Local adaptation of the rare herb *Aster amellus* in fragmented landscape

Jana Raabová

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Charles University, Faculty of Science
Department of Botany

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Supervisor: Mgr. Zuzana Münzbergová, Ph.D.

I declare that no part of this thesis has been used to obtain any other academic degree.

Contents

GENERAL INTRODUCTION

General introduction

Human activities caused massive changes in landscape, including land transformation, habitat deterioration and fragmentation (Saunders et al., 1991; Vitousek et al., 1997). In the process of habitat fragmentation, plant populations become smaller and more isolated from each other. For a number of reasons small populations are expected to face a high risk of extinction.

Small populations are more prone to demographic, environmental, catastrophic and genetic stochasticity than large populations (Ouborg et al., 2006). Demographic stochasticity is a random fluctuation in demographic parameters among individuals within a population. Environmental stochasticity is a random variation in environmental parameters over time and space. Demographic stochasticity is only a threat to very small populations (< 50 individuals), whereas environmental stochasticity will have significant impact on the population growth rate even in average size populations (Lande, 1993). Unpredictable catastrophes, such as hurricanes, flooding and forest fires, are more likely to lead to the extinction of small than of large populations (Lande, 1993; Ouborg et al., 2006). Genetic stochasticity (genetic drift) is the random change in allele frequency that occurs because gametes transmitted from one generation to the next carry only a sample of the alleles present in the parental generation. In small populations (< 100 individuals), allele frequencies may undergo large and unpredictable fluctuation (Ellstrand and Elam, 1993). The genetic stochasticity has attracted the most attention over recent decades (Ouborg et al., 2006).

Moreover, mating of related individuals (inbreeding) increases in small populations (Ellstrand and Elam, 1993). In plants, inbreeding occurs through

selfing and biparental inbreeding when the populations are small or when they exhibit spatial genetic structure (Ellstrand and Elam, 1993). The consequence of inbreeding is reduced individual fitness and is called inbreeding depression (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002). The genetic basis of inbreeding depression has been explained by two hypotheses: (i) overdominance, when both types of homozygotes have lower fitness than heterozygotes and (ii) partial dominance, when inbreeding depression is the result of expression of deleterious recessive alleles at homozygous loci (Charlesworth and Charlesworth, 1987).

In many species, individuals in small populations experience diminished viability and reproduction for nongenetic reasons, known as an Allee effect (Stephens and Sutherland, 1999; Stephens et al., 1999). It is named after an American zoologist and ecologist, W. C. Allee (1885–1955). Allee effect can be caused by the organisms physically or chemically modyfiing their environment, by the social interaction or by density-dependent mating success (Lande, 1988). For plants, Allee effects mainly involve the difficulty of the ovules being fertilised when populations become small and density decreases (Oostermeijer et al., 2003). Small populations of animal pollinated species are less likely to be attractive to pollinators than large populations (Sih and Baltus, 1987). As a consequence, pollinator visitations may be lower, and the degree to which seed production is limited by pollinations may be higher in small than in large populations (Jennersten, 1988; Ågren, 1996).

Many empirical studies have found that plants in small populations exhibit reduced genetic variation and reduced fitness (e.g. Fischer and Matthies, 1998; Fischer et al., 2003; Oostermeijer et al., 1998; Vergeer et al., 2003). Recent meta-analyses have shown that the positive relationships between population size, genetic variation and plant fitness are general (Reed

and Frankham, 2003; Leimu et al., 2006). This strong positive relationship suggests that the small population size negatively affected genetic variation and plant fitness, rather than that habitat quality deteriorates plant fitness, which subsequently reduces population size and genetic variation (Leimu et al., 2006). However, although plant fitness is often reduced due to genetic reasons (e.g. Spielman et al., 2004), the ecological characteristics should also been taken into account in species conservation, instead of viewing ecology and genetics as opposite concepts (Oostermeijer et al., 1998; Ouborg et al., 2006).

It has been assumed that only a very small amount of gene flow is required to reduce the genetic problems of small and isolated populations (Young et al., 1996). Therefore, translocation of individuals between populations has been proposed as a management tool to enhance gene flow between populations (van Groenendael et al., 1998; Storfer, 1999; Tallmon et al., 2004). However, the translocation of organisms during the restoration of native ecosystem provoked new questions concerning the consequences of intraspecific hybridization between locally adapted and transplanted genotypes (Hufford and Mazer, 2003).

Local adaptation

Population isolation together with different selective forces in each population may lead to adaptation to local conditions (Slatkin, 1985). Because plants are sessile and typically have limited gene flow through seeds and pollen, they experience generations of selection by local environmental

conditions (Galloway and Fenster, 2000). This selection may result in adaptive genetic differentiation.

Adaptive evolutionary changes to a wide range of conditions have been reported in plants, including those to soil conditions (Hangelbroek et al., 2003), water stress (Knight and Miller, 2004), flooding (Lenssen et al., 2004), herbivores (Sork et al., 1993), pathogenes (Thrall et al., 2002). Although there is abundant evidence of local adaptation in plants, it is important to remember that not all phenotypic differences between populations growing in different environments are adaptive, and that not all adaptive differences reflect genetic differences (Silvertown and Charlesworth, 2001). Whether population divergence represents an adaptive response to natural selection can be determine with reciprocal transplant technique. Adaptive differences between populations are detected as a home-site advantage, whereby each genotype (ecotype) performs best at its native site (Hufford and Mazer, 2003; Kawecki and Ebert, 2004).

The degree of local adaptation depends on a balance between local selective forces and regional dispersal processes. Gene flow can constrain local adaptation to a spatially heterogeneous environment by preventing local differentiation (Slatkin, 1987). As the distance between populations increases, on average both the degree of environmental and of genetic isolation are expected to increase. Therefore, populations are likely to be less well adapted to sites increasingly distant from their home (Galloway and Fenster, 2000; Montalvo and Ellstrand, 2000). Further, it can be assumed that the degree of local adaptation may vary tremendously between different organisms. Selective forces might result in more persistent adaptive divergence in perennial organisms with lower population turnover than in annual or biennial species (Fenster and Galloway, 2000).

An important mechanism contributing to adaptation is genome multiplication. Differences in ploidy level are commonly observed among closely related plant species and among populations within species (Ramsey and Schemske, 2002). Polyploidy contributes to adaptation by making populations adaptable to a wider range of environmental conditions. Further, polyploidy may induce immediate phenotypic changes that incidentally preadapt plants to a new ecological niche (Ramsey and Schemske, 2002). Thus, polyploidy is widely believed to be a mechanism of local adaptation (Levin, 1983).

Outbreeding depression

Gene flow is usually considered beneficial in conservation biology, because immigrants can infuse new genetic variation that increases fitness (Tallmon et al., 2004). The increase in fitness is due to heterosis in the offspring that result from mating between immigrants and local individuals. Heterosis occurs through two mechanisms. First, immigrant alleles can mask deleterious recessive alleles. Second, mating between immigrants and local individuals produce highly heterozygous offspring, which are often favoured by natural selection. The recent literature suggests that masking of deleterious alleles is the more prevalent mechanism of heterosis (Tallmon et al., 2004).

On the other hand, gene flow can also be detrimental for small populations because, under certain conditions, crossing between genetically distinct populations results in reduced offspring fitness due to outbreeding depression (e.g. Waser and Price, 1994; Hufford and Mazer, 2003; Edmans, 2007). Outbreeding depression can occur already in F1 generation, where it

can be attributed to disruption of local adaptation, underdominance or epistatic interaction (Edmands, 2002). Frequently, however, fitness decline do not occur until the F2 generation, where the original parental gene combinations are broken up by recombination (Edmands, 2002).

While some studies have found outbreeding depression after long distance crosses of several hundred kilometres (Montalvo and Ellstrand, 2001; Galloway and Etterson, 2005), other studies detected outbreeding depression between subsets of individuals within a single population (Waser and Price, 1994; Quilichini et al., 2001). Therefore, outbreeding depression over short distances is not unexpected because it can be a by-product of high local adaptation (Waser and Price, 1989). Evidence of outbreeding depression is a matter of controversy (Frankham et al., 2002). However, some studies suggest that the effects of outbreeding can be as severe as the effects of inbreeding (Edmands, 2007).

This thesis

Local adaptation has become an important issue in nature conservation because mixing of genetic material from populations from different environments may generate genotypes that do not perform well under some or all conditions. The extent of local adaptation in plant populations has been subject of several studies. However, whether adaptive differentiation between populations may arise at a small scale among isolated habitats with little ecological differentiation has rarely been studied. Many threatened species occur in such isolated habitats and the assessment of local adaptation in these situations is important for appropriate conservation strategies of the species.

Many species of dry calcareous grasslands occur in small and isolated populations and are threatened with extinction. Gene flow between populations of these species is restricted and therefore plants in individual populations might be adapted to local conditions. I choose one of theses species, *Aster amellus* as an object of my study. This species occurs in populations of different sizes and is easy to cultivate. Because the species occurs in two ploidy levels in the Czech Republic, I studied the effects of polyploidization on habitat requirements of the species (Chapter 1). I used allozyme markers to quantify gene flow among populations (Chapter 2). I conducted reciprocal transplant experiments to study differentiation between diploid and hexaploid plants (Chapter 1) and to study local adaptation at two spatial scales in diploid populations (Chapter 2). I carried out between-population crosses to assess the extent of outbreeding depression (Chapter 3). I combined field and common garden experiments, pollination experiments with the use of genetic markers in this thesis.

Study species - *Aster amellus* L. (Asteraceae)

Aster amellus is a subcontinental species distributed from western Europe to western Siberia (Meusel and Jäger, 1992). In its whole distribution range it occurs in three ploidy levels (2x, 4x and 6x; x = 9) (Merxmüller et al., 1976). Only diploid and hexaploid plants are known from the Czech Republic (Kovanda 2002; Mandáková and Münzbergová 2006). Kovanda (2002) suggested separation of the two cytotypes into two species and used the name *A. scepusiensis* for the hexaploid cytotype. However, the biosystematic study of Mandáková and Münzbergová (unpublished manuscript) does not support this separation. Because taxonomic status of the species is not clarified, I use the same name, *Aster amellus*, for both cytotypes in this study. Both cytotypes posses many of the same alleles and are morphologicaly similar, suggesting autopolyploid origin of the hexaploid cytotype (Mandáková and Münzbergová, unpublished manuscript). However, more data are needed to confirm this.

Aster amellus is a self-incompatible perennial herb, up to 40 cm high (Kovanda, 2005). Its flowering period lasts from mid-July to mid-October (Kovanda, 2005). Plants reproduce vegetatively and sexually and are mainly pollinated by bees and hoverflies (Raabová, personal observation). The average number of seeds produced per plant is 350 (Raabová, unpublished data). The seeds are usually dispersed over short distances (Münzbergová, unpublished data) and germinate in the spring. Seedlings usually stay at the small rosette stage in the field for several years (Münzbergová 2007a). Its typical habitats, dry calcareous grasslands, have declined over the last

decades, and *A. amellus* became endangered in many parts of Central Europe (e.g. Schönfelder, 1987; Buttler et al., 1997; Holub and Procházka, 2000).

I studied 12 populations of *A. amellus* in two different regions of two ploidy levels. The population names, ploidy levels and coordinates are provided in Table 1.

Table 1. Populations of *Aster amellus* studied in this thesis. S = České středohoří Mts., K = Czech Karst.

Pop.	Population	Ploidy level	Region	Longitude E	Latitude N	Altitude (m)
1	Malíč	2	S	14° 05' 16"	50° 32' 24"	310
2	Holý vrch	2	S	14° 13' 49"	50° 31' 41"	260
3	Encovany	2	S	14° 15' 33"	50° 31' 46"	250
4	Malešov	6	S	14° 18' 58"	50° 30' 03"	200
5	Hošťka	6	S	14° 18' 41"	50° 29' 36"	200
6	Sovice	6	S	14° 18' 23"	50° 27' 56"	190
7	Vrutice sad	6	S	14° 17' 51"	50° 30' 15"	190
8	Vrutice moto	6	S	14° 17' 57"	50° 30' 15"	190
9	Svařenice	6	S	14° 18' 10"	50° 29' 46"	190
10	Koda	2	K	14° 07' 29"	49° 56' 01"	350
11	Karlík	2	K	14° 15' 02"	49° 56' 52"	320
12	Lochkov	2	K	14° 20' 16"	49° 59' 56"	300

Contents of the thesis

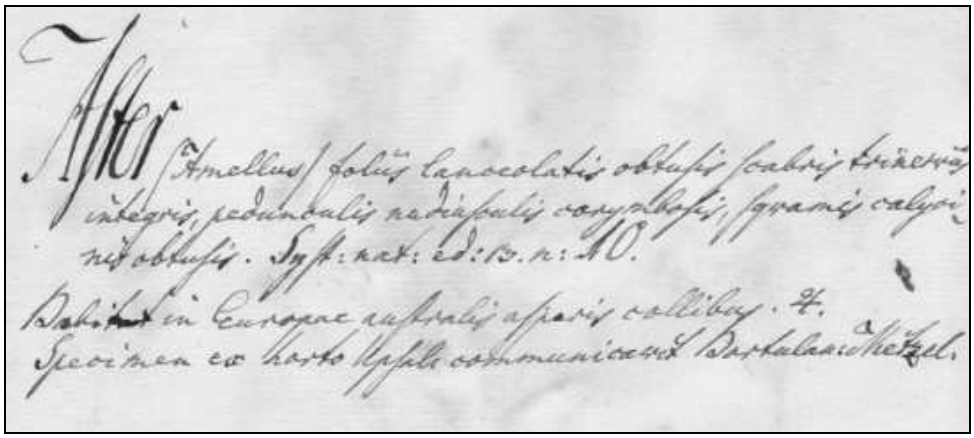
Chapter 1 reports on the study of niche differentiation between diploid and hexaploid *A. amellus*. Diploid and hexaploid *A. amellus* show a strong spatial segregation and no population consisting of both cytotypes has been found in the Czech Republic. I tested the hypothesis that this pattern can be explained by differences in ecological requirements of the two cytotypes. I

analysed habitat characteristics of sites occupied by different ploidy levels. Moreover, I carried out reciprocal transplant experiments with nine populations belonging to two habitat types in the field using both seeds and adult plants.

Chapter 2 deals with the relationship between population differentiation and local adaptation of diploid *A. amellus* on two spatial scales. I present results of three years reciprocal transplant experiment among six populations from two regions. I analysed allozyme variation between populations and characterized the habitat conditions at each site using vegetation composition, soil properties and potential direct solar irradiation. Then, I tested the effects of geographic, genetic and environmental distances between populations on plant performance in the reciprocal transplant experiments.

Chapter 3 explores the consequences of between-population crosses at two spatial scales in *A. amellus*. I conducted three types of crosses: within populations, between populations within regions and between populations from different regions. I assessed seed set, germination percentage, and offspring performance both in the field and in the garden. I asked whether the effect of between-population crosses within regions differ from the one of between-region crosses.

Appendix contain list of the species and their abundance recorded in vegetation surveys in the 12 sites of *A. amellus*.



CHAPTER 1

Niche differentiation between diploid and hexaploid *Aster amellus*

"Things are similar - this makes science possible,

things are different - this makes science necessary."

(Lewontin & Levins)

Chapter 1

Niche differentiation between diploid and hexaploid *Aster amellus*

(with Zuzana Münzbergová)

Abstract

Polyploid and diploid populations of a single species are often spatially segregated but the mechanisms contributing to this segregation are not well understood. In several polyploid complexes, spatial segregation has been related to different ecological requirements of the cytotypes. Although only reciprocal transplant experiments provide direct test of differences in fundamental niche, they have surprisingly rarely been done for diploid and polyploid species pairs. We investigated the role of niche differentiation in spatial segregation of diploid and hexaploid *Aster amellus*. We analysed habitat characteristics of sites occupied by each cytotype and carried out reciprocal transplant experiments with nine populations belonging to two habitat types in the field using both seeds and adult plants. We tested the effects of habitat type, ploidy level and population of origin on plant performance. Sites of diploid and hexaploid populations differ significantly in vegetation and soil properties but much overlap exists in habitat characteristics of the two cytotypes. Seedling survival was higher and transplanted plants had longer leaves at sites of the home ploidy level, suggesting niche differentiation between the two cytotypes. Nevertheless, both seeds and adult plants were able to grow at sites of the foreign cytotype. Furthermore, seedling survival, survival of adult plants and flowering percentage were higher at habitats of

home population than at foreign ones, indicating local adaptation. Subsequent adaptive evolution with the environment could therefore contribute to habitat differentiation of the two cytotypes. We conclude that niche differentiation alone cannot explain spatial segregation of the two cytotypes of *A. amellus*.

Introduction

Genome multiplication is common in flowering plants and is considered as an important mechanism in the origin of evolutionary novelty and the maintenance of diversity in plant populations (Thompson and Lumaret 1992). Polyploid plants often possess new characteristics enabling them to occupy broader range of habitats than their diploid ancestors (Levin 1983). In many cases, polyploid plants are spatially segregated from their diploid progenitors (e.g. Thompson and Lumaret 1992; Burton and Husband 1999).

Spatial segregation of two cytotypes may be explained by three nonexclusive hypotheses. First, spatial segregation might be a consequence of different habitat requirements of the cytotypes. Second, production of infertile hybrids and frequency-dependent minority cytotype exclusion may lead to spatial segregation of cytotypes (Levin 1975, van Dijk and Bakx-Schotman 1997; Baack 2005). Third, segregation of cytotypes may be due to historical factors related to their migration and be maintained by dispersal limitation (Thompson and Lumaret 1992; Baack 2004).

In several polyploid complexes, spatial segregation has been related to different habitat requirements of the cytotypes. Habitat differentiation has been inferred from comparison of habitats in *Dactylis glomerata* (Lumaret et al. 1987) and *Cardamine pratensis* (Arvanitis et al. 2007), from comparison of

associated vegetation in *Anthoxanthum alpinum* (Felber-Girard et al. 1996) and *Galax urceolata* (Johnson et al. 2003) or from different performance of two cytotypes in common garden in *D. glomerata* (Bretagnolle and Thompson 2001). It has been argued that studies based on comparison of habitats may confound competitive displacement with niche differentiation and that only reciprocal transplant experiments provide direct test of differences in fundamental niche (Baack and Stanton 2005).

However, habitat differentiation between two cytotypes may result not necessarily from the polyploidization itself but also from subsequent adaptive evolution with environment (Felber-Girard et al. 1996; Ramsey and Schemske 2002). To distinguish between these two alternatives, it is necessary to compare larger number of populations of each cytotype, covering a range of environments occupied by each of these. To our knowledge, however, only two studies used a larger number of populations in reciprocal transplant experiments with diploid and polyploid pairs: ten populations of *Ranunculus adoneus* (Baack and Stanton 2005) and 12 populations of *Mercurialis annua* (Buggs and Pannell 2007).

The purpose of this study was to investigate the role of niche differentiation in spatial segregation of diploid and hexaploid *A. amellus*. The two cytotypes occur in close proximity in the Czech Republic with the nearest distance of 500 m but they are still strongly spatially segregated and do not occur in mixed populations (Mandáková and Münzbergová 2006). The distribution pattern, absence of intermediate tetraploid cytotype and clear isozyme differentiations suggest secondary contact of the two cytotypes in the Czech Republic (Mandáková and Münzbergová 2006; Mandáková and Münzbergová, unpublished manuscript). Actually, most of the existing contact zones involving diploids and autopolyploids in Europe and North America are

of secondary origin (Hewitt 1988). Various isolating mechanisms have evolved to avoid hybridization between different cytotypes in the contact zones, such as habitat differentiation and shift in flowering phenology (Petit et al. 1999). The flowering phenology of the two cytotypes of *A. amellus* overlaps by more than 50% (Raabová and Münzbergová, unpublished data), enabling hybridization between them. Therefore, niche differentiation might have evolved as an alternative isolation mechanism of the two cytotypes.

Diploid populations of *A. amellus* are confined to habitats with low productivity whereas hexaploids occur in habitats with both low and high productivity. Therefore, we chose three diploid populations and six hexaploid populations in two different habitats. We analysed habitat characteristics of each site. Moreover, we carried out reciprocal transplant experiments in the field using both seeds and adult plants. We asked the following questions: 1. Are the two cytotypes ecologically differentiated? 2. Is there any evidence of niche differentiation due to polyploidization? 3. Can niche differentiation between the two cytotypes lead to their spatial segregation?

Methods

Study species

Aster amellus L. (Asteraceae) is a subcontinental species distributed from western Europe to western Siberia (Meusel and Jäger 1992). In its whole distribution range it occurs in three ploidy levels (2x, 4x and 6x; x = 9)

(Merxmüller et al. 1976). Only diploid and hexaploid plants are known from the Czech Republic (Kovanda 2002; Mandáková and Münzbergová 2006). Kovanda (2002) suggested separation of the two cytotypes into two species and used the name *A. scepusiensis* for the hexaploid cytotype. However, the biosystematic study of Mandáková and Münzbergová (unpublished manuscript) does not support this separation. Because taxonomic status of the species is not clarified, we use the same name, *Aster amellus*, for both cytotypes in this study. Both cytotypes posses many of the same alleles and are morphologicaly similar, suggesting autopolyploid origin of the hexaploid cytotype (Mandáková and Münzbergová, unpublished manuscript). However, more data are needed to confirm this.

Aster amellus is a perennial, up to 40 cm high herb of dry calcareous grasslands with one or few erect stems (Kovanda 2005). One stem produces 100-700 seeds (Raabová, unpublished data). Its flowering period lasts from mid-July to mid-October (Kovanda 2005). It is a self-incompatible insect-pollinated outcrossing plant. Plants of *A. amellus* reproduce vegetatively and sexually and are mainly pollinated by bees and hoverflies (Raabová, personal observation). The seeds are usually dispersed over short distances (Münzbergová, unpublished data) and germinate in the spring. Seedlings usually stay at the small rosette stage in the field for several years (Münzbergová 2007a).

Study sites

We carried out the study in northern Bohemia, Czech Republic (Fig. 1), in the area delimited by the towns of Úštěk, Roudnice nad Labem and

Litoměřice. A measure of total aboveground biomass of all sites in the study area showed that hexaploid populations occur in habitats with both low and high productivity whereas diploid populations are confined only to the habitats with low productivity (Münzbergová 2007b). Thus, we selected three populations belonging two each of the following groups: (1) diploid populations in habitats with low productivity, (2) hexaploid populations in habitats with low productivity and (3) hexaploid populations in habitats with high productivity. Population sizes ranged from 350 to 10 000 flowering stems in 2005.

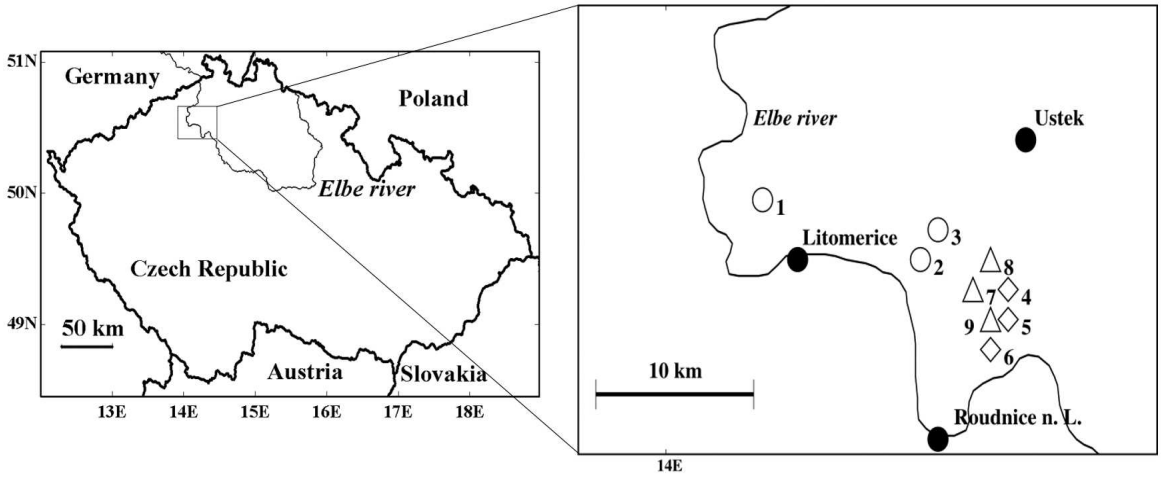


Fig. 1. Distribution of the nine studied populations of *Aster amellus* in the Czech Republic, in the study area delimited by the towns of Úštěk, Roudnice nad Labem and Litoměřice. Circles denote diploid populations (1-3), diamonds denote hexaploid populations in habitats with low productivity (4-6), and triangles denote hexaploid populations in habitats with high productivity (7-9).

Chapter 1

Differences in soil properties and vegetation composition

To characterise the habitats in more detail, we used soil properties and vegetation composition. We collected five to seven soil samples at each site. After transferring samples to the laboratory, they were air-dried, sieved through 2 mm mesh and homogenized. We analysed the concentrations of Ca^{2+}, Mg^{2+}, K^{+} following Moore and Chapman (1986), content of total N and total C following Ehrenberger and Gorbach (1973), carbonate content according to ISO-Standard (10693) and actual (H_2O) and exchangeable (KCl) pH in the laboratory. To assess physical soil properties, we measured maximal water holding capacity of ten samples of 100 cm^3 per site as an amount of water bound in the soil monolith after 24 hours according to Novák (1954). We analysed soil properties with hierarchical analysis of variance (site nested within groups) with S-plus 6.2 (Insightful Corp., Seattle, Washington).

We recorded three vegetation relevés of 2×2 m at each site. We estimated cover of each plant species using the 9-degree Braun-Blanquet scale (1964) and transformed the values according to van der Maarel (1979). We tested the differences in vegetation compositions using canonical correspondence analysis (CCA) with Canoco (ter Braak and Šmilauer 2002). We excluded plant species, which occurred only in one sample, from the analyses. To test for differences among the three groups of sites, we used a split-plot design and permuted whole plots (3 samples from each site together) only. To test for differences among populations within each group, we permuted only relevés within the groups.

Reciprocal transplant of seeds

We sowed seeds from each of the nine populations into each site. We repeated the sowing experiments in 2002 and 2003 to study between-year variation in seedling germination (called experiment 2002 and 2003). We collected ripe seeds from 50 randomly selected individuals from each population in September 2002 and 2003. We sowed the seeds into five 1 × 1.33 m plots divided into 12 grid cells of 33 × 33 cm at each site in October just after natural seed dispersal. In each plot, we placed a bulk sample of 100 seeds from each population on the ground in the middle of nine randomly selected grid cells (9 sites × 9 populations × 5 replications × 100 seeds = 40 500 seeds). The three remaining grid cells in each plot served as controls for natural seed rain. We recorded the percentage of bare soil cover per grid cell in 2005. We counted germinated juveniles in the summer of 2004 and 2005. We did not record germination in experiment 2002 in 2003.

Reciprocal transplant of adult plants

In February 2004, we sowed a bulk sample of c. 300 seeds from each population into plastic trays with garden substrate placed in the greenhouse at 10°C with natural light conditions. In April, we transplanted the seedlings into multipot trays with pots of 3 × 3 cm. We kept the plants in a common garden from June 2004 to April 2005, when we transplanted them into the field. We planted 25 plants from each population into five randomly placed rows at each site (9 × 9 × 5 × 5 = 2 025 plants). We planted the plants 10 cm apart in a random order in a row. We watered them immediately after planting and

measured the length of its longest leaf, because this parameter was found as the best predictor of plant biomass ($r^2 = 0.54$; Plachá et al., unpublished manuscript). The mean number of leaves of the transplanted plants was 6.22 (SE = 0.10) with the mean length of the longest leaf of 13.51 mm (SE = 0.21). Nine percent of plants died in the experimental garden before transplanting (however with no significant differences among populations). Therefore, we excluded these plants from analyses. We recorded survival and flowering percentages and the length of the longest leaf in September 2005 and 2006. Percentage of flowering plants in 2006 was only 1.5% and most of the plants flowered only in one site. Therefore, we used only flowering percentage in 2005 for the analyses.

Data analysis

To analyse differences in plant performance in transplant experiments, we used analyses of covariance for normally distributed variables (longest leaf) and analyses of deviance for binomial variables (germination, survival and flowering percentages). We tested the effects of target site (block nested within site nested within ploidy level and habitat type), population of origin (population nested within ploidy level and habitat type) and population of origin × target site interaction against residuals. We tested the effects of home habitat type, home ploidy level and home population against the population of origin × target site interaction. We also tested the effect of habitat type in hexaploid populations and the effect of ploidy level in habitats with low productivity separately. Because the results of these tests were similar, we show only the results of the tests with the effects of ploidy level, habitat type

and population combined. For significance tests in analysis of deviance we used ratios of mean deviance changes, quasi-F (Francis et al. 1993). To compare the relative importance of the factors, we calculated the percentage variance explained by each factor, R^2 factor as sum of square (deviance) of factor /total sum of square (deviance) × 100. We used the proportion of bare soil cover as a covariate to adjust for different effects of microhabitats on seedling germination. Similarly, we used the initial length of the longest leaf as a covariate to adjust for maternal effects. We performed all analyses with S-plus 6.2 (Insightful Corp., Seattle, Washington).

Results

Differences in soil properties and vegetation composition

The three groups of sites differed significantly in two out of 12 measured soil properties (Table 1). Sites of diploid populations had 68% higher total carbon content than both habitat types of hexaploid populations (Table 1). Carbonate content decreased from sites of diploid populations to habitats of hexaploid populations with high productivity (Table 1). However, all measured soil properties varied largely among sites within the groups (Table 1).

The three groups of sites differed significantly in vegetation composition (15.2% of total variation, $F = 2.15$, $p = 0.002$). Out of 79 recorded vascular plant species, 10 plant species were confined to sites of diploid populations only. Four plant species occurred in sites of hexaploid populations only. Two

species occurred in habitats with high productivity of hexaploid populations only. However, vegetation composition varied also among sites within the groups (27.3% of total variation, $F = 1.65$, $p = 0.002$). A large number of plant species occurred in all three groups of sites. Overall, our results show significant differences between habitats of diploid and both types of hexaploid populations but also large overlap in habitat characteristics of the two cytotypes.

Reciprocal transplant of seeds

Seedling survival percentages were very low in both sowing experiments (0.2% - 1.3%). Seedling survival varied largely among target sites, blocks within a site and populations of origin (Table 2; S1). In both sowing experiments, seedling survival was higher at sites of home than foreign ploidy levels (Table 2; Fig. 2a, b). In experiment 2003, seedling survival was also higher at home than foreign habitat types (Table 2; Fig. 3a, b). However, home ploidy level explained more variability than home habitat type in these cases (Table 2). In experiment 2002, seedling survival was higher at sites with low than high productivity and at sites of home than foreign populations (Table 2; Fig. 4a). Significant positive effects of home habitat type, ploidy level and population provided evidence of local adaptation.

Table 1. Differences in soil properties between different groups of sites and populations of *Aster amellus*.

Source of variation	Groups (d.f. = 2, 6)		Populations (d.f. = 6, 48)		2x low productivity		6x low productivity		6x high productivity	
	F	p	F	p	Mean	SE	Mean	SE	Mean	SE
pH (H_2O)	4.07	0.076	97.25	**<0.001**	7.92±	0.07	7.70±	0.09	7.65±	0.04
pH (KCl)	0.02	0.976	91.46	**<0.001**	7.34±	0.06	7.32±	0.05	7.34±	0.09
Ca^{2+} (mg/g)	2.31	0.181	14.91	**<0.001**	9104.55±	438.75	8448.70±	1242.10	6529.45±	641.43
Mg^{2+} (mg/g)	4.70	0.059	29.72	**<0.001**	64.04±	13.45	68.68±	4.33	96.26±	5.52
K^{+} (mg/g)	3.60	0.094	198.62	**<0.001**	143.66±	30.61	153.26±	32.07	234.87±	15.65
Total N (%)	3.01	0.124	101.44	**<0.001**	0.20±	0.04	0.20±	0.03	0.30±	0.03
Total C (%)	26.70	**0.001**	344.45	**<0.001**	8.44±	0.22^{b}	4.58±	0.33^{a}	4.96±	0.51^{a}
Carbonates (%)	19.78	**0.002**	51.15	**<0.001**	5.90±	0.38^{c}	2.23±	0.50^{b}	1.50±	0.57^{a}
Organic C (%)	1.53	0.290	51.11	**<0.001**	2.53±	0.59	2.35±	0.53	3.46±	0.39
C/N ratio	0.51	0.623	6.84	**<0.001**	13.22±	1.73	11.58±	1.19	11.76±	0.40
Water capacity (%)	3.27	0.110	5.59	**<0.001**	46.26±	3.14	39.08±	1.60	41.06±	1.29

Different superscript letters indicate significant differences according to Tukey's HSD test among groups of sites when applicable.

Table 2. Summary of the analyses of deviance of seedling survival in the reciprocal sowing experiments.

Source of variation		Experiment 2002				Experiment 2003			
		2004		2005		2004		2005	
	df	Quasi-F	R^2	Quasi-F	R^2	Quasi-F	R^2	Quasi-F	R^2
Bare soil	1	1.32		4.24*↓		9.49**↓		26.94***↓	
Target habitat	1	6.48*	20.6	1.95		1.83		1.84	
Target ploidy	1	1.97		1.24		0.58		0.38	
Target site	6	12.19***	19.0	30.45***	36.5	8.11***	25.2	13.05***	25.3
Target block	36	2.86***	9.4	2.89***	7.2	4.95***	18.6	3.27***	11.6
Original habitat	1	4.70		3.80		0.49		0.07	
Original ploidy	1	<0.01		0.01		0.13		0.18	
Original population	6	9.05***	4.9	5.62***	2.3	2.06	1.3	7.01***	4.2
Home habitat	1	0.01		0.63		9.48**↑	1.6	10.02**↑	1.9
Home ploidy	1	4.09*↑	0.6	52.6***↑	5.6	12.62***↑	2.1	26.71***↑	4.9
Home population	1	2.55		6.98*↑	0.7	0.64		1.39	
Tar. site. × orig. pop.	61	1.57**	8.7	1.54*	6.5	1.57**	10.0	1.88***	11.3
Residuals	287								

*** p < 0.001, ** p < 0.01,* p < 0.05. Arrows denote the direction of the effects when applicable (↓ negative effects, ↑ positive effects).

Reciprocal transplant of adult plants

Target site, block within a site and population of origin had a considerable effect on performance of transplanted plants (Table 3; S1). Survival percentage of transplanted adult plants was relatively high (86% in 2005 and 75% in 2006). More adult plants survived at sites of hexaploid than diploid populations (Table 3). Survival percentage was higher at sites of home than foreign populations (Table 3, Fig. 4b). Plants had larger leaves at sites with high than low productivity, at sites of hexaploid than diploid populations and at sites of home than foreign ploidy level (Table 3; Fig. 2c, d). Flowering percentage was quite low (4.8%). Diploid plants and plants from sites with low productivity had higher flowering percentage than hexaploid plants and

plants from sites with high productivity (Table 3). Flowering percentage was higher at sites with high than low productivity and at sites of home than foreign populations (Table 3, Fig. 4c). Overall, reciprocal transplant with adult plants showed evidence of local adaptation only in several traits and only at the level of population and ploidy level, whereas local adaptation in seedling survival was found at all three levels (ploidy level, habitat type and population).

Table 3. Summary of the analyses of variance (leaf length) and deviance (survival, flowering) of plant performance in the reciprocal transplant experiments with adult plants.

Source of variation		Survival				Leaf length				Flowering	
		2005		2006		2005		2006		2005	
	df	Quasi-F	R^2	Quasi-F	R^2	F	R^2	F	R^2	Quasi-F	R^2
Leaf length	1	28.05***↑		85.77***↑		96.09***↑		58.38***↑		419.99***↑	
Target habitat	1	3.49		0.17		6.67*	3.9	17.14**	19.6	8.55*	2.1
Target ploidy	1	11.24*	3.7	1.21		7.30*	4.3	6.61*	7.5	0.01	
Target site	6	2.07		11.8***	9.9	6.57***	3.5	12.46***	6.8	1.02	
Target block	36	3.54***	5.7	3.34***	5.0	1.82**	3.2	2.20***	3.3	6.79***	8.7
Original habitat	1	<0.01		0.10		2.11		0.87		6.22*	1.2
Original ploidy	1	0.80		1.49		5.80		1.88		15.79**	3.0
Original population	6	3.02**	0.8	3.78***	0.9	5.35***	1.6	9.85***	2.5	5.43***	1.2
Home habitat	1	0.11		1.58		2.53		0.05		0.93	
Home ploidy	1	0.03		0.01		1.51		6.36*↑	0.4	0.21	
Home population	1	5.86*↑	0.7	1.33		1.07		0.20		4.96*↑	1.1
Tar. site. × orig. pop.	61	2.65***	7.3	2.18***	5.6	1.50**	4.5	1.59**	4.0	6.53***	14.1
Residuals	1258-1721										

*** p < 0.001, ** p < 0.01,* p < 0.05. Arrows denote the direction of the effects when applicable (↑ positive effects).

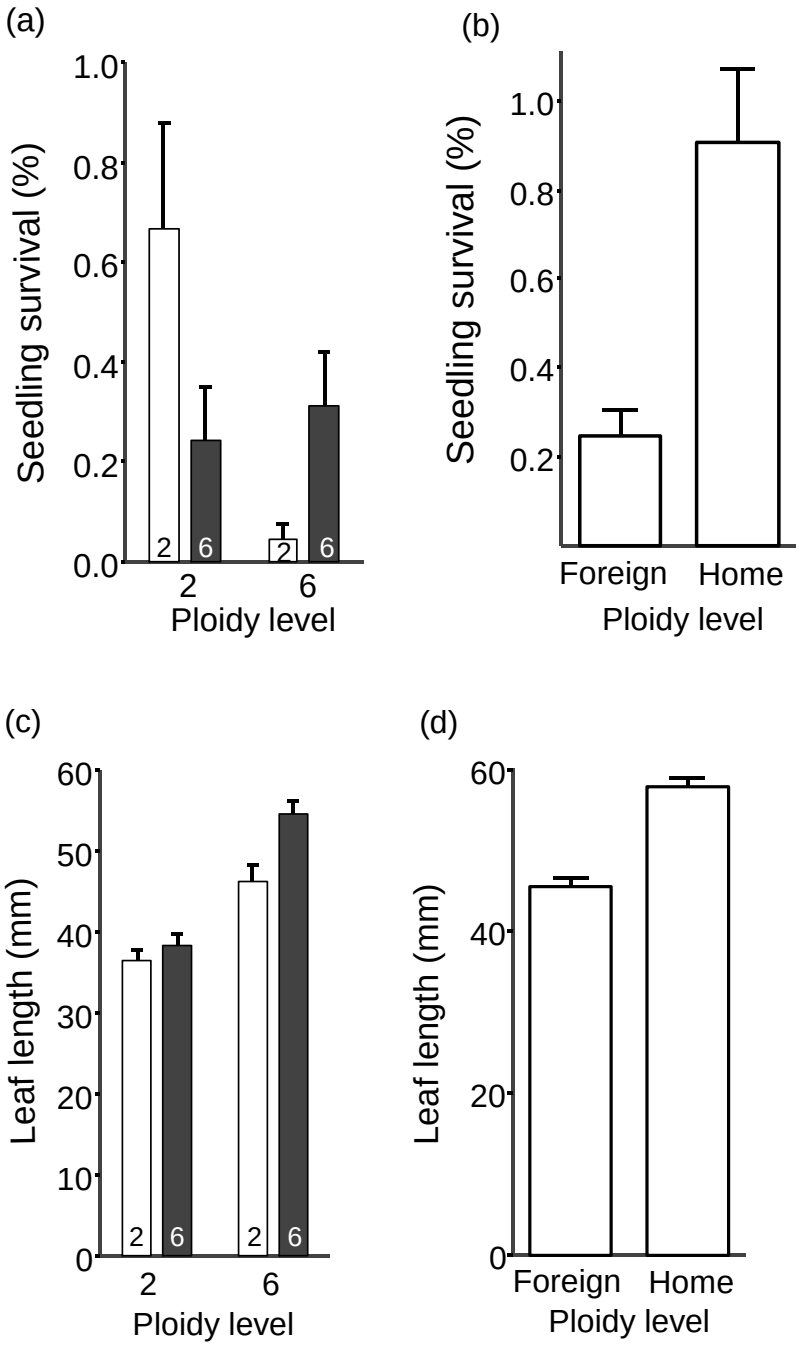


Fig. 2. (a, c) The differences between diploid (open bars) and hexaploid (solid bars) populations at diploid and hexaploid sites and (b, d) the effect home ploidy level in (a, b) seedling survival in experiment 2003 in 2005 and (c, d) leaf length of *Aster amellus* in 2006. Error bars indicate standard error of the mean.

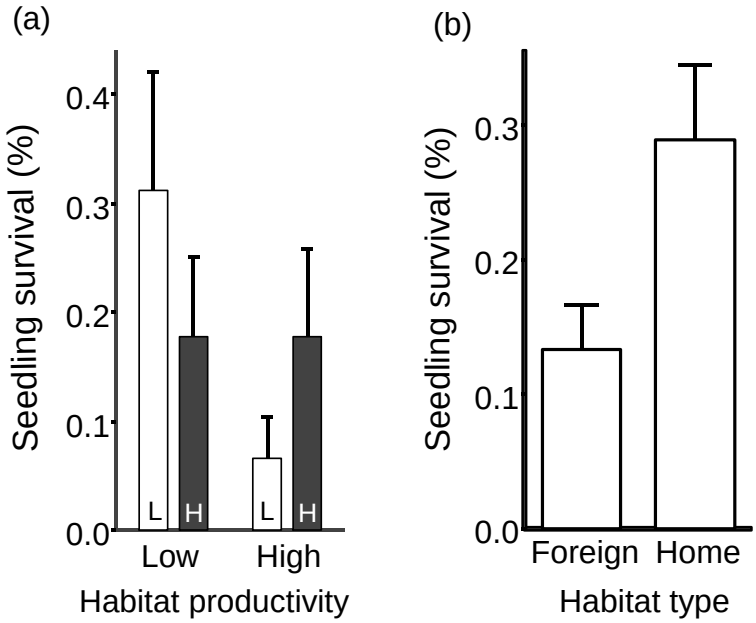


Fig. 3. (a) The differences between plants from sites with low productivity (open bars) and high productivity (solid bars) at sites with low and high productivity and (b) the effect of home habitat type on seedling survival of *Aster amellus* in experiment 2003 in 2005. Error bars indicate standard error of the mean.

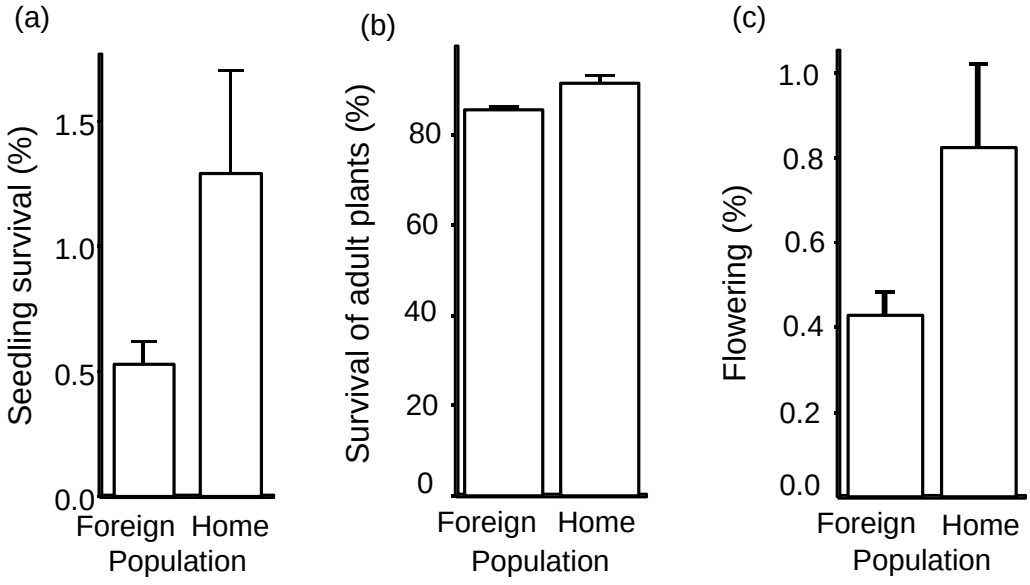


Fig. 4. The effect of home population on (a) seedling survival in experiment 2002 in 2005 (b) survival of adult plants in 2005, (c) flowering in 2005 in *Aster amellus*. Error bars indicate standard error of the mean.

Discussion

Differentiation between sites of diploid and hexaploid populations

Our study shows significant differences among sites of diploid and hexaploid populations of *A. amellus*. Hexaploid populations occupy broader range of habitats (with low and high productivity) that are more acid and host a lower number of specific plant species than sites of diploid populations. Polyploids often possess novel physiological, ecological or phenological characteristics that allow them to colonize a new niche (reviewed in Ramsey and Schemske 1998; Otto and Whitton 2000). Although assessment of habitat productivity suggests larger differences between sites of the two types of hexaploid populations than between sites of diploid and hexaploid populations in low productive habitats, more detailed analyses of vegetation composition and soil properties do not support this. Instead, we found differences between all groups of sites and also among sites within each group. Overall, there are some differences between sites of diploid and hexaploid populations of *A. amellus* but much overlap exists in their habitat characteristics.

Performance of diploid and hexaploid plants

Target site and block within a site largely affected seedling survival and performance of transplanted adult plants, indicating differences among sites and also differences within each site. Target habitat type had a considerable effect on seedling survival, leaf length and flowering and target ploidy level on survival and leaf length. Hence, all groups of target sites affected only leaf

length, with the strongest effect of the target habitat type. The strong effects of site characteristics on leaf length are reasonable because leaf length is more likely to be shaped by environment than the other traits (Nagy and Rice 1997). Our results suggest that *A. amellus* is able of plastic response to heterogeneous environment.

Original population also considerably affected seedling survival and performance of adult plants. In contrast, original habitat type and ploidy level affected only flowering percentage. Diploid plants and plants from sites with low productivity had higher flowering percentage than hexaploid plants and plants from sites with high productivity. In some studies, hexaploid plants outperformed diploid plants as a result of their new characteristics. For example, tetraploids of *D. glomerata* were able to grow in soil of higher pH range than diploids (Lumaret et al. 1987). In other study with *M. annua*, however, diploids were fitter than tetraploids (Buggs and Pannell 2007). Hexaploids of *A. amellus* were found to have higher number of leaves in a garden experiment (Münzbergová 2007b) and higher seed production in field (Münzbergová 2006) than diploids. Nevertheless, previous studies on *A. amellus* have also shown that the species is highly variable and that similar morphological differences can be found both between the two cytotypes and among populations within each cytotype (Mandáková and Münzbergová, unpublished manuscript). We conclude that performance of *A. amellus* differs more among individual populations than between the two cytotypes.

We found significant genotype × environment interactions, indicating local adaptation. Local adaptation was found at all three levels (i.e. ploidy level, habitat type and population) in sowing experiments and at the level of ploidy level and population in experiment with adult plants. Both home

population and home ploidy level affected seedling survival and performance of adult plants. However, plant traits affected by home ploidy level and home population were different. Home habitat type affected only seedling survival in one experiment and its effect was weaker than the effect of home ploidy level. Thus, the two cytotypes show evidence of niche differentiation but at the same time, populations within each cytotype show evidence of local adaptation. Our findings of local adaptation to home population agree with results of our previous study with diploid populations of *A. amellus* in the study area (Raabová et al. 2007), suggesting that subsequent adaptive evolution with environment played an important role in the system. Because the two cytotypes of *A. amellus* have a patchy distribution the divergence between two cytotypes due to selection could evolve. Subsequent evolution with the environment might therefore contribute to habitat differentiation of the two cytotypes.

Conclusions

Diploid and hexaploid populations of *A. amellus* are strongly spatially segregated in the study area. Reciprocal transplant showed some evidence of niche differentiation between the two cytotypes. However, much overlap exists in habitat characteristics of the two cytotypes and both seeds and adult plants are able to grow at sites of the foreign cytotype. Therefore, only niche differentiation cannot explain segregation of the two cytotypes. Our findings of local adaptation indicate restricted gene flow among diploid and hexaploid populations but also among single populations within each cytotype. It is, therefore, likely that the two cytotypes hybridise very rarely in the field,

suggesting that historical factors and dispersal limitations might play an important role in segregation of the two cytotypes.

Acknowledgment

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S 1. Performance of diploid (populations 1-3) and hexaploid (populations 4-9) *Aster amellus* from nine populations at nine sites after reciprocal sowing in 2002 and 2003 over 2 and 3 years (a, b) and after reciprocal transplant of adult plants over two years (c-e). Means ± SE.

Habitat type	Low productivity						High productivity			
Ploidy level	Diploid populations			Hexaploid populations						
Site\Population	1	2	3	4	5	6	7	8	9	Mean
(a) Seedling survival in 2005 in experiment 2002 (%)										
1	1.2 ± 0.6	0.6 ± 0.4	0.2 ± 0.2	0 ± 0	0.2 ± 0.2	0.2 ± 0.2	1.2 ± 1.2	0.8 ± 0.5	0 ± 0	0.5 ± 0.2
2	0.2 ± 0.2	0.8 ± 0.4	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
3	1.6 ± 0.7	2.2 ± 1.3	1.2 ± 0.7	0.2 ± 0.2	0.4 ± 0.2	0 ± 0	0 ± 0	0.4 ± 0.4	0.4 ± 0.2	0.7 ± 0.2
4	0.8 ± 0.5	1.8 ± 0.9	2.0 ± 0.7	7.4 ± 2.1	3.2 ± 2.5	1.2 ± 1	6.8 ± 2.6	5.4 ± 1.8	5.6 ± 2.4	3.8 ± 0.6
5	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0.2 ± 0.2	0 ± 0	0.4 ± 0.4	0.2 ± 0.2	0 ± 0	0.1 ± 0.1
6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
7	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0.2 ± 0.2	0.8 ± 0.5	0 ± 0	0.2 ± 0.2	0.2 ± 0.1
8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
9	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0.2 ± 0.2	0 ± 0	0.4 ± 0.4	0 ± 0	0.1 ± 0.1
Mean	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.9 ± 0.4	0.5 ± 0.3	0.2 ± 0.1	1.0 ± 0.4	0.8 ± 0.3	0.7 ± 0.4	0.6 ± 0.1
(b) Seedling survival in 2005 in experiment 2003 (%)										
1	3.0 ± 1.1	2.2 ± 0.7	0.6 ± 0.4	0.8 ± 0.6	0.2 ± 0.2	1.2 ± 0.6	1.2 ± 0.7	0.4 ± 0.2	0.4 ± 0.2	1.1 ± 0.2
2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0
3	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0
4	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	1.0 ± 0.6	0.4 ± 0.4	0.4 ± 0.4	0 ± 0	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.1
5	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.4	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	0.4 ± 0.4	0.2 ± 0.1
6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
7	0.2 ± 0.2	0 ± 0	0 ± 0	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	0.4 ± 0.2	0 ± 0	0 ± 0	0.1 ± 0
8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.8 ± 0.6	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
9	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean	0.4 ± 0.2	0.3 ± 0.1	0.1 ± 0	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0

Site\ Population	1	2	3	4	5	6	7	8	9	Mean
(c) Survival percentage of transplanted adult plants in 2006 (%)										
1	64 ± 10	54 ± 10	36 ± 10	65 ± 10	92 ± 6	96 ± 4	80 ± 8	62 ± 11	92 ± 6	72 ± 3
2	80 ± 8	96 ± 4	79 ± 9	84 ± 8	80 ± 8	76 ± 9	92 ± 6	64 ± 11	71 ± 10	81 ± 3
3	67 ± 10	77 ± 9	48 ± 11	63 ± 10	45 ± 11	83 ± 8	68 ± 10	56 ± 10	72 ± 9	65 ± 3
4	83 ± 9	75 ± 11	82 ± 10	100 ± 0	91 ± 6	100 ± 0	88 ± 7	83 ± 8	96 ± 4	90 ± 2
5	100 ± 0	86 ± 10	85 ± 8	95 ± 5	91 ± 7	67 ± 13	100 ± 0	82 ± 8	91 ± 6	89 ± 2
6	83 ± 9	71 ± 13	57 ± 11	83 ± 8	53 ± 13	96 ± 5	95 ± 5	74 ± 9	81 ± 9	78 ± 3
7	46 ± 10	22 ± 9	14 ± 8	48 ± 10	29 ± 10	39 ± 10	36 ± 10	38 ± 10	28 ± 9	34 ± 3
8	96 ± 4	92 ± 6	83 ± 8	82 ± 8	100 ± 0	96 ± 4	88 ± 7	92 ± 6	92 ± 6	91 ± 2
9	75 ± 9	91 ± 6	84 ± 8	81 ± 9	96 ± 5	75 ± 9	65 ± 10	74 ± 9	75 ± 9	79 ± 3
Mean	76 ± 3	74 ± 3	63 ± 3	77 ± 3	76 ± 3	82 ± 3	79 ± 3	69 ± 3	77 ± 3	75 ± 1
(d) Length of the longest leaf of transplanted adult plants in 2006 (mm)										
1	32 ± 4	27 ± 3	24 ± 5	32 ± 4	30 ± 2	29 ± 3	24 ± 2	34 ± 5	29 ± 3	29 ± 1
2	41 ± 4	39 ± 3	28 ± 3	52 ± 6	43 ± 4	41 ± 3	44 ± 4	48 ± 7	46 ± 6	42 ± 2
3	46 ± 4	50 ± 4	32 ± 4	36 ± 3	43 ± 7	42 ± 4	36 ± 5	49 ± 4	51 ± 5	43 ± 2
4	50 ± 5	40 ± 4	39 ± 7	71 ± 5	50 ± 4	50 ± 3	44 ± 4	47 ± 3	70 ± 4	53 ± 2
5	60 ± 6	44 ± 7	37 ± 4	61 ± 6	75 ± 5	44 ± 7	50 ± 5	67 ± 4	79 ± 7	59 ± 2
6	51 ± 5	48 ± 9	41 ± 5	43 ± 6	46 ± 9	41 ± 3	56 ± 4	54 ± 5	56 ± 6	49 ± 2
7	47 ± 5	54 ± 10	33 ± 13	63 ± 10	62 ± 6	48 ± 6	48 ± 5	58 ± 7	69 ± 12	55 ± 3
8	68 ± 5	74 ± 6	69 ± 4	78 ± 7	84 ± 8	87 ± 4	78 ± 5	78 ± 6	91 ± 4	79 ± 2
9	48 ± 5	60 ± 6	48 ± 5	61 ± 7	68 ± 7	53 ± 5	52 ± 4	68 ± 6	66 ± 8	58 ± 2
Mean	50 ± 2	50 ± 2	41 ± 2	56 ± 2	57 ± 2	49 ± 2	48 ± 2	57 ± 2	62 ± 2	53 ± 1
(e) Flowering percentage of transplanted adult plants in 2005 (%)										
1	0 ± 0	15 ± 10	0 ± 0	6 ± 6	4 ± 4	0 ± 0	8 ± 6	0 ± 0	0 ± 0	4 ± 1
2	5 ± 5	24 ± 9	0 ± 0	9 ± 6	5 ± 5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5 ± 2
3	11 ± 7	6 ± 6	6 ± 6	0 ± 0	11 ± 11	16 ± 9	6 ± 6	14 ± 10	0 ± 0	8 ± 2
4	6 ± 6	7 ± 7	7 ± 7	17 ± 8	0 ± 0	4 ± 4	4 ± 4	0 ± 0	13 ± 7	7 ± 2
5	5 ± 5	0 ± 0	5 ± 5	0 ± 0	5 ± 5	8 ± 8	8 ± 6	6 ± 6	0 ± 0	4 ± 2
6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5 ± 5	0 ± 0	5 ± 5	1 ± 1
7	5 ± 5	6 ± 6	0 ± 0	4 ± 4	0 ± 0	0 ± 0	0 ± 0	10 ± 7	0 ± 0	3 ± 1
8	13 ± 7	17 ± 8	10 ± 7	0 ± 0	0 ± 0	4 ± 4	9 ± 6	17 ± 8	4 ± 4	9 ± 2
9	0 ± 0	18 ± 8	5 ± 5	6 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 1
Mean	5 ± 2	12 ± 3	4 ± 2	5 ± 2	2 ± 1	3 ± 1	5 ± 2	5 ± 2	3 ± 1	5 ± 1

CHAPTER 2

Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*

"Adapt or perish, now as ever, is nature's inexorable imperative."

(H. G. Wells)

Chapter 2

Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*

(with Zuzana Münzbergová and Markus Fischer; Biological conservation, 139, 348–357)

Abstract

Transferring plants between populations of rare species has often been proposed to increase population size and replenish genetic variation. While this approach has many advantages, it may also disrupt local adaptation. However, the scale over which plants adapt to local conditions is hard to predict. To detect local adaptation, we conducted reciprocal transplant experiments in the field with six populations of the rare perennial herb, *Aster amellus*. We sowed seeds in 2003 and 2004 (called 'Experiment 2003' and 'Experiment 2004') and transplanted adult plants in 2004. We evaluated genetic differences between populations and ecological differences between habitats and tested which differences explain the degree of local adaptation. The number of juveniles from the local populations was 68% and 42% higher than the number of juveniles from the foreign populations in 'Experiment 2003' and 'Experiment 2004', respectively, indicating local adaptation. However, not all populations of *A. amellus* adapted to their local conditions. Differences in local climate and in vegetation composition particularly affected local adaptation. In contrast to transplanted seeds, transplanted adult plants from local populations did not overall perform better than plants from foreign populations. We conclude that transfer of seeds is a more appropriate technique than transfer of adult plants in conservation practice because it more likely prevents non-adapted genotypes from establishing. Material for the transfers should come, not necessarily from the closest, but rather from ecologically similar habitats.

Introduction

Many threatened species are restricted to fragmented habitats. Habitat fragmentation leads to reduced population size and increased isolation of populations. Population isolation together with different selective forces can lead to adaptation to local conditions (Slatkin, 1985). Although many plant populations are adapted to local conditions (e.g. review by Linhart and Grant, 1996), local adaptation is not ubiquitous (Cheplick, 1988; Rapson and Wilson, 1988; Helenurm, 1998). Local adaptation has become an important issue in nature conservation because knowledge of it is necessary for considering effects of transfers between populations. Transfer of individuals is often proposed as a technique to replenish genetic variation and thus reduce negative effects of low genetic variation (e.g. Richards, 2000; Ingvarsson, 2001). However, transferring plants may disrupt local adaptation and lead to reduction in offspring fitness (outbreeding depression; e.g. Hufford and Mazer, 2003).

Plants are considered locally adapted when local plants show higher fitness than foreign plants within each site (Kawecki and Ebert, 2004). The home-site advantage hypothesis also predicts that the relative success of introduced plants will decrease as distance to the population of origin increases (Montalvo and Ellstrand, 2000). The distance between populations can be expressed as geographic distance. However, genetic or ecological differences can be more relevant for local adaptation (Montalvo and Ellstrand, 2000). The relationship between geographic and ecological scales of local adaptation has been poorly investigated (McKay et al., 2005). Nonetheless, knowing the scales of local adaptation would help to determine regions within

which plants could be moved without negative consequences for population fitness (Hufford and Mazer, 2003).

Most previous studies on local adaptation have considered highly contrasting habitats. Local adaptation has been found over the scale of several hundred to several thousand kilometres (Galloway and Fenster, 2000; Joshi et al., 2001; Becker et al., 2006; Bischoff et al., 2006). However, habitats that are a thousand kilometres apart differ largely in climatic conditions and local adaptation can be expected. Local adaptation has also been studied at a small scale in very contrasting habitats, e.g. microhabitats differing in flooding (Prati and Schmid, 2000; Knight and Miller, 2004; Lenssen et al., 2004). In contrast, whether adaptive differentiation between populations may arise at a small scale among isolated habitats with little ecological differentiation has rarely been studied. Many threatened species occur in such isolated habitats and the assessment of local adaptation in these situations is important for appropriate species conservation strategies.

We aimed to explore local adaptations in a system of dry calcareous grasslands in the Czech Republic. We expected restricted gene flow between isolated populations. Moreover, calcareous grasslands differ slightly in several environmental conditions and are stable over time (Studnička, 1980). Therefore time to adapt to local conditions should have been sufficient. We chose *Aster amellus* as a model species because many of the threatened species of dry calcareous grasslands are perennial plants of the Asteraceae (Holub and Procházka, 2000). This species occurs in populations of different sizes and is easy to cultivate. Furthermore, it occurs in two regions with slightly different types of grasslands in the Czech Republic. This allowed us to study local adaptation at two spatial scales.

We carried out reciprocal transplant experiments in the field within and between two different regions. Because local adaptation may vary among years (Rice and Mack, 1991; Jordan, 1992) or among fitness traits (Nagy and Rice, 1997), we studied the entire life cycle of *A. amellus* within three years. We also tested whether genetic, geographic or ecological distances between populations can explain the degree of local adaptation. We asked the following questions: (1) Is there any evidence of adaptation to local conditions? (2) Does plant fitness decrease with increasing geographic, genetic or ecological distance to the population of origin? (3) Is the pattern of local adaptation consistent among traits and years?

Methods

Study species

Aster amellus L. (Asteraceae) is a subcontinental species distributed from Western Europe to Western Siberia (Meusel and Jäger, 1992). Three different ploidy levels occur in the whole area (2x, 4x and 6x; x = 9) (Merxmüller et al., 1976). Its typical habitats, dry calcareous grasslands, have declined over the last decades, and *A. amellus* became endangered in many parts of Central Europe (e.g. Schönfelder, 1987; Buttler et al., 1997; Holub and Procházka, 2000). *Aster amellus* is a perennial, up to 40 cm high herb flowering from mid-July to mid-October (Kovanda, 2005). Generally, the species of the genus *Aster* possess a sporophytic incompatibility system (Richards, 1986). However, partial self-compatibility has been found in *A. curtus* (Giblin and

Hamilton, 1999) and *A. furcatus* (Reinartz and Les, 1994). Plants of *A. amellus* reproduce vegetatively as well as sexually and are mainly pollinated by bees and syrphid flies (J. Raabová, personal observation). The average number of seeds produced per plant is c. 350 (J. Raabová, unpublished data). The seeds are usually dispersed over short distances and can persist in the soil for two years (Münzbergová, 2004). Seedlings usually stay at the small rosette stage in the field for a few years.

Study sites

We selected six diploid populations in two different regions in the central part of the Czech Republic. Ploidy levels of the populations were determined in a previous study (Mandáková and Münzbergová, 2006). The first region is delimited by the towns Litoměřice, Roudnice nad Labem and Úštěk and is characterized by moderate slopes on marl, with frequent soil erosion and plant communities of *Bromion erecti* (České středohoří Mts.; Region S). The second region is delimited by the towns Praha, Dobřichovice and Beroun and is characterized by rocky slopes on limestone and plant communities of *Quercion pubescenti-petraeae* and *Geranion sanguinei* (Czech Karst; Region K). The exact positions of the populations are provided in Fig. 1. The distance between the two regions is 70 km, and the distances between populations within each region range from 2.5 to 17 km. We chose populations consisting of at least 60 individuals to have enough material for transplant experiments. The sizes of the studied populations estimated as the number of flowering individuals in 2005 ranged from 60 to 10,000 (Table 1).

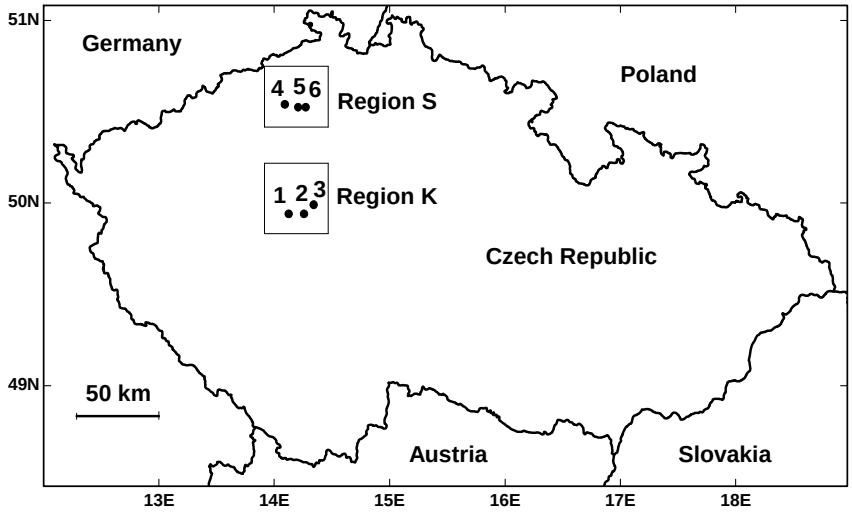


Fig. 1. Map of the Czech Republic showing the locations of the six studied populations of *Aster amellus* in the Czech Karst (Region K) and České středohoří Mountains (Region S). 1 = K1, 2 = K2, 3 = K3, 4 = S1, 5 = S2, 6 = S3.

Isozyme analyses

We used isozyme markers to estimate genetic variation within and between populations. We collected a few green undamaged leaves from 9-14 randomly selected individuals in each population in the field in September 2003. We kept them in the refrigerator and extracted isozymes on the following day in the laboratory. We homogenised approximately 60 mg of leaf material with Dowex-Cl (1-X8) on ice in 0.7 ml tris-HCl extraction buffer: 0.1 M tris-HCl (pH 8.0), 70 mM 2-mercaptoethanol, 26 mM sodium metabisulfite, 11 mM ascorbic acid, 4% polyvinylpyrrolidone. We centrifuged the extracts at 13 000 rpm for 10 min and stored the supernatant at -75 °C for subsequent electrophoresis. We separated the isozymes by polyacrylamide gel electrophoresis (PAGE) using a vertical HOEFER SE 600 (Amersham plc., Amersham, UK): We analysed 30-35 μl samples on polyacrylamide gels (resolving gel: 8% acrylamide, 1.82 M Tris-HCl, pH 8.3; stacking gel: 4%

acrylamide, 0.069 M Tris-HCl, pH 6.9; electrode buffer: 0.02 M Tris, 0.24 M glycine, pH 8.3).

We investigated four enzyme systems: AAT (EC 2.6.1.1), LAP (EC 3.4.11.1), SHDH (EC 1.1.1.25) and 6-PGDH (EC 1.1.1.44). The staining procedures followed Mandák et al. (2005) for AAT and LAP and Vallejos (1983) for 6-PGDH and SHDH, with following modifications: 6-PGDH (0.1 M tris-HCl, pH 8.4, 30 mg 6-phosphogluconic acid), SHDH (0.1 M tris-HCl, pH 8.4).

We obtained banding patterns of five polymorphic loci (AAT-1, AAT-2, LAP-1, SHDH-1 and 6-PGDH-1) that we interpreted allelically. To characterize genetic variation of each population, we calculated mean allelic richness (El Mousadik and Petit, 1996) and gene diversity (Nei, 1987) for each population with FSTAT 2.9.3.2. (Goudet, 2002). We assessed observed heterozygosity (H_O) and expected heterozygosity (H_E) under Hardy-Weinberg equilibrium from the allele frequencies of each population and calculated inbreeding coefficients (F_{IS}). We calculated pairwise F_{ST} statistics for all pairs of populations with Arlequin 3.01 (Excoffier et al., 2005). To estimate distribution of genetic variation, we used analysis of molecular variance (AMOVA) implemented in Arlequin 3.01.

Ecological differences among habitats

We characterized habitats by means of vegetation composition, soil properties and inclination and aspect of the slopes. To obtain vegetation data, we recorded the cover of each plant species in three relevés of 2 × 2 m at each site using the Braun-Blanquet (1964) scale. Then, we transformed the data

according to van der Maarel (1979). To assess soil properties, we collected five samples of the upper soil layer at each site. We transferred them to the laboratory, air-dried them, sieved them through 2 mm mesh and homogenized them. We analysed the concentrations of Ca^{2+}, Mg^{2+}, K^{+} following Moore and Chapman (1986), content of total N and total C following Ehrenberger and Gorbach (1973), carbonate content according to ISO-Standard (10693) and actual and exchangeable pH in the laboratory. To assess physical soil properties, we measured maximal water holding capacity of ten samples of 100 cm^3 per site as an amount of water bound in the soil monolith after 24 hours according to Novák (1954). We used the data on latitude, inclination and aspect of the slopes to calculate potential direct solar irradiation that characterizes the local climate of the sites. This was calculated as the sum of the cosines of the angles at which the solar irradiation reaches the surface on the 21st day of each month from December to June (Jeník and Rejmánek, 1969).

Distance matrices

To determine the differences between populations, we used geographic, genetic and ecological distances. We measured the shortest geographic distances between each pair of populations. The genetic distance consisted of values of $F_{ST}/ (1 - F_{ST})$ between pairs of populations (Slatkin, 1995). To obtain ecological distances between each pair of sites, we calculated Euclidean distances with Statistica (StatSoft, 2001). For each site, we used the mean values of (1) cover of each plant species, (2) each of soil properties, and (3) potential direct solar irradiation for each month. We standardized soil

properties before analysis to eliminate effects of different measurement units. We investigated correlations between all distance matrices with two-tailed Mantel tests in PopTools 2.6 (Hood, 2005).

Local adaptation

Reciprocal sowing experiments

We conducted complete reciprocal transplant experiments, i.e. we sowed seeds from each population into each population. We repeated the sowing experiments in the years 2003 and 2004 to study between-year variation in local adaptation (called 'Experiment 2003' and 'Experiment 2004'). We collected ripe seeds from 50 randomly selected individuals from each population in September 2003 and 2004. We sowed the seeds into five 1×1 m quadrats divided into nine grid cells of 33×33 cm at each site in October just after natural seed dispersal. We placed 100 seeds from each population on the ground in the middle of six randomly selected grid cells. Three remaining grid cells served as controls for natural seed rain. To study germination in natural conditions, we did not disturb the plots before or after sowing as commonly done in other sowing experiments (e.g. Nagy and Rice, 1997; Jakobsson and Dinnetz, 2005), because the habitats in our study naturally contain patches of bare soil that enable seed germination. We recorded the percentage of bare soil cover per grid cell in 2005. We marked each quadrat corner with a nail for later recovery with a metal detector. We counted the germinated juveniles in the summers of 2004, 2005, and 2006.

Reciprocal transplant of adult plants

In February 2004, we sowed c. 300 seeds from each population into plastic trays with garden substrate placed in the greenhouse at 10 °C with natural light conditions. In April, we transplanted the seedlings into multipot trays with pots of 3 × 3 cm. We kept the plants in a common garden from June to October, when we transplanted them into the field. We planted 25 juveniles from each population into five randomly placed rows at each site. We planted the juveniles 10 cm apart in random order in a row. We watered them immediately after planting and measured the length of the longest leaf. We recorded survival and flowering percentage and the length of the longest leaf in September 2005 and 2006. We removed 7% of the juveniles, which had been damaged by roe deer and wild boars, from further analyses. We did not analyse flowering percentage in 2006 because it was less than 1%.

Data analysis

To analyse differences in plant performance in transplant experiments, we used analyses of covariance for normally distributed variables (longest leaf) and analyses of deviance of logistic regression for binomial variables (survival, flowering, number of germinated juveniles linked to number of sown seeds). We tested the effects of target site (block nested within site nested within region), population of origin (population nested within region) and their interaction against residuals. Further, we tested the effects of local vs foreign contrast, linear contrasts of distances between populations and local vs foreign × population interaction against the population of origin × target site

interaction. For significance tests in analysis of deviance we used ratios of mean deviance changes, quasi-F (Francis et al., 1993). We used the proportion of bare soil cover as a covariate to adjust for different effects of microhabitats on seedling germination. In addition, we used the initial length of the longest leaf as a covariate to adjust for maternal effects and effects of different growing conditions before transplanting. We performed all analyses with S-plus 6.2 (Insightful Corporation, Seattle, Washington).

Results

Isozyme analyses

Mean allelic richness per population ranged from 2.531 to 3.135, gene diversity from 0.375 to 0.560 and inbreeding coefficient F_{IS} from -0.212 to 0.459 (Table 1). Mean observed heterozygosity (0.425) was slightly lower than mean expected heterozygosity (0.466), corresponding to a mean inbreeding coefficient F_{IS} of 0.071. Most of the isozyme variation was within populations (83.7%; F_{ST} = 0.16; $p < 0.001$). However, there was also significant variation among populations within a region (7.5%; F_{SC} = 0.08; $p < 0.001$), indicating restricted gene flow. Pairwise $F_{ST}/ (1 - F_{ST})$ values ranged from 0.013 to 0.501 (Table 2). The variation between the two regions was not significant (8.8%; F_{CT} = 0.09; p = 0.118).

Correlations among distance measures

Geographic distance between pairs of populations was positively correlated with soil differences and marginally positively correlated with both vegetation and genetic differences (Table 3). Vegetation differences were positively correlated with soil differences (Table 3). Genetic distance was not significantly related to any of the ecological differences and the differences in local climate were not significantly associated with any of the other distances (Table 3).

Local adaptation

Reciprocal sowing experiments

Germination percentages were very low in both experiments (0.7%-2%) and the number of established juveniles in 'Experiment 2004' was higher in 2006 than in 2005, indicating that some seeds did not germinate until the second year. In 2006, the number of juveniles from the local populations was 68% and 42% higher than the number of juveniles from the foreign populations in 'Experiment 2003' and 'Experiment 2004', respectively (Table 4; Fig. 2). In 2005, the number of juveniles from the local region was 27% higher than the number of juveniles from the foreign region in 'Experiment 2004' (Table 4), indicating local adaptation at a regional scale. The significant local vs foreign × population of origin interaction indicated that only some populations were adapted to their local conditions (Table 4; Fig. 3a, b). The

number of juveniles decreased with increasing differences in local climate and with vegetation distance in ‘Experiments 2003 and 2004’ (Table 4; Fig. 4). Furthermore, it decreased with genetic distance in ‘Experiment 2003’ and with geographic distances in ‘Experiment 2004’ (Table 4). These significant negative effects of various distances also provided evidence of local adaptation.

Reciprocal transplant of adult plants

The leaves of the transplanted adult plants were 16% shorter for plants from local than from foreign populations (Table 4). However, this effect varied among populations as indicated by the significant local vs foreign × population of origin interaction (Table 4; Fig. 3c). Survival probability (Fig. 3d) increased with increasing vegetation distance (Table 4). The contrast between local and foreign transplants in flowering probability differed among populations (significant local vs foreign contrast × population of origin interaction; Table 4; Fig. 3e). In general, however, flowering percentage increased with increasing differences in local climate (Table 4). Overall, we found evidence of local adaptation for seeds but not for adult plants.

Table 1. Isozyme variation in six populations of *Aster amellus*.

Code	Name	Population size 2005	Sample size	Allelic richness (AR)	Gene diversity (GD)	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Inbreeding coefficient (F_{IS})
K1	Koda	1 100	10	2.674	0.458	0.420	0.433	0.030
K2	Karlík	60	9	2.531	0.375	0.356	0.353	-0.007
K3	Lochkov	1 000	10	2.713	0.520	0.420	0.489	0.141
S1	Malíč	10 000	14	2.972	0.527	0.500	0.506	0.013
S2	Holý vrch	1 350	9	2.578	0.481	0.556	0.459	-0.212
S3	Encovany	1 100	10	3.135	0.560	0.300	0.555	0.459
Mean			10	2.767	0.487	0.425	0.466	0.071

Table 2. Matrix of pairwise FST/ (1 - FST) values for six populations of *Aster amellus*.

	K1	K2	K3	S1	S2	S3
K1	0					
K2	0.042	0				
K3	0.013	0.127**	0			
S1	0.095**	0.196***	0.096**	0		
S2	0.239***	0.501***	0.174***	0.118***	0	
S3	0.151***	0.366***	0.120**	0.122***	0.091**	0

For population names, see Table 1. ** $p < 0.01$, *** $p < 0.001$.

Table 3. Correlation coefficients between distance matrices examined with two-tailed Mantel tests.

	Geographic distance	Genetic distance	Vegetation distance	Soil distance	PDSI distance
Geographic distance					
Genetic distance	0.519†				
Vegetation distance	0.700†	0.338			
Soil distance	0.787**	0.388	0.742*		
PDSI distance	0.012	0.227	0.270	0.119	

PDSI is potential direct solar irradiation. Significance levels are based on 999 permutations. † $p < 0.1$, * $p < 0.05$, ** $p < 0.01$.

Table 4. Summary of analyses of plant performance in the reciprocal sowing experiments and the reciprocal transplant experiment with adult plants of *Aster amellus*.

Source of variation		Number of juveniles in sowing experiments					Transplant of adult plants				
		'Experiment 2003'			'Experiment 2004'		Survival		Leaf length		Flowering
	df	2004	2005	2006	2005	2006	2005	2006	2005	2006	2005
Bare soil cover	1	6.94**	80.78***	33.90***	0.13	0.86					
Initial leaf length	1						10.74**	11.94***	1.62	9.78**	87.51***
Target region (Rt)	1	0.58	3.37	1.27	4.32	6.62†	<0.01	0.11	1.49	0.46	1.03
Target population (Pt)	4	2.20†	7.12***	9.43***	5.68**	8.23***	5.08**	6.51**	8.36***	11.06***	2.77†
Block	24	3.89***	5.20***	3.93***	5.15***	2.50***	2.77***	2.26***	2.38***	2.91***	4.14***
Region of origin (Ro)	1	1.52	0.28	0.11	<0.01	0.14	0.22	0.04	1.01	0.13	1.73
Population of origin (Po)	3	1.10	10.42***	10.34***	8.64***	4.49**	1.12	1.48	4.37**	3.88**	8.30***
Local vs foreign	1	2.80	1.81	22.43***↑	3.30	8.38*↑	<0.01	0.79	3.86†↓	7.24*↓	0.25
Genetic distance	1	1.63	<0.01	4.89*↓	<0.01	0.09	2.97	0.02	2.03	0.51	0.58
Vegetation distance	1	0.53	<0.01	3.67†↓	3.04	5.17*↓	3.44†↑	5.40*↑	2.43	2.86	0.06
Soil distance	1	0.03	0.40	<0.01	<0.01	0.70	0.73	0.31	1.70	0.56	1.26
PDSI distance	1	0.05	4.47†↓	12.98**↓	4.82*↓	4.57†↓	0.19	<0.01	1.33	0.32	3.74†↑
Geographic distance	1	<0.01	0.07	0.65	2.04	5.30*↓	0.69	0.02	0.01	0.39	2.26
Local vs foreign × Po	5	1.17	0.89	4.12*	1.15	3.17*	1.15	1.07	1.61	3.35*	3.76*
Rt × Ro	1	0.85	0.30	<0.01	6.09*	0.44	1.33	2.78	0.18	0.69	1.94
Pt × Po	13	2.58**	5.30***	0.79	1.82*	1.05	2.15**	1.50	0.95	1.12	2.52**
Residuals	104-776										

We show F values from the analysis of covariance (leaf length) and quasi-F values from the analysis of deviance (number of juveniles, survival, and flowering) of effects of bare soil cover, initial leaf length, target sites (block nested within population nested within region), original populations (population nested within region), local vs foreign contrast and distances between populations. PDSI is potential direct solar irradiation. Arrows denote the direction of the effects when applicable (↓ negative effects, ↑ positive effects). † $p < 0.1$, * $p < 0.05$., ** $p < 0.01$, *** $p < 0.001$.

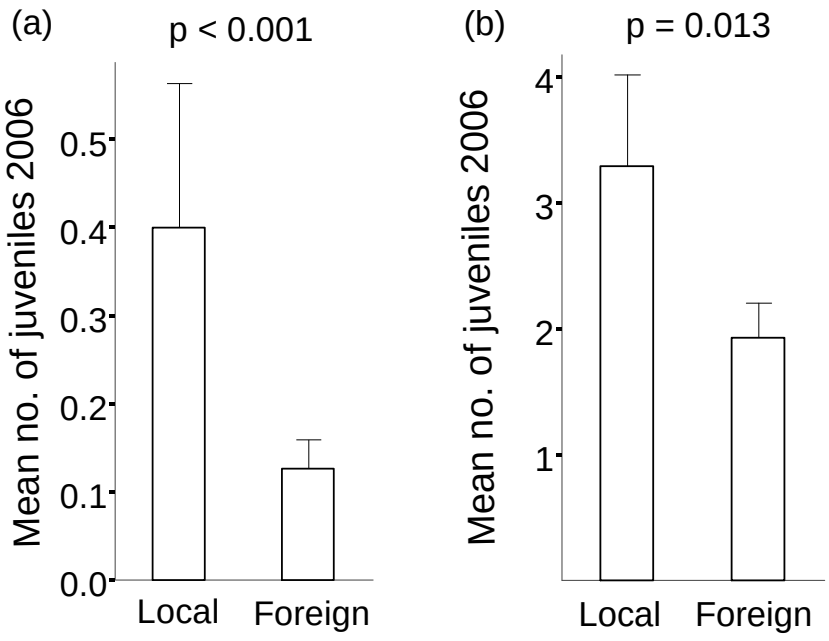


Fig. 2. The effect of the local vs foreign contrast on the mean number of established juveniles of *Aster amellus* in 2006, (a) 'Experiment 2003', (b) 'Experiment 2004'. Error bars indicate 1 SE.

Discussion

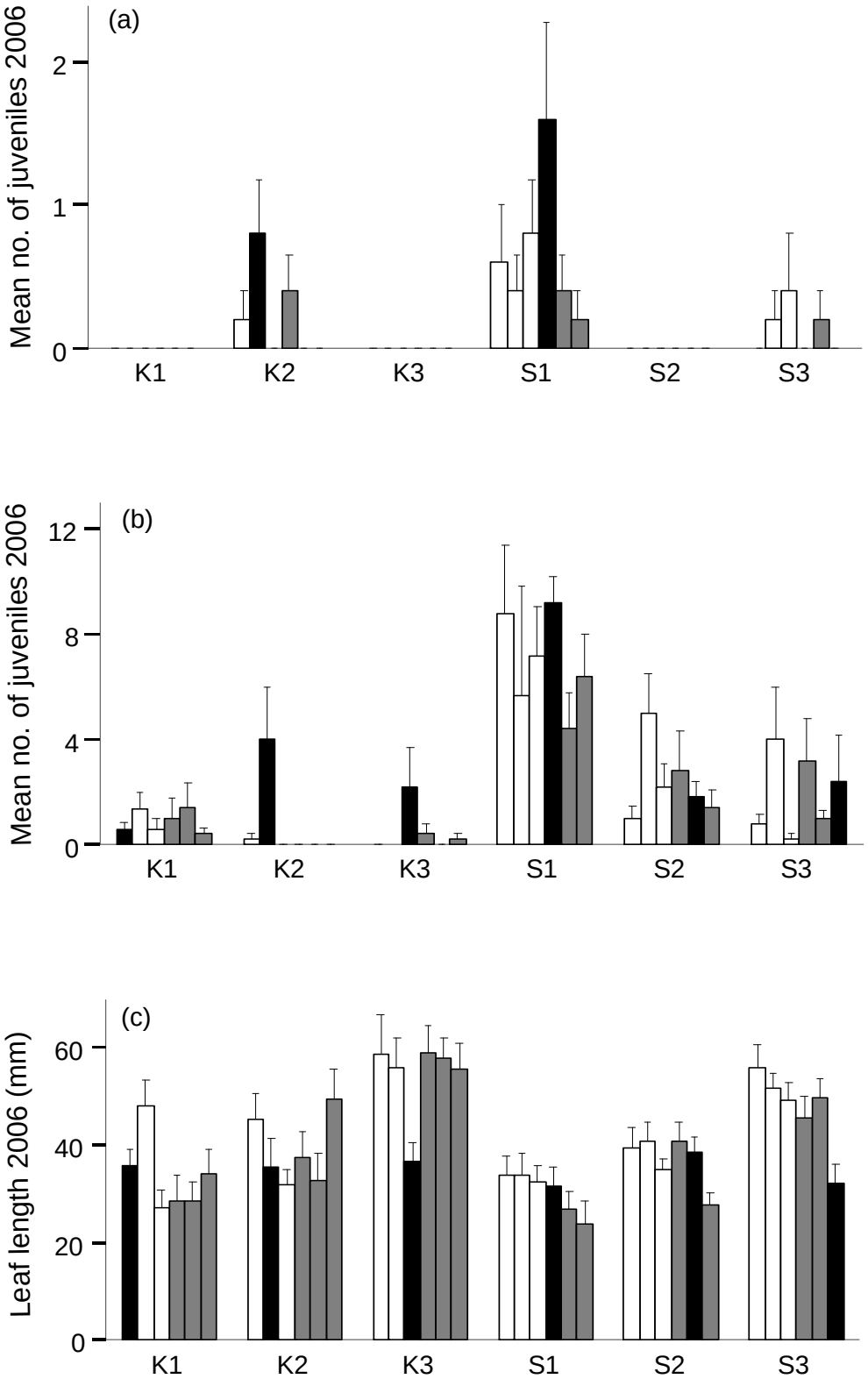
Our results demonstrate adaptation of isolated populations of *A. amellus* to local conditions. This corresponds well with the observed ecological differences and restricted gene flow among populations and suggests that adaptive population differentiation may arise already at a small geographic scale among isolated habitats with relatively little ecological differentiation.

The finding of restricted gene flow among populations was based on isozyme analyses. Molecular markers (e.g. isozymes and microsatellites) are useful tools in detecting genetic divergence that may indicate ecotypic variation (Hufford and Mazer, 2003). Isozyme markers showed little genetic

variation in some studies (e. g. Lesica et al., 1988; but see Lynch et al., 1999; Gravuer et al., 2005) and using microsatellite markers is likely to provide a better resolution for detecting genetic variation within and between populations (e.g. Frankham et al., 2002). In our study, however, isozyme markers turned out to be sufficient for detecting genetic variation and thus were appropriate to compare populations of *A. amellus*.

Aster amellus is a self-incompatible plant with a sporophytic incompatibility system (Richards, 1986). Generally, a higher degree of population differentiation is expected in selfing than in outcrossing species (Ellstrand, 1992). Although the seeds of *A. amellus* bear a pappus that allows potential long-distance dispersal by wind, data on plant height, mean wind speed in the region and terminal velocity strongly suggest that *A. amellus* has a low dispersal ability with a mean dispersal distance of only 3.87 m (Z. Münzbergová, unpublished data). Gene flow by pollen is also expected to occur mainly within populations because bees, which typically pollinate the species, fly mostly between nearest neighbours (e.g. Keasar et al., 1996). Thus, gene flow by seeds and pollen occurs mainly over short-distances in *A. amellus*.

Significant interactions of the local vs foreign contrast with populations of origin indicated that only some populations were adapted to local conditions. This could be due to two reasons. Possibly, only some populations may be able to adapt to local conditions. The ability to adapt increased with population size and isolation in *Carlina vulgaris* (Jakobsson and Dinnetz, 2005). Alternatively, local adaptation may be limited to the most extreme sites (Rice and Mack, 1991). Clearly, more populations should be examined to reliably assess why only some populations of *A. amellus* adapted to local conditions.


(a)
Mean no. of juveniles 2006
0
1
2
K1
K2
K3
S1
S2
S3
(b)
Mean no. of juveniles 2006
0
4
8
12
K1
K2
K3
S1
S2
S3
(c)
Leaf length 2006 (mm)
0
20
40
60
K1
K2
K3
S1
S2
S3

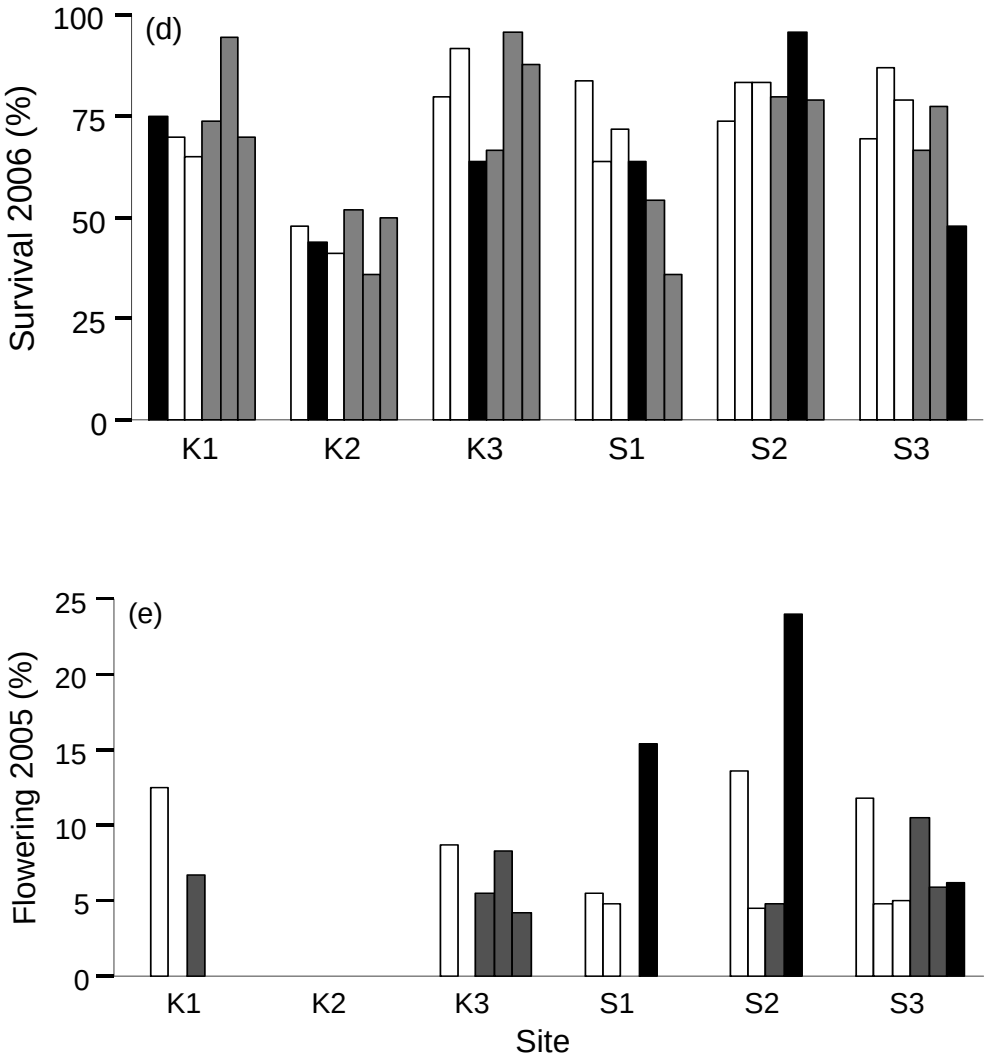


Fig. 3. Performance of *Aster amellus* reciprocally transplanted between six populations in two regions as (a) seeds in 2003, (b) seeds in 2004 and (c-e) adult plants in 2004. Columns denote populations of origin in the same sequence as the sites, i.e. K1, K2, K3, S1, S2 and S3. An empty space indicates that juveniles did not survive or that adult plants did not flower at that site. The shading of the bars denotes regions of origin (white bars: region K, grey bars: region S). Black bars denote the home populations. Error bars indicate 1 SE.

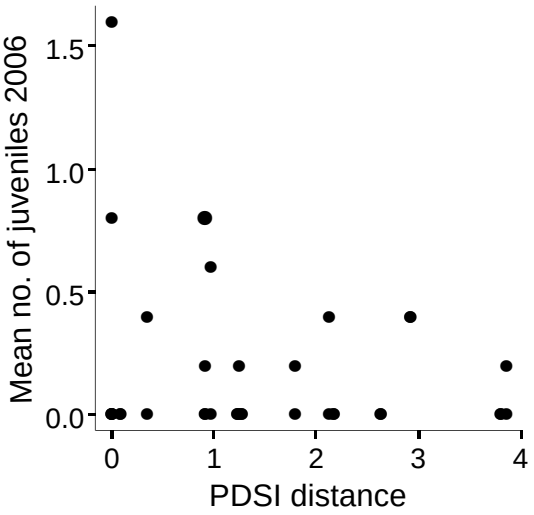


Fig. 4. The effect of differences in potential direct solar irradiation (PDSI) between the population of origin and the target site on the mean number of established juveniles of *Aster amellus* in 'Experiment 2003'.

The numbers of established juveniles that provided evidence of local adaptation were very low in both sowing experiments. The number of established juveniles in Experiment 2003 was negatively influenced by the proportion of bare soil cover at the sites. The mean bare soil cover was 15.1% but it largely differed among sites, ranging from 2.6% to 29%. Higher germination percentage might have been achieved by performing gentle disturbances in the plots (e.g. Jakobsson and Eriksson, 2000). However, sowing seeds into undisturbed areas allows for assessing the processes in natural communities more realistically. The differences in bare soil cover might account for the differences in germination percentage among sites and be a reason why only some populations showed significant local adaptation.

Local adaptation of *A. amellus* was particularly affected by differences in the local climate of the habitats as estimated from the inclination and aspect of the slopes. Similar adaptation has been found in *Quercus rubra.* Seedlings of *Q. rubra* from populations occupying north-, southwest-, and west-facing slopes showed the least damage by herbivores when planted at the site of their maternal plant (Sork et al., 1993). Local adaptation of *A. amellus* was also affected by differences in vegetation composition. In contrast, differences in soil properties did not affect local adaptation of *A. amellus*. This result was unexpected because soil has been found to drive local adaptation in other species (Hangelbroek et al., 2003; Ellis and Weis, 2006). Genetic and geographic distances had only small effects on local adaptation. This result is plausible because genetic distance based on neutral markers reflects past gene flow and genetic drift (Hedrick, 1999) and does not necessarily indicate adaptive divergence between populations. Overall, ecological distances among populations predicted local adaptation of *A. amellus* better than geographic or neutral genetic distance.

The overall evidence of local adaptation of *A. amellus* was detected in both sowing experiments. However, the higher number of juveniles from local than from foreign populations did not become apparent before the second and third year of the experiments. This delayed demonstration of local adaptation may be explained by the delayed germination of *A. amellus* in the field. Similarly, local adaptation of *Carlina vulgaris* was expressed in the second year of the experiment (Becker et al., 2006). Therefore, following plants in the field for more than one vegetation period may be necessary to realistically assess performance of perennial species.

No home-advantage was observed in vegetative traits of transplanted adult plants. Transplanted adult plants showed apparent immigrant advantage

in length of the longest leaf and their survival probability increased with increasing vegetation distance to population of origin. Flowering probability overall increased with increasing differences in local climate. At two sites, however, transplanted adult plants from local populations had greater flowering probability than foreign populations, suggesting local adaptation in reproductive traits. These results are in line with other studies reporting that local adaptation is expressed in reproductive rather than in vegetative traits that may more likely be shaped by the environment (Bennington and McGraw, 1995; Nagy and Rice, 1997; Bischoff et al., 2006).

Sowing seeds and transplanting adult plants led to contrasting results. Only sowing seeds provided overall evidence of local adaptation. This result agrees with other studies that natural selection on seedling germination is extremely effective (e. g. Donohue et al., 2005; Krahulec et al., 2006). Similarly, young vegetative plants were more affected by experimental changes in resource level than reproductive plants (Matthies, 1990). Transplanted adult plants that were able to survive at all sites may fail in establishing viable populations, while natural selection on seedling germination selects appropriate genotypes that have higher chance to survive (Primack and Miao, 1992).

Implications for conservation

Our study shows genetic and habitat differentiation of populations of *A. amellus* at a small spatial scale and evidence of local adaptation. Local adaptation was apparent mainly at the stage of seedling establishment and differed between populations. Transplanted adult plants were able to grow in

all populations in the studied area and to flower in most of them. We suggest that transfer of seeds is a more appropriate technique than transfer of adult plants in conservation practice because it can prevent non-adapted genotypes from establishing. However, the risk of outbreeding depression should be examined by studying consequences of several generations of artificial crosses between populations before using the transfer of plants for conservation purposes.

Our results demonstrate that populations with similar local climate would be the most appropriate source populations for restoration. Our study supports the conclusions of other studies with widely distributed plant species that ecological provenance is more important than geographical provenance for successful plant establishment (Montalvo and Ellstrand, 2000; Smith et al., 2005; Bischoff et al., 2006). We have shown that this principle concerns not only widely distributed but also rare plant species. Because ecological differences tend to be independent of geographical distance, delineating meaningful seed transfer zones, as proposed by Hufford and Mazer (2003), seems not to be possible. We conclude that material for the translocations for nature conservation should come, not necessarily from the closest population, but rather from ecologically similar habitats.

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CHAPTER 3

No first-generation outbreeding depression after between-population crosses at two spatial scales in a perennial herb

"Aldous Huxley once said that an intellectual is a person who has discovered something more interesting than sex. There is a certain irony in this quote for evolutionary ecologist. Many of them would argue, on purely intellectual grounds, that there is in fact nothing more interesting than sex."

(Peter J. Mayhew)

Chapter 3

No first-generation outbreeding depression after between-population crosses at two spatial scales in a perennial herb

(with Zuzana Münzbergová and Markus Fischer; submitted)

Abstract

Many threatened species occur in isolated populations and their fitness is reduced due to low genetic diversity. To increase genetic diversity, increase of gene flow by translocation of individuals between populations is frequently proposed. Previous studies suggest that between-population crosses lead either to higher offspring fitness (heterosis) or to reduced offspring fitness (outbreeding depression). However, little is known about the spatial scale over which these effects may arise, especially at the small spatial scale. We studied the effects of three cross-types: (1) within population, (2) between populations within regions, and (3) between populations from different regions, using four populations of the rare perennial herb, *Aster amellus*. We conducted common garden and field experiments to assess offspring fitness. Between-region crosses (70 km) resulted in 21% higher seed set than between-population crosses within regions (10 km). Between-population crosses led to 18% higher number of flower heads than within-population crosses. However, cross-type did not significantly affect offspring performance in the field. We conclude that outcrossing between populations of *A. amellus* did not lead to immediate outbreeding depression. Therefore, between-population crosses over the distance of 70 km aiming at increasing genetic variation within populations appear as valid management option.

Introduction

Due to habitat fragmentation many plant species occur in small and isolated populations. Species in small and isolated populations are threatened due to genetic, environmental and demographic stochasticity (Ouborg, Vergeer, & Mix, 2006). To reduce genetic problems of small and isolated populations, enhancing gene flow by translocation of individuals between populations is frequently proposed (e.g. Tallmon, Luikart, & Waples, 2004). Translocations of plants may increase genetic variation of isolated populations after interpopulation crosses. Interpopulation crosses can lead, however, not only to higher offspring fitness (heterosis) but also to reduced offspring fitness (outbreeding depression; Lynch, 1991).

Heterosis and outbreeding depression are expected to increase with increasing population divergence. While heterosis is expected when population divergences are caused by genetic drift, outbreeding depression is rather expected when population differentiation is a result of local adaptation (Hufford & Mazer, 2003). Further, heterosis is expected already in the F1 generation (Dudash & Fenster, 2000), while outbreeding depression may be delayed to subsequent generations (Fenster & Galloway, 2000). The extent of outbreeding depression is still a matter of controversy, but some studies suggest that the effects of outbreeding can be as severe as the effects of inbreeding (Edmands, 2007).

The outcrossing distance is of particular interest as a possible predictor of outbreeding depression. However, the geographic or environmental scale over which plant species are adapted is still poorly investigated (McKay, Christian, Harrison, & Rice, 2005) and therefore the risk of outbreeding depression is

hard to predict. Outbreeding depression was found after crosses between populations several hundred kilometres apart (Montalvo & Ellstrand, 2001; Galloway & Etterson, 2005), between nearby populations (Fischer & Matthies, 1997) but also between subsets of individuals within a single population (Waser & Price, 1994; Quilichini, Debussche, & Thompson, 2001). Only a few studies, however, investigated the effects of a broader range of outcrossing distances on offspring fitness (Fenster & Galloway, 2000; Pélabon, Carlson, Hansen, & Armbruster, 2005; Willi & van Buskirk, 2005; Becker, Reinhold, & Matthies, 2006). Particularly studies investigating the effects of various outcrossing distances at a small spatial scale are missing, although such knowledge is important for practical species conservation.

We aimed at determining consequences of between-population crosses in *Aster amellus*, a threatened plant of calcareous grasslands. This species occurs in two different regions about 70 km apart in the Czech Republic, enabling us to study consequences of interpopulation crosses at two spatial scales. Our previous study showed genetic differences between populations of *A. amellus* within and between these two regions and some evidence of local adaptation (Raabová, Münzbergová, & Fischer, 2007). Therefore, crosses between different populations might well lead to outbreeding depression. We grew offspring after within and between-population crosses in substrates from both regions in a common garden, because *Aster amellus* is known to grow very slowly in the field. Moreover, we studied the effects of cross-type on survival and plant size in the field. We asked the following questions: (1) Is there any evidence of outbreeding depression or heterosis? (2) Does the effect of between-population crosses within regions differ from the one of between-region crosses?

Methods

Study species

Aster amellus L. (Asteraceae) is a sub-continental species distributed from Western Europe to Western Siberia (Meusel & Jäger, 1992). Its typical habitats, dry calcareous grasslands, have declined over the last decades, and *A. amellus* has become threatened in many parts of Central Europe (Schönfelder, 1987; Holub & Procházka, 2000). Nowadays, many of its populations are small and isolated.

Aster amellus is a perennial, up to 40 cm high, herb with one or few erect stems. A single plant may have 1-15 flower heads consisting of 40-90 florets (Raabová, personal observation). Its flowering period lasts from mid-July to mid-October (Kovanda, 2005). Plants reproduce both vegetatively and sexually and are mainly pollinated by bees and syrphid flies (Raabová, personal observation). Seeds are usually dispersed over short distances (Münzbergová, personal observation) and germinate in spring. Generally, the species of the genus Aster possess a sporophytic self-incompatibility system (Richards, 1986). However, partial self-compatibility has been found in A. curtus (Giblin & Hamilton, 1999) and A. furcatus (Reinartz & Les, 1994).

Plant and soil material

We collected seeds in four diploid populations in the Czech Karst and in the České středohoří Mountains in the Czech Republic (Table 1). Ploidy levels

of these populations were determined in a previous study (Mandáková & Münzbergová, 2006). We chose four from six populations investigated for local adaptation (Raabová et al., 2007) in a way that the geographic distance between populations within each region was 10 km. The distance between the two regions was 70 km. Isozyme analysis revealed large genetic differentiation between the four populations from our study (F_{ST} = 0.19), and all pairs of populations were significantly different from each other except for the populations in region K (Raabová et al., 2007). Inbreeding coefficient (F_{IS}) of the populations ranged from -0.212 to 0.030, indicating that none of the studied populations suffered from inbreeding due to non-random mating within populations (Raabová et al., 2007). Population sizes estimated as number of flowering individuals in 2005 varied from 60 to 10 000 (Table 1).

To study the effect of different soil conditions on offspring fitness in the garden experiment, we used soil from two different populations in each region (substrate S1 and K1). The substrate S1 (pH 7.29) was more basic than substrate K1 (pH 4.49). Substrate S1 had a 99.7% higher carbonate content and 43% higher calcium concentration. In contrast, substrate K1 had a 70% higher content of organic carbon, 52% higher content of total nitrogen, 61% higher concentration of magnesium and 38% higher concentration of potassium than substrate S1 (for the methods of soil analyses see Raabová et al., 2007).

Table 1. Characteristics of the studied populations of *Aster amellus*.

Code	Population	Longitude E	Latitude N	Population size 2005	Altitude (m)
K1	Koda	14° 07' 29"	49° 56' 01"	1 100	350
K2	Karlík	14° 15' 02"	49° 56' 52"	60	320
S1	Malíč	14° 05' 16"	50° 32' 24"	10 000	310
S2	Holý vrch	14° 13' 49"	50° 31' 41"	1 350	260

Pollinator exclusion

To investigate the possibility of autonomous self-pollination, we randomly selected 20 flowering individuals in each population in the field in July 2003. To exclude pollinators, we covered the flower heads with a bag of fine-mesh nylon before florets opened. We left the flower heads untouched and collected them in September 2003. Although the seeds appeared non-viable we examined their germination ability. We stored the seeds at room temperature until January 2004, when we placed them on wet filter paper in Petri dishes. We kept the Petri dishes in a growth chamber (12 hours at 20°C under light and 12 hours at 10°C in darkness) for a period of 6 weeks and recorded germination weekly. After that period, the seeds had become infested with fungi. Caged flower heads did not produce any viable seeds, indicating that *A. amellus* does not autonomously self-pollinate.

Pollination experiment

In October 2002, we collected seeds of 50 randomly selected individuals per population in the field. In February 2003, we sowed the seeds into plastic trays with garden substrate placed in a greenhouse at 10°C. In May 2003, we transplanted 25 individuals from each population into pots of 10 cm diameter filled with garden substrate and transferred them to an experimental garden in Průhonice, Czech Republic. These plants flowered in August 2004, when we conducted the pollinations. Before the florets started to open, we covered the flower heads with a bag of fine-mesh nylon to exclude pollinators. Hand-pollinations were made by gently rubbing two flower heads together two to four times over the following 5 days to saturate stigmas with pollen. Each flower head served as both pollen donor and pollen recipient. This method has been previously used in the Asteraceae family (e.g. Reinartz & Les, 1994). An alternative method frequently applied in the Asteraceae would have implied the use of a mixture of pollen from a set of plants from each population for all maternal plants (e.g. Pico, Ouborg, van Groenendal, 2004). However, although potentially very good, this alternative approach was not feasible here due to large differences in phenology within flower heads and between plants from different populations. All pollinations were carried out between 11 and 31 August 2004.

We pollinated plants from each of the four populations with plants from each population, resulting in 16 combinations of parental populations. We performed 308 crosses belonging to three cross-types: within population, between populations within regions and between populations from different regions. Generally, we tried to do all cross-types for each plant. In some cases,

the low numbers of flower heads per plant and large differences in phenology between plant populations made it impossible to assign all cross-types to a single plant. To calculate the effect of cross-type on seed set, we selected 5-8 maternal plants per population with successful crosses for all cross-types. For the remaining traits, we selected 68 maternal plants (16-18 plants per population) with successful crosses at least in one cross-type, using 1–6 flower heads per maternal plant and cross-type.

Offspring performance

We assessed seed set for each fruit head separately as proportion of florets that developed ripe seeds 8 weeks after pollination. Ripe seeds were easily recognized because they were filled whereas unripe seeds were flat. In April 2005, we sowed seeds from 10 randomly selected flower heads from each combination of parental populations (10 × 16 = 160 flower heads). We sowed up to 40 ripe seeds from each flower head into 8 × 8 cm pots with a 2:1 mixture of garden substrate and perlite. We placed the pots in a greenhouse at 10°C with natural light conditions in a randomized design. We scored germination 5 and 10 weeks after sowing. In May 2005, we individually planted two randomly selected juveniles per pot into 10 × 10 cm pots with substrates K1 and S1. We grew the plants in the experimental garden in a randomized design and watered them daily. We recorded survival and flowering percentage, length of the longest leaf and number of flower heads in September 2006.

Field experiment

In May 2005, we transplanted 20 seedlings from each combination of parental populations into multipot trays with pots of 3 × 3 cm with garden substrate (in total 320 seedlings). We kept the plants in a common garden until April 2006, when we transplanted them into the field. We planted 80 juveniles at each site, so that populations of their maternal plants corresponded to the target population and populations of their paternal plants were all represented by 20 replicates. We planted the juveniles 10 cm apart in a random order in a row at each site. We watered them immediately after planting and measured the length of the longest leaf. The plants did not flower in the field during the experiment; hence we recorded survival percentage and the length of the longest leaf in September 2007.

Data analyses

We analysed normally distributed variables (length of the longest leaf, number of flower heads) with analysis of variance and binomial variables (seed set, germination, survival and flowering) with logistic regression (analysis of deviance). We log-transformed the numbers of flower heads to achieve normality and homoscedasticity prior to analyses. We tested the effects of population of maternal and paternal plant against residuals and the effects of cross-type against the maternal population by paternal population interaction. Because maternal plants might affect offspring fitness (Roach & Wulff, 1987), we calculated its effect on offspring fitness. Maternal plant

significantly affected only seed germination (quasi-F = 2.34, p = 0.004), however, without changing the effects of the other factors. Therefore, we did not include its effect into the final analyses. We decomposed the effect of cross-type into contrasts of within- and between-population crosses (WP vs BP) and two types of between-population crosses (near BP vs far BP). The analyses of later traits in the garden further included the effects of substrate and its interaction with cross-type. For significance tests in analyses of deviance we used ratios of mean deviance changes, quasi-F (Francis, Green, & Payne, 1993). We performed all analyses with S-plus 6.2 (Insightful Corporation, Seattle, WA, U.S.A.).

Results

Effects on early traits

Maternal plants of different populations differed in seed set and germination (Table 2). Within-population crosses and between-population crosses were not significantly different from each other in seed set and germination (Table 2). However, between-region crosses (70 km) resulted in 21% higher seed set than between-population crosses within regions (10 km; Table 2; Fig. 1), indicating heterosis after far compared to near between-population crosses.

Table 2. Results of analyses of deviance on early traits (seed set and germination) of offspring of *Aster amellus*

	Seed set			Germination		
	d.f.	Quasi-F	p	d.f.	Quasi-F	p
Population of maternal plant (Pm)	3	3.79	**0.012**	3	9.63	**<0.001**
Population of paternal plant (Pp)	3	1.34	0.264	3	0.58	0.627
WP vs BP cross	1	0.29	0.610	1	0.22	0.656
Near vs far cross	1	10.23	**0.015**	1	0.16	0.700
Pm × Pp	7	0.77	0.611	7	1.72	0.109
Residuals	155			144		

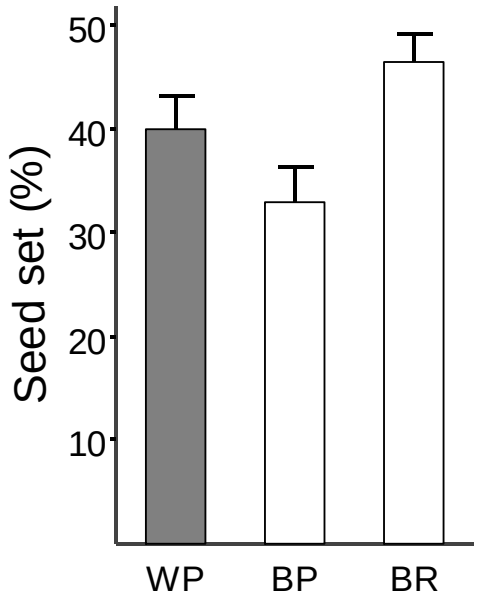

Figure 1. Mean seed set in response to three cross-types in *Aster amellus*. WP = within-population crosses, BP = between-population crosses within regions, and BR = between-population crosses from different regions. Filled and open columns indicate within- and between-population crosses, respectively. Error bars denote 1 SE.

Effects on later traits in the common garden

Survival and flowering percentages in the garden were high (73% and 52%, respectively). Population of maternal plant affected survival percentage, length of the longest leaf and number of flower heads of the offspring (Table 3). Substrate largely influenced offspring performance in the common garden, but did not interact with the effect of cross-type (Table 3). Survival percentage was 24% higher in substrate S1 than in substrate K1. Flowering percentage and number of flower heads, however, were higher in the nutrient-richer substrate K1 than in substrate S1 by 28% and 33%, respectively. The number of flower heads increased after between-population crosses compared to within-population crosses (Table 3; Fig. 2), indicating heterosis.

Effects on later traits in the field

Survival percentage in the field was very high (94%). Population of maternal plant affected survival percentage and length of the longest leaf of the offspring (Table 4). The effect of population of paternal plant differed between populations of maternal plants in survival percentage (Table 4; Fig. 3). Survival percentage was 8% higher after between- than within-population crosses but this effect was only marginally significant (Table 4). Overall, our results do not indicate any evidence of outbreeding depression.

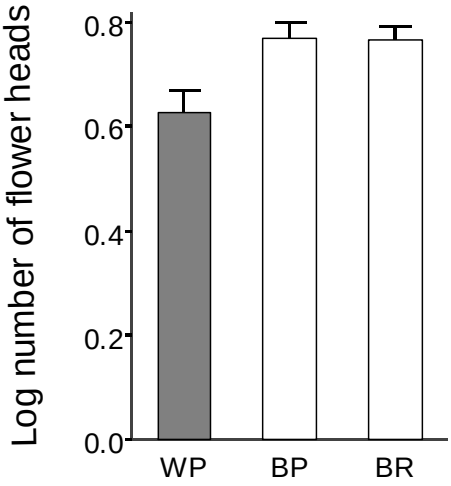

Figure 2. Mean number of flower heads of *Aster amellus* grown in common garden in response to three cross-types. WP = within-population crosses, BP = between-population crosses within regions, and BR = between-population crosses from different regions. Filled and open columns indicate within- and between-population crosses, respectively. Error bars denote 1 SE.

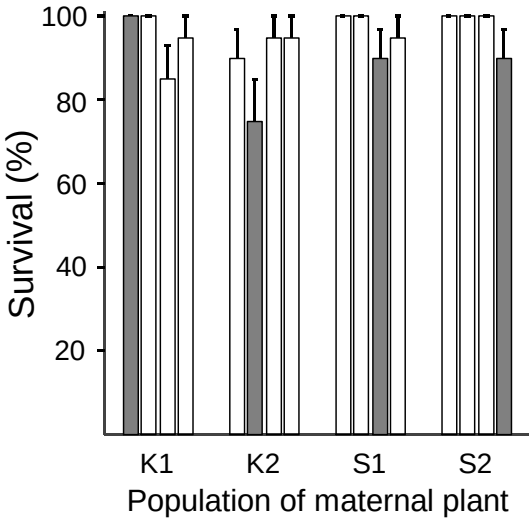

Figure 3. Mean survival percentage of *Aster amellus* reciprocally transplanted between four populations as adult plants in the field. Columns denote populations of paternal plants in the same sequence as the populations of maternal plants, i.e. K1, K2, S1 and S2. Filled bars denote the home population of paternal plants. Error bars indicate 1 SE.

Table 3. Results of analyses of variance (length of the longest leaf and number of flower heads) and deviance (survival and flowering) on later traits of offspring of *Aster amellus* grown in two substrates in the common garden experiment

	Survival			Longest leaf			Flowering			Number of flower heads		
	d.f.	Quasi-F	p	d.f.	F	p	d.f.	Quasi-F	p	d.f.	F	p
Population of maternal plant (Pm)	3	3.53	**0.015**	3	8.94	**<0.001**	3	0.18	0.908	3	3.57	**0.017**
Population of paternal plant (Pp)	3	0.19	0.905	3	1.61	0.187	3	0.28	0.841	3	0.31	0.819
WP vs BP cross	1	0.09	0.777	1	0.29	0.607	1	<0.01	0.967	1	10.95	**0.013**
Near vs far cross	1	2.33	0.171	1	0.01	0.938	1	<0.01	0.985	1	<0.01	0.981
Pair of parental populations	7	0.51	0.826	7	1.22	0.291	7	0.46	0.862	7	0.86	0.540
Substrate	1	16.78	**<0.001**	1	0.12	0.729	1	4.46	**0.036**	1	16.87	**<0.001**
Substrate × WP vs BP	1	0.52	0.483	1	0.45	0.514	1	0.23	0.637	1	0.02	0.886
Substrate × near vs far	1	0.19	0.667	1	0.01	0.930	1	0.09	0.771	1	0.17	0.686
Substrate × Pm × Pp	13	2.64	**0.002**	13	1.58	0.092	13	1.02	0.430	12	0.59	0.846
Residuals	288			202			202			89		

Number of flower heads was log transformed prior to analysis

Table 4. Results of analyses of deviance on later traits of offspring of *Aster amellus* grown in the field

	Survival			Leaf length		
	d.f.	Quasi-F	p	d.f.	F	p
Population of maternal plant (Pm)	3	5.74	**0.001**	3	13.26	**<0.001**
Population of paternal plant (Pp)	3	2.28	0.079	3	0.35	0.790
WP vs BP cross	1	4.51	0.071	1	0.04	0.850
Near vs far cross	1	1.14	0.321	1	0.10	0.765
Pm × Pp	7	4.29	**<0.001**	7	0.94	0.475
Residuals	304			286		

Discussion

Outbreeding depression

Our study did not show any evidence of outbreeding depression in the F1 generation after between-population crosses in *A. amellus*. Survival percentage and plant size were not overall reduced after between-population crosses compared to within-population crosses both in the field and in the common garden. In contrast, we found some evidence of heterosis. The number of flower heads increased after between-population crosses compared to within-population crosses in the common garden. Furthermore, seed set was higher after far between-population crosses than after near ones. These results are in line with our previous reciprocal transplant experiments with *A. amellus*, where transplanted adult plants were able to grow at all sites (Raabová et al., 2007). Nevertheless, we found also evidence of local adaptation in some populations in terms of seedling establishment in our previous study (Raabová et al., 2007). As single populations may differ in

their ability to adapt to local conditions, they might also differ in their vulnerability to outbreeding depression. However, more than four populations need to be investigated to study causes for between-population differences in the consequences of between-population crosses.

We investigated the effects of between-population crosses only in the F1 generation, but outbreeding depression may still become manifest in later generations (Lynch, 1991; Fenster & Galloway, 2000; Keller, Kollmann, & Edwards, 2000). Therefore, our study allows conclusions mainly for short-time effects of heterosis and cannot exclude later outbreeding depression. It is important to note, however, that heterosis needs not only contribute to genetic rescue by affecting the F1 hybrids, but new alleles may also have a selective advantage and therefore spread in the new populations (Ebert**,** Haag, Kirkpatrick, Riek, Hottinger et al., 2002). Finally, positive effects of genetic rescue for rare plant populations need not necessarily be restricted to the first generation but can be maintained beyond the F1 (Willi, van Kleunen, Dietrich, & Fischer, 2007).

Effects of outcrossing distances

The distance between populations affected consequences of between-population crosses in early life-history traits. Crosses between populations from different regions (70 km) resulted in a higher seed set than crosses between populations within regions (10 km). Within-population crosses resulted in intermediate seed set between these two types of crosses. Similarly, seed set and germination percentage were higher after between-regions crosses (444 km) relative to between-population crosses within regions (43 km) in

Hypochaeris radicata (Becker et al., 2006). Results of our study suggest that two types of interpopulation crosses may differ at much smaller spatial scale.

Other studies with threatened and declining species tend to find evidence of heterosis in F1 generation after crosses between populations (Luijten, Kéry, Oostermeijer, & Den Nijs, 2002; Vergeer, Sonderen, & Ouborg, 2004; Paschke, Bernasconi, & Schmid, 2005; Willi & Fischer, 2005). In our study, crosses between populations from different regions were more beneficial for early traits of *A. amellus* than crosses between nearby populations. Thus, our study underlines the importance of spatial scale for interpopulation crosses.

Common garden vs field experiments

The consequences of between-population crosses were similar in both substrates in the common garden and in the field in survival and plant size. However, only the common garden experiment allowed us to study effects of cross type on reproductive traits, representing an important advantage of garden over field experiments with this slowly developing species. Some studies suggest that neither direction nor magnitude of the crossing effects in one environment may be predictable based on results from another environment (Dudash, 1990; Pray, Schwartz, Goodnight, & Stevens, 1994) However, different soil conditions did not interact with the effect of inbreeding in *Hypochaeris radicata* and *Succisa pratensis* (Mix, Pico, van Groenendael, & Ouborg, 2006). In our study, the two investigated traits showed similar pattern in the common garden and in the field. Therefore, results obtained from the garden experiment seem to be applicable to field conditions.

Conclusions

Our study suggests that outbreeding depression does not represent an important threat for *A. amellus*, when transferring plants between different populations and different regions over the distance of 70 kilometres. Over such a distance, there were no large overall differences between offspring resulting from within- and between-population crosses. Moreover, seed set and reproductive traits even showed some evidence of heterosis. Therefore, our study suggests that between-population crosses aiming at increasing genetic variation within populations are a valid management option.

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SUMMARY

Summary

This thesis aimed to test specific hypothesis concerning habitat differentiation, local adaptation and outbreeding depression of the rare herb of dry calcareous grasslands, *Aster amellus*.

Chapter 1 examined habitat requirements of diploid and hexaploid *A. amellus* and their role in segregation of the two cytotypes in the Czech Republic. I chose three diploid and six hexaploid populations belonging to two habitat types (with low and high productivity). To test for differences in fundamental niche between the two cytotypes, I analysed habitat characteristics of sites occupied by each cytotype and used reciprocal transplant experiments. Then, I tested the effects of habitat type, ploidy level and population of origin on plant performance in the experiments.

Sites of diploid and hexaploid populations differed significantly in vegetation and soil properties but much overlap existed in habitat characteristics of the two cytotypes. Diploids had overall higher flowering percentage than hexaploids, suggesting differences between the two cytotypes. However, plants from sites with low productivity also flowered more than plants from sites with high productivity. Moreover, the largest differences in survival, leaf length and flowering were found among plants from different populations. This suggests that overall performance of *A. amellus* differs more among individual populations than between the two cytotypes.

Seedling survival was higher and transplanted plants had longer leaves at sites of the home ploidy level, suggesting niche differentiation between the two cytotypes. Nevertheless, both seedlings and adult plants were able to grow at sites of the foreign cytotype. Furthermore, seedling survival, survival of

adult plants and flowering percentage were higher at sites of home population than at foreign ones, indicating local adaptation. Because the two cytotypes of *A. amellus* have a patchy distribution the divergence between two cytotypes due to local adaptation could evolve. Subsequent adaptive evolution with the environment could therefore contribute to habitat differentiation of the two cytotypes. I conclude that niche differentiation alone cannot explain spatial segregation of the two cytotypes of *A. amellus*.

Chapter 2 aimed to explore local adaptations of diploid *A. amellus* at the small spatial scale. I conducted reciprocal transplant experiments in the field with six populations using seeds and adult plants. To determine the differences between populations, I used geographic, genetic and ecological distances. I used isozyme markers to estimate genetic distance between populations. I analysed vegetation composition, soil properties and potential direct solar irradiation to obtain ecological distances between each pair of sites. Then, I tested which differences explain the degree of local adaptation.

I found evidence of local adaptation in terms of higher seedling survival at home sites than at foreign sites. This result suggests that adaptive population differentiation may arise already at a small spatial scale among isolated habitats with relatively little ecological differentiation. However, not all populations of *A. amellus* adapted to their local conditions. This could be due to two reasons: i) only some populations may be able to adapt to local conditions or ii) local adaptation may be limited to the most extreme sites. Nevertheless, more populations should be examined to reliably assess why only some populations of *A. amellus* adapted to local conditions.

Differences in local climate and in vegetation composition particularly affected local adaptation. This result is plausible because genetic distance based on neutral markers reflects past gene flow and genetic drift and does not

necessarily indicate adaptive divergence between populations. Only sowing seeds provided overall evidence of local adaptation. Therefore, transfer of seeds is a more appropriate technique than transfer of adult plants in conservation practice because it more likely prevents non-adapted genotypes from establishing.

In **Chapter 3** I examined consequences of between-population crosses in *A. amellus*. Crosses between populations may lead either to higher offspring fitness (heterosis) or to reduced offspring fitness (outbreeding depression). The spatial scale, over which these effects may arise, is little investigated. I conducted three types of crosses: within populations, between populations within regions and between populations from different regions. Then, I investigated fitness of F1-hybrids in the common garden and in the field.

Crosses between different populations led to contrasting results depending on the distance between populations. Crosses between populations from different regions resulted in higher seed set, while crosses between populations within regions resulted in lower seed set than within-population crosses. However, the effects of within-population crosses did not significantly differ from the effect of between-population crosses in seed set, not indicating outbreeding depression. Moreover, between-population crosses led to higher number of flower heads in common garden than within-population crosses, indicating heterosis. Plant performance in the field was not affected by the cross-type. I conclude that outcrossing between populations of *A. amellus* did not lead to immediate outbreeding depression.

Implications for conservation

I found only small differences between diploid and hexaploid *A. amellus*. Plants of both cytotypes exhibit large genetic differentiation within and between populations. Habitats of isolated populations of both cytotypes differed in soil properties, vegetation composition and local climate. Such little ecological differentiation together with restricted gene flow led to evolution of locally adapted types. However, local adaptation was apparent mainly at the stage of seedling establishment and differed between populations. In contrast, transplanted adult plants were able to grow in all populations in the studied area and to flower in most of them. Crosses between different populations did not lead to immediate outbreeding depression. Therefore, translocations of individuals between populations over the distance of 70 km aiming at increasing genetic variation within populations appear as valid management option. I conclude that material for the translocations should come, not necessarily from the closest population, but rather from ecologically similar habitats.

Souhrn

Cílem této práce bylo testovat konkrétní hypotézy týkající se stanovištních rozdílů, lokální adaptace a outbrední deprese ohrožené rostliny suchých trávníků, hvězdnice chlumní (*Aster amellus*).

Kapitola 1 se zabývala stanovištními rozdíly mezi diploidními a hexaploidními populacemi *A. amellus* a jejich úlohou v izolaci obou cytotypů v České republice. Pro tuto studii jsem vybrala tři diploidní a šest hexaploidních populací *A. amellus*, které se vyskytovaly ve dvou typech stanovišť (s malou a velkou produktivitou). Rozdíly v nice mezi oběma cytotypy jsem zkoumala pomocí analýzy stanovištních charakteristik a zkřížených přesazovacích pokusů. Potom jsem testovala vliv typu stanoviště, ploidní úrovně a zdrojové populace na úspěšnost rostlin v pokusech.

Lokality diploidních a hexaploidních populací se signifikantně lišily ve vegetaci a půdních vlastnostech, avšak stanovištní podmínky obou cytotypů se také z velké míry překrývaly. Diploidní rostliny kvetly signifikantně více než hexaploidní rostliny, což naznačuje rozdíly mezi oběma cytotypy. Avšak rostliny ze stanovišť s nízkou produktivitou kvetly také signifikantně více než rostliny ze stanovišť s vysokou produktivitou. Navíc největší rozdíly v přežívání, velikosti rostlin a kvetení byly patrné mezi jednotlivými populacemi. To ukazuje, že celková úspěšnost *A. amellus* se lišila více mezi jednotlivými populacemi než mezi oběma cytotypy.

Více semenáčků přežívalo na lokalitách domácí ploidní úrovně než na lokalitách cizí ploidní úrovně. Podobně přesazené rostliny měly delší listy na lokalitách domácí než cizí ploidní úrovně. To naznačuje rozdíly v nice mezi oběma cytotypy. Nicméně semenáčky i dospělé rostliny byly schopné růst i na

lokalitách cizí ploidní úrovně. Dále přežívání semenáčků i dospělých rostlin a procento kvetoucích rostlin byli vyšší na lokalitách domácí populace než na lokalitách cizí populace, což indikuje lokální adaptaci. Protože se oba cytotypy *A. amellus* vyskytují v oddělených populacích, mohla se rozrůzněnost mezi oběma cytotypy v důsledku lokální adaptace vyvinout. Následná adaptivní evoluce s prostředím tak mohla přispět k rozrůzněnosti dvou cytotypů *A. amellus*. Tato studie ukazuje, že rozdílnost nik sama o sobě nemůže vysvětlit prostorovou izolaci diploidních a hexaploidních populací *A. amellus*.

Kapitola 2 měla za cíl zkoumat lokální adaptace diploidních populací *A. amellus* na malé prostorové škále. Lokální adaptace je možné testovat jednoduchým pokusem zkříženého přesazování. Adaptace se prokáže, pokud se přesazeným rostlinám daří lépe na původním než na jiném stanovišti. Proto jsem provedla terénní zkřížený přesazovací pokus s šesti populacemi s použitím semen i dospělých rostlin. Pro stanovení rozdílů mezi populacemi jsem vybrala geografickou, genetickou a ekologickou vzdálenost. Pro určení genetické vzdálenost mezi populacemi jsem použila isozymové markery. Pro získání ekologických vzdáleností mezi lokalitami jsem analyzovala vegetační složení, půdní vlastnosti a potenciální přímou sluneční radiaci. Potom jsem testovala, jaké vzdálenosti mezi populacemi nejlépe vysvětlí míru lokální adaptace.

Lokální adaptace se prokázala vyšším přežíváním semenáčků na domácích než na cizích lokalitách. Tento výsledek naznačuje, že adaptivní rozdíly mezi populacemi se mohou vyvinout již na malé prostorové škále mezi lokalitami s malými ekologickými rozdíly. Nicméně ne všechny populace byly adaptovány ke svým místním podmínkám. To může být způsobeno dvěma důvody: (i) jen některé populace mohou být schopny se adaptovat na lokální

podmínky, (ii) lokální adaptace se může vyvinout jen k extrémním stanovištním podmínkám. Avšak aby bylo možné spolehlivě stanovit, proč jen některé populace *A. amellus* byly adaptovány na lokální podmínky, by bylo nutné studovat vetší počet populací.

Rozdíly v potenciální přímé radiaci (místním klimatu) a vegetaci obzvláště ovlivnily míru lokální adaptace. Tento výsledek je logický, protože genetická vzdálenost, která je založená na neutrálních markerech, reflektuje zejména genový tok v minulosti a genetický drift a neindikuje nezbytně adaptivní rozdíly mezi populacemi. Lokální adaptace byla prokázána jen v pokusu s výsevem semen a nikoli v pokusech s přesazováním dospělých rostlin. To ukazuje, že vysévání semen je vhodnější metoda v ochranářské praxi než přesazování dospělých rostlin, protože lépe zabrání neadaptovaným genotypům před uchycením.

V **kapitole 3** jsem zkoumala důsledky mezipopulačního křížení u *A. amellus*. Křížení mezi populacemi může vést k zvýšení fitness (heteróze) nebo ke snížení fitness (outbrední deprese) potomků. Prostorová škála, na jaké mohou tyto jevy nastat, je však málo prozkoumána. Proto jsem provedla tři typy křížení: v rámci populace, mezi populacemi uvnitř regionu a mezi populacemi z různých regionů. Poté jsem zkoumala fitness F1-hybridů v experimentální zahradě i v terénu.

Křížení mezi populacemi vedlo k odlišným výsledkům v závislosti na vzdálenosti mezi populacemi. Křížení mezi populacemi z různých regionů vedlo k vyšší produkci semen, zatímco křížení mezi populacemi v rámci regionu vedlo k nižší produkci semen než křížení v rámci populace. Nicméně vliv křížení v rámci populace se celkově nelišil od vlivu křížení mezi populacemi, což neindikuje outbrední depresi. Navíc mezipopulační křížení vedlo k vyšší produkci květů v experimentální zahradě, což naznačuje

heterózi. Úspěšnost rostlin v terénu nebyla ovlivněna typem křížení. Tyto výsledky ukazují, že křížení mezi populacemi *A. amellus* nevedlo k bezprostřední outbrední depresi.

Důsledky pro ochranu přírody

Ve své práci jsem nalezla jen malé rozdíly mezi diploidními a hexaploidními populacemi *A. amellus*. Rostliny obou cytotypů vykazovaly velkou genetickou rozdílnost v rámci populace a mezi populacemi. Stanoviště jednotlivých populací obou cytotypů se lišily v půdních podmínkách, vegetačním složení a místním klimatu. Již takové malé ekologické rozdíly dohromady s omezeným genovým tokem mezi populacemi vedly k evoluci lokálně adaptovaných typů. Nicméně lokální adaptace byla nápadná hlavně ve stádiu uchycení semenáčků a lišila se mezi populacemi. Přesazené dospělé rostliny naopak byly schopné růst na všech lokalitách ve studované oblasti a kvést na většině z nich. Křížení mezi různými populacemi nevedlo k outbrední depresi. Proto se přenášení rostlin mezi populacemi na vzdálenost 70 km, které má za cíl zvýšit genetickou proměnlivost, jeví jako oprávněný nástroj ochrany přírody. Tato práce naznačuje, že materiál pro přenášení rostlin by měl pocházet ne nezbytně z nejbližší populace, ale spíše z ekologicky podobných stanovišť.

APPENDIX

Appendix

List of species occurrences and abundance recorded in randomly selected plots of 4 m^2 in 12 populations of *Aster amellus*. Three plots per population were collected, for population names see Table 1 in General introduction. Listed are 153 observed vascular plant species, cover of each species following Braun-Blanquet (1964) and number of species per plot.

Population number	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5
Species\ Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
E3:															
Carpinus betulus					3	+									
Pinus sylvestris						2+									
Quercus pubescens		3	2+			1									
Tilia platyphyllos						3									
E2:															
Cornus sanguinea													+		
Cotoneaster integerrimus	+	+													
Crataegus sp.															
Ligustrum vulgare									r						
Prunus spinosa							+	1							
Quercus pubescens	+	1	r												
Rosa sp.	+						+	+	r						
Sorbus torminalis		+													
E1:															
Achillea millefolium							+	+	+	r		+			
Acinos arvensis															
Agrimonia eupatoria															
Allium montanum			r												
Alyssum montanum		r	+												
Anthericum ramosum	2-		+	2+	+	+	1	+	+						
Anthyllis vulneraria													1		1
Arrhenatherum elatius							+								
Artemisia campestris	+														
Artemisia pontica															
Asperula cynanchica			+	r	+	+			+						
Asperula tinctoria				r											
Aster amellus	1	1	+	1	+	+	+	2-	1	1	2	1	2	3	1
Aster linosyris												r			
Avenula pratensis								+							
Bothriochloa ischaemum			+						+						
Brachypodium pinnatum	+			2-	+	1		2+	+	3	1			1	2
Briza media											+				
Bromus erectus													3	2	1

6	6	6	7	7	7	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12
16	17	18	19	20	21	22	23	24	25	26	27	34	35	36	37	38	39	40	41	42
							1	+	1	1					+					
							1						2		r		r			
																				+
													1							
						+	+				+		+						r	+
																+				
						+	+	3					r	1			+			
3																				r
			+														r			
								+		2										
																				2-
											+								r	r
															r	r	1			
2	1	1	4	2	2	1	3	1	2	2	2	2	1	1						
																		+	2-	+
1	1	1		1	2	2				1	2	2	3	3	r	1	r			
	+	+					+													
			2	4	2							3	1	2				2-	1	1

Appendix

Species\ Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bromus inermis												2			
Bupleurum falcatum						+				+				+	
Calamagrostis epigejos															
Campanula glomerata		r						+	+		1		+	+	
Campanula rotundifolia															
Campanula trachelium		+													
Carex flacca															
Carex humilis	2-	2+	2+	2-	2+	1	2-	3	2+		2				2
Carex montana		+				1									
Carex muricata			+												
Carex tomentosa															
Carlina acaulis														1	
Carlina vulgaris											+				
Carpinus betulus juv.				+											
Centaurea jacea														+	
Centaurea scabiosa			+						r	1	1	1		1	
Centaurea stoebe			r				+	+							
Centaurea triumfettii				+	r	r	+	1	+						
Cirsium acaule				1											
Cirsium arvense															
Cirsium pannonicum											2				
Coronilla vaginalis											1		1		
Cuscuta epithymum			+								+				
Cytisus nigricans						+			+						
Dactylis glomerata												+			
Daucus carota															
Dianthus carthusianorum		+	+												
Dictamnus albus				r											
Elytrigia intermedia															
Elytrigia repens										1		2			
Erigeron acris															
Eryngium campestre								1	+						
Euphorbia cyparissias	+	+	+		r	+	2-	+	+		+				
Festuca rupicola	+				1	1	3	1	+	2		1	+	1	
Festuca valesiaca	+	+	+												
Filipendula vulgaris															
Fragaria moschata				+	+		+								
Fragaria vesca						+		2+							
Fragaria viridis		+	r						+			+			

16	17	18	19	20	21	22	23	24	25	26	27	34	35	36	37	38	39	40	41	42
+	+	+										1			1	r	r	r		r
																	+			
+		+																		
	+																			
+																				
2	4	1			1										r	r	1	3	1	+
																	+			
1					3															
		r		+											r					
+	+	+			+		+	+	1			+		+	r		1	2+	+	2-
	+	+										1	+	1	r	1	1			+
																2+	1	1		
																				r
1																				
												+								
									1											
																			2-	+
																				1
					+							+								
			+																+	+
+	+		+		+	+	+	+					+		1	r	r		+	+
	+	1				1		1					2	2		2-	r	+	2-	2-
																				r
						+	+	+				+							+	r

Appendix

Species\ Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fraxinus excelsior juv.															
Galium glaucum	+	1	1	1	+	+									
Galium verum													1	+	1
Genista tinctoria				+	r	+									
Gentiana cruciata															
Geranium sanguineum	+				+										
Geum urbanum															
Globularia bisnagarica															
Helianthemum grandiflorum	+	r	+	2-	r		+	+	+		+		+		
Hieracium pilosella	2-	+													
Hieracium sp.															
Inula hirta	2-		+	2-	+	+	1	2-	2-						
Inula salicina												1			
Knautia arvensis															
Koeleria macrantha							+								
Koeleria pyramidata			+												
Lactuca perennis	+														
Leontodon hispidus															
Ligustrum vulgare															
Linum catharticum															
Linum tenuifolium															
Lotus corniculatus		+		1				+		+			+	+	+
Medicago falcata								2-	+						
Medicago lupulina			+						+						
Melampyrum cristatum			+												
Melampyrum nemorosum					r	1									
Melica transsilvanica								1							
Onobrychis viciifolia										+	+				+
Ononis spinosa										2	1		1	2	2
Peucedanum cervaria				2-	+	+		+							1
Picris hieracioides															
Pimpinella saxifraga									+						
Plantago lanceolata															
Plantago media															
Poa angustifolia			+				2-	+							
Poa pratensis										+					
Polygonatum odoratum	+	+													
Potentilla arenaria	+	+													
Potentilla heptaphylla		+	+				+	+	1						
Potentilla tabernaemontani							1								

16	17	18	19	20	21	22	23	24	25	26	27	34	35	36	37	38	39	40	41	42
																	r			
				+			+											r	r	+
								+												
																			r	
						3										1				
+	r	r																		
											+				2-		+			
															1	1	1			
																2+		+	2-	2+
2		3					+			+	+			3	3	2-	2-	2-		
				+																
																			+	
+	1	+													2-		2+	1		
															1		2-			
+	+	+	+		+							+						r	r	
+						+												+		
	+		+		+	1	+	+				+		+	r	r	r	r	+	r
																				r
																		1	r	r
1	1	3							1		1							1	2+	1
1	2	2																		
									3											
			+					2							r		r	+		r
												r		+		r				
							+	1								2-			+	+
																	2-	+		
															2-				+	+

Appendix

Species\ Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Primula veris			+												
Prunella grandiflora															
Pyrethrum corymbosum	1	+	1	1	+	+		+	+				+		
Quercus petraea juv.															
Quercus pubescens juv.					r	+									
Quercus robur juv.								r							
Rubus *sp.*															
Salvia nemorosa							+								
Salvia pratensis	+			+	+	1			+	1					
Salvia verticillata															+
Sanguisorba minor							+	+						+	
Scabiosa canescens															
Scabiosa ochroleuca		r								+	+				+
Scorzonera hispanica											1		1	1	
Securigera varia	+	+		+	1	1				1		+		+	
Sedum sexangulare			+												
Senecio jacobaea										+					
Serratula tinctoria					1	1									
Seseli hippomarathrum	+	+	+				+		r						
Sesleria caerulea	1			2-	2+	2-			r				2	1	
Silene nutans			+												
Solidago virgaurea															
Stachys recta														+	+
Stipa pennata	+														
Taraxacum sect. *Ruderalia*															
Tetragonolobus maritimus															
Teucrium chamaedrys	+	+	1			+	+	1	+					+	
Thesium linophyllon				+							2				
Thymus praecox							+								
Thymus pulegioides	+	+	+	2+	+										
Tilia cordata															
Tragopogon pratensis															
Trifolium alpestre	1		+	+	r										
Trifolium medium															
Verbascum lychnitis			+												
Veronica teucrium													+		
Vicia angustifolia															
Vincetoxicum hirundinaria					+	+									
Viola sp.						+					+				
Number of species per plot	28	27	34	24	26	30	23	27	28	15	18	11	15	18	13

16	17	18	19	20	21	22	23	24	25	26	27	34	35	36	37	38	39	40	41	42
1																				
															+	1	2-			
																		r		r
1	+																			
									+											
				+											1	1		r	2+	1
			+			+						+								
	+		+				+	+	+	+		+		+		r	r			
																		+	1	+
+		+				+					1	+							r	
																			1	
							1	+	2			+	+		r	+	r		+	+
											2								+	
	1																	+		+
															r					
	r														r	+				
r																				
																		+	r	
										+	1				2-		1			
1	r																			
																		+	r	r
											+				+		+			
																				+
		+																		
				3																
																+				
															r					
		1																+		
23	21	19	10	8	10	12	15	14	9	7	11	16	11	11	26	22	28	25	29	35

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Working group Biozönoseforschung, Potsdam, 2006 (Photo Birgit Seifert)

Voll Blüten steht der Pfirsichbaum, nicht jede wird zur Frucht,
Sie schimmern hell wie Rosenschaum durch Blau und Wolkenflucht.
Wie Blüten gehen Gedanken auf, Hundert an jedem Tag –
Lass blühen! lass dem Ding den Lauf! Frag nicht nach dem Ertrag!
Es muss auch Spiel und Unschuld sein und Blütenüberfluss,
Sonst wär die Welt uns viel zu klein und Leben kein Genuss.

(Hermann Hesse)

CURRICULUM VITAE

Personal data

Name Jana Raabová

Born 23.3.1978, Prague 5

Education

Since 2002 PhD studies: Department of Botany, Faculty of Science, Charles University in Prague, Thesis: Local adaptations of the rare plant *Aster amellus* in fragmented landscape, supervisor Mgr. Zuzana Münzbergová, Ph.D.

1996-2002 Master studies: Department of Botany, Faculty of Science, Charles University in Prague, Master thesis: Influence of mound-building ants on vegetation pattern and meadow soil, supervisor Prof. Tomáš Herben

1992-1996 Grammar school Nad Štolou, Prague 7

Stays abroad

2006 University of Potsdam (Germany) – Scholarship of the German Federal Environmental Foundation (DBU); 10 months

2001 University of Vienna (Austria) – Erasmus scholarship; 6 months

Work experience

Since 2005 Department of Botany, National Museum in Prague, curator

2005-2006 Department of Botany, Charles University in Prague research assistant

2002-2005 Department of Botany, National Museum in Prague, assistant curator

2002-2003 Prague Botanic Garden, guide

2001-2004 Habitat mapping for the 'Natura 2000'

Teaching experience

2003-2005 Practical exercising in botany

2003 Field exercising in botany

Research grants

2004 Research Grant of the Higher Education Development Fund (FRVS), leader: Local adaptation of the rare plant species

Presentations at conferences

2007 20th Annual Conference of the Plant Population Biology Section of the GfÖ, 17-19 May, Basel, Switzerland. Raabová J., Münzbergová Z.: Niche differentiation between two cytotypes of *Aster amellus* agg., oral presentation

2006 2nd Workshop on rare and endangered plant species, 3-5 November, Lhotka u Mělníka, Czech Republic. Raabová J., Münzbergová Z.: Local adaptation of the rare plant species, oral presentation

2006 1st European Congress of Conservation Biology, 22-26 August, Eger, Hungary. Raabová J., Münzbergová Z., Fischer M.: Relationship between population differentiation, local adaptation and outbreeding depression, oral presentation

2006 19th Annual Conference of the Plant Population Biology Section of the GfÖ, 24-27 May, Halle, Germany. Raabová J.: Influence of mound-building ants on vegetation pattern, poster

2005 17th International Botanical Congress, 17-23 July, Vienna, Austria. Raabová J., Münzbergová Z.: Local adaptation of the rare plant species *Aster amellus* L. (Asteraceae), poster

2005 18th Annual Conference of the Plant Population Biology Section of the GfÖ, 4-8 May, Potsdam, Germany. Raabová J., Mandáková T., Münzbergová Z.: Ecological requirements of two cytotypes of *Aster amellus* (Asteraceae), poster

2005 Central European Workshop in Myrmecology, 24-25 April, České Budějovice, Czech Republic. Raabová J.: Influence of mound-building ants on vegetation pattern, poster

2004 Conference of the Czech botanical society, 20-21 November, Prague, Czech Republic. Raabová J., Münzbergová Z.: Local adaptations of the rare plant species *Aster amellus* L. and *Inula hirta* L. (Asteraceae), poster

2004 34th Annual conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), 13-17 September, Giessen, Germany. Raabová J., Münzbergová Z.: Local adaptations of the rare plant species *Aster amellus* L. and *Inula hirta* L. (Asteraceae), poster

Field experience abroad

Montenegro (2007), Turkey (2004), Austria (2002), Croatia (2001), Italy (2001), Norway (2000), Sweden (2000), Rumania (2000, 2003)

Memberships

Czech Ecological Society - member

Publications

Peer-reviewed publications

Vlasáková, B., Raabová, J., Kyncl, T., Dostál, P., Kovářová, M., Kovář, P., Herben, T.: Ants speed up succession from mountain grassland towards spruce forest. Journal of Vegetation Science, accepted

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Raabová J. (2002): Influence of mound-building ants on vegetation patterns and meadow soils. (MSc. thesis), Depon. in: Library of the Dept. of Botany, Charles University, Prague. [in Czech].