

The growth and survival of three closely related *Myosotis* species in a 3-year transplant experiment

Eva Koutecká and Jan Lepš

Abstract: We studied the growth and survival of three closely related species (*Myosotis caespitosa* C.F. Schultz, *Myosotis palustris* (L.) L. subsp. *laxiflora* (Reichenb.) Schubler et Martens, and *Myosotis nemorosa* Besser) in a 3-year reciprocal transplant experiment. Plants from two populations of each species were transplanted into five experimental localities where one of the three *Myosotis* species was resident. Young plants were planted into three types of competitive microsites as follows: gap, sparse vegetation, and dense vegetation. The experiment demonstrated differences among the species. It also showed large differences among populations within a species. The relative success of the species differed among individual localities and among different microsites, and the favorableness of microsites differed among localities. The *Myosotis* species typically had an advantage in localities where the species was resident. *Myosotis caespitosa* exhibited the highest mortality of its clones, particularly under competition, which corresponds well to its habitat preferences (disturbed and short-term sites), but it also exhibited the ability to spread in gaps by secondary rosettes. *Myosotis palustris* subsp. *laxiflora* spreads best clonally, but its clonal spread was most suppressed by competition. This was consistent with its ability to colonize quickly vegetation-free sites along water, but with weak competitiveness in later stages of succession. *Myosotis nemorosa* exhibited the highest survival rate, which fits with its preference for permanent wet grasslands.

Key words: competition, field experiment, home-site advantage, intraspecific variability, reciprocal transplanting.

Résumé : Les auteurs ont étudié la croissance et la survie de trois espèces étroitement liées (*Myosotis caespitosa* C.F. Schultz, *Myosotis palustris* (L.) L. subsp. *laxiflora* (Reichenb.) Schubler et Martens, and *Myosotis nemorosa* Besser) dans une expérimentation en transplantations réciproques, pendant 3 ans. Ils ont transplanté des plantes de deux populations de chaque espèce dans cinq localités expérimentales, où une des trois espèces de *Myosotis* se retrouve. Les jeunes plantes ont été disposées dans trois types de microsites compétitifs : ouverture, végétation éparse et végétation dense. Les résultats montrent des différences entre les espèces. On observe également de grandes différences entre les populations chez l'espèce. Le succès relatif des espèces diffère entre les localités individuelles et selon les différents microsites, et l'accueil des microsites diffère entre les localités. Typiquement, l'espèce de *Myosotis* jouit d'un avantage dans sa localité de résidence. On observe une plus forte mortalité des clones du *M. caespitosa*, surtout en compétition, ce qui correspond bien à son habitat préféré (sites perturbés et à court terme), mais il montre également une propension à s'étaler par rosettes secondaires dans les ouvertures. Le *M. palustris* subsp. *laxiflora* se reproduit bien par clonage mais l'étalement clonale se trouve fortement supprimé en situation compétitive. Ceci est congruent avec sa capacité à coloniser rapidement des sites sans végétation au bord de l'eau, mais avec une faiblesse compétitive plus tard dans la succession. Le *M. nemorosa* montre le taux de survie le plus élevé, ce qui traduit sa préférence pour les sites herbacés humides permanents. [Traduit par la Rédaction]

Mots-clés : compétition, expérience aux champs, avantage de l'habitat d'origine, variabilité intraspécifique, transplantations réciproques.

Introduction

Closely related species (or subspecies) share evolutionary history; therefore they also share many characters and traits. Comparison of their ecology and environmental adaptations helps to understand and explain processes that led to their separation. Moreover, the comparison of subspecies morphological, physiological, and ecological differentiation could help to disentangle the effects of phylogenetic constraints as well as adaptive evolution on subspecies' ecological behavior (Krahulec 1994; Krahulec et al. 1999).

Related species and (or) polyploid complexes can be investigated with regard to their taxonomy, (eco)physiology, ecology, and phytosociology, but complex studies of more than one aspect are rare and frequently in a review form (e.g., Walck

et al. 2001; Eckstein et al. 2006; Duchoslav et al. 2010). Comparisons of related species are focused, e.g., on reproductive strategy (Urbanska-Worytkiewicz 1980; Moravcová et al. 2001; Moora et al. 2003; Karlsson and Milberg 2008) or on the relationship of species to habitat types and characteristics (Diekmann and Lawesson 1999; Hroudová et al. 1999). Studies of various growth characteristics or phytosociological affinities of pairs or groups of closely related plant species, which should help to explain their rarity or local abundance are relatively common (e.g., Eckstein et al. 2004; Rünk et al. 2004, 2010; Rünk and Zobel 2009). Other studies investigate the effects of selected factor(s) on survival, growth and reproduction of closely related plant species (e.g., Harper and Chancellor 1959; Flegrová and Krahulec 1999; Stöcklin 1999; Dorman et al. 2009; Wu et al. 2010), or the trade-off between

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vegetative and generative reproduction in related species (e.g., Stöcklin and Zoller 1992).

Plant species, but also individual populations within a species are expected to be adapted to local conditions, both the physical environment and biotic interactions in the surrounding community (the home-site advantage hypothesis, e.g., Montalvo and Ellstrand 2000). Reciprocal transplant experiments are often used to quantify this adaptation and to deduce the intensity of selection of genotypes (Silvertown and Charlesworth 2001). Field reciprocal transplant experiments studying the local adaptation of populations, genotypes, or ecotypes are more frequent (e.g., Platenkamp 1990; Bowman et al. 2008; Rice and Knapp 2008) than transplant studies with related subspecies (e.g., Nagy and Rice 1997; Gauthier et al. 1998; Flegrová and Krahulec 1999). Some transplant studies involve several spatial scales, but also these studies compare populations within individual species (e.g., Joshi et al. 2001; Becker et al. 2006, 2008; Bischoff et al. 2006).

We are not aware of any studies that combine transplanting several populations of related plant species to compare variation between these species with variation within species. If closely related species are ecologically significant, their ecological differentiation should be higher than ecological differentiation of individual populations within a species.

The microhabitat of a transplant could play a major role for its survival and reproduction (Bischoff et al. 2006). Adaptive genetic differentiation among plant populations over small scales (a few cm to a few hundred cm) has been documented for plants with different life histories, life forms, and rarity (Linhart and Grant 1996).

The competition of surrounding vegetation is one of the most important microscale factors in the field, which has, however, been rarely manipulated within a reciprocal transplant design (but see Reader et al. 1994; Rice and Knapp 2008). Gaps are places with reduced competition, which represent suitable microsites for seedling germination and establishment (e.g., Mehrhoff and Turkington 1996; Morgan 1997; Špačková et al. 1998). However, this exposes young plants to environmental extremes like drought, standing water, extreme temperatures, and herbivores (Kotorová and Lepš 1999).

For our comparative study, we used plants from different populations of closely related species of swamp forget-me-nots (the *Myosotis palustris* complex) from the southwest region of the Czech Republic as follows: *Myosotis caespitosa* C.F. Schultz, *Myosotis palustris* (L.) L. subsp. *laxiflora* (Reichenb.) Schubler et Martens, and *Myosotis nemorosa* Besser. Although the whole group typically occurs in wet habitats, the individual species differ in their habitat preferences.

In our previous greenhouse and pot studies with the same three *Myosotis* species (each represented by more populations), we demonstrated ecological differences among these species. The short-lived *M. caespitosa* had the highest final germination percentages and the fastest germination rates uniformly in its various populations. *Myosotis nemorosa* showed the highest interpopulation variability and the lowest, but still relatively high final germination in our experiments (Koutecká and Lepš 2009). In a pot experiment (Koutecká and Lepš 2011), *M. caespitosa* exhibited the shortest life span and was also the most sensitive to competition. *Myosotis palustris* subsp. *laxiflora* was the best survivor, producing the most shoots and spreading clones for the longest distances. *Myosotis nemorosa* was intermediate in survival and clonal growth and exhibited the highest among-population variability. In both the experiments, we demonstrated large differences among individual populations within a species in all three species.

Our first aim was to compare the differences between closely related species with the variability among populations within spe-

cies in a reciprocal transplant experiment. We hypothesized that the species should be more successful in their original localities. Our second aim was to evaluate the role of microsite type, offering different competitive pressures from surrounding vegetation, on the survival and growth of experimental plants. We hypothesized that the negative effect of competition on survival and growth should be greatest in *M. caespitosa*, a species growing in disturbed habitats, with the shortest life span. In addition, these three *Myosotis* species differ in their clonal potential and we expected their clonal spread to be influenced by the microsite.

Materials and methods

Studied species

Individual species of *M. palustris* complex are very similar, and their identification in the field requires some experience. The taxonomy of the *M. palustris* group has changed several times in the last decades and was presented differently in different floras (e.g., Schuster 1967; Hegi 1979; Štěpánková 2000), which made interpretation of each published ecological study difficult. We adopted the classification and nomenclature used in Czech Flora (Štěpánková 2000).

For our study, we selected the species *M. palustris* subsp. *laxiflora*, *M. nemorosa*, and *M. caespitosa* (other subspecies in the Czech Republic are *Myosotis palustris* subsp. *palustris* and *Myosotis brevisetacea*). The three species studied differ from each other in their ploidy level; with some experience they can be easily identified. The species were selected because they were known to differ in their habitat preferences, but also because they were sufficiently common in the studied area.

Species of this group are considered to be annual or perennial with variable ability of form stolons, rhizomes, or daughter rosettes (for details, see table 1 in Koutecká and Lepš 2011). In addition, the characteristics vary both among and within populations of the same species. Although all of the species grow in wet sites, they thrive in different habitats and it is rare to see two species growing together in one locality. No comparative ecological study of more species within the *M. palustris* group is available. Phylogenetic studies of the complex based on molecular data are not available. However, differences in morphological characteristics separate *M. caespitosa* species from the rest of *M. palustris* group (Štěpánková 2000).

Reciprocal transplant experiment

Seeds for the reciprocal transplant experiment were collected from six natural populations (two populations of each investigated *Myosotis* species) during the 2002 season (the locality characteristics of each source population are located in Supplementary Table S1¹). In each population, seeds were collected once from hundreds of mother plants. Fruits in each helicoid cymes mature gradually, therefore only a very low number of seeds were collected from each individual mother plant. Only visually mature, dark seeds, falling spontaneously from the calyces, were collected. Seeds were stored under dry conditions at room temperature (about 20 °C) during the winter. Plants used for the experiment were preplanted in a greenhouse in starter (Jiffy) pots. Young *Myosotis* plants were planted in field conditions after they produced four to six leaves.

The field experiment was established in May 2003, when young *Myosotis* plants were transplanted into all five field localities within a 1-week period. Five experimental localities were chosen as typical habitats with the natural occurrence of one of the three experimental *Myosotis* species, two localities of *M. palustris* subsp. *laxiflora* and *M. nemorosa*, and one locality of *M. caespitosa*. We were not able to find another site of *M. caespitosa* that would give a

¹Supplementary data are available with the article through the journal Web site (<http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2012-0214>).

reasonable chance to survive more than one season. Localities are identified by the first letter of the subspecific name, i.e., L1, L2 for *M. palustris* subsp. *laxiflora* etc. Characteristics of experimental localities are summarized in Supplementary Table S2.

Young *Myosotis* plants of all six populations were transplanted to each of the five experimental localities. Each locality contained five 4 m² randomized blocks consisting of homogenous vegetation. Each block contained microsites of three types (presumably differing in competition level), each type in six replicates (one replicate for each experimental population). One young experimental plant was transplanted per microsite; each block contained 18 transplants (6 populations × 3 microsite types). The microsite types were as follows: (i) gap in the vegetation (hereinafter gap) of at least 15 cm in diameter (natural or artificially cleared free space in the vegetation) represented a treatment with presumably lowest aboveground competition; (ii) less dense vegetation (hereinafter sparse vegetation), usually undisturbed microsite, but not in a dense tussock; and (iii) dense vegetation or thick tussock (hereinafter dense vegetation). Treatments were not re-established during the experiment. Plants were watered just once, immediately after transplantation.

Survival and selected growth characteristics were recorded four times over the 2003 growing season (May, 3–4 weeks after planting; June; July–August; and September), three times over 2004 (May, June, and July), and survival was observed once in 2005 (July). The following vegetative and reproductive characteristics were recorded: (i) the number of shoots (stolons or rhizomes) or secondary rosettes (type of clonal organs differed between *Myosotis* species); (ii) the height of the standing aboveground stems representing the height of plant or clone (mm); and (iii) the number of flowers per clone.

The habitat conditions of individual experimental localities were characterized by their aboveground biomass and selected soil characteristics (see Supplementary Table S3 and Supplementary Fig. S1).

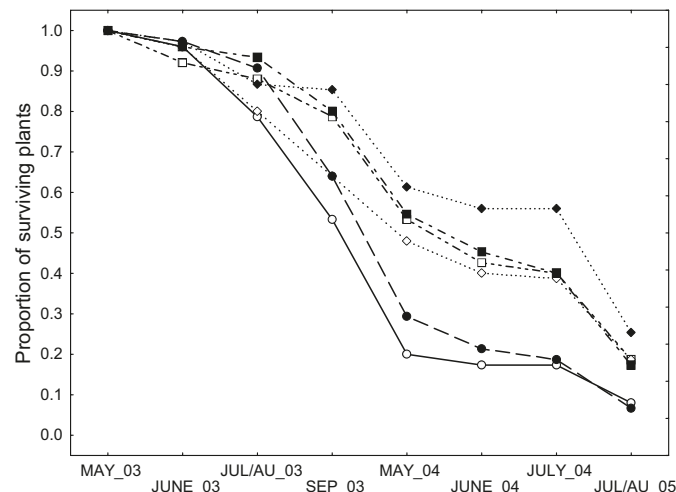
Data analysis

The data were evaluated using various ANOVA models, using the Statistica package (StatSoft, Inc. 1998, 2005), taking into account the repeated observations of individual plants (repeated measures ANOVA) and the hierarchical nature of the experiment. Individual seed source populations (factor Population) were considered as a random factor nested in the Planted Species, while the Locality and Microsite were fixed factors, not nested. If the Planted Species × Locality interaction was significant, we used contrasts to test whether the individual *Myosotis* species grew better in localities where they were native than in localities of the other two species.

The balanced design of the experiment allowed us to compute repeated measures ANOVA for all experimental localities during the first season (calculated using Statistica version 5) and for four localities during the first and second season (site C1 was flooded and therefore excluded from the analysis). Analyzing the number of shoots and rosettes, dead plants were considered in two ways; either as missing observations or as zeroes (in repeated measures). In the first case (missing observations), we compared the state of the surviving plants. Assigning zero to the dead plants was done to compare all plants. We also used an analogical approach to evaluate survival, assigning live individuals a value of one and dead individuals a value of zero. We applied the repeated measures ANOVA (the averages of zero and one values represent the percentage survival for studied category).

Where we were interested in the properties of surviving plants (height of aboveground stem, number of flowers, number of produced shoots or rosettes), we conducted tests for (selected) dates; all surviving individuals were included in the analyses. In this case, the design was necessarily unbalanced and was evaluated using Statistica 7 (which uses the “denominator synthetic degrees

Fig. 1. Proportion of surviving plants of six populations used in reciprocal transplant experiment during three seasons. Abbreviations of species: C, *Myosotis caespitosa*; L, *Myosotis palustris* subsp. *laxiflora*; N, *Myosotis nemorosa*. Planted population numbers and corresponding source localities: open circle, 1 C-Dářko; solid circle, 2 C-Munický rybník; open square, 3 L-Český Krumlov; solid square, 4 L-Trocnov; open diamond, 5 N-Mladějovice; solid diamond, 6 N-Střemily. Averaged over all experimental localities and over all experimental treatments. Differences in survival between species and between populations within a species (i.e., Planted Species × Time and Population × Time interactions) are significant; for full results of repeated measures ANOVA, see Tables 1, 2, and 3 and Supplementary Table S2.



of freedom” approach enabling analysis of the unbalanced hierarchical mixed effect designs). If the main effects were significant, the Tukey's test at $\alpha = 0.05$ (with the mean square corresponding to the denominator MS for the test of corresponding effect used for SE estimation) was used for post-hoc comparisons of species. The numbers of shoots were subjected to square-root transformation ($\sqrt{\text{variable} + 0.5}$) for analyses. However, all figures show the original nontransformed values.

Results

The plants of both *M. caespitosa* populations had the highest mortality during the experiment; plants of the other two species survived significantly longer and in higher numbers. Survival rates of both *M. palustris* subsp. *laxiflora* populations were similar, but we observed large differences between the two populations of *M. nemorosa* (Fig. 1; Table 1; Supplementary Table S5). Some individuals of all the species, including the *M. caespitosa*, survived to the end of the third season.

There were also large differences in survival between experimental localities. Microhabitat treatments consistently affected plant survival. Significant interactions show that the relative performance of individual *Myosotis* species differed according to the locality (Fig. 2). This signified that the relative success in terms of survival differed among the species; however, the differences were dependent upon locality.

The contrast analysis for the end of 2003 (September) showed that, on average, the *Myosotis* species survived better in localities where they were resident in comparison with the other two species ($F_{[1,402]} = 24.01$; $p < 0.001$). The significant interaction of Planted Species × Microsite × Time demonstrated that the *Myosotis* species reacted differently to microhabitat treatments (Fig. 2). *Myosotis caespitosa* plants survived better in gaps than in both sparse and dense vegetation. *Myosotis palustris* subsp. *laxiflora*, and *M. nemorosa* preferred one or other vegetation in each of the ex-

Table 1. Results of repeated measures ANOVA.

Effect	2003						2003 + 2004					
	Effect df	Den. syn. df	Survival		Shoots or rosettes		Effect df	Den. syn. df	Survival		Shoots or rosettes	
			F	P value	F	P value			F	P value	F	P value
Loc	4	12	8.527	0.002	29.621	<0.001	3	9	7.466	0.008	20.848	<0.001
Spe	2	3	1.133	0.430	1.550	0.345	2	3	7.832	0.064	3.294	0.175
Pop	3	360	3.248	0.022	1.659	0.175	3	288	3.294	0.021	2.549	0.056
Micr	2	6	1.477	0.301	36.645	<0.001	2	6	2.896	0.132	29.663	0.001
Time	2	6	39.090	<0.001	11.291	0.009	5	15	156.101	<0.001	22.825	<0.001
Loc × Spe	8	12	3.379	0.029	1.879	0.157	6	9	2.706	0.087	2.274	0.129
Loc × Pop	12	360	0.780	0.671	1.039	0.412	9	288	0.843	0.577	0.912	0.515
Loc × Micr	8	24	3.690	0.006	3.248	0.012	6	18	5.763	0.002	6.398	0.001
Spe × Micr	4	6	5.194	0.037	5.003	0.041	4	6	7.295	0.017	6.211	0.025
Pop × Micr	6	360	0.577	0.748	0.484	0.820	6	288	0.263	0.954	0.396	0.881
Loc × Time	8	24	8.728	<0.001	6.850	<0.001	15	45	11.586	<0.001	25.031	<0.001
Spe × Time	4	6	3.567	0.081	5.475	0.033	10	15	10.162	<0.001	10.417	<0.001
Pop × Time	6	720	3.267	0.004	4.913	<0.001	15	1440	1.565	0.076	2.069	0.009
Micr × Time	4	12	3.644	0.036	3.950	0.029	10	30	1.877	0.089	3.494	0.004
Loc × Spe × Micr	16	24	1.333	0.256	0.796	0.677	12	18	0.939	0.533	1.403	0.250
Loc × Pop × Micr	24	360	0.959	0.521	0.716	0.836	18	288	1.034	0.421	0.867	0.619
Loc × Spe × Time	16	24	4.947	<0.001	4.805	<0.001	30	45	3.265	<0.001	4.116	<0.001
Loc × Pop × Time	24	720	0.883	0.626	0.715	0.839	45	1440	0.839	0.768	0.574	0.990
Loc × Micr × Time	16	48	2.300	0.013	3.833	<0.001	30	90	1.918	0.010	2.810	<0.001
Spe × Micr × Time	8	12	4.938	0.007	3.649	0.022	20	30	3.867	<0.001	2.761	0.006
Pop × Micr × Time	12	720	0.487	0.923	0.734	0.718	30	1440	0.298	1.000	0.554	0.976
Loc × Spe × Micr × Time	32	48	0.919	0.594	1.421	0.133	60	90	1.111	0.322	1.851	0.004
Loc × Pop × Micr × Time	48	720	1.028	0.423	0.768	0.873	90	1440	0.938	0.643	0.733	0.970

Note: Significance of effects and their interactions for response variables: survival of plants and number of secondary shoots or rosettes of clones in five localities during the 2003 season (3 dates) and in four localities during 2003 + 2004 seasons (6 dates). Dead plants are counted as clones with 0 shoots. Summary of all effects. Loc, Locality; Spe, Planted Species; Pop, Population; Micr, Microsite; ×, interaction; Den. syn., denominator synthetic. The Population was random factor nested in the Planted Species factor. Statistically significant results ($p < 0.05$) are in bold. The first date in 2003 (3–4 weeks after planting) was not used, because all the plants were still alive.

perimental localities, no matter if *Myosotis* species was original there or not. Moreover, the suitability of the three different microhabitat treatments for the survival of experimental plants differed between experimental localities (interactions Locality × Microsite and Locality × Microsite × Time).

During the second season, the C1 locality was destroyed by flooding and it was therefore not possible to collect data. Surprisingly, one surviving clone of *M. caespitosa* was found in the C1 locality during the third season even though the site had been flooded by at least 30 cm of water for two seasons. The differences among the four persisting localities and also among experimental populations remained similar to those of the first season. Differences in the survival rates of individual species differed among individual localities (see results to the July 2004, Supplementary Table S4; Fig. 2). Contrast analysis demonstrated that, on average, survival was better in the home-species localities (June 2004, $F_{[1,321]} = 12.06$, $p < 0.001$; July 2004, $F_{[1,321]} = 7.38$, $p = 0.007$), but we cannot say that original *Myosotis* species survived best on its original locality in all cases. In almost all localities (with exception of N2) the survival of *M. nemorosa* was the highest, followed by *M. palustris* subsp. *laxiflora*. *Myosotis caespitosa* species had the lowest survival rate in all four undisturbed localities.

Differences in survival among individual microsite types varied among experimental localities and also among the species. Survival was the highest in gaps in L2 (sandy deposit of brook) and N2 (woody path) localities, both of which had good moisture conditions. By contrast, survival increased with the increasing density of vegetation in microsites on the wet meadow (N1 locality). No differences among microsites were found on the river bank (L1 locality). The positive effect of gaps was most pronounced in *M. caespitosa*, the survival of the other two species was less affected.

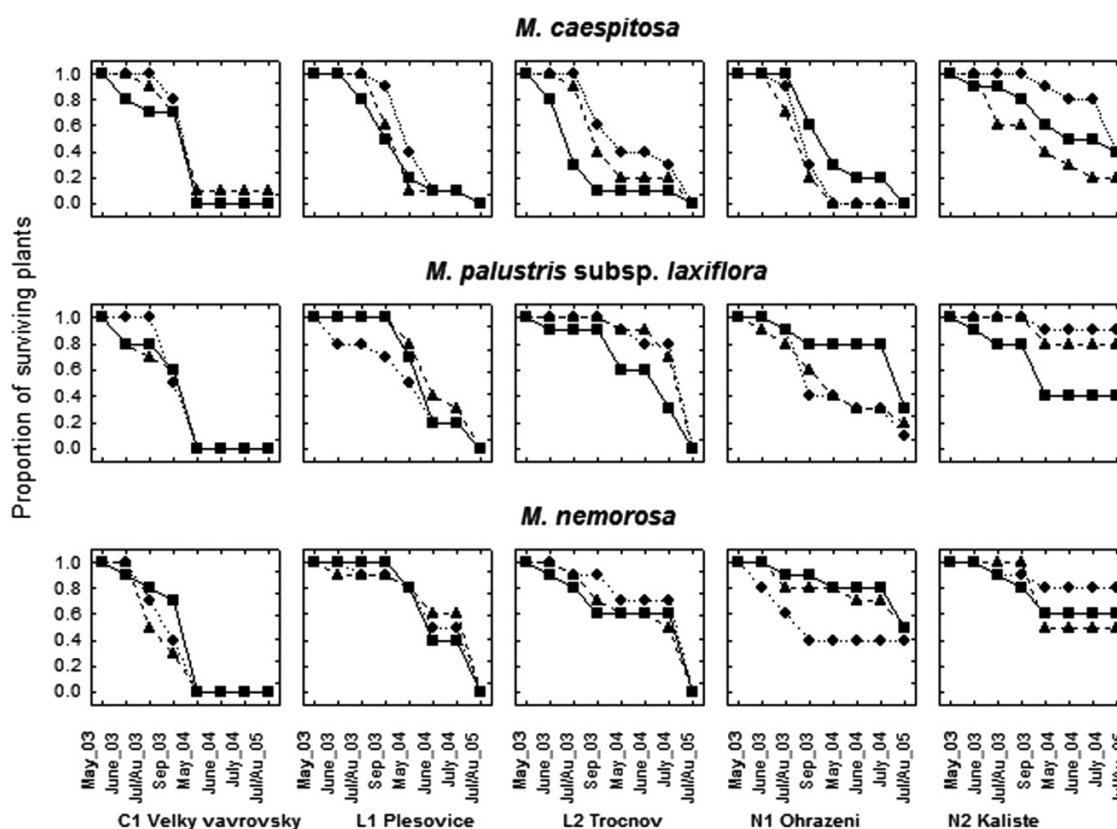
Only the two home localities of *M. nemorosa* (N1 and N2) persisted until the third season. Both *M. palustris* subsp. *laxiflora* localities (L1 and L2) were destroyed by flooding and subsequently overgrown by dense vegetation (mostly *Phalaris arundinacea* L.).

Survival in 2005 was analyzed only for the two persisting localities (to July–August date, see Supplementary Table S4). The species differed significantly, with the highest survival rate in *M. nemorosa* followed by *M. palustris* subsp. *laxiflora*, with only a few surviving individuals of *M. caespitosa*. All plants of *M. caespitosa* died in the N1 locality, whereas *M. nemorosa*, the original species, was the most successful there.

The dynamics of number of shoots–rosettes differed significantly among localities and among species. There were also pronounced differences among the microsites (Table 1; Fig. 3). The number of shoots–rosettes formed per planted individual was also naturally affected by survival; dead individuals do not form shoots. However, this also reflected the clonal behavior of survivors. *Myosotis caespitosa* formed the highest number of secondary rosettes immediately after planting, but they started to die off rather quickly in all experimental localities and survived only in gaps. The number of secondary shoots was most affected by microsites in the *M. palustris* subsp. *laxiflora* (the highest in gaps, the lowest in dense vegetation). Differences increased in the second season. *Myosotis nemorosa* clones had a relatively stable as well as a comparable mean number of shoots throughout the entire experiment.

As for survival results, the effect of microsite type differed considerably among the experimental localities (Fig. 4). The corresponding effects of survival can be found also for shoots and (or) secondary rosettes per living plant (Table 2). In particular, the surviving plants of *M. nemorosa* produced fewer shoots in compar-

Fig. 2. Proportion of surviving plants of three *Myosotis* species on five experimental localities and in three competitive treatments as follows: solid square, dense vegetation; solid triangle, sparse vegetation; solid circle, gap; during three seasons in reciprocal transplant experiment. Averaged over all planted populations of individual species. The interactions Planted Species \times Locality \times Time, Locality \times Microsite \times Time, and Planted Species \times Microsite \times Time are all significant; for full results of repeated measures ANOVA, see Tables 1, 2, and 3 and Supplementary Table S2.



ison with the other two species. Contrasts analysis was not significant for any date of experiment. This meant that individual *Myosotis* species did not produce more secondary shoots or rosettes in their original locality(-ies) compared with the other two species.

The height of living clones differed between individual populations and localities (see Supplementary Table S5). Clones of one *M. caespitosa* population were the shortest, whereas other populations of all the species were comparable. These differences were more obvious during the first (2003) season and gradually disappeared in the second year. The negative effect of competitive microsites on height was observed during the middle of the 2003 season. Differences in height of individual *Myosotis* species clones differed among the experimental localities (significant interaction Locality \times Planted Species in Supplementary Table S5 and Table 3). The contrasts analysis suggested that, on average, the individuals are taller in localities where the species was resident (June 2003, $F_{[1,378]} = 13.98$, $p < 0.001$; July–August 2003, $F_{[1,333]} = 21.20$, $p < 0.001$; June 2004, $F_{[1,130]} = 10.39$, $p = 0.002$; July 2004, $F_{[1,120]} = 7.05$, $p = 0.020$).

The effect of microsites on the height of clones differed among localities (Table 3; Supplementary Table S5), and also changed during time. During the 2003 season, *M. caespitosa* clones were tallest in gaps, but the other two species were tallest in sparse vegetation. All three species were shortest in dense vegetation treatments. At the end of the 2004 season, the clones of all species were tallest in gaps.

Flowering in 2003 differed between localities and microsite treatments. Intensity of flowering was highest in the L1 locality, which also had the greatest shoot height. Interestingly, there were

significant differences between populations of the same species, but not between species. In particular, plants of one *M. caespitosa* population produced the greatest number of flowers in comparison with all other populations (of different species). Despite differences among individual localities in flowering, the intensity of flowering decreased with competition similarly in all the localities (see Supplementary Table S6) and in all the species. We did not detect any home-site advantage for flowering.

Discussion

The three *Myosotis* species differ in their habitat preferences, particularly in the disturbance regime most often encountered in their typical habitats. *Myosotis caespitosa* is typical for rather transient habitats, such as emerged fishpond bottoms. *Myosotis palustris* subsp. *laxiflora* typically occupies disturbed plots on river banks. *Myosotis nemorosa* thrives in a wide range of habitats, where it often faces strong competition from perennial plants of the community matrix (Štěpánková 2000; J. Štěpánková, personal observation).

The fate of our experimental localities followed the typical fate of localities of corresponding species. Our only locality of *M. caespitosa* was flooded after the first season, whereas the two localities of *M. palustris* subsp. *laxiflora* were disturbed by flowing water and then overgrown by a thick bed of tall *P. arundinacea*.

Our previous laboratory and pot studies (Koutecká and Lepš 2009, 2011) have generally demonstrated the adaptive values of individual species traits for their preferred habitats. In particular, *M. caespitosa* is dependent on seed regeneration and its seeds exhibit the highest and the most stable germinability (low interpopulation variability, no decrease of germinability within 2 years).

Fig. 3. Mean number of shoots or secondary rosettes of three *Myosotis* species in three competitive treatments: solid square, dense vegetation; solid triangle, sparse vegetation; solid circle, gap, during 2003 and 2004 seasons. Averaged over all experimental localities. Dead plants counted as clones with 0 shoots. The interactions Planted Species \times Time, Microsite \times Time, and Planted Species \times Microsite \times Time are all significant; for full results of repeated measures ANOVA, see Tables 1, 2, and 3 and Supplementary Table S2.

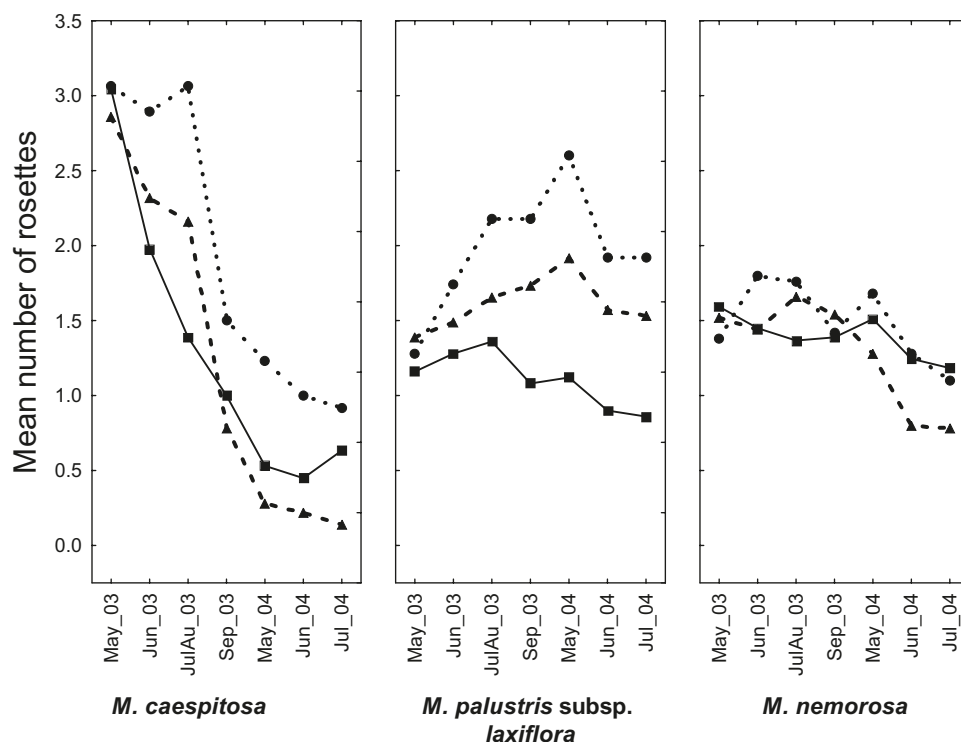


Fig. 4. Mean number of shoots or secondary rosettes in three competitive treatments: solid square, dense vegetation; solid triangle, sparse vegetation; solid circle, gap, on five experimental localities during 2003 and 2004 seasons. Averaged over all three species. Dead plants counted as clones with 0 shoots. The interactions Locality \times Time, Microsite \times Time, and Locality \times Microsite \times Time are all significant; for full results of repeated measures ANOVA, see Tables 1, 2, and 3 and Supplementary Table S2.

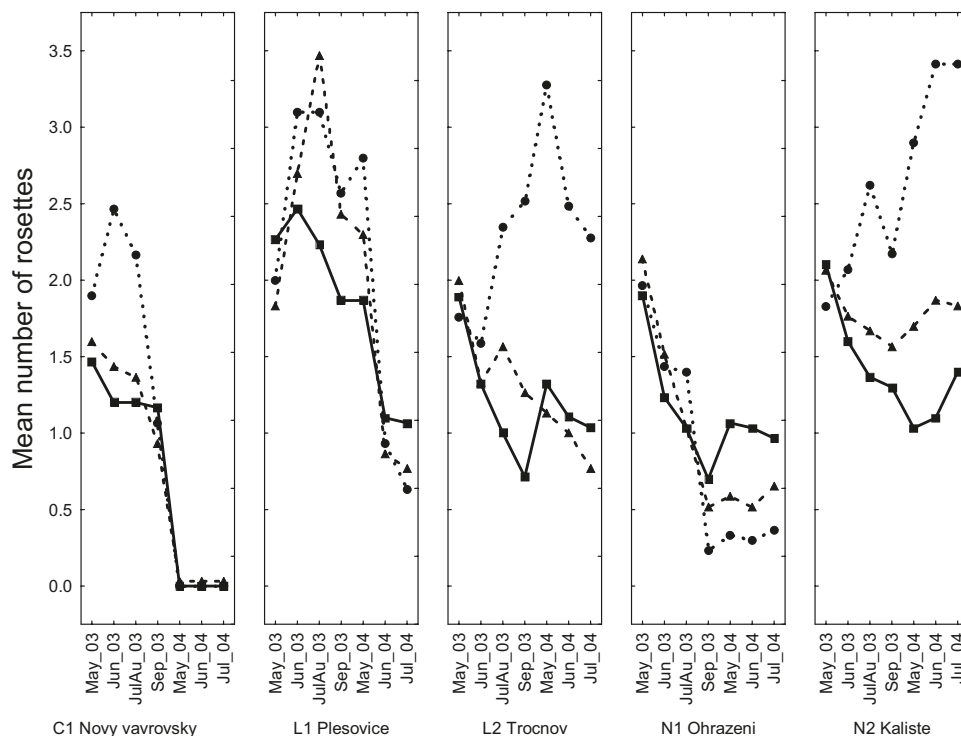


Table 2. Results of hierarchical ANOVA.

Effect	July–August 2003				September 2003				July 2004			
	Effect df	Den. syn. df	F	P value	Effect df	Den. syn. df	F	P value	Effect df	Den. syn. df	F	P value
Loc	4	333.00	18.818	<0.001	4	239.00	10.455	<0.001	3	120.00	4.848	0.003
Spe	2	3.20	6.680	0.072	2	8.69	1.602	0.256	2	15.09	9.259	0.002
Pop	3	333.00	1.201	0.310	3	239.00	0.315	0.814	3	120.00	0.222	0.881
Micr	2	333.00	16.688	<0.001	2	239.00	4.392	0.013	2	120.00	3.207	0.044
Loc × Spe	8	333.00	1.501	0.155	8	239.00	1.701	0.099	6	120.00	1.147	0.340
Loc × Pop	8	333.00	0.751	0.646	8	239.00	0.544	0.823	6	120.00	1.727	0.120
Loc × Micr	4	333.00	1.212	0.306	4	239.00	2.589	0.038	4	120.00	1.696	0.155
Spe × Micr	16	333.00	0.520	0.937	14	239.00	1.151	0.315	10	120.00	1.551	0.130

Note: Significance of effects and their interactions for variables: Number of secondary shoots or rosettes for living plants (dead plants omitted from analysis, therefore design is incomplete) on five localities in 2003 (July–August and September) and on four localities in 2004 season (July). Summary of all effects. Loc, Locality; Spe, Planted Species; Pop, Population; Micr, Microsite; ×, interaction; Den. syn., denominator synthetic. The Population was random factor nested in the Planted Species factor. Statistically significant results ($p < 0.05$) are in bold.

Table 3. (a, b) The mean height of clones of all experimental living plants on five experimental localities in July–August 2003 and (c) the mean number of flowers of all experimental plants on five experimental localities in 2003 season.

Experimental field locality	(a) Clone height (mm)			(b) Clone height (mm)			(c) Number of flowers		
	<i>M. caespitosa</i>	<i>M. palustris</i> subsp. <i>laxiflora</i>	<i>M. nemorosa</i>	Gap	Sparse vegetation	Dense vegetation	Gap	Sparse vegetation	Dense vegetation
C1	93.88	43.59	73.75	65.85	87.60	64.05	7.23	8.77	0.83
L1	162.14	267.32	223.96	246.30	272.24	133.96	20.83	21.80	3.77
L2	123.27	256.21	184.46	251.21	203.14	98.25	8.87	4.57	1.40
N1	55.72	84.68	79.57	54.83	81.71	82.43	2.93	2.20	2.43
N2	122.20	104.85	136.11	126.93	120.77	114.17	8.67	3.57	5.03

Note: Differences between experimental *Myosotis* species (*Myosotis caespitosa*; *Myosotis palustris* subsp. *laxiflora*; *Myosotis nemorosa*) and between three types of competitive microsites (gap; sparse vegetation; dense vegetation). Averaged over all the treatments (a) and over all the species (b, c). Dead plants are omitted. The interaction between locality and species (a) and locality and microsite (b, c) are highly significant ($p < 0.01$). Full results of hierarchical ANOVA in Supplementary Tables S5 and S6.

Myosotis palustris subsp. *laxiflora* seemed to be dependent on vegetative propagation; it demonstrated the highest amount of clonal growth. Its aboveground stolons are typically broken by flood water and severed parts could establish further downstream. *Myosotis nemorosa* clones are able to persist in dense meadow vegetation for years; however, they are spread mainly by short rhizomes. These two experiments (Koutecká and Lepš 2009, 2011) were done under controlled, but rather unnatural conditions. Consequently, we were interested in how the species differed in the natural conditions of their field localities.

As expected, the three *Myosotis* species differed in their survival and growth. The differences corresponded to species habitat preferences. Nevertheless, there were also pronounced differences between individual populations within a species. This clearly demonstrated that comparisons among (not only closely related) species cannot be based on a single population per species alone.

Myosotis caespitosa is a species of transient habitats, typically fishpond bottoms. It is the species with the shortest life span among the studied species, sometimes considered to be annual or short-lived perennial with no clonal potential (Grime et al. 1981; Klimešová and Klimeš 1998; Štěpánková 2000). Our experiment confirmed that mortality of *M. caespitosa* clones was the highest, especially in competitive treatments. Indeed, every individual shoot died after flowering.

On the other hand, during the first season *M. caespitosa* produced the highest number of secondary rosettes and stolons in gaps in comparison with the other two species; some individuals survived for several seasons, particularly in gaps. Still, in our experiment some plants of this species survived and even spread clonally from lateral rosettes in permanent habitats (species rich meadow and woody path) for at least three seasons, confirming the results of our pot experiment (Koutecká and Lepš 2011) in natural conditions. Interestingly, one clone of *M. caespitosa* survived after two seasons and flowered 30 cm under water.

Among our experimental species, *M. palustris* subsp. *laxiflora* is the species with the best developed clonal growth (again, concordantly with Koutecká and Lepš, 2011), forming two types of shoots (stolons and rhizomes). *Myosotis palustris* subsp. *laxiflora* is a long-lived perennial (Štěpánková 2000; Klotz et al. 2002). It occurs in marshlands and alluvia of rivers and streams, is able to grow even in waterlogged sites, and can form floating mats at the edge of water (Grime et al. 1987; Štěpánková 2000; Klotz et al. 2002; Lenssen et al. 2003). Clonal fragments of *M. palustris* subsp. *laxiflora* (even very small modules) are able to disperse downstream for long distances, and colonize and vegetatively expand on flooded banks without competing vegetation. It often colonizes open sites on banks where vegetation was damaged by torrential waters during floods (E. Koutecká, personal observation).

In our field experiment, *M. palustris* subsp. *laxiflora* survived the best in their original localities and its clones were tallest there during the first season. Clonal propagation of this species was most affected by the microsite; in gaps with reduced competition it spreads quite vigorously and fast.

Myosotis nemorosa, of the three *Myosotis* species, occurs in the widest range of habitats. These ranged from wet to dry and from young successional stages to relatively stable communities (Štěpánková 2000). *Myosotis nemorosa* species showed the greatest level of adaptability to different habitats. Data about life history and clonality of *M. nemorosa* differ among individual authors, from annual to biennial, with no or only low clonal potential (Klimešová and Klimeš 1998), to long-lived perennial hemicryptophytes that clonally propagate by short belowground adventitious shoots (Štěpánková 2000; Klotz et al. 2002; Chaloupecká and Lepš 2004).

In our experiment, the surviving plants of *M. nemorosa* produced a few shoots that spread underground only for short distances (about 5 cm) at the beginning of the season. The number of shoots remained relatively stable until the end of the season at all localities

and in all types of microhabitats. Once established, *M. nemorosa* was able to persist in various habitats.

Plant competition is one of the most important forces shaping growth and survival of plants (e.g., the intercontinental multisite *Poa pratensis* transplant study, Reader *et al.* 1994). However, the intensity of competition depends on the plant community and differs between localities.

The survival of experimental plants in our experiment was negatively correlated with biomass production at individual experimental localities (Supplementary Fig. S1 shows biomass production at four localities in the second season (June)). Nevertheless, the competition (or generally the effect of the community) was not homogenous and changed according to the microsites of individuals.

Although the effect of microsites (as the main effect) was rather weak in our experiment, we found strong interaction of microsite treatment with locality. The suitability of microsite types depended on the conditions at individual localities (such as height and type of vegetation, soil type, water regime). The gaps were not always in the most suitable places, particularly for survival.

As already noted (e.g., Kotorová and Lepš 1999), gaps are not just competition free spaces but also areas with pronounced environmental fluctuations. Here, gaps were much more prone to desiccation than were undisturbed areas. Also, individual species were differently affected by microsites as well as various phases of their life cycle. Both the survival of *M. caespitosa* and the clonal spread of *M. palustris* subsp. *laxiflora* were greatly enhanced in gaps in comparison with the other two microsites offering presumably higher competition.

On the contrary, *M. nemorosa* did not show such a pronounced microsite dependence; it was able to cope much better with competition in sparse and even dense vegetation. Additionally, the persistence of microsites, especially gaps, was also influenced by the density and dynamics of surrounding vegetation. Large differences between the effects of microsites on individual localities were found especially during the second season. Gaps were unsuitable microsites for transplant survival in the wet meadow (N1; the locality with stable biomass production, large variations in the water table during a season, and with no shading by surrounding trees). Here, the experimental plants were better protected from herbivores and desiccation outside the gaps.

Reciprocal transplant experiments have typically investigated the adaptation of local populations to a locality. This experiment was a more complicated situation. We were primarily interested in species adaptation to a locality, where the species was resident. Should a species identity have an ecological significance, then it should be adapted to the conditions where it is resident. There are also problems with field experiments with plants adapted to short-term habitats. It was difficult to find suitable populations and localities for long-term experiments. Two of the three species, *M. palustris* subsp. *laxiflora* and *M. caespitosa*, normally grow in short-term habitats. We were interested in an experiment lasting for three seasons, because survival over several seasons was an important factor for population persistence. Moreover, we needed strong source populations (because the seeds mature over a longer period, so we needed large populations to get enough seeds for the experiment), but these populations were often in sites not suitable for the establishment of long-term experiments. For this reason, the seed source and transplanting localities were not always the same. Particularly for *M. caespitosa*, we were able to find just one locality where we believed that it might last for several seasons. However, even this locality was destroyed before the end of the experiment.

The individuals of the resident species populations were not always superior in all the studied parameters. *Myosotis caespitosa* had the best germination in all the localities (our own unpublished data) and *M. nemorosa* had the highest survival rate in most of the localities. Nevertheless, for many of the characteristics

studied, contrast analysis showed that, on average, there was a "home-site advantage", i.e., they survived, germinated, and established more readily in their original localities than in other areas. The plants' final fitness is the result of several processes, including survival, generative reproduction, and clonal spreading. We cannot expect that a species will demonstrate a home-site advantage for each particular process.

Summarizing the results of this combined with our previous experiments (Koutecký and Lepš 2009, 2011), we conclude that a combination of traits determines a species' ecological behavior. *Myosotis nemorosa* persists in a site through long-term survival of individuals (when the flowering stem dies but the genet survives by clonal growth), together with regeneration by seeds. *Myosotis palustris* subsp. *laxiflora* quickly colonizes gaps by clonal growth, supported by long-term dispersal of clonal fragments by flood waters along river banks. *Myosotis caespitosa* produces highly germinable seeds that persist in the seed bank.

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