Univerzita Karlova v Praze
Přírodovědecká fakulta

Studijní program: Botanika

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Dynamika vzácných a ohrožených druhů na místní a regionální prostorové škále – od teorie k praxi

Dynamics of rare and threatened species on local and regional scale – from theory to practice

Disertační práce

Vedoucí závěrečné práce/Školitel: Doc. RNDr. Zuzana Münzbergová, Ph.D.

Praha, 2011

This thesis is based on the following four papers, referred in the text as Papers 1-4:

1. **Population biology of two rare fern species: Long life and long lasting stability**
   Anna Bucharová, Zuzana Münzbergová, Přemysl Tájek
   American Journal of Botany (2010) 97 (8): 1260-1271

2. **Limitation of distribution of two rare ferns in fragmented landscape**
   Přemysl Tájek, Anna Bucharová, *Zuzana Münzbergová*
   Accepted in Acta Oecologica

3. **Gene flow among populations of two rare co-occurring fern species differing in ploidy level**
   Anna Bucharová, Zuzana Münzbergová
   Manuscript

4. **Effect of land use and climate change on future fate of populations of an endemic species of Central Europe**
   Anna Bucharová, Jiří Brabec, Zuzana Münzbergová
   Manuscript

I hereby declare that I made this thesis independently, using the listed references, or in the co-operation with other authors of the papers. I did submit neither the thesis nor its part to acquire any other academic title.

I was responsible for the data analyses and writing of all the papers. In paper 1 and paper 4, I carried out the matrix modeling. I also collected all the data for paper 3 and large proportion of data for paper 1. P. Tájek collected data for the paper 2 and helped with data collection for the paper 1, J. Brabec collected data for paper 4. Z. Münzbergová supervised the whole work. She also developed most of the Matlab scripts used in the matrix modeling in the paper 1 and 4 and helped with data collection for paper 1. Isoenzyme analyses (paper 3) were carried out by the stuff of the Isoenzyme laboratory of the Institute of Botany ASCR.

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Anna Lampei Bucharová

On behalf of all the co-authors, I declare the key participation of Anna Lampei Bucharová in acquiring the results and writing the papers, as described above.

....
Zuzana Münzbergová

Abstract

To assess the status of a rare plant species, we must first understand the factors that affect the size of populations and their numbers. In this thesis, I study processes affecting plant species prosperity on local scale (paper 1), factors influencing species distribution in landscape and ability of species to colonize new habitats (paper 2) and species traits responsible for gene flow between established populations (paper 3). Since I work with rare plants, I also aim to turn theoretical knowledge into practical recommendations for nature conservation (paper 4) to help effectively preserve rare and endangered species.

In the first 3 papers, I work with two rare fern species restricted to serpentine rocks, *Asplenium adulterinum* and *A. cuneifolium*, in a study system covering 10 × 10 km. I found that both species are long living (several decades) and in the study region, populations are in a good state and slowly growing. Even very small populations (10 individuals) have quite high chance to survive. In both fern species, I found dispersal limitation, which might be surprising regarding huge production of small spores in ferns (paper 2). The species differ in ploidy and thus, also mating system. *A. adulterinum* is tetraploid and its main breeding system is intragametophytic selfing. *A. cuneifolium* is diploid and is mainly outcrossing (paper 3). The breeding system has vast influence on colonization ability – the selfing species is better colonist of empty habitats, since it is able of single spore colonization (paper 2), resulting into faster metapopulation dynamics in this species. In the outcrossing species, there is more effective gene flow between already established populations (paper 3).

The two fern species are rare, but since they are not endangered within the study region, there was no need to transfer theoretical knowledge to the conservation practice. This I performed on another species, *Gentianella praecox* subsp. *bohemica*, endemic species of Central European seminatural grasslands with rapidly declining number of populations due to the management change. I carried out population viability analysis using matrix model. I found that management of seminatural grasslands is of crucial importance for survival of this species. If management is performed, even small populations (10 individuals) of this species are able to survive. Climate change (simulated as increased frequency of dry years) will have small impact on the populations. However, unmanaged populations are not able to survive in long-term period (several decades). Extremely small populations may be recovered from the seed bank by management induction (paper 4). Results of the study were implemented into recently prepared action plan for this species in the Czech Republic.

Contents

ACKNOWLEDGEMENT

I am grateful to my supervisor, Zuzana Münzbergová, for her expert advice, help, inspiration, motivation to scientific research and providing adequate working conditions.

I kindly thank to the stuff of Isoenzyme laboratory of the Institute of Botany ASCR for carrying out the isoenzyme analysis. I thank to Ivana Plačková, Jindřich Chrtek and Jan Štěpánek (Institute of Botany ASCR) for helpful advice on interpretation of isoenzyme banding pattern. I thank to my colleagues from the Institute of Botany ASCR for helpful remarks on manuscripts. I thank to Kateřina Štajerová and Petr Vít (Institute of Botany ASCR, PřF UK) for help with the final corrections of the thesis. I thank to Ladislav Tájek for technical help with material preparation for field experiments. I thank to Tomáš Fér and Veronika Kučabová (DNA laboratory, Department of botany, PřF UK) for kind help with AFLP analysis, depsite the fact that these analysis did not provide any usefull results. I thank to AOPK ČR for permission to work with the species. I would like to express my deep thanks to Mark van Kleunen (University of Konstanz) for inspiration and motivation to scientific research and to Katja Tielborger (University of Tübingen) for providing working space during my stays in Tübingen.

This work was supported by projects MŠMT 2B06178, FRVŠ 348/2004 and GAUK 176/2006. It was also partly supported by MŠMT 0021620828 and AV0Z60050516.

GENERAL INTRODUCTION

To assess the status of a rare plant species, we must first understand the factors that affect the size of populations and their numbers. Plants traits are often quite distinct from those of animals. Unique plant characteristics include a seed dormancy, a diversity of mating systems from self-ferilization to complete outcrossing, dispersal due to seeds, transport of haploid cells (pollen, spores) on large distance etc. At some spatial scale, most plant species are patchily distributed due to their sedentary lifestyle and the spatial heterogeneity of the environment (Schemske et al., 1994). The patchy distributions have been further exaggerated by the activities of humans through habitat destruction and fragmentation. Therefore, the assessment of status of a rare plant species requires an ecological and evolutionary information on more that one spatial scale.

On local scale, within a population, the basic unit is an individual – important is the number of individual plants, their fecundity, survival, mortality and processes influencing these vital rates. On the regional scale, the basic unit is a population –the number of populations, their stability, and size, probability of extinction and establishment of a new population. The regional dynamics of a species is critically driven by the number of suitable habitats, their connectivity and quality. The processes on each scale can be driven by totally different factors. For survival of a species in a landscape, both local dynamics and regional dynamics are often equally important.

When the information on rare species status is available and the species is really endangered, the theoretical knowledge on the species should be transferred into the practice. It is often done via so called "conservation action plans" (IUCN/SSC, 2002, IUCN/SSC, 2008). Most species specific conservation action plans currently valid are aimed on animals (typically lion or chimpanzee); considerably less was done for protection of specific plant species.

Population dynamics on the local scale

On the local scale, important information on the population status is if the number of individuals in a population is increasing or decreasing and how are these processes stable in time. From the conservation point of view, it is useful to know the risk of extinction of a population due to the stochastic processes. Prediction of changes in population size and extinction risk requires a demographic analysis (Schemske et al., 1994). The simplest approach is a census of the number of individuals through time, as it is done in a monitoring of many rare and endangered species (web2, 3.11.2010). Although this may be sufficient in some situations, it will not provide an accurate projection of long-time population trends. For example, a long-lived species with poor recruitment may not show a population decline until the oldest individual begin do die (Schemske et al., 1994). Better insight into the population dynamics can be obtained only by collecting detailed information on all the phases of life cycle of a plant and integrating them in models of the whole life cycle of the species.

Two main types of models are generally used in this context: individual based models (IBM) and stage based (matrix) models. The first approach models the fate of single individuals within population and the relation between vital rates (e.g. growth), phases of life cycle (e.g. flowering) and environmental characteristics (e.g. shadow/sun) described by a continuous function. It allows an explicit inclusion of the individual variation in great detail (DeAngelis and Mooij, 2005) and it has been commonly used in animals populations (for review see Grimm, 1999), but its use in plants have been rather limited. Disadvantage of this model is necessity of a large amount of data and it requires good computing facilities. Also lack of defined stages of the life cycle makes the interpretation a bit less intuitive. However, it is recently promising way of plant population modeling (e.g. Buckley et al., 2003, Hoebee et al., 2008, Pico et al., 2009).

On the other hand, matrix projection models (Leslie, 1945, Caswell, 2001) are established, widely used and good approach to evaluating the prospect of a rare plant populations. It is based on stages (age or size categories), which with certain probability survive to another stage, die or reproduce. In contrast to IBM, it works with discrete stages, not with continuous variables. The idea of matrix models is pretty old (Leslie, 1945), but after Menges (1986) discussed the application of matrix model to the conservation and monitoring of rare plants and later described its use for the population viability analysis (Menges, 1990), it started to be a common tool for evaluating the plant population dynamics. Several additional methods were developed to make this tool of wider use – e.g. for calculating mean life span (Cochran and Ellner, 1992) or confidence intervals using bootstrap (Alvarez-Buylla and Slatkin, 1994, Efron and Tibshirani, 1994). It was employed in the research of population dynamics of wide selection of rare vascular plants (Pino et al., 2007, Garcia, 2003, Garcia et al., 2008, Griffith and Forseth, 2005, Jongejans et al., 2008). It was also even used for bryophytes (Okland, 1995). Surprisingly, this approach was never used for ferns or lycophytes. Since many ferns are subject of the interest of nature conservation (web1), detailed information on the population dynamics of ferns is strongly needed.

Population dynamics on the landscape scale

In many plants, factors influencing the number of populations on regional scale may be as important for determining its fate as within-population dynamics. Dynamics of such "population of populations" is described by the classical metapopulation model developed by Ilkka Hanski (e.g. Hanski, 1991, Hanski, 1994) on the basic concept of Levins (1970). It was originally designed for animal populations, but the idea was soon adopted by plant ecologist (e.g. Husband and Barrett, 1998, Vandermeijden et al., 1992, Husband and Barrett, 1996, Eriksson, 1997).

Dynamics of the species in a landscape is determined by available suitable habitats, of which some are occupied and some are free. Pattern of occupancy of these habitat patches by the species is not stable in time. Some of the vacant patches get occupied by colonization, some get free due to the local extinction. Local extinctions are part of the natural process and if new populations arise due to the colonization, species existence on the regional scale is not in danger (Schemske et al., 1994).

Since we are talking about rare plants, a question arises: Why is this species rare? What is the limiting its distribution? In the light of the metapopulation concept, there are basically 2 limiting factors, which may combine: 1. species has not enough habitats, 2. there is enough habitats, but the species is not able to colonize them – it is dispersal limited (Munzbergova and Herben, 2005). In plants, dispersal limitation means lack in availability of diaspores or their bad dispersal ability.

Different types of the distribution limitations for plant species in various systems were studied by many recent studies and they concluded that large majority of the plant species is limited by both seed availability and/or dispersal ability and by availability of suitable habitats (reviewed in Turnbull et al., 2000, Clark et al., 2007).

Suitable habitats

Problematic point of all the distribution limitation and metapopulation studies is identification suitable, but unoccupied habitats. Together with the wider use of Hanski metapopulation models (Hanski, 1991, Hanski, 1994), several methods were developed. The most direct but technically difficult method is to perform a seed addition experiment (Munzbergova and Herben, 2005). While such experiments have been repeatedly used in a variety of plant species (for review see Turnbull et al., 2000, Clark et al., 2007, Moles and Westoby, 2002), it was in fact suggested that such experiments may also not be fully reliable (Ehrlen et al., 2006, Clark et al., 2007). Alternative and also commonly applied methods use different distribution models (Moore and Elmendorf, 2006) parameterized using different environmental variables (Husband and Barrett, 1996, Ouborg, 1993, QuintanaAscencio and Menges, 1996, Wild and Gagnon, 2005) or data on the species composition of the sites (e.g. Milden et al., 2006, Tremlova and Munzbergova, 2007, Munzbergova and Herben, 2004, Romermann et al., 2007). It was shown, that these approaches are able to reliably identify unoccupied suitable localities (Munzbergova and Herben, 2004, Milden et al., 2006, Ehrlen et al., 2006). The best models explaining occupancy of localities and thus the best models for identifying unoccupied habitats (Moore and Elmendorf, 2006) were found to be models including both the abiotic environmental variables and the species composition of the sites (Munzbergova, 2004). It is surprising that this combination of the abiotic factors and the species composition has not been used for predicting of suitable unoccupied habitats until now. Good definition of suitable habitat is useful not only for the scientific purpose. In case of rare species, characteristics of suitable habitats are of key importance for planning the optimal management for the species.

Dispersal abilities

Dispersal abilities are dependent on many factors, e.g. seed size, their adaptation to long distance transport (e.g. wind, zoochory). To a certain level, dispersal abilities are also dependent on the mating system of the species: strictly outcrossing species will disperse worse than species with some ability of selfing. Strictly outcrossing species needs at least 2 seeds arriving to an empty habitat, which have to develop into at least 2 fertile individuals to produce offspring and establish a new population.

Mating system has a vast influence on the genetic structure within and between populations. With increasing selfing, homozygosity increases within populations. In outcrossing species, homozygosity increases with increasing inbreeding (mating between related individuals) (Loveless and Hamrick, 1984).

In the vascular plants, ferns have a bit peculiar position regarding the mating system and the dispersal abilities: In the seed plants, fertilization takes place prior dispersal, before the seed is formed. For the colonization of a new habitat, theoretically one seed is enough. In ferns, fertilization is post dispersal – on haploid gametophyte. For strictly outcrossing species it means, that on a new habitat, two spores have to fall in close proximity in favorable conditions; gametophyte has to develop from both of them with archegonia/anteridia and only than fertilization can occur. On the other hand, if the species is able of an intragametophytic selfing, one spore which develops in a hermaphroditic gametophyte is enough.

There are basicaly 3 types of breeding systems in ferns: 1. true outcrossing – fertilization between gametophytes developed from spores of different parental plants; 2. fertilization between gametophytes from the same parental plant – this is actually equivalent of selfing in seed plants, when two haploid cells from the same parent give arise zygote; 3. intragametophytic selfing – this is an extreme kind of inbreeding, when haploid set of chromosomes actually doubles, resulting in the completely homozygous sporophyte.

As in many other plant groups, in ferns diploids and pollyploids occur. Ploidy has vast influence on the breeding system – while diploids are generally outcrossing, in pollyploids inbreeding is common (Ranker and Geiger, 2008). It is given by the fact that a species with higher ploidy level have fixed heterozygosity (Schneller and Holderegger, 1996) and it reduces the negative consequence of inbreeding depression (Lande and Schemske, 1985). The ability of polyploids of intragametophytic selfing in ferns was experimentally tested (Suter et al., 2000), even in comparison with diploids (Flinn, 2006). Results of experiments with gametophytic crossing suggest that the breeding system of fern species rather fall between the two extremes (intragametophytic selfing and outcrossing). The studies on the genetic diversity, however, suggest a bit different results.

Intragametophytic selfing produces completely uniform offsprings. Some studies on allotetraploids showed almost total lack of the genetic diversity on local (Holderegger and Schneller, 1994) and regional (Jimenez et al., 2009, Vogel et al., 1999) and even continental scale (Ranker et al., 1994). It suggests 100% gametophytic selfing in reproduction. The little present variability is supposed to be given by multiple allopolyploidization (Ranker et al., 1994, Jimenez et al., 2009). Idea of the prevailing intragametophytic selfing in polyploids is supported by the fact that individuals originated from oucrossing produce abortive spores (Schneller, 1996).The clear discrepancy between the experimental studies and the genetic studies may be caused by existence outcrossing in allotetraploids, but it does not produce fully functional sporophytes and the new combination of genes does not continue in the population. Another option is that ability of selfing/outcrossing is species dependent. Most probably, it is combination of both factors.

Ability or disability of a gametophytic selfing has a vast influence on dispersal abilities: a species which is easily able of intragametophytic selfing resulting to a fully functional sporophyte has much bigger potential to spread than a strictly or almost strictly outcrossing species, since it requires 2 gametophytes in the same time in close proximity. Dispersal abilities have big influence on the regional dynamic of the species, which has a big impact in rare species occurring in fragmented habitats, where dynamics on the local scale is of the same importance as dynamics on the regional scale.

Transfer of information to practice

When there is already enough information on the species status and the species is really threatened or endangered, there may be a social request on turning the scientific knowledge to the practice. Process of assessing the population viability and risks for the species existence was named "population viability analysis" (Gilpin and Soulé, 1986). Practical conservation and policy require evaluation state of the populations and probability of their survival/extinction, because the decision making needs to be supported by objective information. Thus, probability of extinction, especially under different conditions (e.g. case of management planning) is of a great value. However, such an extinction probability has to be treated with caution.

There have been several studies (Ludwig, 1999, Fieberg and Ellner, 2000) arguing that it is basically not meaningful to calculate extinction probabilities. Main weak points were defined as not sufficient amount of data per census, not sufficient data (if any) for all the life cycle transitions, short study duration which does not cover whole variability in vital rates and lack of knowledge of effect of catastrophes (Beissinger and Westphal, 1998). However, most of the PVA analysis surveyed in above mentioned studies have been done in animals. Plants have the advantage of sessile life and thus, it is easier to precisely follow a larger number of individuals. It is even possible to carry out an experiment, which can bring information on all the transitions of a life cycle, on effect of different management etc. To estimate sampling error, it is a good idea to compute a confidence intervals using bootstrap (Efron and Tibshirani, 1994). Covering whole variance in vital rates can be done by longer period of the study. Another option is to simulate the demographic and environmental stochasticity using numeric approach (Caswell, 2001).

Results of the PVA is mostly impossible to validate using real data, since they have probabilistic nature – it would require following a large amount of populations, which are often even not available (Beissinger and Westphal, 1998). The results will be always uncertain to some degree and such uncertain information should be used with caution in policy decisions. Thus, absolute interpretations should be avoided or done extremely carefully. More reliable are relative outputs: e.g. to calculate the extinction probability of a rare plant under different management conditions and than compare relative effect of these managements on species persistence (Beissinger and Westphal, 1998).

Critics of computing of extinction probability also stress, that it is pointless to calculate fate of a population in 100 years based on data from several years (Ludwig, 1999). It is again problem of interpretation; long term projection should be viewed as extrapolations of the short term trends for comparing the relative merits of the management options, not as prediction of viability in 100 years (Beissinger and Westphal, 1998).

To conclude, it is not a solution to refuse a PVA analysis when it is needed for creating a species conservation strategy, give no information to the conservation policy and this way give up the responsibility for the future fate of an endangered species. When results of PVA are necessary, including the probability of population survival, it may be performed, but the results should be treated with caution. To estimate sampling error, confidence intervals should be calculated and results of the whole analysis should be correctly interpreted and transformed to policy decision with extreme caution. Preferably, rather relative results (e.g. which management is better) should be used.

My study

In my study, I focused on local and regional dynamics of two rare fern species, *Asplenium adulterinum* and A. *cuneifolium*. In these species, I assessed local (paper 1) and regional dynamics (paper 2), together with the genetic diversity of its populations (paper 3). Since we found these species naturally rare, but not threatened, there was no need to transfer our knowledge to the practice. This I did with other species, *Gentianella praecox* subsp. *bohemica*, where I aimed on the probability of population survival under different management regimes (paper 4).

Study system for* A. *adulterinum and* A. *cuneifolium

Study species

I study two fern species, *Asplenium adulterinum* Milde and *Asplenium cuneifolium* Viv. (Aspleniaceae), both restricted to thee rpentine substrates in Europe (Tutin et al., 1980). A. *adulterinum* occurs also in one population on Vancouver Island, Canada (Ogilvie and Ceska, 1984). The ranges of both species are highly scattered, following the distribution of serpentine rocks in Europe from the Mediterranean to Norway and from Greece to Spain (A. *cuneifolium*, A. *adulterinum* only from Austria to France). In the Czech Republic, the species occur mainly in the Western Bohemia (in the region of Slavkovský les). There are also several localities in the north-eastern Bohemia. In the rest of the country, there are only very few small populations; A. *cuneifolium* is more common in the Czech Republic than A. *adulterinum*.

Both species are rare and of the conservation concern throughout Europe (Tutin et al., 1980). A. *adulterinum* is also a species of interest in the European ecological network Natura 2000 (web1). The species differ in ploidy levels — *Asplenium adulterinum* is an allotetraploid (hypothesized parental species are *A. viride* L. and *A. trichomanes* Huds. subsp. *trichomanes*; Lovis, 1955) whereas A. *cuneifolium* is a diploid (Hejný and Slavík, 1980).

As in other fern species, haploid (gametophyte) and diploid (sporophyte) generation alternate in the study species. In the most cases, ferns reproduce via spores, produced by the sporophyte and germinating to initiate the gametophyte. Clonal reproduction of sporophytes (e.g. *Pteridium aquilinum*; Hejný and Slavík, 1980) and gametophytes (e.g. Hymenophyllaceae; Dassler and Farrar, 2001) is possible in some fern species, but it was not observed in A. *adulterinum* and A. *cuneifolium*.

Study site description

We conducted the study in the region of Slavkovský les, Western Bohemia, Czech Republic. In this region, serpentine rocks are scattered in the landscape dominated by pine and spruce forests and mown grasslands. Both A. *adulterinum* and A. *cuneifolium* are quite common in the area. *Asplenium adulterinum* is more frequent and occupies rocks both in the open landscape and in the forest (dominated by *Pinus sylvestris* and *Picea abies*). In total there are 66 populations ranging from several individuals to nearly 4000 individuals (Tájek, 2003). *Asplenium cuneifolium* occurs on rocks under the forest canopy. Rocks in the open landscape are inhabited rarely, and the populations are very small in the open habitats. In total, there are 48 populations of A. *cuneifolium* were located in the study region, ranging from several individuals up to several hundred individuals (Tájek, 2003)

Study system for *Gentianella praecox* subsp. *bohemica*

Gentianella praecox subsp. *bohemica* is a small, strictly biennial plant. It used to commonly grow on wet pastures and meadows in Central Europe. With increasing intensification of agriculture, extensive pastures were often either abandoned, or changed into (sometimes) mown meadows. It led to considerable decrease of the number populations of *Gentianella* in its whole distribution range. Nowadays, the species has in total approximately 110 populations of the size from several individuals to several thousands. However, sizes of the individual populations highly vary from year to year. The plant is currently of a great interest of nature conservation (web1) and recently, action plan for restoration of this plant have been developed in the Czech Republic.

The study was carried out at 5 localities in Southern Bohemia, in the center of area of the species.

Paper 1

POPULATION BIOLOGY OF TWO RARE FERN SPECIES: LONG LIFE AND LONG-LASTING STABILITY

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American Journal of Botany 97(8): 1260-1271

Population biology of two rare fern species – long life and long-lasting stability.

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ACKNOWLEDGEMENT

We thank to L. Tájek for technical help, Administration of PLA Slavkovský les for permission to work with the species and our colleagues from the Institute of Botany ASCR for helpful remarks to the manuscript. This work was supported by projects MSMT 2B06178, FRVŠ 348/2004 and GAUK 176/2006. It was also partly supported by MSMT 0021620828 and AV0Z60050516.

ABSTRACT

Background and aims This study describes the population dynamics of two rare fern species and evaluates the prospects of their survival. This is the first study of detailed demography of ferns described using transition matrix models. The study species, *Asplenium adulterinum* and *A. cuneifolium*, are restricted to serpentine rocks and differ in ploidy level and partly in habitat requirements. Both species are of interest in nature conservation.

Methods Single life history traits were evaluated and transition matrix models were used to describe the dynamics of the populations. Population growth rates, elasticity values, and life-table response experiments were used to compare the dynamics between species, years, and different habitat types. Predicted population performance based on models was compared with real data on population growth.

Key results All populations of both species are growing. Stable stage distribution based on stochastic simulation corresponds to current stage distribution. The most critical phase of the life cycle is stasis of large adult plants. Reproduction is of low importance. Extinction probability of small populations is low. Mean life span of individuals of both species is 30-50 of years. When compared with real data, the model successfully predicted population performance over 10 years.

Conclusion Populations in the study region are not endangered and current population dynamics are stable. Differences in life history traits between species, probability of extinction between species and habitat, together with different ploidy and thus probably different dispersal ability, suggest the existence of metapopulation dynamics.

Keywords: life cycle; naturally rare species; population dynamics; population growth rate; population viability analysis; ferns; PVA, transition matrix model

INTRODUCTION

A central theme of conservation biology is understanding the causes of species extinction, thereby elucidating the requirements for species persistence (Schaffer, 1990). Gilpin and Soulé (1986) have outlined the conceptual framework of this central theme and named it “population viability analysis”. Since then, population viability analysis has become a cornerstone of conservation science (Bessinger, 2002). An important part of population viability analysis is the detailed description of the full life cycle of a species, i.e., the study of growth, survival, and reproduction of individuals within populations and the effect of these processes on changes in population size.

The most common approach to population viability analysis involves transition matrix models (Leslie, 1945; Schemske et al., 1994; Caswell, 2001). Matrix models have been used for evaluating the population status of rare angiosperms and gymnosperms (e.g., Menges, 1990; Pino et al., 2007; García, 2003, 2008; Griffith and Forseth, 2005; Jongejans et al., 2008) and bryophytes (e.g., Økland, 1995). This approach, however, has not been applied to ferns or lycophytes. Thus, we have only very limited knowledge on critical phases of life cycle and on population dynamics of ferns. Comparison with seed plants is inaccurate because of alternation of haploid and diploid generations in ferns (gametophyte and sporophyte). Since many ferns are subject of interest of nature conservation, detailed information on population dynamics of ferns is strongly needed.

Previous studies on the population dynamics of ferns have described population stage structure of sporophytes (Kuehn and Leopold, 1992; Rünk et al., 2006; Aguraiuja et al., 2008) and growth rate and age of individual sporophytes (Sharpe, 1993; Kelly, 1994; Schmitt and Windisch, 2006). A few studies have described the gametophyte phase of ferns (Shorina, 2001; Watkins et al., 2007). However, to our knowledge, no study has attempted to analyze population viability in the ferns.

In the life cycle of ferns, two very different phases exist — gametophyte and sporophyte. A gametophyte is usually very small and inconspicuous, its ecological requirements often differ from those of a sporophyte (Farrar, 1967; Peck et al., 1990), and it is usually not possible to observe gametophytes in nature. Problems with studying gametophytes have probably prevented researchers from completing detailed assessments of population dynamics, and in particular have limited the use of transition matrix models for ferns. However, studying early phases of the life cycle is not difficult only in ferns. Similar problems can be encountered in seed plants, seeds of which germinate in inaccessible places such as bare rocks or rock crevices (Dinnétz and Nilsson, 2002; Forbis and Doak 2004; García, 2008). In such cases, it is necessary to ignore germination and the early life cycle stages in the models and deal only with transitions between adult fertile plant – seed – small plant, or even between adult fertile plant – small plant (Menges, 1990; Dinnétz and Nilsson, 2002; Forbis and Doak, 2004; García, 2008). A similar approach can be applied to ferns by ignoring the gametophytes and only observing the transition between sporophyte stages, i.e., spore producing plant - very small sporophyte (sporeling).

Relating sporelings to adults from the previous year is accurate only in cases where there is no spore bank, lack of clonal reproduction of gametophyte/small sporophytes and a short duration of the gametophyte phase. Although spores usually germinate very quickly in favorable conditions (Hock et al., 2006; Schneller, 1988) and a sporophyte is produced within several months (Pangua et al., 1994; Lindsay and Dyer, 1996), there is strong evidence for persistent spore banks in ferns (e.g., Lloyd and Klekowski, 1970; Dyer and Linsday, 1992; Arachon and Pangua, 2004). Thus, relating sporelings to adults from the previous year may not be reliable and could work only in relatively stable populations. When using such an approach, it is necessary to estimate the effect of this inaccuracy on the results.

In this study, we assess population dynamics of two serpentine-rock dwelling fern species – *Asplenium adulterinum* and *Asplenium cuneifolium* using transition matrix models. Both of these species are rare across their ranges, often forming only small populations. *Asplenium adulterinum* is listed among the most endangered species in Europe in Annex II of the Habitat directive of NATURA 2000 (Anonymous, 2008). The species are thus of interest to nature conservation (e.g., Holub and Procházka, 2000; Anonymous, 2008), and population viability analysis is especially important.

Populations of rock dwelling species of *Asplenium* are often persistent in time, random fluctuations are low, and their habitats are stable (Holderegger and Schneller, 1994). We thus predicted that ignoring the gametophyte phases and observing only transitions between adult sporophytes – small sporophytes should not lead to large inaccuracies. Moreover, we have additional information on the total number of individuals of the target species from several populations within the study region, confirming their relative stability — changes in population size of a maximum 30 % within the last 10 years (P. Tájek, unpubl. data; Tájek, 2003; Tájek et al., 2006).

The two species occur in the study region on two different habitat types — in open landscapes (i.e., rocks not in forests, but in meadow) and in forests. *Asplenium adulterinum* is equally common in both types of habitats; *A. cuneifolium* is much more common in the forest than in the open landscape. We thus also explored the effect of habitat conditions on population dynamics of the species. Specifically, we asked the following questions: (1) What are the population dynamics of the species and what is the risk of extinction of small populations of the species? (2) What are the most critical phases of the life cycle of the target species? (3) What are the differences in population dynamics of the two species in the two different habitats?

The present study is the first to consider population dynamics of ferns using transition matrix models, evaluating viability of populations, identifying critical phases of the life cycle and assessing stability of the populations. It compares predicted population sizes with real data obtained over 10 years. Moreover, the model species have very similar habitat requirements, but different ploidy levels (*A. adulterinum* is an allotetraploid, *A. cuneifolium* is a diploid; Hejný and Slavík, 1988). Thus, the study contributes to the understanding of the impact of ploidy levels on performance.

METHODS

Study species

We studied two fern species, *Asplenium adulterinum* Milde and *Asplenium cuneifolium* Viv. (Aspleniaceae), both restricted to serpentine substrates in Europe (Tutin et al., 1980). *A. adulterinum* occurs also in one population on Vancouver Island, Canada (Oglivie and Ceska, 1984). The ranges of both species are highly scattered, following the distribution of serpentine rocks in Europe from the Mediterranean to Norway and from Greece to Spain (*A. cuneifolium*, *A. adulterinum* only from Austria to France). In the Czech Republic, the species occur mainly in Western Bohemia (in the region of Slavkovský les). There are also several localities in north-eastern Bohemia. In the rest of the country, there are only very few small populations; *A. cuneifolium* is more common in the Czech Republic than *A. adulterinum*.

Both species are rare and of conservation concern throughout Europe (Tutin et al., 1980). *A. adulterinum* is also a species of interest in the European ecological network Natura 2000 (Anonymous, 2008). The species differ in ploidy levels — *Asplenium adulterinum* is an allotetraploid (hypothesized parental species are *A. viride* L. and *A. trichomanes* Huds. subsp. *Trichomanes*; Lovis, 1955) whereas *A. cuneifolium* is a diploid (Hejný and Slavík, 1988).

As in other fern species, haploid (gametophyte) and diploid (sporophyte) generation alternate in the study species. In most cases, ferns reproduce via spores, produced by the sporophyte and germinating to initiate the gametophyte. Clonal reproduction of sporophytes (e.g. *Pteridium aquilinum*; Hejný and Slavík, 1988) and gametophytes (e.g. Hymenophyllaceae; Dassler and Farrar, 2001) is possible in some fern species, but it was not observed in *A. adulterinum* and *A. cuneifolium*.

Study site description

We conducted the study in the region of Slavkovský les, Western Bohemia, Czech Republic. In this region, serpentine rocks are scattered in the landscape dominated by pine forests and mown grasslands. Both *A. adulterinum* and *A. cuneifolium* are quite common in the area. *Asplenium adulterinum* is more frequent and occupies rocks both in the open landscape and in the forest (dominated by *Pinus sylvestris* and *Picea abies*). In total there are 66 populations ranging from several individuals to nearly 4000 individuals (Tájek, 2003). *Asplenium cuneifolium* occurs on rocks under the forest canopy. Rocks in the open landscape are inhabited rarely, and the populations are very small in the open habitats. In total, there are 48 populations of *A. cuneifolium* were located in the study region, ranging from several individuals up to several hundred individuals (Tájek, 2003).

Field data collection

We collected data annually in August from 2004 to 2007. After careful investigation of individuals of both species, four size categories (stages) were defined. Stage 1 comprised the smallest sporophyte plants; stage 4 comprised fully developed adult plants (Table 1). The stages were selected with the aim of describing the range of sizes found in the species and defining categories with expected similar behavior.

We conducted the study at six localities: *A. adulterinum* was present at all the localities (3 in the forest, 3 in the open landscape), *A. cuneifolium* was present only at 4 of them (3 in forests, 1 in open landscape, Table 2). This disproportion occurred because there was only one population in the open landscape of *A. cuneifolium* in the studied region that was big enough to perform the study (all other populations are very small, with a maximum of only 10 individuals). Localities were further coded as Open 1-3 and Forest 1-3 (Table 2).

Survival and transitions across stages

In each population, 200 to 400 individuals were marked with colored nails using different colors for different stages. To relocate the individuals easily and to be able to identify newly appearing plants, we marked all individuals in a well-delimited part of the population (e.g., a single rock). The census was completed every year in August, and plants were re-marked according to their actual stage. Because we marked all individuals in the well-delimited area, the number of plants in each stage was unbalanced; 10-20 % of individuals were in each stage 1, 2, and 3, and 50-70% of individuals were usually in stage 4. We had, however, at least 15 individuals in each stage (with few exceptions), which is sufficient to obtain reliable information on the transition probability (Münzbergová and Ehrlén, 2005). All individuals in stage 1 without a mark were considered as newly established, and no establishment of a new plant in another stage was observed.

Comparison of single life-history traits

Differences between species and habitats in the probability of growth, survival in the same stage, retrogression, reproduction, and mortality were examined. These parameters were determined in individuals of different size. To remove this effect, the stage from the previous year was used as another independent variable in the tests. Moreover, data were collected in three time periods and from different localities, and thus year and locality (nested within habitat type) were also used as independent variables. Two-way interactions of species with the other independent variables were also included. The effect of these independent variables on probability of growth, survival in the same stage, retrogression, reproduction and mortality was tested using logistic regression in GenStat (Payne et al., 2005). Because six populations were available for *A. adulterinum* (3 in forest and 3 in open landscape) and just four populations for *A. cuneifolium* (3 in forest and 1 in open landscape), two sets of tests were carried out to make sure that the unbalanced design did not bias results of the tests. First, all populations were included. Second, we used only the forest populations and the population of both species from the locality where they co-occur in the open landscape. Given that both tests showed very similar results, only results of the latter test are presented.

Population performance

Demographic data were examined using transition matrix models (Caswell, 2001). Analysis of transition matrices yields a finite rate of population increase, λ. Analysis of transition matrices also generates information on the change in population growth rate, $\delta\lambda$, following a small change in sensitivity a_{ij} (δa_{ij}). Proportional sensitivity (elasticity) is usually used as a measure of the contribution of a matrix element to fitness (de Kroon et al., 2000). Moreover, the right eigenvector of the matrix can be obtained, corresponding to the stable stage distribution of the population (Caswell, 2001).

In this study λ (population growth rate) was calculated for each species, population, and transition interval (Appendix S1, see Supplemental Data with the online version of this article). Furthermore, a stochastic simulation approach (Caswell, 2001) was used to combine all the matrices of one type (species, habitat, population, and year) and estimate overall population growth rate of these combined matrices, as well as their overall elasticity and stable stage distribution.

Each estimate of transition probability, and thus each estimate of population growth rate, as well as of elasticity and stable stage distribution, is confined with an error because of the limited number of individuals that can be sampled. To estimate this error, bootstrap confidence intervals of the population growth rate were calculated (Alvarez-Buylla and Slatkin, 1994). This was done by bootstrapping the original data used to derive the original matrices 10,000 times. Based on the results, confidence intervals of population growth rates were constructed for each population and year (Efron and Tibshirani, 1994). To do this, a MATLAB script developed by Münzbergová (2006, 2007) was used. For estimating the confidence intervals of overall growth rate, elasticity and stable stage distribution of one type (species, habitat, population, and year), the bootstrapped matrices were combined using stochastic simulation (see Münzbergová, 2005).

The confidence intervals allow estimation of variation of single parameters but do not provide estimates of significance of the difference between transition matrices or their combinations (Caswell, 2001). To estimate this, a permutation test was performed, permuting single individuals used to estimate transition probabilities between each pair of populations or between each pair of types of populations (combined matrices of given type, e.g., open landscape – forest, two species). In each permutation run, the difference in population growth rate between the pair of populations or pair of types of populations was estimated, and the number of permutation runs in which the absolute value of the difference was larger than the observed difference was counted. This value was then used to estimate the probability that observed differences between each pair of populations or pair of types of populations could be just random. To do this, a modified MATLAB script developed by Münzbergová (2007) was used.

Because many populations of the species in the region are small (ca. 50 individuals and in some cases even only 10 individuals), the probability of extinction of small populations under conditions of demographic and environmental stochasticity was calculated. Populations of 10 and 50 large individuals (stage 4) were projected by randomly drawing one of the matrices of given type (4 types – 2 species in 2 habitats, 9 matrices per type, except *A. cuneifolium* in open habitat, where only 3 matrices were available). At each time step, the population vector was multiplied with this matrix, and population survival was evaluated after 20 and 50 years. In each step, the resulting population vector was replaced by a value drawn from a Poisson distribution with the given mean to simulate demographic stochasticity. This projection was repeated 1000 times for each habitat type and species (Münzbergová, 2005, 2007).

A life-table response experiment (LTRE) with fixed factorial design was conducted to examine the effect of species and habitat on population growth rate. LTRE is a form of retrospective analysis that allows quantification of factors responsible for the observed variation in population growth rate. We followed the approach described in Caswell (2001), and calculated mean matrix (A). Population growth rate was expressed as follows:

$$\lambda^{py} = \lambda^{\cdot} + \alpha^{p} + \beta^{y} + \alpha\beta^{py}, \tag{1}$$

where $\lambda^{\cdot}$ is the population growth rate of the mean matrix $A^{\cdot}$ and the effects of α^{p} and β^{y} are the main effects of the p^th^ species (*A. adulterinum* and *A. cuneifolium*) and the y^th^ habitat (forested and unforested) on population growth rate λ. The contribution of the transition a_{ij} to the effect of ploidy level/productivity and year on population growth can be expressed as follows:

$$\alpha^{p} = \sum (a_{ij}^{p.+} a_{ij}^{..}) s_{ij}, \tag{2}$$

$$\beta^{y} = \sum (a_{ij}^{y.\,+}\, a_{ij}^{..})s_{ij}, \qquad (3)$$

$$\alpha\beta^{\,py} = \sum (a_{ij}^{py\,+}\, a_{ij}^{..})s_{ij} - \alpha^{p} - \beta^{y} \qquad (4).$$

Here, s_{ij} is the sensitivity of the ij^{th} transition of the mean matrix.

The LTRE analysis indicates the contribution of each life cycle transition to differences between different levels of each factor (species, habitat). Important life cycle transitions are those with large positive contributions at some factor levels and large negative contributions at others. Analogously to ANOVA, the mean of the treatment effects is zero. The interaction term $\alpha\beta^{\,py}$ is calculated as the difference between the actual contribution of a_{ij} to λ^{py} and the difference predicted on the basis of the additive model. Hence, a positive contribution indicates that this interaction increases λ^{py} above the value predicted by the additive model (Caswell, 2001). The significance of LTRE was tested performing a permutation test with 10,000 permutations. A MATLAB script was used to perform the test (details in Münzbergová, 2007).

To test the predictive power of our models, we used data from P. Tájek (unpubl. data) and Tájek et al. (2006) on the real number of individuals in populations of *A. adulterinum*, which were available for two studied localities (Open 3; Open 1 and Forest 1 combined) from 1997 and 2006. The data contained the observed number of individuals in populations, but the smallest plants (stage 1) were not counted. To create an in initial population vector for the simulations, we used stable stage distribution (real stage distribution was not known) and distributed the estimated number of individuals in year 1997 from the given population (representing sum of stages 2, 3 and 4) according to the stable stage distribution of the given population. Number of stage 1 plants, which were not counted during the survey in 1997, was added to the population vectors also based on stable stage distribution. Projection of the obtained population vector (Appendix S2, see Supplemental Data with the online version of this article) was done over 10 years using 10 randomly chosen matrices from a given population (for Open 3, 3 matrices were available, for Open 1 and Forest 1 together 6 matrices). This approach was repeated 1000 times and a 95% confidence interval was constructed. Results were compared with the real number of individuals of a given population in year 2006.

Establishment of new individuals

Transitions corresponding to reproduction are not accurately estimated. Current sporelings are related to the number of spore producing plants in the previous year. This may not be correct because of the existence of a spore bank and the duration of the gametophyte phase of individuals (see above). To assess the influence of this inaccuracy on predicted population dynamics, we varied the reproductive transitions between 0.1 and 2 of the original value and tested the effect of this change on estimates of population growth rate.

Mean life span

Conditional life span is defined as the average age of death for individuals that survive to a certain stage (Cochran and Ellner, 1992). In our study, we were interested in longevity of plants, thus we investigated conditional life span of the biggest plants, i.e., stage 4 (cf. Ehrlén and Lehtilä, 2002). Time spent in the spore bank and time in the gametophyte phase was not regarded as a part of the life span.

The algorithm of Cochran and Ellner (1992) can work only with a single matrix. Our study covered 3 transition intervals for each population of each species, thus it yielded 3 matrices for each population. To obtain total projected life span for each species, we computed the harmonic mean of the life spans for all 3 matrices representing different years within one population as a measure of the average life span for the species at that site (Ehrlén and Lehtilä, 2002). To obtain one value for each species, we calculated the arithmetic mean of life spans at all populations covered by this study (i.e., arithmetic mean of harmonic means). Using means is based upon the logic that life span is inversely related to mortality and that mortality rates ultimately determine life spans. Harmonic means enabled us to also incorporate infinite life spans, which emerged if there was no mortality at all in some transitions. In this case, the infinite value was replaced by maximum value obtained from any other matrices in our dataset for that species (Ehrlén and Lehtilä, 2002). We are aware that this approach provides only a rough estimate of plant age and results are highly dependent on environmental stochasticity in the years of the study. However, it was shown that the value of mean life span obtained in this way corresponds to age measures by alternative methods for many species (Ehrlén and Lehtilä, 2002).

RESULTS

Single life-history traits

Logistic regression revealed a significant difference between species in mortality and stasis, but not in reproduction, growth, and retrogression. A similar pattern was found for differences between localities. The localities, however, also differed significantly in the probability of retrogression. Between habitats, there were no significant differences, probably because there was a strong interaction between species and habitat. Year had significant effect on all vital rates. Interaction between species and year was significant for differences in probability of reproduction and stasis. Effect of locality significantly interacted with species only for the probability of reproduction (see Table 3). There was significant interaction between species and habitat on retrogression, stasis, and mortality. Individuals of *A. adulterinum* had the same mortality in the forest and in the open landscape. Individuals of *A. cuneifolium* had a much higher mortality in the open landscape than in the forest (Fig. 1). Both species had a lower probability of stasis in the open landscape, but the effect of habitat was stronger in *A. cuneifolium*. The probability of retrogression was lower in the forest in both species.

Population growth and risk of extinction

Stochastic population growth rate (calculated using a transition matrix model, stochastic simulation) of each population over three transition intervals was significantly higher than 1 in all cases (Table 4a), indicating that all populations were growing. Populations of the two species significantly differed (p=0.037, permutation test) in mean stochastic growth rate, with a higher value for *A. cuneifolium* (1.067) than for *A. adulterinum* (1.046). Stochastic growth rate of both species varied significantly between years (Table 4b), with the lowest value for both species in the second transition interval (2005-2006). The highest growth rate was observed for *A. adulterinum* in the third transition interval (2006-2007) and for *A. cuneifolium* in the first transition interval (2004-2005). Stochastic growth rates did not significantly differ among individual populations and habitats (forest/open landscape) within species (Table 4). Despite this, survival probabilities of small populations of *A. cuneifolium* in open landscape have a likelihood of extinction twice as high as small populations in the forest. A forest population of 10 large individuals at stage 4 (which corresponds to the smallest population in the region) had a 12% probability of going extinct in 20 years and a 22% probability in 50 (in open landscape it was 22% and 40%, respectively). In *A. adulterinum*, extinction probabilities of a population of 10 individuals were more similar among habitat. In open landscape it was 24% in 20 years and 39% in 50 years, and in forest 25% and 42 %, respectively. Populations of 50 individuals in both habitats had a zero extinction probability within 20 years and less than 5% in 50 years in both species.

Comparison of the predicted and real population growth during a 10 year period (between 1997 and 2006) for both investigated populations of *A. adulterinum* (Open 3; Open

1 and Forest 1 combined) showed that the real number of individuals in populations in 2006 fell into the 95% confidence interval of the number of individuals calculated by projection using population matrices for given populations (Table 5). It suggests that our model is well parameterized and it correctly predicted number of individuals in populations.

Stable stage distribution

Large plants prevail in stable stage distributions of all populations. Stable stage distribution was calculated using matrix model, stochastic simulation. In populations of *A. adulterinum*, there were 50-80% of individuals of stage 4, followed by stage 1 with 10-30% and stage 2 with 5-15%. There were very few individuals of stage 3 (3-13%). In *A. cuneifolium*, there were 50-65% of individuals of stage 4, around 19% of plants from stage 3, 6-19% of stage 2, and 7-17% of stage 1.

Current stage distribution corresponded to stable stage distribution in most populations and years. This means that the populations were stable and the dynamics described by the transition matrices had not changed for a long time. The only exception was the population of *A. adulterinum* on locality “Forest 2”. After the last year of monitoring, there were considerably more plants in stage 1 than were estimated from the calculated stable stage distribution (Appendix S3, see Supplemental Data with the online version of this article).

Importance of vital rates

Analysis of elasticity indicated that the most important transition (with highest elasticity) was the survival of the biggest adult plants (stage 4) in both species. All the other transitions had smaller elasticity values (Appendix S4, see Supplemental Data with the online version of this article). Importantly, transitions corresponding to reproduction had very small elasticity. This means that stasis of the biggest plants was much more important for population dynamics than generative reproduction and stasis of smaller plants. Summed elasticity of stasis is much higher than summed elasticity of growth. The smallest summed elasticity corresponded to reproduction (Fig. 2). This pattern was similar for both species in both habitats.

When comparing species, LTRE analysis revealed that the dynamics of *A. adulterinum* was significantly more driven by stasis and that of *A. cuneifolium* by growth. There was no difference in the importance of reproduction (Table 6). Within species, there were significant differences between habitats, and a common pattern amongst habitats emerged. Dynamics of populations in the forest were significantly more driven by survival and for populations in open habitats by growth (for *A. cuneifolium* marginally significant). When decomposed into single matrix elements (Appendix S5, see Supplemental Data with the online version of this article), the population growth rate of the population of *A. cuneifolium* in the forest was significantly more driven by stasis of plants (stage 2, 3 and 4), by growth from stage 1 to stage 2, and by reproduction of stage 3. The population growth rate of populations in open landscape was driven significantly more by growth of plants from stage 3 to stage 4. In *A. adulterinum*, the population growth rate of populations in the forest was significantly more driven by stasis of plants in stage 2 and 4, and in an open landscape by growth of stage1 to stage 3 and stage 2 to stage 4.

Importance of reproduction

The sensitivity of population dynamics of all populations to changes in reproduction is very low (for details on calculation see methods, chapter “Establishment of new plants”). In most of the populations, a 30 % change in reproduction results in a maximum change in λ of 2.6% (Fig. 3). Total number of adult individuals of both species in Slavkovský les changed by a maximum of 30% in the last 10 years (P. Tájek, unpubl. data; Tájek, 2003; Tájek et al., 2006). Thus, assuming that newly established sporophytes are offspring of adult fertile plants from the previous year will not lead to large inaccuracy of estimating parameters derived from the models.

Mean life span

Mean life span for *A. adulterinum* was 34 years (life span of the populations varied from 26.4 to 56.4), and for *A. cuneifolium* it was 43.9 years (life span of the populations varied from 24.8 to 67.6). For detail on calculation, see methods, chapter “Mean life span”.

DISCUSSION

This study is the first to collect detailed data on the whole life cycle in ferns. No previous study has attempted to perform population viability analysis using transition matrix models of fern populations. This was probably because of the complicated life cycle of ferns (alternating between diploid and haploid phases). We showed that transition matrix models are applicable and useful in ferns, despite some complications. The model allowed us to obtain important information on life cycle and population dynamics of the species, which is of crucial importance in their effective conservation.

We revealed that populations of *Asplenium adulterinum* and *A. cuneifolium* in Slavkovský les, Czech Republic, have stable population dynamics and they are growing. Even very small populations containing only 10 individuals have a reasonable (over 55 %) probability of surviving over the next 50 years. The stability of these populations corresponds to results of other studies indicating that small populations of rock ferns are very stable over time (Holderegger and Schneller, 1994). Such high stability of populations is also common for long-lived species (e.g., Morris and Doak, 1998; García, 2003; Forbis and Doak, 2004; García, 2008), and risk of extinction of small populations is negatively correlated with increasing life span (Pimm et al., 1988). *A. adulterinum* and *A. cuneifolium* clearly belong among long lived species with an estimated life span of at least several decades.

It may be argued that demographic analyses are not sensible in such a long-lived species. However, extinction of populations of long-lived species may be very slow and difficult to detect and demographic analysis is the only option for identifying a possible disequilibrium. In the case of populations in this study, a remarkable similarity of current stage distribution and predicted stable stage distribution suggests that the demographic rates recorded over 3 years are not significantly different from those of the past decades (Doak and Morris, 1999) and that populations are in equilibrium. This shows that populations of both species are not endangered within the region.

Extrapolation of results from studied populations on the whole region is influenced by selection of studied populations. We selected populations with a sufficient number of individuals. As a result, we did not conduct the study in small populations which are sometimes in suboptimal habitats, or even dying out due to habitat changes. However, most of individuals within the study region grow on localities with optimal habitat (Tájek, 2003; Tájek et al., 2006) - in protected areas, which are often very large. Thus, our study describes population dynamics of most individuals of *Asplenium adulterinum* and *A. cuneifolium* in study region and conclusion can be drawn on regional scale.

A. adulterinum and *A. cuneifolium* are long lived species (mean life span is 34 and 44 years, respectively). The estimated life span is an average of all mean life spans over all localities and transition intervals. The variations in projected life span based on single matrices are huge, suggesting that the estimates are very sensitive to environmental stochasticity. The calculated mean life spans in both species correspond to ages observed in the field – around 30 years in *A. adulterinum* and between 40 to 50 years in *A. cuneifolium* (Steffan Jessen, personal communication). Holderegger and Schneller (1994) also noted that individuals of *Asplenium septentrionale* could exceed 30 years. When compared to the longevity with other perennial plants, *A. adulterinum* and *A. cuneifolium* reach a higher age than is the average — 22 years - for non-woody perennials (Ehrlén and Lehtilä, 2002). The long life of individuals is typical for plants growing in harsh habitats such as rock crevices or thin soil (Morris and Doak, 1998; García, 2003; Forbis and Doak, 2004; García, 2008) and *A. adulterinum* and *A. cuneifolium* are no exceptions.

The importance of single vital rates is related to species longevity. In long-lived species, stasis has the greatest importance for population dynamics, whereas fecundity and growth have only low importance (Silvertown et al., 1993; García et al., 2008). Our results agree with this finding: most important is stasis, followed by growth. The importance of fecundity is very small.

Quality of data

The quality of the data on fecundity is a clear weakness of our study. We assessed fecundity as a function of the average number of fertile leaves on plants of each stage and number of sporelings emerging in the area of the study per year. This approach is not new and has been used in plants growing in harsh habitats, such as bare rocks, because of difficulty of finding seedlings (e.g., Dinnétz and Nilsson, 2002; Forbis and Doak, 2004). This approach is based on many assumptions and can contain errors. It was suggested that elasticity of fecundity is low, and thus, the importance of this transition for population dynamics is also low. The elasticity is, however, calculated from the matrices that are potentially incorrect, thus this justification is tautological. To deal with this tautology, we tested directly the effect of change in fecundity on population dynamics and showed that population growth rate barely changes with considerable change in reproduction. In this way we demonstrated that the conclusions based on low elasticity of reproduction are valid.

Moreover, we tested the predictive power of our models using real data on population size over 10 years. To our knowledge, no other study has used real data for testing the predictive power of matrix transition models. The results of this prediction showed that the matrix model can predict population growth over 10 years relatively well. This suggests that our model is insensitive to the possible inaccuracies in estimating fecundity. It corresponds to findings of Forbis and Doak (2004) that in populations of plants with long life span, fecundity has low importance.

Relating newly emerged small plants to diaspore production in previous year may be influenced by density of suitable habitats – rock crevices in this study. Not all spores fall into suitable habitats and establishment of new plants may be dependent on density of rocks. Our localities differ slightly in rock density, but there is no difference in reproduction between localities, suggesting that density of rocks or other characters of each locality (e.g., the size of surrounding population of the same species) has no significant influence on the probability of establishing new plants. Because we follow newly established sporelings at the localities, our transition matrices already include the possible losses of spores due to their long distance dispersal. In this way, the estimated growth rates are the real population growth rates at the localities taking long distance dispersal into account. In contrast to this, studies using sowing experiments to study generative reproduction (e.g., Lennartsson and Oostermeijer, 2001), assume that all diaspores fall within the locality or that diaspore emigration equals to diaspore immigration, which is likely not true in small isolated populations.

Another possible problem with our data is definition of stages. We deal only with sporophytes and some of the stages in one species are functionally similar (e.g., at least two stages produce spores). We define stages as size categories, which is one of the basic options of stage definitions (Caswell, 2001). Size categories are of high informative value when working with one species, e.g., in comparison of stable stage distribution with the real one.

When dealing with more than one species, it is not possible to compare stages between species (e.g. which transition has greater elasticity) because the same stage can have a different position in life cycle of different species. We can, however, still compare probabilities or elasticities of major life history transitions – growth, survival, fecundity. Thus, stages defined as size categories are useful not only when dealing with one species, but even in species comparisons.

Comparison of species

Although the dynamics of the two species is quite similar and populations of both species are increasing in number of individuals, there are some important differences between the two species in details of life cycle, resulting in different sensitivity of the species to environmental and demographic stochasticity. Stasis has the strongest impact on population dynamics of *A. adulterinum*, while growth transitions have the strongest impact on population dynamics of *A. cuneifolium*. Single individuals of *A. cuneifolium* have higher mortality than individuals of *A. adulterinum.* On the other hand, individuals of *A. adulterinum* have higher probability of stasis. However, in spite of the differences in the structure of the life cycle between the two species, there are no significant differences in overall dynamics between the two species.

Distributions of the species are remarkably different within Slavkovský les. *Asplenium adulterinum* is present in both open habitats and forests, *A. cuneifolium* is very rare in open landscapes. Given this, it is surprising that there is no difference in growth rate in *A. cuneifolium* between habitats. In fact, many studies have shown strong differences in population dynamics of a single species in different habitats (e.g., Ehrlén et al., 2005; Ghimire et al., 2008). The different number of populations of *A. cuneifolium* in open landscape and forest can be explained by differences in single vital rates rather than by difference in long-term population growth rate (λ). It leads to higher effects of demographic and environmental stochasticity on population growth rate in the open landscape. Specifically, individuals of *A. cuneifolium* in the open landscape have higher individual mortality than *A. cuneifolium* in a forest and *A. adulterinum* in both habitats, resulting in higher extinction probabilities of small populations due to environmental and demographic stochasticity.

Different extinction probabilities of *A. adulterinum* and *A. cuneifolium* in the open landscape and in the forest may explain the different distribution of both species in the open landscapes. In A. *adulterinum*, extinction probabilities of small populations are about the same in the forest and in the open landscape and the species occur in both habitats with equal frequency. On the other hand, small populations of *A. cuneifolium* have a higher probability of extinction in the open landscape and as a result, this species has very few populations in the open landscape in comparison with the forest. It is interesting to compare extinction probabilities between species. Small populations of *A. cuneifolium* in the forest have a probability of extinction that is half that of *A. adulterinum* and *A. cuneifolium* in the open landscape. Together with distribution in the open landscape it suggests that *A. cuneifolium* is able to maintain its populations when the probability of extinction is low, i.e., in the forest. When assuming that these species form metapopulations, this would suggest lower colonization rates in *A. cuneifolium* than in *A. adulterinum*. This explanation is supported by the fact that colonization for tetraploids (*A. adulterinum* is allotetraploid; Hejný and Slavík, 1988) should be easier, since polyploidization increases the success of self-fertilization of gametophytes and thus single spore colonizations are easier (Masuyama and Watano, 1990; Vogel et al., 1999; Suter et al., 2000; Trewick et al., 2002). Such single spore colonization is much more difficult in diploid species (*A. cuneifolium* is diploid; Hejný and Slavík, 1988). The allotetraploid plants should have higher colonization ability also because polyploids posses much higher heterozygosity and species with higher heterozygosity have higher probability of establishment in harsh conditions (e.g., Lesica and Allendorf, 1992; Kahmen and Poschlod, 2000).

Existence of metapopulation dynamics in the study species was suggested previously by Tájek (2003), based on analysis of the distribution of the species and the identification of suitable but unoccupied localities in the landscape. This study also suggested higher colonization probability in *A. adulterinum* than in *A. cuneifolium*. Additionally, an establishment of one population on previously unoccupied but a suitable locality was documented in *A. cuneifolium*, as well as extinction of 2 populations of *A. cuneifolium* and 1 population of *A. adulterinum*, (P. Tájek unpubl. data; Tájek, 2003; Tájek et al., 2006); extinct populations contained only 1 individual in 1998. Moreover, our unpublished data on genetic diversity of populations of *A. adulterinum* also suggest high genetic similarity between populations that is likely to be caused by frequent spore dispersal between sites. However, further study aimed directly on metapopulation dynamic is needed to confirm the details and differences of metapopulation dynamics of both species.

Only a single population of *A. cuneifolium* was studied in open habitat and 3 populations in the forest. This population is the only one in the open habitat in Slavkovský les that contains more than 10 individuals and thus provided a unique opportunity to study the dynamics of *A. cuneifolium* in an open habitat. We suspect that this population may be a remnant, since this locality used to be in a pine forest and the trees were cleared from the locality 10 years ago (in 1998). Our study did not confirm the expectation that this population is declining and may have an especially low reproduction as would be expected for a remnant population. The population seems to be stable and even increasing. It is large enough not to suffer from negative influences of environmental stochasticity and we identified no threats to its existence.

Implications for species conservation

Populations of *A. adulterinum* and *A. cuneifolium* in Slavkovský les are growing, and the population structure is in a stable state. Many of the populations are quite small due to the small size of the habitat – serpentine rocks. Species restricted to a highly specialized habitat occur naturally in small isolated populations and the populations are able to survive in that state for a very long time (García, 2008). The most important vital rate in both species and all localities is survival of large individuals. Thus, to preserve these species for the future, it is necessary to maintain the current conditions of the habitats. In Slavkovský les, the planting of spruce trees has a major impact. Spruce shades smaller serpentine rocks and its litter fills small rock crevices, which become unsuitable for rock ferns. Thus, the planting of spruce should be avoided at localities with *A. adulterinum* and *A. cuneifolium*.

The smallest populations of both species have a high probability of extinction. However, metapopulation dynamics probably exist within the landscape — some populations go extinct while new one are established on suitable habitats. Thus, even serpentine rocks without presence of *A. adulterinum* or *A. cuneifolium* should not be destroyed or damaged (especially avoid planting spruce trees), because these rocks can be a future home for one or both of the fern species.

There is no need for establishing any preservation program or special treatment. According to our experience, a similar situation is at most of the localities of both species in the Czech Republic. Despite this, both species are considered to be endangered. This is because most red lists do not distinguish between species with declining populations (endangered) and naturally rare species (e.g., Holub and Procházka, 2000). Criteria distinguishing endangered and naturally rare species exists (e.g., IUCN, 2001, 2003), but are rarely used for creating red lists and evaluating species vulnerability (e.g., Cheffings and Farrell, 2005), because of lack of data.

ONLINE SUPPLEMENTAL MATERIAL

Additional materials are available online: Population vectors for projection of real number of individuals over 10 years; stable stage of all populations compared with real stage distribution; elasticities of single life transitions; contribution of each transition to the effect of habitat (forest/open landscape) on population growth rate estimated using LTRE analysis and original transition matrices in for all populations of both species in all year.

LITERATURE CITED

AGURAIUJA, R., M. ZOBEL, K. ZOBEL AND M. MOORA. 2008. Conservation of the Endemic Fern Lineage *Diellia* (Aspleniaceae) on the Hawaiian Islands: Can Population Structure Indicate Regional Dynamics and Endangering Factors? *Folia Geobotanica* 43: 3–18.

ALVAREZ-BUYLLA, E. R. AND M. SLATKIN. 1994. Finding confidence limits on population-growth rates – 3 real examples revised. *Ecology* 75: 255–260.

ANONYMOUS 2008. Natura 2000 network. Website ec.europa.eu/environment/nature/natura2000. [accessed 05.09.2008]

ARACHON, C. F. AND E. PANGUA. 2004. Spore Viability Under Different Storage Conditions in Four Rupicolous *Asplenium* L. Taxa. *American Fern Journal* 94: 28–38.

BESSINGER, S. R. 2002. Population viability analysis past and present. *In* S. R. Bessinger [ed.], Population Viability Analysis, University of Chicago Press, Chicago, IL, USA.

CASWELL H. 2001. Matrix population models: construction, analysis, and interpretation. Sinauer Associates, Sunderland, Massachusetts, USA.

CHEFFINGS, C. M. AND L. FARRELL [eds.], T. D. DINES, R. A. JONES, S. J. LEACH, D. R. MCKEAN, D. A. PEARMAN, et al. 2005: The Vascular Plant Red Data List for Great Britain. Species Status 7: 1–116. Joint Nature Conservation Committee, Peterborough.

COCHRAN, M. E. AND S. ELLNER. 1992. Simple Methods for Calculating Age-Based Life History Parameters for Stage-Structured Populations. *Ecological Monographs* 62: 345–364.

DASSLER, C. L. AND D. R. FARRAR. 2001. Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. *Brittonia* 53 (2): 352–369.

DE KROON H., J. VAN GROENENDAEL AND J. EHRLÉN. 2000. Elasticities: a review of methods and model limitations. *Ecology* 81: 607–618.

DINNÉTZ P. AND T. NILSSON. 2002. Population viability analysis of *Saxifraga cotyledon*, a perennial plant with semelparous rosettes. *Plant Ecology* 159: 61–71.

DOAK D. F. AND W. MORRIS. 1999. Detecting population-level consequences of ongoing environmental change without long-term monitoring. *Ecology* 80(5): 1537–1551.

DYER A. F. AND S. LINSDAY. 1992. Soil spore bank of temperate ferns. *American Fern Journal* 82: 89–123.

EFRON B. AND R. J. TIBSHIRANI. 1994. Introduction to the bootstrap.Chapman and Hall, New York, USA.

EHRLÉN J. AND K. LEHTILÄ. 2002. How perennial are perennial plants? *Oikos* 98: 308–322.

EHRLÉN J., K. SYRJANEN, R. LEIMU, M. B. GARCÍA AND K. LEHTILA. 2005. Land use and population growth of *Primula veris*: an experimental demographic approach. *Journal of Applield Ecology* 42(2): 317–326.

FARRAR D. R. 1967. Gametophytes of Four Tropical Fern Genera Reproducing Independently of Their Sporophytes in the Southern Appalachians. *Science* 155 (3767): 1266–1267.

FORBIS T.A. AND D. F. DOAK. 2004. Seedling establishment and life history trade-offs in alpine plants. *American Journal of Botany* 91(7): 1147–1153.

GARCÍA M. B. 2008. Life history and population size variability in a relict plant. Different routes towards long-term persistence. *Diversity and Distribution* 14: 106–113.

GARCÍA M.B. 2003. Demographic Viability of a Relict Population of the critically Endangered Plant *Borderea chouardii*. *Conservation Biology* 17: 1672–1680.

GARCÍA M.B., F. X. PICÓ AND J. EHRLÉN. 2008. Life span correlates with population dynamics in perennial herbaceous plants. *American Journal of Botany* 95: 258–262.

GHIMIRE S.K., O. GIMENEZ, R. PRADEL, D. MCKEY AND Y. AUMEERUDDY-THOMAS. 2008. Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb *Nardostachys grandiflora*: matrix modeling of harvesting effects in two contrasting habitats. *Journal of Applied Ecology* 45: 41–51.

GILPIN M. E. AND M. E. SOULÉ. 1986. Minimum viable populations: processes of species extinction. *In* SOULÉ M.E. [ed.]: Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts, USA.

GRIFFITH A. B. AND I. N. FORSETH. 2005. Population matrix models of *Aeschynomene virginica*, a rare annual plant: Implications for conservation. *Ecological Applications* 15(1): 222–233.

HEJNÝ S. AND H. SLAVÍK. 1988. Flora of Czech Republic (Květena České republiky). Academia, Praha, Czech Republic. (in Czech).

HOCK Z., P. SZÖVÉNYI AND Z. TÓTHA. 2006. Seasonal variation in the spore bank of ferns in grasslands on dolomite rock, *Plant Ecology* 187: 289–296.

HOLDEREGGER R. AND J. J. SCHNELLER. 1994. Are small isolated populations of *Asplenium septentrionale* variable? *Biological Journal of Linnean Society* 51: 377–385.

HOLUB J. AND F. PROCHÁZKA. 2000. Red list of vascular plants of Czech republic (Červený seznam cévnatých rostlin České republiky). *Preslia*, 72: 187–230 (in Czech).

IUCN. 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

IUCN. 2003. Guidelines for Using the IUCN Red List Categories and Criteria. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

JONGEJANS E., N. DE VERE AND H. DE KROON. 2008. Demographic vulnerability of the clonal and endangered meadow thistle. *Plant Ecology* 198: 225–240.

KAHMEN S. AND P. POSCHLOD. 2000. Population size, plant performance, and genetic variation in the rare plant *Arnica montana* L. in the Rhön, Germany. *Basic and Applied Ecology* 1: 43–51.

KELLY D. (1994): Demography and conservation of *Botrychium australe*, a peculiar, sparse mycorrhizal fern. *New Zealand Journal of Botany* 32 (4): 393–400.

KUEHN D. M. C. AND D. J. LEOPOLD. 1992. Long-term demography of *Phyllitis scolopendrium* (L.) Newm var. *americana* Fern. in central New-York. *Bulletin of Torrey Botanical Club* 119 (1): 65–76.

LENNARTSSON T. AND J. G. B. OOSTERMEIJER. 2001. Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. *Journal of Ecology* 89 (3): 451–463.

LESICA, P. AND F. W. ALLENDORF. 1992. Are small populations of plants worth conserving? *Conservation Biology* 6:135–139.

LESLIE P. H. 1945. On the use of matrices in certain population mathematics. Biometrica 33: 183–121.

LINDSAY S. AND A. F. DYER.1996. Investigating the phenology of gametophyte development: an experimental approach. *In* J. M. CAMUS, M. GIBBY AND R. J. JOHNS [eds.]: Pteridology in Perspective: 633–650. Royal Botanic Gardens Kew, UK.

LLOYD R. M. AND E. J. KLEKOWSKI. 1970. Spore germination and viability in Pteridophyta: Evolutionary significance of chlorophyllous spores. *Biotropica* 2: 129–137.

LOVIS J. D. (1955): *Asplenium adulterinum* and its probable parents. Proc. Bot. Soc. Br. Isl. 1: 389–390.

MASUYAMA S. AND Y. WATANO. 1990. Trends for Inbreeding in Polyploid Pteridophytes. Plant Species Biology 5 (1): 13–17.

MENGES E. S. 1990. Population viability analysis for an endangered plant. *Conservation Biology* 4: 52–62.

MORRIS W. F. AND D. F. DOAK. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. *American Journal of Botany* 85 (6): 784–793.

MÜNZBERGOVÁ Z. 2005. Determinants of species rarity: population growth rates of species sharing the same habitat. *American Journal of Botany* 92: 1987–1994.

MÜNZBERGOVÁ Z. 2006. Effect of population size on prospect of species survival. *Folia Geobotanica* 41: 137–150.

MÜNZBERGOVÁ Z. 2007. Population Dynamics of Diploid and Hexaploid Populations of a Perennial Herb. *Annals of Botany* 100: 1259–1270.

MÜNZBERGOVÁ Z. AND J. EHRLÉN. 2005. How best to collect demographic data for population viability analysis models. *Journal of Applied Ecology* 42: 1115–1120.

OGLIVIE R.T. AND A. CESKA.1984. Alpine plants of phytogeographic interest on northwestern Vancouver Island. *Canadian Jornal of Botany* 62: 2356-2362.

ØKLAND R. H. 1995. Population biology of the clonal moss *Hylocomnium splendens* in Norwegian boreal spruce forest. I. Demography. Journal of Ecology 83: 697–712.

PANGUA E., S. LINDSAY AND A. DYER. 1994. Spore germination and gametophyte development in three species of *Asplenium*. *Annals of Botany* 73: 578–593.

PAYNE, R.W., S. A. HARDING, D. A. MURRAY, D. M. SOUTAR, D. B. BAIRD, S. J. WELHAM, A. F. KANE et al. 2005. The Guide to Genstat Release 8. Part 2: Statistics. VSN International, Oxford.

PECK J. H., C. J. PECK AND D. R. FARRAR. 1990. Influences of life history attributes on formation of local and distant fern populations. *American Fern Journal* 80: 126–142.

PIMM S. L., H. L. JONES AND J. DIAMOND. 1988. On the risk of extinction. *American Naturalist* 132: 757–785.

PINO J., F. X. PICO AND E. DE ROA. 2007. Population dynamics of the rare plant *Kosteletzkya pentacarpos* (Malvaceae): a nine-year study. *Botanical Journal of Linnean Society* 153: 455–462.

RÜNK K., M. MOORA AND M. ZOBEL. 2006. Population stage structure of three congeneric *Dryopteris* species in Estonia. *Proceedings of the Estonian Academy of Sciences*. 55: 15–30.

SCHAFFER M. L. (1990): Population viability analysis. *Conservation biology* 4: 39–40.

SCHEMSKE W. D., B. C. HUSBAND, M. H. RUCKELSHAUS, C. G. GOODWILLIE, I. M. PARKER AND J. G. BISHOP. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75: 584–606.

SCHMITT J. L. AND P. G. WINDISCH. 2006. Growth Rates and Age Estimates of *Alsophila setosa* Kaulf. in Southern Brazil. *American Fern Journal* 96: 103–111.

SCHNELLER J. J. 1988. Spore bank, dark germination and gender determination in *Athyrium* and *Dryopteris*. Results and implications for population biology of Pteridophyta. *Botanica Helvetica* 98: 77–86.

SHARPE J. M. 1993. Plant Growth and demography of the neotropical herbaceous fern *Danaea wendlandii* (Marattiaceae) in Costa Rican rain forest. *Biotropica* 25: 85–94.

SHORINA N.I. (2001): Population Biology of Gametophytes in Homosporous Polypodiophyta. *Russian Journal of Ecology*, 3: 182–187.

SILVERTOWN J., M. FRANCO, I. PISANTY AND A. MENDOZA. 1993. Comparative plant demography – relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology* 81: 465–476.

SUTER M., J. J. SCHNELLER AND J. C. VOGEL. 2000. Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp. *quadrivalens*. *International Journal of Plant Sciences* 161: 233–244.

TÁJEK P. 2003. Serpentine outcrops in Slavkovský les in view of island biogeography. (Hadcové výchozy ve Slavkovském lese z pohledu ostrovní biogeografie). Diploma thesis, Charles University, Czech Republic (in Czech).

TÁJEK P., T. PECKERT, L. ONDRÁKOVÁ, E. SOUČKOVÁ, L. ČECH, A. BUCHAROVÁ AND J. SLADKÝ. 2006. Ladder Spleenwort (*Asplenium adulterinum*) – mapping tables (Sleziník nepravý (*Asplenium adulterinum*) – mapovací karty lokalit.). Ms., 140 pp. [Report from NATURA 2000 research, depon in: AOPK ČR, Praha] (in Czech).

TREWICK S. A., M. MORGAN-RICHARDS, S. J. RUSSELL, S. HENDERSON, F. J. RUMSEY, I. PINTER, J. A. BARRETT et al. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rock fern *Asplenium ceterach*: evidence from chloroplast DNA. *Molecular Ecology* 11: 2003–2012.

TUTIN T.G., V. H. HEYWOO, N. A. BURGES, D. H. VALENTINE, S. M. WALTERS AND D. A. WEBB [eds.]. 1980. Flora Europaea. Cambridge University Press, UK.

VOGEL J. C., F. J. RUMSEY, J. J. SCHNELLER, J. A. BARRET AND M. GIBBY. 1999. Where are the glacial refugia in Europe? Evidence from pteridophytes. *Biological Journal of the Linnean Society* 66: 23–37.

WATKINS J. E. JR., M. K. MACK AND S. S. MULKEY. 2007. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. *American Journal of Botany* 94 (4): 701–708.

Table 1: Criteria for categorization of plant individuals to size stages.

	Asplenium adulterinum	*Asplenium cuneifolium*
stage 1	The smallest plants with one developed leaf; the leaf shorter than 1.5cm	The smallest plants with one developed leaf, the leaf has, either onlyapical frond, or additionally maximum one more pair of fronds (branches, leaflets). It reminds a bit *Trifolium* leaves (apical leaflet plus pair of leaflets)
stage 2	The longest leaf is not longer than 3.5cm.	No leaf has except apical frond more than 2 pairs of fronds plus apical frond.
stage3	Maximum number of leafs is 5, at least one leaf is longer than 3.5cm.	No leaf has more than 4 pairs of fronds (except apical frond).
stage 4	The plant has at least 6 leafs longer than 3.5cm. If the leafs are longer than 7cm, sufficient number of leafs is 4 (adult plants growing in deep crevices with few long leafs).	At least one leaf has more than 4 pairs of fronds (except apical frond)

Table 2: Localities, where the study was carried out and description of the populations.

Code	**coordinates**	**Population size (total number of individuals)**	
		A. adulterinum	*A. cuneifolium*
Open 1	50°04'16" N; 12°47'08" E	Several hundreds	Several hundreds
Open 2	50°03'41" N; 12°43'54" E	~ 300	_________
Open 3	50°03'22" N; 12°44'36" E	~ 1800	_________
Forest 1	50°04'15" N; 12°47'00" E	Several hundreds	Several hundreds
Forest 2	50°01'43" N; 12°43'34" E	Several thousands	Several hundreds
Forest 3	50°03'15" N; 12°46'30" E	Several thousands	Several hundreds

Table 3: Effect of species, year, habitat, stage in previous year and locality nested within habitat and their interaction on probability of reproduction, growth, retrogression, stasis and mortality. Results of logistic regression. Stasis 95 d.f. total, $R^2 = 0.85$; Mortality 95 d.f. total, $R^2 = 0.84$; Reproduction 67 d.f. total, $R^2 = 0.90$; Growth 143 d.f. total, $R^2 = 0.27$; Retrogression 75 d.f. total, $R^2 = 0.47$.

		Stasis		Mortality			Reproduction		Growth			Retrogression	
		R2 = 0.85		R2 = 0.84			R2 = 0.90		R2 = 0.27			R2 = 0.47	
	df	F value	p	F value	p	df	F value	p	F value	p	df	F value	p
Stage previous year	3	117.461	**<0.001**	7.239	**<0.001**	2	187.717	**<0.001**	13.928	**<0.001**	2	2.063	0.135
Species	1	6.164	**0.015**	64.199	**<0.001**	1	0.241	0.610	2.458	0.119	1	1.209	0.274
Habitat	1	0.764	0.383	3.183	0.210	1	2.553	0.110	0.466	0.496	1	0.083	0.759
Locality nested in habitat	2	0.461	0.634	80.915	**<.001**	2	0.024	0.980	0.064	0.938	2	3.606	**0.033**
Year	2	20.047	**<0.001**	10.798	**<.001**	2	31.720	**<0.001**	5.276	**0.006**	2	11.005	**<0.001**
Species × year	2	3.379	**0.039**	2.639	0.078	2	6.961	**<0.001**	1.401	0.250	2	1.688	0.192
Species × stage previous year	3	8.412	**<0.001**	3.328	**0.024**	2	5.829	**0.010**	1.860	0.160	1	7.795	**0.007**
Species × habitat	1	11.934	**<0.001**	138.719	**<0.001**	1	2.023	0.160	0.302	0.583	1	6.128	**0.016**
Species × locality (habitat)	2	2.839	0.065	0.397	0.675	2	3.709	**0.030**	0.549	0.579	2	0.563	0.569

Table 4: Stochastic growth rate (calculated using matrix model) of each population over all transition intervals within populations (a) and within each transition interval over populations (b) of *A. adulterinum* and *A. cuneifolium*. Permutation test was performed to test differences between localities (a) and between transition intervals (b). Significant values are in bold.

a)	***Asplenium adulterinum***			***Asplenium cuneifolium***		
locality	Growth rate	Confidence interval	p	Growth rate	Confidence interval	p
Open 1	1.054	1.054 - 1.083	0.335	1.045	1.045 - 1.066	0.507
Open 2	1.050	1.044 - 1.083	0.673			
Open 3	1.038	1.032 - 1.074	0.756			
Forest 1	1.013	0.997 - 1.038	0.092	1.098	1.093 - 1.123	0.094
Forest 2	1.035	1.029 - 1.069	0.239	1.054	1.053 – 1.083	0.229
Forest 3	1.086	1.071 - 1.118	0.0630	1.067	1.057 - 1.087	0.929

b)

year	Growth rate	Confidence interval	p	Growth rate	Confidence interval	p
2004 - 2005	1.027	1.022 - 1.058	0.624	1.1101	1.108 - 1.131	**0.001**
2005 - 2006	1.000	0.996 - 1.023	**0.006**	1.029	1.026 – 1.043	**0.027**
2006 - 2007	1.104	1.083 - 1.113	**0.005**	1.056	1.053 - 1.087	0.961

Table 5: Real number of adult individuals (stage 2, 3 and 4) in 1997 and 2006 and number of adult individuals of *Asplenium adulterinum* predicted using projection matrices from given populations over 10 years, based on number of individuals from 1997.

	Real no. of individuals		**Predicted no of individuals in 2006 (95% confidence interval)**
	1997	*2006*	
Open 3	1198	1868	1148 - 2215
Open 1 + Forest 1	991	ca 1200	914 - 1290

Table 6: Contribution of each vital rate (growth, stasis and reproduction) to differences in growth rate between species, estimated using LTRE analysis. Positive values mean positive contribution of *A. adulterinum*, negative mean positive contribution of *A. cuneifolium*.

	contribution	**p-value**
Growth	-0.0175	0.002
Survival	0.0122	0.012
Reproduction	-0.0045	0.722

Fig. 1: Mortality, stasis in the same stage and retrogression for *A. adulterinum* and *A. cuneifolium* in open/forested habitat. The plotted values are residuals from logistic regression testing the effect of other main effects (Table 3). In graph, median, 25% and 75% quartiles are shown, whiskers indicate10% and 90 % percentiles, dots are outlying values.

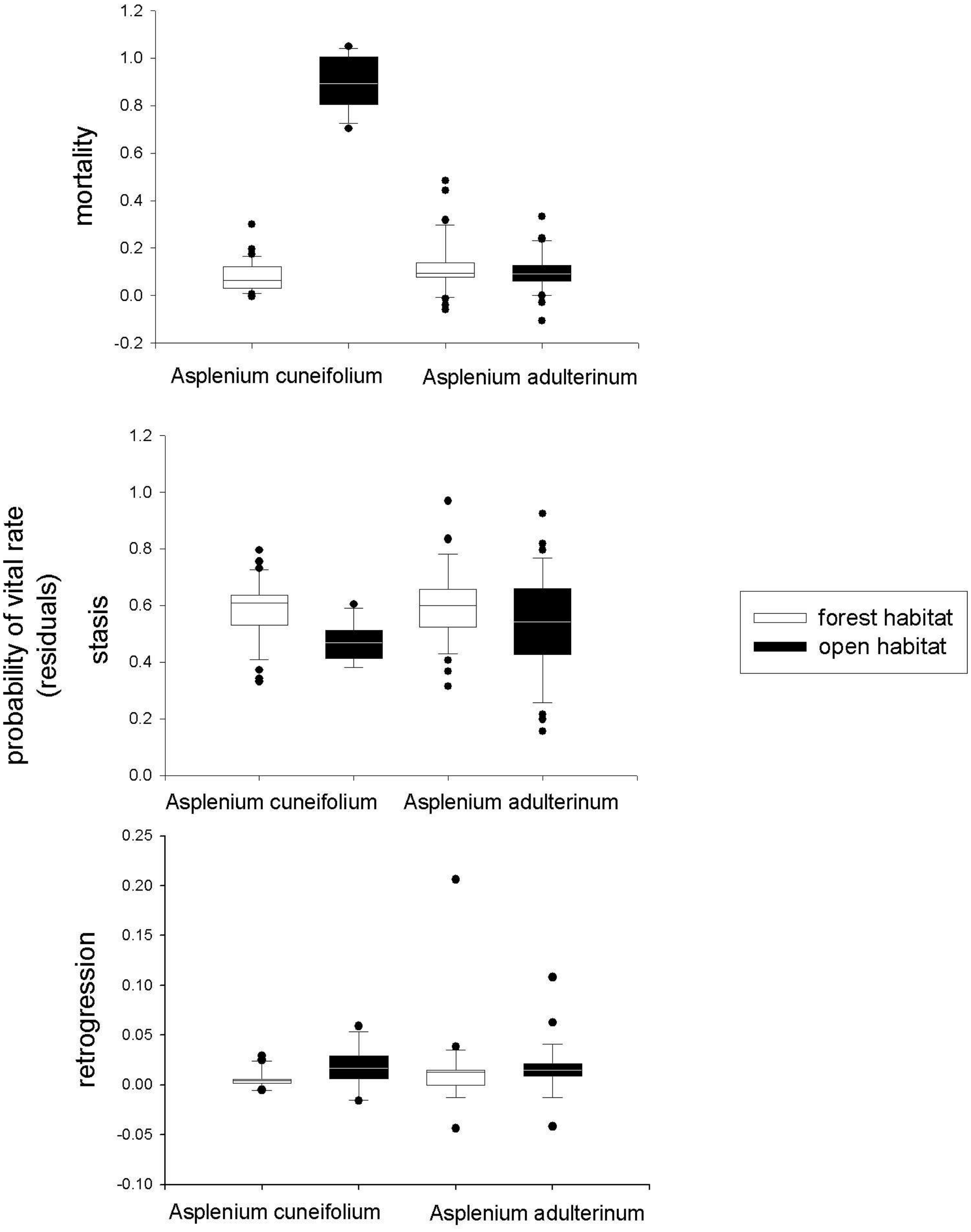


Fig. 2: Summed elasticities, calculated using matrix model, corresponding to vital rates – growth (G), fecundity (F) and stasis (L): a – *A. adulterinum*; b – *A. cuneifolium*. Plotted according Silvertown et al. (1993).

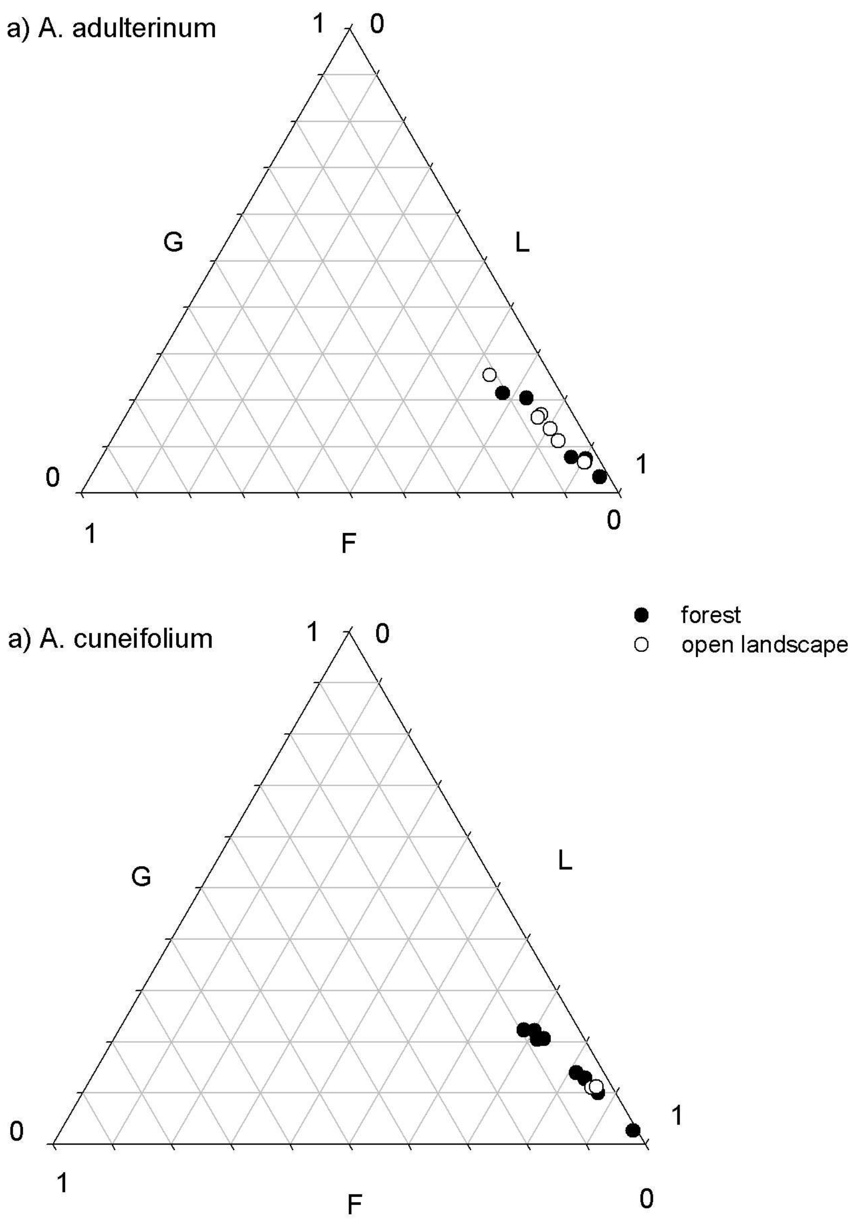
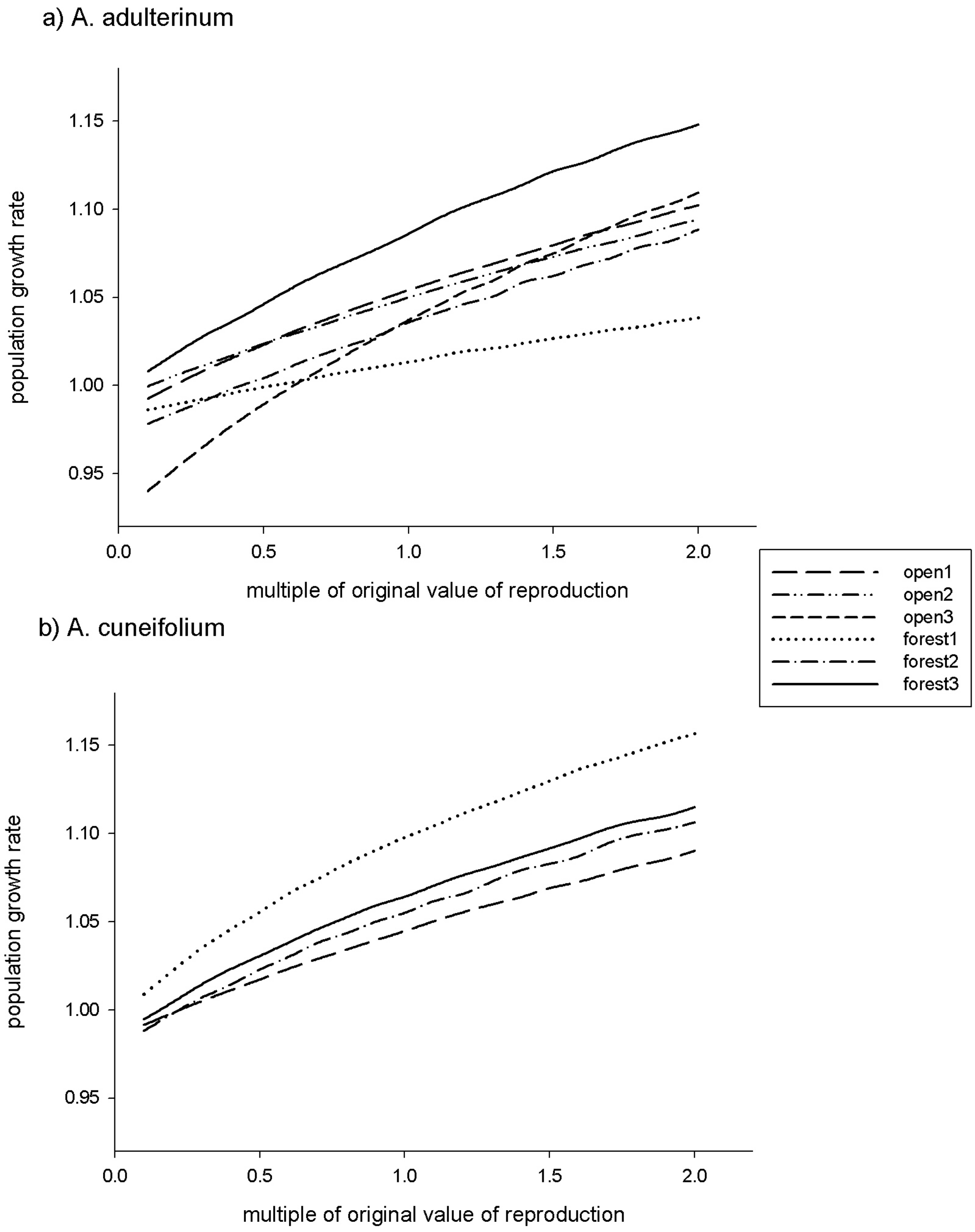


Fig.3: Assessment of inaccuracy of transition parent plant-newly established sporophyte. X axis represents proportional changes in transitions parent plant producing spore-newly established sporophyte (transitions a_{21}, a_{31} and a_{41} in *A. adulterinum* and a_{31} and a_{41} in *A. cuneifolium*) in range 0.05 to 2 multiple of original values for each matrix and calculated growth rate. Y axis represents population growth rate of that population.



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Appendix S1: Original transition matrices with growth rate and its confidence interval given by each matrix.

Asplenium adulterinum

Open 1	2004/2005				2006/2006				2006/2007			
	0.277778	0.008329	0.020841	0.116782	0.555556	0.005519	0.013811	0.077387	0.714286	0.018873	0.047227	0.26463
	0.5	0.45	0	0	0.259259	0.769231	0	0.008065	0.238095	0.76087	0	0.015038
	0	0.1	0.470588	0	0.037037	0.153846	0.769231	0.016129	0	0.043478	0.357143	0.015038
	0.055556	0.375	0.470588	0.969697	0	0	0.230769	0.975806	0	0.130435	0.642857	0.954887
	λ = 1.039, CI 1.032 - 1.086				**λ = 1.025, CI 1.010 - 1.045**				**λ = 1.076, CI 1.047 - 1.115**			
Open 2	2004/2005				2006/2006				2006/2007			
	0.333333	0.000174	0.006216	0.148716	0.285714	0.00013	0.004637	0.110945	0.608696	0.000158	0.005657	0.135353
	0	0	0	0	0.142857	0.25	0	0	0.347826	0.333333	0	0
	0.166667	0	0.25	0.007353	0.285714	0.25	0	0.005376	0.043478	0.5	0.454545	0.027027
	0	0.8	0.75	0.992647	0	0.25	1	0.973118	0	0.166667	0.545455	0.972973
	λ = 1.0336, CI 1 - 1.108				**λ = 1.033, CI 1.024 - 1.073**				**λ = 1.086, CI 1.066 - 1.092**			
Open 3	2004/2005				2006/2006				2006/2007			
	0.25	0.007972	0.018792	0.208187	0.222222	0.004326	0.010196	0.112957	0.458333	0.013678	0.032241	0.35718
	0.5625	0.212121	0	0	0.259259	0.454545	0.071429	0.006667	0.416667	0.827586	0.15	0
	0.0625	0.333333	0.347826	0.01626	0.074074	0.227273	0.25	0.013333	0	0.034483	0.35	0.040462
	0.0625	0.393939	0.478261	0.813008	0.037037	0.181818	0.571429	0.966667	0	0.103448	0.45	0.919075
	λ = 0.990, CI 0.955 - 1.051				**λ = 1.019, CI 1.019 - 1.057**				**λ = 1.078, CI 1.039 - 1.128**			
Forest 1	2004/2005				2006/2006				2006/2007			
	0.25	0.008641	0.020622	0.189617	0.741935	0.00182	0.004344	0.039939	0.444444	0.007093	0.016928	0.155651
	0.125	0.3	0	0	0.096774	0.8	0	0	0.111111	0.85	0	0
	0	0.2	0.235294	0	0	0.066667	0.833333	0.03	0	0	0.333333	0.037037
	0.125	0.5	0.705882	0.993506	0	0	0.083333	0.95	0	0.15	0.611111	0.944444
	λ = 1.050, CI 1 - 1.109				**λ = 0.972, CI 0.930 - 1.006**				**λ = 1.008, CI 0.978 - 1.033**			

Forest 2	2004/2005				2006/2006				2006/2007			
	0.090909	0.001044	0.014252	0.108532	0.578947	0.000354	0.004831	0.036787	0.4	0.003862	0.052715	0.40143
	0.4375	0.363636	0	0	0	0.545455	0.035714	0	0.6	0.818182	0	0
	0.125	0.181818	0.086957	0	0.111111	0.136364	0.178571	0.026667	0	0.181818	0.521739	0.008333
	0	0.130435	0.733333	1	0	0.136364	0.3	0.925	0	0	0.391304	0.966667
	λ = 1.0318, CI 1.022 - 1.066				**λ = 0.941, CI 0.906 - 0.987**				**λ = 1.116, CI 1.004 - 1.161**			
Forest 3	2004/2005				2006/2006				2006/2007			
	0.333333	0.000722	0.003415	0.028386	0.5	0.002124	0.010046	0.083498	0.307692	0.012701	0.060085	0.499399
	0.5	0.535714	0	0	0	0.888889	0	0	0.615385	0.692308	0.25	0
	0	0.035714	0.2	0	0	0	0.5	0.015748	0	0	0.5	0.007463
	0	0.392857	0.7	1	0	0.111111	0.5	0.984252	0	0.307692	0.25	0.985075
	λ = 1.018, CI 1.006 - 1.026				**λ = 1, CI 1-1**				**λ = 1.201, CI 1.141 - 1.252**			

Asplenium cuneifolium

Open 1	2004/2005				2006/2006				2006/2007			
	0.222222	0	0.001198	0.074528	0.238095	0	0.001085	0.067533	0.130435	0.028571	0.004199	0.261296
	0.444444	0.209302	0	0	0.47619	0.37931	0.02381	0	0.608696	0.314286	0	0
	0.111111	0.534884	0.245283	0.020202	0.190476	0.37931	0.595238	0.07483	0	0.514286	0.462963	0.040541
	0	0.116279	0.735849	0.959596	0	0.068966	0.261905	0.918367	0	0	0.481481	0.952703
	λ = 1.021, CI 1.009 - 1.049				**λ = 1.002, CI 0.986 - 1.034**				**λ = 1.076, CI 1.062 - 1.112**			
Forest 1	2004/2005				2006/2006				2006/2007			
	0	0	0.00199	0.233464	0.318182	0	0.001958	0.229727	0.111111	0	0.003075	0.360828
	0.846154	0.307692	0	0	0.5	0.611111	0	0	0.777778	0.590909	0	0
	0	0.615385	0.615385	0	0	0.166667	0.461538	0.027027	0.027778	0.363636	0.7	0.016807
	0	0	0.384615	0.989899	0	0	0.461538	0.963964	0	0.045455	0.3	0.983193
	λ = 1.101, CI 1.080 - 1.128				**λ = 1.036, CI 1.015 - 1.092**				**λ = 1.144, CI 1.106 - 1.164**			
Forest 2	2004/2005				2006/2006				2006/2007			
	0.263158	0	0.002703	0.382757	0.454545	0	0.00027	0.038281	0.384615	0	0.001683	0.238347
	0.315789	0.473684	0	0	0.121212	0.454545	0	0	0.461538	0.583333	0	0
	0.315789	0.421053	0.52	0	0.060606	0.454545	0.535714	0.011236	0	0.25	0.761905	0.033708
	0	0.052632	0.48	1	0	0	0.285714	0.966292	0	0	0.142857	0.932584
	λ = 1.169, CI 1.166 - 1.207				**λ = 0.981, CI 0.980 - 1.015**				**λ = 1.011, CI 0.965 - 1.083**			
Forest 3	2004/2005				2006/2006				2006/2007			
	0.423077	0	0.007474	0.247496	0.148148	0	0.003664	0.121349	0.5	0	0.004867	0.16119
	0.384615	0.125	0	0	0.592593	0.538462	0	0	0.272727	0.722222	0.022727	0
	0.115385	0.75	0.416667	0	0.148148	0.423077	0.677419	0.015873	0.045455	0.111111	0.681818	0.029412
	0.038462	0	0.583333	1	0	0	0.322581	0.984127	0	0	0.227273	0.921569
	λ = 1.130, CI 1.106 - 1.151				**λ = 1.066, CI 1.052 - 1.077**				**λ = 0.986, CI 0.958 - 1.044**			

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Appendix S2: Population vectors for projection of real number of individuals in population

Forest 1 + Open together and Open 3:

population	Stage 1	Stage 2	Stage 3	Stage 4
Forest 1 + Open 1	106	142	95	754
Open 3	129	172	115	912

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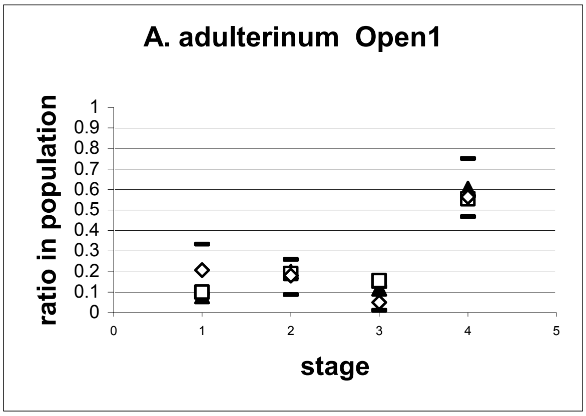
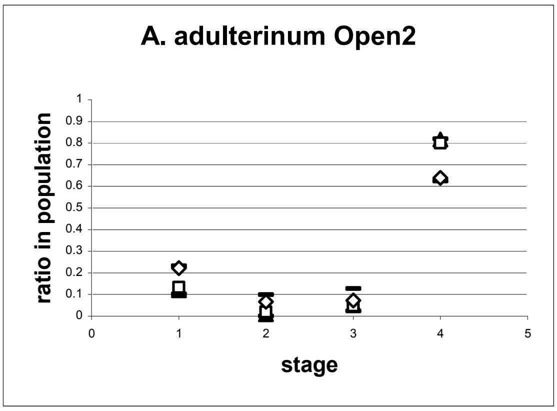
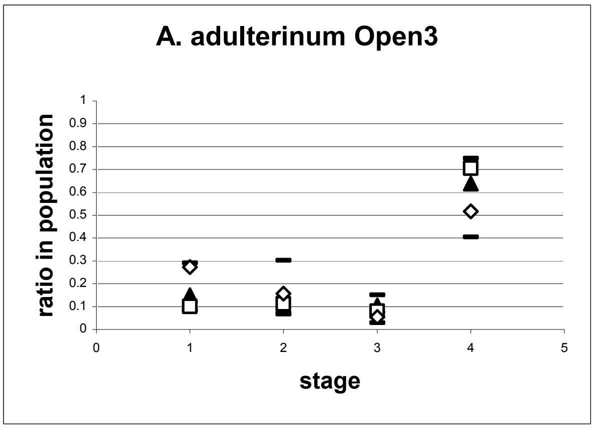
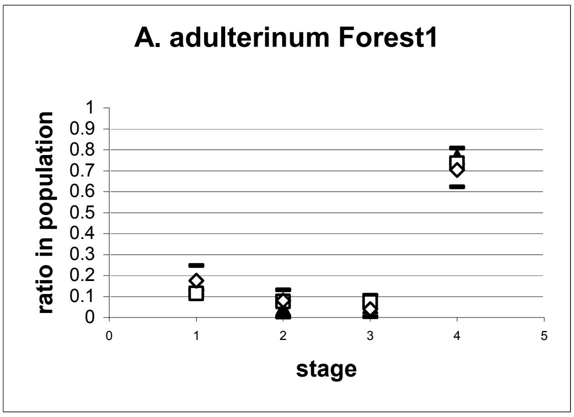
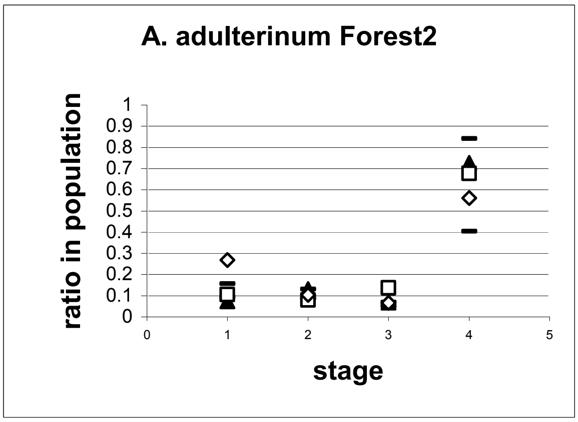
Appendix S3: Stable stage distribution of all populations of both species, in comparison with current stage distribution of populations in each year. Symbols: - range of confidence interval of calculated stable stage distribution, ▲ – year 2005; □ – year 2006; ◊ - year 2007

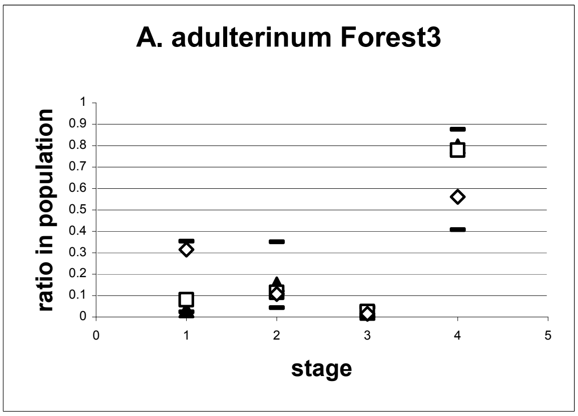


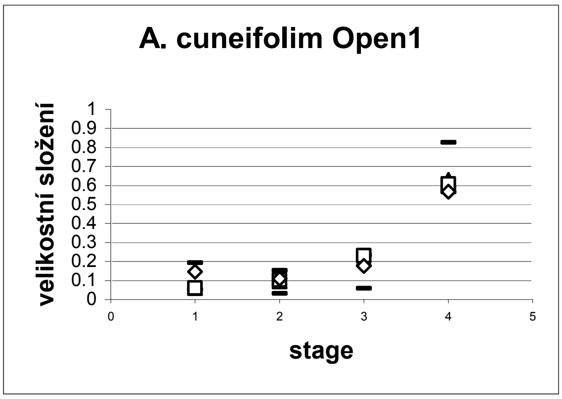


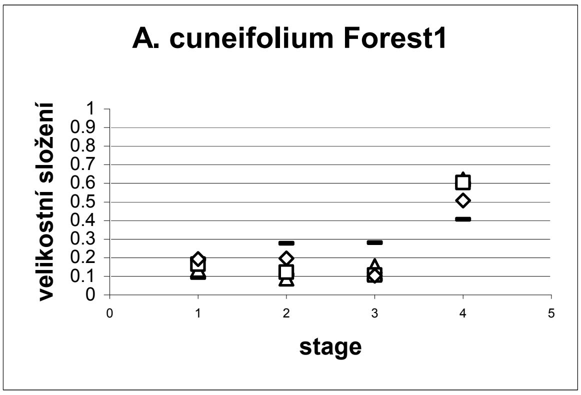


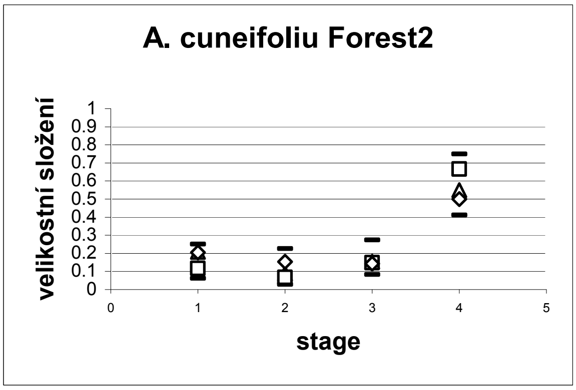


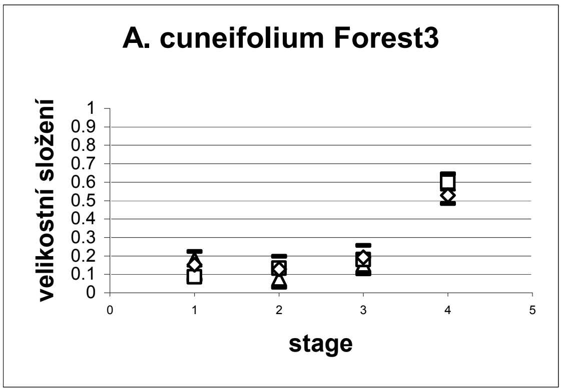





A. cuneifolim Open1
velikostní složení
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5
stage


A. cuneifolium Forest1
velikostní složení
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5
stage


A. cuneifoliu Forest2
velikostní složení
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5
stage


A. cuneifolium Forest3
velikostní složení
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5
stage

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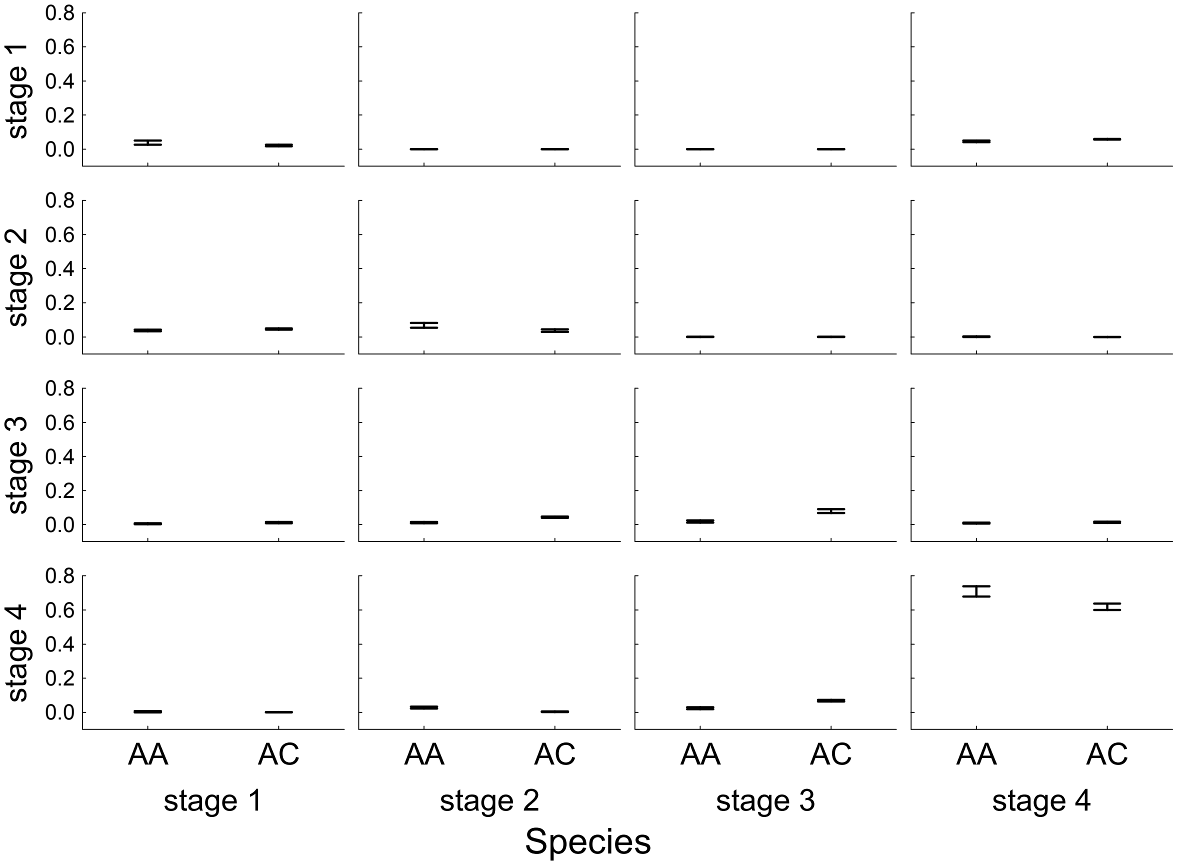
Appendix S4: Elasticity of single life cycle transitions for *Asplenium adulterinum* (AA) and *Asplenium cuneifolium* (AC). The values were obtained using stochastic simulations. The plots within figure are arranged in a form of a transition matrix.



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Appendix S5: The contribution of each transition to the effect of habitat (forest/open landscape) on population growth rate estimated using LTRE analysis. Positive values mean positive effect of forest habitat. The significance was determined using a permutation test and indicate significant difference between effect of forest/open habitat in the respective matrix element. Significant values (<0.05) are in bold. Table is arranged according transition matrix.

Asplenium adulterinum

	stage 1	*stage 2*	*stage 3*	*stage 4*
stage 1	-0.00028	-0.0000605	0.000036	0.000193
stage 2	-0.00182	**0.008579**	0.0000638	-0.00089
stage 3	**-0.00455**	-0.005	0.000372	-0.00061
stage 4	-0.00036	**-0.00486**	-0.0028	**0.009114**

Asplenium cuneifolium

	stage 1	*stage 2*	*stage 3*	*stage 4*
stage 1	-0.00187	0	**0.0000065**	0.021407
stage 2	**0.009048**	**0.008446**	-0.00038	0
stage 3	-0.00584	-0.00552	**0.010466**	-0.00861
stage 4	0	-0.00346	**-0.00934**	**0.012668**

Paper 2

LIMITATION OF DISTRIBUTION OF TWO RARE FERNS IN FRAGMENTED LANDSCAPE

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Accepted in Acta Oecologica

Limitation of distribution of two rare ferns in fragmented landscape

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Author contribution: P. Tájek collected data, A. Bucharová did statistical analysis and wrote the manuscript, Z. Münzbergová supervised the work and helped with manuscript preparation.

ABSTRACT

Species distribution in the landscape is limited either by 1. diaspore production, dispersal and establishment abilities or 2. by availability of suitable habitats; 3. or by a combination of both factors. The relative importance of these factors is species-dependent and has mainly been studied for seed plants. We studied the importance of habitat and dispersal limitation for distribution of two rare fern species, *Asplenium adulterinum* and *A. cuneifolium*, restricted to serpentine rocks, using analysis of their distribution on a regional scale (several kilometers). Within a model region, all 98 serpentine rocks were mapped. We used data on abiotic characteristics and on the presence of all vascular plant species on the rocks to predict which of the rocks were suitable for the two *Asplenium* species. Suitable habitats were positively defined mainly by the presence of appropriate microhabitats and the height of the highest rock, which represents the size of space with lowered concurrence. Other determinants of habitat suitability differed between species. Neither species occupied all suitable localities, indicating dispersal limitation. Locality isolation significantly affected one of the species but not the other. Overall, the results suggest that both fern species have suitable but unoccupied localities in the region and demonstrates that ferns, similar to seed plants, are limited by their dispersal ability in the landscape.

Keywords: unoccupied suitable habitat; dispersal limitation; isolation by distance; fern; polyploidy

1. INTRODUCTION

Species ability to colonize a site depends on both species ability to reach the site and ability to establish at the site. Thus, the species distribution on a regional scale can be limited either 1) by seed availability and dispersal ability of the seeds, 2) by the availability of suitable habitats or 3) by a combination of both factors (Münzbergová and Herben, 2005).

Many recent studies have explored the degree of different types of limitations for species in various systems and concluded that the majority of plant species is limited by both seed availability and/or dispersal ability and by the availability of suitable habitats (reviewed in Clark et al., 2007; Turnbull et al., 2000). Most of the existing studies carried out on plants, however, have dealt with seed-producing plants and considerably less is known about these types of limitations in ferns.

Compared to seed-producing plants, ferns are known for huge production of very small spores which are often considered as “omnipresent”. It was thus expected that ferns are neither spore nor dispersal limited (e.g., Guo et al., 2003; Tryon, 1986, 1970). However, studies using genetic markers to assess genetic differentiation of fern populations showed that there is a certain limitation of dispersal and subsequently of gene flow in ferns on a scale of several to hundreds of kilometers (e.g., Holderegger and Schneller, 1994; Reisch et al., 2007; Stapulionyte et al., 2006). Moreover, the vast majority of fern spores fall in close proximity to the mother plant (Peck et al., 1990; Penrod and McCormick, 1996). Thus, it is not possible to conclude *a priori* that dispersal limitation is out of the question in ferns. Moreover, spores may disperse to many places, but effective species dispersal includes two processes – spore dispersal followed by new plant establishment.

In seed plants, fertilization takes place prior to dispersal, before the seed is formed. For colonization of a new habitat, theoretically one seed is enough. In ferns, fertilization is a post-dispersal process in the haploid gametophyte. For strictly outcrossing species, this means that, on a new habitat, two spores have to fall in close proximity in favorable conditions; a gametophyte has to develop from both of them with archegonia/anteridia and only then can fertilization occur. On the other hand, if the species is capable of intragametophytic selfing, one spore which develops in a hermaphroditic gametophyte is enough. Thus, further, we refer mostly to plant dispersal, including both the processes of spore dispersal followed by successful plant establishment.

Existing studies which have specifically aimed to assess dispersal limitation in ferns have reported contrasting conclusions. Within one population (i.e. at the scale 10-100 m), some studies showed prevailing dispersal limitation (Flinn, 2007; Richard et al., 2000), while others indicated that distribution is limited mainly by microsite availability (Wild and Gagnon, 2005). On the regional scale (i.e. a scale of several kilometers), the only study available on factors determining fern distribution is a study by Murakami et al. (2005), which dealt with the diversity of fern communities in forest fragments. They suggested dispersal limitation, but it is problematic to extrapolate the results of such a study on the features of single fern species, because different species may have different dispersal traits. Logically, the importance of limitation in distribution is species- and scale-dependent (Münzbergová, 2004). Thus, a full understanding of distribution limitation in ferns requires more studies on various species and spatial scales.

Understanding factors limiting the distribution of species can be done in two basic steps. First, we may attempt to identify suitable but unoccupied habitats for the species. The existence of such habitats will indicate that the species is limited by its dispersal ability. Not finding suitable but unoccupied habitats will, on the other hand, suggest that habitat limitation is the main type of limitation of the species (for a similar approach, see Ehrlén and Eriksson, 2000; Münzbergová and Herben, 2005; Wild and Gagnon, 2005). If we confirm the existence of dispersal limitation, we may ask if the absence of species from suitable habitats can be explained by the isolation of these habitats (Dupre and Ehrlén, 2002). The significant effects of habitat isolation suggest that the dispersal ability of the species in the landscape is limited at the given spatial scale. The absence of such a relationship indicates either no limitation by distance, i.e. the ability to disperse to all localities in the landscape, or very limited dispersal that is unlikely even between the closest localities. If the dispersal abilities of a species are very limited even for short distances, its distribution in a landscape is likely the result of random distance-independent dispersal (e.g. occasional zoochory) or of dispersal processes in the past in landscapes with different habitat conditions (Brown and Boutin, 2009; Lindborg and Eriksson, 2004), suggesting that habitat changes may be faster than the rate of species dispersal (Herben et al., 2006).

In the past two decades, together with wider use of metapopulation theory (Hanski, 1994), several methods to identify suitable but unoccupied habitats in a plant species have been developed. The most direct but technically difficult method is to perform a seed addition experiment (Münzbergová and Herben, 2005). While such experiments have been repeatedly used in a variety of plant species (for review see Clark et al., 2007; Moles and Westoby, 2002; Turnbull et al., 2000), it was in fact suggested that such experiments may also not be fully reliable (Clark et al., 2007; Ehrlén et al., 2006). Alternative and also commonly applied methods use different distribution models (Moore and Elmendorf, 2006) parameterized using different environmental variables (Husband and Barrett, 1996; Ouborg, 1993; Quintana-Ascencio and Menges, 1996; Wild and Gagnon, 2005) or data on species composition at the sites (e.g. Milden et al., 2006; Münzbergová and Herben, 2004; Romermann et al., 2007; Tremlová and Münzbergová, 2007). It was shown that these approaches are able to reliably identify unoccupied suitable localities (Ehrlén et al., 2006; Milden et al., 2006; Münzbergová and Herben, 2004). The best models explaining occupancy of localities and thus the best models for identifying unoccupied habitats (Moore and Elmendorf, 2006) were found to be models including both abiotic environmental variables and species composition of the sites (Münzbergová, 2004). It is surprising that this combination of abiotic factors and species composition has not been used for predicting suitable unoccupied habitats until now. A good definition of a suitable habitat is useful not only for scientific purposes. In the case of rare species, the characteristics of suitable habitats are of key importance for planning optimal management of the species.

As mentioned above, a full understanding of dispersal limitation of ferns requires studies on various species. Moreover, studying more species with different traits in one study system allows for easy comparison between species and determining to which extent it is possible to extrapolate results from one species to another one. Being able to do such extrapolation has a practical reason, since many ferns are rare and endangered species (web1), and determining the factors limiting their distribution may help in their effective conservation. Due to the need for a large amount of data to perform such as study, it is not possible to study every single endangered fern species.

In this study, we investigated the factors affecting the distribution of two rare fern species, *Asplenium adulterinum* and *A. cuneifolium*, in one region. Both species are restricted to serpentine rocks. Serpentine rocks form patches in the landscape of the model region, Slavkovský les, Czech Republic. However, not all serpentine rocks are occupied by the species.

Both species have very similar habitat requirements, but they differ in ploidy level. Higher ploidy level implies better colonization ability, because species of higher ploidy level have fixed heterozygosity, resulting in greater selfing ability than diploids (e.g., Flinn, 2006; Masuyama and Watano, 1990; Suter et al., 2000; Vogel et al., 1999) by reducing the negative consequences of inbreeding depression (Lande and Schemske, 1985). As a result, species with higher ploidy level are, in contrast to the diploid species, capable of single spore colonization of a habitat (Trewick et al., 2002, Vogel et al., 1999). Thus, we predict that tetraploid *A. adulterinum* should be a better colonist than diploid *A. cuneifolium* (Hejný and Slavík, 1980; Vogel et al., 1999) and should consequently show a lower degree of dispersal limitation.

Both of the studied species are considered as endangered; *A. adulterinum* is even listed in NATURA 2000, i.e. it is among the species highly protected in Europe (web1). Understanding the factors responsible for the distribution of these species is thus of key importance for their effective conservation. Finding that the species are limited by availability of habitats will suggest that we should try to conserve the present status of the occupied localities, and manage the unoccupied potential localities (serpentine rocks) to make them suitable, if possible. On the other hand, concluding that the species are largely limited by their dispersal ability will suggest that we should try to conserve all the occupied and potential localities in their present state. In the case of a great decline in the number of populations, we may consider active translocation of spores to some of the unoccupied but suitable localities.

In this study, we tested the following predictions. (1) We predicted that the distribution of the two species in the landscape can be explained by environmental conditions of the sites as well as by species composition at these sites. This prediction is based on the fact that the serpentine rocks within the region are very variable, and it is likely that not all the rocks are suitable for the species. We also predicted (2) that the distribution of the two species is partly limited by their dispersal ability as suggested by previous studies dealing with fern distribution on a spatial scale even smaller than the scale considered in this study. Further, we predicted (3) that occupancy of suitable localities will depend on isolation of the habitats. Finally, (4) we predicted that dispersal limitation will be weaker in *A. adulterinum* as this species is an allotetraploid and thus should have better ability in terms of single spore colorization.

To address these predictions, we mapped 98 serpentine rocks (localities) within an area of 100 km^2, and recorded the environmental characteristics and all vascular plant species at each locality. We identified suitable localities, checked if all of them were occupied by the target species and tested whether occupancy of suitable localities depended on isolation and the size of the locality.

2. METHODS

2.1 Study species

The study works with two fern species, *Asplenium adulterinum* Milde and *Asplenium cuneifolium* Viv. (Aspleniaceae), both restricted to serpentine substrates in Europe (Tutin et al., 1980). *A. adulterinum* occurs also in one population on Vancouver Island, Canada (Ogilvie and Ceska, 1984). The ranges of distribution of both species are highly scattered, following the distribution of serpentine rocks in Europe from the Mediterranean to Norway and from Greece to Spain (*A. cuneifolium*, *A. adulterinum* is only found from Austria to France). In the Czech Republic, the species occur mainly in Western Bohemia (Slavkovský les), with several localities in north-eastern Bohemia. In the rest of the country, there are only very few small populations. *A. cuneifolium* is in general more widespread than *A. adulterinum*.

Within a locality, *A. adulterinum* grows mainly in rock crevices, or in scree, where the concurrence of other species is very low. *A. cuneifolium* occurs in rock crevices as well, but due to its larger size, it is able to withstand the concurrence of sparse vegetation of other species on places with deeper soil such as rock terraces or small-grain scree just under the rock. The microhabitats of the species partly overlap (larger rock crevices), but *A. adulterinum* is able occupy much smaller crevices and *A. cuneifolium* can occupy deeper soil.

Competition between the two species is rare and not limiting for species distribution on the landscape scale.

Both species are rare and under the interest of nature conservation throughout Europe (Tutin et al., 1980). *A. adulterinum* is even a species of interest of the European ecological network NATURA 2000 (web1). The species differ in ploidy level – *A. adulterinum* is allotetraploid (parental species *A. viride* L. and *A. trichomanes* Huds. subsp. *trichomanes*, (Lovis, 1955), while *A. cuneifolium* is diploid (Hejný and Slavík, 1980).

2.2 Study site

The study was carried out in the region of Slavkovský les, Western Bohemia, Czech Republic. In this region of ca 10×10 km, 98 serpentine rocks are scattered in the landscape dominated by pine or spruce forests and mown grasslands. The areas of individual rocks range from 1 m^2 to almost 9 ha. Most of the rocks are concentrated in the central part within a 7×4 km area, with a few more distant rocks. The most remote serpentine rock was 4 km far from the closest other one.

Both *A. adulterinum* and *A. cuneifolium* are quite common in the area. *A. adulterinum* is more common and occupies both rocks in unforested habitats and in the forest (dominated by *Pinus sylvestris* and *Picea abies*). In total, there are 66 populations ranging from several individuals to nearly 2000 individuals (Tájek, 2003). *A cuneifolium* prefers rocks under the forest canopy. The unforested rocks are rarely inhabited and the populations are very small here. In total, there are 48 populations of *A. cuneifolium* in the study region, ranging from several individuals up to several hundreds of individuals (Tájek, 2003). *A. adulterinum* and *A. cuneifolium* occur together at 40 localities.

2.3 Field data collection

Field work was carried out from 2001 to 2003. All serpentine rocks (further called localities) identified using geological maps within the study area were mapped in the field using GPS. Also, new rocks not detected on the geological maps were discovered and mapped during the study. A single locality often contained a fine mosaic of rocks and non-rocky places. Localities were considered as distinct when they were separated by a gap of at least 20 m without rock. For all the localities, the characteristics listed in Table 1 were collected. These characteristics may, according our vast field experience, determine species occurrence at localities.

The size of the localities was measured in the field using a measuring tape (up to ca 100 m^2) or in GIS. Because of the presence of the non-rocky places, the area of the locality was adjusted by an educated guess of the cover of the rocky biotope for the purposes of further analysis. In all localities, the height of the highest vertical rock wall was measured, further referred to as “height”. The presence of different microhabitats and their number was recorded at each locality. The following microhabitat types were distinguished: rock crevices, plateau (flat top of the rock with shallow soil), terraces (small terraces in the wall of the rocks, where litter is accumulated), scree (accumulation of big free stones on the slopes of bigger rocks) and slopes (with shallow soil). For details, see Table 1.

For all localities, a complete list of the species of vascular plants was created. Furthermore, the type of vegetation at each locality was recorded using three categories: unforested habitat, pine forest and spruce forest.

2.4 Data analysis

2.4.1 Effect of species composition – Beals index

Species composition at each locality entered further analysis as the Beals index of social favorability (Beals, 1984). This index reflects the fact that some species occur together more often than other species because they need similar abiotic conditions. The Beals index is defined as the probability of occurrence of species j at habitat i:

$$p_{ij} = (1/S_i)\sum_k N_{jk}/ N_k \qquad (1)$$

where p_{ij} is the probability to find species j at habitat i, S_i is the number of species at habitat i (minus 1 if species j is present), N_{jk} is the number of joint occurrences of species j and k ($j \neq k$) and N_k is the number of occurrences of species k in the dataset (Münzbergová and Herben, 2004). To maximize the accuracy of estimation of the Beals index and minimize noise in the data, species rare in the dataset (occurring less than three times) were excluded, since they carried no usable ecological information (De Caceres and Legendre, 2008). We used our species list for all the serpentine localities in the region for calculating the Beals index, always excluding the locality, for which the Beals index was calculated. We obtained one value for each locality and fern species, generating 2 ×98 = 196 values of the Beals index.

2.4.2 Suitability of localities for the target species

The aim was to determine the suitable unoccupied localities and find the features of suitable localities. For this purpose, we built a model (logistic regression) where we explained the presence or absence of the target species (*A. adulterinum* or *A. cuneifolium*) at a locality by abiotic and biotic characteristics of the locality. For each locality, occupied or non-occupied, we calculated the probability of being occupied based on the results of the logistic regression. Logically, most occupied localities obtained a higher fitted value than many unoccupied localities. The unoccupied localities which had a higher probability of being occupied than the occupied locality with the lowest probability of being occupied were considered as suitable but not occupied.

As the model, we used logistic regression in the R program (R Development Core Team, 2009). The dependent variable was the presence/absence of the target species at the locality (binary variable), and the explanatory variables were height of the highest rock at a locality, number of microhabitats, presence of each microhabitat, vegetation type, as well as the Ellenberg indicator values for light, temperature, continentality, moisture, soil reaction (pH) and nutrients (Ellenberg et al. 1992) and the probability of occurrence expressed as the Beals index of social favorability (Beals, 1984; Münzbergová and Herben, 2004) (for details see Table 1). The Ellenberg indicator values represent environmental requirements (light, temperature, continentality, moisture reaction, salinity and nutrients) of individual species and are published for a large set of Central European species. In our dataset, "salinity" was defined only for a few species; thus, it was not included in the analyses. Mean Ellenberg indicator values for each locality were calculated as unweighted means of indicator values for all species recorded at the locality.

To avoid collinearities in explanatory variables, we checked for correlations, and from the correlated explanatory variables, we kept the one with better ecological meaning and excluded the other (Hosmer and Lemeshow, 2000). Further, we excluded variables uniform in the whole dataset (slopes) and fully complementary to other variables (the variable "pine" was complementary to other vegetation types).

All the other variables, which were not excluded because of correlations, were included into the "full model" of logistic regression. The results of the "full model" were used for evaluating the effect of environmental characteristics on the presence/absence of the species at the locality. Since the full model was the same for both species, it allowed for comparisons between species and identifying important differences in the characteristics of locality suitability.

Since the full model also contained variables which were not significant, there was danger of "overfitting" – some variability could be explained by accidental correlations, without any ecological meaning (Hosmer and Lemeshow, 2000). Thus, in the full model, we applied forward-backward stepwise variable selection using the “step” procedure in R (using AIC). We obtained “the best model” for each species. “The best models” were carefully checked to determine if the remaining variables had good ecological sense (Hosmer and Lemeshow, 2000). We verified that the deviance explained by the best model did not significantly differ from the deviance explained by the full model by command “anova” in R (using the test criterion Chi-square).

Fitted values from “the best models” for each species were used for the identification of suitable but unoccupied localities. Localities were considered as unsuitable unoccupied when the fitted value from “the best model” was lower than the lowest value for occupied localities. All occupied localities were considered as suitable.

We are aware that presence and absence does not reflect the number of plants growing at the localities related to the size of localities. From this purpose, we ran also models with the density of plants at the localities as the response variable (GLM with gamma distribution), expressed as the number of species on one square meter of the locality. Those models explained only very little variability in the data and almost no variables showed any effect on the density of the species. We thus stayed with the model with the presence/absence of the species as the response variable, since it proved to be more informative.

2.4.3 Locality isolation

We used two different approaches to calculate locality isolation in this study. First, we calculated isolation of the locality using its distance from all suitable localities in the study region. Second, we calculated isolation only from all occupied localities.

Calculation of locality isolation was based on the centroids of the localities, derived from a digital map of the localities (Tájek 2003). Isolation was expressed as a mass of surrounding localities weighted by its distance to the target locality (Tremlová and Münzbergová 2007). Isolation of a locality was expressed as follows:

$$I_{ij} = \sum^{n}_{k=1}[(P_k/d^2_{jk})\times O_k],\ j\neq k \qquad (2)$$

where I_{ij} is the isolation of locality j for species i, k are all the surrounding localities, P_k is the size of locality k in square meters, d_{jk} is the distance between localities j and k in meters, and O_k is a binary variable (0, 1) coding occupancy of the locality. When calculating isolation from all suitable localities, O_k is always 1. The value of isolation I_{ij} was log transformed and multiplied by -1 before the subsequent analyses. In this way, higher values indicated more isolated localities.

2.4.4 Effect of locality isolation and size

We performed univariate logistic regression with either isolation or area of locality (logarithmically transformed) as the independent variables and the occurrence of species as the response variable. Finally, we performed logistic regression with the occurrence of species as the response variable and both area and isolation as the independent variable (for both types of isolation). We also tested the interaction of isolation and area, but the results were not significant and are not presented. These analyses were done using only the suitable localities for each species. In this way, we in fact tested the effect of habitat size and isolation after taking into account the habitat conditions of the sites.

3. RESULTS

3. 1 Factors determining locality suitability

Due to correlations, several variables including the number of microhabitat types (correlated with scree, plateau and terraces), temperature (correlated with soil reaction) and light (correlated with unforested habitat) were excluded from further models.

For *Asplenium adulterinum*, habitat suitability was, according the full model, positively influenced by the height of the highest rock at a locality, the presence of crevices and scree and increasing continentality (microclimate extremity). It was strongly negatively influenced by nutrient richness. The best model (after variable selection using the forward-backward approach) further revealed that *A. adulterinum* grows rather on localities hosting species preferring soil with higher pH (Table 2). The Beals index and vegetation type (unforested habitat or spruce forest) had no effect.

For *Asplenium cuneifolium*, habitat suitability was, according the full model, influenced by the height of highest rock at the locality and marginally also by the presence of crevices and scree. In contrast with *A. adulterinum*, habitat suitability for *A. cuneifolium* was not influenced by nutrient richness of the locality and continentality, but strongly by the Beals index, the presence of terraces and negatively by unforested habitat. The best model (after variable selection using the forward-backward approach) further revealed that *A. cuneifolium* tends to avoid localities with high moisture, and prefers localities with higher soil reaction (the same as A. *adulterinum*, Table 1).

Based on the fitted values from the final models for both species (Fig. 2), suitable and unsuitable unoccupied localities were identified. For *A. adulterinum*, there were 15 unoccupied but suitable localities and 17 unoccupied and unsuitable localities. For *A. cuneifolium* there were 18 suitable unoccupied localities and 32 unoccupied unsuitable localities. Thus, of 81 suitable localities for *A. adulterinum*, 66 were occupied (81 %). Of 66 suitable localities for *A. cuneifolium*, 48 were occupied (73%); see on-line Appendix.

For *A. cuneifolium*, suitable habitats were located mostly in the central serpentine area with one exception, an occupied serpentine rock approximately 4 km northwest from the main serpentine area. *A. adulterinum* occupied rocks in the central area and also more isolated rocks (up to 4 km from the central area). Some isolated rocks in the southwest were unoccupied but suitable (see Fig 1).

3.2 Locality isolation

Isolation of a locality (calculated either from all occupied localities or all suitable both occupied and unoccupied) was correlated with the area of the locality for both species (correlation coefficient 0.47-0.64; see online Appendix).

In univariate analysis, both types of isolation had a significant negative effect on the occurrence of both target species at a locality. The effect of isolation was stronger for *A. adulterinum* than for *A. cuneifolium*. Isolation explained 26% (when calculated from suitable habitats) or 28% (when calculated from occupied habitats) of the total deviance for *A. adulterinum*, but only 7% and 9%, respectively, for *A. cuneifolium* (Table 3). The area of the locality had a smaller effect on the occurrence of *A. adulterinum* than did isolation, but this was still significant (it explained 15 % of the deviance). However, it had only a marginally significant effect on *A. cuneifolium* (p=0.057, explained deviance 6%).

In the model including both isolation of a locality and its area, isolation calculated from occupied localities (fitted after area) had a stronger negative effect on the occurrence of *A. adulterinum* than isolation based on suitable localities. Any isolation had no effect on the occurrence of *A. cuneifolium* at a locality. The area of the locality had no effect, when fitted after isolation, for both species and both types of isolation (Table 3).

4. DISCUSSION

4. 1 Characteristics of suitable localities

Both *A. adulterinum* and *A. cuneifolium* are restricted to serpentine rocks, but not all serpentine rocks are suitable for these species. There are some environmental characteristics which make a habitat more suitable or unsuitable. Determination of these characteristics is important for effective management of these rare plants.

Both species are positively influenced by the presence of rock crevices and scree. This is logical, since these microhabitats are the most common ones for both species. *A. cuneifolium* further prefers localities with rock terraces, where the soil is deeper and *A. cuneifolium* grows there sometimes with other species (e.g. *Calamagrostis villosa*). On terraces, *A. adulterinum* does not occur since it is not able to withstand competition. The negative effect of competition of other species on the presence of *Asplenium* is illustrated also by the fact that both species prefer higher rocks. On higher rocks, the species better escape competition and shading by plants growing in the neighborhood of the rock.

Serpentine rocks are a very special environment for plant species. The extreme chemistry of the substrate strongly influences plant communities and species composition (Kruckeberg, 1954). We did not analyze the mineral composition of the serpentine rocks at each locality, so it may be argued that this may be an important characteristic which we did not take into account. However, all the other plants at the localities are influenced by the rock quality and, thus, species composition also reflects the chemical quality of the substrate. Most likely, the substrate composition is reflected by the Ellenberg indicator values for soil reaction, since serpentinite is an ultrabasic stone. Both *Asplenium* species are significantly influenced by soil reaction with strong affinity to more alkaline (more serpentine) bedrock.

A. adulterinum tends to avoid localities with high nutrient content. However, this was not the case for *A. cuneifolium*. Higher nutrient content means, in this area, more dense vegetation resulting in higher competition. *A. cuneifolium*, due to its larger size, has better competitive ability than *A. adulterinum* and can thus survive in these high competition conditions. Serpentine outcrops are naturally nutrient-poor (Turitzin, 1982; Chiarucci et al., 1999) and an increased amount of nutrients (mainly phosphorus and nitrogen) means degradation of the habitat (Ricotta et al., 2004). Thus, serpentine species tend to avoid eutrophicated and degraded localities. When conserving suitable localities for these species, sources of nutrients should be restricted at the localities and in close proximity. In the past, the most important source of nutrients at the sites was fertilization of surrounding fields. Most of these are, however, are currently abandoned and field fertilization is thus no longer a problem. Another source of nutrients at present is grazing animals, which are kept at unforested localities as part of the management of these sites. Grazing animals are kept out of the localities overnight to avoid the addition of free nutrients to the ecosystem. Further, airborne nitrogen pollutants may play an important role (for review, see Bobbink et al., 1998). Despite the fact that anthropogenic production of airborne nitrogen has slightly reduced in recent decades, it is still significant and has an effect on ecosystems (Erisman, 2003; Fottová, 2003). In addition, the recovery of vegetation after ceasing nitrogen input is a very slow process (Nordin et al., 2005). Suitable management of serpentine rocks should aim to remove nutrients. This is currently being experimentally assessed at several localities by mowing unforested areas and the herb layer in forests followed by removing the mown biomass.

A. adulterinum is positively influenced by the continentality of the locality, which represents more climate extremity. According to our field experience, *A. adulterinum* is also able to grow, in contrast with *A. cuneifolium*, on very exposed habitats, where the summer is hot and dry, and in the winter, the snow is blown away by wind and no protection from frost is present.

Species composition, expressed as the Beals index, was highly significant for *A. cuneifolium*, but not for *A. adulterinum*. This is probably due to the fact that *A. adulterinum* grows only in rock crevices and does not share any microhabitats with other species. On the other hand, *A. cuneifolium* shares some microhabitats with other species and their presence/absence reflects habitat suitability for this species. It shows that the Beals index is probably not a good predictor for species of extreme microhabitats and, in that case, other environmental variables have to be included.

A. adulterinum occurs with all types of vegetation, whereas *A. cuneifolium* tends to avoid unforested localities. This corresponds to the distribution of *A. cuneifolium* in the landscape and most probably it is given by a higher extinction probability of small populations of *A. cuneifolium* in open landscapes than in forests (Bucharová et al., 2010). Interestingly, there is no significant effect of spruce forest on the probability of occurrence of the target species at a locality. According to our field experience, both *Asplenium* species suffer in spruce forests. In contrast to pines, spruce trees cause deep shadow, supporting moss growth. Moreover, spruce produces a large amount of slowly decomposing litter filling rock crevices, which are the typical microhabitat of both species. It may be argued that some of our populations in the spruce forest may be remnant (Eriksson, 1996), i.e. not reproducing but still surviving at the locality thanks to individual longevity (single individuals may live for up to 34 and 44 years in *A. adulterinum* and *A. cuneifolium*, respectively; Bucharová et al. 2010). In this case, populations in the spruce forest should have the lowest fitted values of the model from of all occupied localities. However, this was not the case in our study. Another objection may be that we worked with presence/absence data only. Thus, we ran also model with the density of species at localities as the response variable (results not shown, as the model showed very poor descriptive value). Most probably, the lack of significance in spruce forest is the result of the coarse spatial scale of our data collection. Spruce trees negatively influence rocks which are directly under them. Among trees, there may be small openings which are actually suitable for species, but on the locality level, spruce forest was recorded.

4.2 Reliability of definition of suitable but unoccupied habitats

We found, in agreement with Murakami et al. (2005) that suitable but unoccupied localities exist for both fern species. This implies the existence of dispersal limitation. However, this conclusion is dependent on the reliability of identification of unoccupied suitable habitats (Münzbergová and Herben, 2005). To identify suitable/unsuitable habitats, it is necessary to develop a model which reflects environmental variability determining species occurrence and thus can reliably distinguish between occupied and unoccupied localities (Moore and Elmendorf, 2006). Our model containing environmental variables and data on species composition explained between 52% and 47% of the variability in the occurrence of *A. adulterinum* and *A. cuneifolium*, respectively. When compared with the study of Ehrlén et al. (2006), our model better described the probability of species occurrence. The models of Ehrlén et al. (2006), using only species composition, explained between 0% and 39% of the variability (with one exception of 78%), and using only environmental variables, explained between 7% and 46% of the variability in the occurrence of the target species. Thus, a combination of environmental variables and data on species composition is useful for explaining species distribution and, thus, for the determination of suitable but unoccupied habitats.

Using only species composition, expressed as the Beals index (Beals, 1984; Münzbergová and Herben, 2004) for predicting suitable/unsuitable localities would be difficult for our species. It significantly and positively explained the occupancy of localities only for *A. cuneifolium*, but had no significant meaning for *A. adulterinum*. There was no significant difference between occupied and unoccupied habitats in this species and thus the Beals index had little informative value in this case (see Ehrlén et al., 2006; Milden et al., 2006; Tremlová and Münzbergová, 2007).

The definition of suitable/unsuitable habitats corresponds well to our vast field experience. In our opinion, the model included most of the key characteristics of the localities.

Beyond that, at one unoccupied locality identified as suitable for *A. cuneifolium* in this study based on data from 2003, this species was found by P. Tájek in 2006. This confirms that this locality really was unoccupied but suitable.

4.3 Habitat isolation

In *A. adulterinum*, the probability of occupancy of a habitat decreases with increasing habitat isolation. This shows that limitation by dispersal in this species is distance-dependent. This result is similar to many previous studies of seed plants (e.g. Franzen and Eriksson, 2003; Kolb and Diekmann, 2005; Ouborg, 1993; Quintana-Ascencio and Menges, 1996). In ferns, dispersal limitation was less expected, due their small, wind-dispersing spores. Previously, it has been suggested that distance-dependent dispersal limitation in a fern species exists, in genetic studies looking at gene flow between fern populations (Holderegger and Schneller, 1994; Reisch et al., 2007; Stapulionyte et al., 2006) or in studies which focused on fern diversity (Murakami et al., 2005).

A significant effect of habitat isolation in *A. adulterinum* means that this species is able to disperse better to closer localities than to more isolated ones. Plant dispersal means dispersal of spores followed by a new plant establishment. The absence of significant effect of habitat isolation in *A. cuneifolium* may have two contrasting explanations. First, *A. cuneifolium* disperses well everywhere (and colonization is no problem). Second, *A. cuneifolium* disperses badly to all localities and current landscape occupancy is due to some other, most probably historical factors (the history of the localities may have a huge influence on species distribution, e.g. Brown and Boutin, 2009; Chýlová and Münzbergová, 2008; Lindborg and Eriksson, 2004). Occupancy of the localities supports the second option, since *A. cuneifolium* occupies fewer suitable localities (73%) than *A. adulterinum* (84%). Mating biology of the two species also supports the second option, since tetraploid *A. adulterinum* has theoretically better colonizing abilities because of its ability of successful gametophyte selfing and thus the ability of single spore colonization of a habitat. In diploid *A. cuneifolium*, gametophyte selfing and single spore colonization is difficult (Flinn, 2006; Lande and Schemske, 1985; Vogel et al., 1999). A similar pattern, i.e. a significant effect of habitat isolation on species with predicted good dispersal abilities and no effect of isolation in species with bad dispersal abilities, was found by Johansson and Ehrlén (2003) in lichens. They concluded, based on other parameters, that their predictions of dispersal abilities were correct and they discussed the suitability of various isolation measures. In the case of their study, the explanation of the pattern can be actually the same as in our study – the badly dispersing species disperses so badly even to closer habitats that the effect of isolation is not visible.

The lack of influence of the size of the habitat area on habitat occupancy is interesting since it is counter to a commonly accepted and repeatedly confirmed theory, that the probability of habitat occupancy increases with its size (e.g. Quintana-Ascencio and Menges, 1996). This is probably due to the fact that our study species are strongly microhabitat-limited and they are also able to grow on very small habitats (even on a stone the size on one square meter, when there are suitable microhabitats). It is important that this habitat is not very isolated from other habitats, since isolation is important.

We showed that distribution of both fern species, *A. adulterinum* and *A. cuneifolium*, is partly limited by availability and dispersal ability of the spores. Plant distribution patterns are usually limited by both dispersal abilities and the availability of environmentally suitable sites. These two limitations are extremes of a continuum and limitation of plant distribution usually lies between these two extremes (Clark et al., 2007; Münzbergová and Herben, 2005). Thus, the availability of suitable habitats and the quality of suitable habitats are most probably to some extent limiting factors for the distribution of target species as well. Because the individuals of our study fern species are very long-lived (exceptionally more than 100 years;

Bucharová et al., 2010), some populations may be in fact remnant (Eriksson, 1996) and an important factor determining species distribution is probably also suitability and occupancy of habitats in the past.

5. CONCLUSION

We showed that both fern species are dispersal-limited on the regional scale, at least to some degree. The importance of dispersal limitation, however, differs a bit between the species due to their different mating strategies. Based on the published literature and the results of our study, we can conclude that dispersal limitation on a regional scale plays an important role in ferns.

To preserve *A. adulterinum* and *A. cuneifolium* in the landscape, current localities should be managed to keep (or improve) their actual state. Attention should also be paid to suitable but unoccupied localities, regardless of their size. They should be managed as occupied localities, since they are future possible localities of the species. The slow dynamics of the species makes the species, on one hand, relatively resistant to negative landscape changes (species persist at a locality for long time). On the other hand, this results in slow responses to positive as well as negative changes in the landscape. The effect of any preservation program thus has to be evaluated from a long-term perspective.

ACKNOWLEGEMENTS

We thank to J. Bartoš, I. Suchara and V. Melichar for technical advice and our colleagues from the Institute of Botany ASCR and two anonymous reviewers for their helpful remarks on the manuscript. This work was supported by MSMT 2B06178, FRVS 348/2004 and GAUK 176/2006 and partly by MSMT 0021620828 and AV0Z60050516.

REFERENCES

Beals, E.W., 1984. Bray-Curtis Ordination: an effective strategy for analysis of multivariate ecological data. Adv. Ecol. Res. 14, 1-56.

Bobbink, R., Hornung, M., Roelofs, J.G.M, 1998. The Effects of Air-Borne Nitrogen Pollutants on Species Diversity in Natural and Semi-Natural European Vegetation. J. Ecol. 86, 717-738.

Brown, C.D., Boutin, C., 2009. Linking past land use, recent disturbance, and dispersal mechanism to forest composition. Biol. Conserv. 142, 1647-1656.

Bucharová, A., Munzbergová, Z., Tájek, P. (2010): Population biology of two rare fern species – long life and long-lasting stability. Am. J. Bot. 97, 1260-1271.

Clark, C.J., Poulsen, J.R., Levey, D.J., Osenberg, C.W., 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. Am. Nat. 170, 128-142.

De Caceres, M., Legendre, P., 2008. Beals smoothing revisited. Oecologia. 156, 657-669.

Chiarucci, A., Maccherini, S., Bonini, I., De Dominicis, V., 1999. Effects of nutrient addition on community productivity and structure of serpentine vegetation. Plant Biol. 1, 121-126.

Chýlová, T., Münzbergová, Z., 2008. Past land use co-determines the present distribution of dry grassland plant species. Preslia 80, 183-198.

Dupre, C., Ehrlén, J., 2002. Habitat configuration, species traits and plant distributions. J. Ecol. 90, 796-805.

Ehrlén, J., Eriksson, O., 2000. Dispersal limitation and patch occupancy in forest herbs. Ecology 81, 1667-1674.

Ehrlén, J., Münzbergová, Z., Diekmann, M., Eriksson, O., 2006. Long-term assessment of seed limitation in plants: results from an 11-year experiment. J. Ecol. 94, 1224-1232.

Ellenberg, H., Weber, H.E., Dull R., Wirth V., Werner W., Paulissen D., 1992. Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica 18, 1-258.

Eriksson, O., 1996. Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. Oikos 77, 248-258.

Erisman, J.W., Grennfelt P., Sutton M., 2003. The European perspective on nitrogen emission and deposition. Environ Int. 29, 311-325.

Flinn, K.M., 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. Am. J. Bot. 93, 1289-1294.

Flinn, K.M., 2007. Microsite-limited recruitment controls fern colonization of post-agricultural forests. Ecology 88, 3103-3114.

Fottová, D. 2003. Trends in Sulphur and Nitrogen Deposition Fluxes in the GEOMON Network, Czech Republic, between 1994 and 2000. Water Air Soil Pollut. 150, 73-87.

Franzen, D., Eriksson, O., 2003. Patch distribution and dispersal limitation of four plant species in Swedish semi-natural grasslands. Plant Ecol. 166, 217-225.

Guo, Q.F., Kato, M., Ricklefs, R.E., 2003. Life history, diversity and distribution: a study of Japanese pteridophytes. Ecography 26, 129-138.

Hanski, I., 1994. A Practical Model of Metapopulation Dynamics. Journal of Animal Ecology 63, 151-162.

Hejný, S., Slavík, H., 1980. Flora of Czech Republic (Květena České Republiky). Academia, Praha.

Herben, T., Münzbergová, Z., Milden, M., Ehrlén, J., Cousins, S.A.O., Eriksson, O., 2006. Long-term spatial dynamics of Succisa pratensis in a changing rural landscape: linking dynamical modelling with historical maps. J. Ecol. 94, 131-143.

Holderegger, R., Schneller, J.J., 1994. Are Small Isolated Populations of *Asplenium septentrionale* variable? Biol. J. Linn. Soc. Lond. 51, 377-385.

Hosmer, D.W., Lemeshow, S., 2000. Applied logistic regression, New York, John Wiley & Sons, Inc.

Husband, B.C., Barrett, S.C.H., 1996. A metapopulation perspective in plant population biology. J. Ecol. 84: 461-469.

Johansson, P., Ehrlén, J., 2003. Influence of habitat quantity, quality and isolation on the distribution and abundance of two epiphytic lichens. J. Ecol. 91, 213-221.

Kolb, A., Diekmann, M., 2005. Effects of life-history traits on responses of plant species to forest fragmentation. Conserv. Biol. 19, 929-938.

Kruckeberg, A.R., 1954. The Ecology of Serpentine Soils .3. Plant Species in Relation to Serpentine Soils. Ecology 35, 267-274.

Lande, R., Schemske, D.W., 1985. The Evolution of Self-Fertilization and Inbreeding Depression in Plants. 1. Genetic Models. Evolution 39, 24-40.

Lindborg, R., Eriksson, O., 2004. Historical landscape connectivity affects present plant species diversity. Ecology 85, 1840-1845.

Lovis, J.D., 1955. *Asplenium adulterinum* and its probable parents. Proc Bot Soc Br Isles. 1, 2.

Masuyama, S., Watano, Y., 1990. Trends for inbreeding in polyploid pteridophytes. Plant Species Biol. 5, 13-17.

Milden, M., Münzbergová, Z., Herben, T., Ehrlén, J., 2006. Metapopulation dynamics of a perennial plant, *Succisa pratensis*, in an agricultural landscape. Ecol Modell. 199, 464-475.

Moles, A.T., Westoby, M., 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. Oikos 99, 241-248.

Moore, K.A., Elmendorf, S.C., 2006. Propagule vs. niche limitation: untangling the mechanisms behind plant species' distributions. Ecol. Lett. 9, 797-804.

Münzbergová, Z., 2004. Effect of spatial scale on factors limiting species distributions in dry grassland fragments. J. Ecol. 92, 854-867.

Münzbergová, Z., Herben, T., 2004. Identification of suitable unoccupied habitats in metapopulation studies using co-occurrence of species. Oikos 105, 408-414.

Münzbergová, Z., Herben, T., 2005. Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. Oecologia 145, 1-8.

Murakami, K., Maenaka, H., Morimoto, Y., 2005. Factors influencing species diversity of ferns and fern allies in fragmented forest patches in the Kyoto city area. Landsc Urban Plan. 70, 221-229.

Nordin, A., Strengbom, J., Witzell, J., Näsholm, T., Ericson, L., 2005. Nitrogen Deposition and the Biodiversity of Boreal Forests: Implications for the Nitrogen Critical Load. Ambio 34, 20-24.

Ogilvie, R.T., Ceska, A., 1984. Alpine Plants of Phytogeographic Interest on Northwestern Vancouver-Island. Can. J. Bot. 62, 2356-2362.

Ouborg, N.J., 1993. Isolation, Population-Size and Extinction: the Classical and Metapopulation Approaches Applied to Vascular Plants Along the Dutch Rhine-System. Oikos 66, 298-308.

Peck, J.H., Peck, C.J., Farrar, D.R., 1990. Influences of Life-History Attributes on Formation of Local and Distant Fern Populations. Am. Fern J. 80, 126-142.

Penrod, K.A., McCormick, L.H., 1996. Abundance of viable hay-scented fern spores germinated from hardwood forest soils at various distances from a source. Am. Fern J. 86, 69-79.

Quintana-Ascencio, P.F., Menges, E.S., 1996. Inferring metapopulation dynamics from patch-level incidence of Florida scrub plants. Conserv. Biol. 10, 1210-1219.

R_Development_Core_Team, 2009. R: A Language and Environment for Statistical Computing. Vienna.

Reisch, C., Mayer, F., Ruther, C., Nelle, O., 2007. Forest history affects genetic diversity - molecular variation of *Dryopteris dilatata* (Dryopteridaceae) in ancient and recent forests. Nord. J. Bot. 25, 366-371.

Ricotta, C., Chiarucci, A., Avena, G., 2004. Quantifying the effects of nutrient addition on community diversity of serpentine vegetation using parametric entropy of type alpha. Acta Oecol. 25, 61-65.

Richard, M., Bernhardt, T., Bell, G., 2000. Environmental heterogeneity and the spatial structure of fern species diversity in one hectare of old-growth forest. Ecography 23, 231-245.

Romermann, C., Tackenberg, O., Scheuerer, M., May, R., Poschlod, P., 2007. Predicting habitat distribution and frequency from plant species co-occurrence data. J. Biogeogr. 34, 1041-1052.

Stapulionyte, A., Lazutka, J.R., Naujalis, J.R., Odland, A., 2006. Assessment of genetic polymorphism in natural populations of ostrich fern (*Matteuccia struthiopteris* L., Dryopteridaceae) in Lithuania by using RAPD markers. Biologija 1, 7.

Suter, M., Schneller, J.J., Vogel, J.C., 2000. Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp. *quadrivalens*. Int. J. Plant Sci. 161, 233-244.

Tájek, P., 2003. Serpentine outcrops in Slavkovský les in view of island biogeography. (Hadcové výchozy ve Slavkovském lese z pohledu ostrovní biogeografie). Diploma thesis. Department of Botany, Charles University, Praha.

Tremlová, K., Münzbergová, Z., 2007. Importance of species traits for species distribution in fragmented landscapes. Ecology 88, 965-977.

Trewick, S.A., Morgan-Richards, M., Russell, S.J., Henderson, S., Rumsey, F.J., Pinter, I., Barrett, J.A., Gibby, M., Vogel, J.C., 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. Mol. Ecol. 11, 2003-2012.

Tryon, R., 1970. Development and evolution of fern floras of oceanic islands. Biotropica 2, 76-84.

Tryon, R., 1986. The Biogeography of Species, with Special Reference to Ferns. Bot. Rev. 52, 117-156.

Turitzin, S.N., 1982. Nutrient Limitations to Plant-Growth in a California Serpentine Grassland. Am. Midl. Nat. 107, 95-99.

Turnbull, L.A., Crawley, M.J., Rees, M., 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88, 225-238.

Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A., 1980. Flora Europaea. Cambridge University Press, UK.

Vogel, J.C., Rumsey, F.J., Schneller, J.J., Barrett, J.A., Gibby, M., 1999. Where are the glacial refugia in Europe? Evidence from pteridophytes. Biol. J. Linn. Soc. Lond. 66, 23-37.

web1. http://ec.europa.eu/environment/nature/natura2000.

Wild, M., Gagnon, D., 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? Ecography 28, 191-196.

Table 1: Environmental characteristics of the localities.

Independent variable	**Values**
Height of the rock	0.2-10.5 m (continuous)
Area (log transformed)	1-89 288 m^2 (continuous)
Number of microhabitat types	1-6; continuous
Beals index (different for each species)	0−1, continuous
*Vegetation type**	
Unforested	0−1, continuous
Pine forest **	0−1, continuous
Spruce forest	0−1, continuous
Microhabitat	
Slopes***	Presence/absence
Crevices	Presence/absence
Scree	Presence/absence
Plateau	Presence/absence
Terraces	Presence/absence
*Ellenberg indicator values*****	
Light	4.8−7.3; continuous
Temperature	3−5.6; continuous
Continentality	3.2−4.5; continuous
Moisture	3.9−6; continuous
Soil reaction	2−6.1; continuous
Nutrients	1−5.8; continuous

Table 2: Effect of locality characteristics on presence/absence of *Asplenium adulterinum* and *A. cuneifolium* based on full model and best model for each species. Presented value for each variable are after evaluating the effect of all the other variables included in the model ("fitted last"). The signs indicate direction of the significant relationships. Significant values p< 0.05 are bold, partially significant (p< 0.1) in italics.

	A. adulterinum						*A. cuneifolium*					
	Full model			The best model			Full model			The best model		
	R2 = 0.52, resid. Df 85			R2 = 0.50, resid. Df 90			R2 = 0.47, resid. Df 85			R2 = 0.44, resid. Df 89		
	p	R2		p	R2		p	R2		p	R2	
Height	**0.003**	**0.073**	(+)	**<0.001**	**0.125**	(+)	**0.026**	**0.036**	(+)	**0.010**	**0.086**	(+)
Beals index	0.762	0.001					**0.020**	**0.040**	(+)	**0.001**	**0.133**	(+)
Vegetation												
Unforested	0.740	0.001					**0.026**	**0.036**	(-)	*0.071*	*0.042*	(-)
Spruce	0.448	0.005					0.32	0.007				
Microhabitat presence												
Crevices	**0.004**	**0.067**	(+)	**0.003**	**0.072**	(+)	*0.078*	0.023	(+)	**0.042**	**0.054**	(+)
Scree	**0.004**	**0.068**	(+)	**0.001**	**0.091**	(+)	*0.059*	0.026	(+)	*0.073*	*0.042*	(+)
Plateau	0.594	0.002					0.245	0.010				
Terraces	0.181	0.014					**0.033**	**0.034**	(+)	**0.018**	**0.073**	(+)
Ellenberg values												
Moisture	0.184	0.014		0.106	0.021		0.164	0.014		**0.030**	**0.062**	(-)
Nutrients	**<0.001**	**0.114**	(+)	**<0.001**	**0.146**	(+)	0.201	0.012				
Soil Reaction	0.113	0.020		**0.032**	**0.037**	(+)	0.134	0.016		*0.079*	**0.040**	(+)
Continentality	**0.006**	**0.060**	(-)	**0.004**	**0.068**	(-)	0.916	<0.001				

Table 3: Influence of the area and isolation (calculated from suitable or occupied localities) of the locality on probability of occurrence of *A. adulterinum* and *A. cuneifolium*. Univariate analysis is presented (a), as well as 2 models including both isolation (in each model one type of isolation) and area of the locality (b).

	A. adulterinum				*A. cuneifolium*			
	df	p	R2		df	p	R2	
a) univariable analysis								
area	79	**0.001**	0.147	(+)	61	0.053		
isolation (suitable)	79	< **0.001**	0.267	(−)	61	**0.016**	0.087	(−)
isolation (occupied)	79	< **0.001**	0.281	(−)	61	**0.032**	0.07	(−)
b) model including isolation and area of the locality								
isolation (suitable)	78	**0.002**	**0.120**	(−)	60	0.163		
area	78	0.94	< 0.001		60	0.859		
isolation (occupied)	78	**0.001**	**0.135**	(−)	60	0.344		
area	78	0.806			60	0.78		

Fig 1: Distribution of studied serpentine rocks in the landscape. Isolated rocks are marked by an arrow.

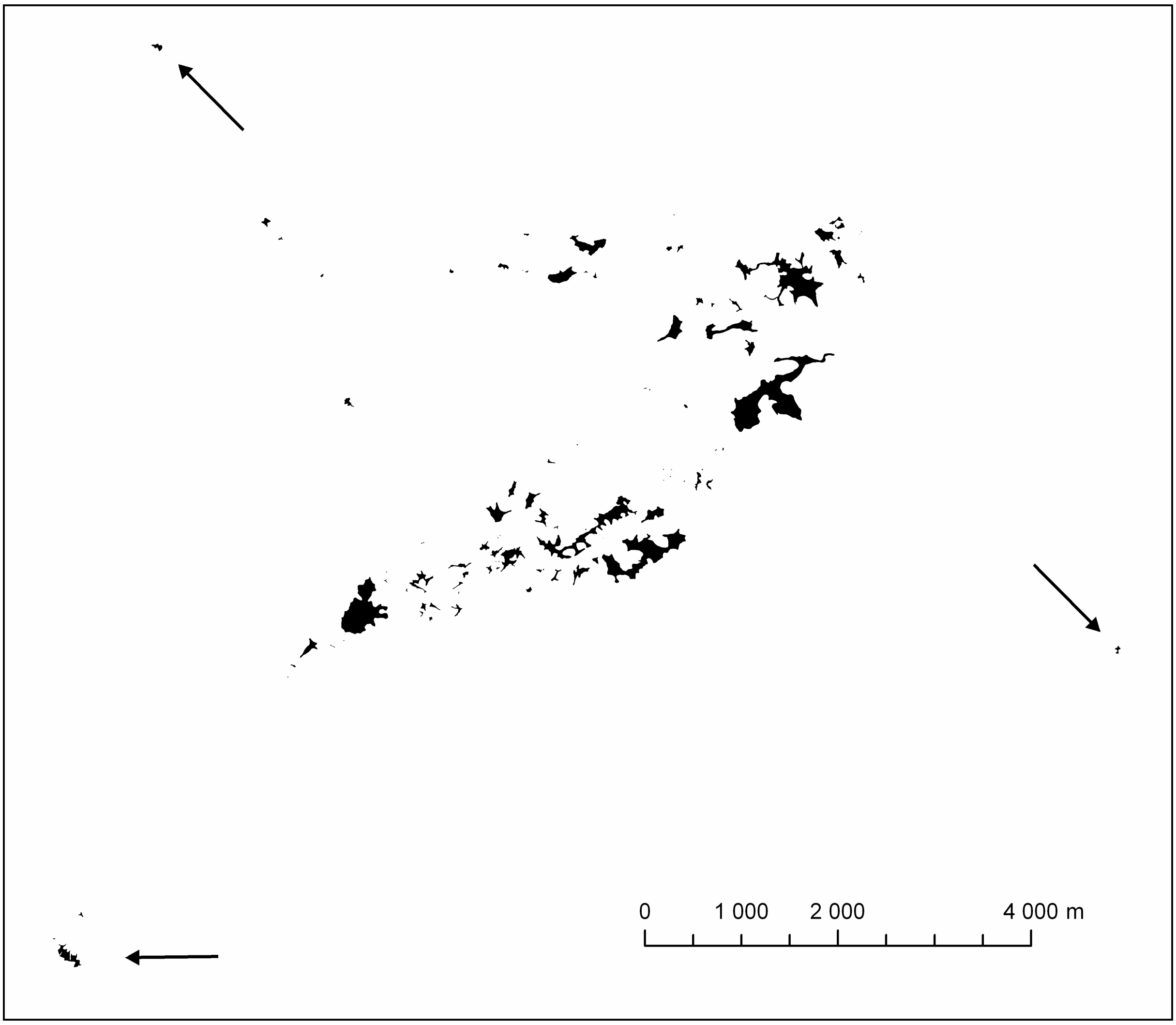
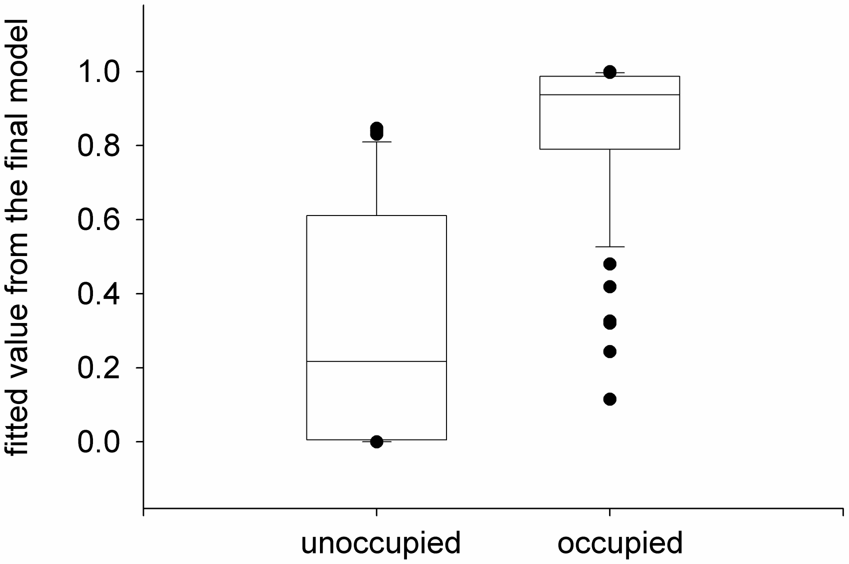
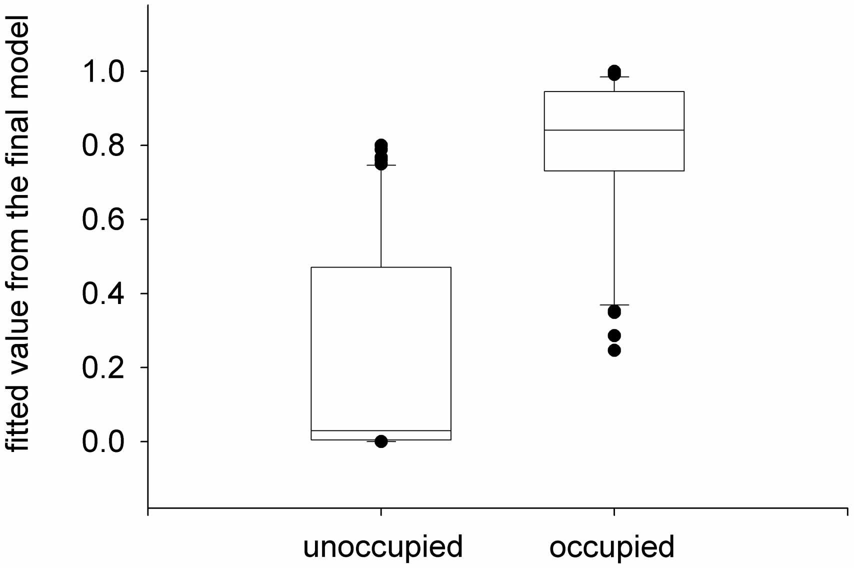


Fig 2: Fitted values from the final models for occupied and unoccupied localities for *A. adulterinum* (a) and *A. cuneifolium* (b). In the graph, the median, 25% and 75% quartiles are shown, whiskers indicate the10% and 90 % percentiles and dots are outlying values.

a) A. adulterinum



b) A. cuneifolium

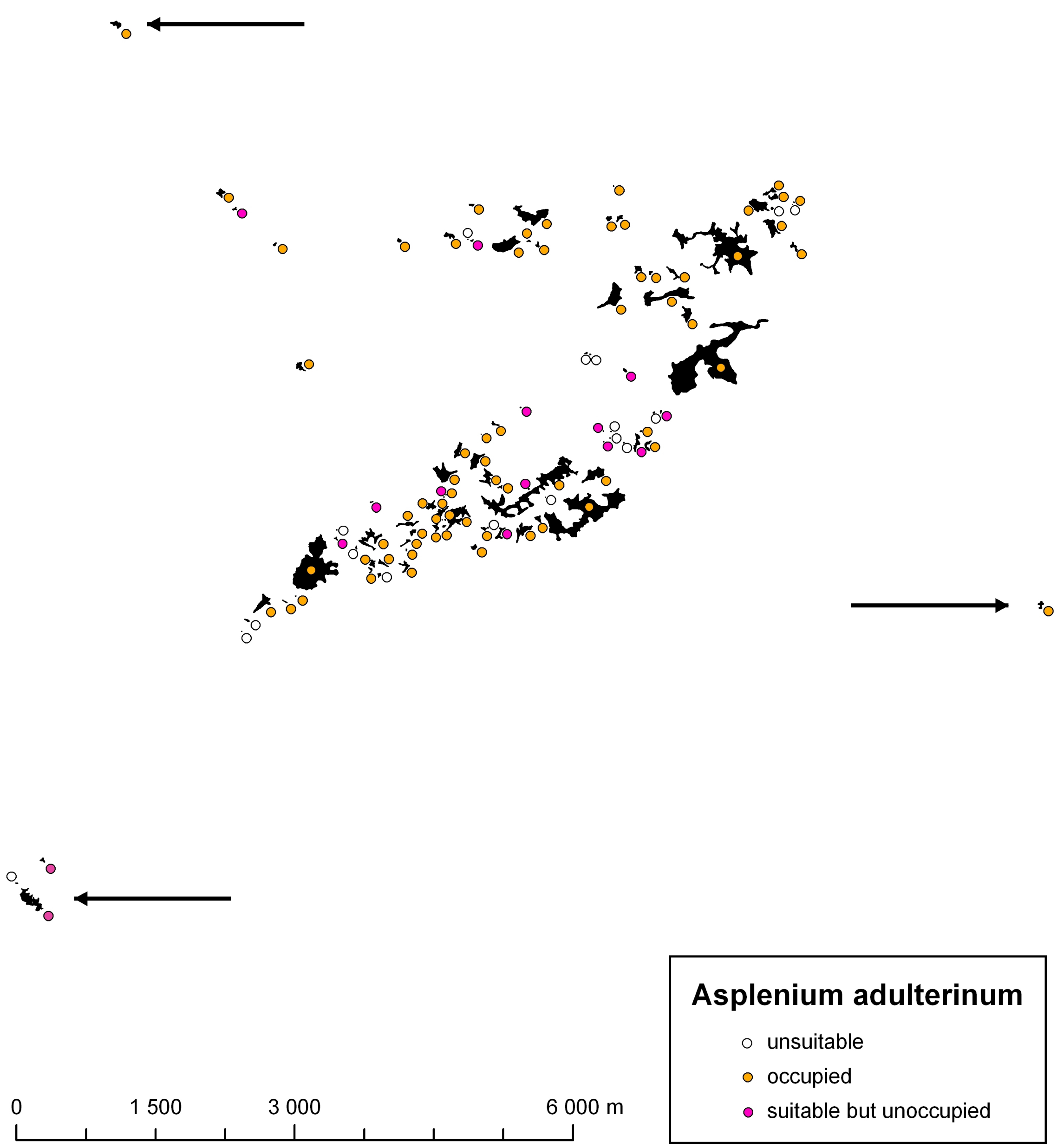


Appendix: Correlations among variables

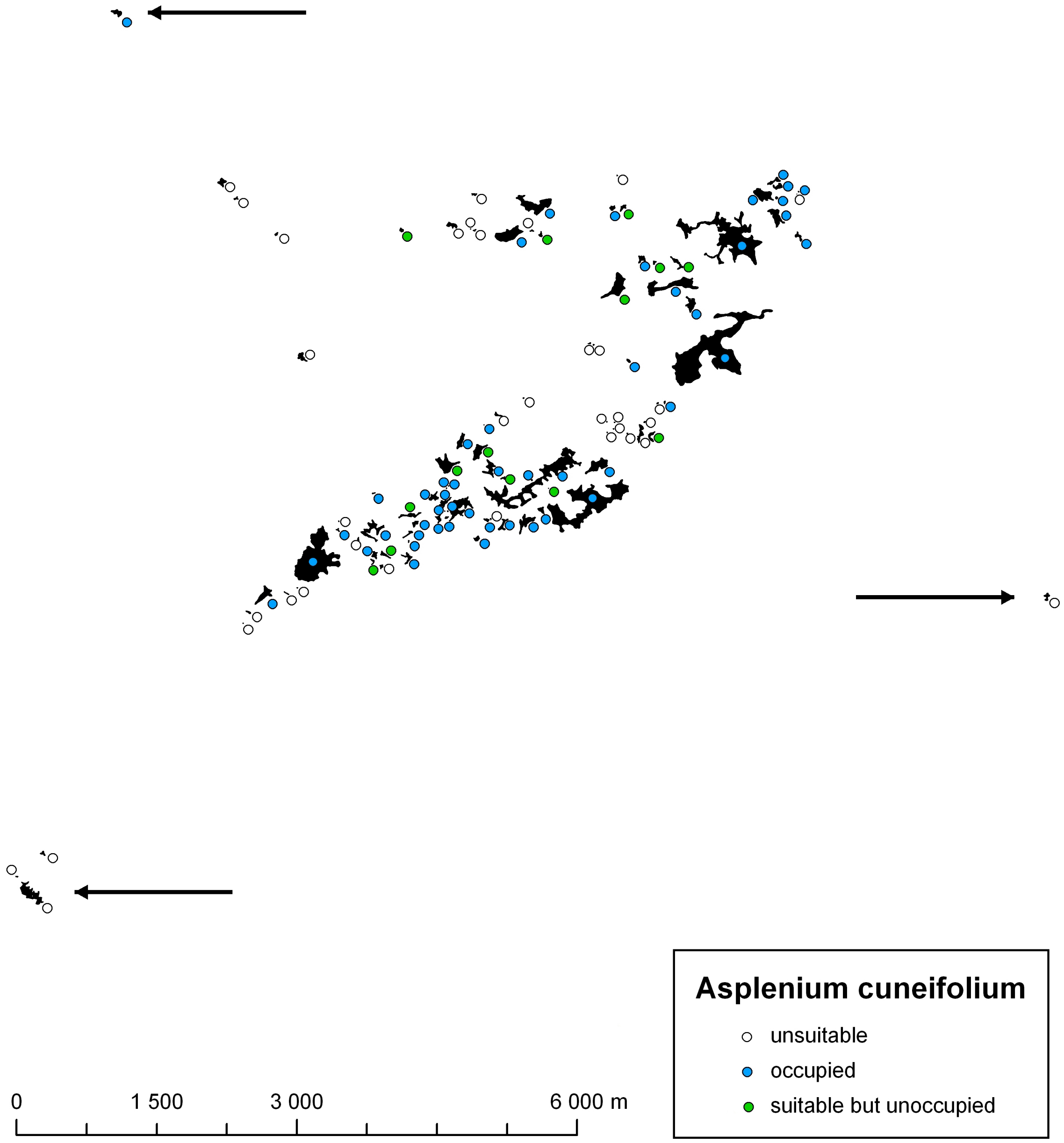
AA - A. adulterinum, AC - A. cuneifolium				**vegetation type**			**presence of species**		**presence of microhabitats**				**Beals index**		**Ellenberg values**						**isolation (log transformed) calculated from**				**habitat suitability**	
		height	**area (log transformed)**	**open**	**spruce**	**pine**	***AA***	***AC***	**crevices**	**scree**	**plato**	**terraces**	***AA***	***AC***	**Light**	**Temperature**	**Continentality**	**Moisture**	**Soil Reaction**	**Nutrients**	**suitable localities for AA**	**suitable localities for AC**	**occupied by AA**	**occupied by AC**	***A. adulterinum***	***A. cuneifolium***
	height		0.64	0.12	0.01	-0.05	0.36	0.28	0.25	0.36	0.47	0.39	0.22	-0.17	-0.05	0.12	-0.10	0.20	0.18	0.39	0.33	0.27	0.45	0.24	0.34	0.29
	area(log transformed)	0.64		-0.07	0.23	0.22	0.55	0.44	0.24	0.58	0.44	0.48	0.39	0.01	-0.04	0.09	0.08	0.18	0.06	0.29	0.57	0.51	0.64	0.48	0.48	0.42
vegetation type	**open**	0.12	-0.07		-0.48	-0.39	-0.09	-0.18	-0.04	-0.03	0.21	0.03	0.06	-0.42	0.58	0.40	-0.34	-0.11	0.45	0.27	-0.08	-0.09	-0.08	-0.09	-0.14	-0.31
	spruce	0.01	0.23	-0.48		-0.09	0.13	-0.06	-0.04	0.23	-0.06	-0.08	-0.08	0.15	-0.52	-0.43	0.30	0.24	-0.35	-0.04	0.10	0.08	0.07	0.08	0.12	-0.01
	pine	-0.05	0.22	-0.39	-0.09		0.09	0.41	0.04	0.11	0.07	0.12	0.13	0.35	-0.05	0.02	0.14	-0.25	-0.17	-0.30	0.31	0.30	0.34	0.29	0.19	0.45
presence of species	***AA***	0.36	0.55	-0.09	0.13	0.09		0.33	0.40	0.41	0.24	0.39	0.10	0.29	-0.05	0.06	0.17	0.09	0.14	-0.04	0.28	0.26	0.32	0.25	0.66	0.53
	AC	0.28	0.44	-0.18	-0.06	0.41	0.33		0.27	0.19	0.30	0.34	0.44	0.36	0.14	0.17	0.00	-0.22	0.20	-0.16	0.35	0.34	0.40	0.31	0.39	0.73
presence of microhabitats	**crevices**	0.25	0.24	-0.04	-0.04	0.04	0.40	0.27		0.08	0.19	0.29	0.04	-0.08	0.00	0.19	0.00	0.19	0.35	0.24	0.11	0.10	0.13	0.10	0.61	0.37
	scree	0.36	0.58	-0.03	0.23	0.11	0.41	0.19	0.08		0.30	0.39	0.25	-0.07	-0.12	0.12	0.04	0.29	0.03	0.33	0.23	0.21	0.26	0.19	0.41	0.22
	plato	0.47	0.44	0.21	-0.06	0.07	0.24	0.30	0.19	0.30		0.25	0.32	-0.05	0.16	0.24	-0.15	-0.01	0.27	0.26	0.22	0.16	0.34	0.14	0.20	0.28
	terraces	0.39	0.48	0.03	-0.08	0.12	0.39	0.34	0.29	0.39	0.25		0.20	-0.05	0.09	0.27	-0.08	0.15	0.18	0.26	0.23	0.21	0.26	0.20	0.41	0.35
Beals index	***AA***	0.22	0.39	0.06	-0.08	0.13	0.10	0.44	0.04	0.25	0.32	0.20		0.21	0.15	0.38	0.00	-0.07	0.27	0.25	0.25	0.22	0.28	0.21	0.09	0.36
	AC	-0.17	0.01	-0.42	0.15	0.35	0.29	0.36	-0.08	-0.07	-0.05	-0.05	0.21		-0.20	-0.17	0.18	-0.43	-0.30	-0.61	0.11	0.12	0.13	0.12	0.10	0.50
Ellenberg values	**Light**	-0.05	-0.04	0.58	-0.52	-0.05	-0.05	0.14	0.00	-0.12	0.16	0.09	0.15	-0.20		0.65	-0.33	-0.52	0.52	0.03	-0.06	-0.06	0.00	-0.06	-0.11	0.06
	Temperature	0.12	0.09	0.40	-0.43	0.02	0.06	0.17	0.19	0.12	0.24	0.27	0.38	-0.17	0.65		-0.39	-0.29	0.62	0.28	0.04	0.04	0.05	0.04	0.04	0.21
	Continentality	-0.10	0.08	-0.34	0.30	0.14	0.17	0.00	0.00	0.04	-0.15	-0.08	0.00	0.18	-0.33	-0.39		0.09	-0.33	-0.20	0.11	0.10	0.07	0.09	0.11	0.05

	Moisture	0.20	0.18	-0.11	0.24	-0.25	0.09	-0.22	0.19	0.29	-0.01	0.15	-0.07	-0.43	-0.52	-0.29	0.09		-0.12	0.52	0.01	0.01	-0.01	0.01	0.22	-0.28
	Soil_Reaction	0.18	0.06	0.45	-0.35	-0.17	0.14	0.20	0.35	0.03	0.27	0.18	0.27	-0.30	0.52	0.62	-0.33	-0.12		0.33	0.06	0.06	0.08	0.05	0.12	0.23
	Nutrients	0.39	0.29	0.27	-0.04	-0.30	-0.04	-0.16	0.24	0.33	0.26	0.26	0.25	-0.61	0.03	0.28	-0.20	0.52	0.33		0.03	0.03	0.02	0.02	0.03	-0.25
isolation (log transformed) calculated from	**suitable localities for AA**	0.33	0.57	-0.08	0.10	0.31	0.28	0.35	0.11	0.23	0.22	0.23	0.25	0.11	-0.06	0.04	0.11	0.01	0.06	0.03		0.99	0.79	0.97	0.19	0.30
	suitable localities for AC	0.27	0.51	-0.09	0.08	0.30	0.26	0.34	0.10	0.21	0.16	0.21	0.22	0.12	-0.06	0.04	0.10	0.01	0.06	0.03	0.99		0.72	1.00	0.17	0.28
	occupied by AA	0.45	0.64	-0.08	0.07	0.34	0.32	0.40	0.13	0.26	0.34	0.26	0.28	0.13	0.00	0.05	0.07	-0.01	0.08	0.02	0.79	0.72		0.69	0.22	0.35
	occupied by AC	0.24	0.48	-0.09	0.08	0.29	0.25	0.31	0.10	0.19	0.14	0.20	0.21	0.12	-0.06	0.04	0.09	0.01	0.05	0.02	0.97	1.00	0.69		0.16	0.26
habitat suitability	***AA***	0.34	0.48	-0.14	0.12	0.19	0.66	0.39	0.61	0.41	0.20	0.41	0.09	0.10	-0.11	0.04	0.11	0.22	0.12	0.03	0.19	0.17	0.22	0.16		0.50
	AC	0.29	0.42	-0.31	-0.01	0.45	0.53	0.73	0.37	0.22	0.28	0.35	0.36	0.50	0.06	0.21	0.05	-0.28	0.23	-0.25	0.30	0.28	0.35	0.26	0.50	

Appendix: Unsuitable, suitable occupied and suitable empty habitats of *A. adulterinum*. Remote habitats are marked by arrow.



Appendix: Unsuitable, suitable occupied and suitable empty habitats of *A. cuneifolium*. Remote habitats are marked by arrow.



Paper 3

GENE FLOW AMONG POPULATIONS OF TWO RARE CO-OCCURRING FERN SPECIES DIFFERING IN PLOIDY LEVEL

Anna Bucharová and Zuzana Münzbergová

Manuscript

Gene flow among populations of two rare co-occurring fern species differing in ploidy level

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ABSTRACT

Differences in ploidy level between different fern species have vast influence on their mating system and consequently on their colonization ability. Specifically, tetraploids, in contrast of diploids, are able of intragametophytic selfing. Because fertilization is a postdispersal process in ferns, ability of selfing results into better colonization abilities in tetraploids, thanks to the ability of single spore colonization.

Better colonization abilities of the tetraploid species were confirmed by our previous study. In the present study, we aimed on gene flow among existing populations. We analyzed genetic structure of selection of population in the study region of size 10 ×10 km using isoenzymes.

Genetic variation in tetraploid species is distributed mainly among populations, genetic distance between populations is correlated with geographical distance and larger populations host more genetic diversity than smaller populations. In the diploid species, most variability is partitioned within populations, genetic distance is not related to geographic distance and genetic diversity of populations is not related to the population size. It suggests that in the tetraploid species, which is expected to be selfing, gene flow is limited. In contrast, in the diploid species, which is likely outcrossing, gene flow is extensive and whole system behaves rather as one large population.

Our results show that in ferns, ability to colonize new habitats and gene flow among existing populations are facilitated by the same species traits (mating system), but in opposite direction.

INTRODUCTION

Breeding behavior and life history peculiarities play an important role in shaping genetic composition of populations. Consequently description of distribution of genetic diversity may help to elucidate these processes (Schneller & Liebst 2007). In this respect, quite a lot of attention was paid to ferns (e.g. Mccauley et al. 1985; Soltis & Soltis 1987a, 1989; Masuyama & Watano 1990; Vogel et al. 1999a), since they are rather attractive due to special life cycle with independently growing haploid and diploid phases, connected with peculiar breeding systems, which have a vast influence on genetic structure of the populations. Three types of fertilization in ferns can be expected (Lloyd 1974): (i) intra-gametophythic selfing (fusion of sperm and egg from the same gametophyte resulting in complete homozygote, which is equivalent to apomixis in seed plants); (ii) inter-gametophytic selfing (fusion of sperm and egg from different gametophytes derived from the same parental sporophyte, which is equivalent to selfing in seed plants); (iii) outcrossing (the fusion of sperm and egg from gametophytes derived from spores of different sporophytes). These three types of fertilization have very different effects on patterns of genetic diversity, thus information on mating system was derived from genetic structure in many studies of homosporous ferns, (e.g. Soltis & Soltis 1992; Vogel et al. 1999a; Vogel et al. 1999b; Pryor et al. 2001; Kang et al. 2008; de Groot et al. 2010).

The occurrence of different types of mating system in ferns was shown to be closely interlinked with ploidy level – outcrossing mainly in diploids and selfing in polyploids (Soltis & Soltis 1992; Vogel et al. 1999b). Higher selfing rates in polyploids are explained by the fact that they are more resistant to inbreeding due to the fixed heterozygosity (Masuyama & Watano 1990). It was even suggested and often repeated in literature that polyploids are strictly selfing and diploid strictly outcrossing.

Fertilization in ferns occurs on gametophyte which originates from spores. It is of crucial importance from dispersal point of view. In seed plants, fertilization takes place prior dispersal, before the seed is formed. For colonization of a new habitat, theoretically, one seed is enough. In ferns, fertilization is a post dispersal process on haploid gametophyte. For fern species that are not able of intra-gametophytic selfing it means that two spores have to fall in close proximity in favorable conditions on a new habitat. Gametophyte has to develop from both of them with archegonia/anteridia and only then fertilization can occur. Colonization of new habitat is problematic in this case (Peck et al. 1990) and spores transported to long distances would be unlikely to establish close enough to allow intergametophytic selfing or crossing, despite huge spore production in ferns. On the other hand, if the species is able of an intra-gametophytic selfing, one spore which develops in a hermaphroditic gametophyte is enough to establish a new, totally homozygous population. Many species, where intra-gametophytic selfing is the main breeding system, are great colonists on continental scale (Schneller & Holderegger 1996a; Suter et al. 2000; Lott et al. 2003); it is especially obvious in comparison of distribution of selfing tetraploids with their outcrossing diploid ancestors (Vogel et al. 1999b).

In general it is expected that most diploid fern species are outcrossing and are thus bad colonists. In contrast, polyploids are selfing and should thus be good colonists (but exception exist, see e.g. Jimenez et al. 2009; de Groot et al. 2010). However, direct comparisons of genetic structure of species representing these two different mating strategies are scarce, and were performed only on continental scale using only a few populations (Jimenez et al. 2009). The only study which aimed on comparison of several species on regional scale is Schneller & Holderegger (1996b). This study, however, used only a very limited number of populations per species, which does not allow any rigorous analysis. In present study, we aimed at comparing distribution of genetic diversity in larger number of populations of diploid and tetraploid fern species co-occurring in the same region.

A clear limit of studies on distribution of genetic diversity within and among populations is that it is difficult to distinguish between gene flow among already established populations and processes during colonization of new habitats (but see de Groot et al. 2010). However, distinguishing between these two processes is crucial. During colonization of empty habitats, selfing species have higher probability of establishing on a vacant place and selfing thus strongly increases the probability of colonization. In contrast, in the case of gene flow between already established populations, outcrossing is more advantageous as it facilitates implementation of new genetic information into gene pool of the given population thus enhancing gene flow among populations. To separate these two types of processes, information on genetic structure of population is not sufficient. Additional information on colonization rates in the system is necessary as both processes in the different phases of population development are combined together in the current patterns of genetic diversity.

In the present study, we employ analysis of genetic structure of populations to investigate gene flow among populations of two rare fern species *Asplenium adulterinum* and *A. cuneifolium* differing in ploidy level and likely also in breeding system. In our model system, the two species occupy very similar habitats – serpentine rocks, which are scattered on area of 10x10km. According our previous study (Tájek et al., in press), vacant but suitable habitats (serpentine rocks) exist in the study system for both species, with higher proportion of suitable unoccupied habitats in diploid *A. cuneifolium* than in tetraploid *A. adulterinum*, suggesting that tetraploid and thus likely selfing species *A. adulterinum* is better colonist of free habitats than the diploid and thus likely outcrossing *A. cuneifolium*. Tetraploid *A. adulterinum* has also faster metapopulation dynamics within the study system than diploid *A. cuneifolium* (Bucharová et al. 2010).

Thus, our study system provides a unique opportunity to distinguish between ability of species to colonize new habitats (based on habitat occupancy analysis in previous study) and gene flow between already established populations (in this study, using analysis of genetic structure of the populations) and compare these processes between the two fern species with different mating system.

We hypothesize, that in tetraploid *A. adulterinum*, selfing is the prevailing mating system while outcrossing is the prevailing mating system in diploid *A. cuneifolium*. Genetic diversity will thus be distributed mainly among populations in *A. adulterinum*, whereas more diversity will be within populations in *A. cuneifolium*, as in other outcrossing species (e.g. Soltis & Soltis 1992; Schneller & Liebst 2007). Gene flow will be more effective between populations of diploid *A. cuneifolium* than between populations of tetraploid *A. adulterinum*, resulting in more profound spatial substructure of genetic variability in *A. adulterinum*. Isolation by distance between populations will thus be detectable rather in *A. adulterinum* than in *A. cuneifolium*. The existence of isolation by distance is, however, not easy to predict as lack of such a relationship can be both no gene flow as well as very extensive gene flow at the given spatial scale.

METHODS

Study species

The study works with two fern species, *Asplenium adulterinum* Milde and *Asplenium cuneifolium* Viv. (Aspleniaceae), both restricted to serpentine substrate in Europe (Tutin et al. 1980). *A. adulterinum* occurs also in one population on Vancouver Island, Canada (Ogilvie & Ceska 1984). Ranges of distribution of both species are highly scattered, following distribution of serpentine rocks in Europe from Mediterranean to Norway and from Greece to Spain (*A. cuneifolium*, *A. adulterinum* only from Austria to France). In the Czech Republic, the species occur mainly in Western Bohemia (Slavkovský les), with several localities in north-eastern Bohemia. In the rest of the country, there are only very few small populations. *A. cuneifolium* is in general more widespread than *A. adulterinum*.

Both species are rare and under the interest of nature conservation throughout Europe

Both species are rare and under the interest of nature conservation throughout Europe (Tutin et al. 1980). *A. adulterinum* is even a species of interest of European ecological network Natura 2000 (web1). The species differ in ploidy level – *A. adulterinum* is allotetraploid - parental species *A. viride* L. and *A. trichomanes* Huds. subsp. *trichomanes*, (Lovis 1955), A. *cuneifolium* is diploid (Hejný & Slavík 1980).

Study site

The study was carried out in the region of Slavkovský les, Western Bohemia, Czech Republic. In this region of ca 10 × 10 km, 98 serpentine rocks are scattered in the landscape. The system is rather isolated from other population of both species, the nearest other population is 50 km to the west. Both *A. adulterinum* and *A. cuneifolium* are quite common in the area. *A. adulterinum* is more common and occupies both rocks in unforested habitat and in the forest (dominated by *Pinus sylvestris* and *Picea abies*). In total there are 66 populations located all over the area ranging from several individuals to nearly 2000 individuals (Tájek 2003). *A cuneifolium* prefers rocks under the forest canopy. The unforested rocks are inhabited rarely and the populations are very small here. In total, there are 48 populations of *A. cuneifolium* in the study region, mostly in the central area with several more distant localities, ranging from several individuals up to several hundreds individuals (Tájek 2003).

For both species, unoccupied suitable habitats exist in the area; *A. adulterinum* occupies 81% of suitable habitats, *A. cuneifolium* 73%, indicating metapopulation dynamics in the study system (Tájek et al., in press).

Sample collection

Samples for isoenzyme analysis were collected from 14 localities in *A. adulterinum* and 12 localities in *A. cuneifolium*. Sampling design follows distribution of the species in the study area (*A. adulterinum* has more populations in total). If available, 20 plants per population were sampled per species. In total, we sampled 268 individuals of *A. adulterinum* and 227 individuals of *A. cuneifolium*.

Samples were evenly distributed over each locality, to represent whole range of variability within the population (under an assumption that geographically more distant plants are less related). We sampled 1-2 young leaves without spores per plant, paying attention not to damage the plant seriously. Position of each plant was recorded using GPS or marked to a map in field, followed by digitalization of the map.

Isoenzyme analysis

Samples collected in field were kept on ice for 24-48 hours till isoenzyme extraction in laboratory. Electrophoresis was performed on crude protein extracts of leaf material. All enzymes were resolved on polyacrylamide gels using 8.16% separating gel and 4% stacking gel.

Nine enzymatic systems were studied, 7 of them provided interpretable pattern and were variable at least for one of the study species: LAP, DIA, 6-PGDH, SHDH, PGM, ADH and AAT. For detailed methodic of isoenzyme extraction, electrophoresis and staining procedure see online Appendix 1.

Band interpretation

Bands were interpreted in two ways. First, only presence/absence of alleles was recorded and the data were further treated as a dominant marker (for similar approach e.g.

(Dias et al. 2008). Dominant data approach was chosen due to the fact that *A. adulterinum* is allotetraploid and it was rarely possible to asses the exact ratio of present alleles on the basis of intensity of the bands. Moreover, allotetraploids have often fixed pairs of alleles segregating always together (Suter et al. 2000). Thus, heritability may be rather disomic than tetrasomic and to reliably distinguish which alleles segregate together, as de facto one allele, is often impossible. As a result, recording only presence/absence of the alleles was the only correct way how to treat the obtained pattern in all enzymatic systems. In *A. cuneifolium*, the same approach of treating the data as dominant marker was used to be able to compare among the species.

Further, we also evaluated the data as co-dominant marker. It was possible in all enzymatic systems in *A. cuneifolium*, but only in one system in *A. adulterinum* – where we have been able to distinguish how the alleles segregate in fixed pairs (see Suter et al. 2000). Despite these data are very limited in *A. adulterinum* and it is necessary to interpret them with caution, they brought interesting insight in comparison of mating systems of the two species.

Statistical analysis

Dominant marker

Binary (presence/absence) data were prepared in program FAMD (Schluter & Harris 2006) for import to program Arlequin (Excoffier & Lischer 2010), where most of the analyses were carried out. Mantel test was performed in program PopTools (Hood 2010).

Mean gene diversity (Nei 1987) was calculated for each population and averaged over species. Further, number of haplotypes (band patterns) for each species and population was calculated. Distribution of genetic variability among and within populations was investigated using AMOVA (Excoffier et al. 1992) and tested using permutation test (1000 permutations). Mantel test (Smouse et al. 1986) performed as comparison of pairwise F_{st} between populations and geographic distance (in meters) between centroids of localities hosting the populations (obtained in study Tájek et al., in press). Relationship between total size of population and its genetic diversity was examined using simple linear regression in program R (R Development Core Team 2009).

Co-dominant marker

We calculated mean expected and observed heterozygosity and inbreeding coefficient (Nei 1987) over all populations for both species in program PopGene (Yeh & Boyle 1997).

RESULTS

Band pattern

The 7 enzymatic systems provided in a total 9 interpretable loci: AAT, ADH-1, ADH-2, DIA, LAP-1, LAP-2, 6-PGHD, PGM and SHDH. In tetraploid *A. adulterinum*, 4 loci were variable: LAP-1, DIA, 6-PGDH, SHDH), In diploid *A. cuneifolium*, 7 loci were variable: AAT, ADH-1, ADH-2, LAP-1, LAP-2, PGM and SHDH. Due to the fact that *A. cuneifolium* and *A. adulterinum* are not closely related species, loci are not always exactly corresponding. However, for purpose of the analyses, it is important that to have the same total amount of loci for both species. Each of the 9 loci had 2 alleles, resulting into data matrix of 18 (presence/absence of an allele) × total number of samples.

In tetraploid *A. adulterinum*, only one locus (LAP-1) could be reliably evaluated as allelic data. This locus showed either fixed heterozygosity (balanced pattern AABB), of fixed homozygosity (pattern AAAA) showing diploid type of inheritance (due to disomic heritability in allotetrapoids). Rarely, clear pattern AAAB was observed (in 2.6% of examined plants, see Appendix 2). This unbalanced pattern was interpreted as heterozygote of fixed allele pairs AA and AB see (Suter et al. 2000). In diploid *A. cuneifolium*, all polymorphic enzymatic systems were evaluated as diploid allelic data.

Dominant marker

Mean gene diversity over localities was 0.94 (ranging from 0.93 to 0.99) in diploid *A. cuneifolium* and 0.47 (ranging from 0.029 to 0.8) in tetraploid *A. adulterinum*. In. *A. adulterinum*, only 14 haplotypes were present in the whole dataset, separate populations containing 2-8 haplotypes. In *A. cuneifolium*, 96 haplotypes were present in the whole dataset, separate populations containing 4-19 haplotypes.

In diploid *A. cuneifolium*, 81.0 % of genetic variation was within populations and only 19.0 % was among populations ($F_{st} = 0.190$, $p < 0.0001$). It contrasts with tetraploid *A. adulterinum*, where 40.6% of genetic variation was within populations and 59.4% among populations ($F_{st} = 0.594$, $p < 0.0001$), Fig 1.

Correlation between geographic and genetic distance between populations was highly significant ($R^2 = 0.112$, $p = 0.001$) in tetraploid *A. adulterinum*. In contrast, in diploid *A. cuneifolium* the relationship was not significant ($R^2 = 0.009$, $p = 0.317$), Fig 2. Larger populations of *A. adulterinum* host more genetic diversity. This relationship was, however, only marginally significant ($R^2 = 0.152$, $p = 0.093$). In *A. cuneifolium* no relationship between size of population and genetic diversity was observed ($p = 0.290$).

Co-dominant marker

In *A. adulterinum*, according to the one enzymatic system which allowed reliable co-dominant scoring, there was very high deficiency of heterozygotes. In *A. cuneifolium*, observed and expected heterozygosity was much more balanced in all enzymatic systems (tab1).

DISCUSSION

In the present study, we estimated genetic variability and structure of two rare fern species differing in ploidy level – allotetraploid *Asplenium adulterinum* and diploid *A. cuneifolium*. Since we analyzed the genetic structure on the same study system (area 10 × 10 km), we can directly compare gene flow between populations in the two species on regional spatial scale.

Mating system

From genetic structure of populations of the two species, it is possible to assess their mating system. In allotetraploid *A. adulterinum*, genetic variation is low and it is partitioned rather within than between populations. Additionally, the one enzymatic system shows striking lack of heterozygotes – we found only 2.6% of heterozygotes in this species, resulting in high inbreeding coefficient (0.86). This inbreeding coefficient is much higher that inbreeding coefficients obtained from any enzymatic systems in (predominantly outcrossing) diploid *A. cuneifolium*. High level of inbreeding corresponds to other tetraploids *Asplenium trichomanes* subsp. *quadrivalens* (Suter et al. 2000) and *A. adiantum nigrum* (Ranker et al. 1994) and similar genetic structure is commonly found in fern species with prevailing intragametophytic selfing (e.g. McCauley et al. 1985; Vogel et al. 1999a). However, we clearly proved existence of individuals originating from outcrossing, which provides evidence for mixed mating system in this species. We are aware that information obtained from co-dominant interpretation of the banding pattern is based on single enzymatic system, but the result is corresponding to literature published on similar species, difference towards diploid *A. cuneifolium* is remarkable and thus, we believe our conclusion is valid.

In diploid *A. cuneifolium*, genetic structure of populations is very different than in *A. adulterinum*. Genetic variation is much higher and it is partitioned rather within than among populations and inbreeding coefficient is low (mean 0.21, varying from -0.13 to 0.38 in single loci), suggesting low level of inbreeding. The results agree with literature that this species, as a diploid, is primarily outcrossing (Masuyama & Watano 1990; Soltis & Soltis 1992).

It was often suggested, that diploids may be obligate, or almost obligate outcrossers (Soltis & Soltis 1992; Vogel et al. 1999b). However, this statement on inability of intragametophytic selfing in diploids is based on indirect data only – on inbreeding coefficient and distribution of the genetic diversity e.g. (Ranker et al. 1994; Vogel et al. 1999b; Hunt et al. 2009; Soltis & Soltis 1987b, 1992; Masuyama & Watano 1990 and literature there). Interestingly, experimental studies on breeding systems in ferns (e.g. Suter et al. 2000; Lott et al. 2003; Flinn 2006; de Groot et al. 2010) show rather opposite. They mostly conclude that given species, regardless if diploid or tetraploid, are able of both – intragametophytic selfing and outcrossing, just in slightly different ratio. One study (Suter et al. 2000) even performed breeding experiments and analysis of genetic structure of populations in the same species, and in breeding experiment found that outcrossing increases the probability of sporophyte formation in comparison with intragametophytic selfing, but genetic structure suggest high predominance of selfing.

The reasons for this clear discrepancy may lay in the fate of sporophytes, since both inbreeding and outbreeding depression was documented in ferns. Schneller (1996) found in *Asplenium ruta-muraria* (tetraploid) presence of outbreeding depression, since sporophytes originating from outcrossing had large number of abortive spores. On the other hand, inbreeding depression on the level of sporophyte performance was documented in originally outcrossing *Athyrium filix-femina*, with lower fitness of individuals originating from gametophytic selfing (Schneller & Holderegger 1997). The study concluded that in natural populations, products of intragametophytic selfing are largely excluded from populations via selection. Thus, to infer obligate ability/disability of intragametophytic selfing of a species on basis of genetic structure of population may be misleading, since genetic structure is result not only selfing abilities, but is also affected by later selection on the level of sporophytes. Ability of intragametophytic selfing is of crucial importance for single spore colonization of new habitats.

Gene flow among populations

Our study revealed that the two species strongly differ in genetic structure of populations on regional spatial scale (ca 10 km). Populations of tetraploid *A. adulterinum* are strongly genetically differentiated (corresponding to other predominantly selfing fern species (e.g. Schneller & Holderegger 1996b; Landergott et al. 2001), genetic distance between populations increases with geographical distance and larger populations contain more genetic variability. Gene flow among populations of this selfing species is rather limited. In contrast, in the diploid *A. cuneifolium* there is a total lack of any relationship between genetic and geographic distance and most of the genetic diversity can be found within populations. It suggests that the whole system of this species functions as one large population with frequent dispersal all over the area and high level of gene flow, as often found in various outcrossing fern species (e.g. Hooper & Haufler 1997; Maki & Asada 1998; Schneller & Liebst 2007; but see Hunt et al. 2009). Another possible explanation for the pattern found in *A. cuneifolium* is that the species disperses extremely badly and populations do not communicate at all. They are product of some processes in the past and genetic drift still did not differentiate the populations. In ferns, with small, well dispersing spores and on scale of several kilometers, rather the first option is probable – extensive gene flow among populations.

Conclusion

In tetraploid *A. adulterinum* we found more genetic variation partitioned between populations than within population and the other way around in *A. cuneifolium*, confirming that *A. adulterinum* is predominantly selfing and *A. cuneifolium* is predominantly outcrossing. Higher colonization ability due to higher selfing rate in *A. adulterinum* is in agreement with our previous conclusion that *A. adulterinum* is a better colonist of empty habitats than *A. cuneifolium* (Tájek et al, in press).

Predominantly selfing tetraploid species *A. adulterinum* is a good colonist of empty patches, but subsequent gene flow between populations is rather limited. Founder effect plays a role here, the patch is occupied by few early arriving genotypes which do not mix (or to a very limited degree) with other genotypes. If outcrossing occurs, its product may be excluded due to outbreeding depression (Schneller 1996). As a result, several independent genotypes exist on patches and reproduce mostly via selfing and populations are thus highly differentiated.

On the other hand, in outcrossing species *A. cuneifolium*, the colonization of empty patches is more difficult in comparison with the selfing species. However, outcrossing facilitates effective gene flow between already established populations, since newly arriving genotype is via crossing with local gametophytes incorporated into population gene pool, as suggested de Groot et al. (2010). Moreover, if inbreeding occurs in predominantly outbreeding species, its product may have disadvantage due to inbreeding depression (Schneller & Holderegger 1997) and may be excluded from the population. Resulting effective gene flow diminishes any spatial structure among populations.

Results suggest, that there are completely opposing processes facilitating colonization of new habitats and gene flow between the existing habitats.

ACKNOWLEDGEMENT:

We thank to Ivana Plačková and staff of Isozyme laboratory of the Institute of Botany ASCR for processing the analysis, to J. Štěpánek and J. Chrtek for precious advices on interpretation of isozyme banging pattern. This work was supported by MSMT [2B06178], FRVS [348/2004] and GAUK [176/2006] and partly by MSMT 0021620828 and AV0Z60050516.

REFERENCES

Bucharová, A., Z. Münzbergová, and P. Tájek. 2010. Population Biology of Two Rare Fern Species: Long Life and Long-Lasting Stability. American Journal of Botany 97:1260-1271.

de Groot, G. A., E. R. J. Wubs, H. J. During, J. C. Vogel, M. Grundmann, P. Bremer, and H. Schneider. 2010. Mixed mating system in the fern Asplenium scolopendrium: implications for colonization potential. Annals of Botany 106:583-590.

Dias, P. M. B., V. F. Pretz, M. Dall'Agnol, M. T. Schifino-Wittmann, and J. A. Zuanazzi. 2008. Analysis of genetic diversity in the core collection of red clover (*Trifolium pratense*) with isozyme and RAPD markers. Crop Breeding and Applied Biotechnology 8:202-211.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. Genetics 131:479-491.

Flinn, K. M. 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. American Journal of Botany 93:1289-1294.

Hejný, S., and H. Slavík, editors. 1980. Flora of Czech Republic (Květena České Republiky). Academia, Praha.

Hood, G. M. 2010. PopTools version 3.2.3. Available on the internet.

Hooper, E. A., and C. H. Haufler. 1997. Genetic diversity and breeding system in a group of neotropical epiphytic ferns (*Pleopeltis*; Polypodiaceae). American Journal of Botany 84:1664-1674.

Hunt, H. V., S. W. Ansell, S. J. Russell, H. Schneider, and J. C. Vogel. 2009. Genetic diversity and phylogeography in two diploid ferns, *Asplenium fontanum* subsp *fontanum* and *A. petrarchae* subsp *bivalens*, in the western Mediterranean. Molecular Ecology 18:4940-4954.

Jimenez, A., L. G. Quintanilla, S. Pajaron, and E. Pangua. 2009. Genetic variation in the allotetraploid *Dryopteris corleyi* (Dryopteridaceae) and its diploid parental species in the Iberian peninsula. American Journal of Botany 96:1880-1886.

Kang, M., H. W. Huang, M. X. Jiang, and A. J. Lowe. 2008. Understanding population structure and historical demography in a conservation context: population genetics of an endangered fern. Diversity and Distributions 14:799-807.

Landergott, U., R. Holderegger, G. Kozlowski, and J. J. Schneller. 2001. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. Heredity 87:344-355.

Lloyd, R. M. 1974. Reproductive Biology and Evolution in Pteridophyta. Annals of the Missouri Botanical Garden 61:318-331.

Lott, M. S., J. C. Volin, R. W. Pemberton, and D. E. Austin. 2003. The reproductive biology of the invasive ferns *Lygodium microphyllum* and *L. japonicum* (Schizaeaceae): Implications for invasive potential. American Journal of Botany 90:1144-1152.

Lovis, J. D. 1955. *Asplenium adulterinum* and its probable parents. Proc. Bot. Soc. Br. Isl. 1:2.

Maki, M., and Y. J. Asada. 1998. High genetic variability revealed by allozymic loci in the narrow endemic fern *Polystichum otomasui* (Dryopteridaceae). Heredity 80:604-610.

Masuyama, S., and Y. Watano. 1990. Trends for inbreeding in polyploid pteridophytes. Plant Species Biology 5:13-17.

McCauley, D. E., D. P. Whittier, and L. M. Reilly. 1985. Inbreeding and the Rate of Self-Fertilization in a Grape Fern, *Botrychium dissectum*. American Journal of Botany 72:1978-1981.

Nei, M. 1987. Molecular Evolutionary Genetics Columbia University Press New York.

Ogilvie, R. T., and A. Ceska. 1984. Alpine Plants of Phytogeographic Interest on Northwestern Vancouver-Island. Canadian Journal of Botany 62:2356-2362.

Peck, J. H., C. J. Peck, and D. R. Farrar. 1990. Influences of Life-History Attributes on Formation of Local and Distant Fern Populations. American Fern Journal 80:126-142.

Pryor, K. V., J. E. Young, F. J. Rumsey, K. J. Edwards, M. W. Bruford, and H. J. Rogers. 2001. Diversity, genetic structure and evidence of outcrossing in British populations of the rock fern Adiantum capillus-veneris using microsatellites. Molecular Ecology 10:1881-1894.

R_Development_Core_Team. 2009. R: A Language and Environment for Statistical Computing, Vienna.

Ranker, T. A., S. K. Floyd, M. D. Windham, and P. G. Trapp. 1994. Historical Biogeography of *Asplenium adiantum-nigrum* (Aspleniaceae) in North-America and Implications for Speciation Theory in Homosporous Pteridophytes. American Journal of Botany 81:776-781.

Schluter, P. M., and S. A. Harris. 2006. Analysis of multilocus fingerprinting data sets containing missing data. Molecular Ecology Notes 6:569-572.

Schneller, J., and B. Liebst. 2007. Patterns of variation of a common fern (*Athyrium filix-femina*; Woodsiaceae): Population structure along and between altitudinal gradients. American Journal of Botany 94:965-971.

Schneller, J. J. 1996. Outbreeding depression in the fern *Asplenium ruta-muraria* L: Evidence from enzyme electrophoresis, meiotic irregularities and reduced spore viability. Biological Journal of the Linnean Society 59:281-295.

Schneller, J. J., and R. Holderegger. 1996a. Colonisation events and genetic variability within populations of *Asplenium ruta-muraria* L. Pages 571-580 in J. M. Camus, M. Gibby, and R. J. Johns, editors. Pteridology in Perspective. Royal Botanic Gardens, Kew.

Schneller, J. J., and R. Holderegger. 1996b. Genetic variation in small, isolated fern populations. Journal of Vegetation Science 7:113-120.

Schneller, J. J., and R. Holderegger. 1997. Vigor and survival of inbred and outbred progeny of Athyrium filix-femina. International Journal of Plant Sciences 158:79-82.

Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple-Regression and Correlation Extensions of the Mantel Test of Matrix Correspondence. Systematic Zoology 35:627-632.

Soltis, D. E., and P. S. Soltis. 1987a. Breeding System of the fern *Dryopteris expansa* - Evidence for Mixed Mating. American Journal of Botany 74:504-509.

Soltis, D. E., and P. S. Soltis. 1987b. Polyploidy and Breeding Systems in Homosporous Pteridophyta - a Reevaluation. American Naturalist 130:219-232.

Soltis, D. E., and P. S. Soltis. 1989. Polyploidy, breeding system, and genetic differentiation in homosporous pteridophytes. Pages 241-258 in D. E. Soltis, and P. S. Soltis, editors. Isozymes in Plant Biology. Dioscorides Press, Portland.

Soltis, D. E., and P. S. Soltis. 1992. The Distribution of Selfing Rates in Homosporous Ferns. American Journal of Botany 79:97-100.

Suter, M., J. J. Schneller, and J. C. Vogel. 2000. Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp quadrivalens. International Journal of Plant Sciences 161:233-244.

Tájek, P. 2003. Serpentine outcrops in Slavkovský les in view of island biogeography. (Hadcové východy ve Slavkovském lese z pohledu ostrovní biogeografie). Diploma thesis. Department of Botany, Charles University, Praha.

Tájek P., Bucharová A., Münzbergová Z. (in press): Limitation of distribution of two rare ferns in fragmented landscape. Acta Oecologica.

Tutin, T. G., V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters, and D. A. Webb. 1980. Flora Europaea. Cambridge University Press.

Vogel, J. C., F. J. Rumsey, S. J. Russell, C. J. Cox, J. S. Holmes, W. Bujnoch, C. Starks, J. A. Barrett, and M. Gibby. 1999a. Genetic structure, reproductive biology and ecology of isolated populations of *Asplenium csikii* (Aspleniaceae, Pteridophyta). Heredity 83:604-612.

Vogel, J. C., F. J. Rumsey, J. J. Schneller, J. A. Barrett, and M. Gibby. 1999b. Where are the glacial refugia in Europe? Evidence from pteridophytes. Biological Journal of the Linnean Society 66:23-37.

web1. ec.europa.eu/environment/nature/natura2000.

Yeh, F. C., and T. J. B. Boyle. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129:1.

Tab1: Expected and observed heterozygosity and inbreeding coefficient (F_{is}) per locus

	locus	He	Ho	F_{is}
A. adulterinum	LAP - I	0.490	0.026	0.862
A. cuneifolium	AAT	0.4185	0.4885	0.0411
	SHDH	0.1454	0.179	0.0759
	LAP - I	0.2335	0.4945	0.368
	LAP - II	0.0308	0.0389	0.1437
	ADH - I	0.1894	0.2938	0.2373
	ADH - II	0.2115	0.4521	0.375
	PGM	0.0837	0.0804	-0.1288
	Mean over 7 loci	0.290	0.188	0.209

Fig 1: Partitioning of genetic variation within and among populations in the two species.

Results of AMOVA, the partitioning was highly significant in both species.

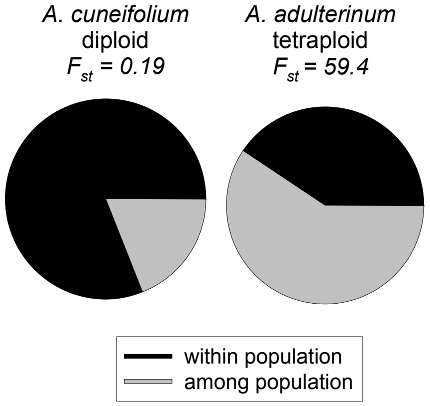
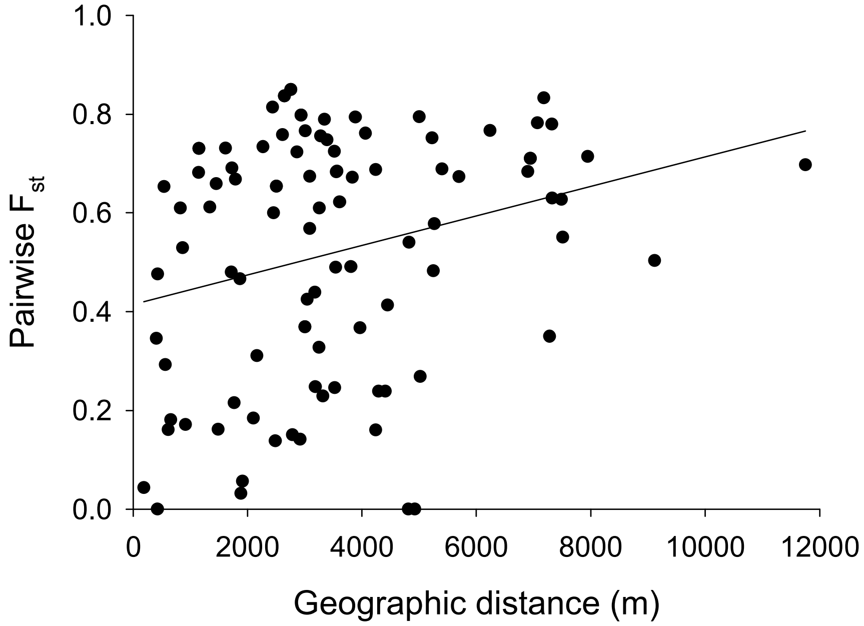


Fig 2: Relationship between geographic distance and pairwise F_{st} between populations. The relationship is significant in tetraploid *A. adulterinum* ($R^2 = 0.112$, $p = 0.001$, Mantel test, A), but not in diploid *A. cuneifolium* ($p = 0.317$, B). The relationship in *A. adulterinum* is significant also when the most remote population is removed from the analysis ($R^2 = 0.084$, $p = 0.035$, Mantel test).

a) *A. adulterinum*



b) *A. cuneifolium*

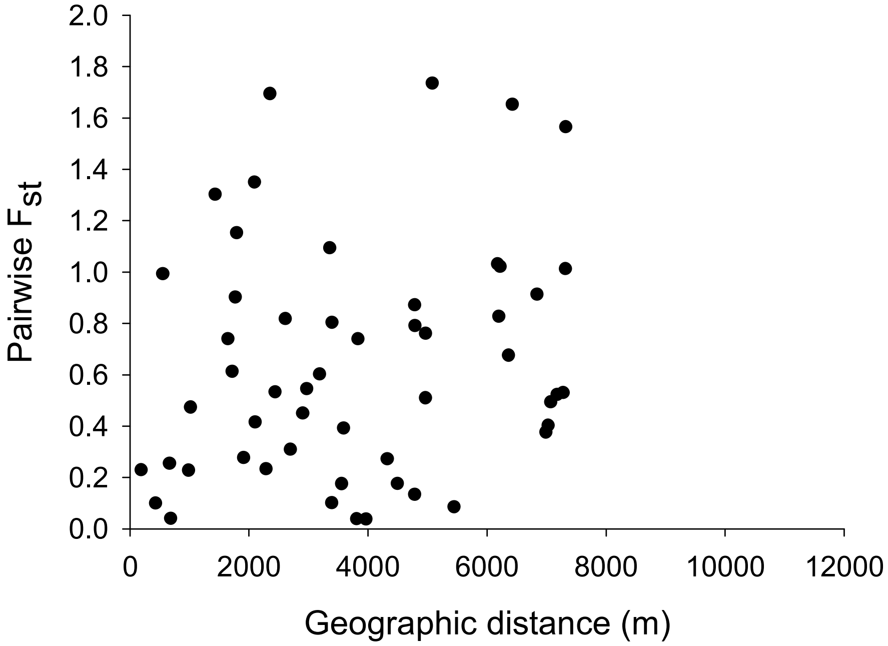


Fig 3: Relationship between population size and genetic diversity (Nei 1987) of individual populations. The relationship is marginally significant in tetraploid *A. adulterinum* (R^2 = 0.152, p = 0.093, linear regression, A), but not in diploid *A. cuneifolium* (p = 0.290, B).

Fig 2: Relationship between geographic distance and pairwise F_{st} between populations. The relationship is significant in tetraploid *A. adulterinum* (R^2 = 0.112, p = 0.001, Mantel test, A), but not in diploid *A. cuneifolium* (p = 0. 317, B). The relationship in *A. adulterinum* is significant also when the most remote population is removed from the analysis (R^2 = 0.084, p = 0.035, Mantel test).

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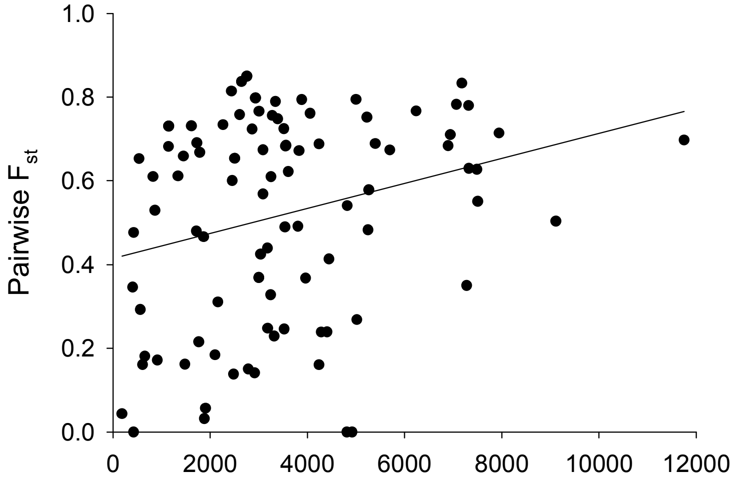
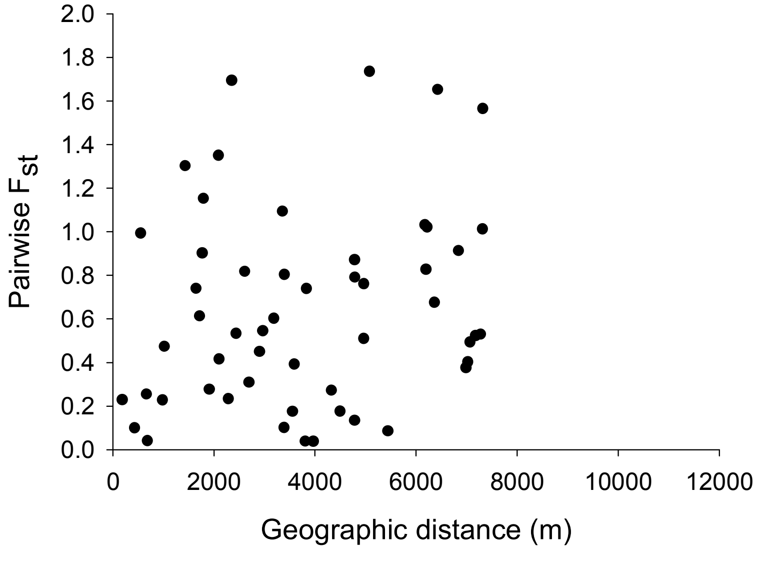


b) *A. cuneifolium*



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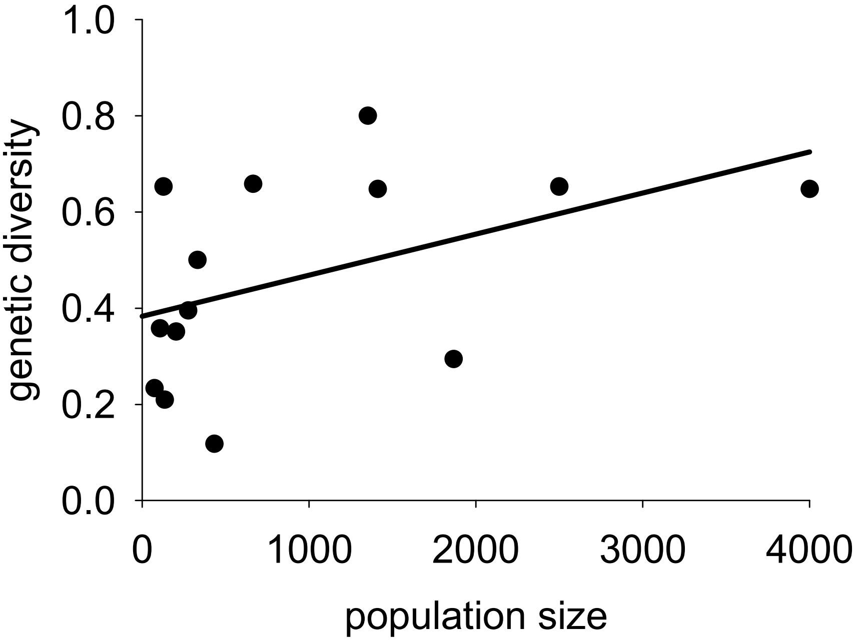
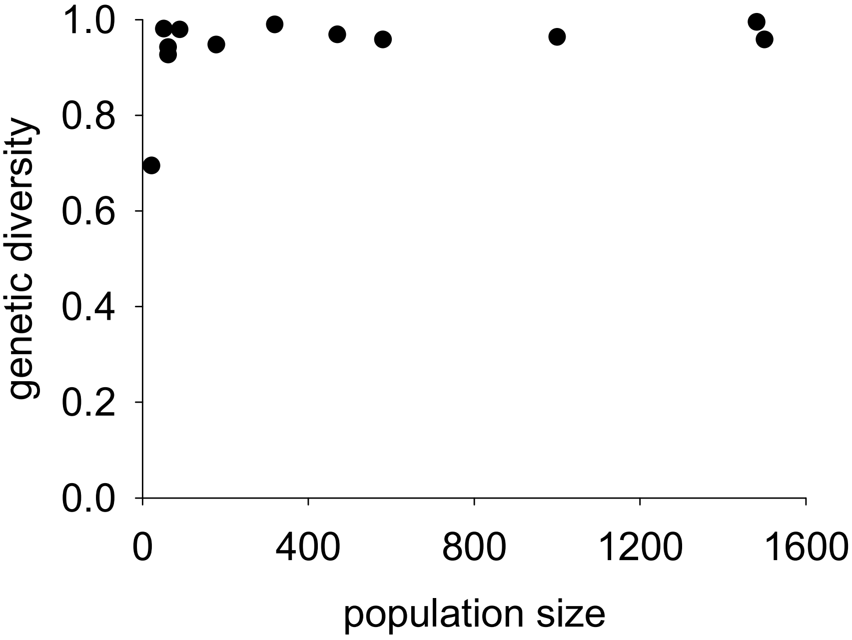
A) *A. adulterinum*



B) *A. cuneifolium*



Appendix 1

Detailed protocol used for the isozyme analysis

Electrophoresis was performed on crude protein extracts of leaf material. Approximately 60 mg of fresh leaf tissue was ground with Dowex-Cl (1-X8) and homogenized on ice in 0.6 ml Tris–HCl extraction buffer (0.1 M Tris–HCl pH 8.0, 78 mM 2-mercaptoethanol, 26 mM sodium metabisulfite, 11 mM ascorbic acid, 4% polyvinylpyrrolidone). The extracts were centrifuged for 10 min at 15,000 rpm and clear supernatants were stored at −75 °C for up to 6 months until electrophoresis. Isozymes were separated on native-PAGE; 15 µl of each sample were employed for electrophoresis in a Hoefer vertical electrophoresis unit. All enzymes were resolved on polyacrylamide gels using 8.16% separating gel and 4% stacking gel. The separating gel was made using a buffer of 1.82 M Tris–HCl, pH 8.9, and the stacking gel using a buffer of 0.069 M Tris–HCl, pH 6.9. The electrode buffer consisted of 0.02 M tris and 0.24 M glycine, pH 8.3.

Nine enzyme systems were investigated in the first step (6-PGDH, AAT, ADH, DIA, IDH, LAP, MDH, PGM, SHDH); variation was found in 4 of them in *A. adulterinum* (LAP, DIA, 6-PGDH, SHDH) and 5 of them in *A. cuneifolium* (AAT, SHDH, LAP 2 loci, ADH 2 loci, PGM), resulting in 4 and 7 variable loci, respectively.

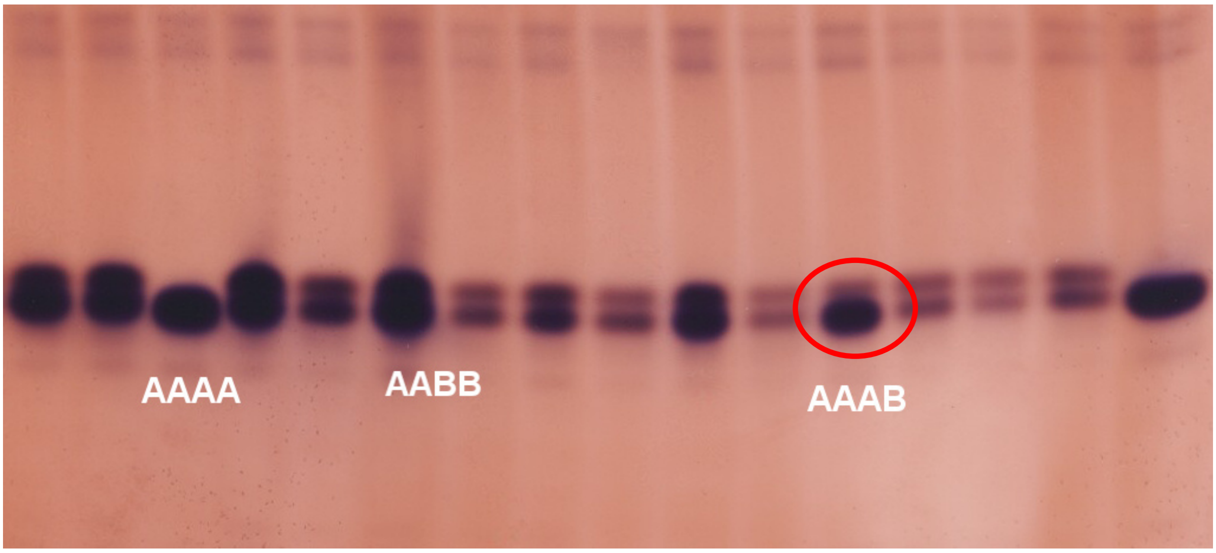
The staining procedures followed Vallejos (1983) to visualize 6-PGDH, ADH, PGM, SHDH and DIA, with the following modifications: 6-PGDH (30 ml 0.1 M Tris–HCl pH 8.4, 10 mg 6-phosphogluconate, 5 mg NADP, 5 mg MTT, 2 mg PMS, 30 mg $MgCl_2$), ADH (40 ml 0.1 M Tris-HCl pH 7.5, 30 mg NAD, 20 mg MTT, 2 mg PMS, 20 ml ethanol), PGM (50 ml 0.05 M Tris-HCl pH 8.5, 100 mg glucose-1-phosphate, 10 mg NADP, 10 mg MTT, 2 mg PMS, 25 mg $MgCl_2$, 80 units glucose-6-phosphate dehydrogenase), SHDH (30 ml 0.1 M Tris-HCl pH 8.4, 30 mg shikimic acid, 5 mg NADP, 6 mg MTT, 2 mg PMS) and DIA (100 ml 0.1 M Tris-HCl pH 8.0, 4 mg 2,6-dichlorophenol-indophenol, 26 mg NADH, 10 mg MTT). Enzyme system AAT was stained using the following method: two staining solutions were prepared, A (20 ml 0.1 M Tris–HCl pH 8.4, 240 mg aspartic acid, 40 mg α-ketoglutaric acid) and B (20 ml 0.1 M Tris–HCl pH 8.4, 25 mg Fast Blue BB Salt, 50 mg Fast Violet B, 25 mg pyridoxal-5-phosphate). Solution A was prepared at least 15 min before the application. The gel was rinsed in water and then in buffer (Tris–HCl pH 7). Solutions A and B were mixed and poured on the gel. The gel was incubated in the dark at 35 °C until bands appeared. Then the gel was rinsed in distilled water and fixed (1:1:3:5, glycerine:acetic acid:H_2O:methanol). Visualization of LAP was done using buffer 0.2 M Tris-maleat pH 6. The gel was rinsed with the buffer and then incubated for 10 min in a solution of 30 ml buffer, 50 mg L-leucyl-β-naphthylamide HCl (in 50% acetone) and 60 mg $MgCl_2$. Then 25 mg Fast Black K Salt in 30 ml of the buffer was added. The gel was incubated in dark, until bands appeared.

References:

Vallejos, C. E., 1983. Enzyme activity staining. In Isozymes in Plant Genetics and Breeding, Part A. Tanksley and Orton eds. p. 469-516.

Appendix 2:

Unbalanced isozyme banding pattern AAAB in locus LAP-1 in tetraploid *A. adulterinum*.



Paper 4

EFFECT OF LAND USE AND CLIMATE CHANGE ON FUTURE FATE OF POPULATIONS OF AN ENDEMIC SPECIES OF CENTRAL EUROPE

Anna Bucharová, Jiří Brabec and Zuzana Münzbergová

Manuscript

Effect of land use and climate change on future fate of populations of an endemic species of Central Europe

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ABSTRACT

Identification of optimal management strategies for a given species is a major challenge of species conservation. It becomes especially intriguing when it is expected that the environmental conditions will change in the future and the optimal management applied today may differ from the management which is optimal under the changed conditions e.g. due to climate change.

This study is dealing with endemic rare plant species of semi-natural grasslands in Central Europe, *Gentianella praecox* subsp. *bohemica*. Number of populations of this species declined rapidly in the last 60 years and currently, conservation action plan has been build built in the Czech Republic, where most populations of this species occur. This study uses periodic matrix models to compare different management regimes under different scenarios of climate change and to identify the optimal management in each case.

Populations of the species without management are not able to survive. Flowering individuals can occur for long time after management cessation, but the extinction of the population is inevitable in several decades. Even very large populations (1000 flowering individuals) will go extinct within less than 50 years without management. Total extinction (including seed bank) follows several years after last flowering plant has been observed. The most suitable management is mowing and disturbance (by harrow), which is also the best one for restoration of populations in bad state. Mowing is less suitable, but it is fully sufficient for large prospering populations. Even small populations (10-15 flowering individuals) are able to survive, when managed. Future climate change may have rather small impact on probability of survival of the species, when management is continuously applied. It will, however, increase the extinction probability of very small populations.

INTRODUCTION

Populations of many species decline and face the risk of extinction because of changes in the environment induced by human activities (Feeley & Silman 2010). A major challenge for ecologists and nature conservation authorities is therefore to identify factors governing population dynamics, forecast effects of environmental change on population viability and design methods to diminish negative effect of these changes (Schemske et al. 1994; Lindenmayer et al. 2008).

One of the main negative factors affecting species survival in the current landscape is land use change (Luoto et al. 2003). It causes habitat fragmentation and habitat loss, which is a major driver of the species loss worldwide (Foley et al. 2005). In Europe, the most dramatic changes came with industrialization of agriculture in second half of 20th century. Some formerly common habitats formed by traditional management decreased significantly and were highly fragmented (e.g. extensive pastures, wet meadows). Thus, plants adapted to such types of habitats became highly endangered (Procházka & Holub 2000; Eriksson et al. 2002). Since these species are dependent on specific human activities, current nature conservation actions are from large proportion aimed on management of these habitats hosting often many species threatened by land-use changes. However, a question arises – what is the best management, when the original management (e.g. extensive grazing) is not feasible on such small fragments of habitats?

Another threat for the species decimated by land-use change is climate change (Travis 2003). Towards the end of 21st century, the climate in Central Europe should be dryer, warmer and extreme rainfall events should be more frequent (Rowell 2005). It means increased probability of severe weather disturbances due to drought or floods. Some species are adapted to periodic catastrophic events and catastrophic events are regular part of the species life cycle (e.g. Roy & Sonie 1992; Blom & Voesenek 1996). Other species, including meadow species, are, however, not adapted to such extremes. The question is, how much such a weather disturbance (e.g. extreme drought), resulting in death of most individuals except seeds in the seed bank affects the probability of survival of the populations. For assessing future prospect of such a species and detecting the most suitable approaches for their management, it is necessary to model population viability under expected disturbance regimes and different management treatments. Because all natural populations experience stochastic variation in vital rates, stochastic demographic models (Caswell 2001; Schleuning & Matthies 2009; Torang et al. 2010) provide an appropriate framework for analyzing the effect of various environmental changes on the population dynamics of species.

Population viability is usually described using population growth rate, λ, or more reliably stochastic population growth rate (Caswell 2001). However, when the environment changes dramatically, the population growth rate reflecting the long term population growth is difficult to use, since it changes from year to year following the environmental change (but see (Horvitz et al. 2005). In such a case, we suggest to use extinction probabilities of small populations instead of population growth rate (Lennartsson 2000; Lennartsson & Oostermeijer 2001).

There have been several studies arguing that it is not meaningful to calculate extinction probabilities (Ludwig 1999; Fieberg & Ellner 2000). The critics are mostly based on studies on animal populations and emphasize, that it is impossible to collect sufficient data set to carry out reliable predictions. Plants have the advantage of sessile life and thus, it is easier to precisely follow a larger number of individuals. It is even possible to carry out experiments, which can bring information on probability of all transitions in the life cycle and on the effect of different management types on these transitions (Ramula 2008). Moreover, Beissinger & Westphal (1998) discuss that problems of extinction probabilities lay rather in the interpretation. Absolute interpretations should be avoided, but relative interpretations are reliable and useful (e.g. comparison under which habitat condition a plant population has higher probability to survive). Such relative information is often the most demanded by practical conservationists as it can e.g. indicate which management to apply to preserve a given species.

Extinction probabilities are often calculated over many years (50 years, Bucharova et al. 2010; Lennartsson & Oostermeijer 2001; or 100 years Menges 1990). It may be argued, that predictions over such a long period are not realistic, since many factors will change during the time. We fully agree with this objection. However, extinction probabilities must not be interpreted as predictions, but rather as illustration of current processes in the populations (Beissinger & Westphal 1998). Calculations over such a long time show the trends under current conditions. If such projections were done over shorter period, e.g. 10 years, the threat of extinction may not be seen in some species such as those forming long-lived remnant populations (Eriksson 1996).

In our study, we aimed on *Gentianella praecox* subsp. *bohemica*, a typical representative of a species endangered by land use and climate change. It used to commonly grow on wet pastures and meadows. With the land use change, number of populations drastically decreased and nowadays, it has only 111 populations in total size from several individuals to several thousands of individuals. However, sizes of individual populations highly vary from year to year. Thus, this plant is currently of great interest of nature conservation (web1) and recently, action plan for restoration of this plant has been developed in the Czech Republic. Creating effective action plan requests reliable scientific information (IUCN/SSC 2002, 2008).

We estimated susceptibility of small populations of *Gentianella praecox* subsp. *bohemica* to extinction under 3 different management regimes: no management, mowing and mowing followed by disturbance by harrow, which was part of traditional management.

Financial resources are always limited, thus, we identified what is the minimal suitable management (the costs for the three managements differs) which will assure existence of the population under given condition (e.g. population size, frequency of extreme events). Since in some areas the populations are in very bad state due to the lack of appropriate management, we also estimated probability of successful restoration from seed bank of almost extinct populations and identified the best management for their restoration. Moreover, we simulated possible influence of future climate change (as increased frequency of extreme drought) on population prospect.

METHODS

Study species

Gentianella praecox (A. et J. Kerner) E. Mayer subsp. *bohemica* (Skalický) Holub is a small (usually 10-25 cm tall), strictly biennial plant. It flowers in autumn (mostly September), followed by seed production. Seeds either germinate in the next spring, or they are stored in the seed bank. Germinated seeds slowly develop during the first season into rosettes. The one year old plant forms a rosette the next year carrying flowering stems in the autumn. Thus, the individuals (except seed in seed bank, which can survive in the same stage) have to either grow, or die (Fig 1). The species is pollinated by insects. Number of individuals in a population is highly fluctuating from year to year.

Gentianella praecox subsp. *bohemica* is an endemic taxon of the Czech Massif. Its historical distribution included the Czech Republic, Bavarian part of the Šumava Mts. (Böhmerwald, Germany), northern part of Austria and southern part of Poland. It typically inhabits secondary grasslands with traditional management of grazing and mowing (Kirschner & Kirschnerová 2000). With agriculture intensification, the semi-natural grasslands with traditional management decreased their size considerably, resulting into the steep decrease of number of populations of the taxon (Brabec 2005). Recently, it was found at only 111 localities all over the distribution range (Brabec, unpublished). Thus, it is rare and under the interest of nature conservation (Procházka & Holub 2000; web1).

Study sites

The experiment was established at 4 localities (Table 1) in the centre of the current distribution of the species - South and South-Western Bohemia, the Czech Republic. All the studied localities were large (> 500 flowering individuals), since we needed sufficient number of individuals to carry out the experiments. Moisture conditions varied from mesic dominated by *Arrhenaterum elatius* to dryer dominated by *Brachypodium pinnatum*, to represent variability of natural habitats of *Gentianella praecox* subsp. *bohemica*. The grasslands are species rich, hosting many rare species such as *Gymnadenia conopsea*, *Platanthera bifolia*, *Botrychium lunaria* or *Lilium martagon*. They were irregularly mown or grazed before beginning of the experiment.

Data collection

Survival of individuals – seed sowing experiment

At the 4 experimental localities, sowing experiment was carried out in 2-5 years (2000-2005). At each locality, 6 plots (60 ×40 cm) divided into 6 subplots (15×15 cm with 2.5 cm buffer zone) were established every year of the sowing experiment (Appendix 1). Three types of management were performed in random design in the subplots; one management type was applied to 2 subplots: unmanaged, clipping (simulation of mowing), clipping and vegetation cover disturbance (simulation of mowing and disturbance by harrow, part of traditional management). Clipped biomass was removed from the plots. Management was carried out in October, after fruiting of the species, since management in time of flowering and fruiting is highly unsuitable for *Gentianella* species (Lennartsson & Oostermeijer 2001).

At one of the subplots with a given management type, 600 seeds of *Gentianella praecox* subsp. *bohemica* collected on the locality were sown in the autumn short after seed maturation to mimic natural release of the seeds as well as possible. This high sowing density approximately corresponds to the situation, when a plant with ripen seeds falls down and all the seeds thus remain in a very limited area, which happens very often in field (one plant produces on average 613 seeds, unpubl. results). The other subplot with the given management type served as a control for natural germination. In every year of sowing, seeds were sown to newly established plots, all the plants present in the subplots thus originated from one sowing event. In each year there were 6 replicates for each treatment.

Germination, seedling establishment and survival of plants in the experimental plots were followed 3 times per year for 2 subsequent years until finishing the whole life cycle (the plant is strictly biennial). At each census, number of plants present in the subplot was recorded. In this way we obtained proportion of established/surviving individuals between censuses. Plants with ripen seeds were collected, number of seeds was counted for assessing the seed production. Further, germination and seedling establishment from the seed bank (i.e. delayed germination of the sown seeds) was followed also the second year after sowing. This was possible as the one year old and two year old plants can be easily morphologically distinguished from each other.

During the period of the experiment, an extremely dry year occurred (2003). The summer was so dry, that almost all plants died and the populations survived only in the seed bank. Such a dry year occurs with probability 1:20 to 1:50 in the study area (period 1950-2007, internal climatic data of our institute). Thus, we were able to record fate of the population in case of a catastrophic event – extreme drought. We subsequently distinguish "dry year" data from 2003, and data from "normal years" coming from all the other years.

Survival in the seed bank

Eighteen fine mash sacks each containing 300 seed (collected at a given locality) were buried at each of 4 localities in autumn 2000 or 2001. In autumn for subsequent 7-8 years, 2 (exceptionally only 1) sacks were excavated and undamaged seeds counted. Undamaged seeds were considered alive (Fischer & Matthies 1998b).

Matrix construction

Periodic matrix model (Caswell & Trevisan 1994) was used for analyzing extinction probability of *Gentianella praecox* subsp. *bohemica*. Periodic matrix models are used to describe the effect of cyclic environmental variation, both seasonal and interannual, on population dynamics. If environmental cycle (e.g. annual) is of length m, with matrices $B^{(1)}$, $B^{(2)}$,..., $B^{(m)}$ describing population growth during the m phases of annual cycle, then population growth over whole cycle is given by the product matrix

$$A = B^{(m)} * B^{(m-1)} * ... * B^{(1)}$$

In the case of this study, we have annual cycle of length 3 (transitions autumn-spring, spring-summer and summer-autumn) and it is thus represented by 3 separate transition matrices. Following stages were distinguished: seed in seed bank, seedling, first year rosette in summer and autumn, two years old plant in spring, summer and autumn. The first matrix represented transition autumn-spring and it comprised transition connected with reproduction (seedling establishment from current seed production and from the seed bank), survival in the seed bank and over wintering of one year old plants. The second matrix represented transition from spring to summer and the third the transition from summer to autumn. These two matrices contained only information on growth of one year old and two year old plants. Survival of seeds in the seed bank was assigned 1 here, since we knew only between year survival, not survival between seasons. The annual set of these 3 transitions following each other described one year of dynamics of the population (Table 2).

Probability of seedling establishment was estimated as number of seedlings in the sowing plots in the spring divided by number of sown seeds in the previous autumn. Probability of transition between all the other size categories was obtained from the sowing experiment, as proportion of individuals in the plot in the current census divided by number of individuals in the plot in the preceding census summed across all subplots with the same type of management in the given year at the given locality (note that all individuals in a single plot are of the same stage). Probability of survival in the seed bank was estimated from the burial experiment as number of viable seeds in the bags in one year divided by number of viable seeds in the bags the year before summed over all localities and years. We estimated this proportion by combining data over all years and localities rather than separate values for each locality and time interval since variability in data was huge, without any strong trend between years/localities.

We started the sowing experiment for estimating individual survival in several subsequent years. For most of the years we knew behavior both of the one year old plants and the two year old plants. For some years, we, however, did not know all the transitions. This happened in the 1st and last year and also due to extreme mortality in the very dry year 2003 (Appendix 1).

Univariate analysis of influence of environmental characteristics on vital rates in our data indicated that the biggest differences are between management types and types of the year (normal/dry year) in most cases (not shown). Missing data were thus replaced by weighted mean (by number of individuals entering the transition) over years with similar weather (normal/dry year) from a given locality and management type. Type of the year had no effect on the seed production per plant (not shown) and missing values for seed production were thus replaced by weighted mean of data over all years from a given locality and management type. This procedure resulted in as many annual set of matrices (each annual set comprising 3 matrices of transitions between seasons) as many life cycles we started in the experiment (by sowing) minus 1 (due to the dry year 2003). In total, we obtained 14 annual sets of matrices for each management type: from 2 localities 3 annual sets of matrices and from 2 localities 4 annual sets of matrices.

Despite high sowing density (3 × 600 seeds per locality, management and year), there were quite little individuals for the construction of the transition matrices in some annual cycles, due to high mortality of seedlings in some years and management types (in extreme cases only several individuals were available, especially under regime of no management). Thus, we decided to construct also pooled sets of matrices over all localities for each type of the year (dry and normal) and for each management type, which were more robust than above described separate sets of matrices. We pooled all individuals followed on all localities in given type of the year and we obtained 2 types of sets matrices for each management type: dry year set of matrices and normal year set of matrices.

Performance of the populations

Population performance under different conditions was expressed as extinction probability of a given population. We employed a periodic matrix model (Caswell and Trevisan, 1994, Caswell, 2001). In contrast with classical matrix models, periodic matrix models allow including more periods within a year, in our case 3 censuses. Thus, it describes population dynamics of the species in more detail and as a result, more precisely.

Stochastic simulation approach, with demographic stochasticity included (Caswell 2001), was used to combine the annual sets of three matrices describing within year dynamics (i.e., the three within-year matrices were always kept together). Populations of certain population vector were projected by randomly drawing one annual set of the matrices of given type for each year. Matrices could be drawn with certain probability, probability of all matrices sums to 1. At each time step, the population vector was multiplied with a given matrix. There were 3 time steps within a year. The resulting population vector after each step was replaced by a value drawn from a Poisson distribution with the given mean to simulate demographic stochasticity. This projection was run over 100 years (i.e. 300 steps). Whole simulation was repeated 1000 times and extinction/survival of the population at the end of each simulation was recorded.

Extinction probabilities in 100 years were used as extrapolation of current processes in populations and thus, for illustration of long-term effects of given population dynamics. Running of the model over 100 years enabled us to see the long term effects especially in unmanaged populations. When run over shorter period, e.g. 20, 30 or 50 years, effect of management absence did not seem to be so lethal, because there were still some seeds present in the seed bank and the population did not go extinct, despite the fact that extinction was matter of only few additional years.

First, we run the model with pooled matrices. Each estimate of transition probability and thus each estimate of extinction probability is confined with an error because of the limited number of individuals that can be sampled. To estimate this error, we bootstrapped the original data used to derive the original matrices 1000 times (Alvarez-Buylla & Slatkin 1994), created new matrices from the bootstrapped data and used these bootstrap matrices to estimate the extinction probabilities of populations as described above. Based on the results, 95%

confidence intervals of extinction probabilities were constructed (Efron & Tibshirani 1994). To do this, a MATLAB script developed by Münzbergová (2006, 2007) was used.

Second, we ran all the models using separate matrices. Separate matrices were based sometimes on too little individuals. It resulted into extremely wide confidence intervals, suggesting that these matrices do not describe reality very precisely. Thus, the results based on pooled matrices are more reliable and are reported first, confidence intervals are reported from pooled matrices only. Differences in the results based on separate matrices are discussed.

The initial population vectors in our simulations were arbitrary. Studies doing such predictions usually use real population vector or a vector derived from stable stage distribution (e.g. Bucharová et al. 2010). None of these approaches were, however, possible as we did not know the initial population vectors (invisible seed bank and hard to detect vegetative rosettes). Also identification of stable stage distribution would not be very sensible due to very high between year variations. To test sensitivity of our conclusions to the distribution of starting population vector, projection with different vectors was also performed (e.g. the number of individuals evenly distributed between one-year rosettes and flowering plants). In result, the difference between managements remained unchanged and absolute results were only slightly different (absolute results are not very reliable anyway). Thus, only results based on vector containing flowering plants only are presented (Model 1 and Model 3, see bellow).

Effect of management and population size on population survival (Model 1)

Populations of *Gentianella praecox* subsp. *bohemica* are often small (reach maximum ca 15 individuals in years with the largest number of individuals). Thus, we ask how small a population can become to still have a reasonable chance to survive under different management conditions. Population vectors of 1 to 100 flowering individuals (only flowering individuals, no seeds and no one year rosettes) were projected by randomly drawing one set of the matrices of a given management type (one set for dry year, one set for normal year), with frequency of dry years of 1:20. For the management type with highest extinction probability (absence of management), we also calculated time to extinction. Time to extinction was calculated as time to total extinction including seed bank and as putative extinction, when no flowering plants appear, but some individuals are still present in form of inconspicuous one year rosettes and/or seed bank.

Population restoration (Model 2)

When a population is unmanaged, it inevitably goes to extinction. Most remarkable sign of upcoming extinction is absence of flowering plants. In this case, it is still possible to restore the population from the seed bank by management introduction. Success of such restoration depends on time, for how long no flowering plant was observed. In the model, we projected population consisting of 50 rosettes and 50 flowering plants with no-management matrices. It inevitably led to population decrease and in certain moment, there were no flowering plants. Model was still projected for predefined time (1 to 15 years) with matrices describing no management, simulating the time until the responsible management office will recognize that something is wrong with the population and induce management. After this predefined time without any flowering plant, we switched the transition matrices to matrices describing a selected management (mowing or mowing and disturbance). We ran the model over 100 years. We recorded in which time step the management was induced and percentage of successfully restored populations. Dry years were again incorporated with probability 1:20.

To test the sensitivity of the results upon the initial conditions, we also ran this model with starting population 100 rosettes and 100 flowering plants or with 100 rosettes and 50 flowering plants. Since results based on different starting vectors did not differ, results only of simulation based on 50 rosettes and 50 flowering plants are shown.

Climate change and population survival (Model 3)

Simulation of climate change, expressed as increased frequency of dry years, on population prospect was performed using the same approach as in Model 1, but we varied frequency of dry years from 1:1 to 1:50. Initial population vector contained 10 flowering individuals.

RESULTS

Effect of management on population survival (Model 1)

Extinction probability of a population decreases with increasing population size (Fig. 2). There were strong differences between management types. Even very small populations (fewer than 10 flowering individuals) have very high probability to survive under management of mowing and disturbance. Mown only, small populations have much smaller probability to survive. For populations larger than 10-20 flowering individuals, both populations mown and disturbed and populations only mown, have almost a zero extinction probability. On the other hand, even very large populations (100 flowering plants), when unmanaged, have zero or very small probability to survive (Fig. 2). However, lower limit of confidence interval decreases with increasing population size, suggesting that even large unmanaged populations may under current conditions extremely exceptionally survive for 100 years.

Interesting is slow disappearance of flowering plants. After the end of management, even small population around 10 individuals will still produce some flowering plants for in average 12 years (Fig. 3). Large populations, e.g. 100 or 1000 flowering plants will produce flowering individuals without being managed much longer after the management cessation – 25 and 39 years on average, respectively. When no flowering plants appear in the population, the population will go extinct completely (including vegetative individuals and seed bank) within approximately 8 years.

When separate matrices were used instead of pooled, the differences between management types were the same. Extinction probability was generally within the confidence interval of pooled matrices, except very small populations on mown and disturbed plots (max. 10 individuals). Here, model based on separate matrices predicted higher extinction probabilities than model based on pooled matrices (online Appendix 2, Fig. 1). Time until extinction was not significantly different when separate matrices were used, resulting time till extinction lies on lower border of confidence interval obtained from pooled matrices (online Appendix 2, Fig. 2).

Population restoration (Model 2)

Probability of successful restoration of a population from the seed bank decreases with time since last flowering plant was observed (Fig 4). When using the most effective restoration management (mowing and disturbance), there is more than 80% probability of successful restoration of the population from the seed bank when no flowering plant was observed for 3 years. With increasing time of no flowering individuals observed, the probability decreases considerably, with only 30% restoration success 6 years since last flowering plant and less than 3% restoration success 10 years since the last flowering plant was observed. (Fig. 4)

When only mowing is used as restoration management, probability of successful restoration is significantly lower from 2 years of absence of flowering plant (confidence intervals do not overlap) and already after 6 years, probability of successful restoration is lower than 10% and after 10 years lower than 1%.

Flowering plants were presented for some time even without management. For illustration, an example of one run of a model of a successful restoration of a population is presented. Fig 5 shows relatively long time, for which a reasonable number of flowering individuals was present before they finally disappeared, and than steep increase in the number of flowering plants after the management introduction.

When separate matrices were used instead of pooled, the differences between management types were the same. Probability of successful restoration using mowing was up to 10% higher than predicted by model based on pooled matrices (online Appendix 2, Fig. 3).

Climate change and population survival (Model 3)

Probability of extinction of populations increases with increasing probability of dry years (Fig. 5). Again, there is an important difference between management types. Unmanaged populations do not survive under any frequency of dry years. In mown populations, the extinction probability is about the same up to frequency of dry years 1:10, than it steeply increases. In mown and disturbed populations, extinction probability is almost 0 until frequency 1:5, than it increases steeply.

When separate matrices were used instead of pooled, extinction under management of mowing and disturbance was higher than in pooled matrices. Under regime of no management on mowing only, results of separate matrices were within confidence interval obtained from pooled matrices (online Appendix 2, Fig. 4).

DISCUSSION

In this study, we used extinction probabilities of small populations to assess the effect of different management types on prospect of populations of a rare and endangered species, *Gentianella praecox* subsp. *bohemica.* We demonstrated that unmanaged populations are not able to survive in the long-term; extinction is inevitable within several decades. Management type (mowing or mowing plus disturbance by harrow) have impact especially on populations in some critical situations (very small number of individuals, population restoration or high frequency of dry years), but in large prospering populations, the management type has rather small impact on probability of population survival.

In contrast to the very little difference in the effect of mowing and mowing and harrowing in the most cases, absence of management had a dramatic effect on the population persistence and population of whatever size was not able to survive without management over long time period. Even very large population (1000 flowering individuals) will go extinct within less than 50 years. This estimation is still rather optimistic, because unmanaged semi-natural grassland will undergo succession including shrub establishment and conditions will became even less suitable for establishment and growth of *Gentianella praecox* subsp. *bohemica*. Since *Gentianella praecox* subsp. *bohemica* belongs among meadow species dependent on regular agriculture treatment, the results are not surprising and they correspond to the conclusions on other species of semi-natural grasslands (e.g. Dullinger et al. 2003; Ockinger et al. 2006). In *Gentianella*, important is that absence of the management can have the same effect as management with bad timing (Lennartsson 2000). In this study, we tested only autumnal management after the fruiting period of the species, which is considered as the most suitable period for performing management actions at *Gentianella* localities.

Interesting is the process of slow disappearance of the plants from the locality after the cessation of the management. Flowering plants still appear for several years; if the population was large, it is possible to observe flowering plants for more than 20 years after the management cessation. It is due to the stochastic environmental and demographic changes and extensive seed bank. Number of flowering plants may sometimes rapidly increase between years even without management. Thus, responsible management officers can easily slip to the feeling, that nothing is wrong because the population prospers even without management. It may result into releasing attention to the given population. As our models show, extinction of such an unmanaged population is inevitable; it usually follows several years after last flowering plants have been observed. For long term prosperity, management induction is thus necessary.

If managed, even very small populations (fewer than 10 flowering individuals) have high chance to survive. Under the best management, mowing followed by disturbance, even one flowering individual has very high chance to establish a new population. Lennartsson (2000) reports similarly high survival probability of *Gentianella campestris* under the best management. In populations larger than 10 individuals, there is almost no difference between management types (mowing or mowing in combination with disturbance). It has practical implications – in large stable populations, the simpler and thus cheaper management (mowing only) should be enough to preserve the species.

Unmanaged populations will inevitably go extinct. A population may seem to be extinct, when no flowering individuals appear. However, there is still some chance for recovery of the population from the seed bank. Our result show, that restoration of a population from seed bank is possible up to 8 years since last flowering plant was observed, but reasonable chance (around 80 %) is up to maximum 3-4 years. For restoration of a population from seed bank, the best management is mowing and disturbance, mowing only is less successful. Here again, absolute interpretation in form of number of years has to be treated with caution for policy decision. We conclude that reasonable chance for restoration of a population (90%) is for up to 3 years since last flowering plant was observed. If longer, chance for success of such an action is disputable.

The above estimated survival probability of small populations is necessary to interpret with extreme caution (Beissinger & Westphal 1998). While the relative interpretation, the difference among the management types is valid, the estimated minimum size of viable population has some limitations. A population consisting only from e.g. 10 flowering individuals, especially if growing on very small area, is vulnerable to local catastrophic events such as small-scale habitat destruction and may thus be lost before it recovers. In addition, predictions concerning small populations also do not include other possible negative consequences of small population size which may be linked to genetic changes in these populations, e.g. inbreeding (Ellstrand & Elam 1993; Ouborg & Vantreuren 1995) or to other factors such as Allee effect, (e.g. Stephens et al. 1999). Negative effects of small population size on plant fitness were documented in *Gentianella* genus (Fischer & Matthies 1997, 1998a). Data in our study were obtained from large populations which suffer from no negative effects described above. In such a population, induction of a management should be followed by rapid increase of population size within 3 years. If there is no such rapid increase in reality, there is something wrong – effect of small population size or environmental changes (e.g. human induced extreme desiccation of the locality). Such a scenario was actually observed recently in field. Experimental management (mowing, mowing and disturbance by harrow) was induced in the last decade on several populations of *Gentianella praecox* subsp. *bohemica*. In most cases, it was followed by rapid increase in population sizes. However, in few small, isolated, populations unmanaged for a long time no appropriate response to management was observed and population sizes remain, despite optimal management, still very small.

Model of population restoration critically depends on precise data, especially data on survival in the seed bank. In *Gentianella* species, both persistent and transient seed bank was reported (Pons 1991; Milberg 1994; Fischer & Matthies 1998b; Kelly 1989; Lennartsson & Oostermeijer 2001). Our study shows, that *Gentianella praecox* subsp. *bohemica* has a persistent seed bank, with 53% probability of survival of a seed in seed the bank over a year. In burial experiment, we assumed that healthy looking seeds were viable, following method of Fischer & Matthies (1998b). We did not perform staining by 2,3,5-tripenthyltetrazolium chloride (further reported as tetrazolium staining) as used by Milberg (1994), since Kelly (1989) showed that this method is not fully reliable in *Gentianella* species. Still, our results may be biased towards overestimating number of surviving seeds especially for seeds buried over a longer period of time. It would mean, that undamaged seeds excavated later after burial should show lower germination, in comparison with earlier excavated seeds. Germination of these seeds sown in the field was, however, proportional to number of viable seeds counted and in some cases even higher in older seeds (Brabec, unpubl.; compare with Fischer & Matthies 1998b).

Impact of increased frequency of dry years on population dynamics of managed populations of *Gentianella praecox* subsp. *bohemica* would be rather small according to our result. Increased frequency of dry years simulates climate change (Lennartsson & Oostermeijer 2001; Torang et al. 2010), since one of the main effect of climate change will be increased weather extremity including extremely dry summers (Rowell 2005). Small impact of dry years, when all the individuals except seeds in seed bank die, is given by high number of seeds stored in the seed bank. Several studies reported increased seedling establishment in the spring following the dry year, since the drought created gaps in dense vegetation, providing additional space for seedling establishment (Gross et al. 1998; Horvitz et al. 1998; Smith et al. 2005; Torang et al. 2010). In our study, we could not assess this effect since we had almost no seeds (except one locality) for sowing experiments the year following the dry year. Establishment from the seed bank was not significantly different the year after dry year to other year. It might be given by the fact that in unmanaged plots almost nothing germinated regardless on year, which is probably given by thick layer of litter which prevents any seedlings from establishment, independently on amount of living biomass. On managed plots, it may be given by the fact, that management treatment itself releases competition and enables seed germination and seedling establishment.

We are also aware, that future climatic change will bring much larger changes than only increased frequency of dry years. It will influence whole set of environmental conditions, resulting in shift in phenology, species distribution, change in community composition and whole context dynamic of biota (Walther et al. 2002). Impact of all these changes, to large degree not known, on population dynamics of a single species is hardly predictable. However, increasing weather extremity is definitely one of major drivers of biota changes, since few extreme events, e.g. drought, can drastically changes species prospect, distribution and even evolution (Easterling et al. 2000).

In our study we used two types of matrices. The first type included separate matrices for each year, locality and management. These matrices reflect temporal and spatial environmental stochasticity. Second, we polled data over localities and types of year (dry and normal, the biggest difference within environmental variance). Thus, we obtained for each management 2 sets of matrices: for normal year and dry year. In this approach, we of course loose part of the environmental stochasticity. However, it is surprising that the use of the separate matrices instead of the pooled ones does not have a large impact on the results. Models estimating extinction probability (Model 1 and Model 3) with separate matrices logically predict higher extinction probability; however, it is largely within confidence intervals calculated from pooled matrices. In model estimating probability of restoration of a population, use of separate matrices resulted even in higher probability of restoration. The relative difference between the management types is comparable, thus, the type of used matrices has no effect on relative interpretations. Absolute interpretations should be taken very carefully and even in this case, the difference between the models using pooled and separate matrices does not give very different results.

Conclusions for nature conservation

Even very small populations (10-15 flowering individuals) of *Gentianella praecox* subsp. *bohemica* are able to survive, if the locality is managed. On the other hand, population of any size is not able to survive if not managed. Management should be carried out in autumn, after fruiting of the species because wrongly timed management has the same or even worse effect as no management (Lennartsson & Oostermeijer 2001).

The best management from the tested experimental managements for populations is mowing and disturbance by harrow. However, in stable and large populations, mowing only is sufficient for preserving species existence at the locality. Mowing and disturbance by harrow should be employed in case, that situation of the population is somehow critical – either it is small, or have suboptimal habitat conditions (too dry), or for restoration of population from the seed bank. Restoration of a population from the seed bank by inducing optimal management has reasonable chance for success (80%) approximately maximum 3 years since last flowering plant was observed.

Unmanaged small populations, or populations restored from a seed bank should respond to induction of management within 2-3 years by rapid increase of number of flowering individuals. If this is not the case, something may be wrong either with the environment (e.g. human induced change in vegetation or moisture) or the population suffers from some negative effects of small population size (inbreeding, allelic loss due to genetic drift, Alee effect). In case of restoration from the seed bank, it may be also simply too late.

The conclusions of this paper were implemented into prepared conservation action plan for *Gentianella praecox* subs. *bohemica* in the Czech Republic.

ACKNOWLEDGEMENT

We thank to number of colleagues (especially M. Štefánek and J. Brabec) for help with data collection. We thank to our colleagues from Institute of Botany AS CR for helpful remarks for the manuscript.

This work was supported from GA UK (268/1999/B-BIO/PřF), VaV 2B06178 and EEA and Norway grants 003/2.

REFERENCES:

Alvarez-Buylla, E. R., and M. Slatkin. 1994. Finding Confidence-Limits on Population-Growth Rates - 3 Real Examples Revised. Ecology 75:255-260.

Beissinger, S. R., and M. I. Westphal. 1998. On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management 62:821-841.

Blom, C. W. P. M., and L. A. C. J. Voesenek. 1996. Flooding: The survival strategies of plants. Trends in Ecology & Evolution 11:290-295.

Brabec, J. 2005. Recent distribution of *Gentianella praecox* subsp. *bohemica* in the Czech Republic. Zprávy České botanické společnosti 40:1-44.

Bucharová, A., Z. Münzbergová, and P. Tájek. 2010. Population Biology of Two Rare Fern Species: Long Life and Long-Lasting Stability. American Journal of Botany 97:1260-1271.

Caswell, H. 2001. Matrix population models: construction, analysis, and interpretation. Sinauer Associates, Sunderland, Massachusetts.

Caswell, H., and M. C. Trevisan. 1994. Sensitivity Analysis of Periodic Matrix Models. Ecology 75:1299-1303.

Dullinger, S., T. Dirnbock, J. Greimler, and G. Grabherr. 2003. A resampling approach for evaluating effects of pasture abandonment on subalpine plant species diversity. Journal of Vegetation Science 14:243-252.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. Science 289:2068-2074.

Efron, B., and R. J. Tibshirani 1994. Introduction to the bootstrap. Chapman and Hall, New York.

Ellstrand, N. C., and D. R. Elam. 1993. Population genetic consequences of small population size - implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Eriksson, O. 1996. Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. Oikos 77:248-258.

Eriksson, O., S. A. O. Cousins, and H. H. Bruun. 2002. Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. Journal of Vegetation Science 13:743-748.

Feeley, K. J., and M. R. Silman. 2010. Land-use and climate change effects on population size and extinction risk of Andean plants. Global Change Biology 16:3215-3222.

Fieberg, J., and S. P. Ellner. 2000. When is it meaningful to estimate an extinction probability? Ecology 81:2040-2047.

Fischer, M., and D. Matthies. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84:1685-1692.

Fischer, M., and D. Matthies. 1998a. Effects of population size on performance in the rare plant *Gentianella germanica*. Journal of Ecology 86:195-204.

Fischer, M., and D. Matthies. 1998b. Experimental demography of the rare *Gentianella germanica*: seed bank formation and microsite effects on seedling establishment. Ecography 21:269-278.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science 309:570-574.

Gross, K., J. R. Lockwood, C. C. Frost, and M. F. Morris. 1998. Modeling controlled burning and trampling reduction for conservation of *Hudsonia montana*. Conservation Biology 12:1291-1301.

Horvitz, C. C., J. B. Pascarella, S. McMann, A. Freedman, and R. H. Hofstetter. 1998. Functional roles of invasive non-indigenous plants in hurricane-affected subtropical hardwood forests. Ecological Applications 8:947-974.

Horvitz, C. C., S. Tuljapurkar, and J. B. Pascarella. 2005. Plant-animal interactions in random environments: Habitat-stage elasticity, seed predators, and hurricanes. Ecology 86:3312-3322.

IUCN/SSC 2002. Species Survival Commission Action Plan Evaluation. IUCN Species Survival Commission, Gland, Switzerland.

IUCN/SSC 2008. Strategic Planning for Species Conservation: A Handbook. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland.

Kelly, D. 1989. Demography of Short-Lived Plants in Chalk Grassland .1. Life-Cycle Variation in Annuals and Strict Biennials. Journal of Ecology 77:747-769.

Kirschner, J., and L. Kirschnerová. 2000. *Gentiana*. Pages 83-98 in B. Slavík, editor. Květena ČR 6, Praha (*In Czech*)

Lennartsson, T. 2000. Management and population viability of the pasture plant *Gentianella campestris*: the role of interactions between habitat factors. Ecological Bulletins 48:111-121.

Lennartsson, T., and J. G. B. Oostermeijer. 2001. Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. Journal of Ecology 89:451-463.

Lindenmayer, D., R. J. Hobbs, R. Montague-Drake, J. Alexandra, A. Bennett, M. Burgman, P. Cale, A. Calhoun, V. Cramer, P. Cullen, D. Driscoll, L. Fahrig, J. Fischer, J. Franklin, Y. Haila, M. Hunter, P. Gibbons, S. Lake, G. Luck, C. MacGregor, S. McIntyre, R. Mac Nally, A. Manning, J. Miller, H. Mooney, R. Noss, H. Possingham, D. Saunders, F. Schmiegelow, M. Scott, D. Simberloff, T. Sisk, G. Tabor, B. Walker, J. Wiens, J. Woinarski, and E. Zavaleta. 2008. A checklist for ecological management of landscapes for conservation. Ecology Letters 11:78-91.

Ludwig, D. 1999. Is it meaningful to estimate a probability of extinction? Ecology 80:298-310.

Luoto, M., S. Rekolainen, J. Aakkula, and J. Pykala. 2003. Loss of plant species richness and habitat connectivity in grasslands associated with agricultural change in Finland. Ambio 32:447-452.

Menges, E. S. 1990. Population Viability Analysis for an Endangered Plant. Conservation Biology 4:52-62.

Milberg, P. 1994. Germination Ecology of the Endangered Grassland Biennial *Gentianella campestris*. Biological Conservation 70:287-290.

Münzbergová, Z. 2006. Effect of population size on the prospect of species survival. Folia Geobotanica 41:137-150.

Münzbergová, Z. 2007. Population dynamics of diploid and hexaploid populations of a perennial herb. Annals of Botany 100:1259-1270.

Ockinger, E., A. K. Eriksson, and H. G. Smith. 2006. Effects of grassland abandonment, restoration and management on butterflies and vascular plants. Biological Conservation 133:291-300.

Ouborg, N. J., and R. Vantreuren. 1995. Variation in Fitness-Related Characters among Small and Large Populations of Salvia-Pratensis. Journal of Ecology 83:369-380.

Pons, T. L. 1991. Dormancy, Germination and Mortality of Seeds in a Chalk-Grassland Flora. Journal of Ecology 79:765-780.

Procházka, F., and J. Holub. 2000. Red list of vascular plants of the Czech Republic (Červený seznam cévnatých rostlin České republiky). Preslia 72:187-230.

Ramula, S. 2008. Responses to the timing of damage in an annual herb: Fitness components versus population performance. Basic and Applied Ecology 9:233-242.

Rowell, D. P. 2005. A scenario of European climate change for the late twenty-first century: seasonal means and interannual variability. Climate Dynamics 25:837-849.

Roy, J., and L. Sonie. 1992. Germination and Population-Dynamics of Cistus Species in Relation to Fire. Journal of Applied Ecology 29:647-655.

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G. Bishop. 1994. Evaluating Approaches to the Conservation of Rare and Endangered Plants. Ecology 75:584-606.

Schleuning, M., and D. Matthies. 2009. Habitat Change and Plant Demography: Assessing the Extinction Risk of a Formerly Common Grassland Perennial. Conservation Biology 23:174-183.

Smith, M., H. Caswell, and P. Mettler-Cherry. 2005. Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. Ecological Applications 15:1036-1052.

Stephens, P. A., W. J. Sutherland, and R. P. Freckleton. 1999. What is the Allee effect? Oikos 87:185-190.

Torang, P., J. Ehrlen, and J. Agren. 2010. Linking environmental and demographic data to predict future population viability of a perennial herb. Oecologia 163:99-109.

Travis, J. M. J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proceedings of the Royal Society of London Series B-Biological Sciences 270:467-473.

Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389-395.

web1. ec.europa.eu/environment/nature/natura2000.

Table 1: Experimental populations in south and south-western Bohemia, the Czech Republic.

Locality	Onšovice	Dobročkov	Chvalšiny	Hroby
Position	N 49º06'46", E 13º46'53"	N 48º54'38", E 14º09'17"	N 48º50'57", E 14º12'10"	N 49º23'36", E 14º51'22"
# of flowering individuals per year (2000-2010)	5-4200	0-3445	219-2825	63-7050
Vegetation type	Dry grassland	Dry grassland	Mesic grassland	Mesic grassland
Altitude (meters)	630	660	610	500

Table 2: Structure of annual set of matrices describing the life cycle of *Gentianella praecox* subsp. *bohemica*. First transition is autumn-spring, when seedling establishment and survival of plants over winter occurs. Survival in the seed bank was also assigned to this transition, since we knew only between-years survival in the seed bank, not between-seasons. **t** is annual life cycle (autumn to autumn). The transitions spring-summer and summer-autumn contain growth only. Numbers (#) refer to the corresponding transitions in the life cycle (Fig 1). Data or one annual matrix set describes behavior of plants in population in one year, thus originate from 3 subsequent experiments established within 3 subsequent years, i.e. 3 experimental life cycles (establishment for autumn seed production from first, survival of one year old plants from second, survival of two years old plants from third experimental life cycle).

		Autumn t		
		seed	**one year old plant**	**two year old plant**
Spring t+1	**seed**	(1) survival in the seed bank		(2) seeds to seed bank
	one year old plant	(3) seedling establishment from the seed bank		(4) seedling establishment from the autumn seed production
	two year old plant		(7) survival over winter	

		Spring t		
		seed	**one year old plant**	**two year old plant**
Summer t+1	**seed**	1		
	one year old plant		(5) growth	
	two year old plant			(8) growth

		Summer t		
		seed	**one year old plant**	**two year old plant**
Autumn t+1	**seed**	1		
	one year old plant		(6) growth	
	two year old plant			(9) growth

Fig 1: Life cycle of *Gentianella praecox* subsp. *bohemica*. The plant is strictly biennial, seedling germinate the first spring, than plant grows, overwinters, and the next autumn produces seeds. Seed give arise either seedling the next spring, or they form seed bank. Each transition (marked by arrow in the picture) was assessed in the experiment and is present in the transition matrices (Table 2).

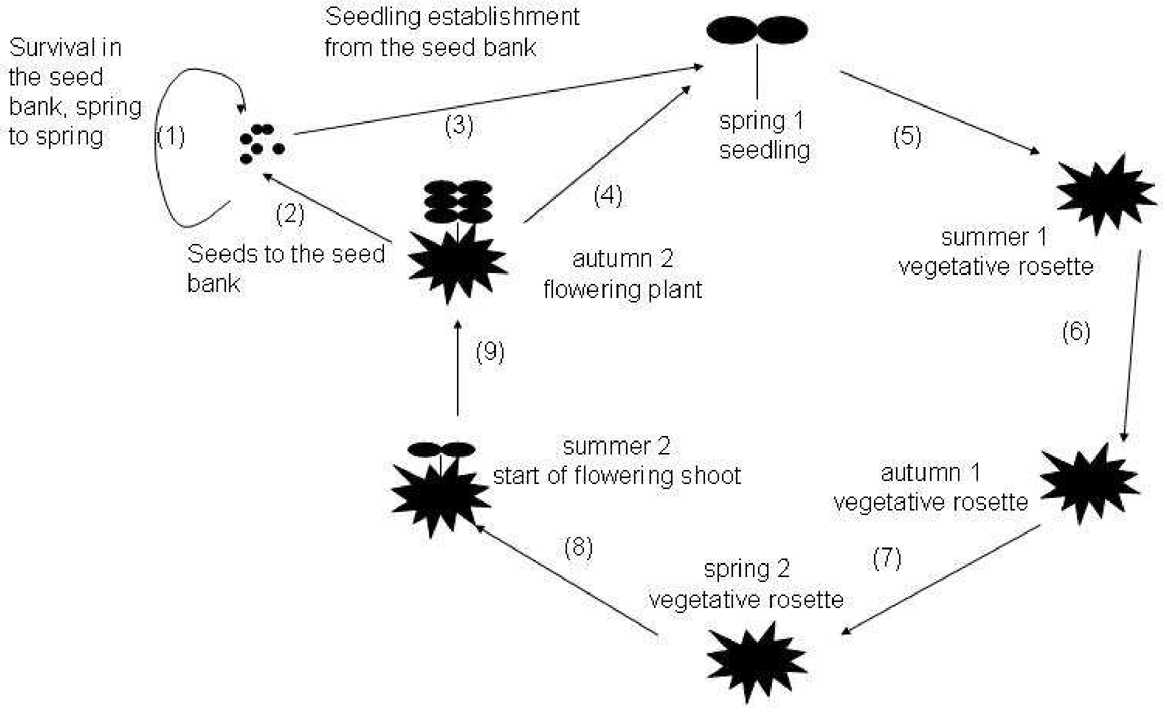


Fig 2: Extinction probability in 100 years as a function of population size (Model 1), based on pooled matrices. 95% confidence intervals are shown in thin lines. In unmanaged populations, wide confidence interval is given by low germination and high mortality of plants in experiment and thus, low number of individuals for assessing transitions.

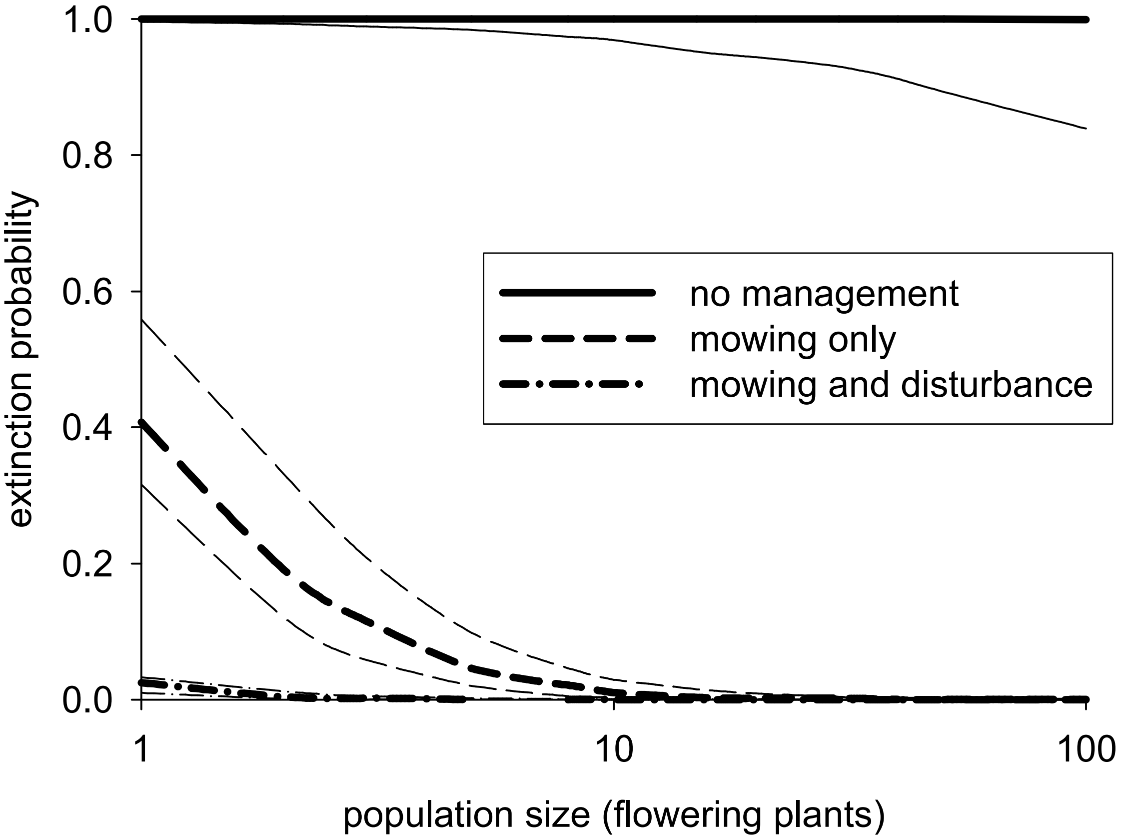


Fig 3: Time to extinction of a population as a function of population size (Model 1), under condition of no management. Time to total extinction and time to extinction of flowering plants is shown. 95% confidence intervals are shown in thin lines.

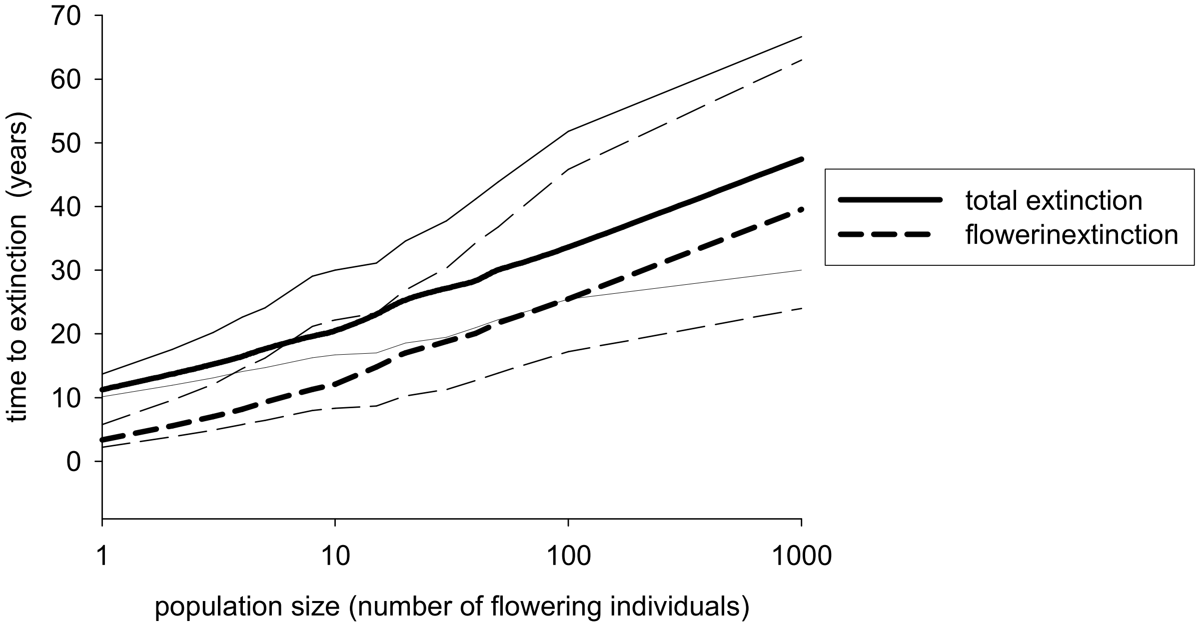


Fig 4: Probability of successful restoration of a population related to the time, for how long no flowering individual was present in the population. Based on pooled matrices, 95% confidence intervals are in thin lines. Model 3.

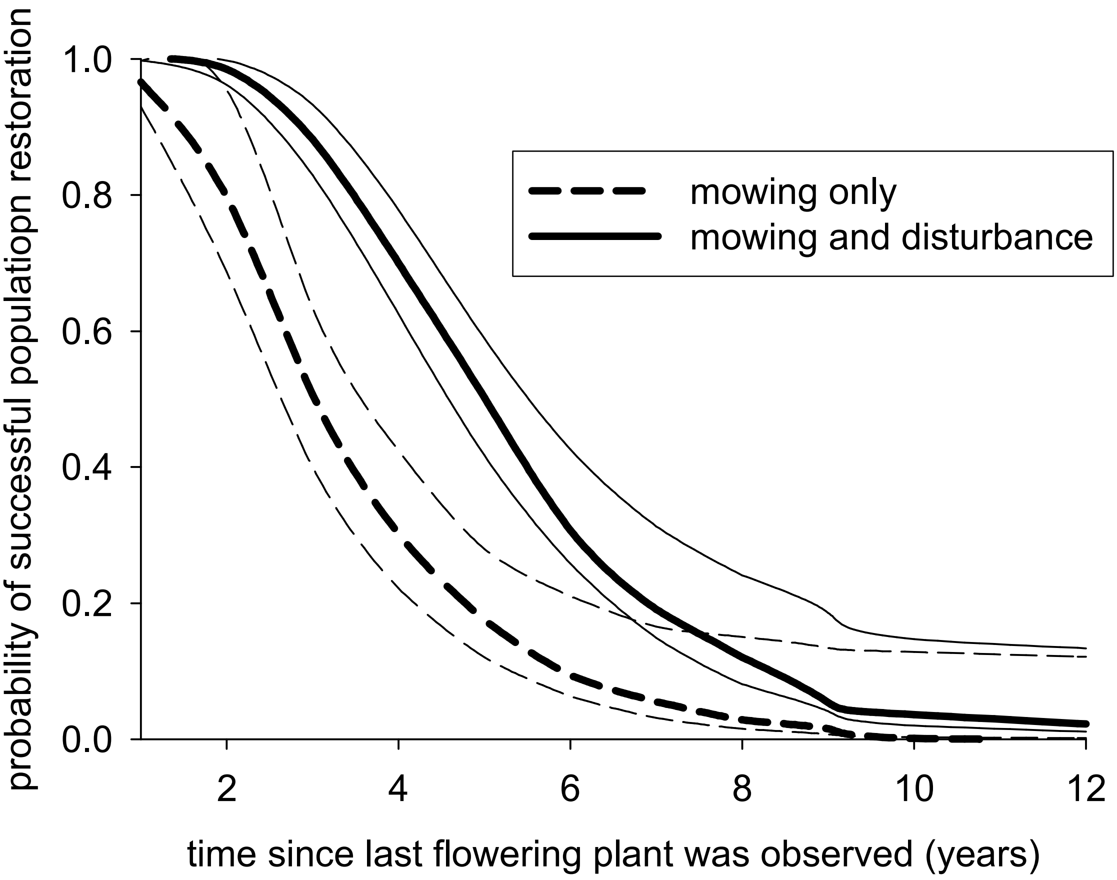


Fig 5: Example of one run of the model for population restoration, based on separate matrices. For estimation of probability of restoration of a population from the seed bank (see fig. 3 in Appendix) 1000 such runs were performed for each “time since last flowering plant was observed”. In this example, “time since last flowering plant was observed” was set 5 years.

Population size varies strongly without management, until flowering individual disappear (year 16), few flowering individuals appear between time 20-25 without any management. At year 25, period after which no flowering plant was observed for 5 years started. After this 5 year of continuous absence of flowering plants management (mowing and disturbance) starts, resulting in a restoration of the population.

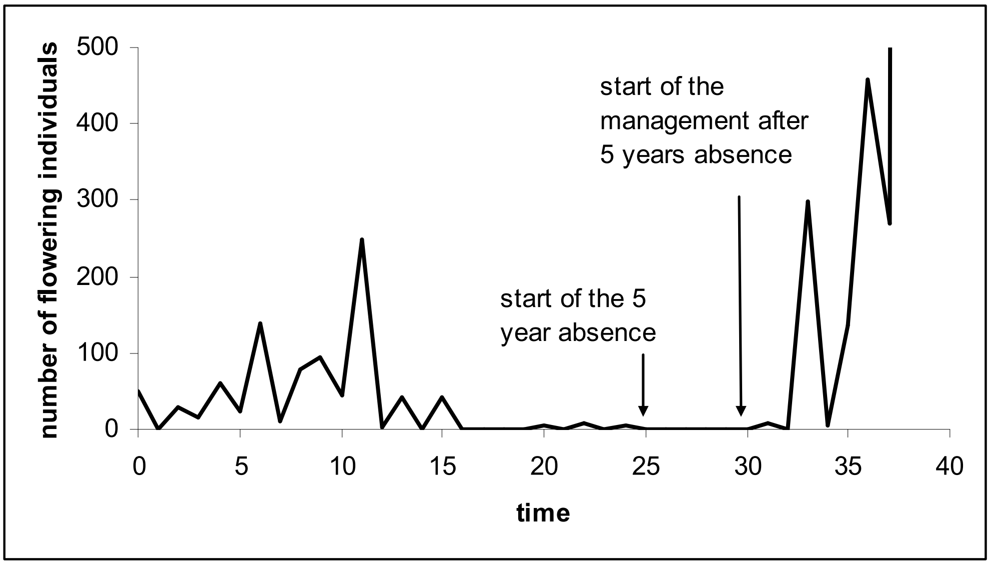
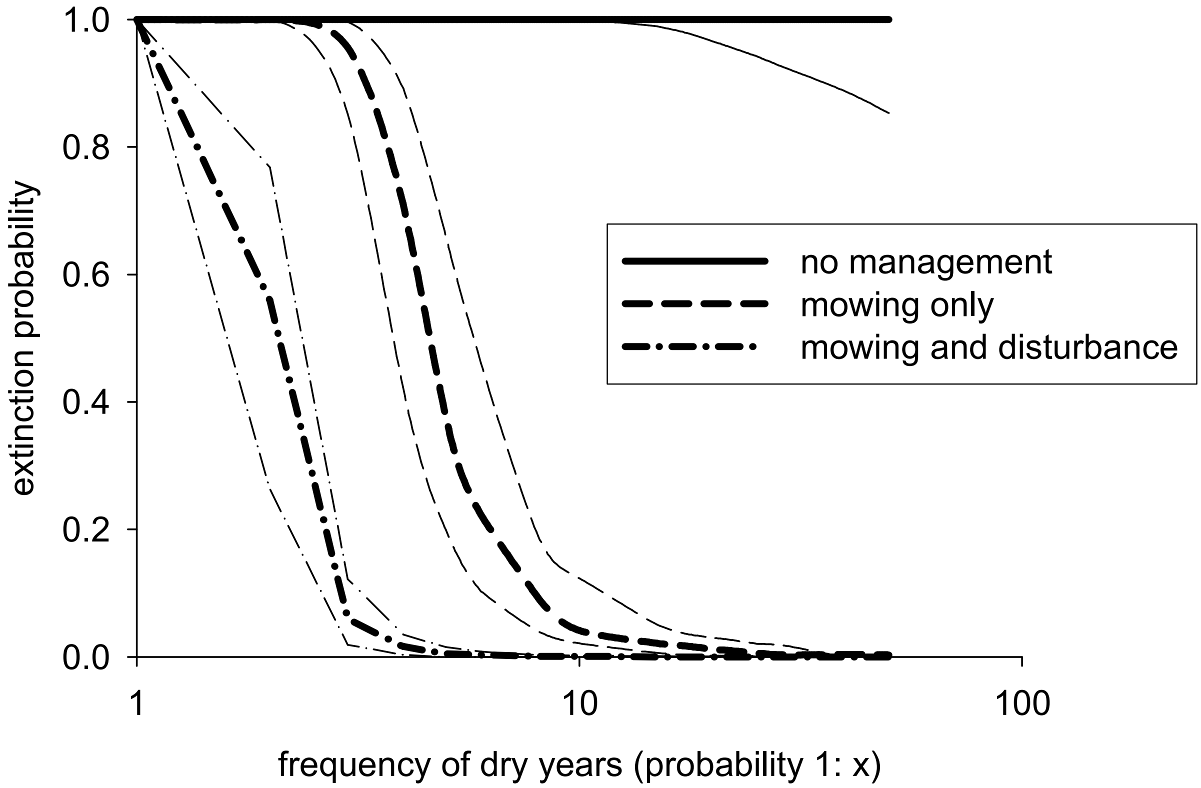


Fig 6: Extinction probability of small population (10 flowering individuals) as function of frequency of extremely dry years. Based on pooled matrices, confidence intervals in thin lines. Model 2.



Appendix 1

List of data available in each year of the experiment. The annual cycle starts in autumn, when seeds have been sown to the soil and next spring, germination and seedling establishment was followed. Symbol “●” means data available, symbol “● all died” means data available, but probability of the transition is zero – all plants died; symbol “NA – dry” means data not available, because all plants died due to the dry summer in 2003. In years 2003 and 2004, no sowing experiment was possible to perform on 3 localities out of 4, because of total lack of seeds because of extreme mortality due to dry summer. On the locality Chvalšiny, few seeds were available in autumn 2003 and 2004 and sowing experiment was carried out in limited size.

				One year old plants			Two years old plants				
locality	**transition interval**	**management**	**seedling establishment from sowing**	**spring-summer**	**summer-autumn**	**autumn-spring**	**spring-summer**	**summer-autumn**	**seed production per plant**	**seeds to seed bank**	**seedling establishment from the seed bank**
Dobročkov	2000-2001	no management	●	●	●					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2001-2002	no management	●	●	●	●	●	●		●	●
		mowing	●	●	●	●	●	●		●	●
		mowing and disturbance	●	●	●	●	●	●		●	●
	2002-2003	no management	●	●	● all died	●	● all died	NA - dry	●	●	●
		mowing	●	●	● all died	●	● all died	NA - dry	●	●	●
		mowing and disturbance	●	●	● all died	●	● all died	NA - dry	●	●	●
	2003-2004	no management				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing and disturbance				NA - dry	NA - dry	NA - dry	NA - dry		●
	2004-2005	no management						NA - dry	NA - dry		
		mowing						NA - dry	NA - dry		
		mowing and disturbance						NA - dry	NA - dry		
	2005-2006	no management	●	●	●					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2006-2007	no management				●	●	●			●
		mowing				●	●	●			●
		mowing and disturbance				●	●	●			●
	2007-2008	no management							●		
		mowing							●		
		mowing and disturbance							●		

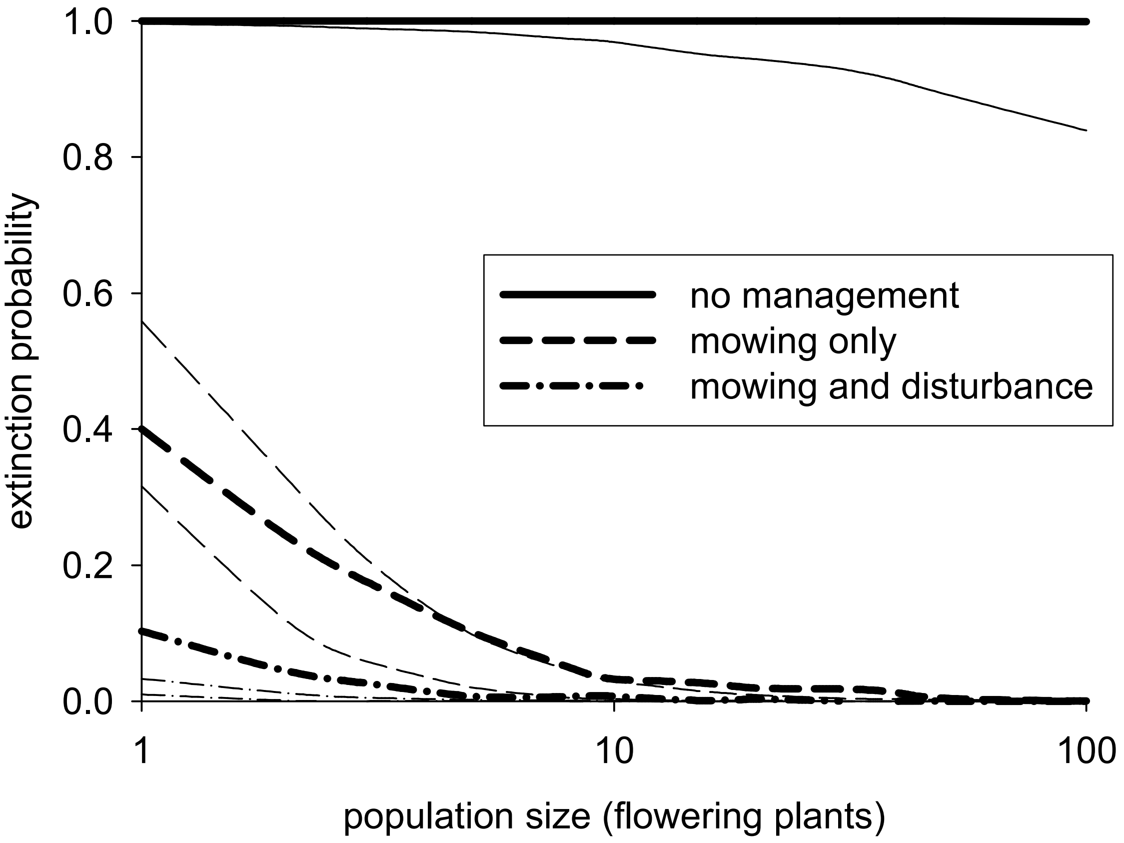
				One year old plants			**Two years old plants**				
locality	**year**	**management**	**seedling establishment from sowing**	**spring-summer**	**summer-autumn**	**autumn-spring**	**spring-ummer**	**summer-autumn**	**autumn first year seed production per plant**	**seeds to seed bank**	**seedling establishment from the seed bank**
Hroby	2000-2001	no management	●	●	●					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2001-2002	no management	●	●	●	●	●	●		●	●
		mowing	●	●	●	●	●	●		●	●
		mowing and disturbance	●	●	●	●	●	●		●	●
	2002-2003	no management	●	●	● all died	●	●	● all died	●	●	●
		mowing	●	●	● all died	●	●	● all died	●	●	●
		mowing and disturbance	●	●	● all died	●	●	● all died	●	●	●
	2003-2004	no management				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing and disturbance				NA - dry	NA - dry	NA - dry	NA - dry		●
	2004-2005	no management	●	●	●				NA - dry	●	
		mowing	●	●	●				NA - dry	●	
		mowing and disturbance	●	●	●				NA - dry	●	
	2005-2006	no management	●	●	●	●	●	●		●	●
		mowing	●	●	●	●	●	●		●	●
		mowing and disturbance	●	●	●	●	●	●		●	●
	2006-2007	no management				●	●	●			●
		mowing				●	●	●	●		●
		mowing and disturbance				●	●	●			●
	2007-2008	no management							●		
		mowing							●		
		mowing and disturbance							●		

				One year old plants			**Two years old plants**				
locality	**year**	**management**	**seedling establishment from sowing**	**spring-summer**	**summer-autumn**	**autumn-spring**	**spring-ummer**	**summer-autumn**	**autumn first year seed production per plant**	**seeds to seed bank**	**seedling establishment from the seed bank**
Chvalšiny	2001-2002	no management	●	●	● all died					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2002-2003	no management	●	●	● all died	●	●	● all died		●	●
		mowing	●	●	● all died	●	●	● all died		●	●
		mowing and disturbance	●	●	●	●	●	● all died		●	●
	2003-2004	no management	●	●	●	NA - dry	NA - dry	NA - dry	NA - dry	●	●
		mowing	●	●	●	NA - dry	NA - dry	NA - dry	NA - dry	●	●
		mowing and disturbance	●	●	●	NA - dry	NA - dry	NA - dry	NA - dry	●	●
	2004-2005	no management	●	●	●	●	●	●	NA - dry	●	●
		mowing	●	●	●	●	●	●	NA - dry	●	●
		mowing and disturbance	●	●	●				NA - dry	●	●
	2005-2006	no management	●	●	●	●	●	●	●	●	●
		mowing	●	●	●	●	●	●	●	●	●
		mowing and disturbance	●	●	●					●	●
	2006-2007	no management				●	●	●	●		●
		mowing				●	●	●	●		●
		mowing and disturbance				●	●	●			●
	2007-2008	no management							●		
		mowing							●		
		mowing and disturbance							●		

				One year old plants			Two years old plants				
locality	**year**	**management**	**seedling establishment from sowing**	**spring-summer**	**summer-autumn**	**autumn-spring**	**spring-summer**	**summer-autumn**	**autumn first year seed production per plant**	**seeds to seed bank**	**seedling establishment from the seed bank**
Onšovice	2000-2001	no management	●	●	●					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2001-2002	no management	●	●	●	●	●	●		●	●
		mowing	●	●	●	●	●	●		●	●
		mowing and disturbance	●	●	●	●	●	●		●	●
	2002-2003	no management	●	● all died	NA - dry	●	●	● all died	●	●	●
		mowing	●	● all died	NA - dry	●	●	● all died	●	●	●
		mowing and disturbance	●	●	● all died	●	● all died	NA - dry	●	●	●
	2003-2004	no management				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing				NA - dry	NA - dry	NA - dry	NA - dry		●
		mowing and disturbance				NA - dry	NA - dry	NA - dry	NA - dry		●
	2004-2005	no management							NA - dry		
		mowing							NA - dry		
		mowing and disturbance							NA - dry		
	2005-2006	no management	●	●	●					●	
		mowing	●	●	●					●	
		mowing and disturbance	●	●	●					●	
	2006-2007	no management				●	●	●			●
		mowing				●	●	●			●
		mowing and disturbance				●	●	●			●
	2007-2008	no management							●		
		mowing							●		
		mowing and disturbance							●		

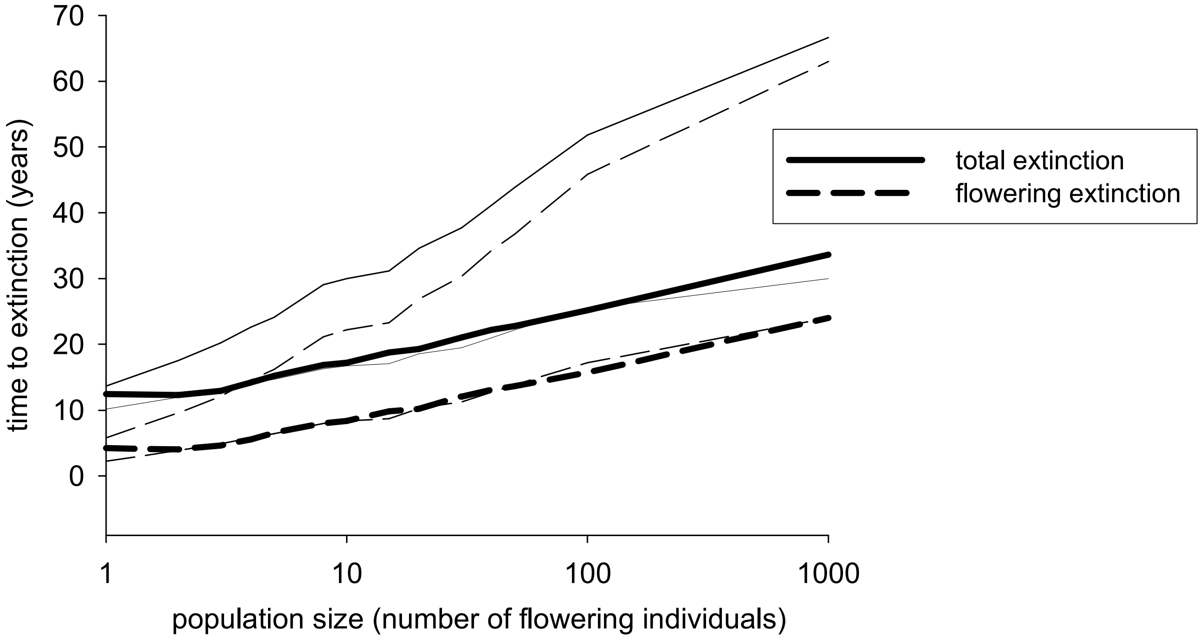
Appendix 2

Appendix 2, Fig 1: Extinction probability in 100 years as a function of population size (Model 1), based on separate matrices. Confidence intervals, in thin lines, based on pooled matrices.



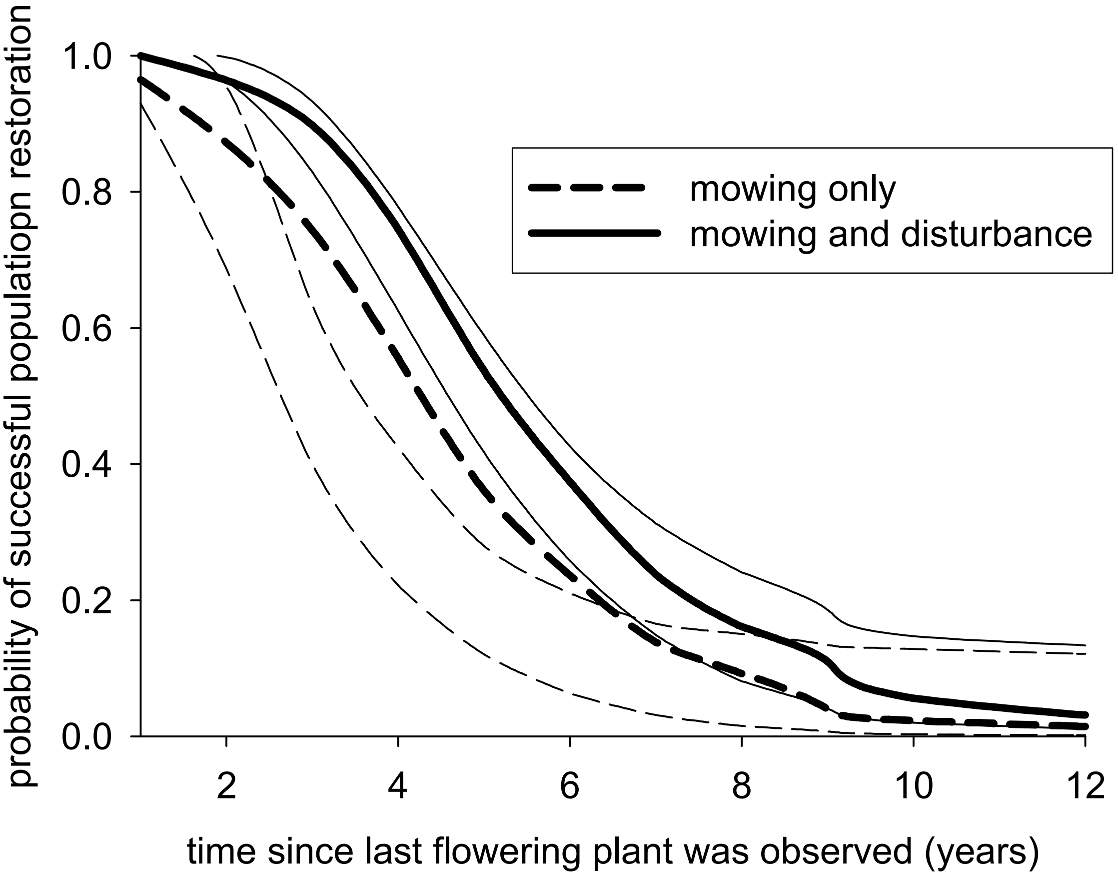
Appendix 2, Fig 2:

Time to extinction of a population as a function of population size (Model 1), under condition on absence of management. In the Graph, putative extinction (end of appearance of flowering plants) a total extinction (extinction of all life stages) is marked. Based on separate matrices. Confidence interval, in thin lines, based on pooled matrices. Model 1.



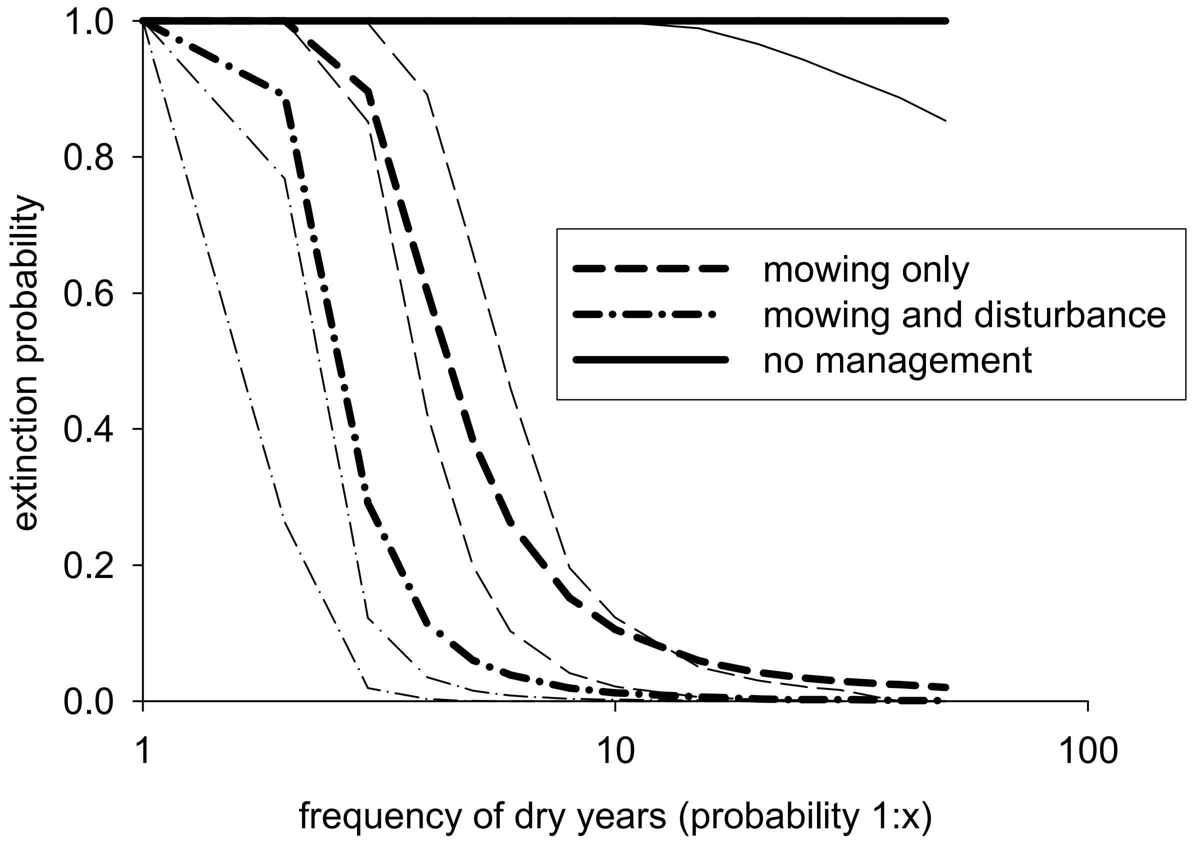
Appendix 2, Fig 3:

Probability of successful restoration of a population related to the time, for how long no flowering individual was present in the population. Based on separate matrices. Confidence interval (in thin lines) based on pooled matrices. Model 2.



Appendix 2, Fig 4:

Extinction probability of small population (10 flowering individuals) as function of frequency of extremely dry years. Based on separate matrices. CI based on pooled matrices. Model 3.



CONCLUSIONS

In this thesis I investigated population dynamics on local and regional scale (area 10x10 km) in two rare fern species restricted to serpentine rocks, *Asplenium adulterinum* and *A. cuneifolium*. Further, I attempted to transfer the theoretical knowledge to nature conservation to practice.

The two fern species are well prospering in the study region. All study populations are slowly growing and even very small populations (10 individuals) have quite high chance to survive. The dynamics of populations is stable in both species and the species are long living – several decades. On the regional scale, distribution of both species is rather limited by dispersal and establishment abilities of the species than by availability of suitable habitats. *A. adulterinum* has better colonization abilities of new habitats than *A. cuneifolium*, since it occupies higher percentage of available suitable habitats. It is in concordance with expectations, since *A. adulterinum* is species with predominant intragametophytic selfing and thus, it is able of single spore colonization, which may be problematic in outcrossing *A. cuneifolium*. On the other hand, gene flow between already established populations is higher in outcrossing *A. cuneifolium* than in selfing *A. adulterinum*, since outcrossing facilitates gene exchange. It illustrates, that colonization of new habitats and gene flow between already established populations both depend on breeding system but in opposite direction.

The two fern species are rare, but since they are not endangered within study region, there was no need to transfer theoretical knowledge to conservation practice. This I performed on another species, *Gentianella praecox* subsp. *bohemica*, endemic species of Central European seminatural grasslands with rapidly declining number of populations due to management change. I found that management of seminatural grasslands is of crucial importance for survival of this species. If management is performed, even small populations (10 individuals) of this species are able to survive, climate change (simulated as increased frequency of dry years) will have small impact on populations. However, unmanaged populations are not able to survive in long-term (several decades). Almost extinct populations may be recovered from the seed bank by management induction. Results of the study were implemented into recently prepared action plan for this species in the Czech Republic.

REFERENCES

Alvarez-Buylla ER, Slatkin M. 1994. Finding Confidence-Limits on Population-Growth Rates - 3 Real Examples Revised. Ecology, 75: 255-260.

Beissinger SR, Westphal MI. 1998. On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management, 62: 821-841.

Buckley YM, Briese DT, Rees M. 2003. Demography and management of the invasive plant species Hypericum perforatum. II. Construction and use of an individual-based model to predict population dynamics and the effects of management strategies. Journal of Applied Ecology, 40: 494-507.

Bucharova A, Munzbergova Z, Tajek P. 2010. Population Biology of Two Rare Fern Species: Long Life and Long-Lasting Stability. American Journal of Botany, 97: 1260-1271.

Caswell H. 2001. Matrix population models: construction, analysis, and interpretation, Massachusetts, Sinauer Associates, Sunderland.

Clark CJ, Poulsen JR, Levey DJ, Osenberg CW. 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. American Naturalist, 170: 128-142.

Cochran ME, Ellner S. 1992. Simple Methods for Calculating Age-Based Life-History Parameters for Stage-Structured Populations. Ecological Monographs, 62: 345-364.

Dassler CL, Farrar DR. 2001. Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. Brittonia, 53: 352-369.

DeAngelis DL, Mooij WM. 2005. Individual-based modeling of ecological and evolutionary processes. Annual Review of Ecology Evolution and Systematics, 36: 147-168.

Efron B, Tibshirani RJ. 1994. Introduction to the bootstrap, New York, Chapman and Hall.

Ehrlen J, Munzbergova Z, Diekmann M, Eriksson O. 2006. Long-term assessment of seed limitation in plants: results from an 11-year experiment. Journal of Ecology, 94: 1224-1232.

Eriksson O. 1997. Colonization dynamics and relative abundance of three plant species (Antennaria dioica, Hieracium pilosella and Hypochoeris maculata) in dry semi-natural grasslands. Ecography, 20: 559-568.

Fieberg J, Ellner SP. 2000. When is it meaningful to estimate an extinction probability? Ecology, 81: 2040-2047.

Flinn KM. 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. American Journal of Botany, 93: 1289-1294.

Garcia MB. 2003. Demographic viability of a relict population of the critically endangered plant Borderea chouardii. Conservation Biology, 17: 1672-1680.

Garcia MB, Pico FX, Ehrlen J. 2008. Life span correlates with population dynamics in perennial herbaceous plants. American Journal of Botany, 95: 258-262.

Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of species extinction. In: Soulé ME ed. Conservation biology: the science of scarcity and diversity. Sunderland, Sinauer Associates.

Griffith AB, Forseth IN. 2005. Population matrix models of Aeschynomene virginica, a rare annual plant: Implications for conservation. Ecological Applications, 15: 222-233.

Grimm V. 1999. Ten years of individual-based modelling in ecology: what have we learned and what could we learn in the future? Ecological Modelling, 115: 129-148.

Hanski I. 1991. Single-Species Metapopulation Dynamics - Concepts, Models and Observations. Biological Journal of the Linnean Society, 42: 17-38.

Hanski I. 1994. A Practical Model of Metapopulation Dynamics. Journal of Animal Ecology, 63: 151-162.

Hejný S, Slavík H. 1980. Flora of Czech Republic (Květena České Republiky). Praha, Academia.

Hoebee SE, Thrall PH, Young AG. 2008. Integrating population demography, genetics and self-incompatibility in a viability assessment of the Wee Jasper Grevillea (Grevillea iaspicula McGill., Proteaceae). Conservation Genetics, 9: 515-529.

Holderegger R, Schneller JJ. 1994. Are Small Isolated Populations of *Asplenium*-Septentrionale Variable. Biological Journal of the Linnean Society, 51: 377-385.

Husband BC, Barrett SCH. 1996. A metapopulation perspective in plant population biology. Journal of Ecology, 84: 461-469.

Husband BC, Barrett SCH. 1998. Spatial and temporal variation in population size of Eichhornia paniculata in ephemeral habitats: implications for metapopulation dynamics. Journal of Ecology, 86: 1021-1031.

IUCN/SSC. 2002. Species Survival Commission Action Plan Evaluation., Gland, Switzerland, IUCN Species Survival Commission.

IUCN/SSC. 2008. Strategic Planning for Species Conservation: A Handbook. Version 1.0, Gland, Switzerland, IUCN Species Survival Commission.

Jimenez A, Quintanilla LG, Pajaron S, Pangua E. 2009. Genetic Variation in the Allotetraploid Dryopteris Corleyi (Dryopteridaceae) and Its Diploid Parental Species in the Iberian Peninsula. American Journal of Botany, 96: 1880-1886.

Jongejans E, de Vere N, de Kroon H. 2008. Demographic vulnerability of the clonal and endangered meadow thistle. Plant Ecology, 198: 225-240.

Lande R, Schemske DW. 1985. The Evolution of Self-Fertilization and Inbreeding Depression in Plants .1. Genetic Models. Evolution, 39: 24-40.

Leslie PH. 1945. On the use of matrices in certain population mathematics. Biometrika, 33: 183-212.

Levins R. 1970. Extinction. Lectures on Mathematics in the Life Sciences, 2: 75-107.

Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics, 15: 65-95.

Ludwig D. 1999. Is it meaningful to estimate a probability of extinction? Ecology, 80: 298-310.

Menges ES. 1986. Predicting the future of rare plant populations: demographic monitoring and modelling. Natural Areas Journal, 6: 13-25.

Menges ES. 1990. Population Viability Analysis for an Endangered Plant. Conservation Biology, 4: 52-62.

Milden M, Munzbergova Z, Herben T, Ehrlen J. 2006. Metapopulation dynamics of a perennial plant, Succisa pratensis, in an agricultural landscape. Ecological Modelling, 199: 464-475.

Moles AT, Westoby M. 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. Oikos, 99: 241-248.

Moore KA, Elmendorf SC. 2006. Propagule vs. niche limitation: untangling the mechanisms behind plant species' distributions. Ecology Letters, 9: 797-804.

Munzbergova Z. 2004. Effect of spatial scale on factors limiting species distributions in dry grassland fragments. Journal of Ecology, 92: 854-867.

Munzbergova Z, Herben T. 2004. Identification of suitable unoccupied habitats in metapopulation studies using co-occurrence of species. Oikos, 105: 408-414.

Munzbergova Z, Herben T. 2005. Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. Oecologia, 145: 1-8.

Ogilvie RT, Ceska A. 1984. Alpine Plants of Phytogeographic Interest on Northwestern Vancouver-Island. Canadian Journal of Botany-Revue Canadienne De Botanique, 62: 2356-2362.

Okland RH. 1995. Population Biology of the Clonal Moss Hylocomium Splendens in Norwegian Boreal Spruce Forests .1. Demography. Journal of Ecology, 83: 697-712.

Ouborg NJ. 1993. Isolation, Population-Size and Extinction - the Classical and Metapopulation Approaches Applied to Vascular Plants Along the Dutch Rhine-System. Oikos, 66: 298-308.

Pico FX, Quintana-Ascencio PF, Milden M, Ehrlen J, Pfingsten I. 2009. Modelling the effects of genetics and habitat on the demography of a grassland herb. Basic and Applied Ecology, 10: 122-130.

Pino J, Pico FX, De Roa E. 2007. Population dynamics of the rare plant Kosteletzkya pentacarpos (Malvaceae): a nine-year study. Botanical Journal of the Linnean Society, 153: 455-462.

QuintanaAscencio RF, Menges ES. 1996. Inferring metapopulation dynamics from patch-level incidence of Florida scrub plants. Conservation Biology, 10: 1210-1219.

Ranker TA, Floyd SK, Windham MD, Trapp PG. 1994. Historoical biogeography of *Asplenium adianthum-nigrum* (Aspleniaceae) in North America and implications for speciation theory in homosporous. American Journal of Botany, 81: 776-781.

Ranker TA, Geiger JMO. 2008. Population genetics. In: Ranker TA, Haufler CH eds. Biology and Evolution of Ferns and Lycophytes. Cambridge, Cambridge University Press.

Romermann C, Tackenberg O, Scheuerer M, May R, Poschlod P. 2007. Predicting habitat distribution and frequency from plant species co-occurrence data. Journal of Biogeography, 34: 1041-1052.

Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG. 1994. Evaluating Approaches to the Conservation of Rare and Endangered Plants. Ecology, 75: 584-606.

Schneller JJ. 1996. Outbreeding depression in the fern *Asplenium ruta-muraria* L: Evidence from enzyme electrophoresis, meiotic irregularities and reduced spore viability. Biological Journal of the Linnean Society, 59: 281-295.

Schneller JJ, Holderegger R. 1996. Colonisation events and genetic variability within populations of *Asplenium* ruta-muraria L. In: Camus JM, Gibby M, Johns RJ eds. Pteridology in Perspective. Kew, Royal Botanic Gardens.

Suter M, Schneller JJ, Vogel JC. 2000. Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium* trichomanes subsp quadrivalens. International Journal of Plant Sciences, 161: 233-244.

Tájek P. 2003. Serpentine outcrops in Slavkovský les in view of island biogeography. (Hadcové výchozy ve Slavkovském lese z pohledu ostrovní biogeografie). Diploma thesis. Praha, Department of Botany, Charles University.

Tremlova K, Munzbergova Z. 2007. Importance of species traits for species distribution in fragmented landscapes. Ecology, 88: 965-977.

Turnbull LA, Crawley MJ, Rees M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos, 88: 225-238.

Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA. 1980. Flora Europaea. Cambridge University Press.

Vandermeijden E, Klinkhamer PGL, Dejong TJ, Vanwijk CAM. 1992. Meta-Population Dynamics of Biennial Plants - How to Exploit Temporary Habitats. Acta Botanica Neerlandica, 41: 249-270.

Vogel JC, Rumsey FJ, Russell SJ, Cox CJ, Holmes JS, Bujnoch W, Starks C, Barrett JA, Gibby M. 1999. Genetic structure, reproductive biology and ecology of isolated populations of *Asplenium* csikii (Aspleniaceae, Pteridophyta). Heredity, 83: 604-612.

web1. ec.europa.eu/environment/nature/natura2000.

web2. 3.11.2010. www.biomonitoring.cz.

Wild M, Gagnon D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? Ecography, 28: 191-196.

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Geselschaft für Oecologie, Basel. Poster: Population biology of rare ferns: *Asplenium adulterinum* and *A. cuneifolium*.

2005:
Geselschaft für Oecologie, Potsdam, Germany. Poster: Population biology and metapopulation dynamics of two rare ferns: *Asplenium adulterinum* and *A. cuneifolium*.

2003:
Geoecological problems of Krkonoše Mts., Sklarska Poreba, Poland. Talk: Management of invasive plant *Rumex alpinus* L.

Publications:
Tájek P., Bucharová A., Münzbergová Z. (in press): Limitation of distribution of two rare ferns in fragmented landscape. Acta Oecologica.

Bucharová A., Münzbergová Z., Tájek P. (2010): Population biology of two rare fern species: long life and long-lasting stability. Am. J. Bot. 97: 1260-1271

Bucharová A. et van Kleunen M. (2009): Introduction history and species characteristics partly explain naturalization success of North American woody species in Europe. Journal of Ecology 97 (2): 230-238

Münzbergová Z., Křivánek M., Bucharová A., Juklíčková V. & Herben T. (2005): Ramet performance in two tussock plants - do the tussock-level parameters matter? – Flora, pp 275-284.

Many publications in local nature conservation magazines.

Unpublished materials:
Bucharová A. (2003): *Rumex alpinus* L. in Krkonoše Mts. – distribution and management of an invasive plant. Master thesis. Ms. dep in. Library of the Department of Botany, Charles University, Prague. (in Czech)
Various materials for nature conservation: surveys of many nature reserves, methods of preservation of *Asplenium adulterinum* etc.

Research visits abroad:
March – August 2008 (6 months) – Institute of Plant Sciences, University of Bern, Switzerland, work with Mark van Kleunen and Markus Fisher.

Others:
2004-2009: head of the editorial office of a regional magazine "Arnika – nature and history of Karlovy Vary region" (in Czech)
2004-2009: member of the editorial office of regional magazine "Český Les" (in Czech)