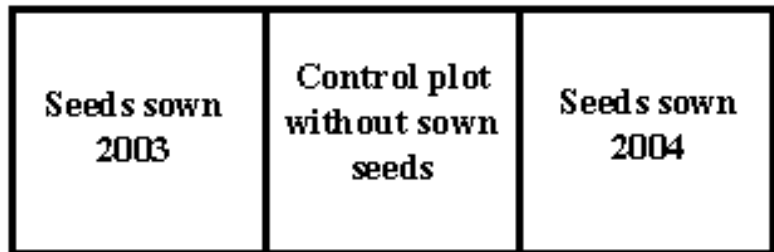


Figure 2.: Design of plots for recording germination of seeds.

transitions from stage of small and large plants to seedlings and transition from seeds in seed bank to seedlings (germinated seedlings in plots after second year). Even though I have sown 1628 and 3919 seeds in Czech and Slovak Karst, respectively, only 14 and 20 seedlings germinated (Table 2). Further more, there were no differences in germination of seeds in the 1st and in the 2nd year after sowing thus providing no clue to what is the mortality of seeds in the seed bank. Therefore I had to compute the probability of staying in the seed bank from matrices without seed bank (described bellow). Specifically, I searched for values of survival

in the seed bank that would result in the same growth rate as was found in matrices without seed bank. From this comparison of matrices with 3 and 4 stages, I obtained value 90% for survival in seed bank. This value is an average of probability of survival in seed bank in populations Haknovec and Kodská stěna (Císařská rokle was excluded from this computation due to low dynamics and inexact estimation of survival in seed bank). For Slovak populations and Císařská rokle I used this average probability of survival in seed bank from two Czech populations.

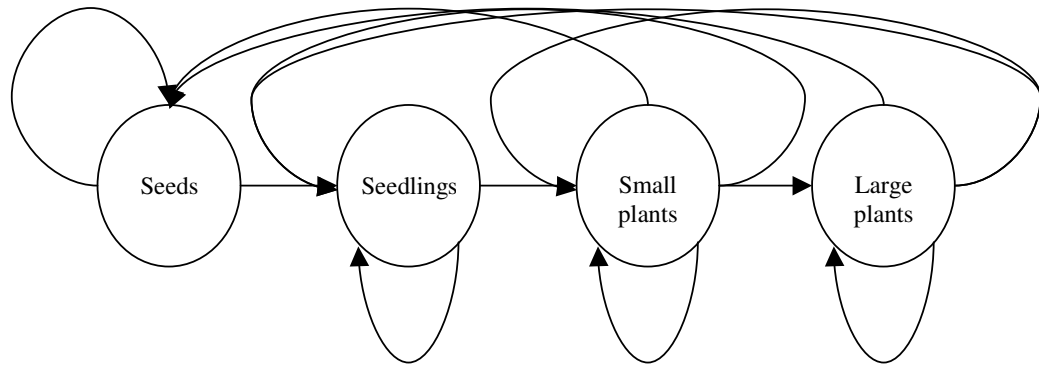


Fig. 3: Diagram of life cycle of *Dracocephalum austriacum* where seeds are included as a separate stage.

2) Matrices do not contain seeds as a separate stage (see Figure 4). These matrices were constructed for better estimation of seed bank survival in matrices with seed bank as a separate stage (described above). In this case, transitions from small and large plants to seedlings were measured indirectly. To do this I set up transects along the longest diameter of each population and on these transects I recorded number of seedlings and small and large plants in 1×1 m squares every 2 metres as long as I had at least 10 plots per locality. Then I computed the ratio of seed production between small and large plants and used this ratio as a

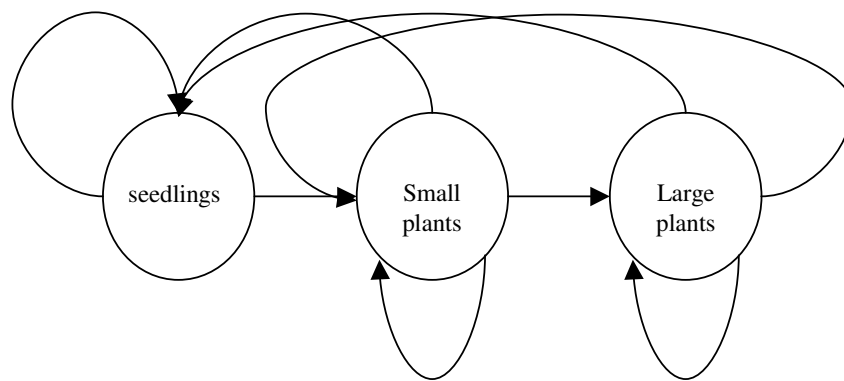


Figure 4: Diagram of life cycle of *Dracocephalum austriacum* without seed bank.

weighting factor to recalculate number of small plants to large plants. I then divided the number of seedlings per plot by the number of plants (recalculated to large plants) per plot and used this value to calculate seedling production by small and large plants. I used the mean of this value over all plots in the population as data on seedling production in the matrix.

These data were recorded in August 2005 (the matrix transition probabilities were recorded to the beginning of July each year) and so the numbers of seedlings had to be increased by their mortality rate from July to August.

The number of seedlings from 2004 was also recorded. Seedlings two years old have one stem, which is higher (up to 10 cm) and thicker. The number of 2004 seedlings had to be increased by their mortality rate from 2004 to 2005 (mortality rate of July 2005 to August 2005 for two years seedlings is insignificant). These data were available only for populations in Czech Karst and so this type of matrices could be used only there.

locality	No. of sowing plots 2003	Total no. of seeds sown 2003	No. of seedlings 2004 from 2003	No. of sowing plots 2004	No. of sown seeds 2004	No. of seedlings 2005 from 2004	No. of seedlings 2005 from 2003
Haknovec	4	313	0	5	160	3	3
Kodská stěna	3	239	0	5	501	3	2
Císařská rokle	3	145	0	4	270	0	3
Zádielský kameň	3	219	3	5	500	3	4
Domické škrapy	6	480	0	12	1200	2	1
Železná vrata	7	560	2	12	960	3	2

Table 2: No. of sown seeds and of resulting seedlings in the localities in 2003-2005 in Czech and Slovak Karst.

2.6.6 Population dynamics

I computed (i) population growth rate and elasticities for each population and each transition interval (ii) stochastic population growth rate and elasticities from matrices 2003-2005 for each population and each region (iii) 95 % confidence intervals of population growth rate and elasticities calculated using bootstrap (iv) extinction probabilities of populations (v) life table response experiments analyses for each population and each region.

Population growth rate is considered a standard measure to predict the future fate of populations given there is no strong between year variation and the growth is density independent (Caswell 1989a).

I computed population growth rate and elasticity for each population and year separately. Then I computed population growth rates and elasticities for the two transition matrices from each population together using stochastic models. I took, at each step, one of

the possible matrices (10 000 replicates). I also computed population growth rate, sensitivity and elasticity for all Czech and all Slovak Karst populations together, respectively, using stochastic simulations (de Kroon et al. 2000). In Czech populations I observed in 2003 and 2004 much fewer seedlings than in 2005 and so for Czech populations I took doubled the number of “poor matrices”.

Each estimate of transition probability and thus each estimate of population growth rate is confined with an error, because of the limited number of individuals that can be sampled. To take this into account I calculated bootstrap confidence intervals (Alvarez-Buylla et Slatkin 1994) of the growth rates, sensitivities and elasticities of each matrix and of each population and region as suggested by (Efron et Tibshirani 1994).

I computed probabilities of extinction of all Czech and Slovak populations by multiplying their population vector with the bootstrapped matrices. For populations for which no matrices were available, I used all matrices of the corresponding region together. Because some populations are endangered by digging up plants by rockgardeners, I computed also extinction probabilities with 1, 3, 5 and 10 digged up large plants per year. At each step, the resulting values of number of individuals per stage was replaced by a value drawn from Poisson distribution with the mean corresponding to the observed value. Also the number of digged up plants was not fixed but was replaced by a value drawn from a Poisson distribution with a given mean.

Further I calculated variation coefficients (Sokal et Rohlf 1995) of all transitions in the bootstrapped matrices and multiplied them by sensitivity for all the matrices together gained using stochastic simulations to see whether the traits with high elasticity are variable, and thus have the potential to change (Zuidema & Franco 2001). The resulting values correspond to values from Life table response experiment (Caswell 1989, 2000).

All analyses were done using program Matlab (Gockenbach 1999).

2.7 Combination of genetic and demographic data

2.7.1 Genetic diversity and population growth rate

The correlation between genetic parametres (Shannon diversity index, effective number of alleles, observed heterozygosity and expected heterozygosity) and number of produced seeds (average through years 2003-2005 and for each year separately) and number of plants in population was tested in program S-Plus (MathSoft 1999).

When positive correlation between genetic diversity (measured using Shannon diversity index) and number of produced seeds was detected, I used this relationship to predict the influence of changes in genetic diversity on population growth rate. According to computed slope of the relationship between genetic diversity and number of produced seed, I calculated the decrease of seed production with decreasing genetic diversity for each population. I added these seed productions into matrices from the populations and computed population growth rate using stochastic models for each population studied for demography separately.

2.7.2 Pollination experiments

In the populations in Czech Karst (Haknovec, Kodská stěna and Císařská rokle) I made also pollination experiments to determine the effect of inbreeding and outbreeding measured as number of produced seeds after different pollination treatments.

I caged five separate flower buds in one plant with monofilament sacks in May 2004. After about three days, when they came into full blossom, I pollinated them in five different ways:

- 1) flower was caged all the time and no transfer of pollen and pollinators was allowed
- 2) flower was marked but not caged; access of natural pollen and pollinators was allowed
- 3) pollinated with its own pollen (manually transferred pollen from stamen to stigma), flower was caged again
- 4) pollinated with pollen from plant from another closest locality, flower was caged again
- 5) pollinated with pollen from nearby plant in the same locality, flower was caged again
- 6) after coming into blossom, sack was removed so that natural pollinators could pollinate the flower

In each out of three localities I treated 10 plants. Treatment no. 4 (transfer of pollen from closest locality) was used only in localities Kodská stěna and Haknovec. Plants from these localities were pollinated reciprocally.

In late June, when the seeds became ripe, I counted number of hard black or dark brown seeds. I sowed these seeds into pots in a greenhouse and recorded their germination and survival.

Differences in number of produced seeds between treatments were tested with GLM with binomial models using program S-plus (MathSoft 1999).

3. Results

3.1 Characteristics of the localities

3.1.1 Vegetation composition

I recorded vegetation composition at 24 plots in the Czech Republic and at 15 plots in the Slovak Republic, they represent 10 and 6 localities, respectively. In plots I recorded 4 species of trees (*Pinus sylvestris*, *Prunus mahaleb*, *Sorbus aucuparia* and *Sorbus aria*), 15 species of shrubs or low trees (*Berberis vulgaris*, *Cornus mas*, *Cornus sanguinea*, *Cotoneaster integerrimus*, *Cotoneaster melanocarpus*, *Cotoneaster tomentosus*, *Euonymus verrucosa*, *Fraxinus excelsior*, *Ligustrum vulgare*, *Prunus spinosa*, *Quercus pubescens*, *Quercus robur*, *Rosa* sp., *Spiraea media* and *Ulmus minor*) and 163 herb species. For vegetation composition on localities see Appendix 1.

In graph of DCA (Figure 5) we can see the differentiation of localities from Czech and Slovak Republic (1. axis explains 9.8 % of variability, 2. axis adds 7.8 %). We can distinguish localities to four groups: Czech Karst, Slovak Karst, Zázmoníky and Deblík.

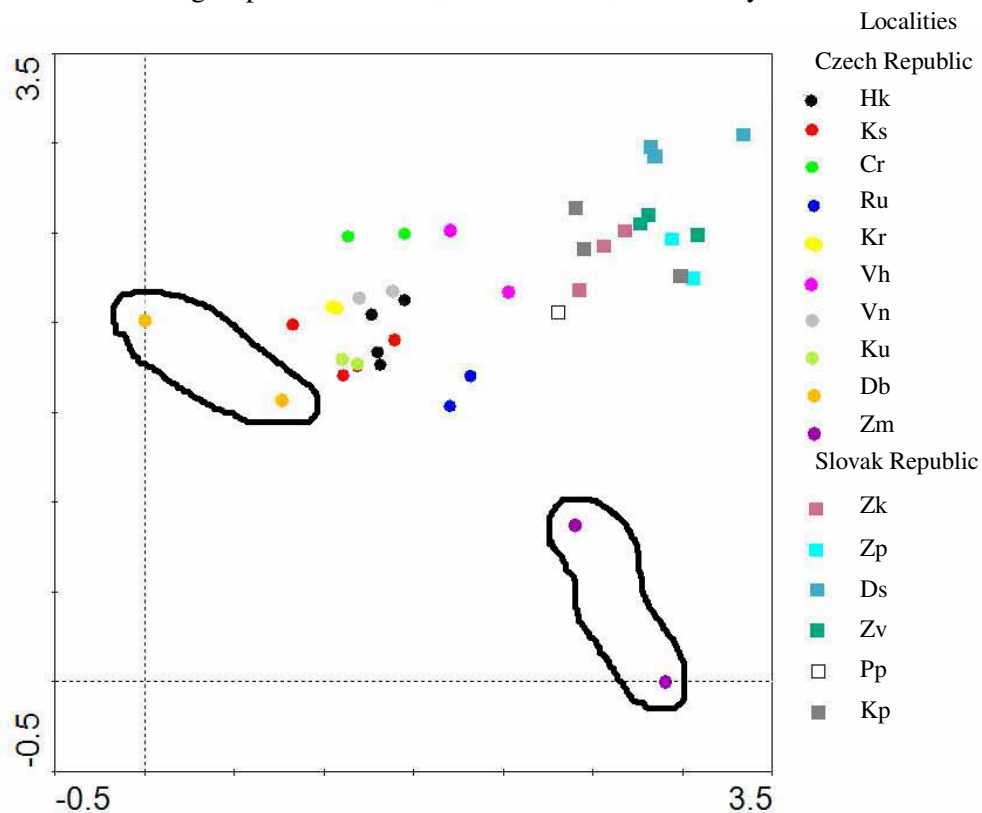


Figure 5: DCA analysis of vegetation composition of Czech and Slovak localities. Circled regions are localities from Zázmoníky and Deblík that are not in any of karst regions. The Czech Republic (Hk – Haknovec, Ks – Kodská stěna, Cr – Císařská rokle, Ru – Radotínské údolí, Kr – Kozelská rokle, Vh – Velká hora, Vn – Vanovice, Ku – Karlické údolí, Db – Deblík, Zm – Zázmoníky), The Slovak Republic (Zk – Zadielský kameň, Zp – Zádielská planina, Ds – Domické škrapy, Zv – Železná vrata, Pp – Plešivská planina, Kp – Koniárska planina).

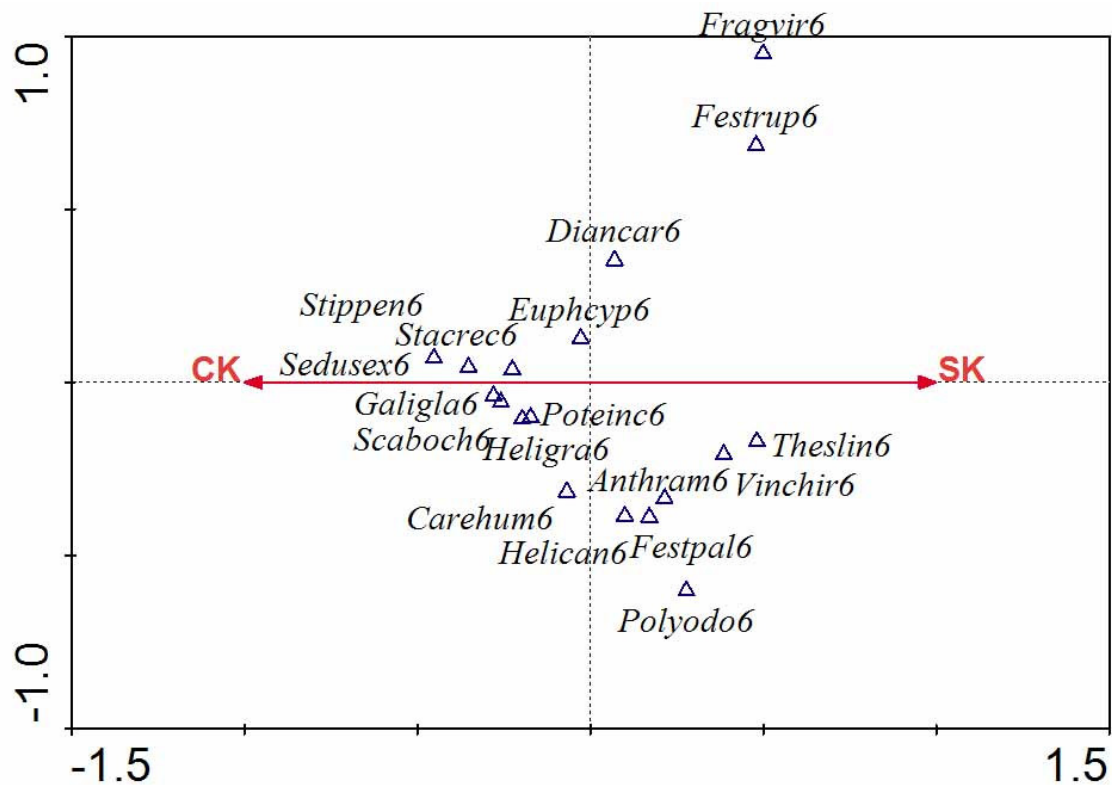


Figure 6: CCA analysis of vegetation composition of Czech and Slovak Karst localities. There are significant differences between vegetation in the the two Karsts ($p = 0.002$). From analysis were excluded species occurring only in flora of one republic. CK = Czech Karst, SK = Slovak Karst.

From DCA analysis it is clear that localities Zazmoníky and Deblík are very different from all the others. Locality in Zázmoníky is in pine forest on deep soil, which is very unusual type of habitat for the species. Population at locality on Deblík is nowadays extinct and overgrown with trees and shrubs such as *Cotoneaster integerrimus*, *Prunus spinosa*, *Ulmus minor* and ruderal species such as *Sysimbrium loeselli*. These localities were excluded from direct analysis of vegetation composition for their differences from Karst regions.

CCA analysis, testing differences in vegetation composition between localities in Czech and Slovak Karst was significant ($F = 3.268$; $p = 0.002$, variability between region explains 9.0 % of total variability in vegetation composition). CCA analysis without species occurring only in flora of one republic explained even more variability in vegetation composition ($F = 3.533$; $p = 0.002$, 9.7 %) – Figure 6. Species most strongly differentiating the localities were *Thesium linophyllum*, *Vincetoxicum hirundinaria* occurring more often in Slovak Karst and *Galium glaucum*, *Sedum sexangulare* and *Stipa pennata* occurring more often in Czech Karst.

3.1.2 Soil analysis

46 samples of soil from 14 localities were analysed. The results show that soils at *Dracocephalum austriacum* localities are neutral to alkaline with pH between 6.55 (Zádielská planina) and 7.61 (Radotínské údolí).

Content of humus (C_{ox}) was relatively high. It ranges from 4.42 % (Zázmoníky) to 27.6 % (locality on Koniárská planina).

Content of total nitrogen (N_t) ranged from 0.34 % (Zázmoníky) to 2.54 % (Koniárská planina). Ratio C:N was quite high in most of the samples. It ranged from 8.41 (Železná vrata) to 13.31 (Vanovice) and soils can thus be considered to have quality humus.

From measured macroelements there was very high contents of available calcium with wide range from 5870 (Domické škrapy) to 22900 (Karlické údolí) mg Ca/kg. Contents of available magnesium were also quite high, from 82 (Císařská rokle) to 2420 (Koniárská planina) mg Mg/kg and contents of available potassium from 123 (Zázmoníky) to 588 (Kodská stěna) mg K/kg of soil. Very low content of available phosphorus was detected (maximum at Velká hora 42 mg P/kg) but almost one third of the samples was under the limit of sensitivity (<10 mg P/kg). Detailed results of soil analyses are in Appendix 2.

There were significant differences ($p < 0.01$) in all soil attributes among the localities within regions. Differences between regions of Czech Karst and Slovak Karst are in Table 3. Locality Zázmoníky was excluded from the test of soil chemistry because it is not situated in Karst region and it would affect the analysis. In Czech Karst there were significantly higher contents of C_{ox} (average values are 8.35 and 16.31 % for Czech and Slovak Karst, respectively), N_t (average values are 1.13 and 1.66 % for Czech and Slovak Karst, respectively) and values of $pH_{(H_2O)}$ (average values are 7.37 and 7.02 for Czech and Slovak Karst, respectively).

Czech isolated locality in Zázmoníky is quite different from others. It has the lowest contents of C_{ox} , N_t and K from all the localities.

region	pH_{H_2O}	C_{ox}	N_t	C : N	Ca	Mg	K	P
df	1	1	1	1	1	1	1	1
df error	13	13	13	13	13	13	13	13
F value	10.09	5.25	8.19	2.57	1.51	1.14	1.36	0.68
p	<0.01	<0.05	<0.05	0.13	0.24	0.31	0.26	0.43
R^2	0.78	0.40	0.63	–	–	–	–	–

Table 3: Results of ANOVA with fixed effects testing differences in soil attributes between the two regions. Statistically significant differences are in bold.

3.1.3 Other abiotic characteristics of the localities

Dracocephalum austriacum grows often on very steep slopes (slope was often about 30 and more degrees) with shallow soils (average soil depth was almost always less than 10 cm (except for Železná vrata and Zázmoníky). Differences in soil depth were significant among localities ($p < 0.001$) but not between regions. The elevation of the localities ranges from 227 (Zázmoníky) to 730 (Železná vrata) metres above sea level (Table 4).

Lokality name	GPS coordinates	Altitude [m.a.s.l.]	Aspect	Slope	No. of plants	Soil depth (SD)
CZECH REPUBLIC						
Haknovec	N 49°56'21,1" E 14°11'25,2"	287	S, SW	40-60°	500	3.2 (4.4)
Kodská stěna	N 49°56'01,6" E 14°07'29,0"	350	S	35-90°	150	6.5 (4.2)
Císařská rokle	N 49°55'46,5" E 14°07'51,6"	270	E	30°-80°	165	2.7 (2.8)
Radotínské údolí	N 49°59'55,9" E 14°18'51,3"	300	NW	40°	55	2.2 (3.6)
Kozelská rokle	N 49°56'56,9" E 14°08'07,7"	241	SE	30- 90°	2	4.3 (3.7)
Vanovice	N 49°55'46,5" E 14°08'59,2"	245	NW	60°	55	3.1 (2.9)
Velká hora	N 49°56'49,3" E 14°09'27,0"	306	S, SW	35-70°	400	4.7 (2.0)
Karlické údolí	N 49°56'56,5" E 14°14'49,3"	347	S, SW	60-80°	7	2.0 (2.3)
Zázmoníky	N 48°56'09,1" E 16°51'09,2"	227	W	4°	1	>30
Deblík	N 50°35' E 14°02'	410	S	5°	0	6.7 (3.7)
SLOVAK REPUBLIC						
Zádielský kameň	–	595	SW	30°	100	4.0 (3.1)
Domické škrapy	–	340	SW	3°	200	9.9 (9.9)
Železná vrata	–	730	S, SW	5-10°	150	12.1 (10.7)

Table 4: List of localities used for measuring of abiotic conditions: their GPS coordinates, altitude, aspect, slope and soil depth, SD = standard deviation. Coordinate system WGS-84.

Zázmoníky is locality with the lowest altitude and highest soil depth. Together with Deblík and Domické škrapy it is also the locality with the shallowest slope (only 5°). The localities are mostly on limestone rocks with organic fillings such as hornstones. The only exceptions are localities Zázmoníky (sandstone, pudding stone) and Deblík (vulcanites). Slovak localities were not studied for geology but all of them are in limestone Slovak Karst.

3.2 Genetic analysis

In allozyme analyses we detected 5 enzymatic systems suitable for scoring. They involved 10 loci – leucine aminopeptidase (LAP, tree loci), superoxid dismutase (SOD, three

loci), glucose-6-phosphate dehydrogenase (G6PDH, two loci), and aspartat aminotransferase (AAT, two loci). Six of the loci were variable. Detailed results of allele scoring are available in Appendix 3. Graphs with numbers of alleles in each variable loci are in Appendix 4.

Polymorphic loci had from 2 to 8 alleles. Proportion of polymorphic loci is in Table 5. Six of ten loci were polymorphic at the species level and 3 (Haknovec, Císařská rokle, Vanovice and Radotínské údolí) to 5 (Kodská stěna) at the population level (Table 5). Genetic diversity, as measured by Shannon diversity index, was 0.4863 at the species level, ranging from 0.1932 to 0.5168 within populations (Table 5). There was no relationship between the level of genetic diversity and size of the population considered ($F_{1,7} = 0.139$; $p = 0.72$).

Locality	Proportion of polymorphic loci [%]	Shannon diversity index	Effective no. of alleles
Haknovec	30	0.27	1.16
Kodská stěna	50	0.29	1.14
Císařská rokle	30	0.20	1.12
Vanovice	30	0.19	1.10
Radotínské údolí	30	0.29	1.20
Velká hora	20	0.26	1.16
Domické škrapy	40	0.40	1.28
Zádielský kameň	40	0.41	1.27
Železná vrata	40	0.52	1.41
All populations	60	0.49	1.25

Table 5: Proportion of polymorphic loci, Shannon diversity index and effective number of alleles for Czech and Slovak populations.

locality	H_{obs}	H_{exp}	F_{is}	p
Haknovec	0.20	0.16	-0.19	0.95
Kodská stěna	0.13	0.17	0.30	0.09
Císařská rokle	0.09	0.12	0.31	0.19
Vanovice	0.06	0.11	0.52	0.02
Radotínské údolí	0.07	0.16	0.59	0.0018
Velká hora	0.10	0.15	0.42	0.05
Domické škrapy	0.17	0.25	0.39	0.02
Zádielský kameň	0.20	0.25	0.25	0.08
Železná vrata	0.28	0.31	0.17	0.18
All populations	0.15	0.19		

Table 6: Observed and expected heterozygosities (H_{obs} and H_{exp}), inbreeding coefficients (F_{is}) for each population and its level of significance (p). Significant values are in bold.

I found significant negative relationship between within population inbreeding coefficient and population size ($F_{1,7} = 7.39$; $p < 0.05$).

Overall fixation index, F_{st} , value was 0.230 for 9 samples per population and 0.370 for all samples (Table 7). Overall inbreeding coefficient, F_{is} , was 0.227 for 9 samples per population and 0.090 for all samples. All these values are significantly different from 0 ($p < 0.001$). F_{is} within populations ranged from -0.189 (Haknovec) to 0.609 (Radotínské údolí) – Table 6. In 3 populations F_{is} was significantly different from 0 ($p < 0.05$) – Table 6. Inbreeding coefficient within populations is negatively correlated with population size ($F_{1,7} = 7.39$, $p < 0.05$). Total inbreeding coefficient, F_{it} , was 0.405 and 0.427 for 9 samples and all samples, respectively. Fixation index for Czech Karst, F_{st-CK} , is higher than index for Slovak Karst, F_{st-SK} , in both types of samples. F_{st-CK} was 0.225 for 9 samples per population (0.333 for all samples) and F_{st-SK} was 0.093 for 9 samples (0.065 for all). F_{st-CSK} (fixation index for Czech and Slovak Karst together, which describes proportion of variability between regions, out of total variability) was 0.001 for 9 samples and 0.065 for all samples. F_{st-CK} and F_{st-SK} and F_{st-CSK} were all significantly different from 0 except for F_{st-CSK} for 9 samples per population (Table 7).

Values of F statistics from 9 samples per population							
No. of alleles	No. of populations	No. of samples		values of F	chisq	DF	p value
19	9	79	Fst	0.230	654.608	144	<0.001
19	9	79	Fis	0.227	644.922	144	<0.001
19	9	79	Fit	0.405	1151.087	144	<0.001
15	6	52	Fst-CK	0.225	327.581	70	<0.001
14	3	27	Fst-SK	0.093	65.117	26	<0.001
19	9	79	Fst-CSK	0.001	3.488	144	>0.999
15	6	52	Fis-CK	0.255	371.956	70	<0.001
15	3	27	Fis-SK	0.206	155.426	28	<0.001
Values of F statistics from all samples							
No. of alleles	No. of populations	No. of samples		values of F	chisq	DF	p value
20	11	120	Fst	0.370	1686.259	190	<0.001
20	11	120	Fis	0.090	411.448	190	<0.001
20	11	120	Fit	0.427	1945.556	190	<0.001
16	8	60	Fst-CK	0.333	600.000	105	<0.001
16	3	60	Fst-SK	0.094	169.135	30	<0.001
20	11	120	Fst-CSK	0.065	295.632	190	<0.001
16	8	60	Fis-CK	0.231	415.385	105	<0.001
16	3	60	Fis-SK	0.256	460.508	30	<0.001

Table 7: Comparison of fixation indices F_{st} , F_{st-CK} , F_{st-SK} and F_{st-CSK} ; inbreeding coefficients F_{is} , F_{is-CK} and F_{is-SK} ; and overall inbreeding coefficients F_{it} from data of 9 samples per each population and all samples per population tested according to Workman et Niswander (1970). F_{st-CK} and F_{st-SK} are fixation indices for separate regions of Czech and Slovak Karst. F_{st-CSK} is a fixation index for Czech and Slovak Karst together, which describes proportion of variability between regions, out of total variability.

Number of unique alleles per population was very low. Only in Kodská stěna, Domické škrapy and Železná vrata 1 unique allele was observed. In Czech and Slovak Karst as well there were 4 unique alleles. Number of all alleles per population was higher in Slovak Karst and ranged from 10 to 16 in Czech Karst and from 16 to 17 in Slovak Karst (Table 8).

Locality/region	Unique alleles	Overall no. of alleles per population (region)
Haknovec	0	14
Kodská stěna	1	15
Císařská rokle	0	14
Vanovice	0	16
Kozelská rokle	0	10
Radotínské údolí	0	15
Karlické údolí	0	13
Velká hora	0	14
Domické škrapy	1	17
Zádiel	0	16
Železná vrata	1	17
Czech Karst	4	20
Slovak Karst	4	20
All together	–	24

Table 8: Number of unique alleles per each population, each region and all population together (the no. of unique alleles was computed from all samples).

3.3 Demography

3.3.1 Transition probabilities

The number of individuals within each population that was followed in the course of the study ranged from 80 to 175 and altogether 838 individuals were used for the matrix analysis (Appendix 6). Average mortality decreased with size of an individual and ranged from 0 – 100 % for seedling, from 2 to 17 % for small plants and from 0 to 5 % for large plants (Appendix 6). In all populations I recorded very high survival of small and large plants. Large plants made a larger contribution to seed production compared to small plants. Germination rates in sowing experiment ranged from 0 to 9.4 % and observed natural germination rates ranged from 0.01 to 0.25 seedlings per 1 small plant and 0.03 to 1.15 seedlings per 1 large plant. Transition matrices are in Appendix 6.

3.3.2 Population growth

Simple projection matrices generated λ -values (population growth rates) from 0.94 to 1.21 (see Figure 7 and Table 9). In transition 2003-2004 populations in Haknovec and Domické škrapy significantly decreased (λ was significantly below 1) and 2004-2005 only population in Haknovec significantly grew (λ was significantly above 1).

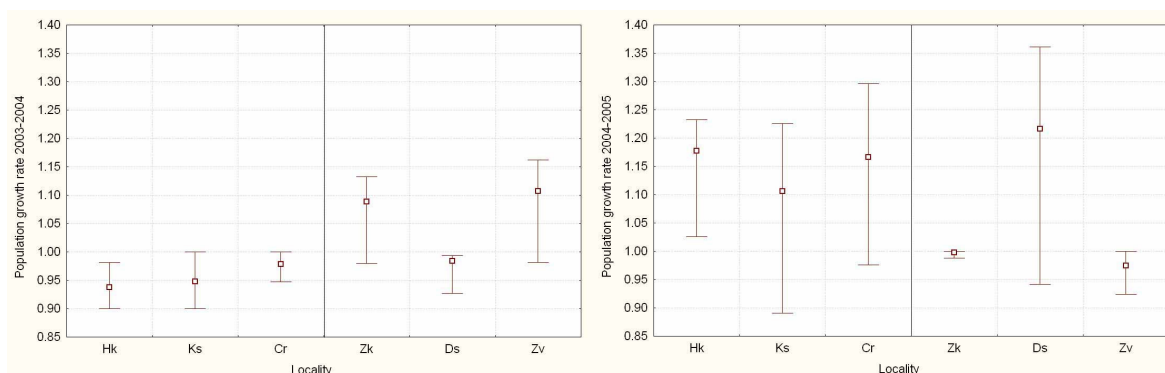


Figure 7: Population growth rate and its 95 % confidence interval at separate populations in Czech and Slovak Karst computed from transition matrices with seed bank for years 2003-2004 and 2004-2005 Hk = Haknovec, Ks = Kodská stěna, Cr = Císařská rokle, Zk = Zádielský kameň, Ds = Domické škrapy, Zv = Železná vrata.

locality	2003-2004			2004-2005		
	95 %CI-L	λ	95 % CI-U	95 %CI-L	λ	95 % CI-U
Haknovec	0.90	0.94	0.98	1.03	1.18	1.23
Kodská stěna	0.90	0.95	1.00	0.89	1.11	1.23
Císařská rokle	0.95	0.98	1.00	0.98	1.17	1.30
Zádielský kameň	0.98	1.09	1.13	0.99	1.00	1.00
Domické škrapy	0.93	0.98	0.99	0.94	1.22	1.36
Železná vrata	0.98	1.11	1.16	0.92	0.97	1.00

Table 9: Values of population growth rate and its 95 % confidence interval at separate populations in Czech and Slovak Karst computed from transition matrices for years 2003-2004 and 2004-2005. CI-L – lower end of the confidence interval, CI-U – upper end of the confidence interval.

There were no significant differences in population growth rates between matrices with and without seed bank constructed for the Czech Karst (Figure 8).

Population growth rate (λ) for years 2003-2005 was computed with stochastic simulation models and mean values ranged from 1.04 to 1.23. All populations are growing but only Císařská rokle, Zádielský kameň and Železná vrata have population growth rates significantly above 1. With bootstrap I computed also 95 % confidence intervals of these values (Figure 9, Table 10).

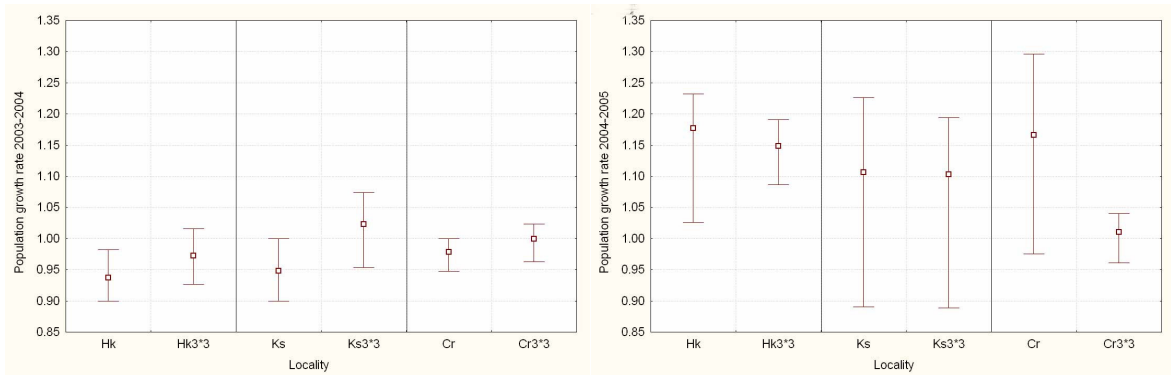


Figure 8: Comparison of population growth rates and their 95 % confidence interval computed from transition matrices for years 2003-2004 and 2004-2005 for populations in Czech Karst Hk = Haknovec, Ks = Kodská stěna, Cr = Císařská rokle. Matrices with mark „3*3“ are without seed bank.

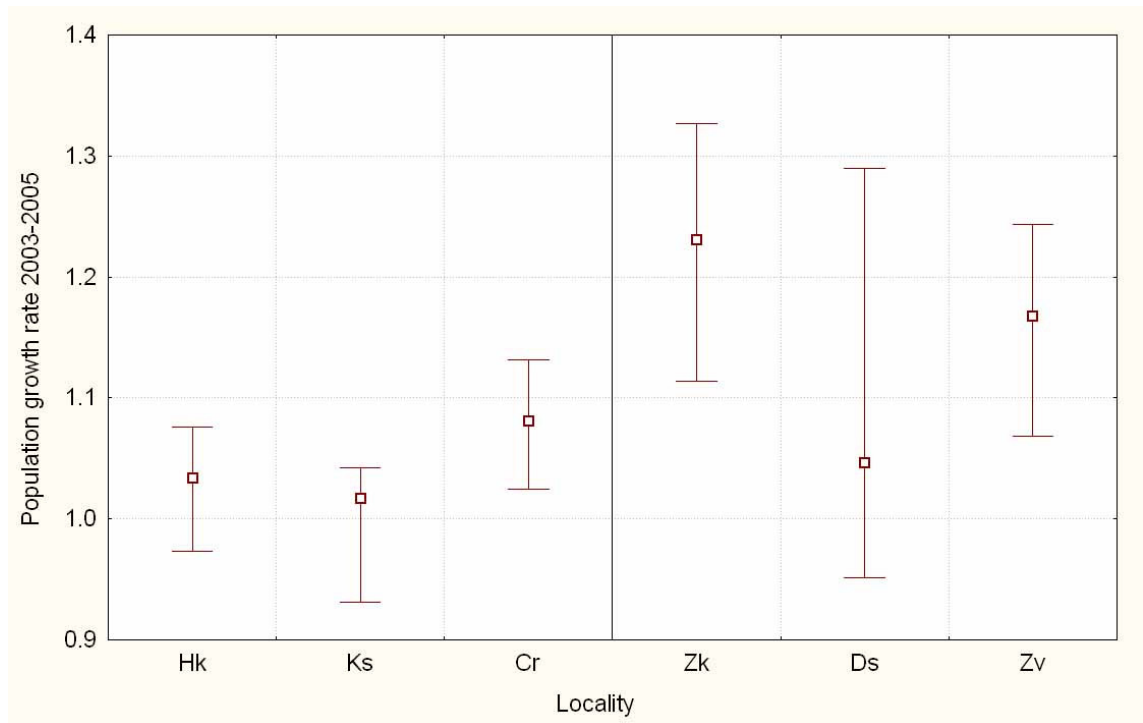


Figure 9: Population growth rate and its 95 % confidence interval at separate populations in Czech and Slovak Karst computed with stochastic simulation models from matrices for years 2003-2005. Hk = Haknovec, Ks = Kodská stěna, Cr = Císařská rokle, Zk = Zádielský kameň, Ds = Domické škrapy, Zv = Železná vrata.

locality	95 %CI-L	λ	95 % CI-U
Haknovec	0.97	1.03	1.08
Kodská stěna	0.93	1.02	1.04
Císařská rokle	1.02	1.08	1.13
Zádielský kameň	1.11	1.23	1.33
Domické škrapy	0.95	1.05	1.29
Železná vrata	1.07	1.17	1.24

Table 10: Population growth rate and its 95 % confidence interval at separate populations in Czech and Slovak Karst computed with stochastic simulation models from matrices for years 2003-2005. CI-L – lower end of the confidence interval, CI-U – upper end of the confidence interval.

I computed also with stochastic simulation models population growth rate of all populations in Czech Karst and all populations in Slovak Karst together. It was 1.08 (95 % CI 1.04; 1.12) for Czech Karst and 1.19 (95 % CI 1.07; 1.28) for Slovak Karst (Figure 10). For Czech Karst I computed also population growth rate for years 2003-2005 without seed bank. Its value, 1.06 (95 % CI 1.05; 1.08), was not significantly different from matrices with seed bank. Population growth rates for both regions were significantly above 1 and populations are growing.

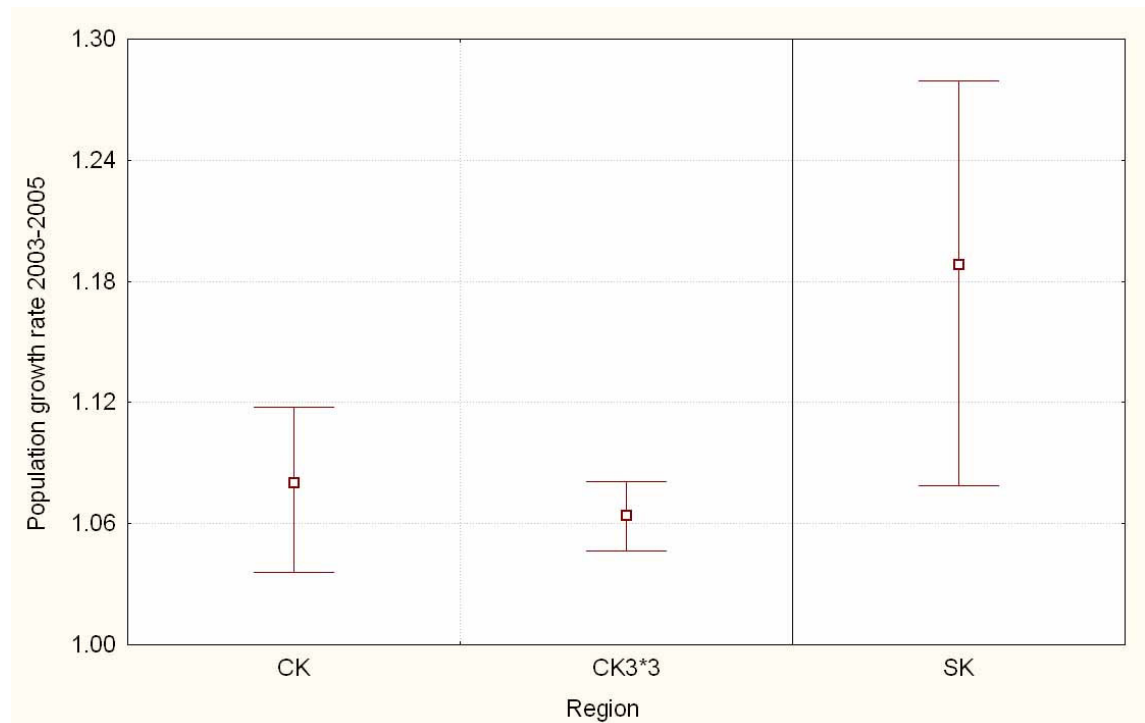


Figure 10: Population growth rates and their 95 % confidence interval for all populations in Czech and Slovak Karst computed with stochastic simulation models from matrices for years 2003-2005. CK = Czech Karst matrices with seed bank, CK3*3 = Czech Karst matrices without seed bank, SK = Slovak Karst matrices with seed bank.

3.3.3 Elasticities and LTRE

In all populations, the highest elasticity was detected for matrix elements representing the likelihood of individuals remaining in the same class (Figure 11). Their summed elasticities were from 0.64 to 0.77. The single most important matrix element was survival in stage of large plants for populations Haknovec, Kodska stěna, Císařská rokle and Zádielský kameň (elasticity ranged from 0.32 to 0.39) and survival in stage of small plants for populations Domické škrapy and Železná vrata (0.47 and 0.30) – see Appendix 5.

The life table response experiments analysis indicated that life stages, which contribute the most to real changes in population growth rates in separate populations, are seed production of large plants and transition from seedlings to small plants. Only in Haknovec and Císařská rokle populations it was only seed production of large plants – see Figure 12. In the Czech Karst it was seed production of large plants and in the Slovak Karst seed production of large and even small plants and growth from seedlings to small plants (Figure 13).

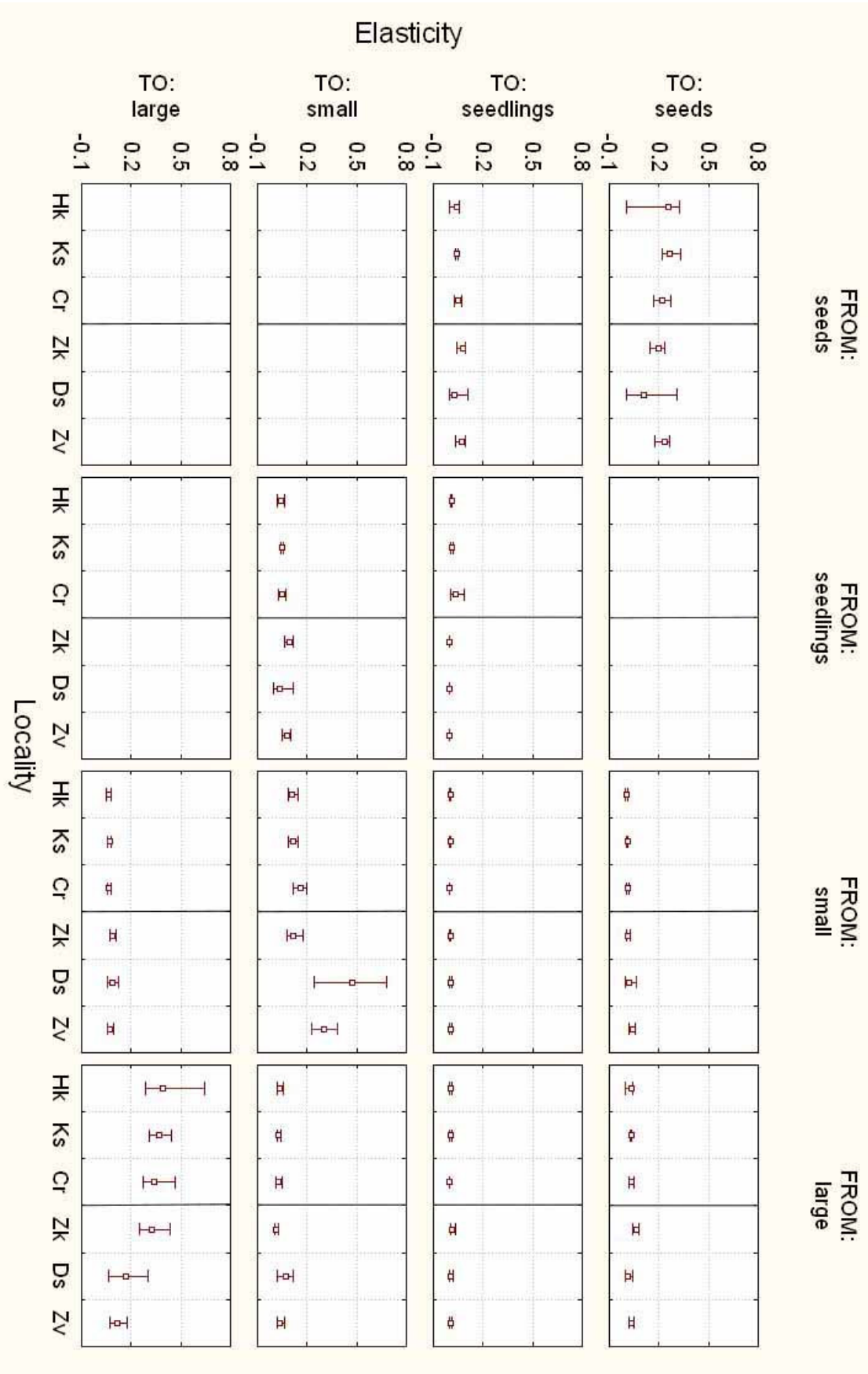


Figure 11: Elasticities and their 95 % confidence interval at separate populations in Czech and Slovak Karst computed with stochastic simulation models from matrices for years 2003-2005. Hk = Haknovec, Ks = Kodská stěna, Cr = Císařská rokle, Zk = Zádielský kameň, Ds = Domické škrapy, Zv = Železná vrata.

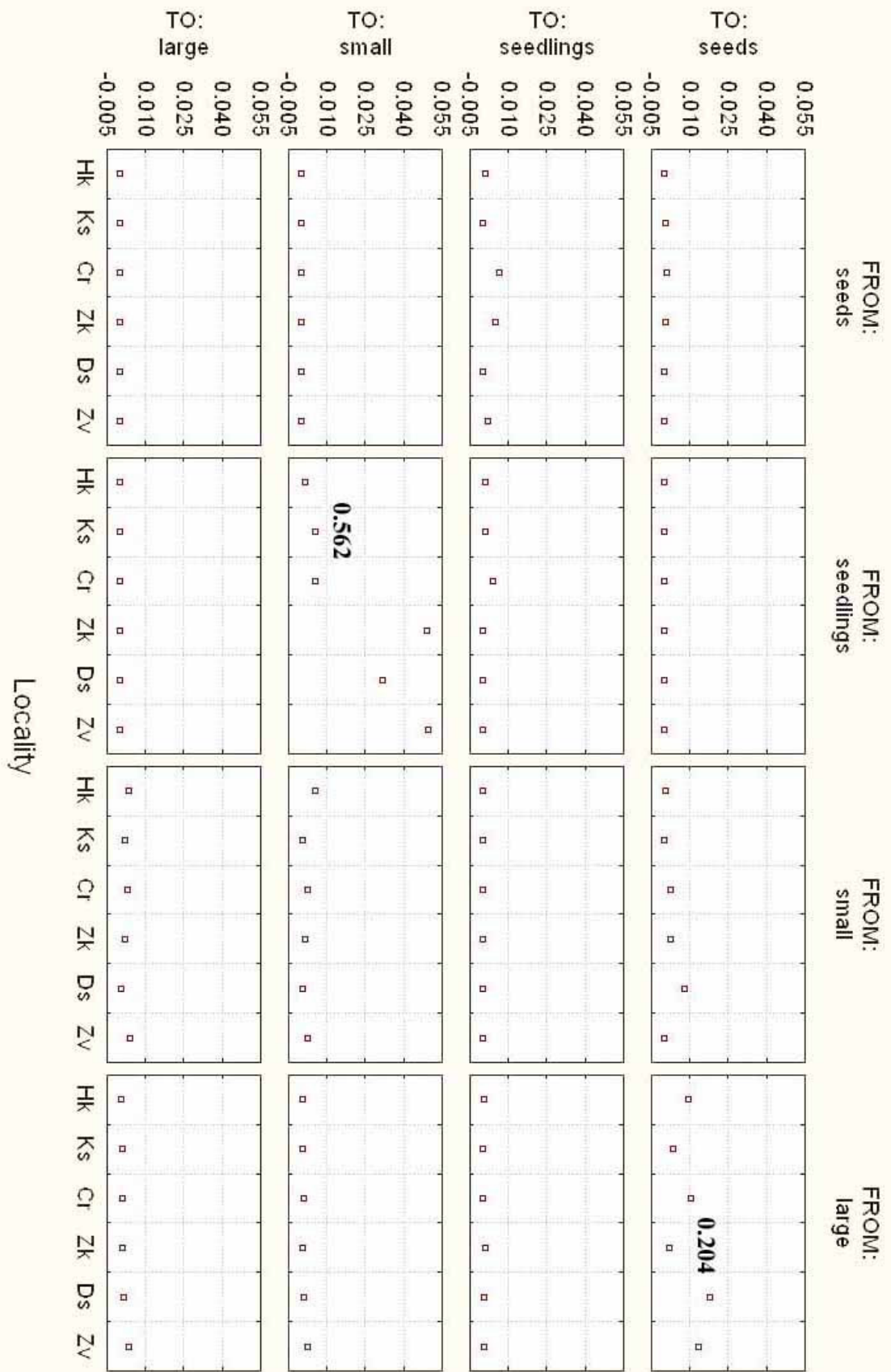


Figure 12: Transitions which contribute the most to real changes in population growth rates in Czech and Slovak Karst populations computed using life table response experiments from matrices for years 2003-2005. Two values was 100× lowered so that other values could be readable. These values are labeled with real values. Hk = Haknovec, Ks = Kodska stena, Cr = Císařská rokle, Zk = Zádielský kameň, Ds = Domické škrapy, Zv = Železná vrata.

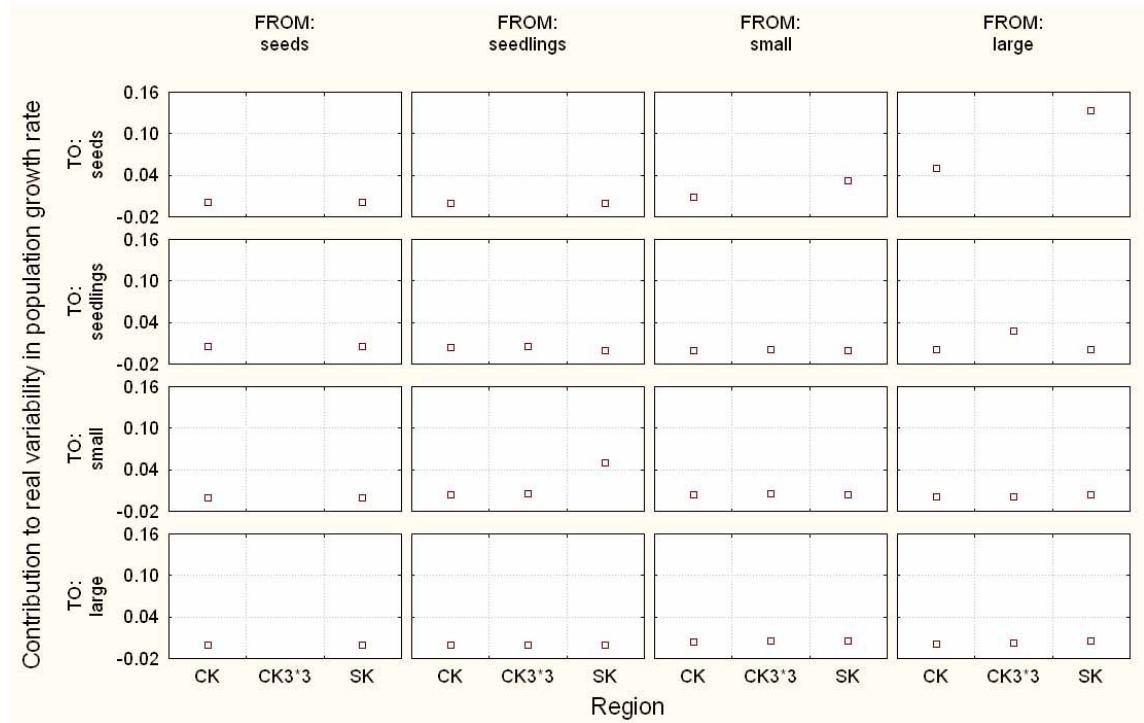


Figure 12: Transitions, which contribute the most to real changes in population growth rates in Czech and Slovak Karst computed using life table response experiments analyses from matrices for years 2003-2005. CK = Czech Karst, SK = Slovak Karst, CK3*3 = Czech Karst without seed bank.

3.3.4 Extinction probabilities

I computed extinction probabilities of the species within 20 and 100 years using stochastic matrix simulation models. All larger populations (larger than 50 individuals) in Czech Karst and all populations in Slovak Karst have almost 0 % extinction probability within 20 years (except 1% in Czech Karst populations Radotínské údolí and Vanovice). This was true even for simulations with digging up of large plants. Small populations in Czech Karst (Karlické údolí and Kozelská rokle) had quite high probabilities of extinction if some plants are dugged up. It ranged from 9 to 17 % when no plants were dugged up to 36 and 62 % when 10 plants were dugged up (see Table 11).

Extinction probabilities within 100 years were 0 % for Czech Karst populations larger than 400 individuals even if 10 large plants per year were dugged up and so was it for Slovak populations larger than 100 individuals. Extinction probabilities at smaller populations in Czech and Slovak Karst increased with number of dugged plants up to 53 % in Slovak Karst and 100 % in Czech Karst smallest populations (Karlické údolí and Kozelská rokle).

Locality	population vector	extinction probability [%] in 20 years					extinction probability [%] in 100 years					
		No. of digged up large plants per year										
		0	1	3	5	10	0	1	3	5	10	
Haknovec	0-0-250-250	0	0	0	0	0	0	0	0	0	0	0
Kodská stěna	0-54-86-53	0	0	0	0	0	0	0	1	13	80	
Čísařská rokle	0-7-78-83	0	0	0	0	0	0	0	0	0	14	
Vanovice	0-0-25-25	0	0	0	0	1	0	0	27	60	95	
Radotínské údolí	0-0-25-25	0	0	0	0	1	0	0	27	60	95	
Karlické údolí	0-0-2-5	9	10	24	35	36	15	76	98	100	100	
Kozelská rokle	0-0-2-2	17	47	47	50	62	49	86	99	100	100	
Velká hora	0-0-200-200	0	0	0	0	0	0	0	0	0	0	
Zádielský kameň	0-10-32-54	0	0	0	0	0	0	0	0	0	1	
Domické škrapy	0-5-94-39	0	0	0	0	0	0	0	0	0	0	
Železná vrata	0-6-80-33	0	0	0	0	0	0	0	0	0	0	
Plešivská planina	0-0-5-5	0	0	0	0	1	0	0	20	40	53	
Koniarská planina	0-0-5-5	0	0	0	0	1	0	0	20	40	53	
Zádielská planina	0-0-25-25	0	0	0	0	0	0	0	0	0	1	

Table 11: Extinction probabilities of populations in Czech and Slovak Karst computed with stochastic simulation models from matrices for years 2003-2005. Extinction probability for populations, I had not transition matrices for, were computed using all matrices from corresponding region (Czech or Slovak Karst). Population vector is number of seeds, seedlings, small and large plants used as initial population vector.

3.3.5 Seed production

None of the measured parameters (soil depth, proportion of rock in plant surroundings, amount of shadow from trees, number of flowering and non-flowering plants within 50 cm and within 100 cm) was significantly correlated with seed production.

3.4 Combination of genetic and demographic data

3.4.1 Genetic diversity and population growth rate

The correlation of genetic parameters (Shannon diversity index, effective number of alleles, observed heterozygosity and expected heterozygosity) and number of developed seeds (average through years 2003-2005) was highly significant ($p < 0.001$) for all the four parameters. I used the slope of the relationship between genetic diversity and number of produced seeds (no. of produced seeds = $23.968 * \text{Shannon diversity index} - 1.811$) to construct relationship between genetic diversity (measured with Shannon diversity index) and population growth rate (Figure 13). Population growth rate was computed using stochastic models, where matrices from all years were combined (for populations in Czech Karst I took double number of „poor“ matrices).

With 50 % decrease in observed genetic diversity population growth rate of none of the studied populations dropped below 1. With 90 % decrease population growth rate of only two populations (Domické škrapy and Haknovec) dropped slightly below 1 and only 99 % decrease cause that all population sizes (except Zádielský kameň) began to decrease.

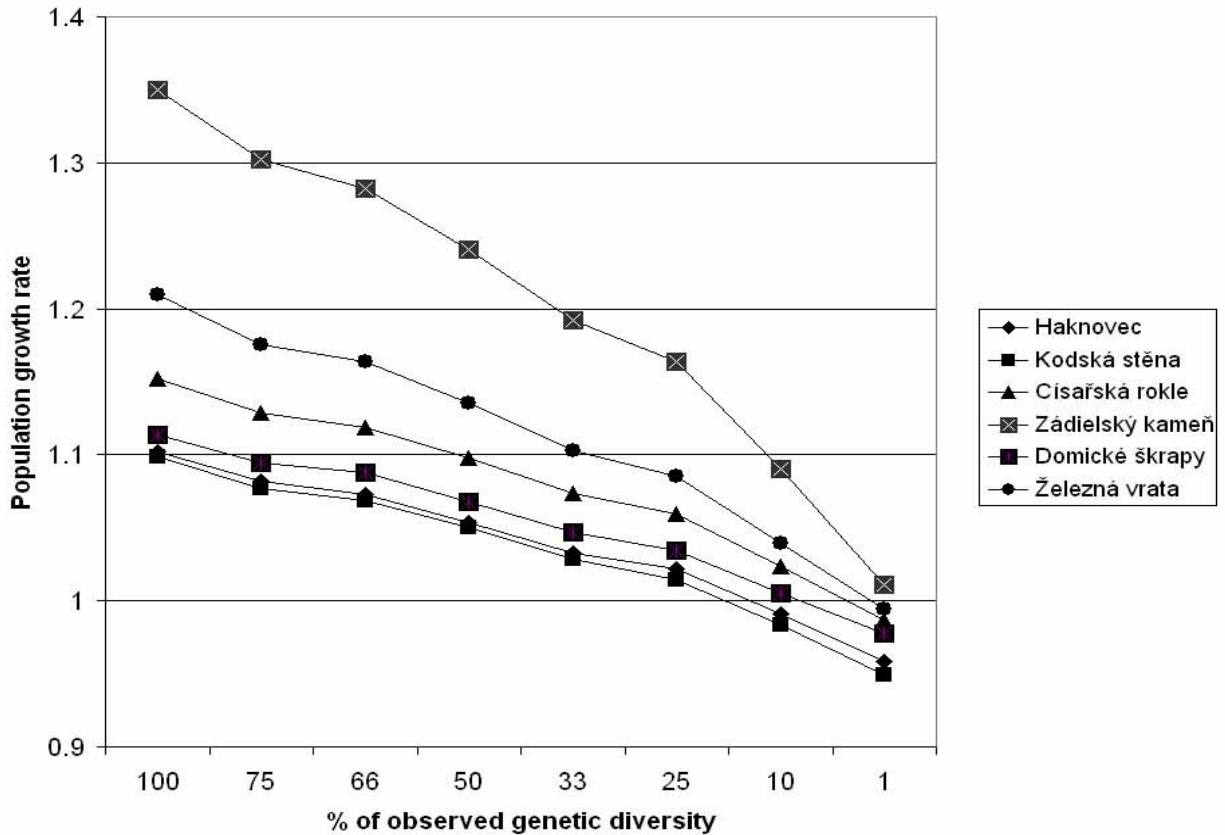


Figure 13: Relationship between genetic diversity (measured with Shannon diversity index) and population growth rate as a result of decreased seed production in populations of *Dracocephalum austriacum*. Population growth rate was computed using stochastic models, where matrices from all years were combined (for populations in Czech Karst I took doubled number of „poor“ matrices).

3.4.2 Pollination experiments

I recorded seed set in 116 flowers out of 170 treated (marking of others were lost). Numbers of flowers for treatments 1-6 were 21, 24, 19, 11, 19 and 22, respectively.

Caged flowers prevented from pollen transfer from other plants or access of pollinators do not produce any seeds and are significantly differed in seed production from all other treatments ($p < 0.001$). The highest number of seeds was produced by flowers pollinated from another locality (1.55 number of seeds per flower on average). This was significantly more than when pollinated with pollen from the same flower or pollen from the same locality

($p < 0.05$). I found no significant differences between non-caged flowers and flowers pollinated with pollen from same flower, another plant in the locality or pollen from another locality (except for preventing from pollen and pollinator access) – Figure 14.

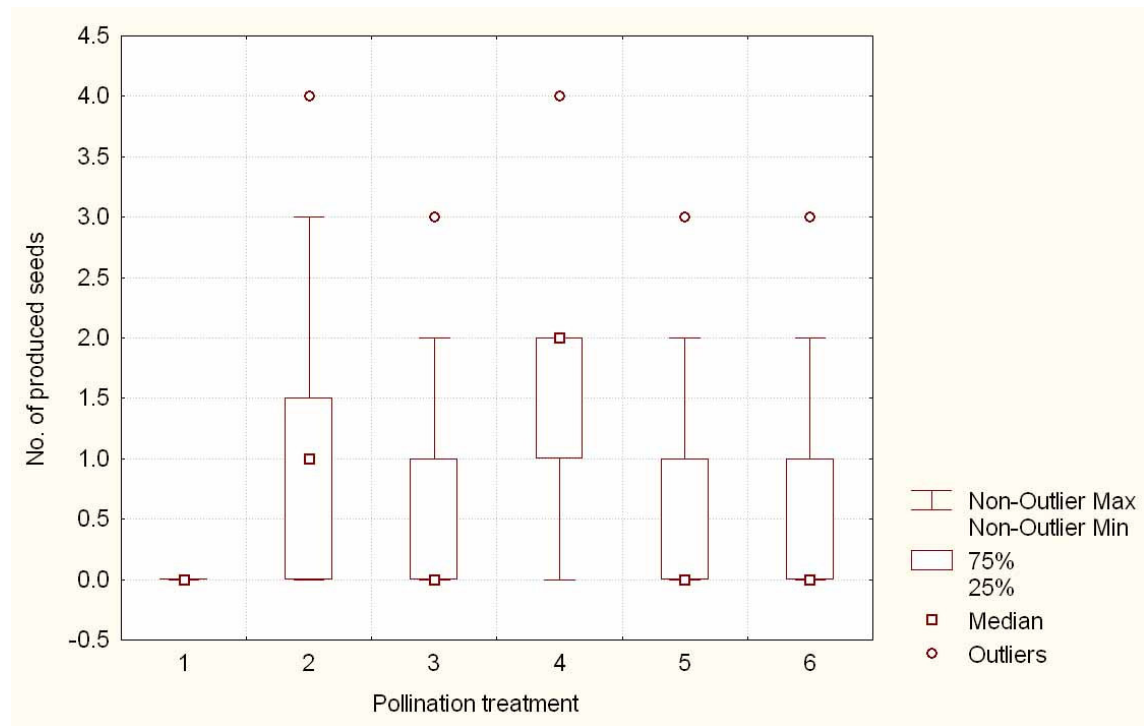


Figure 14: Number of produced seeds in different population treatments. 1 = flower was caged all the time and no transfer of pollen was allowed, 2 = flower was not caged all the time, 3 = pollinated with its own pollen, flower was caged again, 4 = pollinated with pollen from plant from another closest locality, flower was caged again, 5 = pollinated with pollen from nearby plant in the same locality, flower was caged again 6 = after coming into blossom, sack was removed so that natural pollinators could pollinate the flower.

Obtained seeds were sown in glasshouse but they were mixed during watering and so results cannot be used.

4. Discussion

The aim of my study was to compare population dynamics, genetic variation and characteristics of the localities of endangered species *Dracocephalum austriacum* in two distant regions (Czech and Slovak Republic) and to try to evaluate if conclusions on suitable conservation methods from one region are transferable to the other.

The results show that population dynamics of the species in the two regions is different. Population growth rate (λ) differed between regions in both transition intervals in my study. Whereas in first transition of the study (2003-2004) most of Slovak populations had much higher population growth rate (λ), in the second transition interval of the study (2004-2005) the situation was opposite. The reasons for relatively high population growth rate (λ) in the two separate regions were different. While in Czech Karst populations decrease of population growth rate (λ) was caused mainly by very low number of new seedlings, in Slovak Karst populations decrease of λ was caused by high mortality rate of seedlings. And even though population growth rate (λ) in *Dracocephalum austriacum* populations is quite similar between first transition interval in Czech Karst and second transition interval in Slovak Karst and between second transition interval in Czech and first transition interval in Slovak Karst, the reasons for these states are different. Slovak population in Domické škrapy is the only exception in this. Surprisingly it behaves similarly to the Czech populations in the same transition interval.

Published studies on differences in population dynamics are relatively rare. Willems et Ellers (1995) showed variation between two distant regions of *Orchis simia* populations in north-western and south-eastern distributional limit. They found significant differences in fitness traits. In south-eastern distributional limit population fitness is higher due to better climate conditions and vegetation structure. It confirms the importance of environmental variability to population growth rate.

Other studies show variability in population dynamics in centre and periphery of species distribution range but variation in environmental conditions can also play an important role there (Nantel et Gagnon 1999). Kluth et Bruelheide (2005) were interested in population dynamics of annual plant species, *Hornungia petraea*, in distribution centre in Italy and periphery in Germany. Surprisingly, they found lower variation of population growth rate and higher densities of adult plants and in seed bank in the periphery of distributional range. Populations of *Dracocephalum austriacum* in the Czech Republic are

also on the north periphery of its distributional range and I observed here slightly higher variability in population dynamics than in Slovak Karst.

Nevertheless, population growth rate (λ) for separate years was significantly different from 1 only in two localities (Haknovec in Czech Karst and Domické škrapy in Slovak Karst) during 2003-2005. In the first transition interval λ was significantly below 1 in both of these populations as a result of absence of seedlings. New seedlings were, however, missing also in other Czech populations in the first transition interval. In the second transition interval only in Haknovec population growth rate (λ) was above 1. It was due to high number of recorded seedlings in this population.

Total population growth rate computed with stochastic models from years 2003-2005 was higher in the Slovak Karst populations. Higher stasis transitions and on average higher number of produced seeds in Slovak populations is probably the main reason of this. In *Dracocephalum austriacum* better survival ability and higher seed production could be also a result of better habitat conditions of Slovak populations. Although correlation of number of produced seeds and amount of shrubs and trees around single plants within localities was not significant, Czech localities are more overgrown with shrubs and trees and that could result in lower survival probability of plants in Czech populations. Czech and Slovak localities also differ in vegetation composition and soil contents. From the analyzed soil characteristics, higher total nitrogen contents that could be important for plant growth at these localities supports higher fitness of Slovak populations.

Another reason for higher seed production, one of the transitions contributing strongly to population growth rate, in Slovak localities could be much higher genetic diversity of Slovak populations. I showed in my study that genetic diversity explains number of produced seeds very well ($R^2 = 0.71$). Significant positive effect of genetic diversity on plant fitness was also verified in overview study of Reed et Frankham (2003). DeMauro et al. (1993) described low seed production at the lakeside daisy *Hymenoxys acaulis* var. *glabra* in populations with low genetic diversity. They determined inbreeding depression as the main reason of this. On the other hand Lamni et al. (1999) found no correlation between number of produced seeds of rare perennial *Lychnis viscaria* and genetic diversity although they found significant positive correlation between genetic diversity and population size.

Analysis of elasticities showed that transitions that most contribute to population growth rate are transitions of stasis. In Czech Karst it is mainly stasis of large plants and in Slovak Karst of small plants. According to classification of Silwertown et al. (1993) the

species is by its elasticity classified as iteroparous herb of open habitats similarly as *Ranunculus repens*, *Hieracium floribundum* and *Arisaema triphyllum*. Elasticities are often used in conservation management to make predictions about what life stages are critical and may be a target of conservation management. Several recent papers, however, have warned that management decisions based solely on the elasticity analyses may be ineffective because not all transitions can be altered by the same proportion with the same success (de Kroon et al. 2000). Thus because it is often difficult to manipulate life stages with high elasticity values effectively, it can be more effective to try to affect population growth rate through large effects on other life stages. Emery et Gross (2005) revealed highest values of elasticity for survival of non-reproductive adults of an invasive plant *Centaurea maculosa* but when they tried to find change in which transition has the largest effect on changes in population growth rate after fire management, it was reproduction. Such information can be gained from life table response experiments (Caswell 1989b, 2000). From life table response experiments (LTRE) done in this study on *Dracocephalum austriacum* we can see that seed production and growth from seedlings to small plants are transitions, which contribute most to changes in population growth rate. Ehrlén et al. (2005) even showed that the higher the elasticity of particular life cycle transition, the lower the change in that transition rate caused by treatment was observed. This suggests that plants are able partly to buffer the effect of environmental variation by minimizing changes in the life cycle transitions that are most important to population growth rate and on the other hand that elasticity may really not be the best way to determine target traits for conservation management.

Stochastic population growth rate for the whole period (2003-2005) was always above 1 but only in populations in Císařská rokle, Zádielský kameň and Železná vrata it was significantly different from 1 and the populations are really growing. However, the observed variance in λ is quite high and it was suggested that such variation can lead to high population extinction risk (Tuljapurkar et Orzack 1980, Menges 1998). Further more spatial synchrony in the dynamics of the populations could make such species regionally even more vulnerable to extinction (Harrison et Quin 1989, Heino et al. 1997, Matter 2001). Extinction probabilities computed for *Dracocephalum austriacum* were, however, quite low and only in very small populations there was higher probability of extinction. Also only in small populations there were quite high extinction probabilities when digging up of large plants by rockgardeners was simulated. The low extinction probability may also be due to the fact that the evaluated time interval (20 and 100 years) is relatively short compared to the expected life span of the species. Nantel et al. (1996) studied populations of threatened *Panax quinquefolium* and

vulnerable wild *Allium triciccum* that are also endangered by harvesters. They found that number of harvested plants can be quite high (up to 30 % every five years for *Panax* and 1-8 % for *Allium*) but almost all of the recent populations of these species do not have size of minimum viable population. Harvesters prevent these species from reaching appropriate size and both species are going to get more endangered. Another species, *Agave victoriae-reginae*, is endangered with collecting its infrutescences and that is why its populations are declining. The only rescue for this species is that population sizes are so low that nowadays collecting of inflorescences becomes economically impractical (Martínez-Palacios 1999). Populations of *Dracocephalum austriacum* in the Czech Republic do not seem to be largely devastated by harvesters but for smaller populations even digging up of a few plants can have fatal consequences. Large populations have to be often and quite a lot harvested to lower population growth rate. Different situation is in the Slovak Karst where at least one population was almost destroyed by digging up of plants (Karasová in verb.).

Expected survival of *Dracocephalum* in the seed bank is quite high and populations in good climate conditions could reestablish population on the edge of extinction. But note that the data on survival in the seed bank are calculated by comparing the matrices with and without seed bank and that I did not make special experiment for this and so my approximation can be inaccurate. Wardle (2003) found survival in seed bank in herbaceous perennial *Trachymene incisa* 70 % after two years. It can be especially important for small populations of *Dracocephalum austriacum* endangered with low genetic diversity and digging up by rockgardeners. Alexander et Schrag (2003) describes role of seed bank in reestablishment of *Helianthemus annuus*.

I found lower mean genetic diversity in Czech Karst populations. This could be due to position of the populations in northern periphery of the distribution range. Studies show that there are differences in genetic diversity of populations in the centre and in the periphery of the distributional range (Lamni et al. 1999, Faugeron et al. 2004).

I found quite high genetic differences among populations. Population differentiation is much higher in Czech Karst ($F_{st-CK} = 0.333$) than in Slovak Karst ($F_{st-SK} = 0.094$). This difference could theoretically be due to the fact that there were only three populations sampled in Slovak Karst whereas in Czech Karst eight populations were sampled. But even when only three Czech population studied for demography were taken for analysis, F_{st-CK} was 0.243, which is more than 2 times more than in the Slovak Karst. It shows quite low communication between populations in the Czech Karst populations. High differentiation among populations and relatively high genetic variation within populations was found also in

endangered endemic *Agave victoriae-reginae* (Martínez-Palacios 1999). Lamni et al. (1999) describes also quite high among population variation in *Lychnis viscaria* but relatively low intrapopulation variability. *Dracocephalum austriacum* is pollinated mainly by bumblebees and for them it is probably impossible to transfer pollen from one locality to another (at least a few kilometres). And other natural transfers are probably unrealistic, too. Similar situation is common also in other animal pollinated plants (Colas et al. 1997, Martínez-Palacios 1999). On the other hand Brzosko et al. (2002) showed in *Cypripedium calceolus* quite high intrapopulation genetic variability but they found very low interpopulation variability. In their study there was also no significant correlation between genetic and geographical distances, which shows relatively recent origin of the populations studied, or a high level of gene flow among populations. It could be caused by better dispersal abilities of this species. Seeds of *Cypripedium calceolus* (Orchideaceae) are much smaller than those of *Dracocephalum austriacum*.

Genetic differentiation found between regions was quite low ($F_{st}\text{-CSK} = 0.065$). And it was not even significant when I computed it for 9 samples per population and not for all samples. Godt et al. (1995) examined genetic variability in *Helonias bullata*, a threatened perennial plant species and even though genetic diversity was low for the species, they found quite high level of genetic variation among populations and positive correlation between genetic diversity and geographical distance. Reisch et al. (2003) recorded with RAPD markers quite high genetic variability in *Saxifraga paniculata* between disjunct populations in central Europe but RAPD markers are much more sensitive to variability. Even when using isozymes (expected to be much less variable) I still found relatively high genetic diversity within populations and regions indicating that the genetic marker used in this study has the potential to detect variation. The absence of differentiation between regions in *Dracocephalum austriacum* is thus quite surprising and indicates that although populations in these regions are divided for a very long time, they do not seem to be yielded to genetic drift quickly.

Neither seed production nor population growth rate was significantly correlated with inbreeding coefficient, F_{is} , of a population. But population size is negatively correlated with inbreeding coefficient in my data ($p < 0.05$). Tarayre et Thompson (1995) found in 23 *Thymus vulgaris* populations higher inbreeding coefficient and proved at 11 of them heterozygote deficiency. I observed significant heterozygote deficiency only in 3 of 9 populations of *Dracocephalum austriacum*. But in pollination experiments I found higher seed set in flowers pollinated from other populations than in plants pollinated with its own pollen or with pollen from the same population. Flowers, which were prevented from natural pollen and pollinators

access, produced no seeds. Flower pollinated manually by pollen from the same plant produced seeds, which proves that the species is self-compatible. Luijten et al. (2002) described also high number of produced seeds after inter population crosses at self-incompatible *Arnica montana*. Routley et al. (1999) showed that self-compatible *Aquilegia canadensis* can achieve full seed set in the absence of pollinators via automatic self-pollination. The proportion of seeds produced through outcrossing was generally low and varied widely among populations.

Oostermeijer et al. (2003) shows the importance of integrating demographic and genetic approaches for effective plant conservation. Luijten et al. (2002), Richards (2000) and Lamni et al. (1999) and many others are searching for influence of inbreeding, Allee effect and genetic diversity on plant fitness, usually measured as seed production. None of these studies has, however, shown direct influence of genetic diversity on population growth rate. In *Dracocephalum austriacum* populations genetic diversity has strong positive effect on seed set and this positively influences population growth rate. Decrease of genetic diversity about 50 % resulted in 50 % decrease in seed production. This decrease in seed production, however, results only in little decrease in population growth rate and only strong decrease of genetic diversity (up to 1% of nowadays observed) lowered population growth rate below 1 and populations become to decrease in size. This result indicates that decrease in seed production does not necessarily lead to direct endangerment of the populations. Such quantification of the effect of genetic diversity directly on population growth rate may be thus usable in creating decisions on species conservation.

Mean higher seed production, higher stochastic population growth rate and lower inter population genetic variability in Slovak *Dracocephalum austriacum* populations found in this study could be caused by the fact that Czech populations are at the northern distribution limit of the species distribution range. Lamni et al. (1999) also found higher genetic diversity of *Lychnis viscaria* populations in the centre of its distribution range but they did not find positive correlation between position in distribution range and fitness traits. Faugeron et al. (2004) describe in their study on *Gigartina skottsbergi* (Rhodophyta) higher genetic variability and lower genetic population differentiation in the centre of species distribution range in southern Chile. Nantel et Gagnon (1999) showed differences in population dynamics in clonal herb *Heliathus divaricatus* and clonal shrub *Rhus aromatica* in central and peripheral part of its distributional range in North America. Peripheral populations showed higher variability in population growth, which results in possibly higher probability of extinction.

Conservation implications

Populations of *Dracocephalum austriacum* are nowadays endangered with fragmentation of original habitats and overgrowing with grasses, shrubs and trees (Machová et Kubát 2004, Karasová in verb., personal observation). Due to low number of studied populations I could not study directly correlation of population growth rate with shrubs and trees. It can be, however, seen that vitality of plants in populations overgrown with shrubs and trees is lower. In studied larger populations in the Czech and Slovak Karst the stochastic population growth rates are never significantly below 1 and so populations are not decreasing. It is, however, necessary to maintain at least the present state of the habitats and thus limit the ongoing expansion of shrubs and trees. Stages of small and large plants are stages with high elasticity values that most contribute to changes in population growth rate. But they do not seem to change very much under current conditions and unless the plants are dug up by gardeners or damaged by wild boar survival of these plants does not seem to be a problem in the populations. Transitions, variation in which nowadays contributes most to variation in population growth rate, are growth from seedlings to small plants and seed production. Low seed production can be a result of inbreeding as indicated by the results of the pollination experiment. Because population size is negatively correlated with inbreeding coefficient it is clear that further decrease in population size can further limit seed production in the populations. This can lead to extinction vortex. It is thus very important to preserve the species in large populations.

Small populations of *Dracocephalum austriacum* are also more endangered with demographic and environmental stochasticity. But even populations with about 50 plants are not expected to get extinct in the future if current conditions on localities do not change. Populations with 10 and less plants are endangered with demographic and environmental stochasticity quite a lot and if also their genetic diversity will decrease further it can cause lower seed production and extinction probability will increase.

Another serious danger, particularly for small localities, is digging up plants by rockgardeners. A few localities are nowadays extinct because of this (Deblík in České středohoří mountains, locality in Zádielská planina). Digging up of plants with environmental stochasticity and low genetic diversity leads to high extinction probabilities.

A possibility to support the small populations is transferring of plants (or seeds) from other populations with higher genetic diversity (Rottenberg et Parker 2003). I did not found outbreeding depression (Lynch 1991) in my pollination experiments but it could express in

the latter stages of individual development or even in next generations (Quilichini et al. 2001) and so the danger of outbreeding depression should be still tested.

5. Conclusions

Population growth rates in both regions differ. Czech Karst populations showed higher population growth rates in the second transition interval of the study and Slovak Karst populations in the first transition interval of the study. Population growth rate (λ) for separate years was significantly different from 1 only in two localities (Haknovec in Czech Karst and Domické škrapy in Slovak Karst) during 2003-2005.

Analysis of elasticities showed that transitions that most contribute to population growth rate are transitions of stasis. In Czech Karst it is mainly stasis of large plants and in Slovak Karst of small plants. This indicates that in both regions we have to try to maintain vitality of flowering small and large plants. But from life table response experiments (LTRE) we can see that seed production (for all populations but mainly for Zádielský kameň in Slovak Karst) and growth from seedlings to small plants (for Slovak populations and Kodská stěna in Czech Karst) are transitions, which contribute most to changes in population growth rate. The relatively comparable results of elasticity analysis indicate that information about population dynamics is transferable between regions. The results of LTRE, however, indicate different transitions as the most important transitions for observed variation in population growth rate in different populations. All the transitions are, however, related to seed production and seedling establishment indicating that even here knowledge of dynamics in one population may help to design conservation action in the other.

Slovak populations showed higher genetic diversity within populations. Differences in genetic diversity among Czech populations ($F_{st-CK} = 0.333$) were higher than among Slovak populations ($F_{st-SK} = 0.090$). There were small or even no genetic differences between regions. In three populations (Vanovice, Radotínské údolí and Domické škrapy) I found significant heterozygote deficiency.

Genetic diversity has strong positive effect on seed set and this positively influences population growth rate. Decrease of genetic diversity by about 50 % resulted in 50 % decrease in seed production. But although there is positive correlation between population growth rate and genetic diversity, only large decrease of genetic diversity lowered population growth rate below 1 and populations start to decrease in size.

In pollination experiments I found higher seed set in flowers pollinated by pollen from other population than in plants pollinated by their own pollen or by pollen from the same population. Flowers, which were prevented from natural pollen and pollinators access, produced no seeds.

Mean higher seed production, higher stochastic population growth rate and lower inter population genetic variability in Slovak *Dracocephalum austriacum* populations found in this study could be caused by the fact that Czech populations are in the northern distribution limit of the species distribution range. Although the whole distribution range of the species is discontinuous position in the distribution limit can be quite important.

In studied larger populations in the Czech and Slovak Karst the stochastic population growth rates are never significantly below 1 and so populations are not decreasing. It is, however, necessary to maintain at least present state of the habitats by cutting shrubs and trees. Small populations of *Dracocephalum austriacum* are more endangered with demographic and environmental stochasticity. This is true especially for populations with 10 and less plants. Genetic diversity of small populations is decreased and it results in lower seed production. Transfers of plants (or seeds) from other population with higher genetic diversity could be considered to support them but proper test for possibly outbreeding depression must be done first.

6. Summary

In this study I wanted to compare population dynamics of an endangered species, *Dracocephalum austriacum* L., in two distant regions (the Czech and Slovak Karst). I also wanted to estimate genetic diversity in this species and assess the importance of genetic diversity for population dynamics of this species. The species is one of critically endangered species in the Czech Republic and effective conservation strategies are very needed nowadays.

Population dynamics was studied using analysis of population transition matrices from years 2003-2005. For estimating of genetic diversity I used allozyme analysis and analysed 10 variable loci in 4 enzymatic systems.

Population growth rates in both regions differ. Czech Karst populations showed higher population growth rates in the second transition interval of the study and Slovak Karst populations in the first transition interval of the study. Population growth rate (λ) for separate years was significantly different from 1 only in two localities (Haknovec in Czech Karst and Domické škrapy in Slovak Karst) during 2003-2005.

Analysis of elasticities showed that transitions that most contribute to population growth rate are transitions of stasis. In Czech Karst it is mainly stasis of large plants and in Slovak Karst of small plants. This indicates that in both regions we have to try to maintain vitality of flowering small and large plants. But from life table response experiments (LTRE) we can see that seed production (for all populations but mainly for Zádielský kameň in Slovak Karst) and growth from seedlings to small plants (for Slovak populations and Kodská stěna in Czech Karst) are transitions, which contribute most to changes in population growth rate. The relatively comparable results of elasticity analysis indicate that information about population dynamics is transferable between regions. The results of LTRE, however, indicate different transitions as the most important transitions for observed variation in population growth rate in different populations. All the transitions are, however, related to seed production and seedling establishment indicating that even here knowledge of dynamics in one population may help to design conservation action in the other.

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In pollination experiments I found higher seed set in flowers pollinated by pollen from other population than in plants pollinated by their own pollen or by pollen from the same population. Flowers, which were prevented from natural pollen and pollinators access, produced no seeds.

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In studied larger populations in the Czech and Slovak Karst the stochastic population growth rates are never significantly below 1 and so populations are not decreasing. It is, however, necessary to maintain at least present state of the habitats by cutting shrubs and trees. Small populations of *Dracocephalum austriacum* are more endangered with demographic and environmental stochasticity. This is true especially for populations with 10 and less plants. Genetic diversity of small populations is decreased and it results in lower seed production. Transfers of plants (or seeds) from other population with higher genetic diversity could be considered to support them but proper test for possibly outbreeding depression must be done first.

7. Literature

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Appendix 1: Vegetation composition of the localities. Names of species are according to Kubát et al. (2002). Species were surveyed using the Braun-Blanquet abundance scale. Localities in the Czech Republic (Hk – Haknovec, Ks – Kodská stěna, Cr – Císařská rokle, Ru – Radotínské údolí, Kr – Kozelská rokle, Vh – Velká hora, Vn – Vanovice, Ku – Karlicklé údolí, Db – Deblík, Zm – Zázmoníky), in the Slovak Republic (Zk – Zadielský kameň, Zp – Zádielská planina, Ds – Domické škrapy, Zv – Železná vrata, Pp – Plešivská planina, Kp – Koniárska planina).

	Hk				Ks				Cr		Ru		Kr		Vh		Vn		Ku		Db		Zm		Zk			Zp			Ds			Zv			Pp		Kp	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
Releve number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
Aspect (degrees)	210	203	210	210	180	180	180	180	90	90	315	315	135	135	203	203	203	203	203	203	255	255	252	252	225	225	225	250	250	240	240	240	200	210	210	275				
Slope (degrees)	30	4	20	45	40	12	35	30	15	20	35	20	15	9	8	8	8	8	10	50	40	5	4	4	6	30	30	30	20	1	2	1	5	2	2	40	20	40	50	
Relevé area (m2)	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Altitude (m)	287	277	287	287	350	350	350	350	270	270	300	300	241	241	306	310	245	245	347	347	405	410	227	227	595	595	595	590	590	340	340	340	730	730	730	470	460	460	460	
Cover tree layer (%)	0	0	20	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	
Cover shrub layer (%)	5	30	10	15	0	0	0	0	20	30	0	7	0	5	0	0	0	0	10	15	5	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cover herb layer (%)	40	50	50	45	45	35	50	50	50	60	60	40	30	40	40	20	50	35	30	25	60	50	85	90	35	40	40	60	85	85	95	85	40	20	50	55	50	40	40	
Cover moss and lichen layer (%)	20	20	30	20	20	15	30	35	30	15	30	20	50	40	50	40	50	30	20	7	10	2	0	5	20	16	20	10	10	50	50	50	40	50	25	14	30	25	25	
Cover bare rock (%)	10	5	10	5	10	7	10	10	30	10	5	25	25	30	25	50	20	30	70	70	10	6	0	0																
Year 20..	04	04	04	04	04	04	03	03	04	04	03	04	04	04	04	04	04	04	04	04	04	05	05	04	04	03	03	03	03	03	04	04	04	04	04	03	03	03	03	03
month	7	7	7	7	6	6	6	6	6	6	6	8	8	8	8	8	8	8	8	9	9	8	8	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
E3																																								
<i>Pinus sylvestris</i>	4	
<i>Prunus mahaleb</i>	2
<i>Sorbus aria</i>	.	2	2
<i>Sorbus aucuparia</i>	1
E2																																								
<i>Berberis vulgaris</i>	.	2	2	+
<i>Cerasus fruticosus</i>	2	.	1	
<i>Cornus mas</i>	.	+	+	
<i>Cornus sanguinea</i>	2	1	

Releve number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39			
<i>Dracocephalum austriacum</i>	1	2	2	2	1	1	1	1	2	2	2	1	+	+	2	2	1	+	+	+	.	.	r	.	+	1	1	r	1	2	2	2	2	1	2	1	1	2	1			
<i>Echium vulgare</i>	.	.	+	.	.	.	+	r	2	.	.	r	+	+		
<i>Elymus intermedia</i>	+	+	+		
<i>Erigeron annuus</i>	+		
<i>Erophila verna</i>	+	+		
<i>Eryngium campestre</i>		
<i>Erysimum odoratum</i>	+		
<i>Euphorbia cyparissias</i>	+	+	1	+	.	.	+	.	+	+	1	+	+	+	+	+	+	+	+	.	+	.	+	.	+	r	.	.	+	+	2	2	+	+	+	+	r	r	.			
<i>Fallopia convolvulus</i>	+	r		
<i>Festuca pallens</i> s.lat.	+	.	+	2	1	1	1	+	1	1	2	1	1	.	+	+	+		
<i>Festuca rupicola</i>	.	+	+	.	.	2	1	+	2	2	1	1		
<i>Festuca valesiaca</i>	r	1	+		
<i>Filipendula vulgaris</i>	1	1	+		
<i>Fragaria viridis</i>	1	+	.	2	3	2	1	+	+	.	.	.		
<i>Fraxinus ornus</i>		
<i>Galium album</i> s.lat.		
<i>Galium glaucum</i>	+	+	+	+	1	+	1	.	1	1	+	+	2	+	+	+	+	+	+	+	+	.	.	+	+	1	+	r	+			
<i>Genista pilosa</i>	+	+	+		
<i>Genista tinctoria</i>		
<i>Geranium sanguineum</i>	+	+	+	+	2	.	.	.	+	1	1	+	2	1	+	.	1	.	.	1	2	1	+	.	.			
<i>Glechoma hederacea</i>		
<i>Helianthemum canum</i>	+	.	.	+	+	1	2	+	r	+	.	+	2	+	
<i>Helianthemum grandiflorum</i> s.lat	+	+	+	1	1	r	+	+	+	r	1	+	+	+	+	+	+	+	.	+	+	+	+	+	.		
<i>Hieracium pilosella</i>	.	r	.	.	.	+	.	.	+	+		
<i>Hylotelephium maximum</i>	r		
<i>Hypericum maculatum</i>	r		
<i>Hypericum perforatum</i>	+	+	r	.	+		
<i>Chamaecytisus ratisbonensis</i>		
<i>Chamaecytisus supinus</i>	r	
<i>Inula ensifolia</i>	+	+	+

Releve number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39				
<i>Inula hirta</i>	+		
<i>Iris aphylla</i>	+	r	.	.	.	1	+	+	1	.		
<i>Isatis species</i>	+		
<i>Jovibarba globifera</i>	+	+	+	+	+	+	+	+		
<i>Jovibarba hirta</i> ssp. <i>glabrescens</i>	+	.	.	.	r	+	+	+	r	+	1	.		
<i>Juniperus communis</i>	+	2		
<i>Jurinea mollis</i>	r	.	.		
<i>Knautia arvensis</i>	+		
<i>Koeleria macrantha</i>	+	+	+	+	+	+	r		
<i>Lactuca perennis</i>	+	+	r		
<i>Laserpitium latifolium</i>	+		
<i>Linum austriacum</i>	+	+		
<i>Medicago falcata</i>	1	
<i>Medicago minima</i>	.	+	.	.	r	+	+	
<i>Melampyrum arvense</i>	+	.	r	1	.	+
<i>Melica ciliata</i>	1	+	.	.	+	+	+	1		
<i>Melica transsilvanica</i>	1	.	+	+	
<i>Onobrychis species</i>	
<i>Origanum vulgare</i>	r	.	1	+	+	
<i>Orobancha species</i>	r	
<i>Peucedanum cervaria</i>	1	1	+	
<i>Phleum phleoides</i>	+	1	2	+	+	
<i>Pimpinella major</i>	+
<i>Poa bulbosa</i>	+
<i>Polygonatum odoratum</i>	1	1	.	+	.	+	+	+	2	1	.	1	1	2	+	1	2		
<i>Polygonum aviculare</i> agg.	+	
<i>Potentilla arenaria</i>	2	2	2	2	2	2	1	2	+	.	+	1	2	2	2	.	+	+	+	1	+	.	.	1	1	+	1	.	.	+	+	2	2	1	+			
<i>Potentilla heptaphylla</i>	+	+	
<i>Potentilla inclinata</i>	+	+	
<i>Pseudolysimachion spicatum</i>	+	+	+	+	

Appendix 2: Results of soil analyses. Analyses of pH_(H2O) and C_{ox} were done according to ČSN ISO 10390, N_t according to Kjeldahl and Ca, Mg, K and P – according to Mehlich III. For protocols of soil analyses see Appendix on enclosed CD.

Locality	pHH2O	Cox	Nt	C : N	Ca	Mg	K	P
		(%)	(%)		(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Císařská rokle 1	7.42	9.33	0.85	10.98	10900	213	389	10
Císařská rokle 2	7.51	7.81	0.77	10.14	11500	182	392	< 10
Císařská rokle 3	7.51	7.90	0.74	10.68	10800	237	562	24
Domické škrapy 1	7.09	7.43	0.76	9.78	7190	349	323	< 10
Domické škrapy 2	7.05	7.49	0.79	9.48	7670	342	432	< 10
Domické škrapy 3	7.17	8.31	0.87	9.55	8370	306	280	< 10
Domické škrapy 4	6.92	6.13	0.58	10.57	5870	311	444	< 10
Domické škrapy 5	6.78	5.82	0.58	10.03	6170	318	392	< 10
Domické škrapy 6	6.97	5.23	0.56	9.34	6260	291	318	< 10
Haknovec 1	7.13	16.80	1.63	10.31	15000	347	418	27
Haknovec 2	6.93	19.90	1.85	10.76	11700	511	324	23
Haknovec 3	7.29	19.20	2.02	9.50	16900	239	252	23
Karlické údolí 1	7.34	8.92	0.94	9.49	22900	236	300	19
Karlické údolí 2	7.36	7.47	0.80	9.34	9030	263	414	14
Kodská stěna 1	7.32	10.90	1.07	10.19	13300	302	588	15
Kodská stěna 2	7.57	8.43	0.91	9.26	10300	200	341	11
Kodská stěna 3	7.46	9.52	1.02	9.33	11100	201	536	11
Koniářská planina 1	insuficient sample	27.60	2.54	10.87	10800	2420	369	19
Koniářská planina 2	7.09	23.70	2.05	11.56	12000	1910	339	23
Koniářská planina 3	6.59	22.50	2.40	9.38	13200	1020	285	14
Koniářská planina 4	6.96	21.30	2.08	10.24	13700	919	317	28
Kozelská rokle 1	7.17	9.71	1.08	8.99	11100	215	308	< 10
Plešivská planina 1	7.43	8.29	0.70	11.84	9950	329	182	< 10
Plešivská planina 2	7.39	9.28	0.90	10.31	10600	362	217	< 10
Plešivská planina 3	7.47	8.93	0.82	10.89	11600	283	191	< 10
Radotínské údolí 1	7.40	13.00	1.33	9.77	15100	350	457	19
Radotínské údolí 2	7.28	20.30	1.77	11.47	14100	701	409	32
Radotínské údolí 3	7.61	11.30	1.05	10.76	13100	383	268	20
Vanovice 1	7.55	14.90	1.22	12.21	13700	990	297	12
Vanovice 2	7.52	18.50	1.39	13.31	17100	1130	349	12
Velká hora 1	7.46	14.10	1.38	10.22	12600	414	354	27
Velká hora 2	7.48	15.10	1.64	9.21	15100	308	312	42
Zádielský kameň 1	6.98	25.20	2.49	10.12	12200	439	292	17
Zádielský kameň 2	7.21	21.70	2.32	9.35	12800	501	435	15
Zádielský kameň 3	7.24	23.30	2.45	9.51	14100	377	312	19
Zádielská planina 1	7.41	11.40	1.19	9.58	11000	402	300	28
Zádielská planina 2	7.29	11.00	1.19	9.24	9940	305	224	11
Zádielská planina 3	7.05	13.30	1.36	9.78	10100	514	263	11
Zádielská planina 4	6.55	24.20	2.52	9.60	12300	595	457	16
Zádielská planina 5	6.59	20.60	2.17	9.49	11600	571	281	< 10
Zádielská planina 6	insuficient sample	25.90	2.51	10.32	11300	524	208	14
Zázmoníky	7.31	4.42	0.34	13.00	12000	343	123	< 10
Železná vrata 1	7.10	16.30	1.71	9.53	12100	512	174	11
Železná vrata 2	6.80	15.00	1.65	9.09	10700	538	217	13
Železná vrata 3	6.67	14.50	1.60	9.06	9910	479	223	< 10
Železná vrata 4	6.93	18.00	2.14	8.41	12900	449	275	21

Appendix 3: Result of allele scoring of 5 enzymatic systems from allozyme analyses (leucine aminopeptidase (LAP), superoxid dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PDH), and aspartat aminotransferase (AAT) –“a”, “b”, “c”, ... are scored alleles, where “a” is the fastest one; “?” means hardly scored locus. Not all samples were used for all analyses as described in methods.

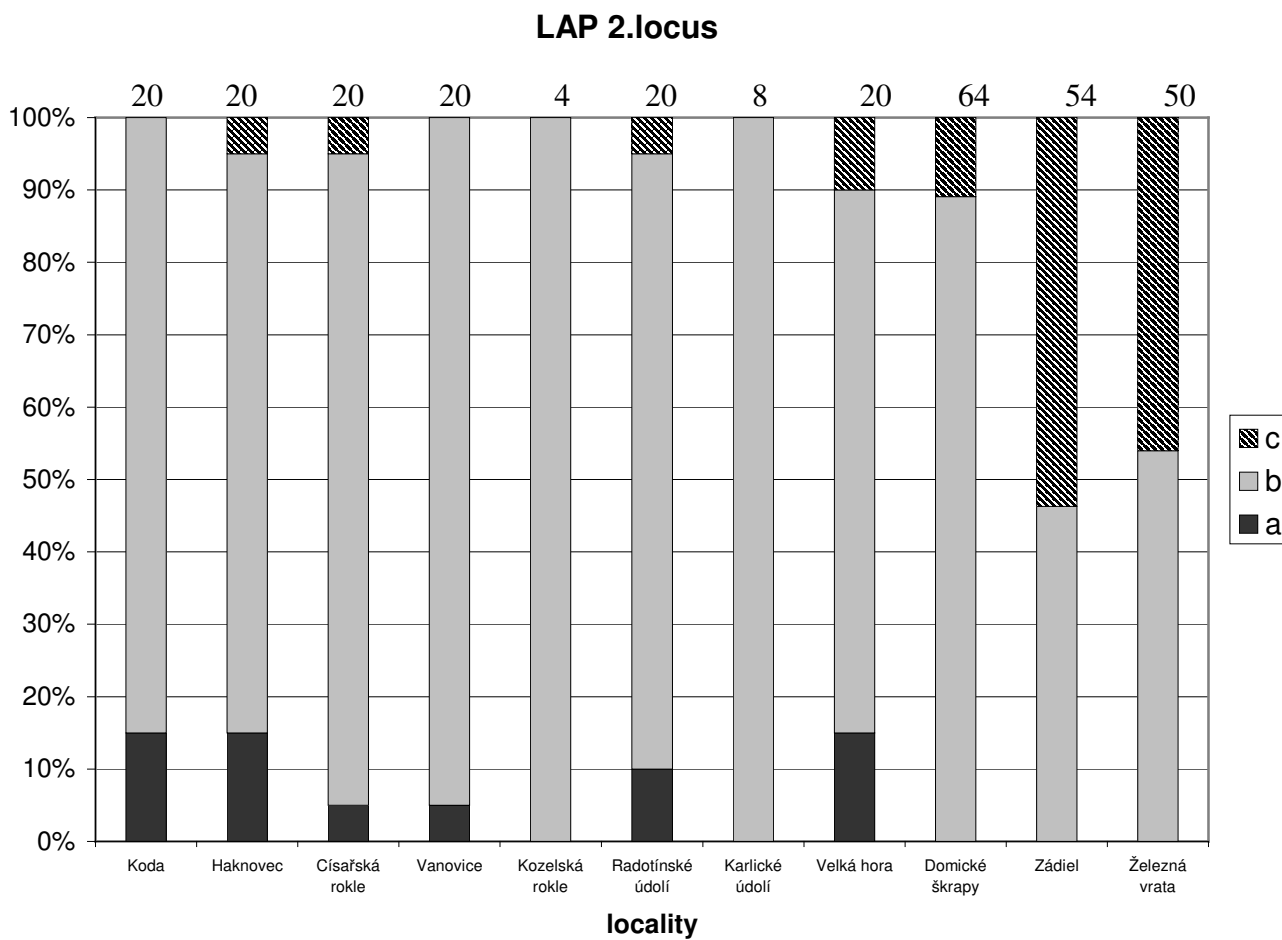
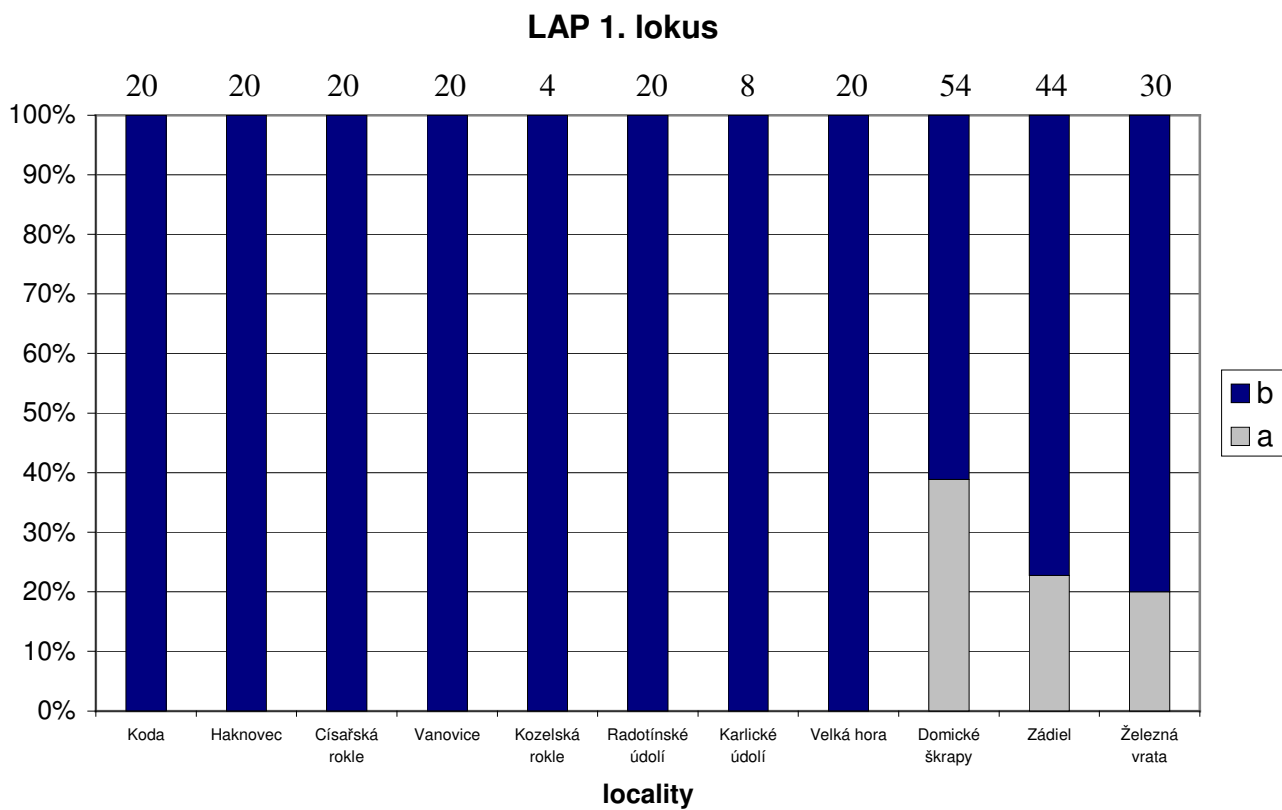
No. of sample	Locality	Enzymatic system									
		LAP			SOD			6PGDH		AAT	
		1	2	3	1	2	3	1	2	1	2
15	Kodská stěna	bb	bb	cc	bc	aa	aa	aa	aa	aa	bb
16	Kodská stěna	bb	bb	cc	bb	aa	aa	aa	ab	aa	bb
17	Kodská stěna	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
18	Kodská stěna	bb	ab	cc	bb	aa	aa	aa	ab	aa	bb
19	Kodská stěna	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
20	Kodská stěna	bb	bb	bb	bb	aa	aa	aa	ab	aa	bb
21	Kodská stěna	bb	bb	cc	bc	aa	aa	aa	aa	aa	ab
22	Kodská stěna	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
23	Kodská stěna	bb	aa	?	bb	aa	aa	aa	aa	aa	bb
24	Kodská stěna	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
25	Haknovec	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
26	Haknovec	bb	bb	bb	cc	aa	aa	aa	aa	aa	bb
27	Haknovec	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
28	Haknovec	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
29	Haknovec	bb	ab	?	bb	aa	aa	aa	aa	aa	bb
30	Haknovec	bb	bc	bb	bb	aa	aa	aa	aa	aa	ab
31	Haknovec	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
32	Haknovec	bb	ab	bb	bc	aa	aa	aa	aa	aa	bb
33	Haknovec	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
34	Haknovec	bb	bb	bb	bc	aa	aa	aa	aa	aa	ab
35	Čísařská rokle	bb	ab	cc	bc	aa	aa	aa	aa	aa	bb
36	Čísařská rokle	bb	bc	?	bb	aa	aa	aa	aa	aa	bb
37	Čísařská rokle	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
38	Čísařská rokle	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
39	Čísařská rokle	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
40	Čísařská rokle	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
41	Čísařská rokle	bb	bb	bb	cc	aa	aa	aa	aa	aa	bb
42	Čísařská rokle	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
43	Čísařská rokle	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
44	Čísařská rokle	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
45	Vanovice	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
46	Vanovice	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
47	Vanovice	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
48	Vanovice	bb	bb	bd	bb	aa	aa	aa	aa	aa	bb
49	Vanovice	bb	bb	cc	bc	aa	aa	aa	aa	aa	bb
50	Vanovice	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
51	Vanovice	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
52	Vanovice	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
53	Vanovice	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
54	Vanovice	bb	bb	gg	bb	aa	aa	aa	ab	aa	bb
55	Kozelská rokle	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
56	Kozelská rokle	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb

No. of samples	Locality	Enzymatic system									
		LAP			SOD			6PGDH		AAT	
		1	2	3	1	2	3	1	2	1	2
57	Radotínské údolí	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
58	Radotínské údolí	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
59	Radotínské údolí	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
60	Radotínské údolí	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
61	Radotínské údolí	bb	bb	ee	bb	aa	aa	aa	aa	aa	bb
62	Radotínské údolí	bb	bc	dd	bb	aa	aa	aa	aa	aa	bb
63	Radotínské údolí	bb	bb	dd	bb	aa	aa	aa	aa	aa	bb
64	Radotínské údolí	bb	bb	dd	bb	aa	aa	aa	aa	aa	bb
65	Radotínské údolí	bb	bb	dd	bc	aa	aa	aa	aa	aa	bb
66	Radotínské údolí	bb	bb	dd	bb	aa	aa	aa	aa	aa	bb
67	Karlické údolí	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
68	Karlické údolí	bb	bb	aa	bb	aa	aa	aa	aa	aa	bb
69	Karlické údolí	bb	bb	aa	bb	aa	aa	aa	ab	aa	bb
70	Karlické údolí	bb	bb	cc	bb	aa	aa	aa	aa	aa	bb
71	Velká hora	bb	bb	dd	bb	aa	aa	aa	aa	aa	bb
72	Velká hora	bb	bb	?	bb	aa	aa	aa	aa	aa	bb
73	Velká hora	bb	bc	?	bb	aa	aa	aa	aa	aa	bb
74	Velká hora	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
75	Velká hora	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
76	Velká hora	bb	ab	bb	bb	aa	aa	aa	aa	aa	bb
77	Velká hora	bb	bb	gg	bb	aa	aa	aa	aa	aa	bb
78	Velká hora	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
79	Velká hora	bb	bc	bb	bb	aa	aa	aa	aa	aa	bb
80	Velká hora	bb	bb	?	bb	aa	aa	aa	ab	aa	bb
81	Domické škrapy	?	bb	bb	bb	aa	aa	aa	aa	aa	bb
82	Domické škrapy	aa	bb	bb	ac	aa	aa	aa	aa	aa	bb
83	Domické škrapy	?	cc	be	bb	aa	aa	aa	aa	aa	bb
84	Domické škrapy	bb	bb	be	bb	aa	aa	aa	aa	aa	bb
85	Domické škrapy	?	bb	?	bb	aa	aa	aa	aa	aa	bb
86	Domické škrapy	aa	bc	bb	bb	aa	aa	aa	aa	aa	bb
87	Domické škrapy	?	bc	?	ab	aa	aa	aa	aa	aa	bb
88	Domické škrapy	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
89	Domické škrapy	aa	bb	bb	bb	aa	aa	aa	aa	aa	bb
90	Domické škrapy	bb	bb	bb	cc	aa	aa	aa	aa	aa	bb
91	Zádielský kameň	?	bb	?	bb	aa	aa	aa	aa	aa	bb
92	Zádielský kameň	?	bc	?	bb	aa	aa	aa	aa	aa	bb
93	Zádielský kameň	bb	cc	be	bc	aa	aa	aa	aa	aa	bb
94	Zádielský kameň	bb	bc	bb	bb	aa	aa	aa	aa	aa	bb
95	Zádielský kameň	bb	bc	?	bb	aa	aa	aa	aa	aa	bb
96	Zádielský kameň	bb	bc	bb	bb	aa	aa	aa	aa	aa	bb
97	Zádielský kameň	aa	cc	?	bc	aa	aa	aa	aa	aa	bb
98	Zádielský kameň	bb	bb	bg	bb	aa	aa	aa	aa	aa	bb
99	Zádielský kameň	?	bc	?	bc	aa	aa	aa	aa	aa	bb
100	Zádielský kameň	?	?	?	bc	aa	aa	aa	aa	aa	bb
101	Železná vrata	?	bb	bb	bc	aa	aa	aa	aa	aa	bb
102	Železná vrata	?	cc	bb	bc	aa	aa	aa	aa	aa	bb
103	Železná vrata	?	bb	bb	bb	aa	aa	aa	aa	aa	bb
104	Železná vrata	?	bc	bb	bc	aa	aa	aa	aa	aa	bb
105	Železná vrata	?	bc	?	bb	aa	aa	aa	aa	aa	bb

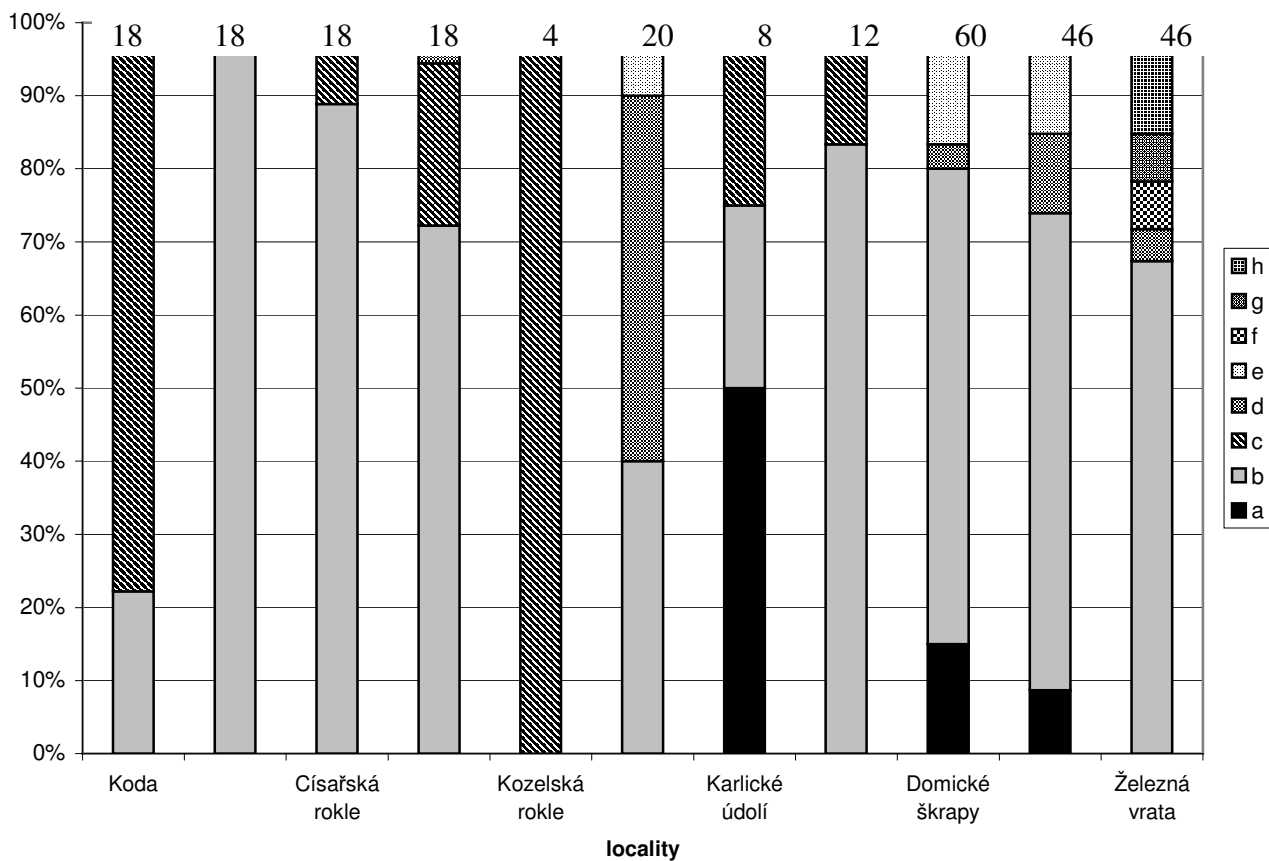
No. of samples	Locality	Enzymatic system									
		LAP			SOD			6PGDH		AAT	
		1	2	3	1	2	3	1	2	1	2
106	Železná vrata	?	bc	?	bc	aa	aa	aa	aa	aa	bb
107	Železná vrata	?	cc	bb	bc	aa	aa	aa	aa	aa	bb
108	Železná vrata	?	bb	bb	bb	aa	aa	aa	aa	aa	bb
109	Železná vrata	?	bb	bb	bb	aa	aa	aa	aa	aa	bb
110	Železná vrata	?	?	?	bb	aa	aa	aa	aa	aa	bb
111	Zádielský kameň	aa	cc	bb	bb	aa	aa	aa	aa	aa	bb
112	Zádielský kameň	bb	cc	ee	bb	aa	aa	aa	aa	aa	bb
113	Zádielský kameň	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
114	Zádielský kameň	bb	cc	be	bc	aa	aa	aa	aa	aa	bb
115	Zádielský kameň	bb	bb	bh	bc	aa	aa	aa	aa	aa	bb
116	Zádielský kameň	?	?	aa	bc	aa	aa	aa	aa	aa	bb
117	Zádielský kameň	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
118	Zádielský kameň	bb	cc	dd	bb	aa	aa	aa	aa	aa	bb
119	Zádielský kameň	?	bc	bb	bb	aa	aa	aa	aa	aa	bb
120	Zádielský kameň	aa	cc	ad	bc	aa	aa	aa	aa	aa	bb
121	Zádielský kameň	aa	cc	ad	bb	aa	aa	aa	aa	aa	bb
122	Zádielský kameň	bb	bb	bb	bc	aa	aa	aa	aa	aa	bb
123	Zádielský kameň	bb	bc	bb	bb	aa	aa	aa	aa	aa	bb
124	Zádielský kameň	aa	cc	de	bc	aa	aa	aa	aa	aa	bb
125	Zádielský kameň	bb	bc	ee	bb	aa	aa	aa	aa	aa	bb
126	Zádielský kameň	?	bc	bb	bb	aa	aa	aa	aa	aa	bb
127	Zádielský kameň	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
128	Zádielský kameň	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
129	Zádielský kameň	bb	bb	bb	bb	aa	aa	aa	aa	aa	bb
130	Železná vrata	?	cc	bh	bb	aa	aa	aa	aa	aa	bb
131	Železná vrata	aa	bc	ff	bc	aa	aa	aa	aa	aa	bb
132	Železná vrata	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
133	Železná vrata	bb	bc	bg	bb	aa	aa	aa	aa	aa	bb
134	Železná vrata	bb	cc	bg	bc	aa	aa	aa	aa	aa	bb
135	Železná vrata	bb	bb	fg	bc	aa	aa	aa	aa	aa	bb
136	Železná vrata	bb	bb	bd	bb	aa	aa	aa	aa	aa	bb
137	Železná vrata	?	?	?	bc	aa	aa	aa	aa	aa	bb
138	Železná vrata	aa	bb	bh	?	aa	aa	aa	aa	aa	bb
139	Železná vrata	?	?	?	bc	aa	aa	aa	aa	aa	bb
140	Železná vrata	?	?	?	bc	aa	aa	aa	aa	aa	bb
141	Železná vrata	bb	cc	bb	bb	aa	aa	aa	aa	aa	bb
142	Železná vrata	aa	bc	bh	bb	aa	aa	aa	aa	aa	bb
143	Železná vrata	bb	bc	bh	bb	aa	aa	aa	aa	aa	bb
144	Železná vrata	bb	bc	bh	bb	aa	aa	aa	aa	aa	bb
145	Železná vrata	bb	bb	bh	bb	aa	aa	aa	aa	aa	bb
146	Železná vrata	bb	cc	bd	bc	aa	aa	aa	aa	aa	bb
147	Železná vrata	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
148	Železná vrata	bb	bc	bh	bc	aa	aa	aa	aa	aa	bb
149	Domické škrapy	bb	bc	bb	bc	aa	aa	aa	aa	aa	bb
150	Domické škrapy	aa	bb	bb	ab	aa	aa	aa	aa	aa	bb
151	Domické škrapy	bb	bb	be	bb	aa	aa	aa	aa	aa	bb
152	Domické škrapy	bb	bb	ae	bc	aa	aa	aa	aa	aa	bb
153	Domické škrapy	bb	bb	aa	ac	aa	aa	aa	aa	aa	bb
154	Domické škrapy	aa	bb	ab	ac	aa	aa	aa	aa	aa	bb

No. of samples	Locality	Enzymatic system										
		LAP			SOD			6PGDH		AAT		
		1	2	3	1	2	3	1	2	1	2	
155	Domické škrapy	aa	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb
156	Domické škrapy	aa	bb	ee	bc	aa	aa	aa	aa	aa	aa	bb
157	Domické škrapy	ab	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb
158	Domické škrapy	bb	bb	ae	aa	aa	aa	aa	aa	aa	aa	bb
159	Domické škrapy	bb	bb	bb	ab	aa	aa	aa	aa	aa	aa	bb
160	Domické škrapy	bb	bc	ae	?	aa	aa	aa	aa	aa	aa	bb
161	Domické škrapy	bb	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb
162	Domické škrapy	ab	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb
163	Domické škrapy	aa	bb	be	bc	aa	aa	aa	aa	aa	aa	bb
164	Domické škrapy	bb	bb	be	ab	aa	aa	aa	aa	aa	aa	bb
165	Domické škrapy	ab	bb	bb	ac	aa	aa	aa	aa	aa	aa	bb
166	Domické škrapy	bb	bb	dd	bc	aa	aa	aa	aa	aa	aa	bb
167	Domické škrapy	aa	bb	aa	ab	aa	aa	aa	aa	aa	aa	bb
168	Domické škrapy	ab	bb	ab	bc	aa	aa	aa	aa	aa	aa	bb
169	Domické škrapy	ab	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb
170	Domické škrapy	?	bb	bb	bb	aa	aa	aa	aa	aa	aa	bb

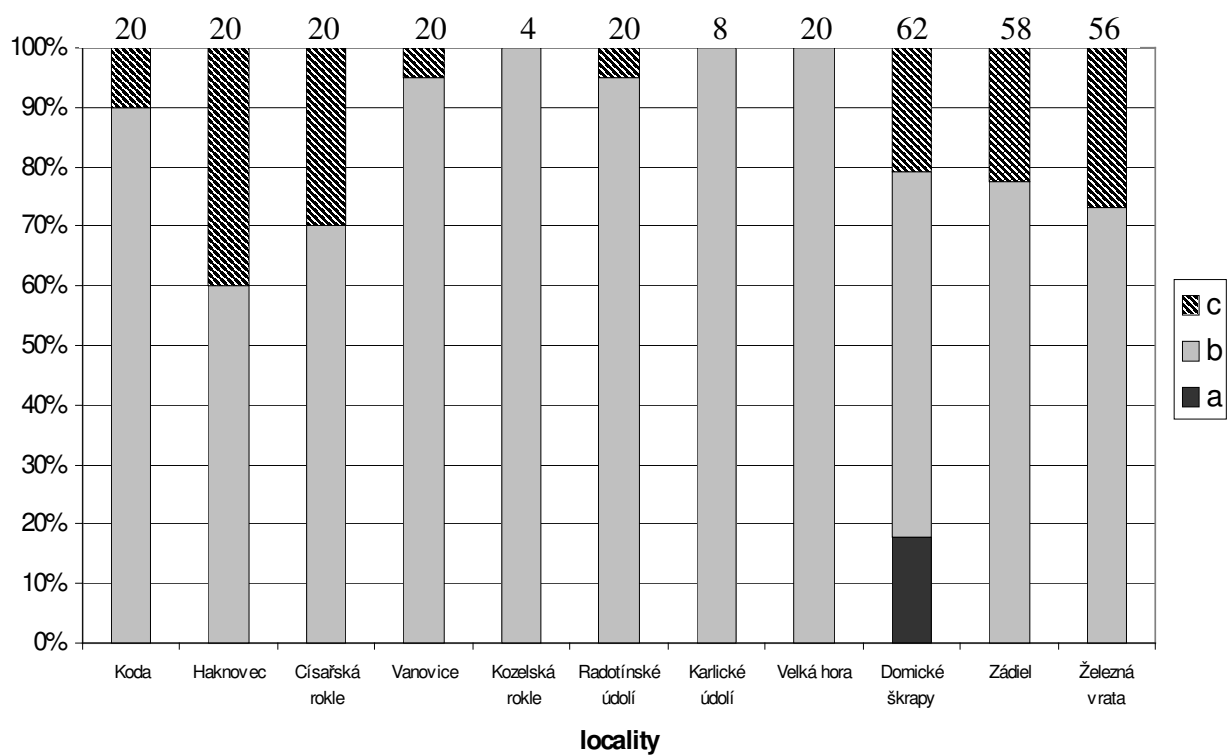
Appendix 4: Graphs with alleles proportions in separate variable loci (values above columns are total no. of alleles)



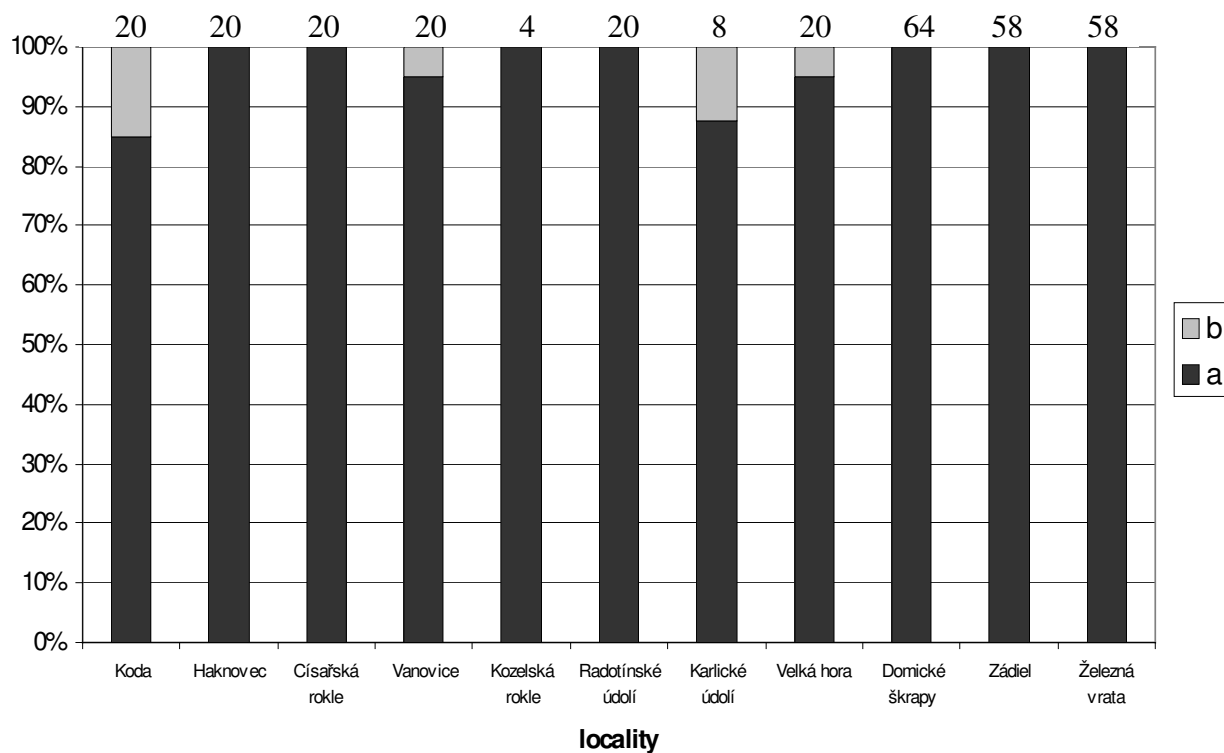
LAP 3.locus



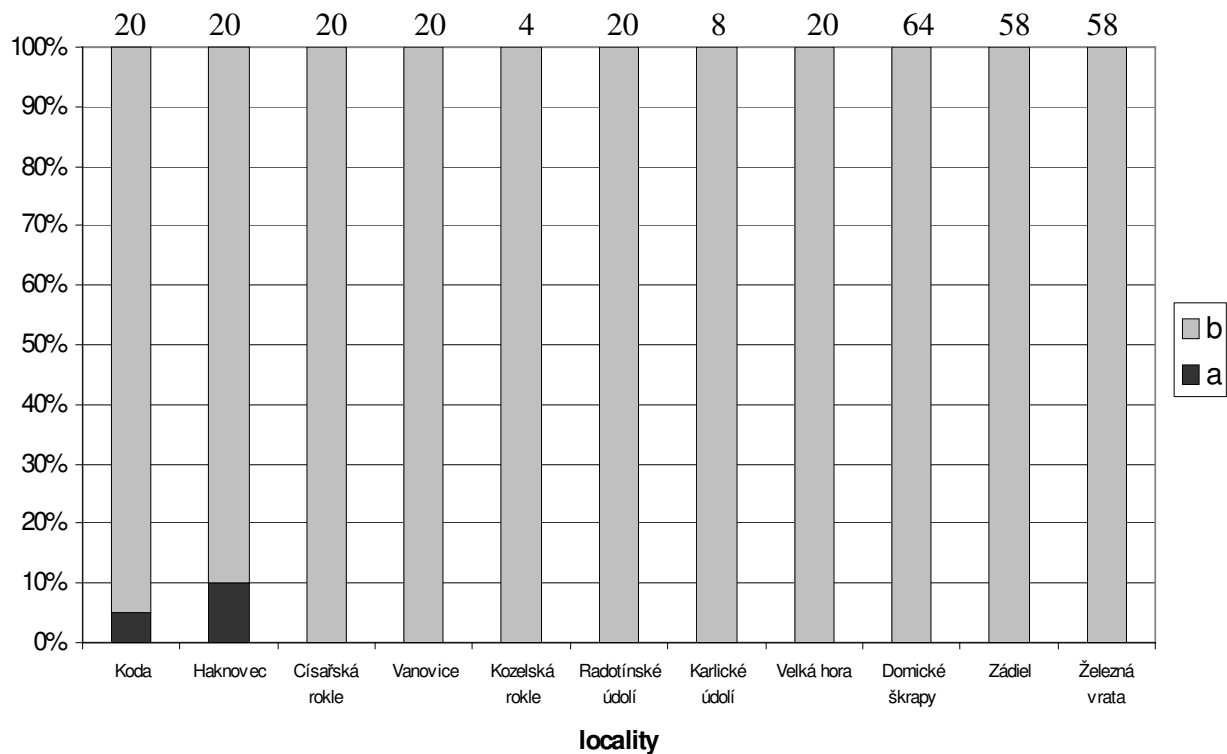
SOD 1.locus



6PGDH 2.locus



AAT 2.locus



Appendix 5: Matrices of elasticities for *Dracocephalum austriacum* populations and their 95 % confidence intervals from years 2003-2005. 1 = seeds, 2 = seedlings, 3 = small plants, 4 = large plants, 95 % CI-L = lower end of the confidence interval, 95 % CI-U = upper end of the confidence interval.

					95% CI-L	95% CI-U	95% CI-L	95% CI-U	95% CI-L	95% CI-U	95% CI-L	95% CI-U		
					1	2	3	4	1	2	3	4		
Haknovec	1	0.26	0.00	0.01	0.03	1	0.00	0.32	0.00	0.00	0.01	0.00	0.04	
	2	0.04	0.01	0.00	0.00	2	0.00	0.06	0.00	0.02	0.00	0.01	0.00	0.01
	3	0.00	0.05	0.11	0.03	3	0.00	0.00	0.02	0.06	0.08	0.15	0.02	0.06
	4	0.00	0.00	0.07	0.39	4	0.00	0.00	0.00	0.00	0.05	0.08	0.29	0.64
Kodská stěna	1	0.27	0.00	0.01	0.03	1	0.22	0.33	0.00	0.00	0.01	0.01	0.03	0.04
	2	0.04	0.01	0.00	0.01	2	0.04	0.05	0.00	0.02	0.00	0.01	0.00	0.01
	3	0.00	0.05	0.11	0.03	3	0.00	0.00	0.04	0.06	0.09	0.15	0.02	0.04
	4	0.00	0.00	0.07	0.37	4	0.00	0.00	0.00	0.00	0.06	0.08	0.31	0.44
Císařská rokle	1	0.22	0.00	0.01	0.04	1	0.17	0.27	0.00	0.00	0.01	0.02	0.02	0.05
	2	0.05	0.03	0.00	0.00	2	0.03	0.07	0.01	0.09	0.00	0.00	0.00	0.00
	3	0.00	0.05	0.16	0.03	3	0.00	0.00	0.03	0.07	0.12	0.20	0.02	0.05
	4	0.00	0.00	0.07	0.34	4	0.00	0.00	0.00	0.00	0.06	0.08	0.27	0.47
Žádielská kameň	1	0.20	0.00	0.01	0.06	1	0.14	0.24	0.00	0.00	0.00	0.02	0.04	0.08
	2	0.08	0.00	0.00	0.01	2	0.04	0.10	0.00	0.00	0.00	0.01	0.00	0.03
	3	0.00	0.09	0.12	0.01	3	0.00	0.00	0.06	0.11	0.08	0.18	0.00	0.03
	4	0.00	0.00	0.09	0.32	4	0.00	0.00	0.00	0.00	0.07	0.11	0.25	0.44
Domické škrapy	1	0.11	0.00	0.02	0.01	1	0.00	0.31	0.00	0.00	0.00	0.07	0.00	0.04
	2	0.03	0.00	0.00	0.00	2	0.00	0.11	0.00	0.00	0.00	0.01	0.00	0.02
	3	0.00	0.04	0.47	0.07	3	0.00	0.00	0.00	0.11	0.25	0.68	0.02	0.12
	4	0.00	0.00	0.08	0.17	4	0.00	0.00	0.00	0.00	0.06	0.12	0.07	0.31
Železná vrata	1	0.23	0.00	0.04	0.03	1	0.18	0.27	0.00	0.00	0.02	0.05	0.02	0.05
	2	0.07	0.00	0.01	0.00	2	0.04	0.10	0.00	0.00	0.00	0.01	0.00	0.01
	3	0.00	0.08	0.30	0.04	3	0.00	0.00	0.05	0.10	0.23	0.39	0.02	0.06
	4	0.00	0.00	0.08	0.12	4	0.00	0.00	0.00	0.00	0.05	0.10	0.07	0.18

Appendix 6: Transition probabilities of 4 classes of *Dracocephalum austriacum* individuals during 2 time-intervals. Each matrix contains the probabilities that individuals in one class year t (columns in the matrix) will enter a class in year t+1 (rows in the matrix). n = the number of individuals starting in a size class in the 1st year. 1 = seeds, 2 = seedlings, 3 = small plants, 4 = large plants and † = dead.

2003-2004					2004-2005				
	1	2	3	4		1	2	3	4
Haknovec									
n=	*	38	81	59	*	16	65	84	
1	0.90	0.00	2.56	6.73	0.88	0.00	3.29	3.86	
2	0.00	0.14	0.00	0.00	0.02	0.31	0.12	0.14	
3	0.00	0.35	0.51	0.07	0.00	0.50	0.85	0.13	
4	0.00	0.00	0.36	0.88	0.00	0.00	0.13	0.87	
†	0.10	0.51	0.13	0.05	0.10	0.19	0.02	0.00	
Kodská stěna									
n=	*	49	86	30	*	7	91	51	
1	0.90	0.00	0.80	2.47	0.85	0.00	1.23	4.09	
2	0.00	0.00	0.00	0.00	0.05	0.57	0.05	0.15	
3	0.00	0.59	0.54	0.07	0.00	0.29	0.75	0.14	
4	0.00	0.00	0.32	0.89	0.00	0.00	0.08	0.84	
†	0.10	0.41	0.14	0.04	0.10	0.14	0.17	0.02	
Císařská rokle									
n=	*	8	73	58	*	3	73	58	
1	0.90	0.00	0.45	0.99	0.81	0.00	1.17	2.30	
2	0.00	0.38	0.00	0.00	0.09	0.50	0.00	0.00	
3	0.00	0.50	0.65	0.07	0.00	0.33	0.85	0.17	
4	0.00	0.00	0.31	0.91	0.00	0.00	0.12	0.83	
†	0.10	0.12	0.04	0.02	0.10	0.17	0.03	0.00	
Zádielský kameň									
n=	*	8	34	34	*	3	31	42	
1	0.90	0.00	1.51	3.19	0.84	0.00	2.80	13.55	
2	0.00	0.00	0.07	0.15	0.06	0.00	0.06	0.27	
3	0.00	1.00	0.61	0.09	0.00	0.00	0.65	0.02	
4	0.00	0.00	0.33	0.91	0.00	0.00	0.32	0.98	
†	0.10	0.00	0.06	0.00	0.10	1.00	0.03	0.00	
Domické škrapy									
n=	*	8	113	32	*	1	103	40	
1	0.90	0.00	4.65	13.69	0.89	0.00	8.64	25.11	
2	0.00	0.14	0.00	0.00	0.01	0.00	0.05	0.14	
3	0.00	0.71	0.84	0.19	0.00	1.00	0.83	0.31	
4	0.00	0.00	0.13	0.81	0.00	0.00	0.13	0.67	
†	0.10	0.14	0.03	0.00	0.10	0.00	0.04	0.02	
Železná vrata									
n=	*	7	87	36	*	4	71	49	
1	0.90	0.00	5.56	17.01	0.87	0.00	5.23	12.26	
2	0.00	0.00	0.06	0.20	0.03	0.00	0.05	0.12	
3	0.00	1.00	0.75	0.17	0.00	0.00	0.89	0.40	
4	0.00	0.00	0.23	0.83	0.00	0.00	0.08	0.60	
†	0.10	0.00	0.02	0.00	0.10	1.00	0.03	0.00	

Appendix 7: Permissions from Ministry of Environment of The Czech and Slovak Republic for manipulation with *Dracocephalum austriacum* and entrance to its localities.

MINISTERSTVO ŽIVOTNÉHO PROSTREDIA
SLOVENSKEJ REPUBLIKY
812 35 BRATISLAVA, NÁMESTIE LUDOVÍTA ŠTÚRA 1

Odbor ochrany prírody a krajiny

Bratislava 07.05.2003
Číslo: 1927/497/03-5.1
Vybavuje: RNDr. Dzubinová

Rozhodnutie

Ministerstvo životného prostredia Slovenskej republiky, odbor ochrany prírody a krajiny ako príslušný orgán štátnej správy podľa § 2 zákona SNR č. 595/1990 Zb. o štátnej správe pre životné prostredie v znení neskorších predpisov a § 65 ods. 1 písm. h) a § 83 ods. 1 zákona č. 543/2002 Z.z. o ochrane prírody a krajiny, na základe žiadosti Tomáša Dostálka, Dobřešovická 119, 252 43 Průhonice, ČR (ďalej len "žiadateľ") zo dňa 08.04.2003, v súlade s § 46 zákona č. 71/1967 Zb. o správnom konaní

A. povoľuje výnimku

zo zákazov ustanovených § 14 ods. 1 písm. c), h), § 16 ods. 1 písm. a) v znení § 14 ods. 1 písm. c); h) a § 34 ods. 1 písm. a), b) a c) zákona č. 543/2002 Z.z. o ochrane prírody a krajiny (ďalej len „zákon“). Výnimkou sa povoľuje pohybovať sa mimo vyznačeného turistického chodníka alebo náučného chodníka za hranicami zastavaného územia obce a zbierať semená a listy chránenej rastliny *Dracocephalum austriacum* v NPR Domické škrapy, NPR Zádielská dolina a Plešivecká planina za účelom vykonávania výskumu uvedeného v časti B,

B. vydáva súhlas

podľa § 56 ods. 1 zákona na výskum za účelom vypracovania diplomovej práce „Identifikácia kritických fáz životného cyklu druhu *Dracocephalum austriacum*“ v NPR Domické škrapy, NPR Zádielská dolina a Plešivecká planina na území NP Slovenský kras od začiatku mája do konca augusta v rokoch 2003 – 2005.

Podmienky rozhodnutia v zmysle § 82 ods. 12 zákona:

1. Všetky práce budú robené nedeštručnou metódou a o ich priebehu bude vedená podrobná dokumentácia,
2. pri výskume bude odoberaný rastlinný materiál (semená a listy) za prítomnosti pracovníka Správy národného parku Slovenský kras, spočítané semená sa vysejú na určenú lokalitu, odber semien na iný než uvedený projekt nie je povolený,
3. v rámci výskumu nie je možné odoberať iné druhy rastlín,
4. plechové a plastové štítky budú po ukončení projektu z územia odstránené,
5. potenciálnych opeľovačov je potrebné determinovať na mieste, na ich prípadný odchyt je potrebné osobitné povolenie,
6. pred začatím terénnych prác sa žiadateľ osobne alebo telefonicky ohlási na ŠOP SR, Správe NP Slovenský kras, Biely kaštieľ č. 188, 049 51 Brzotín (tel. č. 058/7326815, fax: 058/7346769).

Osobitné predpisy, ako aj ostatné ustanovenia zákona, ostávajú vydaním tohoto rozhodnutia nedotknuté.

Výnimka platí do konca augusta 2005.

Pracovníci Štátnej ochrany prírody Slovenskej republiky - Správy NP Slovenský kras, ktorí majú podľa § 79 zákona oprávnenia členov stráže prírody, sú podľa § 77 ods. 1 písm. b) zákona oprávnení dozerat' na dodržiavanie podmienok tohto rozhodnutia.

Odôvodnenie:

Listom zo dňa 03.04.2003 požiadal žiadateľ Ministerstvo životného prostredia Slovenskej republiky, odbor ochrany prírody a krajiny (ďalej len "ministerstvo") o povolenie výnimky zo zákazov ustanovených zákonom, spočívajúcej v umožnení vykonať výskum chráneného druhu *Dracocephalum austriacum* v NPR Domické škrapy, NPR Zádielská dolina a Plešivecká planina na území NP Slovenský kras v rokoch 2003 – 2005. Včelník rakúskej (*Dracocephalum austriacum*) je jedným z kriticky ohrozených taxónov nielen slovenskej ale i českej kveteny. Súčasný klesajúci stav populácií tohoto druhu na území Slovenského krasu je veľmi znepokojivý, a preto daný projekt, ktorý prinesie nové poznatky o biológii druhu je veľmi potrebný pre obe republiky. Výsledky výskumu sa použijú v praxi s cieľom posilnenia populácie.

K žiadosti zaslali odborné stanovisko ŠOP SR - Správa NP Slovenský kras list č. NP SK 468/2003/Ka zo dňa 29.04.2003.

Ministerstvo si rozhodovanie vo veci vydania súhlasu podľa § 56 ods. 1 zákona vyhradilo v zmysle § 83 ods. 1 zákona.

Vzhľadom na to, že vykonávanie činností bude prebiehať pod dohľadom odbornej organizácie ochrany prírody a krajiny, čo je predpokladom, že nedôjde k narušeniu prírodného prostredia, rozhodlo ministerstvo tak, ako je uvedené vo výrokovvej časti tohto rozhodnutia.

Ministerstvo žiadateľa upozornilo, že vydaním rozhodnutia ostávajú nedotknuté osobitné predpisy (napr. povolením výnimky nie sú dotknuté vlastnícke vzťahy), ako aj ostatné ustanovenia zákona, napr. povinnosť vopred oznámiť KÚ v Košiciach, odboru životného prostredia začatie a ukončenie výskumu a do 60 dní po jeho ukončení podať ŠOP SR, Lazovná 10, P.O. Box 5, 974 01 Banská Bystrica správu o jeho výsledku podľa § 56 ods. 3 zákona a § 29 vyhlášky MŽP SR č. 24/2003 Z.z., ktorou sa vykonáva zákon č. 543/2002 Z.z. o ochrane prírody a krajiny.

Poučenie:

Proti tomuto rozhodnutiu možno podľa § 61 zákona č. 71/1967 Zb. o správnom konaní podať rozklad do 15 dní odo dňa jeho doručenia na Ministerstvo životného prostredia Slovenskej republiky, Nám. Ľ. Štúra 1, 812 35 Bratislava.



Jusková
RNDr. Anna Jusková
riaditeľka odboru

Rozhodnutie dostanú:

Tomáš Dostálek, Dobřešovická 119, 252 43 Průhonice, ČR

Po nadobudnutí právoplatnosti na vedomie:

Krajský úrad, odbor životného prostredia, Komenského 52, 041 26 Košice
ŠOP SR - Správa Slovenský kras, Biely kaštieľ č. 188, 049 51 Brzotín
SIŽP - inšpektorát OP, Rumanova 14, 040 00 Košice



MINISTERSTVO ŽIVOTNÍHO PROSTŘEDÍ

Vršovická 65, 100 10 Praha 10

odbor zvláště chráněných částí přírody
tel.: 267 121 111, fax: 267 311 096

Dle rozdělovníku

Č.j.
MŽP 21862/03-620/4550/03

Praha
23. 01. 2004

Věc: Rozhodnutí o udělení výjimky podle zákona 114/1992 Sb., o ochraně přírody a krajiny, ze základních podmínek ochrany zvláště chráněného kriticky ohroženého včelníku rakouského (*Dracocephalum austriacum*) a výjimky ze základních ochranných podmínek NPR Karlštejn a NPR Koda v CHKO Český kras

Ministerstvo životního prostředí jako ústřední orgán státní správy ochrany přírody podle ustanovení § 79 odst.1 zákona ČNR č. 114/1992 Sb., o ochraně přírody a krajiny v platném znění (dále jen "zákon"), posoudilo žádost T. Dostálka, Mgr. Z. Münzbergové z katedry botaniky PřF UK Praha a Mgr. V. Rybky, koordinátora monitoringu vybraných rostlinných druhů soustavy Natura 2000 a pracovníka Pražské botanické zahrady, postoupenou dne 30. 07. 2003 Správou CHKO Český kras a vydává po zvážení všech okolností, na základě provedeného správního řízení toto

ROZHODNUTÍ

Výjimka podle ustanovení § 56 odst. 1 zákona ze základních podmínek ochrany zvláště chráněných rostlin uvedených v § 49 odst.1 zákona a podle § 14 odst.1 vyhlášky MŽP č. 395/1992 Sb. a přílohy č. II této vyhlášky kriticky ohroženého včelníku rakouského (*Dracocephalum austriacum*) a výjimka podle § 43 zákona, ze základních ochranných podmínek zvláště chráněných území NPR Karlštejn a NPR Koda dle ustanovení § 29 písm. d), i) zákona za účelem provádění výzkumu a identifikace kritických fází životního cyklu výše uvedeného druhu (tj. k nedestruktivnímu označení rostlin, izolaci části květenství v rámci sledování samoopylení, sběru a zpětnému výsevu semen a odběru vzorku listů k provedení isoenzymové analýzy aj.) v rámci projektu, jehož součástí je diplomová práce T. Dostálka na katedře botaniky PřF UK Praha, se žadatelům

u d ě l u j e

za těchto podmínek:

1. Tato výjimka se vztahuje na T. Dostálka, Mgr. Z. Münzbergovou z katedry botaniky PřF UK Praha a Mgr. V. Rybku z Pražské botanické zahrady.
2. Vstup do výše uvedených zvláště chráněných území bude omezen na nejnižší míru nutnou k výzkumné činnosti.

3. V rámci výzkumu bude dbáno pokynů pracovníků Správy CHKO Český kras a v jeho průběhu nedojde k negativnímu ovlivnění území, ani jiných zvláště chráněných rostlin nebo živočichů. Přesné místo výsevu sebraných semen v rámci jednotlivých populací bude rovněž předem konzultováno se Správou CHKO Český kras.
4. Žadatelé zajistí odborné provedení všech prací v rámci výzkumu tak, aby nedošlo k poškození rostlin, nebyla ohrožena jejich vitalita a byla minimalizována všechna související rizika. Vzorek 1 listu z 10 rostlin v každé populaci bude odebírána šetrně, s ohledem na výše uvedené.
5. Průběh výzkumu bude průběžně dokumentován a vyhodnocován. Vždy nejpozději k 31. 03. následujícího roku bude Správě CHKO Český kras předána zpráva o výsledcích za uplynulý rok. Veškeré výsledky výzkumu (obhájená diplomová práce T. Dostálka a všechny související odborné publikace, popř. souhrnná závěrečná správa) budou v kopii nebo v podobě separátních otisků, nejpozději do 30 dnů od jejich publikování, zaslány Správě CHKO Český kras, Odboru zvláště chráněných částí přírody MŽP a do ústředního seznamu ochrany přírody na Agenturu ochrany přírody a krajiny ČR, Praha 3, Kališnická 4-6.
6. Toto rozhodnutí má platnost od nabytí právní moci do 31. 12. 2005 a nenahrazuje povolení či rozhodnutí potřebná podle jiných právních předpisů.

ODŮVODNĚNÍ

Odbor zvláště chráněných částí přírody Ministerstva životního prostředí obdržel žádost T. Dostálka, Mgr. Z. Münzbergové z katedry botaniky PřF UK Praha a Mgr. V. Rybky, koordinátora monitoringu vybraných rostlinných druhů soustavy Natura 2000 a pracovníka Pražské botanické zahrady, postoupenou dne 30. 07. 2003 Správou CHKO Český kras, o výjimku ze základních podmínek ochrany zvláště chráněného kriticky ohroženého včelníku rakouského (*Dracocephalum austriacum*) a výjimku ze základních ochranných podmínek zvláště chráněných území NPR Karlštejn a NPR Koda v CHKO Český kras za účelem výzkumu a identifikace kritických fází životního cyklu uvedeného druhu v rámci projektu, jehož součástí je diplomová práce T. Dostálka na katedře botaniky PřF UK Praha. Součástí žádosti byl detailní popis plánovaného výzkumného projektu se specifikací jeho jednotlivých částí a metod. Při výzkumu bude využíváno nedestruktivních metod, s výjimkou odběru omezeného množství vzorků z listů rostlin k provedení analýzy isoenzymů v rámci studia genetické variability.

Na základě výše zmíněného podání oznámil odbor zvláště chráněných částí přírody Ministerstva životního prostředí zahájení správního řízení v souladu s § 70 a 71 zákona příslušným obcím, tj. obci Svätý Jan pod skálou, obci Srbsko, Bubovice, Mořina, Hlásná Třebáň, Karlštejn (k.ú. Budňany), Korno, Měňany (k.ú. Tobolka), Tetín a městu Beroun (k.ú. Hostim) a registrovaným občanským sdružením. K účasti v tomto správním řízení se v řádném termínu přihlásilo pouze občanské sdružení Děti Země, ale nezaslalo žádné stanovisko nebo vyjádření. Obec Tetín s žádostí souhlasí a doporučuje jí vyhovět, ostatní obce v rámci správního řízení nevyužily svého práva účastníka řízení a k řízení se nijak nevyjádřily. Správa CHKO Český kras ve svém odborném stanovisku doporučuje žadatelům výjimku udělit a uvádí základní podmínky, jež musí být dodrženy.

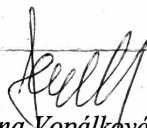
Včelník rakouský (*Dracocephalum austriacum*) je druhem kriticky ohroženým jak podle přílohy II vyhlášky 395/1992 Sb., tak dle aktuálního červeného seznamu a je rovněž druhem uvedeným v příloze II Směrnice 92/43/EHS, o ochraně přírodních stanovišť, volně žijících živočichů a planě rostoucích rostlin. Vzhledem k stupni ohrožení a celkové situaci populací tohoto druhu v ČR byl včelník rakouský rovněž zařazen v Metodice záchranných

programů rostlin a živočichů (AOPK ČR, 2002) do pracovního seznamu druhů navržených k vypracování a realizaci záchranného programu. Zamýšlený výzkumný projekt tak umožní získat důležité informace o biologii a ekologii tohoto druhu, především kritických fází jeho životního cyklu, které budou vhodným podkladem pro cílenou péči a případně doplní poznatky potřebné k vypracování kvalitního a smysluplného záchranného programu podle § 52 zákona.

Realizace výzkumného projektu „Identifikace kritických fází životního cyklu včelníku rakouského (*Dracocephalum austriacum*)“ přispěje k prohloubení znalostí o tomto druhu, výsledky budou vhodným podkladem pro zpracování a uplatňování patřičného managementu a stanou se rovněž vstupními informacemi pro zpracovávání záchranného programu. Veřejný zájem v oblasti ochrany přírody tak v tomto případě výrazně převyšuje zájmy chráněné zákonem. Protože výzkumná skupina tvořená žadateli je předpokladem dostatečného odborného zajištění záměru a v obdobných činnostech mají žadatelé dostatečné zkušenosti, a protože nebylo ze strany dalších účastníků správního řízení vzneseno dalších připomínek k žádosti a s výzkumem při dodržení podmínek tohoto rozhodnutí souhlasí i Správa CHKO Český kras, bylo žádosti o udělení výjimky ze základních podmínek ochrany zvláště chráněného kriticky ohroženého včelníku rakouského (*Dracocephalum austriacum*) vyhověno a bylo rozhodnuto tak, jak je uvedeno ve výroku.

POUČENÍ O ROZKLADU

Proti tomuto rozhodnutí lze podat do 15 dnů od jeho doručení rozklad podle § 61 zákona č.71/1967 Sb., o správním řízení, a to u Ministerstva životního prostředí, Vršovická 65, Praha 10.

V. Z. 

RNDr. Alena Kopálková
ředitelka odboru
zvláště chráněných částí přírody



Appendix 8: Photos of *Dracocephalum austriacum* and its localities



Photo 1: Haknovec is the biggest locality in the Czech Republic with cca 500 flowering plants. The locality is overgrowing with shrubs and trees in some places.



Photo 2: At locality Haknovec in the Czech Karst a few pink-flowering plants were seen.



Photo 3: Localities of *Dracocephalum austriacum* are often situated on the top of rock walls. In the picture There is locality on Kodská stěna in the Czech Karst.



Photo 4: Locality Kodská stěna in the Czech Karst is on the sunny steep top of the rocky wall. It seems to be good position for generative reproduction.



Photo 5: One of the most overgrown parts of locality in Císařská rokle in the Czech Karst.



Photo 6: Locality on Zádielský kameň in the Czech Karst.



Photo 7: Domické škrapy is the biggest Slovak locality. This locality is on the meadow with limestone rocks. It is one of a few localities which are not on the steep slope.



Photo 8: Locality in Železná vrata in the Slovak Karst is overgrowing with grasses and *Juniperus communis*.



Photo 9: Plant of *Dracocephalum austriacum* with very good seed production in Zádielký kameň in the Slovak Karst.



Photo 10: Detail of the flower of *Dracocephalum austriacum*.