Population and metapopulation biology of perennial plants in fragmented grasslands

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Statement of contribution

The seven papers presented in this thesis are not work of only myself. The first paper is my joint work with Johan Ehrlén, the third paper profited from cooperation with Ivana Plačková, the fourth and sixth paper is a joint project with Tomáš Herben and the seventh paper is part of cooperation with Johan Ehrlén, Mikael Mildén and Tomáš Herben. All of these people contributed to the papers. I therefore ask the reader to replace the word I used throughout the introduction with WE wherever applicable. The word I was used here only for consistency. See the authorship statements enclosed at the back of the thesis for specific contributions.

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Introduction

Factors affecting survival of species in fragmented landscapes belong among the central issues in species ecology and conservation biology (van Groenendael et al. 1998). Traditional studies on this issue focus on studying single traits of species or dynamics of local populations (Morris and Doak 2002). With the recent development of metapopulation theory (Hanski 1989), it has been recognized that regional scale processes may also be important for survival of species in the landscape (e.g. Carroll et al. 2003, du Toit et al. 2004). In such cases studying the viability of local populations only is of little use.

Recently several theoretical models have been developed to assess the prospects for species survival at the landscape level (e.g. Gustafson and Gardner 1996, With et al. 1997, Hanski and Ovaskainen 2000, Casagrandi and Gatto 2002a, Dreschler et al. 2003). Most of the models assume that the current distribution of species in habitat fragments represents equilibrium between local colonization and extinction. Under this assumption, data on local population dynamics are not necessary and the pattern of distribution of the species in the landscape alone can be used to estimate the prospects for landscape level survival of the species (Hanski and Ovaskainen 2000).

The critical point in this reasoning is the assumption that colonization/extinction dynamics operate fast enough to keep the current distribution close to equilibrium and that local population dynamics is thus unimportant. Whereas this assumption is likely to be correct in short-lived highly dispersible organisms such as many insects (Hanski et al. 1994, Baquette 2003, Purse et al. 2003), it is less likely to apply in sessile organisms such as plants that are known to have very slow local population dynamics with low extinction and immigration probabilities (Eriksson 1996, Freckleton and Watkinson 2002). Therefore models based on the assumption that species are in equilibrium and that local population dynamics are thus unimportant are of little use in plants. Unfortunately, plants (precisely due to their limited migration capacities) are directly threatened by habitat destruction and predictions of the effects of fragmentation are needed (Eriksson and Kiviniemi 1999). Applying metapopulation theory to plants thus essentially means taking into account processes operating on the local scale and integrating them with processes occurring at the regional scale.

At the local scale species dynamics are determined by the behaviors of individuals. Individuals are however not independent and are influenced by their interaction with other conspecific individuals as well as with other species and the environment. Describing this thus essentially includes studying a range of processes from density dependence in seedling germination to patterns of pollination within the community. Already at this scale population dynamics are affected by landscape changes. The factors include both changes in habitat quality as well as the changes in population size. Habitat quality may affect performance of single individuals as well as their interaction (e.g. Eisto et al. 2000; Vergeer et al. 2003). Population size has important consequences mainly for patterns of mating via attraction of pollinators and by providing a source of pollen, leading to reduced genetic diversity, and consequently to lower fitness (e.g. Mustajarvi et al. 2001; Lienert et al. 2002; Paschke et al. 2002). These direct negative effects are further enhanced by genetic drift and population stochasticity, which play an important role in dynamics of small populations (Ellstrand & Elam 1993; Young et al. 1996).

At the regional scale species dynamics are affected by all the local scale processes as well as by a range of other processes related to both attributes of the species, such as their dispersal ability, and attributes of the landscape, such as the spatial arrangement of suitable habitats and their size. Studies at this scale thus necessarily include studying factors determining habitat suitability for the species, the dispersal ability of the species in the landscape and combine them with information on local population dynamics.

In this thesis I present a series of papers aimed at understanding the population biology and survival potential of perennial plants in fragmented grasslands. In the **first paper** I explore methodological issues related to demography of local populations. Studies of local population dynamics usually rely on following the performance of single individuals over multiple years and use it to predict the performance of the whole population. This is done by means of matrix population models, which allow combining information from many single individuals to predict their overall behavior. In recent years matrix population models have become a standard method to assess the viability of structured populations (Morris and Doak 2002). Repeated iterations of a matrix result in a projection of a population's equilibrium growth rate that provides a measure of the overall performance of populations. Moreover, sensitivity or elasticity analysis of matrix models can identify the life history stages most critical for the persistence of a species. There is a large literature concerned with the techniques to analyze collected data to make projections or predictions (e.g. Horvitz et al. 1997, Ehrlén and van Groenendael 1998, Ludwig 1999, Mills et al. 1999, de Kroon et al. 2000, Caswell 2001, Ehrlén et al. 2001, Calder et al. 2003). In contrast, very little attention has been paid to how the primary data are actually sampled. This is surprising because the quality of the resulting matrices is primarily a function of how the data were collected and no post-collection manipulations can overcome this.

Almost all recent plant demographic studies have sampled all individuals within plots (Münzbergová, unpubl. data). The most likely explanation for this is that sampling within plots is easy to apply in the field and also provides information on the actual stage distribution. However, for any given sampling effort this strategy is unlikely to provide the most accurate estimates of demographic parameters, since it often results in a very unequal number of individuals per stage. In the literature it is, in fact, not rare to encounter transition probabilities that are estimated using only one or a few individuals (Münzbergová, unpubl. data). Any sampling strategy that is able to decrease the problems with strongly unbalanced sample sizes at the stage of data collection has therefore a much greater potential to increase the accuracy of estimates than post-collection approaches. Gross (2002) suggested that this problem may be partly overcome by using previous knowledge of the relative importance of different life cycle transitions to sample individuals more efficiently. He also gives two examples showing that an alternative method, efficient sample composition (ESC), can considerably increase the precision of population growth rate estimates. However to apply the ESC-method, we need to make an educated guess of the relative importance of different transitions in the life cycle for population growth. This educated guess can be made using data from studies of the same or a related organism, the investigator's prior knowledge, pilot data or comparative demographic studies (Gross 2002), but we need to know how sensitive the sampling efficiency is to the appropriateness of the guess. Hence a method that is both simple to apply and does not require any previous knowledge of the demography, but that can provide significantly more precise and accurate measures than conventional methods would be of a large potential value.

In the first paper I explore this issue. Specifically I first examine how sensitive the ESC-method is to the quality of our previous knowledge of the species demography. Secondly, I investigated if a relatively simple approach, sampling an equal number of individuals per stage, can provide better estimates than plot-based sampling and if these estimates are as good as those based on the ESC-strategy.

To estimate the effectiveness of three different sampling strategies, the traditional plot based sampling, the ESC sampling and sampling equal number of individuals, on the accuracy and precision of properties of the resulting matrix, I used data for 32 different plant species collected from the literature.

The results show that the ESC method is sensitive to the reference matrix used to estimate the sampling proportions. In contrast, sampling equal numbers of individuals per stage provide estimates that were relatively robust and overall more accurate than both the

ESC-method and the conventional plot-based method. I therefore conclude that collecting demographic data from an equal number of individuals per stage may constitute a simple and accurate method that is likely to improve the quality of demographic studies in many cases.

In the **second paper** I move on to studying local population dynamics. Specifically I compare population dynamics of two congeneric species that inhabit dry grasslands and differ in rarity, and I try to identify the key differences between them and thus to determine what factors are important in determining species rarity and commonness. Factors responsible for the commonness and rarity of closely related species have been a topic of many previous studies. However these studies suffer from two important drawbacks. (i) Most of the studies compare species that are closely related phylogeneticaly but occupy different habitats, so any observed difference may be due to habitat differences. (ii) Most studies also concentrate only on single life history traits, with unknown relevance for the population growth rates of the species, though knowledge of the growth rates is necessary to demonstrate that the factor is really responsible species rarity.

In the second paper I compare the complete demography of two *Cirsium* species sharing the same habitat, one of them being very rare and the other very common. Because seed herbivory is very common in this genus, I hypothesize that it may be one important factor in the rarity of the rare species.

The results show that population growth rate is slightly lower in the rare species, and this translates into a large difference in the local extinction probability. Seed predation does not differ between the species. However I demonstrate that in connection with the data on complete demography seed predation is the key factor causing the lower population growth rate in the rare species.

These results are the first estimation of factors responsible for commonness or rarity of plants in terms of population growth rate without confounding differences in habitat. It is also the first demonstration of differential effects of seed herbivory on the life cycles of two congeneric species.

Local population dynamics of species are also a topic of the **third paper**. Here I explore the effect of population size and genetic diversity on the performance of a plant species. Decreases in population size and genetic diversity are important concerns for conservation in fragmented landscapes. Therefore many studies have recently studied and demonstrated negative effect of low population size and genetic diversity on single plant traits. However nothing is known on the effects of these differences on the long-term probability of species survival.

In the third paper I study the effect of population size and genetic diversity on population growth rate in a rare perennial herb occurring in fragmented grasslands. Its performance was measured using several seed traits. The data were then connected with data on the mean demography of the species. Three different matrix models differing in the number of transitions based on measurements in a range of populations differing in size were used to explore the relationship between population size, genetic diversity and population growth rate.

The results show that genetic diversity itself is only weakly related to plant performance. It is however positively related to population size and population size is positively related to most of the seed traits. All the three matrix models showed that despite the decline in seed production in small populations, the population growth rate is always positive. The model using only data on seed production per flower head from each population in the matrix model, and the model using these data plus data on number of flower heads per plant and field germination rates indicated a positive relationship between population size and population growth rate. Contrary to this, according to model using realistic data on number of seeds per flower head and number of flower heads per plant in the matrix model no such relationship exists.

I conclude that declines in some plant traits in small populations do not necessarily lead to the species‘ direct endangerment. I thus suggest that wider use of matrix population models in this type of studies may provide new insight into the real effect of population size on species demography. The results also demonstrate that conclusions about the effect of habitat fragmentation on demography are crucially dependent on the parameters used to estimate plant performance and use of different parameters may lead to different conclusions.

Moving from the local scale to the regional scale incorporates questions about the importance of different local and regional processes for species distribution. This essentially means asking questions about what factors affect species distribution at different spatial scales. Answers to these questions enable determination of whether the distribution of a species is limited by availability of seeds and sites. Recently there have been an increasing number of studies concerned with the effect of various types of limitations on species' local population sizes and distribution patterns at the landscape scale. The terminology used to describe these limitations is however inconsistent. Since the terms are often used as a part of conclusions of papers, the inconsistency in their use obscures the message of these papers. In the **fourth paper** I thus review the current uses of these terms, identify the basic concepts involved in the discussion of a *limitation* and link the concepts with the term. Finally I discuss the experimental approaches that are used to assess these limitations.

I differentiated four basic concepts resulting from combinations of limitation by environment versus ability to grow and spread, and two spatial scales (local and regional). The two concepts at each spatial scale are expected to form a gradient of all possible combinations of the two respective types of limitations. In the considerations of various experimental approaches used to assess these limitations, I conclude that sowing experiments, meaning seed addition into existing population or seed introduction into unoccupied habitats, are the only reliable types of evidence for these limitations.

In the **fifth paper** I explicitly explore the above-mentioned limitations for species distribution. In concordance with paper four I start with the notion that distribution of species in fragmented landscapes is a result of combined seed and site availability at different spatial scales. At the local scale species may be limited either by seed numbers and germination ability (seed limitation) or by the availability of microsites suitable for germination (microsite limitation). At the regional scale species may be limited by ability to reach the site (dispersal limitation) or by availability of suitable habitat (habitat limitation).

While a lot is known on importance of these limitations for species distribution on one spatial scale, it is not known how their importance differs among different spatial scales. Additionally, while there is a lot of information on the effect of environmental factors on both pattern of seedling establishment and distribution of adult plants, the correspondence between these patterns is unclear, as is the effect of spatial scale on this relationship.

In this study I sowed seeds of seven species of dry grasslands into twenty-two localities that differed in occupancy by these species. I followed seedling establishment of these species over three years. The number of resulting seedlings was then compared at two regional scales, between occupied and unoccupied localities and occupied and unoccupied blocks within occupied localities, and at a local scale, between plots with and without seed addition within occupied blocks. Furthermore, I examined relationships among environmental factors and the number of seedlings and distributions of adult plants at the two regional scales.

The results show that both seed and microsite availability are important in structuring the distribution of these plant species. Their relative importance however depends on the spatial scale considered. Also the relationship between environmental factors and patterns of seedling recruitment and adult occurrence is clearly scale dependent.

Paper five shows that many species are dispersal limited and thus that there are suitable habitats that are currently unoccupied. This expectation is a prerequisite of studies of metapopulation dynamics of species. Using approaches such as that in paper five is the most direct approach to identify suitable unoccupied habitats. To get a good estimate of suitability using this method one should follow the whole life cycle of the species as population bottlenecks may occur at later stages of recruitment (Losos 1995, Gustafsson et al. 2002). This can, however, take much longer than any research project can last (Ehrlén and Eriksson 2000). Therefore, alternative indirect ways to estimate habitat suitability are sought (e.g. Husband and Barrett 1996).

It the **sixth paper** I therefore propose a new quantitative technique to identify suitable but unoccupied habitats for metapopulation studies in plants. It is based on species composition in a habitat and knowledge of species co-occurrence patterns. It uses data from a large phytosociological database as a background for estimating species co-occurrence patterns. If such a database is not available, the technique can still be applied using the data for which the prediction is done simultaneously to estimate the species co-occurrence pattern. Using the technique I was able to indicate suitable unoccupied habitats and differentiate them from the unoccupied unsuitable ones. I also identified habitats with low probability of being suitable that were occupied. Compared to a direct approach of identification of suitable habitats, which involves introduction of a species to the habitat and studying its performance, the approach presented here is much easier to apply and can provide extensive information on habitat suitability for a range of species with much less effort and time.

In the **seventh paper** I integrate the knowledge about local population demography of species (papers 2 and 3) with information on availability of suitable unoccupied habitats (papers 5 and 6) and with information on species dispersal ability and spatial structure of habitats in the landscape in a framework of a spatially explicit metapopulation model. I use this model to estimate the effect of habitat fragmentation on survival probability on a species occurring in fragmented grasslands. The motivation of this paper is that common approaches to estimating the effect of habitat destruction on species survival probability, such as metapopulation capacity, are based on the assumption that the current distribution of species in habitat fragments is a result of equilibrium between local colonization and extinction. This may be a reasonable assumption for short lived, highly dispersible organisms, but is unrealistic in sessile long-lived species such as most plants. Here I use an alternative approach, a realistic dynamic landscape-level model that does not use this assumption. It enables estimation of effect of habitat destruction using realistic field data on biology of a species and on landscape structure. Since the approach relies on direct comparison of changes in population size and survival probability due to habitat changes, it can be easily extended to other conservation questions, such as the effect of local population destruction or searches for optimal reintroduction strategies.

I apply this method to a perennial herb *Succisa pratensis* that is a typical representative of fragmented low-production grasslands. The results show that habitat destruction alone has only little effect on its prospects for survival. The effect however increases when combined with population destruction, which is expected to play a significant role in the study system. Using the same approach I was also able to design optimal reintroduction strategy for the species, assuming the species was extinct from the landscape. Given the biology of this species, I argue that only a dynamical model with local events as the one presented here makes it fully possible to evaluate its survival perspectives in fragmented landscapes and to design the most appropriate reintroduction strategies.

I.

Simpler is better – data collection for demographic studies

Zuzana Münzbergová and Johan Ehrlén

Abstract

Matrix population models have become important tools in many fields of ecology and conservation biology. There is a large literature concerned with different aspects of matrix model analysis, but relatively little attention has been paid to how data are collected. In most demographic studies data are sampled in permanent plots, resulting in poor representation of some stages. In a recent paper Gross (2002) suggested a method to sample demographic data more efficiently. The idea is that with a previous knowledge of species demography it is possible to assess the most efficient sample composition (ESC). By using demographic data from 32 species we examined how sensitive the ESC-method is to the quality of the previous demographic information. We also investigated if a relatively simple approach, sampling equal number of individuals per stage, can provide better estimates than the plot based method and ESC-method.

Our examples show that the ESC method is sensitive to the reference matrix used to estimate the sampling proportions. In contrast, sampling equal number of individuals per stage provided estimates that were relatively robust and overall more accurate than both the ESC-method and the conventional plot-based method. Collecting demographic data from an equal number of individuals per stage may thus constitute a simple and accurate method that is likely to improve the quality of demographic studies in many cases.

Keywords: matrix population model, sampling, population growth rate, demography

Introduction

Matrix population models have become a standard method to assess the viability of structured populations (Morris and Doak 2002). Repeated iterations of a matrix result in a projection of a population's equilibrium growth rate that provides a measure of the overall performance of populations. Moreover, sensitivity or elasticity analysis of matrix models can identify the life history stages most critical for the persistence of a species. There is a large literature concerned with the techniques to analyze collected data to make projections or predictions (e.g. Horvitz et al. 1997, Ehrlén and van Groenendael 1998, Ludwig 1999, Mills et al. 1999, de Kroon et al. 2000, Caswell 2001, Ehrlén et al. 2001, Calder et al. 2003). In contrast, very little attention has been paid to how the primary data are actually sampled.

Almost all recent plant demographic studies have sampled all individuals within plots (Münzbergová, unpubl. data). The most likely explanation for this is that sampling within plots is easy to apply in the field and also provides information of the actual stage distribution. However, for any given sampling effort this strategy is unlikely to provide the most accurate estimates of demographic parameters, since it often results in a very unequal number of individuals per stage. In the literature it is, in fact, not rare to encounter transition probabilities that are estimated using only one or a few individuals (Münzbergová, unpubl. data). Sampling individuals within plots may be disregarded also because of the problems with spatial autocorrelation. However, even other ways to sample individuals within populations, e.g. along transects, face similar problems. Any sampling strategy that is able to decrease the problems with strongly unbalanced sample sizes already at the stage of data collection has therefore a much larger potential to increase the accuracy of estimates than post-collection approaches. Gross (2002) suggested that this problem may be partly overcome by using previous knowledge of the relative importance of different life cycle transitions and sample individuals more efficiently. He also gives two examples showing that an alternative method, efficient sample composition (ESC), can considerably increase the precision of population growth rate estimates. However to apply the ESC-method, we need to make an educated guess of the relative importance of different transitions in the life cycle for population growth. This educated guess can be made using data from studies of the same or a related organism, the investigator's prior knowledge, pilot data or comparative demographic studies (Gross 2002), but we need to know how sensitive the sampling efficiency is to the appropriateness of the guess. Hence a method that is both simple to apply and does not require any previous knowledge of the demography, but that can provide significantly more precise and accurate measures than conventional methods could be of a large potential value.

In this study we first examined how sensitive the ESC-method is to the quality of our previous knowledge of the species demography. Secondly, we investigated if a relatively simple approach, sampling equal number of individuals per stage, can provide better estimates than plot-based sampling and if these estimates are as good as those based on the ESC-strategy.

Methods

To estimate the effect of different sampling strategies on the accuracy and precision of properties of the resulting matrix, we used data for 32 different plant species collected from the literature (see Appendix). We selected species for which at least 3 different matrices were available. We arbitrarily selected one of the matrices (the last one in each paper) as the target matrix and kept the others as reference matrices. We used data from the reference matrices, i.e. from the same species but from other years or other populations, to make an *educated* *guess* (sensu Gross 2002). This is most likely to provide a conservative estimate of the sensitivity, as guesses in practice often would have to be based on other species (cf. Gross 2002).

Three strategies were used to determine the sampling proportions. First, estimating ESC's from the reference matrices, following Gross (2002). One sampling proportion was calculated for each reference matrix. Second, sampling an equal number of individuals per stage. Third, the conventional plot-based strategy was investigated by estimating proportions from the projected stable stage distribution for the target matrix. We used these proportions as estimates of the actual stage distribution in the field, since the latter was not available for all species. Sampling an equal number of individuals per stage and plot-based sampling thus always had one sampling proportion per strategy and species, whereas the ESC-strategy resulted in as many sampling proportions per species as there were reference matrices (N = 2 – 27).

To estimate the quality of the matrices resulting from the different strategies we simulated 2000 hypothetical data sets in which individuals of each stage had average transition probabilities equal to those in the target matrix (Gross 2002). The simulations were done by sampling 25 individuals times the dimension of the matrix (number of stages). These individuals were distributed according to the respective sampling proportions derived by the three strategies. Calculations of ESC or projections of stable stage distributions occasionally resulted in a very low (< 5) number of sampled individuals for a stage. In these cases, the sampling distribution would be strongly skewed. Therefore we applied an arbitrary correction of this and sampled 5 individuals in that stage, and reduced the number of individuals in the other stages proportionally to maintain the total number of individuals sampled.

Separate transition matrices for each simulation run were created based on the fates of the sampled individuals. Transitions involving reproduction in our matrices were estimated from separate experiments and therefore kept constant at the values of the target matrix over the simulation runs.

The precision of these new matrices was estimated as variation in lambda as well as mean variation of elasticity values. Not only the precision but also the accuracy of population growth rate and elasticity values are a main concern. We therefore also examined how the accuracy of matrix properties were influenced by the different sampling strategies, by estimating the mean deviation of simulated values from values based on the target matrix.

To compare the accuracy and precision between different strategies we selected the median ESC-value, as well as the lowest and highest, for deviations and variance in population growth rate and elasticities. The lowest values are referred to as ESC-best, the median as ESC-median and the highest as ESC-poorest. The ESC-best provides an estimate of performance of ESC sampling if the reference matrix is very similar to the target matrix. The ESC- poorest is an estimate of the worst result using available data from the same species. The three ESC-values, -median, -best and -poorest, were then compared to the equal number strategy and the plot-based strategy.

To assess the significance of pair-wise differences between the three measures of the ESC-strategy, the equal number strategy and the plot-based strategy we used Tukey multiple comparison test. All the simulations were done using Matlab, version 5.3.1 (Anonymous 1999). Statistical testing was carried out with SPSS version 11.0 (Anonymous 2002).

Results

The differences between different sampling strategies were similar for variances in projected population growth rate and elasticities, as well as their respective differences from the actual values of the target matrices (Fig. 1). The ESC-poorest estimations were significantly worse than any other results, whereas the ESC-best estimations gave very precise results, often being the best of all possible strategies. Sampling an equal number of individuals per stage performed on average better than ESC-median in all parameters. It was as good or better than ESC-median for 59 percent of species in variation in lambda, for 66 percent of species in differences of lambda, 78 percent of species in variation in elasticity and 69 percent in differences of elasticity. Equal number strategy was not significantly worse than ESC-best in any comparison, but it was significantly better than ESC-poorest for variation in population growth rate and for accuracy of population growth rates and elasticities. Plot-based sampling performed slightly worse than equal number sampling and was comparable to ESC-median (Figure 1). It was worse that equal number and ESC-median strategy in 57 and 50 percent of species in variation in lambda, in 67 and 53 percent of species in differences of lambda, 63 and 43 percent of species in variation in elasticity and 70 and 40 percent in differences of elasticity respectively. However, it did significantly better than ESC-poorest for accuracy of population growth rate and elasticities.

Discussion

Our simulations showed that sampling according to the ESC-method is very sensitive to the quality of the previous knowledge. The median performance of the ESC-method was only slightly better than plot-based sampling but ESC-poorest was worse for all comparisons. This shows that the ESC-approach is potentially useful if we have a very good previous knowledge. However, as long as there is no certain way to assess how appropriate the reference matrix is, there is a high risk that the ESC-approach yields estimates that deviates more from the true value than estimates based on sampling all individuals within plots. Moreover, in our study we used matrices from the same species as educated guesses. In many cases such information will not be available and we instead have to use information from related species, or from comparative demographic studies (Gross 2002). In practice the performance of the ESC approach may be even worse than suggested by our results. Lastly, our study used 5 individuals per stage even for distributions yielded by the ESC and plot-based strategies that suggested sampling of a smaller number. Allowing for fewer than 5 individuals would have decreased the precision and accuracy of these methods further (unpubl.). This correction was done in order to mimic behavior of a person not blindly relying on the formalized estimation of sampling proportions.

Equal proportion sampling was on average better than plot-based sampling and the ESC-median for all estimates of performance, although it was slightly worse than the best-performing ESC-distribution. Hence, the equal number sampling strategy is not necessarily the best strategy in every case, but it performs on average better than both plot-based and ESC sampling strategies. Moreover, it was a safer strategy in the sense that it never produced sampling distributions that varied or deviated as much as the ESC-strategy. Lastly, sampling an equal number of individuals is much simpler and more straightforward than the ESC strategy as it does not depend on any prior knowledge of population processes, does not make any assumptions and is easy to apply in most situations.

Total number of individuals sampled in our simulations (twenty-five individuals per stage on average) is slightly higher than median number of individuals per stage sampled in demography studies (17, N = 45 published demographic studies 1977 – 2001, Münzbergová, unpubl. data). This shows that our conclusions are done using realistic numbers. Changing the total number of sampled individuals however does not qualitatively affect the conclusions (results not shown).

In conclusion, sampling an equal number of individuals in demography studies appears to be both a simple and accurate way to collect data for demographic studies. Applying this method, instead of using the common practice to sample all individuals within plots, is likely to considerably improve the quality of demographic information and the predictions of population viability models for most species.

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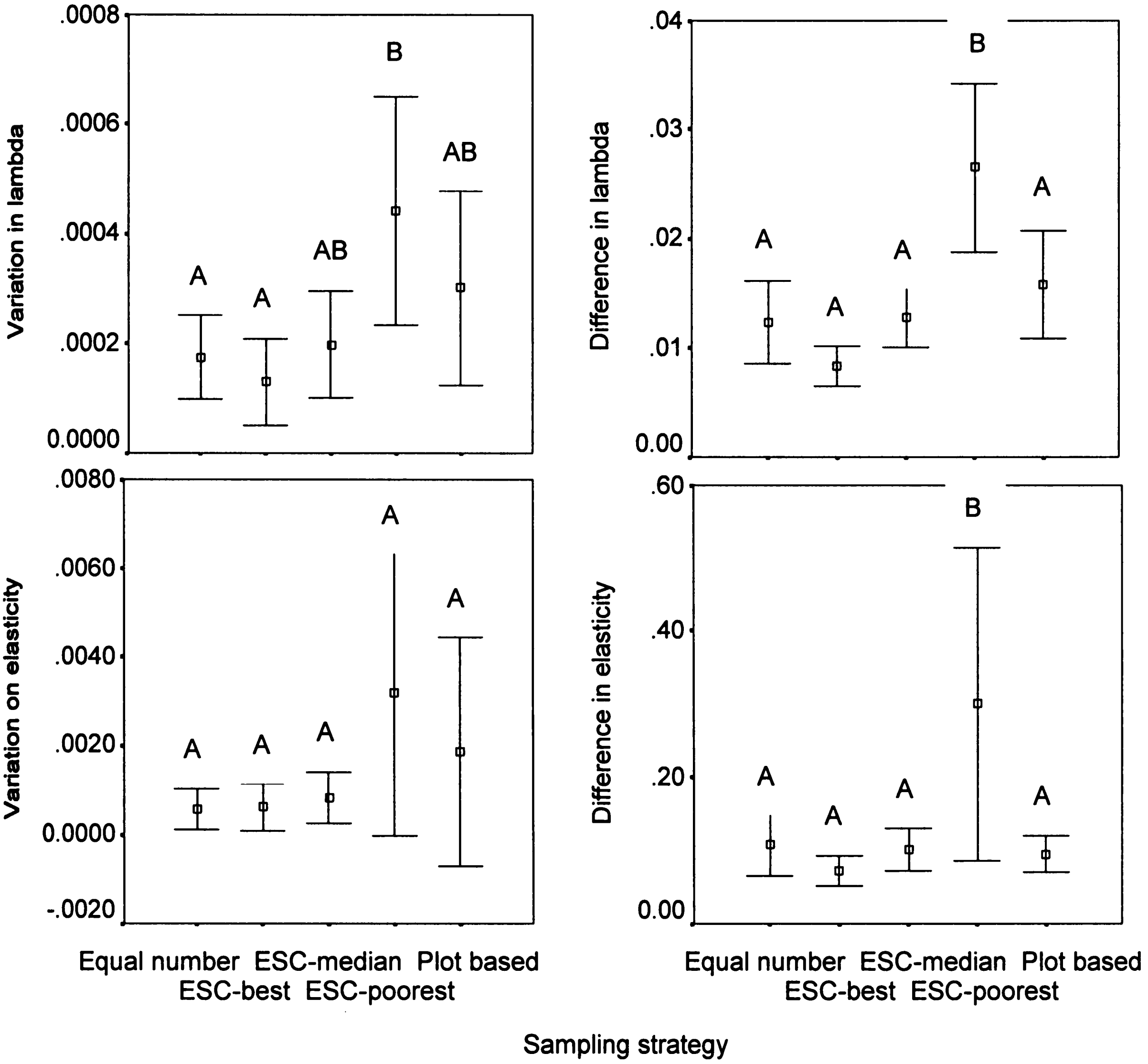
Figure 1. The effect of different sampling strategies on the precision and accuracy of population growth rate and elasticity values of the resulting matrix for 32 plant species. Precision is expressed as the coefficient of variation, and accuracy as the mean proportional difference between simulated and actual values based on the target matrix. The comparison is done for both lambda (population growth rate) and elasticity. Different letters denote pair-wise differences between groups that are significant at P = 0.05, using Tukey multiple comparison test. Bars represent mean and 95% confidence interval of the mean.



Appendix. Matrices used in the study. No. of matrices shows how many matrices of that species were used in this study. Number of excluded matrices provides number of matrices in the original paper that could not be used because they were missing some stages or have transition probabilities in some stages equal to 1, and are therefore unreliable.

Species	Source	No. of matrices	Note
Agrimonia eupatorium	Kiveniemi (2002)	11	
Allium tricoccum	Nault and Gagnon (1993)	4	
Alnus incana	Huenneke and Marks (1987)	6	
Arabis fecunda	Lesica and Shelly (1995,1996)	24	
Arisaema triphyllum	Bierzychudek (1982)	4	
Asarum canadense	Damman and Cain (1998)	20	ramet level
Asarum canadense	Damman and Cain (1998)	12	clone level
Astragalus cremnophylax	Machinski et al. (1996)	6	
Astragalus scaphoides	Lesica (1995)	10	
Calathea ovandensis	Horovitz and Schemske (1995)	16	
Calochortus albus & obispoensis	Fielder (1987)	4	4 stages
Calochortus pulchellus & tiburonensis	Fielder (1987)	4	3 stages
Carlina vulgaris	Löfgren et al. (2000)	8	
Cecropia obtusifolia	Alvarez-Buylla (1994)	5	
Danthonia sericea	Moloney (1988)	9	1 excl.
Daucus carota	Verkaar and Schenkeveld (1984)	3	
Epilobium latifolium	Doak (1992)	13	1 excl.
Fumana procumbens	Bengtsson (1993)	6	
Geum rivale	Kiveniemi (2002)	3	1 excl.
Lathyrus vernus	Ehrlén (1995)	28	2 excl.
Miconia albicans	Hoffmann (1999)	3	
Myrsine guianensis	Hoffmann (1999)	3	
Panax quinquefolium	Nantel et al. (1996)	12	
Panax quinquefolium	Charron and Gagnon (1991)	6	
Plantago media	Eriksson and Eriksson (2000)	8	
Potentilla anserina	Eriksson (1988)	3	
Primula veris	Ehrlén (unpubl.)	7	
Primula vulgaris	Valverde and Silvertown (1998)	14	
Roupala ontana	Hoffmann (1999)	3	
Rourea induta	Hoffmann (1999)	3	
Saxifraga cotyledon	Dinnetz and Nilsson (2000)	8	
Scabiosa columbaria	Verkaar and Schenkeveld (1984)	3	

II.

Population growth rates of rare and common *Cirsium* species sharing the same habitat

Zuzana Münzbergová

Abstract

Many studies have attempted to identify factors responsible for the commonness and rarity of closely related species. However these studies suffer from two important drawbacks. (i) Most of the studies compare species that are closely related phylogeneticaly but occupy different habitats, so any observed difference may be due to differences in the species' ecology. (ii) Most studies also concentrate only on single life history traits, with unknown relevance for the population growth rates of the species, though this knowledge is necessary to demonstrate that the factor is really responsible species rarity.

Here, I compare the complete demography of two *Cirsium* species sharing the same habitat, one of them being very rare and the other very common. Since seed herbivory is very common in this genus, I hypothesize that it may be one important factor in the rarity in the rare species.

The results show that population growth rate is slightly lower in the rare species, and this translates into a large difference in the local extinction probability. Seed predation does not differ between the species. However it can be demonstrated that in connection with the data on complete demography seed predation is the key factor causing the lower population growth rate in the rare species.

These results are the first estimation of factors responsible for commonness or rarity of plants in terms of population growth rate without confounding differences in ecology. It is also the first demonstration of differential effects of seed herbivory on the life cycles of two congeneric species.

Keywords: population growth rate, rarity, seed predation, population biology, seed size, congeneric species, dry grasslands, *Cirsium acaule*

Introduction

Processes determining the commonness or rarity of species are one of the key issues in ecology (Bevill & Louda 1999). It is of special interest also for conservation biology, as knowledge of these processes can provide the understanding necessary for effective conservation of rare plant species. Given the same environmental requirements and human influence, species rarity can basically be influenced by factors related to species demography, colonization ability and ability to adapt to changing environments.

As in other fields of population biology, essential insights on rarity can be gained by comparing closely related species. Comparisons of pairs of species that differ in their rarity can identify ecological and genetic traits that are associated with rarity. While this kind of comparison seems to be an attractive way to approach the processes underlying patterns of rarity, information gained from comparative studies of common-rare species pairs until now is surprisingly scattered and inconsistent and defies generalization (Bevill & Louda 1999). This is due to two main drawbacks in the existing studies.

First, many of these studies are concerned with species life history traits, e.g. differences in reproductive attributes and vegetative attributes, competitive ability or habitat preferences (e.g. Rabinowitz & Rapp 1981, Fiedler 1987, Karron 1989, Byers & Meagher 1997, Menges et al. 1999). Such studies are usually able to identify some differences between the two species; however, as only one or few aspects of the life cycle are usually addressed, the observed difference can hardly be interpreted as the one responsible for the rare versus common difference (but see Lloyd et al. 2002).

Second, most of the existing studies on this topic compare species that are closely related phylogenetically, but that occupy different habitats. In such case the observed patterns can easily be due to differences in species ecology and cannot be safely used to explain the common versus rare difference (but see Brown et al. 2003, Moora et al. 2003, Simon and Hay 2003).

In this paper, I compare the complete demography of a pair of closely related rare and common *Cirsium* species sharing the same habitat viz. chalk grasslands in northern Bohemia, Czech Republic. Using this approach I answer the following questions: (i) What are the differences in population biology between the rare and common species? (ii) Are these differences able to explain the differences in rarity of these species?

Genus *Cirsium* is common model in studies of herbivory, and herbivory is commonly documented to have strong impact on plant performance (see reviews in Crawley 1989, 1997, Louda 1989, Gange 1990, Marquis 1992). It may be therefore hypothesized that herbivory is the key factor responsible for the rarity of the rare species in this study. While many studies have evaluated the effects of herbivory on single plant traits, very little is known about its effect on the whole life cycle of the species (Ehrlén 1995, Maron et al. 2002). Additionally, no comparison has yet been done to estimate the life cycle effects of herbivory in closely related species sharing the same habitat but differing in rarity. Only such analysis, in comparison with analysis of other traits, can unequivocally determine whether herbivory is really the key factor responsible for species rarity.

To explore this issue I addressed the following questions: (iii) What is the effect of seed herbivory on the performance of the two species? (iv) Can herbivory be used as an explanation for the difference in rarity between the two species?

To answer these questions, I studied two *Cirsium* species, common *C. acaule* and rare *C. pannonicum*. I collected data on the complete demography of each species in three populations over two transition periods. Further, I collected data on the level of seed predation in at least ten populations of each species over two years, as well as data on several other traits possibly responsible for the common versus rare difference (ability to survive in the seed bank, seed germination rate and ability to self fertilize). The differences in single traits between the species were compared using ANOVA. Further, I used matrix population models to explore the differences in population growth rate between the two species and how it is affected by herbivory.

Methods

Studied species and study region

Cirsium pannonicum (L. fil.) Link is a perennial herb up to 80 cm high with leaves entire or denticulate with rigid spinules 1-5 mm long. The distribution range covers eastern central and northeast Europe, extending to northern Italy and northwards to 55°N in Russia (Tutin et al. 1964-1983). In the Czech Republic, the main distribution is in the eastern and central part of the country, with the western border of the distribution in the western part of the country (Dostál 1989).

Cirsium acaule Scop. subsp. *acaule* is a perennial acaulescent herb with leaves having 2-5 spinose-dentate lobes. The distribution ranges from north England and Estonia southwards to southern Spain and central Yugoslavia (Tutin et al. 1964-1983). In the Czech Republic the main distribution is in the western and central part of the country, with a few isolated localities in the eastern part, extending to Central Slovakia (Dostál 1989).

Both species are restricted to chalk grasslands in the study region, i.e. in the České středohoří Mountains in the northern Bohemia, Czech Republic. For both species this region is the area with one of the highest concentrations of localities within the Czech Republic. Both of the species are attacked by a suite of inflorescence feeding insect herbivores, mainly *Rhinocyllus conicus* Frölich and *Larinus planus* (F.). All these inflorescence feeding insects consume flower heads and developing flowers and seeds prior to or after full seed development.

Survival and transition into other stages

To describe the whole life cycle of the species, I marked 150 individuals, with at least 30 individuals in each stage (seedling, vegetative, reproductive) at three localities in 2001. The stage division was done arbitrarily, based on subjective classification in the field. The idea of this was to assure that a more or less equal number of individuals in each stage is marked, which is the most accurate method of demography data collection (Münzbergová and Ehrlén submited). The individuals were marked in three permanent plots per locality, taking all individuals in the plot initially. If there were already twenty marked individuals of one stage per plot, further individuals of this stage were skipped to make sure that each stage was represented by at least ten individuals per plot. Each individual was marked with plastic and metal labels that were dug just behind it. A census was carried out once a year in June from 2001 to 2003. At that time survival of each individual and its size were recorded. If there were fewer than thirty living marked individuals per stage at the time of the census, new individuals were marked to maintain the minimum number of individuals per stage. At each census the number of leaves, the length of the longest leaf, and the number of inflorescences were measured.

Seed production and germination

At the time of fruiting, 20 flowering ramets were randomly selected at each locality and number of fruits per ramet and number of seeds per fruit were estimated. Afterwards fifteen 20 cm × 20 cm plots were marked on a transect at each locality and 100 seeds were sown in each plot. Seedling germination was recorded in each plot, as well as in a control plot adjacent to each sowing plot, in the following June.

Survival in the seed bank

To estimate species ability to survive in seed bank 50 nylon bags each containing 100 seeds were buried at one of the three demography localities in late September 2001. Ten bags were buried at each of five different places at the locality. Ten bags (2 from each place) were excavated in late September in 2002 and 2003 and the seeds were checked for viability.

Viability of seeds was estimated by putting all seeds from one bag into a petri dish. The seeds were regularly watered with distilled water and kept under a fluctuating regime (12 hours light at 20 °C, 12 hours dark at 10 °C) in a growth chamber. The germinating seeds were regularly removed. The dishes were kept until all the seeds germinated or decayed (approximately 6 months). The same procedure was used to estimate the viability of fresh seeds in the first year. This value was then used as a starting value to estimate the decline in seed germination rate over time. This procedure provided information on potential survival in the seed bank given that the seed did not germinate in the previous year. High survival in the seed bank thus does not imply that not all seeds germinate the first year.

To get information on real delay of germination, I monitored seedling germination in the seedling recruitment plots also in the subsequent years. It provided information on delayed germination for seeds sown in 2000 in 2002 and 2003 – 1 or 2 year delay and for seeds sown in 2001 in 2003 – 1 year delay.

Stage division

In *C. pannonicum*, plants with leaves shorter than 3 cm or with less than 3 leaves never flowered in the subsequent year. Therefore this size was used as an upper limit for seedling definition. There was very high variation in leaf length of larger individuals both between sites and years, and it was clearly strongly affected by surrounding vegetation height. On the other hand, there was very low variation in number of leaves in the rosettes of these larger individuals. Therefore none of these parameters could be used to define any reasonable size classes, and so all vegetative individuals larger than a seedling were considered one size class. Reproductive plants were considered a third size category.

In *C. acaule*, plants with leaves shorter than 5 cm or with less than 3 leaves never flowered in the subsequent year. Therefore this size was used as an upper limit for seedling definition. There was very little variation in leaf number and length between the larger rosettes, so only one size class of vegetative and one size of reproductive plants was distinguished.

This stage division resulted in three stages in each species (seedling, vegetative adult, reproductive adult), with comparable definitions of the stages between the species. This allows direct comparison of stage performance between the species.

Seed predation

To estimate the importance of seed predation in the species, 100 flower heads per population were sampled in each of population of *C. pannonicum* of *C. acaule* in 2002 and 2003 in the time of fruiting of the species. Altogether 24 population samples were collected for *C. pannonicum* and 12 population samples for *C. acaule* over the study period. Each flower head was opened and number of fully developed seeds without any evident damage was counted. Further evidence for seed predation (presence of insect larvae, adult beetle or feces of these) was recorded for each flower head.

Other seed related traits

Seed weight was estimated by weighing 10 groups of 10 seeds from each of three source populations with the pappus removed before weighing. Seed production per square meter was estimated as number of flowering plants per square meter counted in five quadrats in each of three populations of the species, and multiplying it by seed production per plant estimated in these three populations.

Habitat requirements

To compare habitat requirements of the two species I used relevés from dry grasslands from the Czech national phytosociological database (Chytrý & Rafajová 2003). I used all relevés of dry grasslands from the area delimited by longitude 13°15'-15°30' E a latitude 50°00'-50°40' N. All together there were 2984 relevés. Data on species composition of these relevés were summarized using detrended correspondence analysis (DCA) using Canoco (ter Braak and Šmilauer 1998). DCA is a multivariate technique assuming a bell-shaped species distribution along an underlying environmental gradient that enables the extraction of a few ordination axes that summarize maximum variation in species composition. The two *Cirsium* species were excluded from the dataset before the analysis. Positions of the relevés on the 1st and 2nd ordination axis from this analysis were then used to compare species composition at localities of the two *Cirsium* species.

Data analysis

The difference in proportion of flower heads affected by seed predation was tested using ANOVA with number of predated flower heads per population as the dependent variable (this was possible as an equal number of flower heads was sampled in each population) and with year, species and their interaction as independent variables. Differences in the number of developed seeds per flower head between species, year and flower heads with and without seed predation were tested using GLM with Poisson distribution.

To analyze data on the local population dynamics of the species, data on transition probabilities from each single population and year were separately combined with mean seed predation data from each population and each year to yield as many matrices as there were seed predation data on single populations/years. This was done because seed predation data came from more populations than the data on transitions between stages, and also the intensity of seed predation was expected to be the most variable component of the life cycle of the species. This procedure yielded 6 (3 populations × 2 transition intervals used to study full species demography) times 12 for *C. acaule* and 24 for *C. pannonicum* (number of populations in single years used to estimate seed predation) demography matrices all together. All these matrices were combined using stochastic simulations as described in Caswell (2001), with equal probabilities of drawing each matrix. The model yielded the dominant eigenvalue of the matrices for each species (λ) and the elasticity of single life cycle transitions of each species.

Further, conditional total lifespan and time to first flowering were calculated using each transition matrix separately (as described in Cochran and Elster 1992). Here, only six transition matrices were available for each species, as this calculation does not incorporate data on reproduction.

Further, I estimated the fate of a local population that was randomly assigned one of the matrices each year. The population started with 10 flowering and 90 vegetative individuals (which is close to stable stage distribution in both *Cirsium* species) and the population growth was projected over 100 years. The number of runs (out of 1000) when the population went extinct was counted and used to estimate the probability of local population extinction in each species.

To estimate confidence intervals of population growth rate, elasticities, expected life span, time to first flowering and extinction probability, I bootstrapped the original field data on transitions of single individuals between stages. This yielded 100 new transition probability matrices for each species and population. The procedures described above were applied to each resulting matrix to yield 100 new estimates of each value. These were used to construct confidence intervals for each of the values as described in Efron and Tibshirani (1994). All the matrix calculations were done using Matlab, ver. 5.3.1.

Results

There is a large overlap in species composition of sites with the two *Cirsium* species (Fig. 1). It is also clear that *C. pannonicum* has stricter habitat requirements than *C. acaule*. Still the species share many habitats, and also all the study populations were at localities that fall into the overlapping region in Fig. 1.

There was no difference in the proportion of predated flower heads between years or species, nor was there an interaction between species and year (effect of species $F_{1,32} = 2.98$, $p = 0.09$; effect of year $F_{1,32} = 2.77$, $p = 0.11$; interaction species × year $F_{1,32} = 0.36$, $p = 0.55$). There was a significant difference in the number of developed seeds between flower heads with and without seed predation, between species and also between years. Also all the interactions were significant (Table 1). Number of developed seeds per flower head was equal in the two species in 2002 but was lower in *C. acaule* 2003 (Fig. 2).

Contrary to the results for number of seeds, population growth rate was generally higher in *C. acaule* than in *C. pannonicum*. There was however no difference between the two species when assuming no seed predation. The difference between the growth rates increased when assuming mean seed predation, and was the highest when full seed predation was assumed (Fig. 3). The difference in growth rate also translated in large differences in extinction probability of local populations of the species (Table 2).

Growth transitions and the survival of individuals in the flowering stage were higher in *C. acaule*, whereas transition from flowering individuals to vegetative stage and the other way round were higher in *C. pannonicum*. Elasticities of the single life cycle transitions showed the opposite pattern, with higher importance of survival transitions in *C. acaule* and higher importance of reproduction and seedling survival in *C. pannonicum* (Table 2).

Both species are able to self-fertilize without any decrease in seed production. Also, ability to survive in the seed bank is comparable between the species.

All the results comparing the two species are presented in Table 2.

Discussion

Habitat requirements

One prerequisite of this study was the assumption that the species share the same habitat. The comparison of species composition of localities of the two species confirmed that they are very similar. This is further supported by the existence of several localities where these two species co occur. However, it is still possible that there are some differences in habitat requirements between the two species at the microsite level, since no detailed study on microsite requirements of these two species was carried out. Figure 1 shows that the range of localities of *C. acaule* is wider than that of *C. acaule*. In spite of this, the habitat requirements of these species are probably as similar as possible in two different species, and thus life history traits of these species can be compared without confounding the comparison by different ecology, the common drawback of many of such comparisons.

Reproductive traits

Reproductive traits both supporting and opposing the rarity of *C. pannonicum* could be identified. Similarly to patterns in other rare-common species pairs (e.g. Rabinowitz & Rapp 1981, 1985, Fiedler 1987, Aizen & Patterson 1990, Baskin et al. 1997), the common *C. acaule* has larger seeds, which is expected to result in faster seedling development of this species. This trait also corresponds to overall faster development of the common species predicted by the demography analysis. On the other hand seed production was higher in *C. pannonicum*, showing that, contrary to conclusions many others (e.g. Fiedler 1987, Baskin et al. 1997, Young & Brown 1998), this trait alone cannot explain the rarity of the species. However in connection with full demography, it could be demonstrated that seed production is the key factor favoring the common species. This shows that the context of a trait, not its absolute value, is important when searching for traits responsible for species rarity.

Ability to self-fertilize, considered as another possible factor responsible for the common-rare difference between species (e.g. by Purdy et al. 1994, Baskin et al. 1997 and Young & Brown 1998), did not differ in this study.

Population growth rate

Mean stochastic population growth rate of both species was slightly bellow 1, showing that populations of both species are declining. Population growth rate of *C. pannonicum* was significantly lower than that of *C. acaule*, but the difference was very small. However this difference translated into a much larger difference in the probability of population extinction over 100 years and can serve as an explanation of the much lower number of existing populations of *C. pannonicum*.

The life cycles of both species were divided into comparable categories. This enables direct comparison of the importance of these categories. Growth transitions and the survival of individuals in the flowering stage were higher in *C. acaule*, whereas transition from flowering individuals to vegetative stage and the other way round were higher in *C. pannonicum*. Overall *C. acaule* is a species with faster population turnover, as confirmed also by its lower expected lifespan. This trait could favor survival of the species in a system with occasional disturbances (e.g. small scale landslides occurring at the study localities), as shorter life span corresponds to better ability to recover after disturbance. However the expected time to flowering that could support this pattern was not different between the two species.

Elasticities of the single life cycle transitions showed a greater importance of survival transitions in *C. acaule* and of reproduction and seedling survival in *C. pannonicum*. This is congruent with the observed stronger effect of seed predation on population growth rate in *C. pannonicum*.

Effect of seed herbivory

Herbivory is considered a factor strongly affecting the population viability of many plant species. The genus *Cirsium* is well know to be strongly affected by herbivory and is a common model in studies of the effect of herbivory in plant populations (e.g. Louda & Potvin 1995, Guretzky & Louda 1997, Palmisano & Fox 1997, Stanforth et al. 1997, Edwards et al. 2000, Rowland & Maun 2001). In agreement with these studies, seed predation had a strong impact on the population growth rates of both species.

Without seed predation, the population growth rates of both species were almost the same, as were the population extinction probabilities. Also the probability of seed predation was the same in the two species, and seed production when assuming mean seed predation was higher in the rare *C. pannonicum*. However when incorporating the seed predation effects to the demography matrix, it appears that seed predation has much stronger effect on *C. pannonicum* than on *C. acaule*, causing large differences in population growth rate of the species in the field. This shows that equal rate of seed predation due to the same predators in two closely related species sharing the same habitat can have dramatically different effects on population growth rate. Thus the higher importance of seedling recruitment in the life cycle of *C. pannonicum*, in combination with seed predation, seems to be the explanation for its rarity.

Conclusions

The combination of full demography with data on the intensity of herbivory in two related species differing in rarity and sharing the same habitat demonstrated significantly lower population growth rate in the rare species, resulting in a higher probability of local population extinction. This was a result of different importance of generative reproduction in the two species, in combination with seed loss due to seed predation. This result is the first quantification of differences between any rare and common species at the population level that provides quantitative conclusions about the effects of single traits, which would not be possible if the traits were not connected in a common framework. It is also the first demonstration of differential effects of seed herbivory on the life cycles of two congeneric species.

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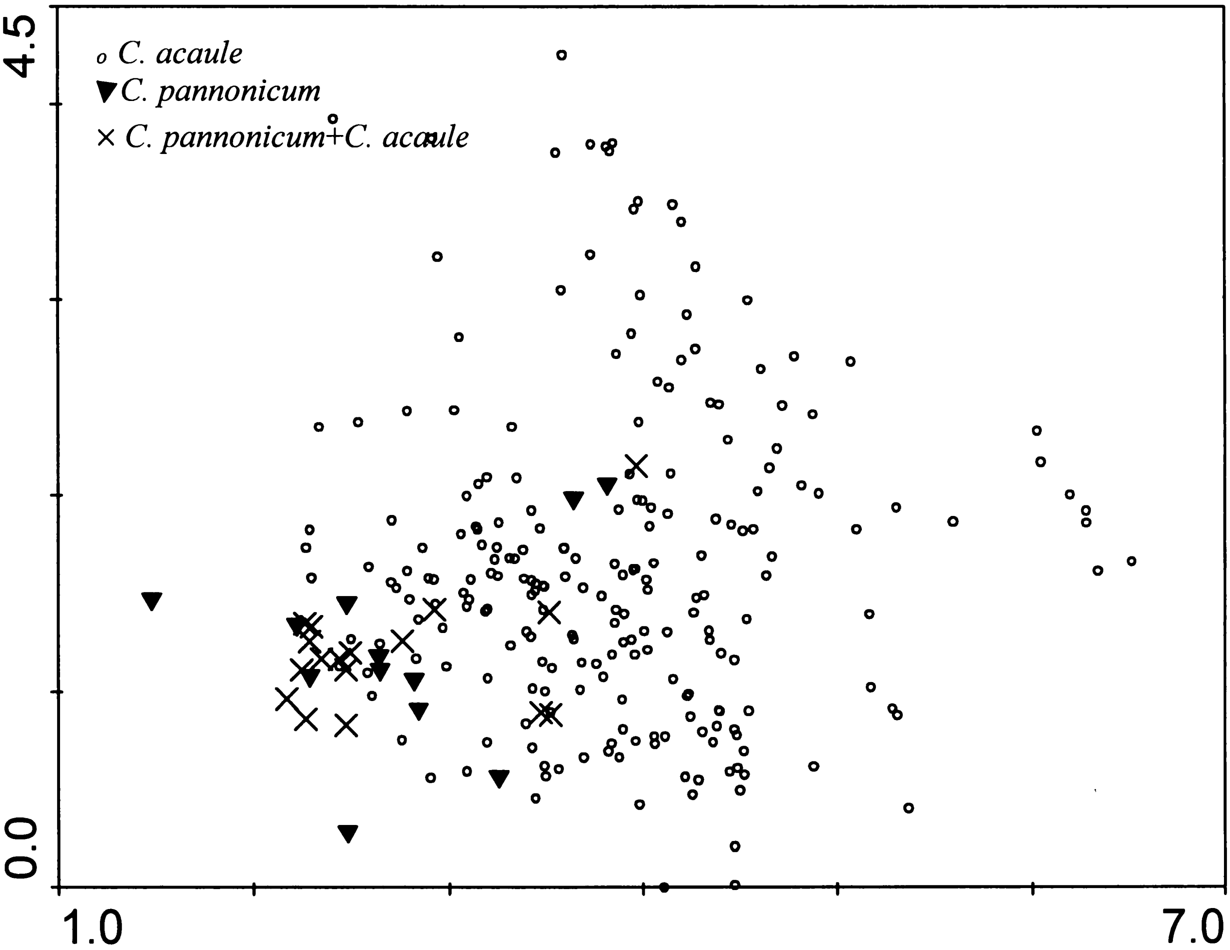
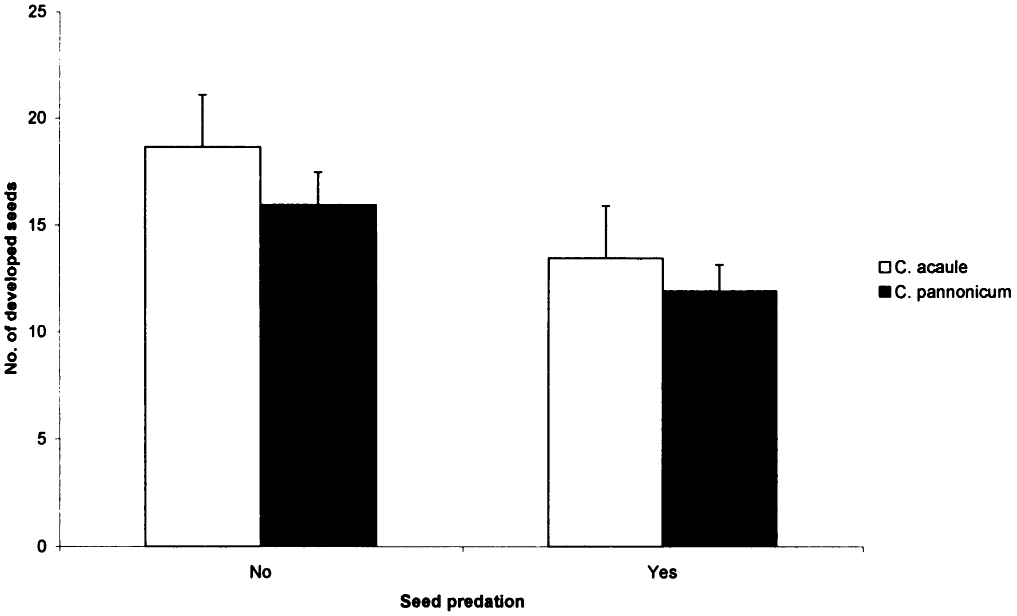
Figure 1. Comparison of species composition of sites with occurrence of the species of interest. Graph shows position of samples with *Cirsium* on the first and second ordination axis based on DCA analysis of 2984 relevés from dry grasslands. Samples without any *Cirsium*



species are not shown.

Figure 2. Number of developed seeds in flower heads with and without seed predation in different years: in A) 2002 and B) 2003.


25
20
15
10
5
0
No. of developed seeds
No
Yes
Seed predation
□ C. acaule
■ C. pannonicum

B)

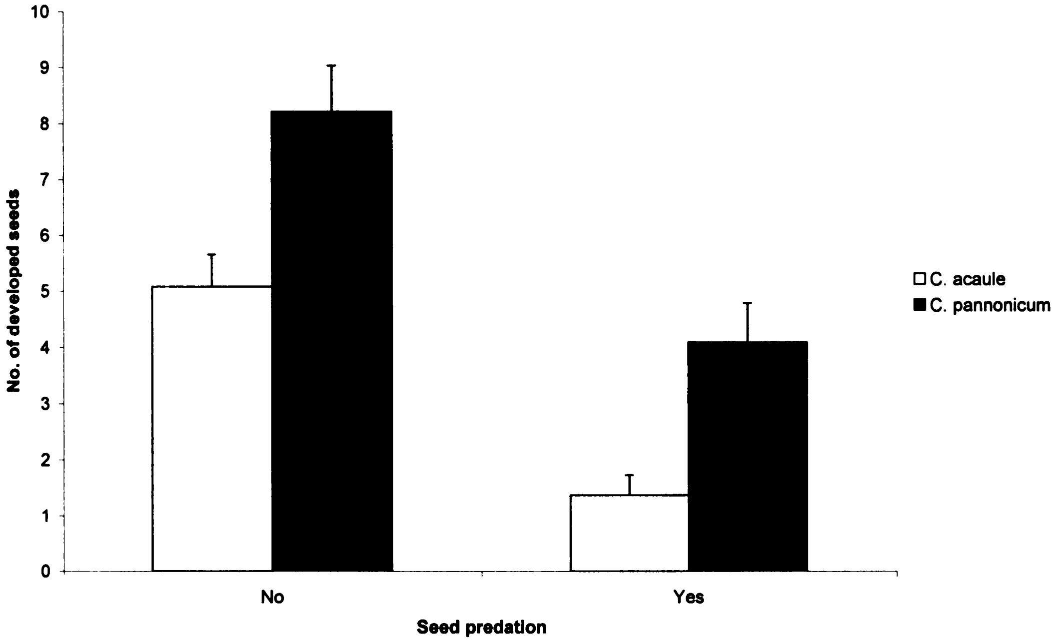

10
9
8
7
6
5
4
3
2
1
0
No. of developed seeds
No
Yes
Seed predation
□ C. acaule
■ C. pannonicum

Figure 3. Local population growth rate of populations of *C. acaule* and *C. pannonicum* estimated using data on full demography of the species. Seed production was estimated as number of seeds per flower head when no flower head was predated, when mean field proportion of flower heads was predated and when all flower heads were predated. Means and standard errors of the means are shown.

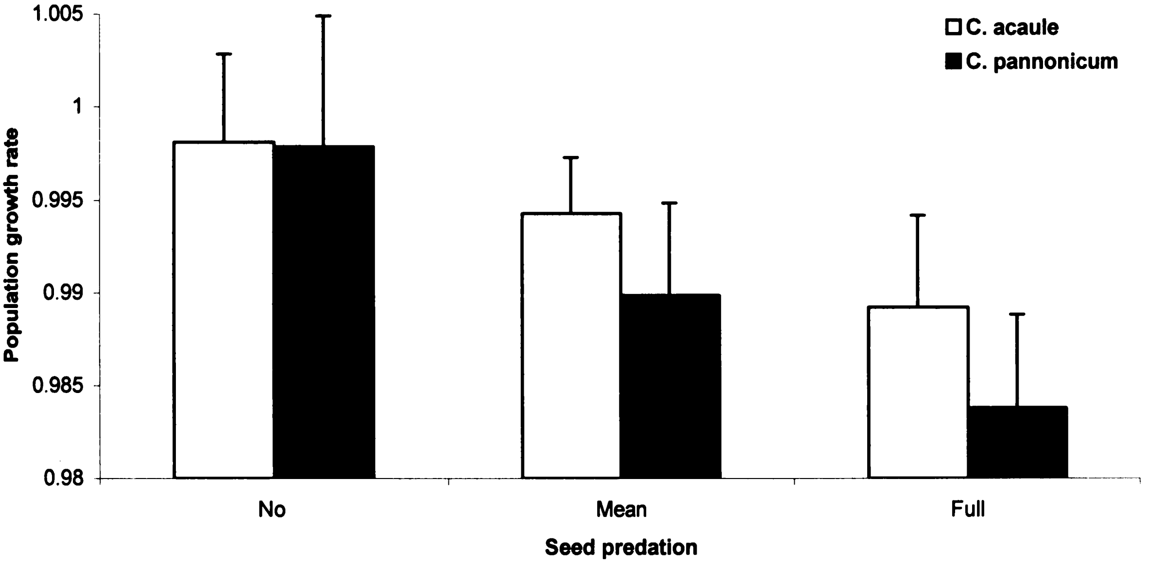


Table 1. Effect of seed predation on number of developed seeds in different years in the two *Cirsium* species. For all tests d.f.= 1.

	d.f. error	Chi	p
Species	1243	25545.7	<0.001
Year	1242	22700.1	<0.001
Predation	1241	22158.89	<0.001
Species × Year	1240	21810.7	<0.001
Species × Predation	1239	21774.24	<0.001
Year × Predation	1238	21679.51	<0.001
Species × Year × Predation	1237	21657.37	<0.001

Table 2. Comparison of traits that could be interpreted as the key parameters responsible for the common-rare difference in the two *Cirsium* species. Table provides mean of each value plus standard deviation (if applicable). Sign + means that the trait is significantly better for the common *C. acaule*, sign – means, that the trait is significantly worse, 0 means no difference between the species, +- means that it is not clear whether the trait is positive or negative (details are explained in the text). In transition probabilities and elasticities sign + indicates values that simply are higher.

	C. acaule	*C. pannonicum*	difference
Reproductive attributes			
Decrease of seed production after self-fertilization	0	0	0
Germination rate	0.12	0.19	0
Seed weight (mg)	2.8+-0.16	1.6+-0.42	+
Survival in seed bank	0.139	0.105	0
Seed production per plant	8.76+-0.738	11.12+-0.656	-
Herbivory			
Probability of flowerhead predation	0.29+-0.7	0.42+-0.5	0
Percentage decrease of seeds per flowerhead due to herbivory	27+-8	30+-6	0
Demography			
Population growth rate	0.9943+-0.003	0.9898+-0.005	+
Extinction probability after 100 year	0.158	0.488	+
Age at first reproduction	17+-3.7	14+-1.5	0
Expected life span	84+-5	144+-8	-
Elasticity of life cycle-transitions (x 1000)			
seedling→seedling	16+-0.04	41+-0.02	-
vegetative→seedling	11+-0.04	0.000000	+
reproduction	7+-0.06	24+-0.15	-
seedling→vegetative	17+-0.05	33+-0.14	-
vegetative→vegetative, inc. clonal growth	850+-0.23	724+-0.49	+
reproductive→vegetative	39+-0.05	67+-0.07	-
seedling→reproductive	1+-0.0005	0.000000	+
vegetative→reproductive	45+-0.08	91+-0.08	-
reproductive→reproductive, inc. clonal growth	14+-0.04	12+-0.01	+
Life cycle transitions			
seedling→seedling	0.416+-0.027	0.605+-0.003	-
seedling→vegetative	0.320+-0.030	0.048+-0.001	+
seedling→reproductive	0.023+-0.007	0.000	+
vegetative→seedling	0.032+-0.003	0.000	+
vegetative→vegetative	0.870+-0.019	0.899+-0.012	0
vegetative→reproductive	0.041+-0.005	0.071+-0.002	-
reproduction	0.531+-0.038	0.441+-0.080	0
reproductive→vegetative	0.744+-0.023	0.838+-0.016	-
reproductive→reproductive	0.220+-0.02	0.131+-0.016	+
clonal growth of vegetatives	0.052+-0.003	0.035+-0.002	+
clonal growth of reproductives	0.023+-0.002	0.031+-0.001	-

III.

Long-term population growth rate in populations differing in size and genetic diversity

Zuzana Münzbergová and Ivana Plačková

Abstract

1) Effect of habitat fragmentation on performance of plant species is a topic of many recent studies. Many studies have demonstrated negative effect of low population size and genetic diversity on single plant traits. However nothing is known on the effects of these differences on long-term species survival perspectives.

2) We studied the effect of population size and genetic diversity on population growth rate in a rare perennial herb occurring in fragmented grasslands. Its performance was measured using several seed traits. The data were then connected with data on mean demography of the species. Three different matrix models differing in the number of transitions based on measurements in a range of populations differing in size were used to explore the relationship between population size, genetic diversity and population growth rate.

3) The results show that genetic diversity itself is only weakly related to plant performance. It is however positively related to population size and population size is positively related to most of the seed traits. All the three matrix models showed that despite the decline in seed production in small populations, population growth rate is always positive. The model using only data on seed production per flower head from each population in the matrix model, and the model using these data plus data on number of flower heads per plant and field germination indicated a positive relationship between population size and population growth rate. Contrary to this, according to model using realistic data on number of seeds per flower head and number of flower heads per plant in the matrix model no such relationship exists.

4) *Synthesis and application.* The results show that decline in some plant traits in small populations does not necessarily lead to the species direct endangerment. We thus suggest that wider use of matrix population models in this type of studies may provide new insight into the real effect of population size. Our results also demonstrate that conclusions on the effect of

habitat fragmentation are crucially dependent on the parameters used to estimate plant performance and use of different parameters may lead to different conclusions.

Keywords

fragmented habitats, matrix models, plant performance, population viability, rare species, *Scorzonera hispanica*, seed production

Introduction

Due to recent changes in land-use, many landscapes are becoming increasingly fragmented. As a consequence, populations of many species are becoming small and isolated. Species in small populations are strongly influenced by demographic stochasticity (e.g. Dinnetz & Nilsson 2002; Saether et al. 2002; Kéry et al. 2003; Snyder 2003). They may also suffer from low pollinator attraction (Aizen & Feinsinger 1994; Ågren1996; Kunin 1997) that reduces the level of out crossing and increases the rate of self-fertilization (Jennersten 1988; van Treuren et al. 1994). Due to this and the overall higher probability of mating with closely related individuals, small populations are expected to suffer from fixation of deleterious alleles due to genetic drift (Ellstrand & Elam 1993; Young et al. 1996) and to be strongly affected by inbreeding depression (e.g. Charlesworth & Charlesworth 1987; Menges 1991; Husband & Schemske 1996).

These possible dangers for small populations motivated recent studies that explored the effect of population size and/or genetic diversity on plant performance measured as seed production, seed germination rate or plant growth (e.g. Mustajarvi et al. 2001; Lienert et al. 2002; Paschke et al. 2002). Most of these studies indicate poorer performance of individuals in small populations. This information, however, is inadequate for making predictions for future population growth; this is feasible only after the data are connected with the full demography of the species. The only study that attempted this in plants was Fischer & Matthies (1998). They studied a strict biennial species whose dynamics are simpler than most other perennial plant species.

The aim of this study is to explore the effect of population size and genetic diversity on the population growth rate of a species occurring in fragmented habitats. When doing this, collecting full demographic information on each population would be the most accurate method. This is however not feasible, since there are too few individuals in small populations to get sensible estimates of all life cycle transitions. Also collecting such an amount of data would be extremely time consuming. An attempt to estimate population growth rates of populations of different sizes thus requires finding the appropriate level of generalization that makes the estimation feasible and still generates the necessary information. Therefore, the second aim of this study is to estimate the effect of different levels of generalization on the conclusions about the effect of population size and genetic diversity on the population growth rate.

Specifically, we studied the effects of population size and genetic diversity on population growth rate in an iteroparous perennial plant, *Scorzonera hispanica*, occurring on fragmented dry grasslands. We used 21 populations ranging from 4 to 2500 flowering individuals. Using isozyme analysis we estimated genetic diversity of these populations. We also estimated total seed production per flower head, number of flower heads per plant and germination rate under field conditions in each population. In three populations we studied the full demography of the species over two transition periods. The effect of population size and genetic diversity on plant performance may interact with the effect of local environmental conditions. Therefore we also estimated several environmental variables of the localities and studied the relationship between these variables and population size, genetic diversity and single plant traits.

We used matrix population models to combine the data on single traits with the full demography and to estimate population growth rate of each population. We constructed three types of matrix models, differing in the amount of data from single populations used in the model. Specifically, we used either only data on seed production per flower head, these data plus data on number of flower heads per plant, or combined these data also with data on seed germination in the field. For all three cases we connected these data with data on mean transition probabilities of the remaining stages. These three types of models were used to explore the relationship between population size and genetic diversity and population growth rate as well as the effect of different levels of generalization of the model on these conclusions.

Materials and methods

Study species and study area

Scorzonera hispanica L. (*Asteraceae*) is a rare perennial herb occurring on dry grasslands in central and southern Europe. In the Czech Republic it occurs in central and northern Bohemia and southern Moravia. It has a single rosette and one flowering stalk carrying one to seven flower heads. Occasionally it is cultivated for its edible rootstock and locally naturalized (Tutin et al. 1964-1983).

This study was carried out in the České středohoří Mountains, an area of fragmented dry grasslands in northern Bohemia, Czech Republic, Central Europe. For this study we used 21 populations of the species (Table 1), i.e. all populations that were found in a field survey of the landscape, ranging from 4 to 2500 flowering individuals (Table 1). The distance between the two most distant populations is 38 km. Given the mean dispersal distance of seeds of the species, 1.2 to 7 m, all the populations may be considered isolated; the probability that a tail of the dispersal curve reaches neighbouring population is close to zero. The mean dispersal distance of seeds was estimated using data on plant height, mean wind speed and terminal velocity estimated by Münzbergová (submitted).

Seed production and plant size

To estimate seed production we sampled 40 flower heads, one per plant, in each population, at the time of fruiting in early July 2003. Both the plants within populations and flower heads within plants were selected randomly. If there were fewer than 40 individuals in a population all individuals were sampled. Plant height and total number of flower heads were recorded for each sampled plant. In each sampled flower head we counted the numbers of developed and aborted seeds, and estimated total weight of all developed and all aborted seeds. The weight of one seed was than estimated as the total seed weight divided by number of seeds.

Life cycle

To describe the whole life cycle of the species we marked 150 individuals with at least 30 individuals in each stage (seedling, vegetative, reproductive) at three localities in 2001. The individuals were marked in three permanent plots per locality, including all individuals in the plot at the beginning. If there were already twenty marked individuals of one stage per plot, further individuals of this stage were skipped to make sure that each stage was represented by at least ten individuals per plot. Each individual was marked with plastic and metal labels that were placed just behind it. The census was carried once a year in June from 2001 to 2003. At each census survival and size of each individual was recorded. If there were fewer than thirty living marked individuals per stage at the time of census, new individuals were marked to keep the minimum number of individuals per stage. At each census number of leaves, length of the longest leaf, maximum leaf width and number of inflorescences were measured.

The life cycle of the species was divided into three stages. These were (i) seedlings, i.e. vegetative plants with leaf width up to 1 cm and length up 15 cm (size of the seedling was defined as the maximum size than an individual could reach within a season), (ii) vegetative plants, i.e. non flowering individuals larger than seedlings and (iii) flowering individuals.

Germination rate

To estimate germination rate of the species in the field, ten 33 cm × 33 cm plots were marked at each locality where the full demography was studied in two subsequent years (2001 and 2002) and 50 seeds were sown in each plot. Seed germination was recorded in each plot as well as in a control plot, a plot with no seeds sown adjacent to each sown plot, in the following June. The plots were checked for new seedlings a year later to record delayed germination.

Further, we used data on seed germination and seedling survival from another experiment (Münzbergová submitted), that followed these parameters over the same period for several of the other study *Scorzonera* populations. This experiment was set up in exactly the same time and in the same way as that to follow germination at the localities with demography plots. This experiment provided data for ten additional *Scorzonera* populations.

Isozyme analysis

If available, we randomly selected ten adult individuals, they could be both vegetative and flowering, in each population. The selection of individuals was done by selecting random points at the locality (determined by a random distance and angle from the centre of the locality) and by collecting an individual within 20 cm of this point. If no individual were found in this distance the point was skipped. The 20 cm limit was used in order not to give a higher chance of being sampled to isolated individuals. From each selected individual we took a sample of a fresh leaf approximately 10 cm^2. The samples were kept on ice after sampling and the isozymes were extracted on the following day.

Electrophoresis was performed on crude protein extracts of leaf material. All enzymes were resolved on polyacrylamide gels using 8.16 % separating gel and 4 % stacking gel. Eight enzyme systems were investigated in the first step; four of them yielded clear patterns and were analysed further. They included 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), aspartate aminotransferase (AAT, EC 2.6.1.1), alcoholdehydrogenase (ADH, EC 1.1.1.1) and leucinaminopeptidase (LAP, EC 3.4.11.1). Two independent loci were identified and scored in ADH, two in AAT, one in 6-PGDH and three in LAP, i.e. data for eight independent loci were obtained together. The detailed protocol of the analysis is provided in the appendix.

Environmental variables of the localities

Environmental variables were selected to capture the most important differences between localities. In the study communities the most important factors are assumed to be productivity and water availability. The following parameters were measured: inclination, aspect, soil water holding capacity and bulk density and above ground biomass. Aboveground biomass was collected in 40 plots 15 cm × 15 cm placed over the locality at peak standing crop in June 2002. There were five measurements of the other parameters within each locality; the mean of these values was used in the analysis. Data on inclination and aspect were used to calculate potential direct solar radiation. It was done by calculating the sum of the cosines of the angles between the sunrays and the locality over the whole day at 15-minute intervals. The calculation was done on the 21st day of each month between December and June. As some of these values are highly correlated, only data on direct radiation in January, June and the average over the whole period were used. Data on aspect were strongly negatively correlated with potential direct radiation in January and were therefore not used in the further analysis. Soil water holding capacity was measured as the amount of water bound in the soil monolith after it stood on a constantly wet filter paper for 24 hours. It was expressed as the amount of water retained per gram of dry soil.

Data analysis

To compare genetic parameters of the populations we calculated proportion of polymorphic loci (95% criterion) and the observed and expected heterozygosity (Levene 1949) using POPGENE version 1.31 (Yeh et al. 1997).

Linear regression was used to study the effect of population size on genetic variation. In one of the populations only four individuals were sampled for genetic analysis instead of ten individuals due to small population size, which could bias the analysis. Therefore both tests including this population and without it were performed; since the trends remained the same only results without the exclusion are shown further.

Linear regression was used also to study the relationship between population size and environmental variables, between seed related parameters (total weight of developed seeds, weight of one developed seed, proportion of developed seeds and number of developed seeds) and environmental variables and between seed related parameters and population size and genetic variation. Seed number per flower head was also combined with number of flower heads per plant and this was further combined with data on seed germination. These two combined parameters represented cumulative fitness parameters, and were used in the tests of the effect of population size and genetic parameters. For all seed related parameters the tests were also done for the dataset with populations with less than 40 sampled individuals excluded. Since the trends in were the same in these two tests, only results without this exclusion are shown further.

All the seed parameters were square root transformed before the analysis to gain normality. Population size was log-transformed in all analysis. All statistical analyses were performed using S-Plus (2000). In all test of the effect of population level parameters on plant traits, population code was used as an error term to take into account that number of degrees of freedoms should reflect number of populations and not number of plants.

Matrix population models

The magnitude of the effect of changes in plant traits for long-term population behaviour was estimated using population matrix models (Caswell 1992). Data from each population where the demography data were collected were used to construct a separate demographic matrix for each transition period. This resulted in six source matrices (Table 2). These source matrices were taken as estimates of mean performance of *Scorzonera* in the study region. To estimate the population growth rate of each population in the area these source matrices were connected with data on plant performance from each population. This was done in three ways, differing in what part of the demographic matrix was replaced with population specific information yielding three different specific models per source matrix. (i) Replacing only data on seed production per flower head, (ii) replacing data on both seed production per flower head and flower head number per plant and (iii) using the same data as in (ii) plus data on seed germination and seedling survival. Connecting the data mentioned in (i) to (iii) with the six source matrices resulted in eighteen demography matrices for each population, six for each level of generalization. Projections of the population growth rate of these populations was then done for all six matrices corresponding to one level of generalization together using stochastic simulations with a time limit of 10 000 as described in Caswell (1992).

For each population growth rate we also calculated bootstrap confidence interval. This was done by 100 times bootstrapping the original data used to derive the source matrices and to estimate the population specific reproduction related transitions. For each population we constructed 100 new matrices for each of the three specific models (level of generalization) and for each of the six source matrices. The 100 groups of six source matrices for each population and each specific model were again combined using stochastic simulations. The resulting 100 estimates of population growth rate were then used to derive the confidence intervals for each specific model for each population. This was done according to Efron & Tibshirani (1994).

Results

Effect of environmental variables

Environmental variables of the localities had little effect on population size (all parameters df = 1, 14, range of p = 0.021-0.55 with significant positive effect of inclination). In several cases the environmental variables also affected seed related parameters (all tests df = 1, 14, for total seed weight range in p = 0.41-0.83; for weight of one seed range of p = 0.0003-0.48, with a significant negative effect of inclination and significant positive effect of potential direct solar radiation in January and water holding capacity; for proportion of developed seeds range of p = 0.08-0.70 and for number of developed seeds range of p =

0.0004-0.6 with a significant negative effect of inclination and significant positive effect of potential direct solar radiation in January and water holding capacity). Also plant height, number of flower heads per plant and germination rate was related to environmental variables. For plant height and number of flower heads per plant df = 1, 14, for plant height range of p = 0.0007-0.97 with significant negative effect of inclination and significant positive effect of biomass, for number of flower heads range of p = 0.02-0.91 with significant negative effect of inclination, significant positive effect of biomass and significant positive effect of bulk density. For seed germination rate df =1,7 and range in p = 0.059-0.79 with marginally significant negative effect of biomass.

Population size and genetic diversity

There was a positive significant relationship between population size and expected heterozygosity (Fig. 1) and proportion of polymorphic loci but not between population size and observed heterozygosity (Table 3).

Single plant traits

Population size had a positive significant effect on total seed weight, proportion of developed seeds and number of developed seeds but not on the weight of one developed seed. Genetic diversity only affected seed weight, which was significantly negatively related to expected heterozygosity of the population. Total seed weight and weight of one seed also increased with plant height, while total seed weight and number of developed seeds decreased with number of flower heads per plant (Table 4). There was also a significant negative relationship between population size and plant height ($F_{1,17} = 8.9$, $p = 0.008$) and between population size and number of inflorescences per plant ($F_{1,17} = 22.3$, $p < 0.001$).

Contrary to the effect on number of seeds per flower head there was no effect of population size and genetic parameters on the product of number of seed per flower head and number of flower heads per plant (df = 1,19, range of p = 0.1-0.29). However the relationships became positive again after combining this value with data on seed germination (df = 1,6, range of p = 0.02-0.37 with significant positive effect of population size and of proportion of polymorhic loci.

Long-term projection of population growth rate

All the population growth rates were above one, and none of the confidence intervals overlapped with the one line (Figure 2), showing that all the populations are expected to grow. When only data on seed production per flower head from the target populations were used to parametrize the matrix models, population size had a significant positive effect on population growth rate ($F_{1,18} = 19.37$, $p < 0.001$). The population growth rate ranged between 1.03 and 1.09 (Fig. 3A). When both data on seed production per flower head and number of flower heads were used the relationship became non-significant ($F_{1,18} = 0.0008$, $p = 0.98$). In this case population growth rate is maximum for medium population size and declines to both directions (Fig. 3B). Adding data on seed germination and seedling survival returned the relationship to being significant and positive ($F_{1,10} = 28.38$, $p < 0.001$). In this case the range of lambda was the largest (between 1.01 and 1.09) (Fig. 3C).

Similarly to the effect on single plant traits, the effect of genetic diversity on population growth rate was weaker than that of population size. The only significant effect was marginally significant effect of expected heterozygocity on population growth rate when all data on seed production per flower head, number of flower heads per plant and germination rate were used in the model. When only data on seed production per flower head from the target populations were used to parametrize the matrix model df = 1,16 and range of p = 0.8-0.95. When both data on seed production per flower head and number of flower heads per plan were used, df = 1,16 and range of p = 0.32-0.55. When also data on seed germination were used df = 1,8 and range of p = 0.06-0.99.

Discussion

In this study we used matrix population models to explore the effect of population size and genetic diversity on plant performance. We did this by using full demography from a few populations of the target species and connecting it with data on individual traits from all populations under study. This approach enabled us to estimate the long-term effects of changes in single traits on species survival perspectives. Furthermore we were able to demonstrate the effect of different levels of generalization in the data, when different fractions of the demography matrix were based on data from the spectrum of populations differing in size, on conclusions on the effects of population size on long-term population performance.

Effect of environmental variables

Habitat quality is commonly regarded an important factor responsible for population decline (e.g. Eisto et al. 2000; Vergeer et al. 2003). Moreover habitat quality may also be the key factor affecting plant performance. In this study larger populations were found on localities with higher inclination, i.e. on localities with overall lower productivity that are less prone to degradation. Environmental variables also affected plant height, number of flower heads per plant, germination rate and several seed related traits. There was also a significant negative correlation between plant height and population size.

These patterns show a complex relationship between habitat quality, plant traits and population size. The main direction in these relationships is positive effects of high productivity on several plant traits and negative relationship between high productivity and population size. Thus negative effect of small population size may be masked by positive effect of higher productivity. Finding positive relationship between population size and plant performance in this study therefore means that the effect of population size is really strong, and that it is independent of environmental conditions.

Population size and genetic variation

A significant positive relationship was found between population size and both expected heterozygosity and proportion of polymorphic loci but not between population size and observed heterozygosity. This demonstrates that lower population size leads to a strong decrease in genetic variation as was previously shown e.g. by van Treuren et al. (1991), Raijmann et al. (1994), Sun (1996) and Schmidt & Jensen (2000). However the genetic diversity of populations is lower than expected, as shown by the large difference between observed and expected heterozygocity. This is probably due to highly non-random patterns of mating that can be due to pollinator behavior (see e.g. Mitchell & Marshall 1998; Skogsmyr & Lankinen 1999).

Genetic diversity, commonly reported as an important factor affecting plant performance (e.g. Oostermeijer et al. 1995; Buza et al. 2000), had only a limited direct effect on any of the seed related traits in this study. The only effects were a negative relationship between expected heterozygosity and weight of one seed and positive effect of percentage of polymorhic loci on the product of number of seeds per flower head, number of flower heads per plant and seed germination rate.

In contrast to genetic diversity, population size had a significant positive effect on the number of developed seeds, proportion of developed seeds and total weight of developed seeds. It had no effect on the product of number of seeds per flower head, number of flower heads per plant, but it had a significant positive effect on the product of these two variables and seed germination rate. This reversal in results shows that number of flower heads per plant partly compensates the negative effect of lower population size. This is due to the above-mentioned higher productivity in smaller populations.

Positive effect of population size on several plant traits found in this study is in agreement with many other studies relating population size and plant performance (Menges 1991; Raijmann et al. 1994; Heschel & Paige 1995; Kéry et al. 2000; Fisher et al. 2000). The absence of this relationship when data on the number of seeds per flower head and the number of flower heads per plant were used shows that the conclusion of the no relationship studies (e.g. Widén 1993; Morgan 1999; Costin el at. 2001) may not be due to an inherent nature of the species and that selection of different parameters may lead to positive conclusions. Therefore it is crucial that not one but several parameters are used in assessing the effect of population size on plant performance if the results are to be used to assess endangerment status of the species.

Long-term projection of population growth rate

The projected long-term population growth rate of all the populations was above one in all cases, so even small population are expected to survive in the long run if the conditions remain the same. A decrease in seed production of smaller populations is therefore not so important as would be assumed if the full demography data were not available. It should be however kept in mind that this conclusion is based on the assumption of constant environmental conditions and that adding more years to the study may change the pattern.

Population size had a significant positive effect on population growth rate when only data on seed production per flower head were used from the target populations. This was a clear result of the positive relationship between population size and seed production per flower head. Similarly to pattern in single plant traits, this effect disappeared when both data on seed production per flower head and number of flower heads per plant were used. Adding data on seed germination resulted again in a positive relationship. If only data on the number of flower heads per plant and not on seed numbers per flower head were used, the relationship between population size and population growth rate would even become negative (data not shown).

These results show, similarly to the results for single plant traits, that the conclusions of such a study crucially depend on the level of detail to which each single population is studied. Even this study contains some generalizations, as data on adult survival are not available from a larger spectrum of populations. So even here it is not known whether some important information was missed and it can only be speculated, that adult survival is less sensitive to population size than reproductive traits.

Conclusions

This study has demonstrated positive effects of population size but not of genetic diversity on plant performance. These effects are partly compensated by higher productivity in smaller populations. This results in reversal of conclusions depending on what parameter is used. It demonstrates that selection of parameters to be measured could strongly affect the conclusions of such a study. By connecting the individual performance data with demographic analysis, we were able to show that in spite the decline in plant performance due to lower population size, population growth rate of even the smallest population is above one. The populations have thus the potential to survive in the long run. This result is a first direct quantification of the importance of single plant traits for long-term population growth rate. We argue that this kind of quantification can provide valuable information and that wider use of matrix models in studies of the effect of habitat fragmentation on species performance may provide additional insight into survival perspectives of rare species.

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Table 1. Geographic positions and sizes of populations used in the study.

Population	Longitude	Latitude	Population size
1	14° 13' 59.1"	50° 33' 0.4"	12
2	14°15' 12.6"	50° 31' 44.6"	2464
3	14° 15' 47.7"	50° 31' 52.1"	10
4	14°14' 6.7"	50° 32' 2.9"	398
5	14° 14' 7.4"	50° 32' 2.1"	60
6	14° 14' 13.9"	50° 31' 46.6"	1632
7	14°16' 39.9"	50° 32' 42.9"	200
8	14° 20' 5.2"	50° 30' 5.2"	3
9	13° 58' 39.6"	50° 29' 45.9"	48
10	14° 8' 40.6"	50° 33' 26.6"	54
11	14° 5' 23.7"	50° 33' 1.1"	106
12	14° 5' 19.7"	50° 32' 34.8"	1980
13	14° 5' 21.9"	50° 32' 58.2"	1920
14	14° 10' 16.4"	50° 33' 10.4"	387
15	14° 7' 51.6"	50° 33' 23.2"	1716
16	14° 8' 35.7"	50° 33' 34.6"	63
17	14° 13' 59.3"	50° 32' 11.6"	690
18	13° 52' 42.8"	50° 28' 19.1"	76
19	14° 5' 16.4"	50° 32' 25.0"	2475
20	14° 14' 50.2"	50° 31' 59"	27
21	13° 48' 3"	50° 31' 56.8"	12

Table 2. Source demography matrices used in the study. Transition generative -> seedlings provides only data on germination rate. It was used to multiply data on seed production for the first and the second model; it was replaces by a population specific value in the third model. For population two seed germination and seedling survival values in both matrices come from the first transition interval, since the seed sowing plots for the second transition interval were lost. Population numbers in this table correspond to those in Table 1. Note that the non-zero vegetative->seedling transitions in some matrices are due to shrinking of larger plants.

Population	Transition interval		Seedling	Vegetative	Generative
2	2001-2002	Seedling	0.8840	0.0000	0.0460
		Vegetative	0.0100	0.9063	0.3043
		Generative	0.0000	0.0938	0.6957
			Seedling	Vegetative	Generative
	2002-2003	Seedling	0.8840	0.0147	0.0460
		Vegetative	0.0800	0.8971	0.4634
		Generative	0.0000	0.0882	0.5366
			Seedling	Vegetative	Generative
6	2001-2002	Seedling	0.9129	0.0000	0.0450
		Vegetative	0.0100	0.6571	0.4000
		Generative	0.0000	0.3143	0.6000
			Seedling	Vegetative	Generative
	2002-2003	Seedling	0.8300	0.0106	0.0320
		Vegetative	0.0100	0.7340	0.3333
		Generative	0.0000	0.2340	0.6154
			Seedling	Vegetative	Generative
13	2001-2002	Seedling	0.6000	0.0286	0.0350
		Vegetative	0.0700	0.8714	0.3182
		Generative	0.0000	0.0857	0.6818
			Seedling	Vegetative	Generative

2002-2003	Seedling	0.8900	0.0000	0.0630
	Vegetative	0.0100	0.6875	0.2500
	Generative	0.0000	0.3125	0.7500

Table 3. Regression analysis of genetic parameters vs. population size (log-scale) in 21 populations of *Scorzonera hispanica*. In all tests df = 1,18. Significant results are in bold.

Genetic parameter	R	F	P
Observed heterozygosity	0.056	0.057	0.814
Expected heterozygosity	0.552	7.9	**0.012**
Proportion of polymorphic loci	0.63	11.84	**0.003**

Table 4. Effect of population size and genetic variation on parameters related to seed production in *Scorzonera hispanica*. Significant results are in bold, significant negative relationships are underlined.

		df	Error df	Mean Sq.	F	p
Total seed weight	Population size	1	12	0.4	8.9	**0.011**
	Observed heterozygosity	1	12	0	0.5	0.495
	Expected heterozygosity	1	12	0.1	3	0.108
	Proportion of polymorphic loci	1	12	0	0.3	0.615
	Plant height	1	576	0.1	11.4	**<0.001**
	Number of flower heads	1	576	0	4.5	**0.034**
Weight of one seed	Population size	1	12	0	0	0.872
	Observed heterozygosity	1	12	0	0.1	0.748
	Expected heterozygosity	1	12	0	11	**0.006**
	Proportion of polymorphic loci	1	12	0	2.6	0.131
	Plant height	1	576	0	29.8	**<0.001**
	Number of flower heads	1	576	0	1.3	0.264
Proportion of developed seeds	Population size	1	12	3.3	12.6	**0.004**
	Observed heterozygosity	1	12	0.1	0.4	0.556
	Expected heterozygosity	1	12	0.2	0.6	0.437
	Proportion of polymorphic loci	1	12	0.3	1	0.341
	Plant height	1	576	0	0	0.913
	Number of flower heads	1	576	0.1	2.1	0.148
Number of developed seeds	Population size	1	12	3926.1	8.6	**0.013**
	Observed heterozygosity	1	12	209.8	0.5	0.511
	Expected heterozygosity	1	12	73.3	0.2	0.696
	Proportion of polymorphic loci	1	12	529.7	1.2	0.303
	Plant height	1	576	28.1	0.5	0.48
	Number of flower heads	1	576	228.2	4.1	**0.044**

Figure 1. Relationship between population size and expected heterozygosity of the population for 21 populations of *Scorzonera hispanica*. The relationship is significant.

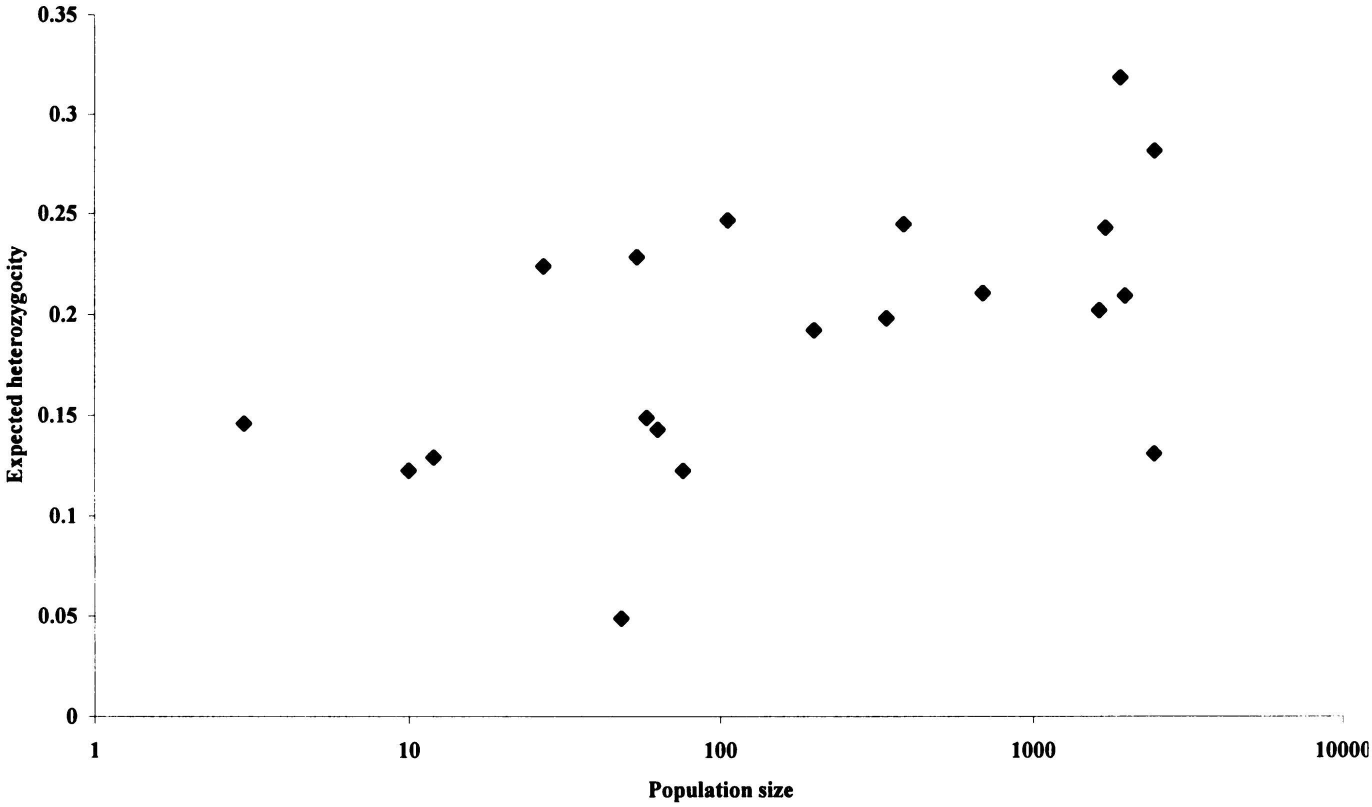
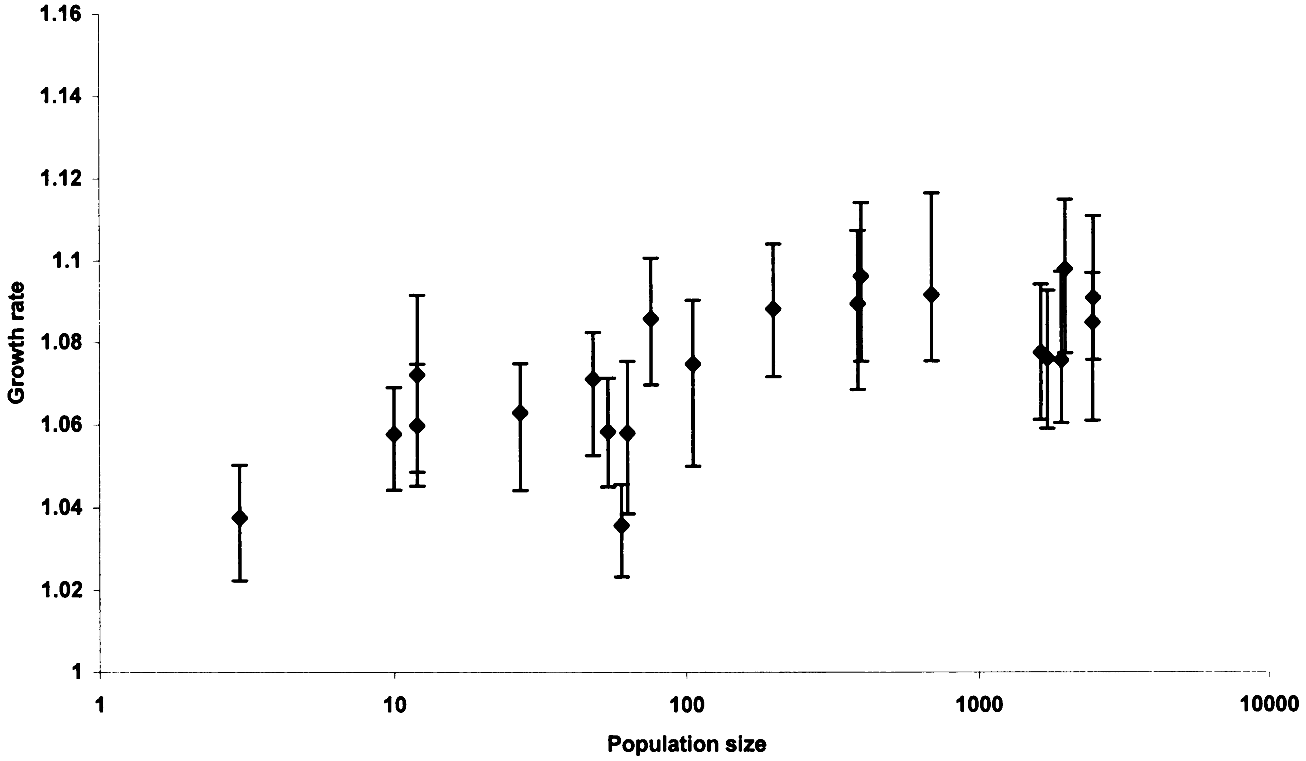
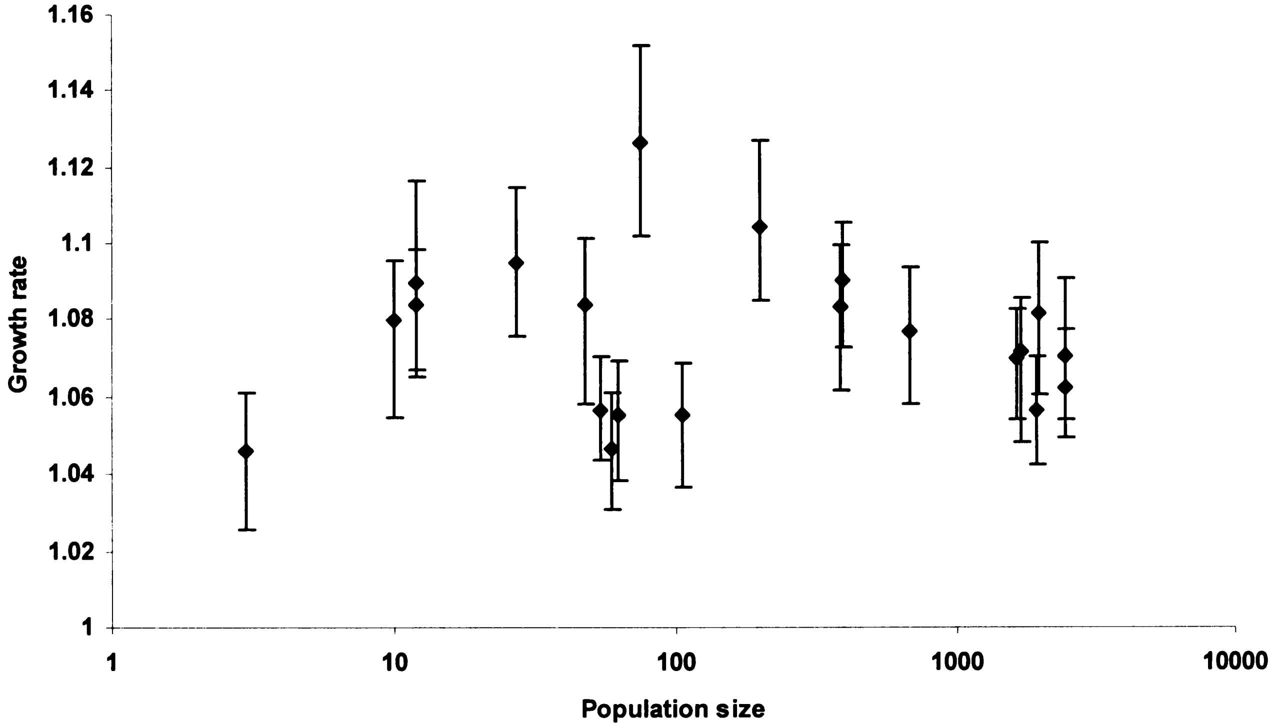


Figure 2. Relationship between population size and population growth rate (based on full demography of *Scorzonera*) with increasing realism of the estimates. In graph A population growth rate was estimated using only data on seed production per flower head from the target population, in graph B data on seed production and flower head number were used, in graph C also data on seed germination and seedling survival from the target populations were used. Graph C includes only a subset of populations for which the seed germination data were available. Graphs show stochastic lambda with 95% confidence intervals. The relationship in graph B is not significant.

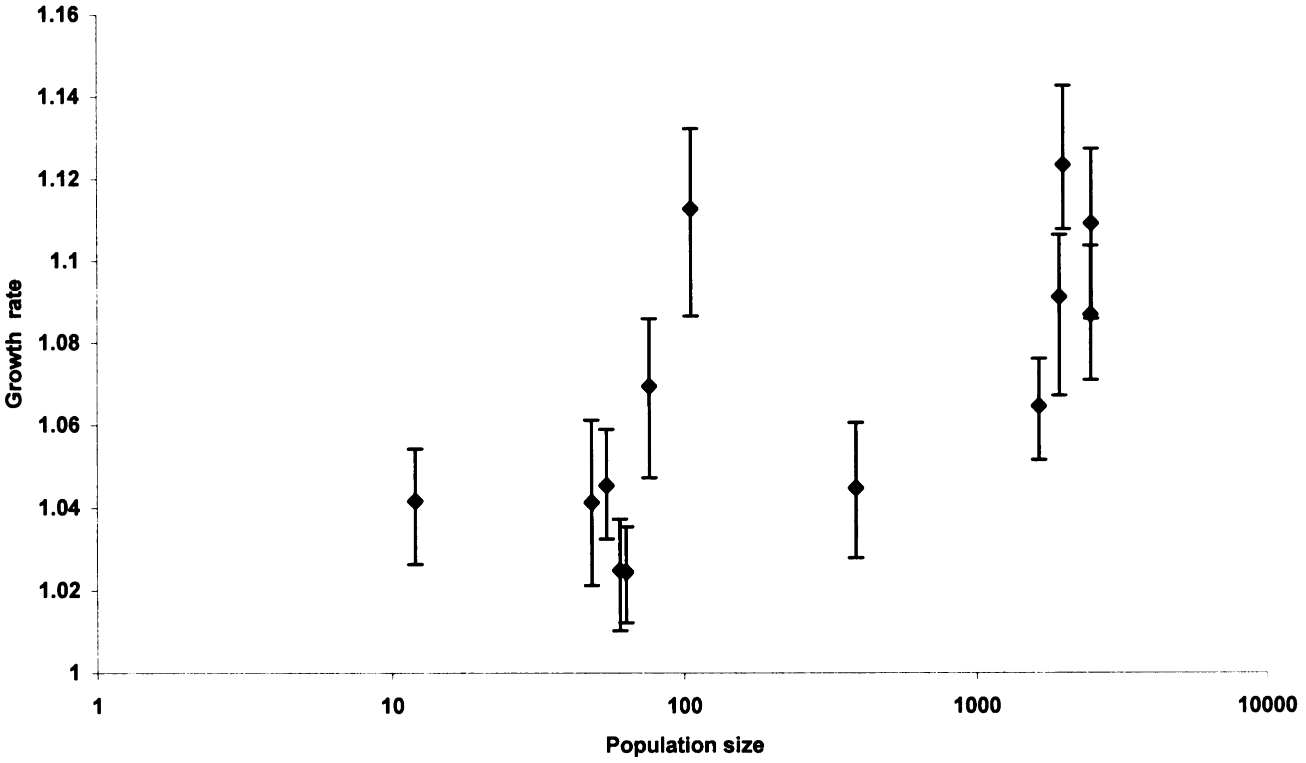
A)



B)


1.16
1.14
1.12
1.1
1.08
1.06
1.04
1.02
1
Growth rate
1
10
100
1000
10000
Population size

C)


1.16
1.14
1.12
1.1
1.08
1.06
1.04
1.02
1
Growth rate
1
10
100
1000
10000
Population size

Appendix

Detailed protocol used for the isozyme analysis

Electrophoresis was performed on crude protein extracts of leaf material. Approximately 60 mg of fresh leaf tissue was ground with Dowex-Cl (1-X8) and homogenized on ice in 0.7 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. The extracts were centrifuged for 10 min at 13,000 rpm and clear supernatants were stored at –75 °C for up to 6 months until investigated in electrophoresis. Isozymes were separated on native-PAGE; 30 µl of each sample were employed for electrophoresis in a Hoefer vertical electrophoresis unit. All enzymes were resolved on polyacrylamide gels using 8.16 % separating gel and 4 % stacking gel. The separating gel was made using a buffer of 1.82 M tris-HCl, pH 8.9, and the stacking gel using a buffer of 0.069 M tris-HCl, pH 6.9. The electrode buffer consisted of 0.02 M tris and 0.24 M glycine, pH 8.3.

Eight enzyme systems were investigated in the first step; four of them yielded clear patterns and were analysed further. They included 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), aspartate aminotransferase (AAT, EC 2.6.1.1), alcoholdehydrogenase (ADH, EC 1.1.1.1) and leucinaminopeptidase (LAP, EC 3.4.11.1). The staining procedures followed Vallejos (1983) to visualize 6-PGDH and ADH, with the following modifications 6-PGDH (0.1 M tris-HCl pH 8.4, 30 mg 6-phosphogluconic acid) and ADH (20 ml ethanol). Enzyme system AAT was stained using the following method: two staining solutions were prepared, A (20 ml 0.1 M tris-HCl pH 8.4, 240 mg aspartic acid, 40 mg α-ketoglutaric acid) and B (20 ml 0.1 M tris-HCl pH 8.4, 50 mg Fast Blue BB Salt, 50 mg Fast Violet B, 25 mg pyridoxal-5-

phosphate). The solution A was prepared at least 15 min before the application. The gel was rinsed in water and then in buffer tris-HCl pH 7. Solutions A and B were mixed and poured on the gel. After incubating in the dark at 32°C until the bands appeared, the gel was rinsed in distilled water and fixed (1:1:3:5, glycerine : acetic acid : H_2O : methanol). Visualization of LAP was done using buffer 0.2 M tris-maleat pH 6. The gel was rinsed with the buffer and then incubated for 10 min in a solution of 30 ml of the buffer, 40 mg L-leucyl-β-naphthylamide.HCl (in 50 % acetone) and 60 mg $MgCl_2$. Then 25 mg Fast Black K Salt in 30 ml of the buffer were added. The gel was incubated in dark, until bands appeared.

Two independent loci were identified and scored in ADH, two in AAT, one in 6-PGDH and three in LAP, i.e. data for eight independent loci were obtained together.

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IV.

Seed, dispersal, microsite, habitat and recruitment limitation – identification of terms and concepts in studies of limitations

Zuzana Münzbergová and Tomáš Herben

Abstract

Recently there is an increasing number of studies concerned with the effect of various types of limitations on species local population size and distribution pattern at the landscape scale. The terminology used to describe these limitations is however very inconsistent. Since the terms are often used as a part of conclusions of papers, the inconsistency in their use obscures the message these papers are making. In this study we are reviewing the current uses of these terms, identify the basic concepts involved in the discussion of a *limitation* and link the concepts with the term. Finally we discuss the experimental approaches that are used to assess these limitations.

We differentiated four basic concepts resulting from combination of limitation by environment vs. ability to grow and spread, and two spatial scales (local and regional scale). The two concepts at each spatial scale are expected to form a gradient of all possible combinations of the two respective types of limitations. In the considerations of various experimental approaches used to assess these limitations, we conclude, that sowing experiments, i.e. seed addition into existing population or seed introduction into unoccupied habitats, are the only reliable types of evidence for these limitations.

Keywords

carrying capacity, species distribution, habitat occupancy, population size, sowing experiment

Introduction

Knowledge of factors affecting distribution of species provides necessary background for understanding conditions for species survival in the landscape. Understanding of these factors is especially important in fragmented landscapes where total abundance of species is an outcome of its distribution at several independent scales. These factors have been described using several related terms, viz. seed-, dispersal-, microsite-, habitat- and recruitment- limitation; popularity of these terms has been increasing in recent years (Fig. 1).

Term recruitment limitation is the prevailing term in zoological studies; it is especially common in studies of marine organisms. Since the origination the term is quite consistently used in marine zoology to express relative importance of pre- and post settlement processes in population dynamics. Even this term, obviously due to the development of the field, has been criticized as that *the term has undergone ambiguous changes in definition since its origin that threatens its usefulness* (Hixon 1998).

This is even more true in plant ecology. In plant ecology papers, all the terms can be found, but as far as we are aware, no clear and consistent definition of them has been made (but see Nathan and Muller-Landau 2000, Muller-Landau et al. 2002). Insistence on formal definitions may be unnecessary in many cases; however, these terms often appear as a part of the conclusions that state that the species is/is not seed/dispersal/microsite/habitat/recruitment limited. Use of these terms sounds very conclusive, but may have very different meanings in different studies.

In this study we first aim briefly review the current uses of these terms and the experimental approaches that are used to assess these limitations. Further we identify the basic concepts involved in the discussion of a *limitation*. In most cases this goes back to simple concepts of classical population dynamics. Then, we try to link these concepts with the existing terms. Finally we consider the different experimental approaches and evaluate whether they are suitable way to test the processes described by these concepts.

The terms

Dispersal limitation

Term dispersal limitation was first mentioned by Harrison et al. (1992) and was used as a general term to describe limited ability of species to disperse over large spatial scale. According to Eriksson (1998) dispersal limitation appears when recruitment is equally good at habitats with and without established populations; similarly Eriksson (1998), Ehrlén and Eriksson (2000) and Gustafsson et al. (2002) define dispersal limitation as a situation when successful germination and establishment can be found at unoccupied patches. Other papers use relationship between distance from the seed source or age of the habitat and species occurrence, or spatial aggregation of species to test for dispersal limitation (Table 1). Even though most of these studies consider patterns of seedlings or adult plants, some studies are concerned with seeds only (e.g. Muller-Landau et al. 2002).

Seed limitation

Term seed limitation first appeared in 1992 in papers by Eriksson and Ehrlén (1992) and van der Meijden et al. (1992). In the former paper seed limitation is one of two components of recruitment limitation. Higher recruitment at patches with added seeds compared to patches without seed addition within occupied habitats is a proof for this limitation. According to the latter paper seed limitation includes all factors secondary to availability of habitats suitable for germination and establishment (safe-site limitation) namely those limiting survival, growth and seed production. In their review on seed limitation of plant populations, Turnbull et al. (2000) define seed limitation as increase in population size following seed addition regardless the fact whether the habitat was originally occupied or not. According to Dalling et al. (2002), seed limitation is a general term expressing lack of seeds at a site. This can be due to source limitation, i.e. limitation by the amount of seeds produced and dispersal limitation, i.e. limitation by the ability to disperse. Other studies using this term usually compare seedling number at habitats with naturally or experimentally varying seed densities (Table 1).

Microsite limitation

Term microsite limitation comes from Eriksson and Ehrlén (1992) paper and seems to be the most confusing term (Table 1). According to them seed and microsite limitation are two aspects of recruitment limitation. Presence of microsite limitation is defined as increase of germination after disturbance assuming that disturbance is the important factor limiting recruitment of a species. Only four out of the eleven papers using this term follow this definition and compare seed germination between disturbed and undisturbed habitats or habitats with different management regimes. According to Grieshop and Nowierski (2002) microsite limitation is a term denoting competition for safe sites for germination which is studied by assessing seed germination and seedling establishment after seed addition and herbivore exclusion; it is an opposite to seed limitation. Ackerman et al. (1996) also define microsite limitation as an absence of suitable microsites for extra seeds. Hilton and Boyd (1996) studied microsite limitation by comparing performance of seeds added to occupied and unoccupied habitats. Their definition is thus an exact opposite of dispersal limitation sensu Eriksson (1998). Zamora (2002) use term microsite limitation to express the observation that there is a low number of suitable microsites for the species of interest.

Even though not mentioned by the authors, we consider terms safe-site limitation (van der Meijden et al. 1992), or niche-based limitation (Plotkin et al. 2002) synonyms to microsite limitation.

Habitat limitation

Habitat limitation is the second most common term in zoological studies. In plants it has been first used by Douglas and Illingworth (1998) to describe lack of available habitats for species growth at the regional scale. According to Mulligan and Gignac (2001) habitat limitation is lack of suitable unoccupied habitats determined by a transplant experiment, Singleton et al. (2001) and Butaye et al. (2002) use this term to describe reasons for absence of species in newly established forest fragments.

Recruitment limitation

First use of the term in plants comes from the transplant experiment of Garbary et al. (1991) who use term recruitment limitation to describe successful transplantation of the species into unoccupied habitats, e.g. in the sense of dispersal limitation as defined by Eriksson (1998). Eriksson and Ehrlén (1992) use recruitment limitation as a general term that includes both seed and microsite limitation. Tilman (1997) uses the term to describe successful seed addition into an occupied habitat, i.e. in the sense of only seed limitation of Eriksson and Ehrlén (1992). Other authors (e.g. Barrett and Silander 1992, Ribbens et al. 1994, Wright and Steinberg 2001, Bruna 2003) use this term to express importance of seed production and dispersal compared to the rest of species lifecycle based on observation of population dynamics being closest in the meaning to the original definition used in marine biology. Other uses of the term include effect causing spatial aggregation of individuals (Svenning 2001), i.e. dispersal limitation by other (see above), spatial or temporal variation in recruitment (Clark et al. 1998, Conell and Green 2000, de Steven and Wright 2002) termed seed limitation above and a general term for low recruitment (Symstad and Tilman 2001). According to Verheyen and Hermy (2001) and Honnay et al. (2002) recruitment limitation is absence of recruitment due to unsuitable habitat, i.e. microsite limitation in view of others.

Term recruitment limitation is also used in theoretical literature in models explaining maintenance of local species diversity (Hurtt and Pacala 1995). In this framework recruitment limitation is defined as the failure of the species to have any viable juveniles at an available habitat.

The concepts

First, it should be made clear that we are interested only in limitation on population size (not on population growth rate). Population size reflects changes in population growth rate; at any moment of time however, population size is the variable that is most readily observed. In any local population, population size may be limited either by habitat carrying capacity or by the intrinsic ability of the population to grow.

In the former case, the population is at its carrying capacity, i.e. its population growth rate equals exactly zero and the zero population growth rate is maintained by a feedback process. This means that the habitats are fully saturated by individuals. In such a case, the population size would change only if the habitat structure changed (e.g. if more microsites became available). Adding individuals to the population (such as sowing seed) will not have any effect on the total population size in the long run.

A contrasting situation occurs when the habitats are not fully saturated by individuals. In such a case the population is in transient state and its size is limited by the ability of the population to grow. Such population has not yet managed to attain its maximum density at its habitats due to disturbance, very low population growth rate or similar adverse processes. Adding individuals to the population (such as sowing seed) is thus likely to have an effect on the total population size.

In fragmented landscapes, these two factors can act at two distinct scales, at the local scale (e.g. at a single occupied habitat as discussed above) and at the regional scale, when one takes into account also constraints of the physical space. Whereas population size is the prime parameter of interest at the local scale, proportion of occupied habitats is the interesting parameter at the regional scale.

At the regional scale limitation by carrying capacity thus means that all suitable habitats in the landscape are occupied; further increase of number of populations is possible only by making new habitats available for the species. The limitation by ability of population to grow in spatially extended case is a result of restricted seed dispersal, due to which some habitats have not (yet) been colonized by the species. Further increase in number of populations of the species can be achieved by enhancing dispersal of the species.

Using this distinction, we may identify four major concepts given by combination of limitation by environment vs. ability to grow and spread, and the two spatial scales (the local and the regional scale) (Table 2). The two concepts at each spatial scale are expected to form a gradient of all possible combinations of the two respective types of limitations.

(A) Local patches are not fully saturated by seeds

The main idea of this concept is that species distribution is primarily limited by seed availability at the local scale. This is a non-equilibrium situation of a population with a low long-term population growth rate. This may be either due to low seed production of the species, or due intensive seed or seedling predation. We propose that this concept is called **seed limitation**.

Two types of evidence are presented in literature as evidence for this concept: (A1) number of seedlings increases after adding extra seeds or excluding herbivores feeding on seeds or seedlings, (A2) number of seedlings varies between patches and years.

Evidence (A1) can be obtained from an experiment in a single population. The conclusion on seed limitation then refers to that population and can differ between populations. Contrary to this, evidence (A2) relies on a comparative approach, and only comparing more populations or more years enables drawing any conclusions. It should take into account size and quality of the habitat in each population and each year as the differences can be not only due to seed limitation but also due to microsite limitation (see bellow), i.e. due to the fact that different habitats in different years can support different maximum seedling densities. Differentiation between these two options is however not possible without an additional seed sowing experiment which provides evidence (A1).

(B) Local population size is limited by availability of opportunities for establishment/growth

This concept refers to an equilibrium situation of population at its carrying capacity (i.e. growth rate = 0). We propose that this concept is called **microsite limitation**.

Three types of evidence related to this concept can be found in the literature: (B1) no increase after seed addition is observed, (B2) maximum number of seedlings is reached when adding seeds in different densities, (B3) number of seedlings at a patch is related to disturbance regime, management or other habitat conditions.

The evidence (B1) uses the same approach as evidence (A1). While evidence (A1) is positive for a gradient from full seed limitation to partial seed limitation, evidence (B1) is positive only at the extreme case of full microsite limitation. This inconsistency is solved by the evidence (B2). This evidence is a search for shape of the relationship between number of seeds added and number of resulting seedlings. Finding non-linearity in the relationship is the proof of a combination of seed and microsite limitation, and evidence (B2) is thus the method to determine the position of the species on the seed-microsite limitation gradient.

The main idea of evidence (B3) is that population size of the species is primarily limited by availability of suitable microsites for seed germination and seedling establishment. This can be changed by modifying conditions for seed germination and seedling establishment, e.g. by disturbance (i.e. by reducing above ground competition) or other treatment such as mowing or fertilizing. This evidences is crucially dependent on the selected treatment and identification of the most appropriate one is often not trivial. Identification of such treatment is commonly regarded as an evidence that it is the microsite and not seeds what limits population size of the species. As mentioned above we define full microsite limitation as one end-point of the seed-microsite limitation gradient, as such definition the only consistent way to approach the limitation issue. Contrary to this, evidence (B3) brings in another dimension of microsite limitation. This evidence depends on the management regime considered. It can be expected that for any species there may be an optimal management regime given all possible alternations of the locality (e.g. turning the locality into a fully managed garden). Due to this, conclusion of evidence (B3) is predetermined by the management regime selected. Contrary to (B1) evidence failure to prove microsite limitation in this sense thus does not mean that the species is seed limited, but that we failed to find the appropriate management regime. Therefore we suggest that this evidence should be framed as search for optimal management regime of the population, rather than a search for evidence of microsite limitation in the species, and should be excluded from the limitation concepts.

(C) Landscape is not fully saturated with species

The main idea of this concept is that species distribution is primarily limited by seed availability at the landscape level. This is again the non-equilibrium situation of a species whose number of populations is limited by a very low ability to spread over space. We propose that this concept is called **dispersal limitation**.

The reviewed papers provided four types of evidence for this concept: (C1) adding seeds to unoccupied habitats results in successful germination and establishment, (C2) species does not occur at habitats that are newly created or distant, (C3) distribution of species is clustered and it is not due to any underlying environmental factor and (C4) seed densities and seedling recruitment is correlated with distance from seed source.

Evidence (C1) and (C2) are concerned with fragmented landscapes with clearly distinct habitats, contrary to this evidence (C3) and (C4) are applicable also in continuous habitats at any spatial scale.

Evidence (C1) is the direct proof of the metapopulation concept that unoccupied habitats do exist (Hanski 1997). Evidence (C2) is a natural experiment searching for evidence similar to (C1). In contrast to evidence (C1), this evidence concerns established individuals and thus does not suffer the problem of working only with seedlings which is a common drawback of (C1) evidence. The basic difference in evidence (C1) and (C2) is that the prime interest of evidence (C1) lies in identification of suitable unoccupied habitats with the notion of metapopulation theory that at any moment there are some habitats that are suitable but unoccupied, regardless on the reason of the absence, evidence (C2) concerns only a special case of absence due to large distance from the source or short time since becoming available. Important aspect of evidence (C2) is that it is necessary to proof that the unoccupied habitats are unoccupied really due to dispersal limitation and not due to different environmental conditions. This is not easy as the range of environmental conditions that can be measured is very wide and there is no good way how to decide which are the important ones. Attempt to gain evidence (C1) is a solution of this problem; it however discards the usefulness of evidence (C2) alone.

Evidence (C3) can be regarded an extension of evidence (C2) for continuous landscapes. Evidence (C4) is a special case of (C3). While evidence of seed limitation at the local scale is concerned only with seedling or adult plant numbers, evidences for dispersal limitation may include both grown plants and seeds (evidence C4). However difference in number of seeds between plots does not necessarily imply differences in resulting number of seedlings. Therefore conclusions on importance of seed distribution for species distribution can be drawn only after considering habitat suitability. This is however fully feasible only using evidence (C1).

(D) Regional distribution of species is limited by availability of suitable habitats

The main idea of this concept is that species distribution in the landscape is limited by availability of suitable habitats. This is the equilibrium situation of species occupying all suitable habitats in the landscape. Number of populations of such species can be increased by increasing number of suitable habitats. We propose that this concept is called **habitat limitation**.

Evidence for this concept is usually mixed with evidence for microsite limitation. In the literature we found two types of evidence related to this concept: (D1) that species occurrence depends on abiotic factors of the habitats, or altering the conditions can make the habitat suitable and (D2) that species does occur at all suitable habitats.

Evidence (D1) is extension of evidence (B3), that applying appropriate management can increase size of the population, to the regional scale. Similarly to this it is crucially dependent on the management applied or range of habitats considered. Taken to the extreme, this is always true as no terrestrial species will grow in water and any unsuitable place can be turned into suitable given enough time and money. Therefore, using the same argument as for evidence (B3), we suggest that this evidence should be rather framed as the search for habitat requirements of the species or appropriate landscape management, and should not be mixed with the limitation concepts.

Evidence (D2) is evidence for the full habitat limitation being one of the extreme ends on the dispersal-habitat limitation gradient. Contrary to seed-microsite limitation gradient (evidence B2), there is no specific method to determine position of the species on the dispersal-habitat limitation gradient. However we suggest that proportional habitat occupancy, determined using (C1) evidence, is a possible solution.

In most sowing experiment studies any number of seedlings at unoccupied habitats is considered as evidence for dispersal limitation in the species (e.g. Ehrlén and Eriksson 2000). However the absolute number of seedlings between occupied and unoccupied habitats may significantly differ (Münzbergová unpubl.). Then equal number of seedlings at both types of habitats may be considered pure dispersal limitation, while lower but non-zero number of seedlings at the unoccupied habitats could be indication of combination of dispersal limitation at the regional scale with microsite limitation at the local scale (as discussed at evidence A2).

Restrictions of the concepts

The concepts presented here rely on strict distinction between local and regional scale. Local scale in our definition refers to a homogeneous area occupied by continuous population of the species of interest. Regional scale is usually concerned with clearly distinct suitable habitats surrounded by matrix of unsuitable habitats. The regional scale concepts can be however applied also to continuous large habitats, given that distribution of the species is patchy rather than continuos. The habitats can be of any size, so we suggest applying the regional and not the local scale concepts also to what could be called patchy population (i.e. homogeneous habitats with patchy occurrence of the species of interest), and to keep the local scale concepts only for continuos stands of the species.

Most types of evidence mentioned above are based on results of seed sowing experiments. The conclusions regarding total population size and habitat occupancy based on these are therefore restricted by the fact that only the initial stages and not the full lifecycle are usually captured, as development of most plant species is very slow (Ehrlén and Eriksson 2000 and Gustafsson et al. 2002). This problem might be solved by using indirect approaches that compare actual distribution of species (e.g. evidence C2). However such data include other possible confounding factors and the seed sowing method is thus the best available option for determining these limitations.

Conclusions

Our analysis of the concepts of limitations for population size and species distribution differentiated patterns at the local and regional scale.

At the local scale gradient from full seed limitation to full microsite limitation can be defined, with each population lying somewhere in between. While full microsite limitation is feasible in the field (in case of strong density dependence), full seed limitation is only a hypothetical point, however newly established populations are very close to this situation. Seed addition into an existing population is the only appropriate evidence to differentiate between seed and microsite limitation, adding seeds in different densities enables identification of the position of the population on the seed-microsite limitation gradient. Effect of experimental modification of habitat conditions that is commonly used as evidence for microsite limitation should rather be framed as search for appropriate management regime of the population, and not be included in the limitation framework.

Similarly to local scale a gradient between dispersal and habitat limitation can be defined at the regional scale. Proportional habitat occupancy may serve here to determine position on the gradient. Seed introduction experiment is the most appropriate evidence for this distinction. Similarly to local scale we suggest that evidence (D1) that species occurrence depends on abiotic factors of the habitats, or that altering the conditions can make the habitat suitable, is not considered habitat limitation, and is rather framed as the search for habitat requirements of the species or appropriate landscape management.

The distinction between seed and microsite limitation makes sense in each homogenous population of the species, and may differ between populations. Similarly conclusions on dispersal-habitat limitation are bounded to the area and spatial scale considered and shifting to another area and spatial scale may result to very different conclusions. Therefore description the population or the area and scale is a necessary part of any limitation study.

This study defined four out of the five limitation terms mentioned in the introduction, skipping the explicit definition of recruitment limitation. As briefly mentioned in Table 1, we suggest that recruitment limitation is retained as a general term describing low seed germination and seedling establishment relative to other parts of species lifecycle sensu Hixon (1998). Even though it is not the case in most studies using this term, we suggest that explicit analysis of the whole species life-cycle should be the basis for such conclusion.

Appendix: Methods

To determine the context in which these terms are used we searched Web of Science for papers using these terms. From all the papers found we excluded papers in which the key term was only used as a key word plus, i.e. not mention by the authors. Further we excluded those that were concerned only with animals; this meant excluding 89 papers mainly concerned with recruitment limitation in marine ecosystem. In all the other papers (107) we checked what type of evidence for any type of limitation the paper provides. Then we grouped these types of evidence into categories and assigned each paper to one or more of these categories. Further we divided the papers according to the term (type of limitation) used to describe the result. Separately we counted papers in which the term was used without any evidence provided.

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Figure 1. Number of papers using the limitation terms over time. Studies concerned with habitat limitation are not shown in the graph as there are only four plant ecology studies using this
term.

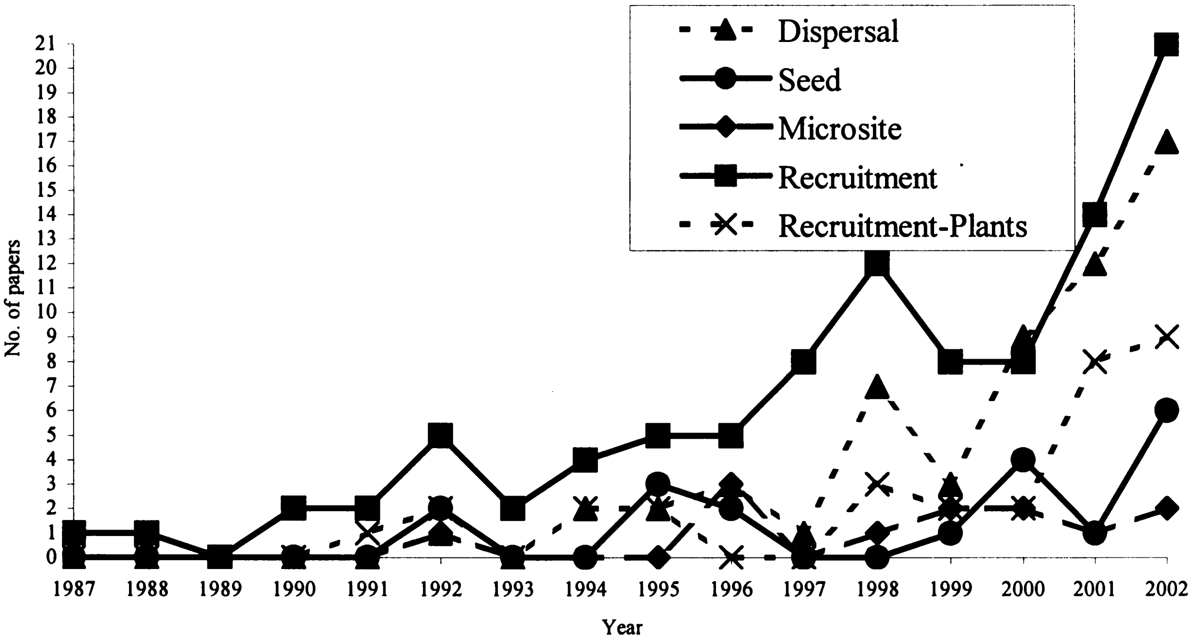
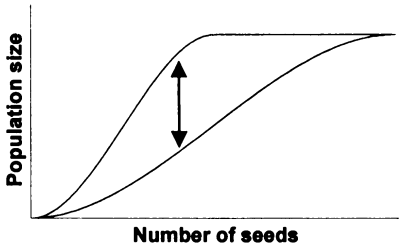
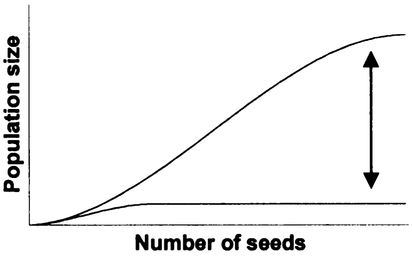


Table 1. Summary of the literature search determining the published evidence for different types of limitations and the term used to described that limitation in the paper. Codes in brackets behind the evidence refer to codes in the text. Figures in the table show number of studies of each kind. Last collumn gives the suggested term for that kind of evidence.

Published evidence	Recruitment limitation	Dispersal limitation	Seed limitation	Microsite limitation	Habitat limitation	Spatial scale	Suggested term
Number of seedlings increases after adding extra seeds or excluding herbivores feeding on seeds or seedlings. (A1)	4		16			local	Seed limitation
Number of seedlings varies between patches and years. (A2)	3		3			local	Doubtful - see text for explanation
Absence of increase of population size after seed addition. (B1)				2		local	Microsite limitation
Maximum number of seedlings is reached when adding seeds in different densities. (B2)				2		local	Determination of position on seed-microsite gradient
Number of seedlings at a patch is related to disturbance regime, management or other habitat conditions. (B3)	1			9		local	Not part of limitation concepts - see text for explanation
Adding seeds to unoccupied habitats results in successful germination and establishment. (C1)	1	14	2			regional	Dispersal limitation
Species does not occur at habitats that are newly created or distant (C2)	1	13				regional	Doubtful - see text for explanation
Distribution of species is clustered and it is not due to any underlying environmental factor. (C3)	1	4				regional	Doubtful - see text for explanation
Seed densities and seedling recruitment is correlated with distance from seed source. (C4)	4	3				regional	Doubtful - see text for explanation
Species occurrence depends on abiotic factors of the habitats. (D1)	2			3	3	regional	Not part of limitation concepts - see text for explanation
Species does occur at all suitable habitats. (D2)					1	regional	Habitat limitation
Seed germination and seedling survival are much lower than any other transitions in plant demography	4					local	Recruitment limitation
General term	23	23	1			?	?

Table 2. Classification of the four limitation terms according to the process of interest and spatial scale.

Scale		
Local	Seed limitation	Microsite limitation
Regional	Dispersal limitation	Habitat limitation

V.

Factors limiting distribution of dry grassland species at different spatial scales

Zuzana Münzbergová

Summary

1. Distribution of species in fragmented landscapes is a result of combined seed and site availability at different spatial scales. While a lot is known their importance on one spatial scale, it is not known how the importance differs among different spatial scales.

2. Importance of site availability implies that species distribution should depend on environmental conditions of the locality. Therefore relationship between species distribution and environmental conditions of the localities is often sought in this context. While there is a lot of information on the effect of environmental factors on spatial pattern of both seedling establishments in sowing experiment and distribution of adult plants, the correspondence between seedlings and adults is unclear. Also nothing is known on the effect of spatial scale on this relationship.

3. I sowed seeds of seven species of dry grasslands into twenty-two localities that differed in occupancy by these species. I followed seedling establishment of these species over three years and compared number of emerging seedlings at three scales: between occupied and unoccupied localities, between occupied and unoccupied blocks within occupied localities and between plots with and without seed addition within occupied blocks. Furthermore, I related the number of seedlings and distributions of adult plants at the two larger scales to environmental factors.

4. Both seed and site availability are important in structuring the distribution of these plant species. Their relative importance however depends on the spatial scale considered. Also the relationship between environmental factors and pattern of seedling recruitment and adult occurrence is clearly scale dependent.

5. The result imply that conclusions on importance of seed and microsite limitation in natural communities is scale dependent and the studies of these thus need to explicitly considered it.

Key-words: community structure, seed dispersal, species diversity, distribution patterns, grassland, limitation, metapopulation dynamics, patch occupancy, perennial herbs, sowing experiment

Introduction

Pattern in species distribution is determined by the balance between colonization and extinction (Mac Arthur and Wilson 1967, Grubb 1977, Tilman 1993, Hanski 1997). The ability to colonize a site consists of two groups of traits: a species ability to reach the site and the species ability to establish there. These two groups of traits are reflected in two groups of hypotheses. One of them is assuming that species communities are unsaturated and that species diversity is limited mainly by availability of the seeds (Eriksson 1996, Eriksson & Ehrlén 1992, van der Meiden et al. 1992, Hanski 1997, Ackerman et al. 1996, Ehrlén & Eriksson 2000, Foster and Tilman 2003). The other assumes that communities are potentially saturated and it is the local biotic interactions and site availability that primarily determine species diversity (Grubb 1977, Brown 1984, van der Maarel & Sykes 1993, Forster 2001).

Species distribution in a landscape is a result of these processes at two basic spatial scales, local and regional. At both of these scales, both processes related to availability of seeds and sites for germination play a role. At the local scale these processes include seed production, seed predation and density dependence, at the regional scale these local scale processes further interact with seed dispersal ability and habitat availability at the landscape level. Because there is no a clear link between the different scales, the conclusions on the importance of seed and site availability are expected to be scale dependent. Recently an increasing number of studies have used sowing experiments to determine the relative importance of seed and site availability for single species distribution (e.g. Houle & Phillips 1989, Eriksson & Ehrlén 1992, van der Meiden et al. 1992, Ackerman et al. 1996, Ehrlén & Eriksson 2000, Turnbull et al. 2000, Foster & Tilman 2003). Most studies have, however, carried out the sowing experiments at only one spatial scale, and the relative importance of different types of limitations at different spatial scales is still largely unknown (Ehrlén & Eriksson 2000).

Importance of site availability implies that species distribution should depend on environmental conditions of the locality. Therefore the ultimate aim of many sowing experiments is to identify environmental factors that affect germination success of the species (e.g. Burke & Grime 1996, Ehrlén & Eriksson 2000, Franzén 2002). These can then serve to explain species distribution patterns as well as to identify suitable unoccupied habitats for metapopulation studies. Identification of such factors is, however, also not straightforward. First, it has been repeatedly shown that habitat requirements of seedlings can differ from those of adult plants and that critical phases may appear only later in the species development (Losos 1995, Ehrlén & Eriksson 2000, Gustafsson et al. 2002). Separate information on either seedlings or the adults thus will not fully capture the real effect of the factors. Second, many plant species are able to modify their environments; therefore, differences in the abiotic and biotic factors between the occupied and unoccupied habitats may be a consequence, and not the cause, of the difference (e.g. Charley & West 1975, Franco-Pinaza et al. 1996, Münzbergová & Ward 2002). Even though there have been many attempts to relate seedling numbers and adult distribution to environmental variables (e.g. Ehrlén & Eriksson 2000, Verheyen & Hermy 2001), these have often been done in separate studies and the relationship between these two is often unknown (but see Howard & Goldberg 2001). Again, the difference between seedling and adult relationship to environment may depend on spatial scale.

The objective of this study is to answer the following questions: (i) What is the importance of availability of seeds and sites for species distribution at the three spatial scales (at a patch, between patches within occupied localities and between localities)?

(ii) What is the difference in the relationship between seedling numbers and environmental factors at different spatial scales? (iii) What is the difference in the pattern in (ii) between seedlings and established plants at each scale?

To answer these questions I performed a sowing experiment using eight dry grassland species. I followed seedling recruitment of these species at twenty-two localities over three years. Recruitment success was compared at three spatial scales, between localities occupied and unoccupied by focal species, between blocks occupied and unoccupied by focal species within occupied localities and between plots with and without seed addition within occupied blocks. Seedling recruitment and adult distribution was also related to environmental parameters and actual species occurrence at the block and locality scales.

The vegetation type used in this study, dry calcareous grassland, has been the subject of many recent studies on the importance of site and seed availability for species diversity on the local scale (e.g. Hutchings & Stewart 2002, Kalamees & Zobel 2002, Tofts & Silvertown 2002). Identification of the importance of these effects at the regional scale as well as the difference between the scales can thus contribute not only to knowledge of scale dependency in general, but also to specific issues of conservation of these dry grasslands.

Methods

Studied area

The study was carried out in northern Bohemia, Czech Republic. The region is characterized by the occurrence of chalk grasslands developed on Tertiary marine sediments. Their geology and geomorphology (usually steep slopes on foothills) results in low water permeability and continuous erosion. The localities form distinct patches surrounded mainly by agricultural fields. All these localities have been abandoned for at least the last 30 years. The region including all the study localities was approximately 870 km^2; the distance between the two most distant localities was 41 km.

Study species

Eight species were used for the study. The species were selected to meet the following criteria: (i) the species are restricted to chalk grasslands in the study region, i.e., they do not occur at any other habitat type, (ii) the species are not very common, so suitable unoccupied localities are likely, (iii) the species are not too rare, so that it was possible to collect enough seeds for the experiment without affecting the source populations. The selected species were *Anemone sylvestris*, *Aster amellus*, *Coronilla vaginalis*, *Cirsium pannonicum*, *Globularia punctata*, *Linum flavum*, *Linum tenuifolium* and *Scorzonera hispanica.* All the species are perennial forbs; details on the biology of the species are in Table 1. Nomenclature of the species follows Tutin et al. (1964-1980).

Sowing experiments

The sowing experiment was established at twenty-two different localities in the study area (Table 2). The localities were selected subjectively to cover the whole range of types of chalk grasslands that could be found in the region.

At each locality I subjectively selected five distinct patches of homogenous vegetation and established one block in each of these. Each block consisted of four 1 m^2

plots, separated by 1 m, that were divided into nine subplots 0.33 m × 0.33 m for a total of 36 subplots in each block. These subplots were randomly assigned to nine different treatments, sowing of one of the eight species or control, to yield four subplots with each treatment. The experiment was started in two subsequent years, 2000 and 2001, with half of the subplots, two for each treatment, used in each year.

Seeds for the experiment were collected from two source populations for each species. Seeds from different source populations were used as the two replicates within each block in each year. *Coronilla* and *Linum flavum* were exceptions, with only one source population each. In each subplot I sowed 100 seeds (50 seeds for *Coronilla* and *Scorzonera*) in late September either in 2000 or in 2001. The seeds were sown into undisturbed vegetation. Germination and survival of seedlings were followed yearly in late May/early June from 2001 to 2003. At each census, I counted the number of target seedlings in the subplot as well as the number of seedlings of the other study species (these values served as controls). The number of seedlings of all studied species was also counted in the control subplots. Controls at the unoccupied localities enabled estimation of possible secondary dispersal of seeds between the subplots that could have affected the results. No secondary dispersal was detected for any of the species. In 2003, each seedling in the plots was assigned into one of three size categories of equal intervals. The categories were defined by leaf length (*Aster*, *Cirsium*, *Globularia*, *Scorzonera*) or plant height (*Coronilla* and both *Linum* species).

Environmental parameters

At each locality I recorded a set of parameters to capture the most important differences between localities and blocks. They were also expected to serve as correlates of past management regimes of the localities, because they can be both its cause (vineyards are always at south or south west facing slopes) and its consequence (altered productivity and physical soil properties).

Parameters at the block scale were above-ground biomass collected in 15 cm × 15 cm plots at peak standing crop, inclination, aspect, soil water holding capacity, bulk density, species composition and percentage of bare ground. Each parameter was measured four times per block, once at each of the four 1-m^2 plots. In further analysis means of these values for each block were used. Data on inclination and aspect were used to calculate potential direct solar radiation. It was done by summation of cosines of angles between sun and the locality surface over the whole day with an interval of 15 minutes. The calculation was done for the 21st day of each month between December and June. As some of these values are highly correlated, only data on direct radiation in January, June and average over the whole period were used. Soil water holding capacity was measured as the amount of water bound in the soil monolith after it stood on a constantly wet filter paper for 24 hours. It was expressed as amount of water retained per gram of dry soil. Parameters at the locality scale were presence of the target species and total species composition of the locality. I also used mean values of the block scale parameters to describe between locality differences.

Data on species composition of both the localities and the blocks were used to calculate probability to encounter the species at a locality using Beals index of sociological favourability (Beals 1984, further called Beals index value, see Münzbergová & Herben 2004 for its use). This index calculates probability to encounter a species at a locality using data on presence of other species at that locality and information on patterns of co occurrence of the target species with other species. To estimate the species co occurrence patterns, I used 2984 relevés on species composition of dry grasslands in the Czech Republic from the Czech national phytosociological database (Chytrý & Rafajová 2003). Data on species composition at both spatial scales were also summarized using detrended correspondence analysis (DCA) using the program CANOCO (ter Braak & Šmilauer 1998). DCA is a multivariate technique assuming bell-shaped species distributions along an underlying environmental gradient that enables one to extract a few ordination axes that summarize maximum variation in species composition. Positions of the samples on the 1st and 2nd ordination axis (later referred to as AX1 and AX2) from this analysis were used as additional environmental variables in the analysis; these are used as data on summarized species composition of the localities and blocks. The AX1 and AX2 was calculated for each focal species separately, with that species excluded from the analysis.

Parameters of the species

To be able to interpret the observed differences between the species, I collected data on life history traits. The traits sampled were traits related to seed production, seed germinability, seed bank, and seed dispersal. To estimate species ability to survive in the seed bank 50 nylon bags each containing 100 seeds were buried at three localities in late September 2000. Ten bags were buried at each of five different places at each locality. Ten bags (two from each place) were excavated in late September in 2002 and 2003 and the seeds were checked for viability. Viability of these seeds was estimated by putting all seeds from one bag into a petri dish. The seeds were regularly watered with distilled water and kept under a fluctuating regime (12 hours light at 20 °C, 12 hours dark at 10 °C) in a growth chamber. Germinated seeds were regularly removed. The dishes were kept until all the seeds germinated or decayed (approximately six months). The same procedure was used to estimate the viability of fresh seeds in the first year.

This value was then used as a starting value to estimate the decline in seed germination rate over time. Seed weight was estimated by weighing ten groups of ten seeds from three source populations. Seed production per square meter was estimated as number of flowering plants per square meter counted in five quadrates in each of three populations of the species, and multiplying it by seed production per plant estimated in these three populations.

To estimate species dispersal ability, I estimated each species' terminal velocity and attachment ability. Data on terminal velocity for *Aster*, *Coronilla*, *Globularia* and *L. tenuifolium* are taken from the DIASPORUS database (Bonn et al. 2000), the terminal velocity of the others was measured using the method described in Tackenberg et al. (2003). Attachment ability, used as an estimate of the ability to disperse via exozoochory was assessed as ability of the seeds to attach and stay on sheep fur (see Fischer et al. 1996). This was measured in the following way: 100 seeds were placed in a plastic tray, the fur was gently placed over the tray and number of attached seeds was counted.

Data analysis

To take into account natural seedlings at the occupied blocks, average number of seedlings of target species in all subplots within block where the target species was not sown was subtracted from the number of seedlings found in the subplot with that species added. All the analyses were done both with and without this subtraction. As there was little difference in the overall patterns between these two types of tests, only results without the subtraction are shown. There was also little difference between results of the experiment started in the two subsequent years, so only results for the experiment started in the first year are presented.

All tests (see below) were done separately at two spatial scales, block and locality; the tests on the factors limiting species distribution were also done on the local scale. Tests of the effect of the environmental variables were done separately for seedlings and naturally occurring adult plants (further referred to as adults). To adjust for reduced number of degrees of freedom in tests at the locality scale, sum of all seedlings or frequency of adults was calculated for each locality and used as dependent variable. All tests including seedling numbers were done using generalized linear model with Poisson distribution, tests evaluating adult occurrence used model with binomial distribution. All tests were performed using S-PLUS (2000).

Altogether only 7 seedlings of *Anemone* were found in all the sowing plots. Therefore this species could not be used in any analysis and is not mentioned further.

Factors limiting species distribution

Results of the sowing experiment in relationship to habitat occupancy were evaluated by comparing the number of seedlings at three spatial scales: (i) at occupied and unoccupied localities, (ii) at occupied and unoccupied blocks within occupied localities, (iii) at subplots with and without seed addition (controls) within occupied blocks. Equal number of seedlings between the two groups in comparison (i) and (ii) was considered an indication of dispersal limitation at that spatial scale. Lower but non-zero number of seedlings in the latter variant in each pair was considered an indication of dispersal and habitat limitation. Absence of recruitment in the latter variant of each pair and not in the former one was considered an indication of habitat limitation. An equal number of seedlings in the two groups in the third comparison was considered indication of microsite limitation, higher number in plots with seeds added is considered an indication of seed limitation.

Terminology

There is little consistency in use of the terms seed, dispersal, microsite and habitat limitation in the literature (Münzbergová & Herben unpubl.). Here I use seed limitation to describe increase of number of seedlings after extra seeds were added into places were the species does occur (sensu Eriksson & Ehrlén 1992) and microsite limitation to describe absence of increase of number of seedlings at occupied places after seed addition (sensu Grieshop & Nowierski 2002). I use dispersal and habitat limitation as alternative terms to seed and microsite limitation defined at the regional scale. A species is dispersal limited if it can establish at sites where it does not occur (sensu Eriksson 1998) and habitat limited if it cannot (sensu Mulligan & Gignac 2001).

The terminology used here strictly relies on a distinction between local and regional scales. Local scale in this definition refers to a homogeneous area occupied by a continuous population of the species of interest and assumes that the dispersal curve is flat over that area. Regional scale refers to distinct patches at least partly isolated from other patches (dispersal curve is not flat). It can be both distinct localities and distinct patches within one locality.

Effect of environmental variables

To identify to what extent it is possible to explain number of seedlings and occurrence of adults by environmental factors at the two regional scales I performed stepwise regression combining back and forward approaches, determined the optimal model and recorded the percentage of total variance explained by that model. This was done for all the environmental parameters together, as well as separately for biotic (Beals index value, AX1, AX2) and abiotic (all the others) parameters. While the percentage of variance explained by the model was not sensitive to definition of the original model, the exact formulation of the optimal model was (cf. Graham 2003). Comparison of the single factors included in the optimal model between the two scales and between seedlings and adults could thus be misleading, and I therefore also tested the significance of single factors. This was done using log-likelihood ratio estimated by comparing a baseline model and model including the term of interest. The baseline model included source of the seeds and year of experiment establishment; for the test of differences in environmental variables between blocks, it also included the locality code. To correct for the high number of tests on the same data the significance level of these tests was adjusted using the Bonferroni correction.

To estimate the consistency of the effect of single environmental variables between the two scales separately for seedlings and adults and between seedlings and adults separately for the two scales, I calculated the Jaccard similarity coefficient for each contrast by comparing the number of parameters having a significant effect in both tests of that contrast and the total number of significant tests in that contrast. The relationships between proportional habitat occupancy and plant traits were tested using linear regression, using single species as data points. Because there are only eight species in this study the test is rather weak and the results should be taken only as an indication of possible patterns that need further exploration.

Results

In the course of the experiments all the species except for *Anemone* successfully germinated and established at least at some localities. Several species also flowered, there were 31 flowering individuals of *L. tenuifolium* in the second and 29 in the third year, there were two *Coronilla* individuals flowering in the second and 20 in the third year, and there was one *Cirsium* individual flowering in the third year.

Factors limiting species distribution

Three different types of pattern of seedling numbers were distinguished at the locality scale. (i) In *Aster* and *Cirsium* there was no difference in number of seedlings between occupied and unoccupied localities in any of the three years (Fig. 1), indicating dispersal limitation, (ii) in *Coronilla*, *Globularia*, *L. tenuifolium* and *Scorzonera* there was a higher number of seedlings at the occupied localities in the first years of the experiment, but the difference disappeared in all of them in the last year (Fig. 1), possibly also indicating dispersal limitation, (iii) in *L. flavum* there was a higher number of seedlings at the unoccupied than at the occupied localities in the second and third year (Fig. 1), suggesting an effect of some other factor determining species distribution.

Four different types of pattern of seedling numbers were distinguished in the comparison between blocks within occupied localities. (i) Higher number of seedlings at occupied than at unoccupied blocks in all three years in *Coronilla* (Fig. 2), indicating a combination of dispersal and habitat limitation, (ii) higher number of seedlings at occupied than at unoccupied blocks only in the first year in *Globularia* (Fig. 2), probably also indicating a combination of both types of limitation, (iii) lower number of seedlings at occupied blocks in *L. flavum* in the second and third year (Fig. 2), probably a result of some other type of limitation, (iv) no difference between occupied and unoccupied blocks in the other species (Fig. 2), indicating dispersal limitation.

Two different types of patterns could be distinguished in the comparison between subplots with seeds added and without seed addition within occupied blocks. (i) Higher number of seedlings could be found at subplots with seeds added than at subplots without seed addition in *Aster*, *Cirsium*, *L. tenuifolium* and *Scorzonera* (Fig. 3), indicating seed limitation, (ii) no difference the between number of seedlings between subplots in *Coronilla*, *Globularia* and *L. flavum* (Fig. 3), indicating microsite limitation.

Effect of environmental variables

Percentage of total variation in adult distribution explained by environmental factors was higher than that for seedlings (Table 3). This difference was especially strong at the block scale. While the abiotic parameters contributed to the variance explained mainly in number of seedlings, biotic parameters contributed much more to explanation of adult species distribution (Table 3). There was no consistent trend in the correlations of seedlings with environmental variables over time, it could be decreasing, increasing as well as be highest in the second year. This pattern also differed between the block and the locality scale (Table 3).

There was little consistency in the significance of single environmental factors between seedlings and adults, and between the two scales (Table 4). The consistency was lowest for the seedling versus adult comparison at the locality scale, and it was the highest between the scales for adults (Table 4).

Overall the Beals index value was the most successful factor explaining number of seedlings and adult occurrence at the two scales; it was significant in 46 % of all comparisons. It was followed by percentage of bare ground (significant in 39 % of all comparisons), bulk density (36 %), biomass (32 %), soil water holding capacity (29 %), vegetation composition on AX1 (21 %), vegetation composition on AX2 (18 %), potential solar direct radiation in June (18 %), mean potential solar radiation (14 %), inclination (11 %) and potential solar radiation in January (7 %), see appendix.

Locality occupancy and species traits

Actual locality occupancy, estimated based on the results of the sowing experiment, varied between 23 and 48 per cent. There was no relationship between this proportion and any of the plant traits measured (for terminal velocity $F_{1,5}$=0.97, p=0.37, for attachment ability $F_{1,5}$=0.95, p=0.38, for seed weight $F_{1,5}$=0.32, p=0.59, for seed production $F_{1,5}$=1.28, p=0.31, see Table 1 for absolute values).

Discussion

Factors limiting species distribution

Seven out of eight species used in this study recruited well both at occupied and unoccupied patches within occupied localities as well as at the unoccupied localities. The patterns of recruitment however differed markedly between the three spatial scales as well as between the species. Hence any conclusion about importance of different types of limitations for species distribution is scale dependent.

At the locality scale three different patterns of seedling numbers could be distinguished. No difference in seedling germination and survival between occupied and unoccupied localities in *Aster* and *Cirsium* can be considered an unequivocal evidence for dispersal limitation. Surprisingly, these are the two species with the best wind dispersal ability in the dataset. In *Coronilla*, *Globularia*, *L. tenuifolium* and *Scorzonera* seedling numbers were higher at the occupied localities in the first year, but this difference disappeared with time. Although this may be also considered as an indication of dispersal limitation, it can be explained as follows. If the occupied localities are better, and if this results in faster development of seedlings growing here, they may reach a phase of high post establishment mortality earlier. In contrast, seedlings at unoccupied localities may just stay very small over the whole period and never reach this phase. In the study system this effect cannot however be fully identified as the species often differ markedly in size between localities, but size does not seem to be a good indicator of plant maturity. In *L. flavum* the relationship between number of seedlings and locality occupancy was negative (higher number of larger seedlings could be found at the unoccupied localities). Similar pattern, i.e. higher recruitment at unoccupied than at some occupied localities was found by Ehrlén and Eriksson (2000) for *Lathyrus vernus.* Possible explanations of this pattern may be either presence of specific pathogens or predators that are present only at the occupied localities, or a correlated change of environmental conditions since establishment of the species at the occupied localities. In the latter case adult distribution would reflect past but not present habitat suitability (see also bellow).

At the block scale, recruitment was predominantly dispersal limited; clear evidence for this limitation was found in *Aster*, *Cirsium*, *L. tenuifolium* and *Scorzonera*. In *Coronilla*, a higher number of seedlings at occupied than at unoccupied blocks provides an evidence for habitat limitation, while dispersal limitation also plays a role in this case. In *Globularia,* a higher number of seedlings was found at occupied blocks in the first year only. This pattern may be interpreted as an indication for dispersal limitation, or using the argument used for *Coronilla*, *Globularia*, *L. tenuifolium* and *Scorzonera* at the locality scale, as an indication of combination of dispersal and habitat limitation. In *L. flavum* there was, as at the locality scale, a higher number of seedlings at the unoccupied than at the occupied blocks.

Within occupied blocks, seed addition enhanced recruitment in most of the species, indicating seed limitation. This is congruent with findings in many other studies (e.g. Cavers & Harper 1967, Clark et al. 1998, Eriksson & Ehrlén 1992, Ehrlén & Eriksson 2000, Jakobsson & Eriksson 2000). On the other hand, absence of this limitation, as observed in *Coronilla, Globularia* and *L. flavum* in this study, has rarely been reported. In *Coronilla* three years of the study may not be long enough to break its dormancy, so that most of the extra seeds may just germinate later (Thompson et al. 1997). However no dormancy was observed in the other two species and the pattern can therefore be regarded as true microsite limitation.

Comparison between different scales demonstrates that conclusions about importance of limitation by seed and site availability for species distribution depend on the spatial scale used. Limitation by site availability became more important with decreasing spatial scale, showing that the spatial scale used can predetermine an outcome of a study. There was however also strong interaction between scale and species. The seven different species used in this study have similar ecology and patterns of distribution. Comparison between them shows the high variation of mechanisms behind the distribution pattern. This weakens the predictive power of patterns of distribution for the underlying processes (e.g. Quintana-Ascencio & Menges 1996, Bastin & Thomas 1999, Dupré & Ehrlén 2002).

Effect of environmental variables

Different environmental factors were important for distribution of seedlings and adult plants at both spatial scales. This discrepancy may be due the fact that (i) small and large plants are affected by different factors, or selective forces appear later in species development (Losos 1995, Ehrlén & Eriksson 2000, Gustafsson et al. 2002), (ii) adult plant distribution may reflect random dispersal events in the past, (iii) adult plants may modify their environment (Charley & West 1975, Franco-Pinaza et al. 1996). Reason (i) and (iii) would predict higher variation explained by environmental factors for adults, whereas reason (ii) would predict higher variation explained in seedlings. At the block scale higher variation explained in adult plants thus supports explanation (i) or (iii). If these explanations hold, the percentage of variation in number of seedlings explained by the environment should increase with time, but at block scale this was true only for *L. tenuifolium*. However since *L. tenuifolium* was the first species to reach maturity, it successfully flowered in several localities already in the second year of the experiment, similar patterns may appear later also in other species. At the locality scale environmental variables explained comparable percentage of variation in seedlings and adults. The percentage was overall higher than that at the block level. Here seedlings of three species, *L. tenuifolium*, *Coronilla* and *Cirsium*, showed an increase of variation explained with time. Since *Coronilla* and *Cirsium* are the species that rank second and third in the number of flowering individuals in the experiment, this pattern again supports the expectation that a similar increase would appear later on also in the other species (see Ehrlén & Eriksson 2000 for a similar pattern).

Variation in seedling numbers was explained mainly by abiotic factors, while variation in presence of naturally occurring adults was much better described by biotic parameters. Similar pattern can be observed also in the results of Beals index value alone, predicting probability of species occurrence based on species co occurrence patterns. It was much more successful in predicting adult occurrence that in predicting seedling numbers. Both these pattern show a clear difference in the importance of biotic versus abiotic parameters for seedlings and adults. This could indicate that different factors affect survival of seedlings and adults and that seedling success can be described by simple abiotic parameters determining basic requirements of all seedlings such as water balance while survival of adults is determined by more complex factors that are captured in species composition. If this were true, one would expect an increase in the importance of biotic parameters over time. In reality it is the importance of abiotic factors that is increasing in several species over time. One explanation for this could be that species composition of the sites is a result of past events or past environmental conditions at the localities that affected establishment of all the species in the past but have already changed (Eriksson 1996). Detailed information on the past habitat structure would be however needed to confirm this.

Studies that relate germination success at the locality scale to environmental variables usually conclude that no such factors can be identified (e.g. Eriksson & Ehrlén 1992, Ehrlén & Eriksson 2000), whereas studies with the motivation to demonstrate effect of environmental factors within localities usually succeed (e.g. Kelly 1989, Peart 1989, Robinson et al. 1995, Tilman 1997, Franzén 2002, Xiong et al. 2003). The reverse pattern, higher success in explaining patterns at the between locality scale, in this study is possibly an outcome of the fact that the localities used in this study cover a much wider range than they usually do in the between-locality comparisons. All the localities are however relatively small and homogeneous so the within-locality differences are on the other hand rather small. This discrepancy demonstrates that importance of different types of limitations and environmental variables presented here are dependent on selection of the experimental localities for the sowing experiment. Larger variation between localities would increase importance of habitat limitation as well as the importance of environmental variables. Similar pattern would have held if the localities used in the study had been more heterogeneous. This scale dependency is usually neglected, but unavoidable, parts of any sowing experiment that have to be taken into account when interpreting the results. This is very important since most published studies of this type do not explicitly state what spatial scale they used, and how relevant this scale was for the question they studied (e.g. Ehrlén & Eriksson 2000, Gustafsson et al. 2002).

Conclusions

This study demonstrated that conclusions about importance of limitation by seed and site availability for species distribution depend on the spatial scale used. Limitation by site availability became more important with decreasing spatial scale, showing that the spatial scale used can predetermine an outcome of a study. Any conclusions of this type should therefore explicitly state what spatial scale was used. The same holds for the interpretation of the effects of environmental variables. Comparison of seven different species with similar ecology and distribution patterns also demonstrates that similar patterns can result from different processes and thus that the current distribution of species cannot be easily used to reveal the underlying mechanisms.

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Figure 1. Seedling numbers at occupied and unoccupied localities over the three years of the sowing experiment. Graph shows mean ± SD. Asterisks above points indicate significant differences between occupied and unoccupied localities in that species and year. Significances were estimated using generalized linear model with Poisson distribution, separately for each species, using sum of seedlings in all plots within one locality as dependent variable. Each test was based on 22 observations.

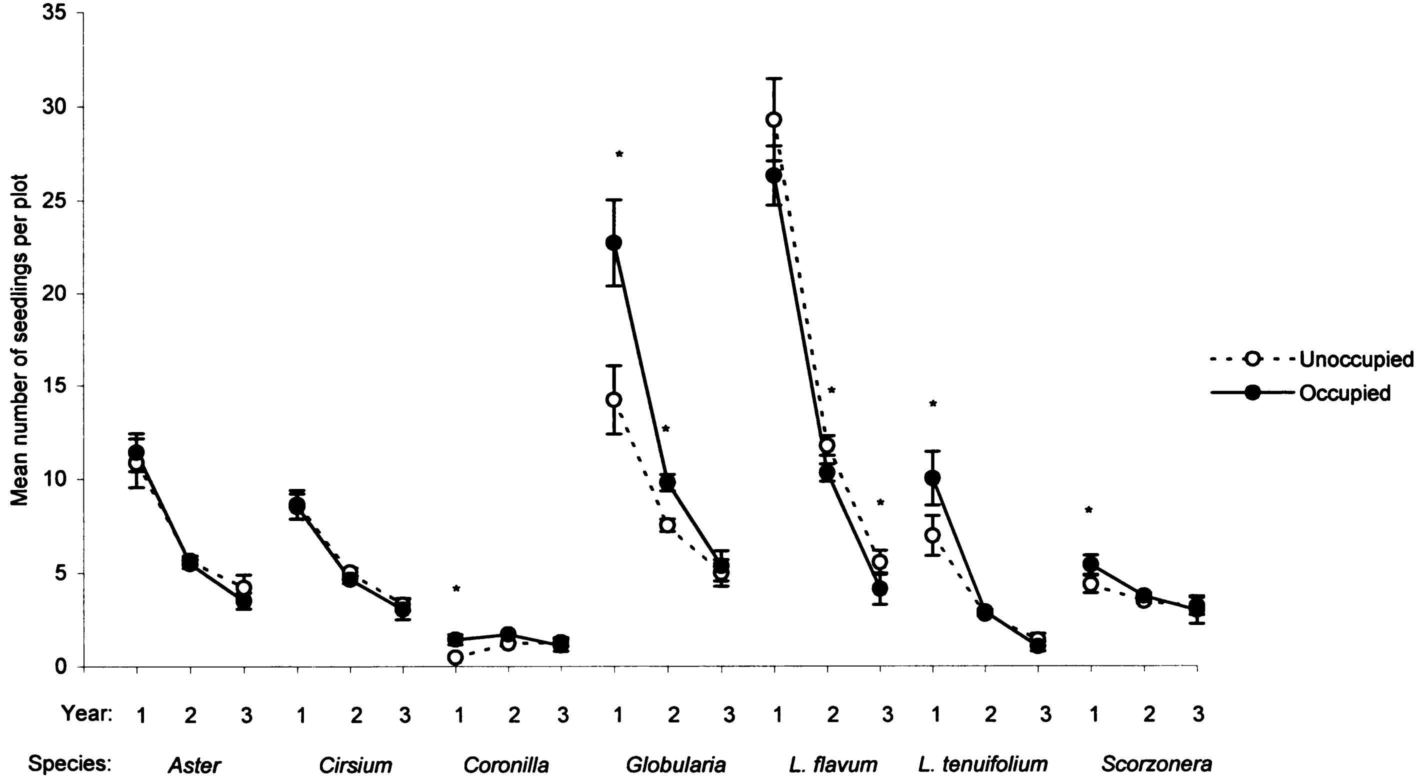

Figure 2. Seedling numbers at occupied and unoccupied blocks within occupied localities over the three years of the sowing experiment. Graph shows mean ± SD. Asterisks above points indicate significant differences significant differences between occupied and unoccupied blocks in that species and year. Significances were estimated using generalized linear model with Poisson distribution, separately for each species, using number of seedlings per plot as dependent variable. There were 90 observations for *Aster*, 50 for *Cirsium*, 100 for *Coronilla*, 50 for *Globularia*, 50 for *L. flavum*, 70 for *L. tenuifolium* and 70 for *Scorzonera*.

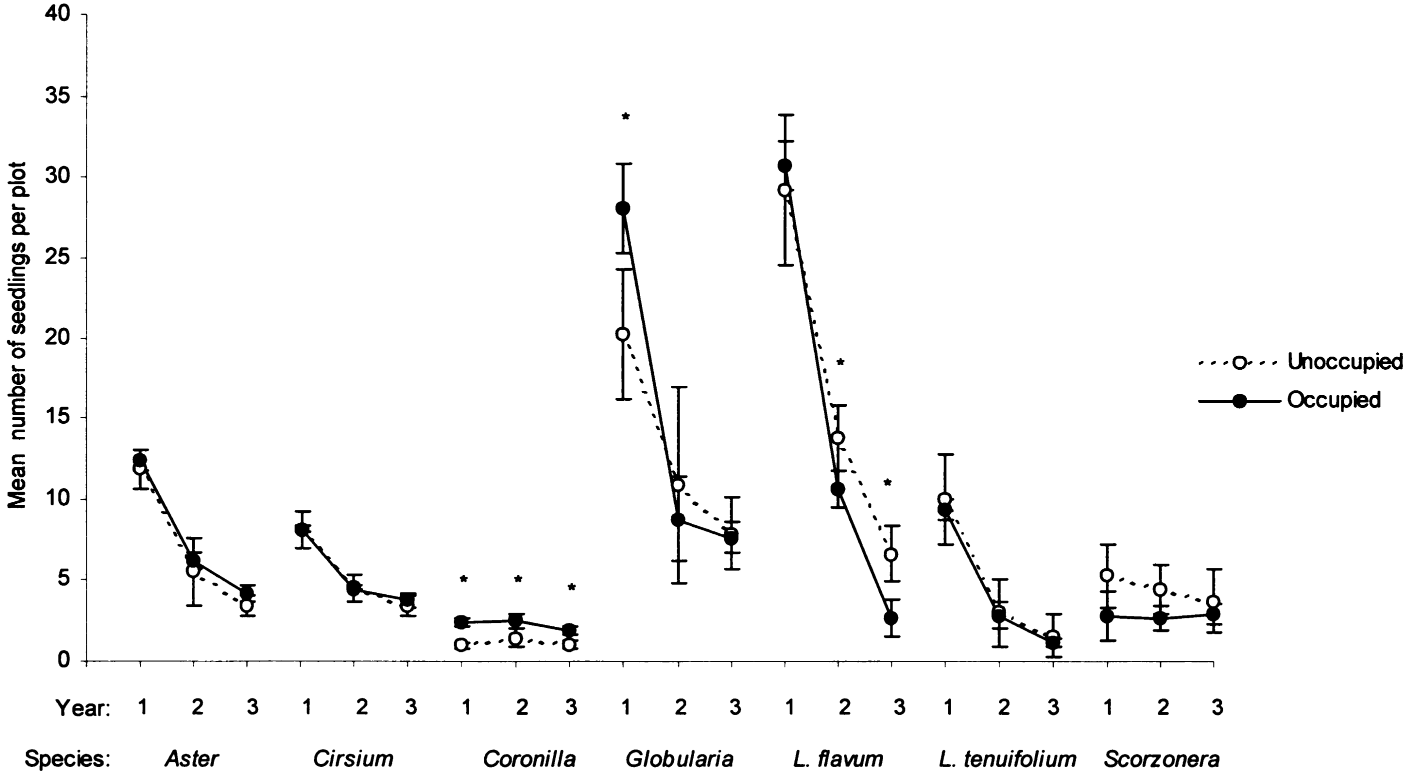

Figure 3. Seedling numbers at plots with seeds added and without seed addition (controls) within occupied blocks over the three years of the sowing experiment. Graph shows mean ± SD. Asterisks above points indicate significant differences between plots with seed added and without seed addition within occupied blocks. Significances were estimated using generalized linear model with Poisson distribution, separately for each species, using number of seedlings per plot as dependent variable. There were 22 observations for *Aster*, 14 for *Cirsium*, 36 for *Coronilla*, 26 for *Globularia*, 20 for *L. flavum*, 24 for *L. tenuifolium* and 34 for *Scorzonera*.

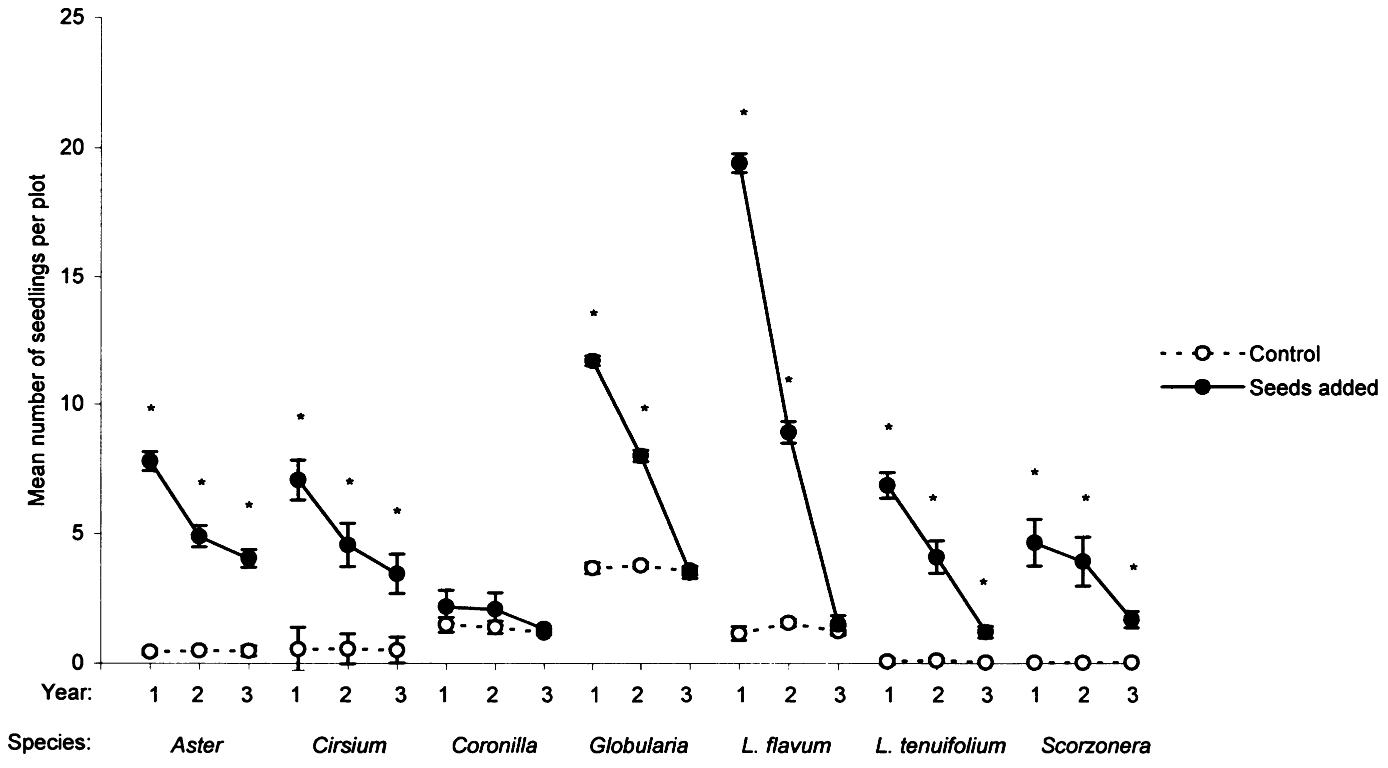

Table 1. List of localities used in the sowing experiment. Table provides geographical position of the localities, and estimates of number of flowering individuals at occupied localities. Unoccupied localities are marked with zero.

Latitude	Longitude	*Anemone*	*Aster*	*Cirsium*	*Coronilla*	*Glubularia*	*L. flavum*	*L. tenuifolium*	*Scorzonera*
50° 32' 22.8"	14° 18' 48.1"	0	0	0	0	1850	0	330	0
50° 32' 25.8"	14° 19' 9.3"	0	0	3	0	5	20	0	0
50° 23' 28.3"	14° 3' 36.5"	12	0	0	55	80	0	0	2
50° 33' 0.4"	14° 13' 59.1"	14	0	0	49	0	0	0	0
50° 31' 39.1"	14° 19' 40.3"	420	1	0	0	750	0	540	0
50° 31' 50.8"	14° 15' 36.4"	0	550	0	1100	0	0	730	0
50° 31' 46.6"	14° 14' 13.9"	43	0	520	0	0	0	0	1630
50° 29' 45.9"	13° 58' 39.6"	0	66	0	0	0	0	340	48
50° 24' 39.4"	13° 56' 41.4"	0	0	0	0	0	0	0	0
50° 33' 26.6"	14° 8' 40.6"	0	145	80	5	0	0	0	54
50° 30' 25.4"	14° 19' 20"	0	290	0	0	0	0	670	0
50° 32' 34.8"	14° 5' 19.7"	120	3450	960	1400	1400	6000	0	1980
50° 23' 24.5"	14° 5' 9.8"	650	0	0	0	0	0	0	0
50° 33' 10.4"	14° 10' 16.4"	0	0	0	0	0	280	0	387
50° 23' 55.8"	14° 10' 42.8"	0	0	0	5	0	0	0	0
50° 28' 19.1"	13° 52' 42.8"	80	0	0	40	0	0	0	76
50° 28' 1.7"	14° 18' 37.7"	0	220	0	95	0	0	420	0
50° 33' 54.7"	14° 20' 55.8"	3	0	0	0	0	0	0	0
50° 25' 47.6"	13° 52' 58.6"	0	0	0	30	0	0	80	0
50° 31' 56.8"	13° 48' 3"	20	230	80	0	0	350	0	12
50° 31' 42.7"	14° 20' 40.8"	0	180	0	0	40	40	0	0
50° 23' 28.4"	14° 9' 8.1"	30	0	0	560	0	0	0	0
No. of occupied localities		10	9	5	10	5	5	7	8

Table 2. Main characteristics of the studied species and the importance of different types of limitations for species distribution at three spatial scales based on results of this study. D indicates dispersal limitation, S indicates seed limitation, H indicates habitat limitation, M indicates microsite limitation and other indicates limitation by other factors. Last row contains data on proportion of occupied localities from all suitable localities. Suitable locality is defined as locality with at least one seedling surviving in the 3rd year of the experiment.

Species	*Anemone sylvestris*	*Aster amellus*	*Cirsium pannonicum*	*Coronilla vaginalis*	*Globularia punctata*	*Linum flavum*	*Linum tenuifolium*	*Scorzonera hispanica*
Descriptive parameters								
Family	*Ranunculaceae*	*Asteraceae*	*Asteraceae*	*Fabaceae*	*Globulariaceae*	*Linaceae*	*Linaceae*	*Asteraceae*
Main dispersal agent	Wind	Wind	Wind	No	No	No	No	Wind
Clonal propagation	Yes	Yes	Yes	No	No	No	No	No
Seed germination ability (%)	21	24	19	36	45	80	65	100
Decrease in germination after 3 years	90	58	100	19	22	100	98	100
Scarification required	No	No	No	Yes	No	No	No	No
Terminal velocity (m/s)	0.67	0.80	0.42	2.40	1.35	1.91	1.50	1.78
Attachment ability	97	79	76	5	58	30	21	30
Seed weight (mg)	0.5	0.6	1.6	3.8	0.2	0.8	0.6	8.0
Seed production per 1m2	1736	1866	141	425	3868	10510	692	189
Results								
Locality scale	No germination in the field	D	D	D,H?	D,H?	D, other	D, other	D,H?
Block scale		D	D	H,D	D,H?	D, other	D	D
W/out seed addition		S	S	M	M	M	S	S
Proportional habitat occupancy		0.41	0.23	0.48	0.23	0.24	0.35	0.32

Table 3. Percentage variatiance in seedling numbers in the 1^{st}, 2^{nd} and 3^{rd} year of the experiment and in adult occurrence explained by two groups of environmental variables, abiotic parameters and parameters describing species composition. Total denotes total variation explained by both of these groups of parameters together. The increase of this value compared to sum of the two previous columns is due to variance that could possibly be explained by both of the two factors. Variance decomposition at the block scale is based on 220 observations; variance decomposition at the locality scale is based on 22 observations. Bold values mark the higher value of the two neighbouring columns showing contribution of species composition and abiotic parameters.

		Year 1			Year 2			Year 3			Adults		
Scale	Species	Species comp.	Abiotic par.	Total	Species comp.	Abiotic par.	Total	Species comp.	Abiotic par.	Total	Species comp.	Abiotic par.	Total
Block	*Aster*	0	**1**	4	0	0	1	0	**2**	4	**35**	0	45
	Cirsium	0	**1**	2	0	**1**	1	0	0	0	**35**	0	60
	Coronilla	0	**3**	3	0	**7**	7	0	**3**	4	0	**24**	68
	Globularia	1	**8**	10	0	**16**	17	0	**12**	14	**1**	0	33
	L. flavum	0	0	2	0	**12**	15	0	**12**	14	**25**	23	64
	L. tenuifolium	0	**16**	19	4	**18**	29	0	**26**	26	0	**2**	7
	Scorzonera	0	**1**	1	0	0	0	0	0	0	**43**	0	70
Locality	*Aster*	2	**25**	33	2	**15**	17	0	**20**	22	**10**	2	12
	Cirsium	2	**18**	20	0	**28**	29	0	**31**	32	**9**	2	42
	Coronilla	0	**12**	14	**12**	10	27	6	**31**	49	2	**7**	35
	Globularia	3	**35**	41	3	**18**	25	0	**28**	28	**3**	0	74
	L. flavum	0	**29**	29	1	**46**	49	0	**43**	43	**60**	0	60
	L. tenuifolium	2	**29**	34	0	**57**	61	0	**48**	79	0	**10**	17
	Scorzonera	5	**14**	19	10	**11**	21	**13**	8	21	**7**	2	32

Table 4. Correspondence between importance of single environmental factors for explaining number of seedlings in the third year of the experiment and adult occurrence at block and locality scale. Jaccard is a value of Jaccard coefficient comparing significances of single factors between seedlings and adults at the block and locality scales, and between the scales for seedlings and adults, N gives number of significant tests on which the comparison is based. The comparison is based on 11 environmental variables.

	Block scale		Locality scale		Seedlings		Adults	
	N	Jaccard	N	Jaccard	N	Jaccard	N	Jaccard
Aster	7	0.57	4	0.00	7	0.57	4	0.50
Cirsium	4	0.00	5	0.00	4	0.00	5	0.80
Coronilla	3	0.00	10	0.00	12	0.33	1	0.00
Globularia	10	0.40	6	0.00	12	0.67	4	0.50
L. flavum	4	0.50	8	0.00	10	0.40	2	1.00
L. tenuifolium	4	0.00	6	0.00	10	0.80	0	-
Scorzonera	2	0.00	3	0.00	3	0.00	2	0.00

Appendix

Table A. Results of analysis of the effect of selected variables on number of seedlings at block and locality scale in the third year of the experiment. Table shows significance values from log-likelihood tests comparing the baseline model with the model including the term of interest. Significant values are in bold (significance level is adjusted using Bonferoni correction). If the direction of the response makes sense, the sign of the relationship if significant is given in brackets. Results at the block scale are based on 220 observations; results at the locality scale are based on 22 observations.

	Aster		*Cirsium*		*Coronilla*		*Globularia*		*L. flavum*		*L. tenuifolium*		*Scorzonera*	
Variable	block	locality	block	locality	block	locality	block	locality	block	locality	block	locality	block	locality
Beals index value	0.040	0.014	0.170	0.728	0.025	**<0.001** (+)	**<0.001** (+)	0.275	**<0.001** (-)	0.212	0.258	0.008	0.452	**<0.001** (+)
AX1	0.025	**<0.001**	0.830	0.272	0.334	**<0.001**	0.008	0.086	0.880	0.302	0.180	0.019	0.206	**<0.001**
AX2	0.390	0.648	0.064	0.014	0.098	**<0.001**	0.760	0.013	0.520	**<0.001**	0.058	**<0.001**	0.583	0.016
Biomass	**0.002** (+)	0.017	0.950	0.003	0.009	**<0.001** (-)	**<0.001** (-)	**<0.001** (-)	0.003	**<0.001** (-)	**<0.001** (-)	**<0.001** (-)	0.698	0.245
Inclination	0.380	0.745	0.250	0.121	0.303	**<0.001** (+)	0.004	**<0.001** (-)	0.382	0.250	0.498	**<0.001** (-)	0.336	0.900
Potential solar radiation in January	0.360	0.772	0.740	0.100	0.946	**<0.001** (+)	**<0.001** (+)	0.011	0.146	0.054	0.012	0.024	0.483	0.074
Potential solar radiation in June	**<0.001** (+)	**<0.001** (+)	0.037	**<0.001** (+)	0.662	0.539	0.910	0.041	0.238	**<0.001** (+)	0.360	0.019	0.312	0.001
Bulk density	**<0.001** (+)	**<0.001** (+)	0.990	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	0.010	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	0.900	0.054
Soil water holding capacity	0.730	0.014	0.089	**<0.001** (+)	0.341	**<0.001** (+)	**<0.001** (-)	**<0.001** (+)	0.111	**<0.001** (-)	**<0.001** (-)	**<0.001** (-)	0.570	**0.002** (-)
Mean potential solar radiation	0.410	0.234	0.200	0.008	0.452	**<0.001** (-)	**<0.001** (-)	0.008	**<0.001** (+)	**0.002** (+)	0.798	0.324	0.665	0.015
Percentage of bare ground	**0.001** (+)	0.075	**<0.001** (+)	0.017	**0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	0.113	0.313

Table B. Results of analysis of the effect of selected variables on occurrence of adult plants at the block and locality scale. Table shows significance values of log-likelihood test in logistic regression comparing the baseline model with the model including the term of interest. Significant values are in bold (significance level is adjusted using Bonferoni correction). If the direction of the response makes sense, the sign of the relationship if significant is given in brackets. Results at the block scale are based on 220 observations; results at the locality scale are based on 22 observations.

	Aster		*Cirsium*		*Coronilla*		*Globularia*		*L. flavum*		*L. tenuifolium*		*Scorzonera*	
Factor	block	locality	block	locality	block	locality	block	locality	block	locality	block	locality	block	locality
Beals index value	**0.003** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	0.293	**0.002** (+)	**<0.001** (+)	**<0.001** (+)	**<0.001** (+)	0.221	0.184	0.006	0.212
AX1	0.155	0.699	**<0.001**	**<0.001**	0.008	0.602	0.049	0.095	0.134	0.040	0.720	0.063	**<0.001**	0.103
AX2	0.531	0.204	0.007	0.374	0.825	0.013	**<0.001**	0.066	0.632	0.730	0.565	0.038	**0.002**	0.016
Biomass	0.541	0.008	**0.004** (-)	0.589	0.925	0.220	**0.004** (-)	0.058	0.708	0.082	0.794	0.006	0.155	0.355
Inclination	0.024	0.297	0.574	0.377	0.162	0.185	0.311	0.524	0.993	0.157	0.139	0.388	0.623	0.899
Potential solar radiation in January	0.098	0.145	0.013	0.732	0.530	0.261	0.555	0.076	0.143	0.100	0.515	0.034	0.276	0.930
Potential solar radiation in June	**<0.001** (+)	0.641	0.514	0.389	0.381	0.158	0.292	0.078	0.560	0.033	0.640	0.685	0.298	0.053
Bulk density	0.736	0.354	0.942	0.917	0.908	0.040	0.858	0.668	0.588	0.300	0.965	0.605	0.083	0.180
Soil water holding capacity	0.242	0.069	0.566	0.908	0.058	0.062	0.684	0.558	0.586	0.168	0.179	0.649	0.880	0.228
Mean potential solar radiation	0.570	0.167	0.176	0.605	0.787	0.720	0.827	0.241	0.445	0.410	0.193	0.897	0.608	0.642
Percentage of bare ground	**0.004** (-)	0.956	0.151	0.991	0.010	0.942	0.349	0.420	0.094	0.668	0.878	0.244	0.261	0.028

VI.

Identification of suitable unoccupied habitats in metapopulation studies using co-occurrence of species

Zuzana Münzbergová and Tomáš Herben

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This study proposed a new quantitative technique to identify suitable but unoccupied habitats for metapopulation studies in plants. It is based on species composition at the habitat and knowledge of species co-occurrence patterns. It uses data from a large phytosociological database as a background for estimating species co-occurrence patterns. If such a database is not available, the technique can still be applied using the same data for which the prediction is done to estimate the species co-occurrence pattern. Using the technique we were able to indicate suitable unoccupied habitats and differentiate them from the unoccupied unsuitable ones. We also identified habitats with low probability of being suitable that were occupied. Compared to a direct approach of identification of suitable habitats, which involves introduction of a species to the habitat and studying its performance, the approach presented here is much easier to apply and can provide extensive information on habitat suitability for a range of species with much less effort and time needed.

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One of the key predictions of metapopulation theory is the occurrence of suitable, but unoccupied habitats (Levins 1969, Hanski and Simberloff 1997); due to the ongoing colonization and extinction, a certain fraction of suitable habitats should be unoccupied if the system is in equilibrium. Identification of unoccupied but suitable habitats is therefore a part of many empirical metapopulation studies. It is, however, often difficult to tell what constitutes a suitable habitat (Ehrlén and Eriksson 2000). This difficulty was recently used as one of the important arguments in the critique of application of metapopulation theory in plants (Freckleton and Watkinson 2002).

The most direct but technically difficult way for estimation is to introduce the species into the habitat and follow its performance (Eriksson and Ehrlén 1992, Turnbull et al. 2000). To get a good estimate of suitability using this method one should follow the whole life cycle of the species as population bottlenecks may occur at later stages of recruitment (Losos 1995, Gustafsson et al. 2002). This can, however, take much longer than any research project can last (Ehrlén and Eriksson 2000).

Therefore, alternative indirect ways to estimate habitat suitability are sought (Husband and Barrett 1996). A common indirect way to define suitable habitats is to use environmental variables (Ouborg 1993, Husband and Barrett 1996, Quintana-Ascencio and Menges 1996). However, the array of environmental variables that can be measured is very wide (e.g. soil properties, microclimate, occurrence of extreme events) and there is no good way to decide which variables are the right ones.

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Measuring all of them is very laborious and time consuming so suitability estimation using this approach is of limited use (Ehrlén and Eriksson 2000).

An alternative approach is to use information on presence of other species as a measure of habitat suitability. It is based on the common experience that occurrences of species are correlated. Combination of a species list from a habitat and data on species co-occurrence then provides estimation of habitat suitability for the species of interest. Compared to the use of environmental variables this enables much better exploitation of information about conditions at a habitat (Ewald 2002) as species composition is an outcome of the important environmental variables. Reasoning of this type is the basis of phytosociology and is sometimes applied for smoothing of phytosociological databases (i.e. adding species that might have been overlooked) (Beals 1984) or gap filling of flora databases (Witte 1998). Recently a similar approach has been used to define species pool composition (Ewald 2002, Chytrý et al. in press). However, no attempt has been made to use it in a metapopulation context to define suitable unoccupied habitats.

The aim of this study is to explore patterns of habitat suitability for a particular species predicted from its co-occurrence with other species present at that habitat. This can be done by calculating the probability of species occurrence at a habitat regardless of its actual occurrence. If there are suitable but unoccupied habitats available, one would expect to get high probabilities of species occurrence at occupied habitats and both high and low probabilities at unoccupied habitats. Our prime question is therefore (i) is it possible to find the predicted pattern of estimated probabilities at occupied and unoccupied habitats (high probability at occupied and both high and low probability at unoccupied habitats) in a system of plant species on habitat islands?

Further, we are asking two methodological questions. First, a typical approach to calculate such probability is to use the same data-set for calibration and for prediction (Beals 1984, Witte 1998). However, circularity can be avoided if different data-sets are used to estimate species co-occurrence patterns and for predictions. In some countries there are large phytosociological databases that could be used as reference databases for this prediction (Ewald 2002, Chytrý and Rafajová 2003). When using a reference database there are many options of how large range of communities should be included in it. Therefore we ask (ii) how does the prediction of habitat suitability depend on the spectra of communities covered by the reference database?

Second, in some cases such a reference database may however not be available. Therefore, our last question is (iii) does the prediction change if the same database is used for both calibration and prediction?

To estimate habitat suitability we used Beals index of sociological favourability (Beals 1984), which defines probability of species occurrence based on co-occurrence of that species with other species. This index provides an estimate of the probability to encounter a species at a habitat. The estimate is independent of the fact whether the species really occurs there. To predict species occurrence we used data from dry grasslands in northern Bohemia, Czech Republic. Relevés from the Czech national phytosociological database were used as a reference database (Chytrý and Rafajová 2003).

Methods

Field data

To investigate the pattern of estimated probability at occupied and unoccupied habitats we used data from twenty-two localities of dry grasslands of the *Bromion* community. At each locality presence of all species was recorded in ten 1 × 1 m plots selected to cover the whole range of the locality. These data were then pooled and used as information on species composition of the locality.

Reference database

To estimate species co-occurrence patterns we used relevés from the Czech national phytosociological database (http://www.sci.muni.cz/botany/database.htm). We used relevés from the area delimited by longitude 13°15′–15°30′ E and latitude 50°00′–50°40′ N. To estimate the effect of reference database width on the prediction we used two different definitions of the reference database, a wider definition including all dry and mesophylous grassland communities and a narrower definition including only broad leaf dry grasslands (*Bromion* community).

The wider database included all relevés of classes *Festuco-Brometea*, *Trifolio-Geranietea*, *Molinio-Arrhenatheretea*, *Artemisietea vulgaris*, *Agropyretea repentis* and alliance *Agrostietalia*. It included 2784 relevés. The narrower database included only relevés of *Bromion* community in a narrower sense. The inclusion of relevés into this selection was based on presence of at least 5 diagnostic species of this community. The diagnostic species were selected as diagnostic species as defined by Chytrý (unpubl.) having the Phi > 0.30 (Chytrý et al. 2002). It included 785 relevés.

To simulate a situation when reference database is not available we also used the calibrated set itself as the reference database. In this case the sample being calibrated was excluded from the data-set.

Probability of species occurrence

Probability of species occurrence at a habitat was estimated using Beals index of sociological favourability (Beals 1984). The probability of occurrence of species j at a habitat i is defined as

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

where p_{ij} is probability to find species j at habitat i, S_i is number of species at habitat i (minus 1 if species j is present), N_{jk} is number of joint occurrences of species j and k, $j \neq k$ in the reference database, N_k is number of occurrences of species k in the reference database. It is important to note that the Beals index value is independent of the actual occurrence of the species at a habitat. We calculated Beals index for each species in the data-set in two ways, using the external database and without it. We compared Beals index values for 45 species that were present in at least 3 localities.

Probability of occurrence at unoccupied habitats

Probability of occurrence at unoccupied habitats was estimated from a cumulative frequency distribution of Beals index values of each species at all occupied habitats. We used it to demonstrate where in this distribution each unoccupied habitat lies, i.e. where it would rank if it were occupied. This recalculation was done in order to define threshold value for suitability of the unoccupied habitats as the absolute values of the Beals index value strongly depend on the frequency of the species in the external database. These values (later called percentiles of Beals index value) can be directly interpreted in terms of suitability: an unoccupied habitat having Beals index value lower than any occupied habitat had a 0% probability of being occupied; a habitat having Beals index value equal to median of the Beals index values at the occupied habitats had 50% probability etc. (Fig. 1).

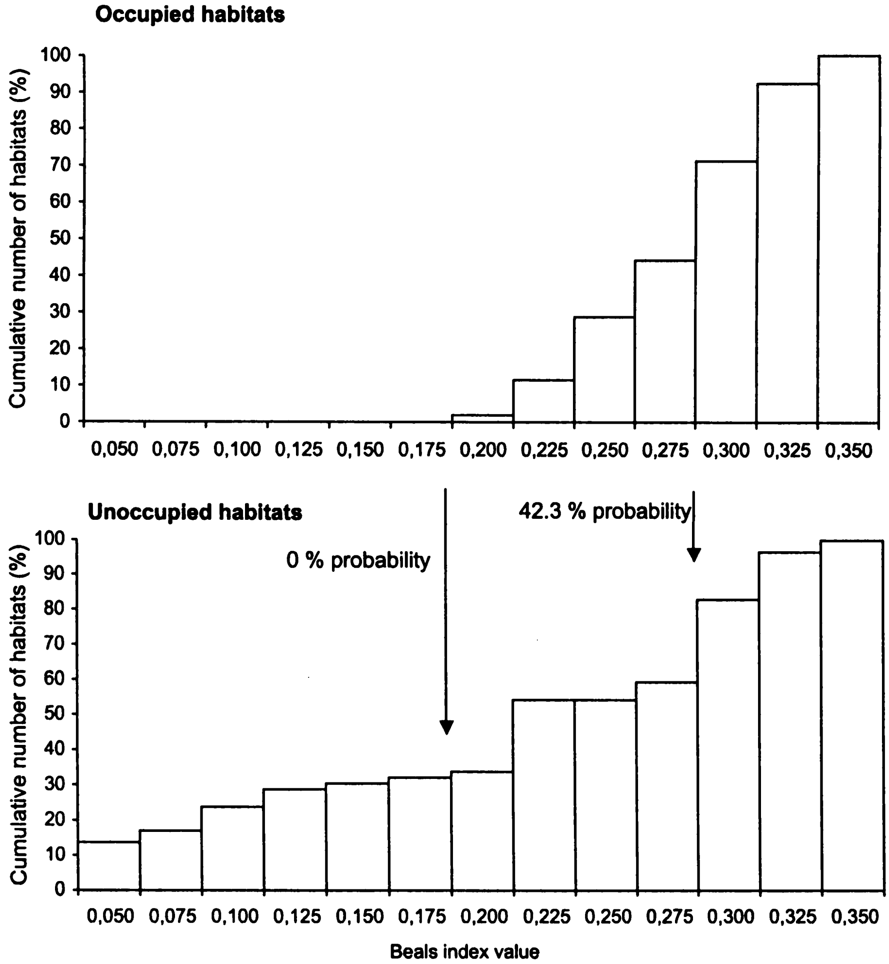

Fig. 1. An example of translation of Beals index values at unoccupied habitats into percentiles of Beals index values at occupied habitat. The upper histogram represents cumulative distribution of Beals index values at occupied habitats. The lower represents distribution of Beals index values at unoccupied habitats. The arrows indicate the probability that an unoccupied habitat with given probability will be occupied that was derived from the distribution of Beals index values at the occupied habitats. The scale used in actual calculations was much finer.

Reliability of estimation

To see how sensitive the Beals index value is to composition of the reference database we calculated 95% bootstrap confidence intervals of the value based on 100 resamplings of each reference database. Using these data we also calculated 95% confidence intervals of percentiles of Beals index value at unoccupied habitats.

Data analysis

We used linear regression to estimate the relationship between the three types of predictions (using two types of external database and without it). Beals index value and percentile of Beals index value calculated without external database were used as dependent variables (one data point corresponds to one species at one locality) and the respective value calculated using wider external database and species were used as independent variable. Comparison of the two external databases was done in the same way. The calculations were done using SPSS version 11.0.1.

Results

Estimation of probabilities

In all cases the Beals index value was on average higher at occupied than at unoccupied habitats (Fig. 2, Table 1), but there were some unoccupied habitats with percentile of Beals index value equal to 100 (the Beals index value was as high or higher than the highest Beals index value at any occupied habitat). This pattern was obtained from calculations using all three ways to estimate species co-occurrence patterns. In a few cases we also identified localities with low probability of being suitable that were actually occupied.

There is only a very weak but significant relationship between percentiles of Beals index values estimated using wider external reference database and using the calibrated set as reference database ($R^2 = 0.02$, df = 1,675 $F = 51.07$, $p = 0.024$). The relationship was however much stronger between the original Beals index values ($R^2 = 0.12$, df = 1,989, $F = 222.283$, $p < 0.001$). There was strong relationship between both the percentiles of Beals index value ($R^2 = 0.74$, df = 1,675, $F = 1351$, $p < 0.001$) and Beals index values ($R^2 = 0.99$, df = 1,989, $F = 7444$, $p < 0.001$) estimated using the two types of external reference databases.

Reliability of estimation of the probabilities

When the external reference database was not available the standard error values were larger than when external

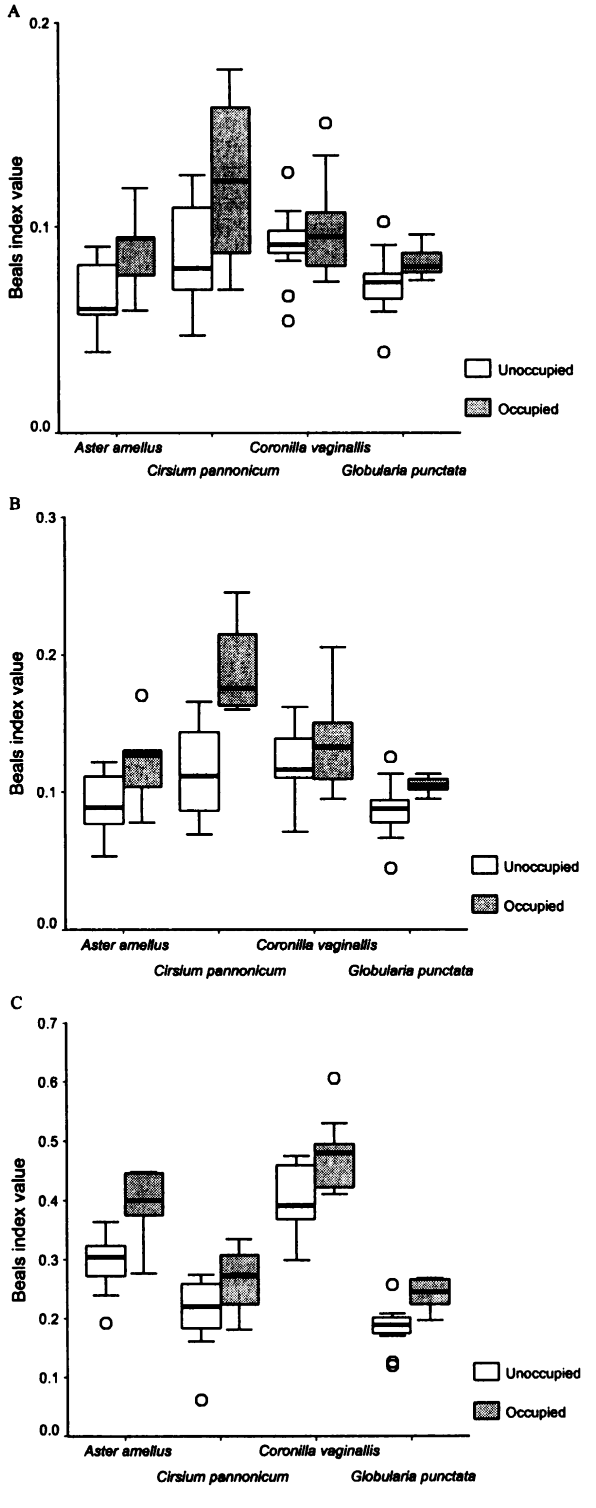

Fig. 2. Beals index values plotted separately for four species of dry grasslands. Values are separated for occupied and unoccupied habitats. The absolute values of Beals index depend on the frequency of the species in the database; therefore only comparisons within species make sense. Reference database: (A) all dry grasslands, (B) only *Bromion* dry grasslands, (C) the same database for which the prediction was done.

Table 1. Median Beals index values for actually occupied and unoccupied habitats calculated separately for each species. The values were calculated using either narrow or wide external reference database or without any external reference database (using data itself).

	Narrow		Wide		No	
	Unoccupied	Occupied	Unoccupied	Occupied	Unoccupied	Occupied
Agrimonia eupatoria	0.176	0.187	0.131	0.133	0.165	0.145
Anemone sylvestris	0.055	0.055	0.041	0.044	0.385	0.334
Anthericum ramosum	0.188	0.233	0.162	0.202	0.327	0.246
Anthyllis vulneraria	0.126	0.128	0.096	0.096	0.422	0.420
Asperula cynanchica	0.366	0.356	0.329	0.331	0.294	0.374
Aster amellus	0.089	0.127	0.060	0.095	0.304	0.401
Astragalus onobrychis	0.011	0.018	0.009	0.012	0.107	0.120
Briza media	0.243	0.270	0.200	0.233	0.117	0.081
Bromus erectus	0.208	0.254	0.157	0.182	0.106	0.049
Carex humilis	0.402	0.456	0.350	0.410	0.303	0.255
Carex tomentosa	0.034	0.036	0.029	0.033	0.348	0.288
Carlina vulgaris	0.257	0.262	0.184	0.189	0.593	0.579
Centaurea scabiosa	0.306	0.342	0.251	0.275	0.549	0.484
Cirsium acaule	0.370	0.404	0.256	0.294	0.759	0.761
Cirsium eriophorum	0.032	0.025	0.024	0.017	0.239	0.175
Cirsium pannonicum	0.111	0.176	0.080	0.123	0.221	0.273
Coronilla vaginalis	0.116	0.132	0.092	0.095	0.391	0.481
Eryngium campestre	0.229	0.274	0.197	0.243	0.385	0.382
Falcaria vulgaris	0.077	0.087	0.067	0.080	0.207	0.198
Galium verum	0.291	0.376	0.250	0.313	0.228	0.240
Geranium sanguineum	0.100	0.133	0.099	0.146	0.105	0.070
Globularia punctata	0.088	0.105	0.073	0.081	0.190	0.245
Helianthemum grandiflorum	0.290	0.296	0.203	0.218	0.329	0.296
Inula hirta	0.073	0.088	0.050	0.058	0.207	0.174
Knautia arvensis	0.344	0.373	0.262	0.276	0.173	0.114
Koeleria macrantha	0.123	0.111	0.097	0.079	0.375	0.333
Linum catharticum	0.274	0.291	0.211	0.219	0.126	0.107
Linum flavum	0.063	0.083	0.043	0.057	0.239	0.258
Linum tenuifolium	0.111	0.129	0.083	0.097	0.359	0.302
Melampyrum arvense	0.031	0.030	0.034	0.034	0.169	0.090
Ononis spinosa	0.250	0.293	0.189	0.223	0.445	0.469
Peucedanum cervaria	0.097	0.131	0.076	0.100	0.272	0.209
Plantago media	0.407	0.422	0.304	0.320	0.337	0.317
Primula veris	0.097	0.115	0.082	0.106	0.269	0.269
Prunella grandiflora	0.180	0.225	0.137	0.172	0.596	0.575
Salvia pratensis	0.388	0.400	0.297	0.306	0.411	0.335
Salvia verticillata	0.122	0.108	0.097	0.087	0.366	0.289
Scorzonera hispanica	0.087	0.094	0.062	0.069	0.333	0.290
Seseli hippomarathrum	0.144	0.181	0.149	0.168	0.190	0.120
Sesleria varia	0.138	0.186	0.133	0.169	0.362	0.347
Stachys recta	0.141	0.164	0.161	0.187	0.363	0.371
Tanacetum corymbosum	0.109	0.150	0.090	0.119	0.406	0.368
Tetragonolobus maritimus	0.083	0.105	0.064	0.077	0.144	0.136
Teucrium chamaedrys	0.309	0.297	0.249	0.260	0.458	0.387
Thesium linophylon	0.040	0.056	0.038	0.058	0.196	0.166

reference database was used (Fig. 3). The relative width of the confidence intervals was larger for the percentiles of Beals index value than for the Beals index values themselves. This is due to two sources of stochasticity combined in the estimation of the percentiles – stochasticity of estimation of the Beals index values from occupied and unoccupied habitats.

Discussion

Patterns of Beals index values

There was a clear difference in the range of Beals index values estimated for occupied and unoccupied habitats. Whereas most occupied habitats had a high Beals index value, unoccupied habitats had both high and low values for most of the species. This is an indication that the method used is able to differentiate between habitats and has therefore a predictive power to decide on habitat suitability. In case of several species there were a few occupied habitats having very low Beals index value. It can be speculated that these could be remnant populations, where the species survives just thanks to its longevity and is already not able to reproduce (Eriksson 1996). However other reasons, such as occurrence of the species on the margins of its ecological range or biased composition of the reference database, might also explain this pattern.

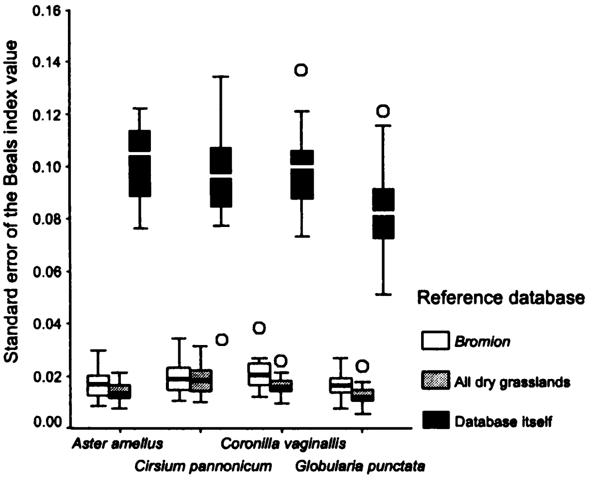

Fig. 3. Standard errors of Beals index values calculated by bootstrapping the database used to estimate species co-occurrence patterns. Two different external databases and the data itself were used for this purpose.

Predictions if the reference database is not available

Predictions without external reference database produced quite wide confidence intervals of the Beals index value. Still there was a clear difference between Beals index values estimated for unoccupied and occupied habitats and the prediction seems reasonable. The relationship between percentiles of Beals index value at unoccupied habitats estimated using external reference database and without it was however quite weak, even though there was quite good correspondence between the Beals index values. This shows that a rather small variation in Beals index values can cause quite a large variation in percentiles of Beals index value. This is due to a relatively low number of occupied habitats used to estimate the percentiles. It shows that even if external reference database is not available, one can gain quite reasonable estimates of the Beals index value. If the percentiles of Beals index values are to be calculated using this approach, one has to make sure that there are enough occupied habitats to construct a reliable cumulative frequency distribution. In our case they were too few.

Potentials and drawbacks of the method

The method presented here was originally developed for adding missing species to large databases. It worked with the assumption that species occupy all habitats they can and their absence in the database is just caused by not having been recorded. Their incidence in the database is therefore expected to be high. Metapopulation theory, however, predicts that not all possible species are present at a habitat. The use of the method in the metapopulation context can therefore be limited depending on the fraction of habitats that are actually occupied; the lower the actual incidence of the species used for the prediction, the lower the possibility to make a good prediction. This brings a kind of contradiction into the issue, as one has to assume that the incidence of a species is reasonably high so that its occurrence patterns have predictive power, and still to expect a fraction of habitats to be unoccupied.

The width of the confidence interval of the Beals index values was quite wide even in case when the large external reference database was used. One could speculate that the width of the confidence interval will be a product of definition of external database (range of communities cover by the database) and range of species lists from the localities of interest. If both species lists are too wide, one may expect wide confidence intervals due to presence of a high number of species without any predictive power. On the other hand, if it is too narrow, the confidence intervals would be wide due to very few species used for the prediction. Our comparison of two different external databases however demonstrated that there is a very good correspondence between Beals index values estimated using these two and that also the confidence intervals of these two are comparable. This shows that selection of external database is not a major issue, and any reasonably large database can be used for this purpose. Detailed exploration of this issue is however beyond the scope of this paper.

This method is suggested as an easier to apply and possibly more informative counterpart to use of environmental variables. However given the longevity of many plant species, it may be argued that environmental factors such as soil conditions may be more informative when the environment had recently dramatically changed and the plants have not responded yet.

Conclusions

Our study provides a first insight into the possibilities to use species co-occurrence patterns to identify habitat suitability. Further exploration is needed to provide test of the method by comparing it with other approaches. Further work also needs to be done to evaluate the effect of various external database definitions and habitat definitions on the prediction.

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VII.

Population viability and reintroduction strategies: a spatially explicit landscape-level approach

Zuzana Münzbergová, Mikael Mildén, Johan Ehrlén and Tomáš Herben

Abstract

Common approaches estimating effect of habitat destruction on species survival perspectives, such as metapopulation capacity, are based on the assumption that current distribution of species in habitat fragments is a result of equilibrium between local colonization and extinction. This may be feasible in short lived highly dispersible organism, but is surely far from reality in sessile long-lived species such as most plants. Here we use an alternative approach, a realistic dynamic landscape-level model that does not use this assumption. It enables estimation of effect of habitat habitat destruction using realistic field data on biology of a species and on landscape structure. Since the approach relies on direct comparison of changes in population size and survival probability due to habitat changes, it can be easily extended to other conservation questions, such as the effect of local population destruction or search for optimal reintroduction strategy.

We apply this method to a perennial herb *Succisa pratensis* that is a typical representative of fragmented low-production grasslands. The results show that habitat destruction alone has only little effect on its survival perspectives. The effect however increases when combined with population destruction, that are expected to play a significant role in the study system. Using the same approach we were also able to design optimal reintroduction strategy for the species, assuming the species was extinct from the landscape. Given the biology of our species, we argue that only a dynamical model with local events as the one presented here makes fully possible to evaluate its survival perspectives in fragmented landscapes and to design the most appropriate reintroduction strategies.

Keywords

extinction threshold, habitat fragmentation, metapopulation capacity, plants, population viability analysis, reintroduction, *Succisa pratensis*, survival probability

Introduction

Understanding factors affecting survival of species in fragmented landscapes, and the possibility to restore their populations once they are gone, belong among the central issues in species conservation (van Groenendael et al. 1998). The simplest way of doing this is to perform population viability analysis at the level of local populations by examination of total species demography, or predicting the effect of single species traits on species survival perspectives (Morris and Doak 2002). With the recent development of metapopulation theory (Hanski 1989), it has been recognized that also regional scale processes may be important for survival of species in the landscape (e.g. Carroll et al. 2003a, du Toit et al. 2004). In such cases the single local population approach to population viability analysis alone is of little use. In contrast, population viability analysis has to be performed at the multi-site level (Morris and Doak 2002) and should take into account both present distribution of the species in the landscape as well as the number and distribution of patches that are potentially available for recolonization.

Fragmented landscapes present a difficulty also for species reintroductions. While the single site approach again relies on assessing the optimal local conditions that would maximize species survival perspectives (Schemske et al. 1994, Hodder and Bullock 1997, van Groenendael et al. 1998, Smulders et al. 2000, Kauffman et al. 2003), in fragmented landscape it is necessary to evaluate the success of a reintroduction by its ability to spread and survive at the landscape scale (Eriksson 2000, Carroll et al. 2003b). This has to take into account the spatial structure of the landscape, in terms of the arrangement of suitable habitat patches around the habitat patch were the species was reintroduced, and the capacity of the species to reach it.

Recently several theoretical models have been developed to assess the landscape level perspectives of species survival (e.g. Gustafson and Gardner 1996, With et al. 1997, Hanski and Ovaskainen 2000, Casagrandi and Gatto 2002a, Dreschler et al. 2003). These models assume that the current distribution of species in habitat fragments represents equilibrium between local colonization and extinctions. Under this assumption, present pattern of distribution of the species can be used to estimate expected threshold number of habitat patches needed to ensure metapopulation survival (Hanski and Ovaskainen 2000).

The critical point in this reasoning is the assumption that colonization/extinction dynamics operate fast enough to keep the current distribution close to equilibrium. This assumption is rather difficult to test directly as long-term data on immigration and extinction rates are necessary (Hanski et al. 1994) and may be directly violated if the landscape has recently undergone major change in fragmentation. While this assumption is likely to be correct in short-lived highly dispersible organisms such as many insects (Hanski et al. 1994, Baquette 2003, Purse et al. 2003), it is much less likely in sessile organisms such as plants that are known to have very low extinction and immigration probabilities (Eriksson 1996, Freckleton and Watkinson 2002). Consequently the expected time to equilibrium is so long that it can hardly be attained in realistic time frames over which external environment may be assumed to be constant (Freckleton and Watkinson 2002). Therefore models based on the assumption of equilibrium are of little use in plants. Unfortunately plants (precisely due to their limited migration capacities) are directly threatened by habitat destruction and predictions of the effects of fragmentation are surely needed (Eriksson and Kiviniemi 1999).

Moreover even if the equilibrium assumption holds, the above-mentioned models are basically designed to assess effect of habitat destruction only; inclusion of other possible interacting factors such population destruction is much less straightforward in this framework. While many species are able to survive for a long time even on a single patch (Eriksson 2000), this may not be true in systems with local population destructions (such as insect outbreaks, fires, short-term change in management practices) that may wipe out the population while leaving the habitat essentially unchanged and ready for recolonization. These population destructions may shift the system even further out of the equilibrium and thus make an equilibrium-based analysis impossible. Still such processes may often interact with habitat destruction (Casagrandi and Gatto 2002b); therefore it is important to evaluate not only the effect of habitat destruction itself, but also its possible interaction with population destruction.

To study species survival perspectives of species in fragmented landscapes and possibilities of species reintroduction, it is thus important to use an approach that can represent both transient and equilibrium phases of local population development and of its regional distribution. This can be easily attained using a spatially explicit dynamic landscape-level model. Such model can work with realistic data on landscape structure and take into account actual species distribution in the landscape and data on local population dynamics. Such approach does not rely on the equilibrium assumption and enables incorporation of much more site-specific information. It can be used to extrapolate changes in population sizes over time under different landscape scenarios both in transient and equilibrium phases; this has direct relevance for conservation, as it enables conservationists to assess the consequences of expected landscape changes for species over different time frames. The same approach can be used both for a population viability analysis and for designing an optimal strategy to reintroduce a species into a landscape consisting of several suitable habitat patches.

The aim of this paper is twofold. First, we want to demonstrate the usability of a dynamic model to perform viability analysis and design optimal reintroduction. Specifically we attempt to assess the effect of habitat destruction, population destruction and their interaction on species survival and the optimal reintroduction strategy if the species has gone extinct at the regional scale. Second, we want to address specific conservation concerns for a group of plant species that are restricted to low-production grasslands that often decline under current landscape management. Many of these species are long-lived perennials with restricted clonal growth and low dispersal; equilibrium assumption especially at the regional scale is likely to be untenable for their populations. We therefore use *Succisa pratensis*, a long-lived perennial plant with very slow dynamics, as the model species. This species is a rather typical representative of a rare grassland species in an agricultural landscape in southern Sweden. It has recently been extensively studied (Mildén et al. unpubl.) and we can rely on the wealth of landscape and population biology information that has been collected for it.

Methods

Study species and system

Succisa pratensis is a perennial, polycarpic, rosette herb with a life span of at least 25 years (Hooftman et al. 2002). In August to September it produces one to several, 20-80 cm high, flowering stems with one to several pale violet flower heads. The flowers are self-compatible, but crossing enhances seed set considerably (Vergeer et al. 2003). Inbreeding may affect small and isolated populations negatively (Vergeer et al. 2003). Clonal propagation sometimes occurs through side rosettes. In Sweden, *S. pratensis* is most commonly found in dry to wet semi natural grasslands. It benefits from grazing and the present distribution in the landscape largely depends on the management history, i.e. is closely correlated with former grazing and moving. After abandonment of management of a site, populations of *S. pratensis* can survive for long times, though with lowered performance (Mildén et al. unpubl.).

We collected the field data in the northern part of the Nynäs nature reserve, 100 km south of Stockholm, Sweden (58°50'N, 17°24'E). This area is a well-documented agricultural landscape with a long management history (Cousins and Eriksson 2001). The study area was a landscape fragment of 3.1 by 2.3 kilometers (7.1 km) containing managed agricultural land, and surrounded by coniferous forests that were considered unsuitable for *S. pratensis*. The oldest grasslands were probably well above sea level already 4000 years ago. Since, the establishment of two villages in the area around 1500 BP, management has been continuous and slowly expanding. In the 17th and 18th century 60% of the area was covered with grassland and 32% was arable land (Cousins and Eriksson 2001). From early 19th century to 1945 a large proportion of grasslands were converted to arable field due to changing practices of producing winter fodder for livestock. Since 1945 many of the grasslands have been abandoned and are now turning into forests whereas a proportion of former fields have been turned into pastures. At present, grasslands constitute 18% of the study area of which almost half are more than 200 years old.

Landscape data collection

We carefully inventoried the area and recorded the size, shape and position of all habitat patches potentially suitable for *S. pratensis*, and recorded whether they were occupied or not and population sizes (number of flowering and vegetative individuals) within a patch, from 2000 to 2002. Suitability of habitats was assessed (i) using a subjective classification into "suitable", "probably suitable" and "not suitable", (ii) using complete floristic surveys of habitats and in all "suitable" and "probably suitable" patches, and in a fraction of "not suitable" patches. We then used the index of sociological favourability (Beals 1984, see also Münzbergová and Herben, 2004) to identify correlation of *S. pratensis* occurrence with other species and to predict suitability of the patches based on their floristic composition. Lastly, (iii) we confirmed suitability for a random subset of each of the three categories by sowing seeds into patches (Mildén et al. unpubl.). By combining these three approaches we derived a map of all suitable habitats in the area and used it as an input for the model.

Demographic data collection and analysis

Demographic data were collected at five populations both at grazed and non-grazed sites. At each population we recorded at least 250 plants with a minimum of 50 flowering individuals. Individual plants were mapped, using a grid frame with mesh size of 10 cm × 10 cm. Each individual plant was recorded once a year and followed from 2000 through 2002. At each visit emerged seedlings were searched for, recorded and included in the further study. During flowering, we measured length and width of the largest leaf and flower number. The product of length and width of the largest leaf was used as an estimate of plant size. The number of seeds per flower head was estimated by examination of 30 flower heads outside the permanent plots in each year and population. Seed germination experiments were carried out at each site. At the time of seed ripening each year, fifty seeds were sown into six plots of 10 cm × 10 cm. The plots were visited twice a year, in early June and mid September, and the number of new and surviving seedlings was recorded.

To build a matrix model of the dynamics of the different populations, *S. pratensis* individuals were assigned to one of five stages: (1) seeds in the seed bank; (2) seedlings; (3) small vegetative individuals (estimated area of the largest leaf 30-449 mm^2); (4) large vegetative individuals (estimated area of the largest leaf more than 449 mm^2); (5)

flowering individuals. First-year germination rates were estimated from the seed sowing experiments in 2000 and 2001 as the proportion of sown seeds that germinated. Germination rates of seeds in the second and subsequent year after sowing were assumed to be equal to germination in the first year. Survival in the soil seed bank was calculated from the number of seedlings that emerged in the second year after sowing based on the assumption of constant germination rates. Seedling survival was estimated from seedlings in the seed sowing experiments. For both survivals of seeds in the soil and of seedling we used the estimate for the 2000-2001 transition also for the 2001-2002 transition.

The model

The model simulates a set of habitat patches explicitly located on the simulation plane. In our example, the simulation plane represented a rectangle of an area of 7.13 km^2, which covers the entire habitat surveyed in the field study. Absorbing boundaries (i.e. diaspores passing over the boundary are lost) were used because forests largely surround the region modeled. The sizes and shapes of patches were taken from the field inventory. The patches were represented as sets of contiguous cells (cell size 5 m × 5 m) with explicit positions in the simulation plane. Each patch was assigned a habitat quality (grazed or ungrazed) based on the field data.

Local population dynamics was simulated at the level of patches. Each patch was assigned a population vector that contained numbers of individuals in each size class. Population growth at the *k*th patch was modeled as

$$\mathbf{x}'_k(t+1) = \mathbf{A}_{Q(k)} \cdot \mathbf{x}_k(t), \tag{1}$$

where elements of $\mathbf{A}_{Q(k)}$ are stage-specific transition probabilities of the habitat quality state Q(k) (grazed or ungrazed). One-year transition interval was used.

Density dependence within habitats was modeled using a logistic growth equation. We hence used the difference between total population size and maximum population size at the patch as the logistic term. In our example, maximum population size was estimated as a product of the third highest density of *S. pratensis* found in the field (the two most extreme densities were excluded) and patch size. The population vector resulting from the matrix multiplication was thus replaced by the following term

$$\mathbf{x}''_k(t+1) = \mathbf{x}'_k(t+1)\ \frac{1}{\lambda}[1+(\lambda-1)(1-\frac{N(t)}{K})] \tag{2}$$

where λ is the dominant eigenvalue of the matrix $\mathbf{A}_{Q(k)}$, $N(t)$ is the total population size (sum of all stages) at the time t (before the matrix multiplication), and K is the maximum number of individuals at the patch of the given size. Finally, each vector element is replaced by a Poisson-distributed deviate with the mean of x_i. to represent demographic stochasticity.

Environmental stochasticity was simulated by using several matrices for one habitat quality. At each step, one matrix was drawn from this set at random with specific probabilities assigned to each matrix (see the next section). Environmental stochasticity was assumed to be uncorrelated over space and time.

Two components of dispersal were modeled: distance-dependent and distance-independent. Distance-dependent dispersal was assumed to affect most of the seeds, while distance-independent dispersal was used to model rare dispersal events. For distance-dependent dispersal, seeds produced at each patch were divided among the cells that comprise that patch and dispersed from these cells (the grid size corresponded to the size of the grid used to represent patches). Number of seeds arriving at a cell was proportional to the value of the exponential function of distance between the source and target cells. Diaspores that fell into the space in between the patches were considered lost. Distance-independent dispersal was modeled by removing a fraction of diaspores at each step from each patch and redistributing them over the whole simulation plane.

Parameter estimation and initial conditions

There were 6 matrices from grazed sites (3 populations × 2 transition periods) and 4 matrices from ungrazed sites (2 populations × 2 transition periods). To represent environmental stochasticity, all demographic matrices collected at all plots of one habitat quality (grazed and ungrazed) in both transition periods were pooled. In this way we pooled spatial environmental variation within one habitat quality (grazed or ungrazed) with the temporal variation obtained by recording demography over two transition periods. For the simulation, matrices were drawn from the pool of matrices of the given habitat quality in proportions derived from the field data on occurrence of these habitat types.

Dispersal distance was estimated as the product of plant height and wind speed divided by the terminal velocity, using the formula given in Soons and Heil (2002). Given the variation in terminal velocity (1.7 – 3.1 m/s, data from Soons and Heil 2002), variation of wind speed at the locality (4.3 – 7.8 m/s, data from Landsort Metereological Station 1991-1995) and variation in height of seed release in *S. pratensis* (0.25 – 0.60 m), the values obtained ranged from 0.35 to 2.77 m. In the simulations we used only one value, 0.94 m, since our previous tests showed this value has almost no effect on the outcomes (Mildén et al. unpubl.). Since no good estimation of the proportion of seeds dispersing independently of distance was available, simulations were run with two values (0.0001 and 0.001), covering the range where we considered that the true value of this parameter most likely will fall. Since the qualitative results did not change depending on this value, only results dispersing 0.0001 of seeds are shown.

Habitat and population destruction

To assess species survival perspectives due future landscape changes we studied effects of two different kinds of changes likely to be associated with ongoing habitat fragmentation and deterioration. These were effects of habitat destruction, i.e. of permanent removal of some suitable habitat patches for the system, and the effects of population destruction, i.e. of single removal of the population from a habitat patch while keeping the habitat essentially intact and ready for recolonisation. Habitat destruction mimics situations when some available habitats disappear for the landscape due to being turned into arable fields, becoming unsuitable due to ongoing succession, or being directly destroyed due to construction activities. Finding a proportion of habitats that can be destroyed without affecting species survival probability at a landscape level is essentially an attempt to find the threshold value in metapopulation capacity models. On the other hand population destruction brings in another dimension, viz. the assumption that a population may be wiped out of its habitat without essentially affecting the habitat.

Effect of habitat destruction was assessed by randomly removing a fraction ranging from 10 to 90 % of all habitat patches from the system. Effects of local population destruction were simulated by removing all individuals (including seeds) from a given habitat patch; this was attained by drawing a zero transition matrix to a habitat patch with a specified probability. This probability was set so that mean local population destruction frequency (per habitat patch) be 20, 30, 40 or 50 years. This was used to mimic large-scale non-correlated local population destruction events, such as single plowing event, construction work, burning, ditching or intensive trampling, that result in sudden disappearance of local populations. Moreover, effects of population destruction were combined with habitat destruction. In all simulations of effects of population and habitat destruction, parameter combination without any population and habitat destruction was used as a reference.

In all simulations of effects of local population destruction and habitat destruction, we used actual patch occupancy and population sizes in 2002 as initial conditions. Since the field data did not contain numbers of seeds and seedlings, these were calculated from the numbers of adult individuals assuming a stable stage structure. In all cases, the response variables (total population size, proportion of occupied patches and probability of extinction at the landscape level) were evaluated after simulation running for 100 years. For each parameter combination, 100 runs were done and the results averaged.

Reintroduction strategies

The efficiency of different types of reintroduction strategies was studied by taking a fixed number of seeds and distributing them in different ways over an unoccupied landscape. Here we used the same realistic landscape as above and assumed that the species has gone extinct. We studied consequences of introducing either 1000, 10 000 or 10 000 seeds into the whole landscape, corresponding to seeds approximately of 1, 10 and 100 individuals. Five reintroduction strategies were evaluated in each case: (i) introducing equal number of seeds into each habitat patch, (ii) introducing equal number of seeds into ten habitat patches systematically distributed over the landscape, (iii) introducing equal number of seeds into ten largest habitat patches, (iv) introducing equal number of seeds into the three largest habitat patches and (v) introducing all the seeds into the largest habitat patch only. Further we combined these strategies with population destruction occurring on average every 20 or 50 years. This mimicked large-scale non-correlated population destructions that could interfere with the reintroduction attempts. The response variables (total population size, number of occupied habitat patches) were evaluated after 20 and 100 years. Results were evaluated in the same way as for habitat destruction and local population destruction.

Results

Habitat and population destruction

Total population size strongly declined due to both habitat destruction and local population destruction (Fig. 1). In the case of habitat destruction, this is however simply due to lower carrying capacity of the environment. The species has 100% survival probability even if only 20% of the habitat patches were left (Fig. 2) showing that habitat destruction alone does not affect species survival perspectives over 100 years. Also patch occupancy was little affected by habitat destruction alone (Fig. 3).

In contrast to the small effect of habitat destruction, local population destruction occurring every 20 years resulted in very high probability of extinction even if no patches were removed. Also proportional patch occupancy was clearly dependent on local population destruction frequency (Fig. 3).

When habitat destruction and local population destruction were combined, there was a strong interaction of these two effects resulting in an increasing number of habitat patches needed to sustain the species in the landscape with the increase in local population destruction frequency (Fig. 2). For example the species had only 30 % survival probability when only 40% of patches were left under local population destruction occurring every 20 years.

Reintroduction strategies

Conclusions on the optimal reintroduction strategy strongly differed between the two parameters to be maximized (total population size and frequency) as well as between different starting seed numbers (Figs. 4 and 5). On the other hand there was little difference between the results after 20 and 100 years, and also the population destruction frequency had little effect.

When maximizing population size, introducing the species to three largest habitat patches would be the best strategy if only 1000 seeds were available, introducing to ten largest habitat patches would be the best strategy if 10 000 seeds were available and introducing to all habitat patches would be the best strategy if 100 000 seeds were available (Fig. 4).

For maximizing number of occupied habitats, introducing seeds into the ten largest habitat patches is the best strategy if only 1000 seeds are available, introducing seeds to all habitat patches is on the other hand the best strategy if at least 10 000 seeds are available (Fig. 5).

Discussion

Population viability

In this study we demonstrate an approach for estimating the effect of habitat destruction on likelihood of the species survival that is not based on the assumption of colonization-extinction equilibrium. Relaxing this assumption enables estimation of the effect of habitat destruction under realistic situations in the field and is able to take into account detailed data on biology of a species and on landscape structure.

Our results show that habitat destruction was important in combination with population destruction. These results correspond well to the biology of the species, which is a long-lived slowly growing perennial with seeds without any specific adaptation to wind dispersal (Grime at al. 1989). It has rather low colonization ability and is strongly affected by demographic stochasticity in early stages of the population development, but is highly persistent at a patch once the population gets established. If there are no population destructions operating in the system, the species is capable of surviving even if only one population is left, given that local conditions remain the same.

In reality, however, local destructions do occur and have to be taken into account when assessing the species survival in the landscape. A methodological difficulty here is that local destructions occur at different temporal and spatial scales. Large-scale population destruction that has the strongest effect is often so rare that realistic estimates of their frequency are rarely available. In the course of our study, two local population destructions due to construction work that resulted in total disappearance of a local population were observed (Mildén pers. obs.). Appearance and disappearance of many patches can readily result from shifting plowing regimes that are obvious from old maps of the area (Cousins and Eriksson 2002). Therefore we believe that local population destructions need to be taken into account when assessing the risks for long-term survival of *S. pratensis*. The data presented indeed show that for a slow growing plant with low rate of spreading, rate of local population destruction is *the* important force that determines whether the species will be able to survive. The effect of habitat destruction has to be thus assessed in interaction with population destruction.

While interpreting these results it should be kept in mind that the predictions are based on the assumption that local demography is stable over time. This is clearly a weak point of the model, since it does not incorporate possible negative effects of lower population size, greater isolation and habitat deterioration on local demography. Such effects have been repeatedly shown to play an important role in many species (Lienert et al. 2002, Paschke et al. 2002, Vergeer et al. 2003). Incorporating these effects would be feasible, however we yet do not have any realistic estimates of these effects for *S. pratensis.*

Optimal reintroduction strategy

Using our model, we were able to design optimal reintroduction strategy for the species. From the modeling perspective, reintroduction is essentially identical to viability analysis of existing populations with only initial conditions different. It again requires following species local population dynamics and its spread over landscape over time. Therefore once the model is parameterized for the species it can be used for both studying the effects of habitat and population destruction as well as for designing the optimal reintroduction strategies. It allows to combine these two as well as to include other possible effects.

Assessment of an optimal reintroduction strategy in a fragmented landscape depends on the population parameter, total population size or number of occupied patches that one wants to maximize. The parameter however also interacts with the original number of seeds that are available. For example, if there are enough seeds available, number of occupied habitat patches is maximized by distributing them equally among all existing habitat patches. If the number is low, this strategy leads to very high initial local population extinctions, and it is much better to introduce the seeds to ten largest habitats only. Similar pattern holds for local population size, where introducing seeds to ten or all habitats proved to be the best strategy depending on number of seeds available.

Decision on the parameter to be maximized depends on the need to spread the risk. This, in turn, largely depends on the probability of local population destruction as shown by simulations of species survival. If frequency of such events is high, the reintroduction strategy should be designed to maximize number of patches occupied. While the results on optimal reintroduction strategy did not qualitatively depend on whether local population destructions were introduced in the system, the results clearly confirm that habitat occupancy is less sensitive to these alternations than total population size.

It should be also noted that conclusions on optimal strategy did not differ much when evaluated after 20 and 100 years. This is clearly the result of slow dynamics of the species since it is far from equilibrium even after 100 years. Detailed analysis of the model predictions shows that the species can attain equilibrium in the landscape only at a time frame of several thousand years (Mildén et al. unpubl. data).

The difference between the conclusions on the optimal reintroduction strategy based on the parameter to be maximized alone may seem trivial. Its interaction with number of available seeds is however not straightforward and shows that this kind of simulation experiment is necessary if a realistic reintroduction strategy is to be designed at a landscape level. In combination with considerations on optimal reintroduction strategy at a local level (e.g. Schemske et al. 1994, Hodder and Bullock 1997, van Groenendael et al. 1998, Smulders et al. 2000, Kauffman et al. 2003), this approach is likely to improve design of future reintroduction plans.

Conclusions

Compared to other methods, the approach presented here makes possible estimation of the effect of habitat destruction irrespective of whether the species distribution is in transient stage or equilibrium. For this reason, it also enables to include other processes such as population destruction. By including population destruction in the model we demonstrate that population destruction plays a key role in survival of perennial plants in the landscape. Not including the effect, as done in other similar models, would lead to misleading conclusions on species response to habitat destruction.

A further strength of the approach is its ability to incorporate a whole range of landscape and species specific information and evaluation of the outcome over any time frame. On the other hand, the same feature makes this approach data demanding; it requires information on explicit position of all available patches, present distribution of the species, magnitude of dispersal and on local population dynamics. Therefore, the results are more difficult to obtain, and are necessarily more context-dependent and more difficult to generalize. However given the slow dynamics of *S. pratensis*, as well as of many similar long-lived perennial plants, only a dynamical model as the one presented here makes fully possible to perform population viability analysis for the species in fragmented landscapes and to design the most appropriate reintroduction strategy.

While the data available in this study were very detailed, simplified information on a system such as habitat positions derived from an aerial photograph and rough estimates of species demography, should be enough enable application of this approach in other species and systems. Even with rough estimates, the information gained using this approach, compared to traditional approaches, is expected to be much more detailed and realistic.

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Figure 1. Effect of proportion of habitat patches destroyed and yearly rate of local population destruction on total population size after 100 years. The points are means for 100 simulations. After local population destruction no living individual of *S. pratensis* remains at the patch. In all simulations proportion of seeds dispersing by distance independent dispersal was 0.0001.

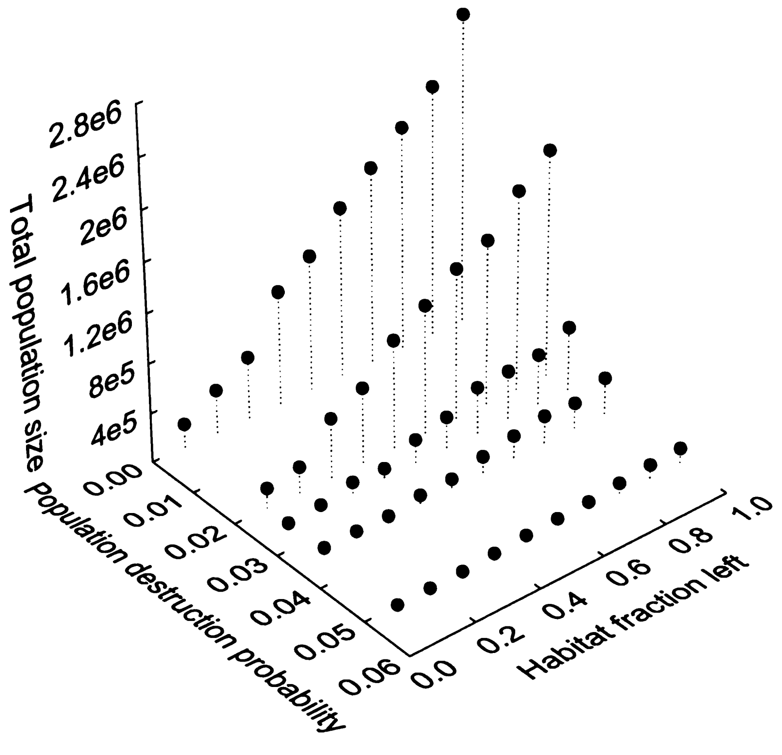


Figure 2. Effect of proportion of habitat patches destroyed and yearly rate of local population destruction on probability of extinction on the regional scale; the vertical axis indicates the proportion of realizations (out of 100) where the species persisted. After local population destruction no living individual *Succisa* remains at the patch. In all simulations proportion of seeds dispersing by distance independent dispersal was 0.0001.

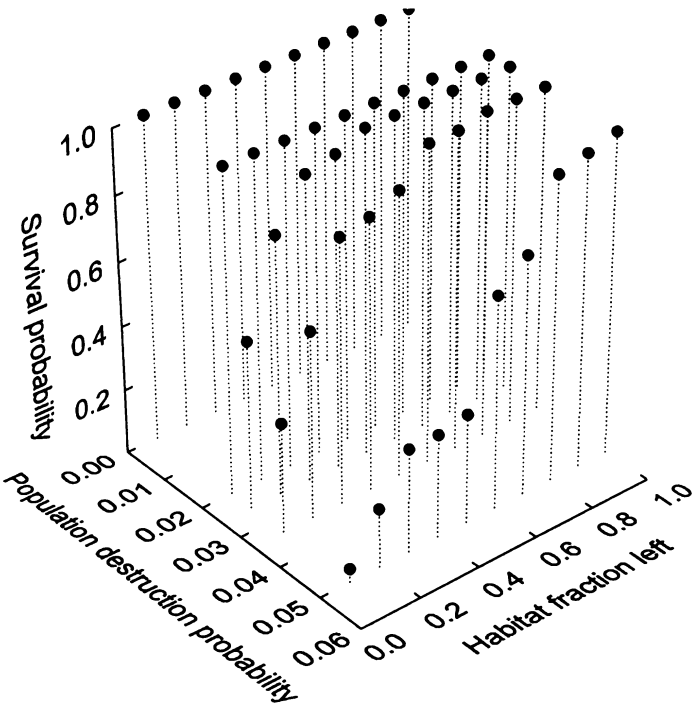


Figure 3. Effect of proportion of habitat patches destroyed and yearly rate of local population destruction on relative habitat occupancy. After local population destruction no living individual *Succisa* remains at the patch. In all simulations proportion of seeds dispersing by distance independent dispersal was 0.0001.

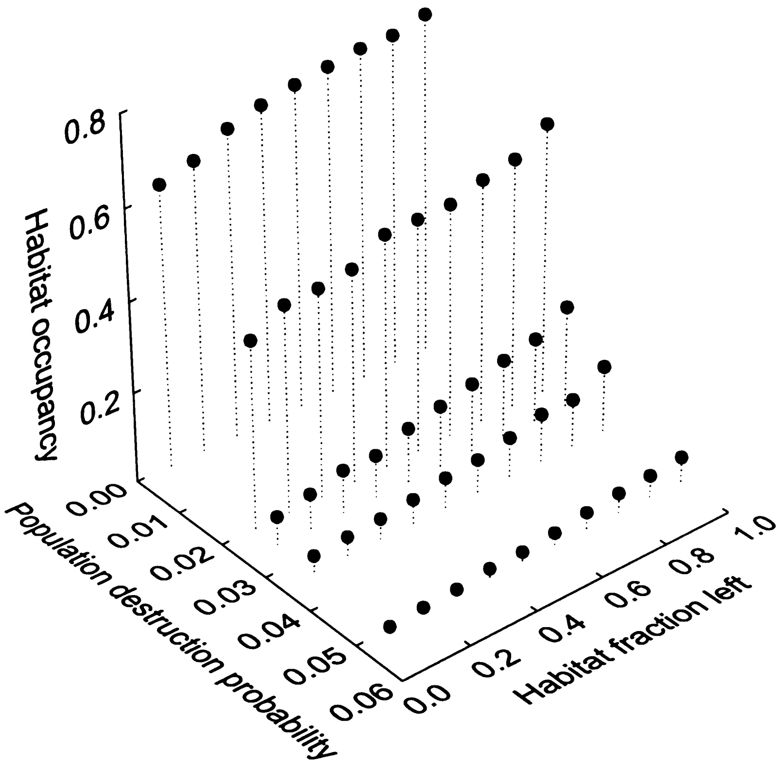


Figure 4. Total population size of *S. pratensis* 100 years after reintroducing different numbers of seeds using different reintroduction strategies. Results are based on 100 simulation runs.

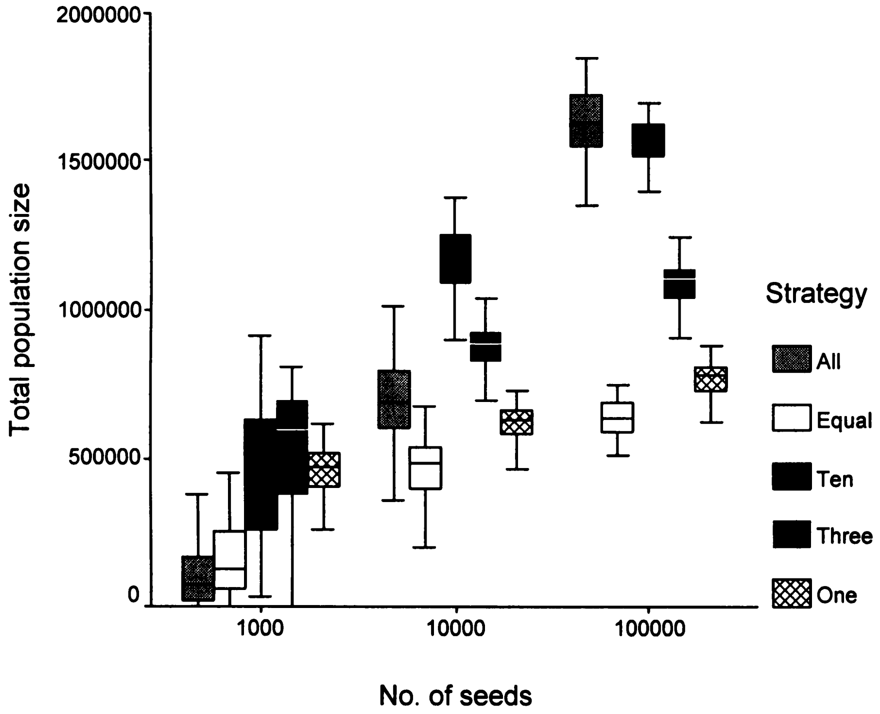
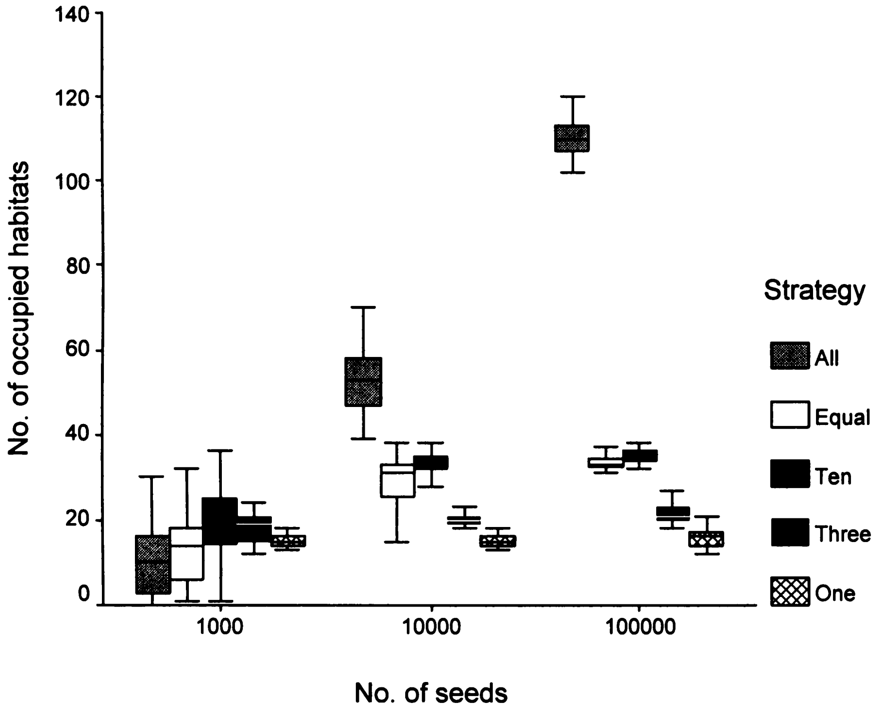


Figure 5. Number of occupied habitats 100 years after reintroducing different numbers of seeds using different reintroduction strategies. Results are based on 100 simulation runs.



Conclusions

In this thesis I have investigated local and regional population dynamics of species of fragmented grassland habitats. I have explored both methodological and terminological aspects of this type of study (papers 1,4, and 6) and provided specific examples of factors affecting local (papers 2 and 3) and regional (papers 5 and 7) species dynamics.

From the methodological point of view this thesis has suggested a new way of sampling data for demographic studies (paper 1) that is expected to considerably increase precision of our knowledge on species population biology. It has also suggested a new method for identifying suitable unoccupied habitats for metapopulation studies (paper 6) that is expected to be much more easily applicable and at least as informative as the preceding ones. Difficulties in identifying suitable unoccupied habitats are one of the major concerns in application of metapopulation theory to plants (Freckleton and Watkinson 2002). Since this method provides an easy to apply tool for identification of suitable habitats, it has the potential to increase our understanding of metapopulation dynamics of plant species.

Another important part of this study is the exploration of local population biology of species in fragmented grasslands (papers 2 and 3). These papers used the methodology proposed in paper 1. Both of these papers explore important topics related to estimating of endangerment status of rare species. Both of the topics have been explored in many previous studies. The strength of the studies presented here compared to all the previous studies is the connection of studies on single species traits with species population models. By doing this I demonstrate the quantitative importance of changes in single traits due to low population size and genetic diversity for long-term population growth rate (paper 3) and to demonstrate which differences in single traits of species have the potential to result in differences in species commonness in the landscape (paper 2).

To connect the local population dynamics of species to their regional distribution I explored the importance of different factors determining species distribution on different spatial scales. Because the terminology used to denote these different types of limitations is very variable, I first identify the basic concepts in these studies, and linked them to appropriate terms and suggested methods of their study (paper 4). Then I use this approach and demonstrate the importance of different types of limitation for species distribution is scale dependent. This shows an important weakness of previous studies of the importance of different types of limitation for species distribution, since these are concerned with one spatial scale only.

In the final paper (paper 7) I perform population viability analysis of a perennial herb restricted to fragmented grassland habitats at a landscape level. The method applied here extends the traditional methodology of assessing the effects of habitat fragmentation on prospect for species survival that is based only on species distribution patterns and assumes that species distribution is in equilibrium. The major strength of the approach used here is that it is applicable also to non-equilibrium situations and it enables to fully use also information on local population dynamics. In this analysis I combine data on local species dynamics such as those gained in paper 2 and 3, with information on factors affecting species distribution at the landscape level analyzed in papers 5 and 6.

The results of this thesis suggest that both local and regional processes are crucial for dynamics of perennial plants in fragmented grassland habitats. They show that our knowledge of factors determining species dynamics in fragmented habitats is, in spite of the large number of studies on this topic, very incomplete. They also demonstrate that incorporation of knowledge on local population dynamics to studies of the importance of population size and genetic diversity for the prospects for species survival, on the factors determining species rarity as well as to studies on regional dynamics species can provide new valuable insight into these topics.