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Diverzita, rozšíření a mezidruhová a vnitrodruhová variabilita růstových a reprodukčních charakteristik druhů suchých trávníků

Species diversity, distribution and interspecific and intraspecific variation in performance of dry grassland species

Disertační práce
Ph.D. Thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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TABLE OF CONTENTS

ABSTRAKT

Přežití a dynamika rostlinných druhů ve fragmentované krajině je dána nejen lokálními stanovištními podmínkami, ale také strukturou krajiny, která se v průběhu času mění, a také vlastnostmi jednotlivých druhů a jejich lokální populační dynamikou. Porozumění faktorům a procesům, které určují diverzitu, rozšíření a mezidruhovou a vnitrodruhovou variabilitu růstových a reprodukčních charakteristik druhů fragmentovaných stanovišť, je proto velmi složité a komplexní. Většina předchozích studií se zaměřila pouze na některé faktory. Studie, které zkoumaly relativní význam všech těchto faktorů pro diverzitu druhů i druhové složení, jsou však vzácné a to zejména v suchých trávnících. Studie zkoumající rozdíly v růstových a reprodukčních charakteristikách druhů ve vztahu k fragmentaci krajiny se často zabývají pouze jedním druhem a často byly provedeny v odlišných studijních systémech, což znesnadňuje zobecnění. Cílem této disertační práce bylo prozkoumat faktory, které jsou zodpovědné za diverzitu, rozšíření a mezidruhovou a vnitrodruhovou variabilitu růstových a reprodukčních charakteristik druhů suchých trávníků (tzv. pleší) v jinak lesnaté krajině.

V **1. kapitole** jsem zkoumala význam současné a historické struktury krajiny a lokálních stanovištních podmínek pro druhové bohatství suchých trávníků (pleší) a zhodnotila jsem jejich relativní význam. Údaje o současné i historické struktuře krajiny jsem získala z leteckých snímků území z let 1938, 1973, 1988, 2000 a 2007, na základě nichž jsem pro každou lokalitu vypočetla její plochu a izolovanost v současnosti i v minulosti a také jsem vyjádřila kontinuitu lokalit. Zjistila jsem, že lokální stanovištní podmínky a následně pak historická struktura krajiny mají největší vliv na druhové bohatství studovaných suchých trávníků. Současná krajinná struktura byla nejméně důležitá. Největší druhové bohatství bylo pozorováno na větších a heterogenních lokalitách s mělkou půdou a výskytem skalek, které byly velké a dobře propojené již v roce 1938 nebo alespoň v roce 1988.

V **2. kapitole** jsem zjistila, že také druhové složení suchých trávníků (pleší) je významně ovlivněno jak současnou, tak historickou strukturou krajiny, přičemž historická struktura krajiny v jednotlivých časových obdobích byla přibližně stejně důležitá jako struktura současná. Na lokality s velkou kontinuálně existující plochou již od roku 1973 nebo 1988 a také na aktuálně dobře propojené lokality se svým výskytem přednostně vážou vytrvalé druhy, které jsou opylovány hmyzem nebo větrem, kvetou po určitou omezenou dobu, jsou šířené zvěří, mají nízké nároky na živiny a vyznačují se kompetitivní nebo částečně stres-tolerantní strategií. Naproti tomu ruderální druhy, schopné samoopylení převažují v současně izolovaných lokalitách.

Ve **3. kapitole** jsem zkoumala vliv populační velikosti a konektivity na růstové a reprodukční charakteristiky rostlin u 21 vybraných druhů suchých trávníků lišících se svými vlastnostmi. Zjistila jsem, že klesající populační velikost i konektivita významně ovlivnila schopnost růstu a reprodukce všech studovaných druhů, avšak ne u všech druhů negativně. Tyto efekty byly pozorovány nejen v terénu, ale i ve standardních podmínkách zahradního pokusu (zde nebyly tak výrazné), což naznačuje, že rozdíly v růstu a reprodukční schopnosti jedinců u většiny druhů suchých trávníků nejsou dané jen odlišnými podmínkami na jejich stanovištích, ale také jejich populační velikostí a konektivitou jako takovou. Na základě

porovnání reakcí jednotlivých druhů jsem také identifikovala, jaké vlastnosti mají druhy, které jsou nejvíc citlivé ke zmenšující se populační velikosti a konektivitě.

Velké množství dat získaných v **kapitole 3** mi navíc umožnilo zabývat se obecnější otázkou a to jakým způsobem různé druhy alokují svou biomasu do nadzemních a podzemních částí svého těla, čemuž jsem se věnovala ve **4. kapitole**. Zjistila jsem, že některé druhy alokují biomasu izometricky a jiné alometricky do nadzemí vs. podzemí. Také jsem potvrdila, že alokační „pattern" je druhově a věkově specifický a že vlastnosti rostlin mohou mnohem lépe vysvětlit rozdíly ve způsobu alokace biomasy u různých druhů než jejich fylogenetická příbuznost na úrovni druhů vyskytujících se ve stejném typu prostředí. Způsob alokace biomasy lze tedy použít jako doplňující vlastnost druhů k vysvětlení jejich citlivosti ke krajinné struktuře, což jsem otestovala v **kapitole 3**. Ukázalo se, že druhy s nižším alokačním sklonem, tj. alokující proporčně více do podzemí než do nadzemí s rostoucí velikostí, který je typický pro časně kvetoucí druhy s vyšším SLA, jsou více citlivé ke snížené konektivitě populací.

Celkové výsledky ukazují, že znalost krajinné struktury v současnosti a v minulosti je důležitá pro pochopení současné druhové diverzity i rozšíření druhů a že vlastnosti druhů nám mohou pomoci předpovídat, které druhy budou více citlivé ke změnám ve struktuře krajiny a také jakým způsobem budou které druhy alokovat biomasu.

ABSTRACT

In fragmented landscapes, survival and dynamics of plant species may be determined not only by local habitat conditions but also by landscape structure and its changes over time as well as by species life-history traits and their local population dynamics. Understanding the factors and processes determining diversity, distribution and variation in performance in species of fragmented habitats is thus very complex. Most previous studies focused only on some particular factors. However, studies that explored the relative importance of all these factors for species diversity and composition are rare, especially in dry grassland communities. Also studies exploring variation in species performance in relation to landscape fragmentation usually deal only with a single species and were often done in different study systems making generalization difficult. The aim of this Ph.D. thesis was to explore factors that are responsible for species diversity, distribution and interspecific and intraspecific variation in performance of dry grassland species occurring in a system of dry grassland-like forest openings in the forested landscape.

In **Chapter 1** I examined the effect of current and past landscape structure and local habitat conditions on species richness at dry grassland-like forest openings and assessed their relative importance. I analyzed information on past and present landscape structure using aerial photographs from 1938, 1973, 1988, 2000 and 2007 and calculated the area and isolation of each locality in the present and in the past and the continuity of localities. I found that local habitat conditions had the strongest effect on species richness, followed by historical landscape structure. Current landscape structure had the weakest effect. The highest species richness was observed on larger and more heterogeneous localities with rocks and shallow soils, which were already large and well connected to other localities in 1938 or at least in 1988.

In **Chapter 2** I found that also species composition was determined by landscape structure in the past and at present, and the past landscape structure in each time period separately was equally important as the present landscape structure. Perennial species that are insect or wind pollinated, flower over limited periods of time, are dispersed by animals, have low nutrient requirements and have competitive or partly stress-tolerant strategies were found to be restricted to continuous localities existing at least since 1973 or 1988 and to localities that are currently well interconnected. In contrast, self-pollinated, ruderal species prevail in currently less-connected localities.

In **Chapter 3** I explored the effect of population size and connectivity on plant performance of 21 dry grassland species differing in their life-history traits. I found that population size and connectivity significantly affected the performance of all the studied dry grassland species, but the effect of decreasing population size and connectivity was not always negative. These effects were detected not only in the field, but also in the common garden environment (but they were weaker there). This indicates that the differences in plant performance of most dry grassland species are not only due to differences in the actual habitat conditions but also due to effects of their population size and connectivity *per se*. The

between species comparisons identified range of species traits determining species sensitivity to changes in population size and connectivity.

Extensive data obtained in **Chapter 3** also allowed to explore more general question related to determinants of above- and below-ground biomass allocation patterns in the different species which I aimed in **Chapter 4**. I found isometric as well as allometric patterns of biomass allocation in the studied species. I confirmed that biomass allocation pattern is species- and age-specific and that plant life-history traits are much more important in explaining allocation pattern among species than is phylogenetic relatedness among species within a habitat. The pattern of biomass allocation can thus be used as additional species trait explaining species sensitivity to landscape structure. Species with lower shoot–root allocation slope at adult plants, which is typical for earlier flowering species with higher SLA, are more sensitive to decreasing population connectivity as was shown in **Chapter 3**.

The overall results indicate that knowledge of landscape structure in the present and in the past is important for understanding the current species diversity and distribution and that species traits can be used as useful predictors of species responses and sensitivity to the landscape structure as well as of biomass allocation patterns.

GENERAL INTRODUCTION

Understanding the factors and processes determining species diversity, distribution and variation in performance in species of fragmented habitats is fundamental challenge in ecology. The question why some species occur or perform well somewhere and not elsewhere is very complex and can be seen from different complementary perspectives. The survival and dynamics of plant species in the landscape level, and the variation in their performance, may be determined not only by local habitat conditions but also by landscape structure and its changes over time as well as by species life-history traits characterizing the key processes of dispersal, recruitment, establishment and survival of species at localities and their local population dynamics (e.g. Eriksson 1996; Ehrlén and Eriksson 2000; Dupré and Ehrlén 2002; Kolb and Diekmann 2005). Many plant and animal species may live in naturally fragmented habitats and can be well capable of coping with the special conditions associated with habitat fragmentation (Lienert 2004). However, due to extensive changes in land use in the European landscape during the past few centuries, many previously opened habitats, mainly semi-natural grasslands, that have been maintained by human activity, such as mowing or keeping grazing animals, are slowly disappearing and are consequently becoming smaller and more fragmented (e.g. Eriksson et al. 2002; Jacquemyn et al. 2003; Soons 2003; Hérault and Honnay 2005; Piessens et al. 2005; Adriaens et al. 2006, 2009; Helm et al. 2006; Cousins et al. 2007; Chýlová and Münzbergová 2008).

Landscape fragmentation is a process where originally large and continuous localities are divided into a series of smaller and less continuous patches (Soons 2003). As a consequence, also populations of many species are becoming small and isolated. Species in small populations are strongly influenced by demographic and environmental stochasticity and are thus prone to extinction (Graae and Sunde 2000; Dupré and Ehrlén 2002; Kolb and Diekmann 2004; Münzbergová 2006a; Jackson and Sax 2010). Also changed abiotic and biotic conditions at localities and thus deterioration of habitat quality (e.g. encroaching by shrubs and eutrophication (Eriksson et al. 2002; Soons and Heil 2002)) can reduce ability of species to survive at such localities (Adriaens et al. 2009; Leimu 2010; Tsaliky and Diekmann 2010; Lauterbach et al. 2011; Busch and Reisch 2016; Roque et al. 2017). Moreover, reduced connectivity between the patches can limit the spread of the species due to the increasing distance or presence of barriers between patches and therefore lead to a lower possibility of recolonization of those patches (Ehrlén and Eriksson 2000; Jacquemyn et al. 2001; Dupré and Ehrlén 2002; Soons 2003; Ozinga et al. 2005, 2009). The distribution and dynamics of plant species in such patchy landscape are thus determined not only by local habitat conditions, but also by the balance between local extinction and colonization (Mac-Arthur and Wilson 1967; Hanski 1998). All these processes have a strong impact on the regional survival of the species (Jacquemyn et al. 2002) and thus lead to reduction of overall species richness and also to the changes in species composition of particular localities.

One possible explanation of species richness and diversity in fragmented habitats is provided by the equilibrium theory of island biogeography (Mac-Arthur and Wilson 1967). This theory postulates that that number of species on the island is determined by the dynamic balance between immigration and extinction. The number of species on the island will decrease with increasing distance of the island from the mainland or from the other islands, as the species occurrences will be limited by its ability to reach the island. On the contrary, more species can be kept on the island when its area is larger. This positive species-area relationship has been demonstrated by a large number of studies (e.g. Kohn and Walsh 1994; Ricklefs and Lovette 1999; Ney-Nifle and Mangel 2000; Pyšek et al. 2002; Peintinger et al. 2003; Turner and Tjorve 2005; Cousins et al. 2007; Knappová et al. 2012) and has been explained by several theories where the area can affect species richness either directly or indirectly via increasing habitat heterogeneity (Mac-Arthur and Wilson 1967; Connor and McCoy 1979; Ricklefs and Lovette 1999). Similarly, numerous studies have demonstrated the negative effects of habitat isolation on species diversity (e.g. Dupré and Ehrlén 2002; Eriksson et al. 2002; Soons and Heil 2002; Kolb and Diekmann 2004, 2005; Ozinga et al. 2005; Adriaens et al. 2006; McEuen and Curran 2006; Knappová et al. 2012). The occurrence of individual species in an isolated locality therefore depends on its ability to disperse. The species with limited dispersal will leave many suitable habitats unoccupied. The degree of species dispersal limitation is primarily determined by their traits (Ozinga et al. 2005), such as the amount of seeds produced and their weight (i.e. a colonization-competitive "trade-off" between the ability of the species to reach the locality and then to maintain there) as well as the ability of species to disperse, establish and maintain on the site (Ehrlén and Eriksson 2000; Dupré and Ehrlén 2002; Tremlová and Münzbergová 2007). Especially for occurrence of species in isolated habitats, the long distance dispersal is important, which is mediated by wind, large mammals, birds or water (Soons 2003). At the regional scale, the degree of dispersal limitation can also be influenced by the abundance of species in the regional "species pool" and the spatial configuration and connectivity of suitable habitats (Ozinga et al. 2005).

The dynamics of species in fragmented landscape can also be described by the theory of metapopulation dynamics (Hanski 1998), where fragmented populations may be seen as a certain kind of metapopulation. Metapopulation represents a system of local populations and unoccupied but potentially suitable habitats within a larger area/region, which are connected through species dispersal and are maintained by a dynamic balance between colonization and extinction (Eriksson 1996). However, when fragmented populations are insufficiently connected through dispersal, extinction prevails over colonization (Lienert 2004) and the metapopulation is no longer viable. In a dynamically changing landscape, assuming that the distribution of plant species is in equilibrium may be therefore misleading (e.g. Eriksson 1996; Ehrlén and Eriksson 2000; Fahrig 2003; Herben et al. 2006; Münzbergová et al. 2013) and it is thus necessary to consider also the history of the landscape (mainly the historical area and isolation of the localities and their persistence at the same place continuously). For the purpose of this study, the landscape history is considered only in the short-term, of only the last one hundred years, and therefore the long-term processes, such as processes of speciation, are not included (Stuessy et al. 2003; Outlaw and Voelker 2008). The historical landscape structure is especially important for explanation the distribution pattern of long-lived species or species with extensive seed banks that tend to build up remnant population systems (Eriksson 1996). Many plant species can survive in habitat fragments under suboptimal conditions for decades (Eriksson 1996) and thus they respond to changing conditions with a certain time lag (Eriksson et al. 2002; Lindborg and Eriksson 2004; Helm et al. 2006; Vellend et al. 2006; Krauss et al. 2010; Münzbergová et al. 2013), which may lead to extinction debt (Cousins 2009; Kuussaari et al. 2009; Jackson and Sax 2010). On the other hand, many species may be missing from suitable habitats due to dispersal limitation (Ehrlén and Eriksson 2000; Münzbergová 2004; Münzbergová and Herben 2005; Ehrlén et al. 2006; Ozinga et al. 2009). As a result, distribution of some species reflects historical habitat configuration rather than current habitat configuration (Helm et al. 2006; Herben et al. 2006; Lindborg 2007).

The dynamics of species in the landscape is thus determined not only by the current habitat conditions and landscape structure, but also by the conditions and landscape structure in the past. Since each landscape is changing over the time, new suitable habitats can appear and the existing habitats can disappear. The species occurrence will thus depend not only on the actual habitat conditions of the locality, but also on species ability to survive even under the conditions which are deteriorating, and especially on its ability to disperse elsewhere (Eriksson 1996; Ehrlén and Eriksson 2000). Therefore, the specific spatio-temporal landscape structure is essential for species dispersal and survival in the landscape.

The effect of landscape history on plant species diversity has been studied mainly in forest fragments (e.g. Graae and Sunde 2000; Jacquemyn et al. 2001; Honnay et al. 2004; Kolb and Diekmann 2004; Hérault and Honnay 2005). Studies on other habitat types are relatively rare and were carried out in rural or agricultural landscape and focused mainly on changes of land use at particular habitats (Bruun 2000; Bruun et al. 2001; Cousins and Eriksson 2001, 2002; Krauss et al. 2004; Chýlová and Münzbergová 2008). Much less is, however, known about the effects of the past landscape structure (in terms of habitat size and isolation) on patch-scale species richness (e.g. Lindborg and Eriksson 2004; Helm et al. 2006; Cousins et al. 2007; Öster et al. 2007; Krauss et al. 2010) and even less is known about these effects on species composition (the only such studies are Purschke et al. 2012; Hemrová and Münzbergová 2015) in fragmented grassland systems. The species composition and diversity in fragmented localities is a result of the current and the past landscape structure and their local habitat conditions. Most studies focused only on some of these factors. However, studies that examined the relative importance of all these processes for species diversity as well as species composition are very rare, especially in dry grassland communities.

In fragmented landscape, however, not only species distribution and species diversity are changing and decreasing, but even before it, the individual populations of species may become smaller and more isolated, which can lead to reduction of their fitness and subsequently to their disappearance from the locality. Many species are able to persist in the locality for a long time, even under the suboptimal conditions, but they may not be performing very well there. Poor performance of individuals in small and isolated localities, expressed especially as reduced growth and reproductive ability of individuals of such species, may be seen much earlier and may subsequently result in a gradual reduction of population sizes and thus higher extinction risk of the local population (Fischer and Matthies 1998; Dupré and Ehrlén 2002; Münzbergová 2006a; Jackson and Sax 2010). Recognizing the causes of poor performance of species in small and isolated populations is thus crucial for effective conservation of plant species diversity.

Smaller, less dense and more isolated populations are often less attractive for pollinators (Ågren 1996; Groom 2001; Aguilar et al. 2006; Kolb 2008; Peterson et al. 2008; Dauber et al. 2010), making them easily overlookable and their reproduction is thus limited by availability of pollinators as well as of compatible pollen (i.e. pollen quality and quantity), leading to a pattern referred as the Allee effect (Groom 1998, 2001; Ghazoul 2005; Jakobsson et al. 2009). As a consequence of poor pollination quality, especially due to mating of closely related individuals or through selfing, plant individuals in small and/or isolated populations often suffer from increased level of inbreeding (Jacquemyn et al. 2002; Kolb 2005; Leimu et al. 2006; Van Geert et al. 2008). In a short term, genetic erosion leading to inbreeding depression can have immediate impact on local populations by reducing individual performance, especially their survival and reproduction (Lienert 2004; Frankham 2005). In the long term, lower level of genetic diversity may reduce the evolutionary potential of the populations and thus greatly reduce their ability to respond to changing environmental conditions through adaptation and selection (Frankham 2005; Bowman et al. 2008).

Among these genetic and ecological mechanisms, the performance of plants in small and isolated populations may be affected by other factors. Mainly changed actual abiotic and biotic conditions at some localities due to deterioration of habitat quality (e.g. encroaching by shrubs and eutrophication, as a consequence of the ongoing landscape fragmentation (Eriksson et al. 2002; Soons and Heil 2002)) can reduce plant growth and reproduction (Adriaens et al. 2009; Leimu 2010; Tsaliky and Diekmann 2010; Lauterbach et al. 2011; Busch and Reisch 2016; Roque et al. 2017). On the other hand, plants in small and isolated populations may be less attacked by herbivores or pathogens (Soons 2003; Groom 2001; Münzbergová 2006b) and thus the seed production and proportion of developed seeds may not be reduced by seed predators. In some cases, however, plants in small populations may be attacked by herbivores much more, as their populations can be already large enough to maintain viable population of herbivores but still too small to maintain populations of their natural enemies – predators and parasitoids of herbivores (Lienert 2004; McEuen and Curran 2006; Matesanz et al. 2015). Moreover, thanks to inbreeding depression and low level of genetic diversity, plants in small populations are less able to adequately respond to herbivory or regrow after being damaged (Leimu et al. 2008; Kolb 2008; Leimu and Fischer 2010).

For these reasons, it would be expected that plants in small and isolated populations should be more negatively affected by habitat fragmentation in their performance. A large number of studies have been carried out on this topic, but their results are rather ambiguous. Many of these studies have revealed a positive relationship between population size and performance of individuals or their genetic diversity (reviewed in Leimu et al. 2006; than e.g. Hensen and Wesche 2006; Kolb and Lindhorst 2006; Honnay et al. 2007; Jacquemyn et al. 2007; Peterson et al. 2008; Dostálek et al. 2010; Hornemann et al. 2012; Lauterbach et al.

2011; Putz et al. 2015; Busch and Reisch 2016; Münzbergová et al. 2018). There are, however, many other studies that have found no relationship (e.g. Oostermeijer et al. 1994; Ouborg and Treuren 1995; Lammi et al. 1999; Eisto et al. 2000; Leimu and Mutikainen 2005; Honnay et al. 2006; Severn et al. 2011) or even negative relationship (e.g. partly Hooftman et al. 2003 and Schleuning et al. 2009). Most of these studies, however, deal only with a single plant species, usually rare and endangered. The different study systems can also be very heterogeneous and differ in spatial scale, environmental conditions, habitat type, age of the localities etc. Moreover, most studies surveyed the species performance only in the field and not also in the common garden environment (but e.g. Oostermeijer et al. 1994; Ouborg and Treuren 1995; Fischer and Matthies 1998; Kéry et al. 2000; Hooftman et al. 2003; Kolb 2005, 2008; Schleuning et al. 2009) and thus the differences in individual performance due to small population size and isolation can be masked by the actual habitat conditions at the localities.

Importance of species life-history traits

The degree to which species are affected by fragmentation, and also in which way, may vary between species and depend on their specific habitat requirements and biological attributes (Dupré and Ehrlén 2002; Kolb and Diekmann 2004, 2005). The distributional patterns as well as inter- and intraspecific variation in plant performance of dry grassland species can thus largely depend on plant species life-history traits, mainly those related to dispersal and survival. It should thus be possible to identify which life-history traits have species that prevail in a certain habitat type (according to its present and past habitat configuration) and which life-history traits have species that are more or less sensitive to landscape fragmentation.

Although the species distribution and their intra- and interspecific performance variation in relation to landscape structure is driven by different processes and factors, we can see a certain similarity in the life-history traits of species occurring and also well-performing in a certain habitat type (with a certain habitat configuration).

A lot of attention was paid mainly to persistence traits of species in relation to distribution as well as plant performance variation. It has been shown that short-lived plants are more sensitive in their occurrences to landscape changes and respond much faster to such changes than long-lived plants. As a result, the distribution of short-lived species should be affected mainly by the current landscape structure (Lindborg 2007; Lindborg et al. 2012). In contrast, long-lived plants with clonal propagation and plants with long-lived seed banks tend to build up remnant population (Eriksson 1996) and their distribution is thus mainly affected by historical landscape structure (Lindborg 2007; Purschke et al. 2012). Such long-lived species may thus persist at the site for a long time and benefit from large area of locality in the past and from its long continuity. However, if the habitat quality deteriorates, these species still persist at the site, but they may not perform very well there. Their populations size may be decreasing and they thus often suffer from lack of pollinators (as they are mainly outcrossing) and subsequent inbreeding depression (Angeloni et al. 2011), which leads to a significant reduction in fitness. On the other hand, many short-lived species have traits that enable them to persist well also in fragmented landscape (i.e. they are often self-pollinated species with ruderal strategy, that produce large amount of small well-dispersed seeds, and thanks to purging effect, they have significantly lower level of inbreeding depression than long-lived species) (Adriaens et al. 2006; Angeloni et al. 2011; Hemrová and Münzbergová 2015) and thus when the habitat conditions at the locality become unsuitable for them, they easily disperse elsewhere – to another suitable, usually unoccupied locality. They may thus build up "true" metapopulations (Eriksson 1996). From the point of view of one population, however, short-lived species may appear to be more sensitive to landscape changes, as they are mainly semelparous and thus respond quickly to the changes, which was supposed by several previous studies, which finally did not find any difference in performance of annuals and perennials (Aguilar et al. 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007).

Also the pollination mode/type and thus the relationship with and the degree of dependence on pollinators are very important especially for plant species performance variation and subsequently also for species distribution. It was shown that obligate and mainly outcrossing species are much more negatively affected by declining population sizes or their isolation (Aguilar et al. 2006; Honnay and Jacquemyn 2007), which leads to limited pollination and consequently to a reduction of genetic variation in populations (Leimu et al. 2006). On the other hand, self- or wind-pollinated species can be less susceptible to landscape fragmentation (Kolb and Lindhorst 2006). For this reason, it was also found that isolated localities are more often occupied mainly by self-pollinated species (e.g. Groom 2001; Purves and Dushoff 2005).

Whereas it is commonly assumed that distribution of species at the landscape scale is largely determined by species dispersal traits (e.g. Ehrlén and Eriksson 2000; Münzbergová 2004; Ozinga et al. 2005, 2009), most previous studies testing the effect of various species traits in fact concluded that persistence traits are better predictors of species distribution than dispersal traits (e.g. Graae and Sunde 2000; Lindborg 2007; Lindborg et al. 2012; Hemrová and Münzbergová 2015). There are only several studies, that show significant effects of dispersal traits on the dependence of species occurrences on current habitat configurations (Adriaens et al. 2007; Schleicher et al. 2011; Saar et al. 2012; Hemrová and Münzbergová 2015) or on historical habitat configurations (Purschke et al. 2012). I am in fact not aware of any study, which would attempt to find such relationships also for plant performance. Nevertheless, it could be interesting to explore whether also relationship between plant performance and population size or isolation could be affected by species dispersal traits.

Also, many other species life-history traits could affect the distributional pattern as well as variation in plant performance of dry grassland species, for example seed weight, that could have impact through a colonization-competitive "trade-off" between the ability of the species to reach the locality and then to maintain there (Ehrlén and Eriksson 2000) as well as could affect the germination success and subsequent growth of new individuals (Weiner et al. 1997; Münzbergová and Plačková 2010), clonality (Lindborg 2007; Lienert 2004; Kolb and Lindhorst 2006), CSR-strategy or specific leaf area (Hemrová and Münzbergová 2015).

For species of fragmented habitats also pattern of resource allocation into the above- and below-ground biomass can be extremely important, as small and isolated habitats often suffer from encroaching by shrubs and eutrophication leading to changes in resource availability for species living here (Soons and Heil 2002). Pattern of shoot–root biomass allocation of species may also vary according their life-history traits and during their life (ontogeny) (McConnaughay and Coleman 1999; Weiner 2004; Gedroc et al. 1996) and also according to their phylogenetic relatedness (as was shown by McCarthy et al. (2007) and McCarthy and Enquist (2007) in a global dataset at higher taxonomic levels) and could thus also have effect on plant performance and population size or isolation relationship and subsequently also on species distribution in fragmented landscape. It was shown that some species are able to change their allocation strategy in relation to environmental changes (resource limitations in the environment) and thus preferentially allocate biomass to the organ that acquires the most limiting resource (Bloom et al. 1985; Shipley and Meziane 2002; McCarthy and Enquist 2007). Such species feature "true" plasticity and can thus perform well even under impaired habitat conditions of small and isolated habitats. On the other hand, other species may not change their allocation strategy in relation to different resource levels, but the changes in their allocation pattern occur under the normal course of growth and development (Coleman et al. 1994; McConnaughay and Coleman 1999; Weiner 2004), and such changes reflect the changing priorities of an organism during its development. Such species thus usually respond to the environmental conditions deterioration through slower growth (i.e. they are smaller) as they feature size-dependent allometric patterns ("apparent" plasticity; McConnaughay and Coleman 1999; Weiner 2004; McCarthy and Enquist 2007). And because worse habitat conditions cause smaller size, these species may be more sensitive to habitat fragmentation. Few studies found that perennials exhibited "apparent" plasticity whereas annuals exhibiting "true" plasticity (Mao et al. 2012; Zhou et al. 2014), but these studies were done on very few (1–3) species within each category. Moreover, the differences in performance of annuals and perennials (or short- and long-lived species) may be due to their different life strategies, which can reflect changes in their allocation pattern during their ontogeny. For annual plants, it was found that the proportion of allocation to roots declines during their growth and development (Coleman et al. 1994; Gedroc et al. 1996; McConnaughay and Coleman 1999), as annuals finish their life cycle after fruiting and seeding. The direction of preferential allocation to roots or shoots over the course of ontogeny is more complicated and less consistent among perennial plants (e.g. Niinemets 2004 vs. Janeček et al. 2014), as the perennials need not only reproduce but also store resources for the next season. Differences in perennials allocation pattern may thus probably depend on in which stage of their life cycle the studied plants are.

A useful approach for understanding species distribution in a changing landscape is to analyse the relationships between species occurrences in habitats with different configurations and life-history traits. Earlier studies, however, have not attempted to link species life-history traits directly to landscape characteristics (but see Purschke et al. 2012; Hemrová and Münzbergová 2015). Instead, they have focused only on the relationship between mean trait values (at the site level) (Lindborg 2007) or emergent groups of functionally similar species, and site or landscape descriptors (Kolb and Diekmann 2004, 2005; Adriaens et al. 2006; Lindborg et al. 2012). However, this approach cannot answer the question of how individual traits contribute to species dynamics in the landscape. Moreover, in case of intra- and inter-specific performance variation, most of the between species comparisons were done only as meta-analyses or reviews (Aguilar et al. 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007; Angeloni et al. 2011 for plant performance–population size or isolation relationship, Poorter et al. 2012; Weiner et al. 2009; McCarthy et al. 2007; McCarthy and Enquist 2007 for biomass allocation). But the individual studies can be very heterogeneous, as they were often done only on single or few species and also in different studied systems making the results of such different studies hardly comparable. Studies studying performance variation of more than single species (but still only very few species) coexisting in the same habitat type are still very rare and did not directly tested the species intraspecific performance variation against their life-history traits. We thus do not know whether and how the plant performance variation (expressed as species sensitivity to landscape fragmentation or as biomass allocation pattern) could differ between species, which encompass a range of life forms/strategies, breeding systems, pollination and dispersal characteristics and coexisting within the same habitat type in fragmented landscape.

Model system (study area and species)

The study was carried out in Týřov Site of Community Importance (**Chapter 1–3**). It is part of Křivoklátsko Protected Landscape Area and Biosphere Reserve in the Czech Republic. The region is characterised by patchy occurrence of dry grassland-like forests openings with typical dry grassland plant communities (Kolbek at al. 2001; details in **Chapter 1**), which represent the places with the highest plant and animal species richness and are thus the places of high conservation interest (Kučera 1997). The individual patches are often relatively small and isolated from each other and for this reason it can be assumed that the occurrence and survival of species will be largely influenced by the ability of these species to spread in the landscape. In addition, during the last century, these places experienced several changes in the landscape structure. The main factors responsible for changes in the landscape are shrub invasions likely initiated by increased nutrient deposition since the 1990s (Kolbek 1996; Petřík et al. 2011) and outbreak of hoofed game (Ungulates), especially mouflons (*Ovis musimon*), in the region in the 1970s and 1980s (Kolbek 1996; Kolbek et al. 2003). The landscape structure of this region is thus changing over time and therefore, it can be assumed that the current species diversity and distribution could be influenced also by historical factors.

To study the effect of the landscape structure on plant performance (**Chapter 3**) I selected 21 dry grassland species from a wide taxonomic range strictly occurring only in these forest openings and differing in their life-history traits. The species were selected to occur on at least 15 out of a total of 110 localities in the studied area. I also made sure to select species growing in populations differing in their size and connectivity. For the purpose of this study, I was able to collect material usually from 20 (12–20) localities per species differing in their population size and connectivity. Based on these criteria, the following model species were selected: *Acinos arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Asperula cynanchica, Dianthus carthusianorum agg., Echium vulgare, Fragaria viridis, Geranium columbinum, Hieracium pilosella, Inula conyza, Jasione montana, Melica transsilvanica, Myosotis ramosissima, Origanum vugare, Phleum phleoides, Potentilla argentea, Scleranthus perennis, Teucrium botrys, Triforium arvense, Veronica dillenii, Vicia hirsuta.* The seeds, collected in the field and coming from several individuals from each studied population were used to establish an experiment in an experimental garden. These cultivated plants were used to assess the effects of the landscape structure on plant performance when planting in common environment (**Chapter 3**) as well as to study the pattern of above- vs. below-ground biomass allocation of these species (**Chapter 4**).

Aims

The aim of this Ph.D. thesis was to explore factors that are responsible for species diversity, distribution and interspecific and intraspecific variation in performance of dry grassland species occurring in a system of dry grassland-like forest openings in the forested landscape, especially in relation to the degree of landscape fragmentation at present and in the past as well as in relation to species life-history traits. Specifically, I asked these questions:

1) What is the effect of current and past landscape structure and habitat conditions on species richness at dry grassland-like forest openings in a forested landscape and what is their relative importance for species richness?

2) What is the importance of current and historical landscape structure for plant species composition in dry grassland-like forest openings in forest matrix and which life-history traits explain species response to current and past landscape structure?

3) What is the effect of population size and connectivity on plant performance of dry grassland species and how does the relationship differ between species and in relation to their life-history traits?

Extensive data obtained when attempting to answer Question 3 also allowed to explore more general question related to determinants of allocation patterns in the different species.

4) How does allocation to above- vs. below-ground biomass differ between seedlings and adult plants within and among species and can the between species differences in allocation pattern be explained by species life-history traits and by phylogenetic relatedness of species?

By answering this question, I could in turn use the knowledge on the allocation patterns as additional species trait explaining species sensitivity to landscape structure in Question 3.

Main results

In **Chapter 1** I examined the effect of current and past landscape structure and local habitat conditions on species richness at dry grassland-like forest openings in a forested landscape and assessed their relative importance for species richness. I mapped all current dry grassland-like forest openings in the study region and collected data on plant species distribution and abiotic conditions at each locality. I also analyzed information on past and present landscape structure using aerial photographs from 1938, 1973, 1988, 2000 and 2007 (the habitat configuration in the year 2007 represents the current landscape structure). In GIS I calculated the total area of each locality and its isolation in the present as well as its continuity, isolation and area in the past and also some other parameters describing local habitat conditions. Subsequently, I assessed the effect of each individual factor of current and past landscape structure and local habitat conditions as well as the relative importance of all of these factors for species richness.

I found that the current species richness of dry grassland-like forest openings was significantly affected by all three studied groups of variables: the local habitat conditions and the landscape structure in the present and in the past. Each of these three groups explained different but important deal of species richness variance. Local habitat conditions had the strongest effect on species richness, followed by historical landscape structure (in all the studied years). Current landscape structure had the weakest effect. The substrate heterogeneity seems to be the most important overall factor. From historical parameters the area of habitats in 1938 was the most important and from current parameters it was the current area of habitats (but it was in fact the only current landscape predictor, as current isolation had no significant effect on species richness). The current area of localities was however the second most important overall predictor of species richness, but after removing the effect of habitat heterogeneity and historical parameters of landscape structure, its effect became much weaker but still significant. The highest species richness was thus observed on larger and more heterogeneous localities with rocks and shallow soils, which were already large and well connected to other localities in 1938 or at least in 1988. The changes in the landscape structure in the past can thus have strong effects on current species richness. Future studies attempting to understand determinants of species diversity in fragmented landscapes should also include data on past landscape structure, as it may be even more important than the present structure.

This study is important also from methodological point of view because all the parameters of the landscape structure and local habitat conditions that have been calculated there, are subsequently used in the other related chapters **(Chapter 2–3)**. Moreover, in this chapter I also demonstrated that the changes in the landscape structure of the study region during the last several decades, were substantially different and the reduction of the locality area was not so dramatic (only by 27% since 1988 up to now) in comparison with other studies on semi-natural dry grasslands performed in Belgium (Adriaens et al. 2006) or Sweden (Eriksson et al. 2002).

In **Chapter 2** I assessed the importance of current and historical landscape structure for species distribution in dry grassland-like forest openings and identified which species life-history traits are related to species dependence on habitat configuration in different time periods. Although a number of studies identify plant life-history traits responsible for species distribution in fragmented landscape, our understanding of the effects of individual traits on species dependence on landscape structure is still rather limited. I examined the effect of current (2007) and past landscape structure (area and isolation) in 1938, 1973, 1988 and 2000, habitat continuity (as proportion of area of each locality that persisted at the same place as in the past) and habitat conditions on species composition. I also assembled data on life-history traits related to seed dispersal, species life strategy and habitat requirements and I used these traits to explain the species dependence on landscape structure in different time periods.

I found that the current distribution of dry grassland species in the dry grassland-like forest openings has been significantly affected not only by local habitat conditions but also by landscape structure in the present and in the past, and the past landscape structure in each time period separately was as important as the present landscape structure. Specifically, from current landscape structure, the only important factor was the isolation of localities. Among factors of historical landscape structure, the most important factor was the proportion of area of each locality that persisted at the same place since 1938 or at least since 1973, 1988 or 2000. Localities with large proportion of continual area since a certain period in the past could provide better conditions for species survival and thus support larger populations. Many typical dry grassland species could thus profit from the large area and high connectivity already in the past and persist at such localities for a long time.

I also found that the typical dry grassland species, i.e. long-lived species that are insect or wind pollinated, flower over limited period of time, are tall, are dispersed by animals, are not ruderal, have low nutrient requirements, and maintain competitive or partly stress-tolerant strategies, were restricted to continuous localities existing at least since 1973 or 1988 and to localities that are currently well interconnected. In contrast, short, self-pollinated, ruderal species prevail in currently less-connected localities. Although most previous studies, testing the effect of various species traits, in fact concluded that persistence traits are better predictors of species distribution than dispersal traits, I found relatively high importance of zoochory for species distribution in the landscape. Seed dispersal by animals is very likely in my study area, as it is known for high numbers of hoofed game, especially mouflons, preferably concentrating their feeding and relaxation activities at the dry grassland localities.

The results indicate that a knowledge of the past landscape structure is important for understanding the current species distribution and that species traits can be used as useful predictors of species responses to the landscape structure.

In **Chapter 3** I focused on 21 carefully chosen target species within the same habitat type of dry grassland-like forest openings differing in their life-history traits and growing in populations differing in their size and connectivity in fragmented landscape and I explored the effect of population size and connectivity on plant performance of individual species and I subsequently asked whether the between species differences in these relationships can be explained by species life-history traits. I collected data on plant performance (reproductive and growth parameters) of all these selected species directly in the field as well as in the experimental garden where I planted the plants from the seeds coming from the same populations. I tested the effect of population size and connectivity on individual performance parameters of each species separately. Then I compared the results of these analyses for plants growing in the field and planted in the garden. I also assembled data on life-history traits related to pollination, seed dispersal, species abundance in the landscape and species life strategy and used these traits to explain the between species differences in the plant performance–landscape structure relationship.

I found that population size and connectivity significantly affected the performance of all the studied dry grassland species. Contrary to my expectation, the effects of decreasing population size and connectivity were not always negative, but at some species they were even positive. Effects of population characteristics were detected not only in the field, but also in the common garden, even though they were weaker in the garden. This indicates that the differences in plant performance of most dry grassland species are not only due to differences in the actual habitat conditions (incl. biotic interactions, mainly with pollinators) at the localities but also due to effects of their population size and connectivity *per se*. The between species comparisons showed that species more sensitive to decreasing population size and connectivity are insect-pollinated species, dispersed by wind or water, with lower terminal velocity as well as more common species. In addition, species more sensitive only to decreasing population size are long-lived species with lower SLA that are dispersed also by animals. Species more sensitive only to decreasing population connectivity are species with lighter seeds, higher SLA and lower shoot–root allocation slope at adult plants and also species with less persistent seed bank, that are not dispersed by animals. Such results indicate that reduction in population size represent an immediate danger for long-lived species likely due to reduced pollinator availability and more intense inbreeding depression. In contrast, the effects of habitat isolation are slower and the long-lived species may respond to it just with a certain time lag. Seed dispersal characteristics seem to be one of the best predictors of the plant performance in relation to different population size and connectivity, though their effects have not been studied previously. Especially, the effects of dispersal by animals are interesting and indicate the importance of local game (mainly mouflons) in effective spread of seeds within the landscape, which probably ensure sufficient gene flow between populations.

The results thus indicate that species life-history traits are useful predictors of species responses to population size and connectivity. They also indicate that studies performed directly in the field likely provide stronger patterns than common garden studies. Common garden studies, are however useful to identify the long-term effects of landscape changes that are not connected to current habitat conditions and/or pollinator behaviour.

In **Chapter 4**, large amount of data on above- and below-ground biomass production at seedlings and adult plants of a spectrum of dry grassland species differing in their life-history traits, coming from garden experiment in Chapter 3, motivated me to explore how does allocation to above- vs. below-ground biomass differ between seedlings and adult plants within and among species and whether the between species differences in allocation pattern can be explained by species life-history traits and phylogenetic relatedness.

I collected data on above- and below-ground biomass of seedlings and adult plants of 20 species from a common garden experiment, which was set up in study described in Chapter 3. In collaboration with Prof. Jacob Weiner I tested whether the slope of the relationship between allocation into above- and below-ground biomass of each species and each age category is significantly different from one (isometry) and whether the allocation slopes differ between seedlings and adult plants of each species. Then I used the values of allocation slopes and tested them against species life-history traits and phylogenetic distances.

In line with allometric biomass partitioning theory, I found isometric as well as allometric patterns of biomass allocation in dry grassland species, regardless of whether they were annuals or perennials. This is in contrast to the expectation of isometric allocation in annuals and allometric allocation in perennials (Mao et al. 2012; Zhou et al. 2014). Seedlings and adult individuals of more than half of the species differed in their above- vs. below-ground biomass allometric exponents. Seedlings and adult individuals of the remaining species differed in their allometric coefficients (intercepts). Annual species usually decreased their shoot–root allometric slopes from young to adult individuals, whereas later flowering perennial species usually increased their shoot–root slopes from seedlings to adults. Differences in species' life span had the strongest effect on intraspecific biomass allocation in seedlings. For adult plants, the most important traits affecting intraspecific allocation patterns were the age of first flowering and month in which the species usually start to flower. Leaf traits, such as SLA, also had important effects on biomass allocation. Plant life-history traits are much more important in explaining allocation pattern among species than is phylogenetic relatedness among species within a habitat. This suggests that allocation patterns vary greatly among closely related species but can be predicted based on species life-history traits. This could be because biomass allocation patterns are evolutionarily labile and can evolve quickly.

Summary of main results and answers to main questions

In the studies outlined above, I attempted to gain insights into the factors and processes that are responsible for species diversity, distribution and interspecific and intraspecific variation in performance of dry grassland species occurring in a system of dry grassland-like forest openings in a forested landscape. Even though it was not possible to investigate all the factors and processes, the results showed many interesting findings:

1) The current species richness of the dry grassland-like forest openings in the landscape was significantly affected by all three studied groups of variables: the local habitat conditions and the landscape structure at present and in the past. Local habitat conditions had the strongest effect on species richness, followed by historical landscape structure. Current landscape

structure had the weakest effect. Substrate heterogeneity seems to be the most important factor overall. The highest species richness was observed on larger and more heterogeneous localities with rocks and shallow soils, which were already large and well connected to other localities in 1938 or at least in 1988.

2) Also species composition in the dry grassland-like forest openings was determined by landscape structure in the past and at present, and the past landscape structure in each time period separately was as equally important as the present landscape structure. The typical dry grassland species, i.e. long-lived species that are insect or wind pollinated, flower over limited periods of time, are relatively tall, are dispersed by animals, are not ruderal, have low nutrient requirements, and maintain competitive or partly stress-tolerant strategies were found to be restricted to continuous localities existing at least since 1973 or 1988 and to localities that are currently well interconnected. In contrast, short, self-pollinated, ruderal species prevail in currently less-connected localities.

3) Population size and connectivity significantly affected performance of all the studied dry grassland species. However, the effect of decreasing population size and connectivity was not always negative, but at some species it was even positive. Effects of population characteristics were detected not only in the field, but also in the common garden, even though they were weaker in the garden. The between species comparisons showed that species most sensitive to decreasing population size and connectivity are insect-pollinated species, dispersed by wind or water, with lower terminal velocity as well as more common species. In addition, species more sensitive only to decreasing population size were long-lived species with lower SLA that are dispersed also by animals. Species more sensitive only to decreasing population connectivity are species with lighter seeds, higher SLA and lower shoot–root allocation slope at adult plants (which was assessed when answering Question 4) and also species with less persistent seed bank, that are not dispersed by animals.

4) Also shoot–root biomass allocation pattern significantly differed among species with different plant traits and also between seedlings and adult plants. The results are consistent with allometric biomass partitioning theory, as I found isometric as well as allometric patterns of biomass allocation in dry grassland species, regardless of their life span. Annual species generally allocated proportionally more to above- than below-ground biomass as seedlings than as adults, whereas later flowering perennial species showed the opposite pattern. Plant life-history traits, such as plant life span, age of first flowering, month in which the species begin flowering, and specific leaf area (SLA) were much more important in explaining differences in shoot–root allometry among species than were phylogenetic relationships among species.

As the shoot–root biomass allocation pattern is species-specific, it also could be used as additional species trait explaining species sensitivity to landscape structure in Question 3, showing that species with lower shoot–root allocation slope at adult plants, which is typical for earlier flowering species with higher SLA, are more sensitive to decreasing population connectivity.

Conclusions

The overall results demonstrate that although the changes in the structure of the studied area were not as dramatic as in other European countries (e.g. Eriksson et al. 2002; Adriaens et al. 2006), landscape structure in the present and also in the past together with local environmental conditions, have important effects on diversity and distribution of dry grassland species. The results thus confirmed the positive species-area relationship, in which the area has direct as well as indirect effect on species richness via increasing habitat heterogeneity at larger localities **(Chapter 1)**. On contrary, the current species distribution was affected only by current habitat isolation, which indicate that many species are missing in isolated localities due to strong dispersal limitations (Münzbergová 2004; Ehrlén et al. 2006; Ozinga et al. 2009) or can be replaced by other, well dispersed species, usually with a partly ruderal strategy **(Chapter 2)**.

Thanks to the relationship between the current species richness and distribution and landscape history, the results also demonstrated a delay in the species response to fragmentation and changes in the landscape structure. Many species can be maintained at localities due to high species survival since the periods when the locality has been larger and more connected, indicating an extinction debt (Kuussaari et al. 2009; Jackson and Sax 2010). Species can survive either in the aboveground vegetation (long-lived species) or in the seed bank. In my study system, the historical area of the localities strongly correlated with their continuity, and thus, the localities with larger areas in the past had longer uninterrupted development. Such localities have higher probabilities of being colonized due to the longer time available for species colonization and also could provide better conditions for long-term species survival. In such localities, species-rich communities with many typical dry grassland species well adapted to live in specific habitats could develop and they survive there up to now **(Chapter 1–2)**.

The results of this study also demonstrated that also performance of all the studied dry grassland species, i.e. common habitat specialist co-occurring in the same habitat type, was significantly affected by habitat configuration **(Chapter 3)**. Lower performance of plants in small and isolated habitats may be caused by pollen and pollinator limitation and/or low level of genetic variation and inbreeding depression (Groom 1998, 2001; Hooftman et al. 2003; Kolb 2005). Also changed abiotic and biotic conditions at some localities (e.g. shrub encroachment and eutrophication, as a consequence of the ongoing landscape fragmentation (Eriksson et al. 2002; Soons and Heil 2002)) can reduce their growth and reproduction. In this study, I also demonstrated that to identify factors responsible for variation in plant performance in relation to habitat fragmentation, it is important not only carry out the field survey, as was done in many previous studies, but also compare such results with results from common garden experiment (Fischer and Matthies 1998). Such approach enables to identify the long-term effects of landscape changes that are not connected to current habitat conditions and/or pollinator behaviour and help to recognize the causes of poor performance of species in small and isolated populations which is crucial for effective conservation of plant species diversity. In this way I in fact found that most of the studied species were significantly affected by the population size and/or connectivity not only in the field, but also in the garden. This suggests that not only actual abiotic and biotic conditions (incl. Allee effect) at the localities but also other processes (such as genetic changes or possible maternal as well as epigenetic trans-generational effects) can significantly affect performance of dry grassland species. The effects of decreasing population size and connectivity were, however, not always negative, but at some species they were even positive, indicating that some species perform better in small and/or isolated habitats.

I also confirmed that distribution as well as variation in plant performance of dry grassland species largely depend on plant species life-history traits, mainly those related to dispersal and survival **(Chapter 2–4)**. Based on comparison of the results from **Chapter 2** and **Chapter 3** I found that species most sensitive to decreasing population size are long-lived, insect-pollinated species that are dispersed mainly by animals and these species were also found to be restricted to localities with large continual area since the past, especially since 1973 or 1988. Such habitats that already encompassed large areas in the past, or at least had large proportion of area persisting at the same place, could provide better conditions for species survival and thus support larger populations, which are less influenced by demographic and environmental stochasticity and are thus less prone to extinction (Münzbergová 2006; Jackson and Sax 2010). Many typical dry grassland species could thus persist and also perform well at such localities for a long time. In contrast, self-pollinated species perform better in isolated populations and they also prevail on currently isolated localities. This can be because these species are less dependent on their pollinators, and are thus less susceptible to small population connectivity and thus less susceptible to Alee effect and, subsequently, pollen and pollinator limitation in small and isolated populations (Ågren 1996; Groom 1998; Kolb 2005, 2008; Peterson et al. 2008; Dauber et al. 2010).

Whereas it is commonly assumed that distribution at the landscape scale is largely determined by species dispersal traits (e.g. Ehrlén and Eriksson 2000; Münzbergová 2004; Ozinga et al. 2005, 2009), most previous studies testing the effect of various species traits in fact concluded that persistence traits are better predictors of species distribution than dispersal traits (e.g. Graae and Sunde 2000; Lindborg 2007; Lindborg et al. 2012; Hemrová and Münzbergová 2015). I confirmed that not only persistence traits, but also dispersal traits are highly important predictors of species distribution as well as variation in plant performance of dry grassland species in my study area **(Chapter 2–3)**. Especially, dispersal by animals seems to be important in dry grassland openings in a forest matrix, as seed dispersal by animals is directed towards suitable habitats, and thus it is much more effective than dispersal using other vectors (Purves and Dushoff 2005; Adriaens et al. 2007). Also pollinator vector and from persistence traits, especially plant longevity and specific leaf area were important for explanation of species distribution as well as variation in plant performance. Also shoot–root biomass allocation pattern (expressed in **Chapter 4**), as additional species trait, significantly helped to explain species sensitivity to landscape structure.

Species traits are also important for explaining patterns of resource allocation into the above- and below-ground biomass **(Chapter 4)**. The results are consistent with allometric biomass partitioning theory, as I found isometric as well as allometric patterns of biomass allocation in dry grassland species, regardless of their life span (i.e. in contrast to previous studies (Mao et al. 2012; Zhou et al. 2014)). This indicates that some species can feature true plasticity and other species can feature apparent plasticity, in response to environmental variation. The results confirmed that biomass allocation pattern is species- and age-specific and that plant life-history traits are much more important in explaining allocation pattern among species than is phylogenetic relatedness among species within a habitat. This is in contrast to previous studies (McCarthy et al. 2007; McCarthy and Enquist 2007), who showed that phylogenetic relatedness is important predictor of allocation pattern in a global dataset at higher taxonomic levels. The pattern of biomass allocation can thus be used as additional species trait explaining species sensitivity to landscape structure. Species with lower shoot–root allocation slope at adult plants, which is typical for earlier flowering species with higher SLA, are more sensitive to decreasing population connectivity as was shown in **Chapter 3**.

Even though the results showed a range of significant predictors of species diversity, distribution and interspecific and intraspecific variation in performance of dry grassland species occurring in a system of dry grassland-like forest openings in the forested landscape, a lot of the variation still remains unexplained. For more accurate conclusions regarding the future of the dry grassland species in fragmented landscape, it would make sense to explore other factors and processes, such as mycorrhiza, root or shoot herbivory, pollinator mutualism, genetic diversity of the populations etc. Also more detail study of species dispersal, especially by animals, in the study region, could provide important novel insights.

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CHAPTER 1

Relative importance of current and past landscape structure and local habitat conditions for plant species richness in dry grassland-like forest openings

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ABSTRACT

In fragmented landscapes, plant species richness may depend not only on local habitat conditions but also on landscape structure. In addition, both present and past landscape structure may be important for species richness. There are, however, only a few studies that have investigated the relative importance of all of these factors.

The aim of this study was to examine the effect of current and past landscape structures and habitat conditions on species richness at dry grassland-like forest openings in a forested landscape and to assess their relative importance for species richness. We analyzed information on past and present landscape structures using aerial photographs from 1938, 1973, 1988, 2000 and 2007. We calculated the area of each locality and its isolation in the present and in the past and the continuity of localities in GIS. At each locality, we recorded all vascular plant species (296 species in 110 forest openings) and information on abiotic conditions of the localities.

We found that the current species richness of the forest openings was significantly determined by local habitat conditions as well as by landscape structure in the present and in the past. The highest species richness was observed on larger and more heterogeneous localities with rocks and shallow soils, which were already large and well connected to other localities in 1938. The changes in the landscape structure in the past can thus have strong effects on current species richness. Future studies attempting to understand determinants of species diversity in fragmented landscapes should also include data on past landscape structure, as it may in fact be more important than the present structure.

Keywords Species diversity; Tree-less patch; Forest matrix; Habitat fragmentation; Connectivity; Area; Isolation

INTRODUCTION

At the landscape level, the distribution and dynamics of plant species are determined not only by local habitat conditions but also by landscape structure and its changes over time (Eriksson 1996; Ehrlén and Eriksson 2000). In a landscape, new habitats can appear, and the existing habitats can disappear. In the European landscape, many previously opened habitats that have been maintained by human activity, such as mowing or keeping grazing animals, are slowly disappearing and are consequently becoming smaller and more fragmented (Eriksson et al. 2002; Soons 2003; Helm et al. 2006).

As a consequence of landscape fragmentation, numerous species populations are becoming small and isolated. Species in small populations are strongly influenced by demographic and environmental stochasticity and are thus prone to extinction (Graae and Sunde 2000; Dupré and Ehrlén 2002; Kolb and Diekmann 2004; Münzbergová 2006; Jackson and Sax 2010). Moreover, reduced connectivity between the patches can limit the spread of the species due to the increasing distance or presence of barriers between patches and therefore lead to a lower possibility of recolonization of those patches (Jacquemyn et al. 2001; Ozinga et al. 2005). All of these processes can result in reduced habitat species richness. To understand the importance of landscape structure changes for species richness, we need to separate these effects from other factors driving the species richness of a habitat.

Among the basic drivers of habitat species richness are the habitat area and isolation. The existence of a relationship between habitat area and species richness was traditionally postulated by the equilibrium theory of island biogeography (Mac-Arthur and Wilson 1967). A positive species-area relationship has been demonstrated by a large number of studies (e.g., Kohn and Walsh 1994; Peintinger et al. 2003; Cousins et al. 2007; Knappová et al. 2012) and has been explained by several theories (see Mac-Arthur and Wilson 1967; Connor and McCoy 1979; Ricklefs and Lovette 1999). Similarly, numerous studies have demonstrated the negative effects of habitat isolation on species richness (e.g., Dupré and Ehrlén 2002; Kolb and Diekmann 2004; 2005; McEuen and Curran 2006; Knappová et al. 2012), which also provided a range of explanations for this pattern (see Soons and Heil 2002; Eriksson et al. 2002; Ozinga et al. 2005; Adriaens et al. 2006).

The species richness of a habitat patch can also depend on habitat conditions at the sites, such as the type of substrate, type of vegetation, soil depth, slope (Adriaens et al. 2006; Löbel et al. 2006; Turtureanu and Dengler 2012), insolation or geology (Chýlová and Münzbergová 2008), soil pH (Löbel et al. 2006; Merunková et al. 2012), and, in some cases, the disturbance regime (Kolb and Diekmann 2004) or habitat heterogeneity in terms of land use history (Kraus et al. 2004; Chýlová and Münzbergová 2008). However, the species richness of a habitat patch can also depend on the habitat diversity in the surrounding landscape (Janišová et al. in press; Zulka et al. in press). This last parameter is important especially at a larger spatial scale and in the landscape with more habitat types, which are common for agricultural landscapes with different land use types.

The studies assessing the effects of current habitat size, isolation and habitat conditions on species richness implicitly assume that the system is in equilibrium so the species richness can reflect the status of the landscape (Fahrig 2003). Such an assumption is likely to be valid in oceanic islands that have maintained their size and isolation for thousands or even millions of years, where the habitat conditions at these sites are relatively stable (Kohn and Walsh 1994; Ricklefs and Lovette 1999; McMaster 2005). In the mainland, the landscape is much more dynamic, leading to strong changes in habitat size and isolation over time. Therefore, assuming that the distribution of plant species is in equilibrium may be misleading (e.g., Eriksson 1996; Ehrlén and Eriksson 2000; Fahrig 2003; Herben et al. 2006; Münzbergová et al. 2013). To understand the drivers of plant species richness in such a landscape, we need to consider the history of the landscape. In this study, we considered the landscape history of only the last one hundred years and did not use a longer-term perspective. Thus, we do not include the processes of speciation (Stuessy et al. 2003; Outlaw and Voelker 2008).

The effect of the last few hundred years of landscape history on plant species richness has been studied mainly in forest fragments (Graae and Sunde 2000; Jacquemyn et al. 2001; Honnay et al. 2004; Hérault and Honnay 2005). Studies looking at other types of habitats are relatively rare, were performed in rural or agricultural landscapes and focused mainly on changes of land use in particular habitats (Bruun 2000; Bruun et al. 2001; Cousins and Eriksson 2001; 2002; Krauss et al. 2004; Chýlová and Münzbergová 2008). Much less is, however, known about the effects of the past landscape structure (in terms of habitat size and isolation) on patch-scale species richness in fragmented grassland systems (Lindborg and Eriksson 2004; Helm et al. 2006; Krauss et al. 2010).

The above-mentioned studies have demonstrated that the plant species richness in fragmented localities is a result of the current and the past landscape structure and their habitat conditions. In spite of the relatively high number of studies addressing some of these factors, there is surprisingly little information on the relative importance of all of these factors, especially in dry grassland communities. Such knowledge is however crucial for effective conservation of plant species richness.

The aim of this study was to assess the relative importance of current and past landscape structures and habitat conditions for plant species richness in dry grassland-like forest openings, below noted only as “forest openings”, in the forested landscape of Křivoklátsko Biosphere Reserve, Czech Republic. In this area, the forest openings are places with the highest plant and animal species richness and are thus of conservation interest (Kučera 1997). During the last century, these places experienced several changes in the landscape structure, which was at first larger and more connected but is now smaller and more isolated. The main factors responsible for changes in the landscape are shrub invasions likely initiated by increased nutrient deposition since the 1990s (Kolbek 1996; Petřík et al. 2011) and outbreak of hoofed game (Ungulates), especially mouflons (*Ovis musimon*), in the region in the 1970s and 1980s (Kolbek 1996; Kolbek et al. 2003). To identify determinants of patch-scale plant species richness in this landscape, we asked the following questions: (i) Which factors are responsible for plant species richness in forest openings in the forest matrix? (ii) What is the relative importance of three groups of factors, namely, current landscape structure, past landscape structure and habitat conditions, for plant species richness in the patches?

To answer these questions, we mapped all of the current forest openings and collected data on plant species distribution and abiotic conditions at each locality. We analyzed information on past and present landscape structure using aerial photographs from 1938, 1973, 1988, 2000 and 2007. In GIS we calculated the total area of each locality and its isolation in the present as well as its continuity, isolation and area in the past and also some parameters describing habitat conditions. We assessed the relative importance of all of these factors for species richness.

METHODS

Permits and approvals for the field work were obtained from the Protected Landscape Area (PLA) Administration Křivoklátsko. We did not sample any of the protected vascular plant species or perform any experiments with them.

The study system

The study was performed in the Protected Landscape Area and Biosphere Reserve Křivoklátsko in the Czech Republic, specifically at the Site of Community Importance (SCI) Týřov (49°58’10’’ N, 13°48’40’’ E) (Figure 1). The study area was approximately 4 km^2 and mainly forested. Approximately 2% of the area was represented by forest openings, a mean area of 635 m^2 and a mean nearest distance between localities of 48 m. In the study region, we recorded 110 forest openings with the area ranging from 20 m^2 to 11,123 m^2. The vegetation of these localities is composed of a specific mosaic of different xerophilous vegetation units ranging from chasmophytic vegetation of rocks and rock crevices (*Asplenietea trichomanis, Festuco-Brometea*), vegetation of primitive soils (*Sedo-Scleranthetea*) and mobile screes (*Thlaspietea rotundifolii*) to vegetation of dry grasslands (*Festuco-Brometea*) and dry herbaceous fringes (*Trifolio-Geranietea*) (Kolbek et al. 2001). At some localities, the vegetation includes ruderal plant communities (*Artemisietea vulgaris, Chenopodietea*) (Kolbek et al. 2001). The surrounding forests are represented by ancient dry acidophilous oak forests (*Quercion petraeae*, *Genisto germanicae-Quercion*), which have been barely managed due to their specific and badly accessible position (Kolbek et al. 2003). Additionally, all of our localities were not managed (no removal of young trees and shrubs colonizing grassland areas, no mowing, grazing cattle, etc.).

For the purpose of the study, we defined the locality of the forest opening as every open patch with less than 30% tree cover (i.e., there could be some individual trees inside the locality, but they were isolated from the forest matrix by at least 7 m of the opening), which was isolated from other patches by at least 20 m of forest. The localities were thus delimited by the trunks of trees of forest matrix growing around each patch (Figure 2). The border between the grassland and the forest was always abrupt without a transient zone.

The occurrence of localities in the area is defined by a combination of summit and river phenomenon (Ložek 2005), by exposition, climate, geological and soil conditions and by human activities in the past.

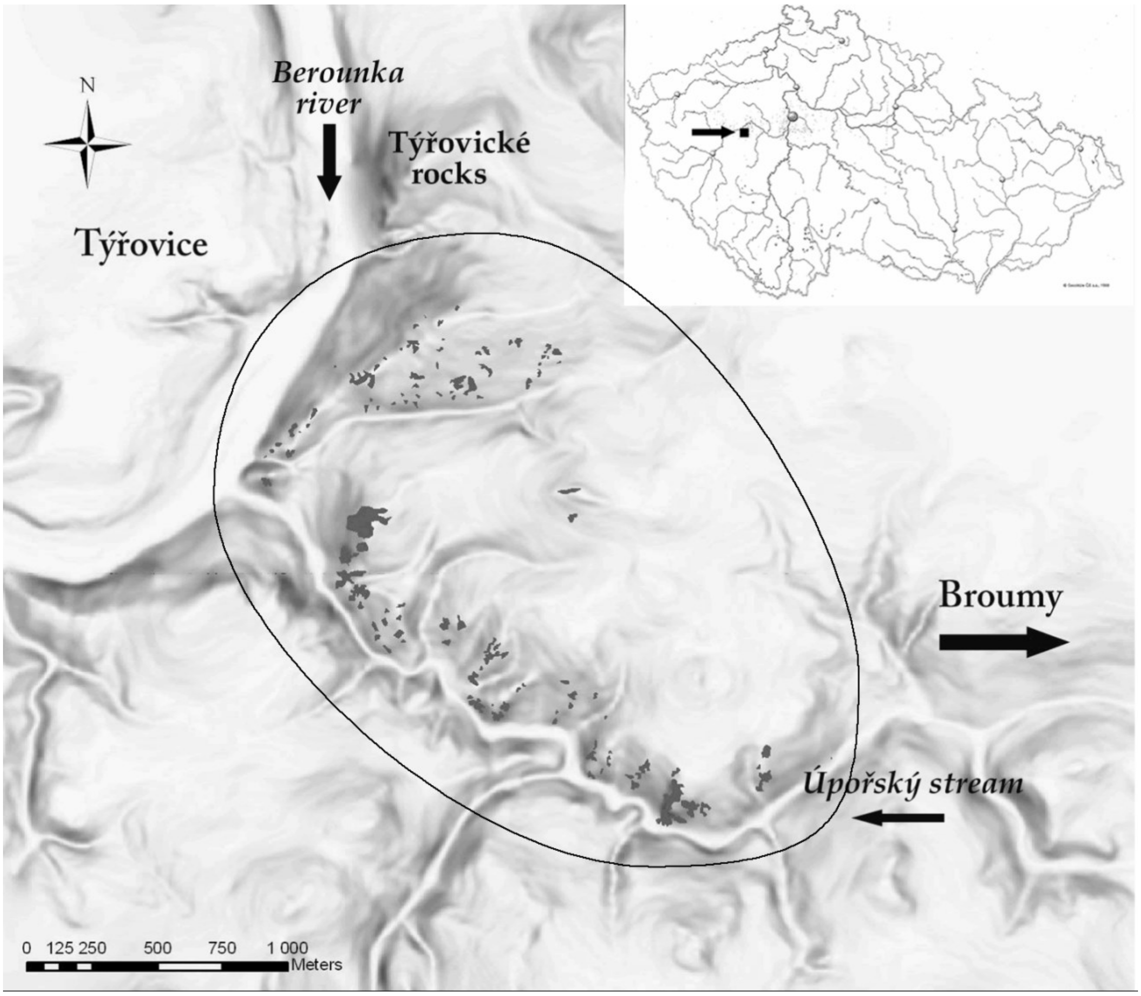


Figure 1. Study area with all studied localities. Study area with all studied localities (gray) in Site of Community Importance (SCI) Týřov (49°58’10’’ N, 13°48’40’’ E) and the position of study area within the Czech Republic. The line defines the border of our study area.

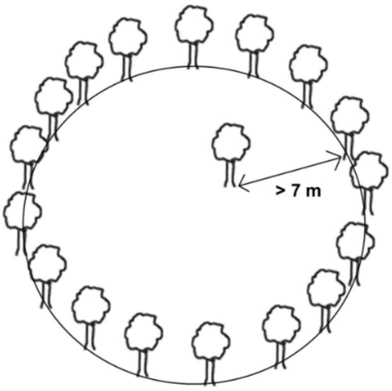


Figure 2. Definition of locality and its separation from the forest matrix.

Data collection

Field data collection

In the field, we recorded all vascular plant species at each locality. We surveyed each locality twice a year (from April to August 2005–2007) to include both the spring and summer plant flowering period. We used the plant nomenclature defined by Kubát et al. (2002).

During the field survey, we observed that some species were growing not only in the forest openings but also in the surrounding forests. To study the effects of isolation and habitat size on species richness, we thus decided to exclude these species from the analyses. This was important for the species occurring in the forests, as the localities are in fact not isolated. Studying the effects of isolation on the forest species would thus not be sensible. To exclude these species, we generated a list of species growing both in the study localities and in the surrounding forests in the study region. The species that were included in the analysis belonged to the xerophilous vegetation units (*Asplenietea trichomanis, Festuco-Brometea, Sedo-Scleranthetea, Thlaspietea rotundifolii, Trifolio-Geranietea*) and to ruderal vegetation units (*Artemisietea vulgaris, Chenopodietea*), whereas the excluded species belonged to the vegetation of ancient dry acidophilous oak forests (*Quercion petraeae*, *Genisto germanicae-Quercion*). In this way, we excluded 34% of the 296 species recorded at the studied localities (Table S1). The tests not excluding these species provided similar results and are presented in Table S2.

At each locality, we recorded several abiotic conditions. First, we measured the height of the horizon from the center of each locality (at the ground level) in eight main directions using an inclinometer (in degrees) including the surrounding trees. We used these values to calculate the potential solar insolation of each locality (see below). We also recorded the proportion of each substrate category type on the localities (below noted as "substrate") according to their soil depth into four categories: rock, shallow soil, deeper soil and scree. Rock was defined as a site with exposed rocks without any soil cover with several microhabitats, such as rock platforms and rock crevices, with specific conditions. Shallow soil was less than 10 cm deep and hosted vegetation of primitive soils and narrow-leaved dry grasslands. Sites with deeper soils (> 10 cm) typically hosted vegetation of broad-leaved dry grasslands and dry herbaceous fringes. Scree was created by a moving substrate of relatively small stones. Although we originally measured the soil depth at the localities using a 2 mm diameter metal rod, the four categories (rock, shallow soil, deeper soil and scree) were in fact easy to distinguish visually. Therefore, we were able to differentiate and estimate the proportion of each substrate category type visually without exact mapping. To confirm our estimations, we measured the substrate depth by inserting a 2 mm diameter metal rod several times into the substrate at places with shallow and deeper soil at each locality with these substrate category types until it reached bedrock. To describe the substrate heterogeneity, we calculated the Shannon diversity index according to the proportions of substrate category types at each locality.

The study area is not the only area with forest openings in the region. To avoid the edge effects when calculating habitat isolation, we mapped all forest openings up to 500 m from the edge of the study area and used these only to calculate habitat isolation (see below).

Preparation of historical data

To assess the historical distribution of forest openings, we used aerial photographs of the study region from 1938, 1973, 1988 and 2000, which were provided by the Military and Hydrometeorological Institute of the Czech Republic. To input the historical forest openings into the GIS and calculate their geometry, we first rectified aerial photographs using PCI Geomatics 10.0 (PCI Geomatics Enterprises 2006). We used current orthophoto maps that provide accurate positions and cadastral maps that provide information about altitude to set control points. We used the same points from several aerial photographs of the same age as the tie points. Both types of points served to place aerial photographs into a system of coordinates. Then, we created a digital terrain model using a digital contour line map (with 2 m between contour lines and a raster cell size of 5 m × 5 m), which we used to create historical orthophoto maps from the aerial photographs.

We classified the historical orthophoto maps using program Definiens (Definiens AG 2006). This program can classify landscape structure based on the pixel value and on the structure and texture of classified subjects. Therefore, it is suitable for classifying black-and-white aerial photographs. We classified the historical landscape structure of the study region into two categories: forest and tree-less area. The relatively bad quality of some of the aerial photographs precluded the resolution of more categories. We manually verified and corrected the identified tree-less areas according to present-day conditions and our own field experience. We excluded all tree-less areas in positions unlikely hosting dry grasslands (e.g., a meadow in the valley next to a stream). From the old maps, we also excluded regularly shaped localities (i.e., squared), as these were likely to be artificial clearings that were subsequently reforested. The remaining tree-less areas were considered to represent the localities of forest openings.

In the same way, we also classified the current landscape structure of the study region using current orthophoto maps (2007) available from Geoportal Cenia (http://geoportal.cenia.cz). Additionally, we corrected the distribution and shape of all current localities according to our own field experiences because we thoroughly mapped them.

Landscape structure and local habitat conditions

Using GIS (ArcGIS 9.2 (ESRI 2006)), we calculated the geometry for each current locality: area, distance between localities and their isolation. Due to a high topographic heterogeneity, we calculated the surface area for each locality according to a digital elevation model of the terrain (created using a digital contour line map with a 2 m distance between contour lines and a raster cell size of 5 m × 5 m and orthophoto maps). The isolation was calculated using a formula provided by Tremlová and Münzbergová (2007):

$$I_i = -\sum_{j=1}^{n} \frac{S_j}{d_{ij}^{2}} \;;$$

where I_i represents the isolation of the *i*-locality, *j* represents all other localities in the circuit of 500 meters around the *i*-locality, *n* is the total number of localities in the circuit of 500 meters, S_j is the area of *j*-locality (in m^2), d_{ij} is the distance between locality *i* and all other localities *j* (measured in m, as distance between locality centers because the localities do not exhibit an elongated shape). We considered the circuit of 500 meters because this distance was the best according to a sensitivity analysis of different distances (100 m, 200 m, 300 m, 500 m and 1000 m). Löbel et al. (2006) also identified a circuit of 500 meters as the best for calculating isolation. The value of I_i increases with increasing isolation of the locality. We also calculated the isolation of each current locality from past localities in each time period separately using the same formula.

According to the digital elevation terrain model (see above), we calculated several topographic heterogeneity parameters for each locality: slope and aspect using GIS and topographic wetness index (TWI) using SAGA GIS (http://www.saga-gis.org). The median, minimum, maximum and standard deviation values of the slope were used as a basis for subsequent analyses. We also used the slope and aspect values and the above mentioned values for the horizon height to calculate the potential direct solar insolation (PDSI) of each locality on the 21st day of every month from December to June (Herben 1987). We also considered calculating the heat load index (McCune and Keon 2002) for the localities but did not include it at the end, as it highly correlated with PDSI, and the algorithm for calculating these two values is extremely similar. Moreover, PDSI takes shading by adjacent topography and the surrounding trees into account and is therefore a better predictor of species richness especially at relatively small localities such as in our study system (McCune and Keon 2002). Moreover, Turtureanu and Dengler (2012) observed a close correlation between the heat load index and the locality slope, and the slope was also included in our model. For calculating the TWI, we first calculated the slope (using a local morphometry function with a fit 2 degree polynom) and specific catchment area (using a parallel processing function with multiple flow direction method and number one convergence) according to the digital elevation terrain model (DEM). According to these parameters, we calculated the topographic wetness index (TWI) for each locality (with an area conversion of 1/cell size) (see Kopecký and Čížková 2010 for details).

According to the geological map of the area (Czech Geological Institute 1997; 1:50,000), we classified the geological conditions of each locality into two categories: andesites and dacites.

Other parameters we calculated using the GIS served to describe a historical landscape structure: the historical area of each current locality (expressed as the area of historical localities around each current locality in a 30 m circuit, also calculated as surface area), historical isolation (see above) and continuity of localities (expressed as the percentage of overlap of each current locality with past locality in each time period separately). It is also possible that one historically large locality was divided into several smaller localities in the present, which is also represented by the parameter of the historical area of the locality (area 1938, area 1973, area 1988 and area 2000).

As supplementary information, even though we did not obtain direct measurements of some other habitat conditions, we calculated Ellenberg indicator values (Ellenberg et al. 1991) for light, temperature, soil reaction pH and nutrient at each locality using the species compositions at the localities. Numerous studies have used this approach for indirectly estimating the habitat conditions (Diekmann 1995; 1996; 2003; Pyšek and Pyšek 1995).

Statistical analysis

The statistical analyses were performed using S-plus 4.6 (MathSoft 1999) and Statistica 7.0 (StatSoft 2004). The dependent variable, the number of species, was normally distributed, and so no transformation of the data was needed.

To reduce the number of independent PDSI and slope variables, we used a pair-wise correlation matrix (Statistica 7.0 (StatSoft 2004)) and selected the least correlated ones for use in subsequent analyses (for PDSI – median value for December and June; for slope – median and maximum value). To assess the relationship between all variables and their categories, we also calculated a pair-wise correlation matrix (Table S3) in Statistica 7.0 (StatSoft 2004).

To take into account the linear spatial trends (spatial auto-correlation) of parameters describing localities across the study area, we used variation partitioning according to Borcard et al. (1992) and Legendre and Legendre (1998), which enabled us to separate the pure spatial component from the pure environmental component and their shared contribution. To identify complex spatial trends, seven derived geographical variables were constructed by including all quadratic and cubic combinations of x and y as suggested by Borcard et al. (1992): x, y, x^2, xy, y^2, x^3, x^2y, xy^2, y^3. We used the values selected in the stepwise regression (x, y, xy) as covariates in all subsequent tests to remove the effect of spatial position of the localities (spatial auto-correlation) because we wanted to study only the pure effect of the environment and not of the spatial component.

The tests of the effects of all the independent variables (Table 1) on species richness were performed in three steps. First, we tested the effect of each variable on species richness separately without any covariates, which represents the fraction of variation explained by non-spatial environmental variation and spatially structured environmental variation together (e.g., shared contribution of environmental and spatial variation). Second, we used the geographical coordinates of the center of each locality (those selected in the stepwise regression) as covariates and tested the effect of each independent variable separately, which represents the fraction of variation explained by non-spatial environmental variation (Borcard et al. 1992, Legendre and Legendre 1998). Finally, to obtain the pure effect of each variable after removing spatially structured environmental variation and any other shared variation with all other parameters, we used geographical coordinates and all significant factors from the second analyses as covariates (according to Borcard et al. 1992 and Økland and Eilertsen 1994). We used an analysis of variance (ANOVA) with type III sum of squares (S-plus 4.6) to identify a real effect of particular factors without the effect of all other factors. In the case of categorical variables with more than one category (substrate) and variables with multiple levels (slope, PDSI), we analyzed these categories or levels together using the difference between the models with and without all of the categories or levels. The means, medians and ranges of each predictor and dependent variable per locality are presented in Table 2.

Table 1. All studied variables enrolled in subsequent analyses.

Group of variables	Description
Coordinates	x, y, xy
Substrate	Four categories: Rock, shallow soil, deeper soil, scree (%)
Substrate heterogeneity	Shannon diversity index of substrate types
Slope	Slope (median+max) = median and maximum values of slope; Slope - STD = standard deviation values of slope
PDSI	PDSI (Dec+June) = median values of PDSI in December and June; PDSI - STD (Dec+June) = standard deviation values of PDSI in December and June
Geology	Andesites or dacites
TWI	Topographic wetness index
Area	log area: historical (1938, 1973, 1988, 2000) and current (2007)
Isolation	log isolation: historical (1938, 1973, 1988, 2000) and current (2007)

For assessing the species-area relationship, we compared the fit provided by two alternative functions, a logarithmic function and a power function, using SPSS (SPSS Inc. 2002). The test revealed that the logarithmic function explained a higher amount of variation in our data (R^2 = 0.36) than the power function (R^2 = 0.22). We thus decided to use the logarithmic function in the following analyses.

Alternatively, it is also possible to test the effect of studied factors on species richness using a multimodel inference analysis based on AIC (Crawley 2002), which would alleviate the problem of testing many partially correlated variables using p-values. The comparison of models based on AIC yielded extremely similar results to our first approach, and thus, only the results based on the p-values are presented.

To assess the relative importance of the three groups of factors (current landscape structure, historical landscape structure and habitat conditions) for species richness, we analyzed the effect of each of these three groups of factors alone. We also tested the effect of each of these groups of factors after using the other two groups of factors as covariates. Because each group contained too many independent variables, we chose only those factors that were significant when testing their independent effect on species richness using only coordinates as covariates. We used variance partitioning (according to Økland and Eilertsen 1994) to calculate the proportion of variance explained by each group of factors. We expressed the portion of explained variance as a relative part of the total explained variance.

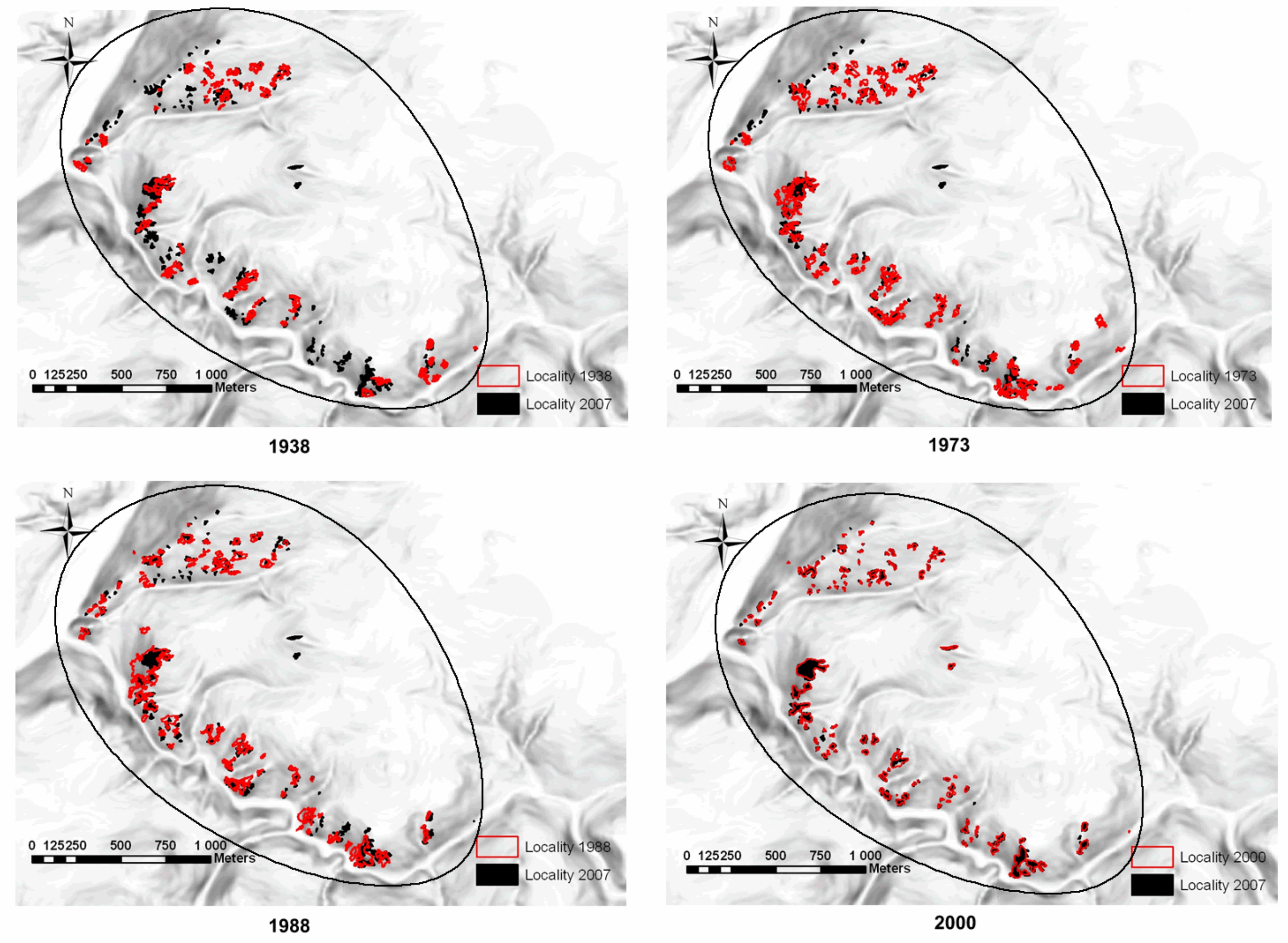
Table 2. Means, medians, minima and maxima of species numbers (per locality) and the independent variables. For the abbreviation explanations, see Table 1.

Variable		Mean	Median	Min	Max	Units
Total species number		58.30	60.5	4	153	
Number of dry grassland openings species		37.58	37.5	1	115	
Local habitat conditions	Substrate - rock	0.34	0.25	0	1	proportion
	Substrate - shallow soil	0.30	0.1	0	1	proportion
	Substrate - scree	0.22	0	0	1	proportion
	Substrate - deeper soil	0.14	0	0	1	proportion
	Substrate heterogeneity	0.47	0.50	0	1.28	
	Slope - median	27.32	28	8	41	degrees
	Slope - max	30.14	30	13	48	degrees
	Slope - STD	1.82	1.24	0	9.52	degrees
	PDSI - median - Dec	0.92	0.54	0	4.89	
	PDSI - median - June	5.87	6.22	0	8.48	
	PDSI - STD - Dec	0.33	0.20	0	1.22	
	PDSI - STD - June	5.47	5.57	0	8.14	
	Geology	0.83	1	0	1	
	TWI	4.76	4.52	3.27	10.48	
	Ellenberg - L	7.36	7.38	6.71	8	
	Ellenberg - T	5.91	5.89	5.29	7	
	Ellenberg - R	6.18	6.29	3.50	8	
	Ellenberg - N	3.16	3.15	1.86	4.50	
Current landscape structure	Log area 2007	2.50	2.46	1.42	4.10	m^2
	Log isolation 2007	0.15	0.14	-0.47	1.28	
Historical landscape structure	Log area 1938	1.43	1.80	0	3.16	m^2
	Log area 1973	2.29	2.71	0	3.46	m^2
	Log area 1988	2.08	2.50	-1.20	3.90	m^2
	Log area 2000	2.56	2.63	0	3.47	m^2
	Log isolation 1938	0.33	0.28	-1.10	5	
	Log isolation 1973	0.06	-0.03	-0.69	5	
	Log isolation 1988	0.07	0.06	-0.69	5	
	Log isolation 2000	0.15	0.15	-0.49	1.29	

RESULTS

Changes in landscape structure

During the 20th century and at the beginning of the 21st century, the landscape structure of the study region changed. Specifically, there were fewer patches of forest openings in the past, but they were larger and more interconnected in comparison with the current landscape (Table 3). Today, we can observe a gradual reduction in the area of forest openings and an increase in their isolation (Figure 3).



1938

1973

1988

2000

Figure 3. Distribution of forest openings in 1938, 1973, 1988, 2000 (red) in comparison with the current distribution – 2007 (black). The line defines the border of our study area.

Table 3. Numbers and mean areas of forest openings in each studied period in the past and in the present.

Year	Number of forest openings	Mean area of forest openings (m^2)
1938	66	650
1973	105	840
1988	89	1080
2000	103	720
2007	110	635

Correlations between parameters

We identified many strong correlations within the group of historical parameters. The highest correlation was between the continuity of localities and their historical area. We thus decided to include only the historical area parameter into subsequent analyses because of its higher predictive power. There was no significant correlation within the group of current parameters and within the group of habitat conditions and between all of the three groups of parameters. The current area and isolation of the localities were also not significantly correlated, whereas the historical area and isolation were slightly correlated (Table S3).

The effect of studied factors on species richness

The local habitat conditions, especially the substrate and substrate heterogeneity, exhibit strong significant effects on the species richness even after removing the effect of other significant independent parameters (Table 4). The substrate heterogeneity was the most important overall predictor of species richness, where it alone accounted for 40% of the total variation and 6.5% of the variation after removing all other significant factors. The most heterogeneous localities thus hosted the highest number of dry grassland species (Figure 4). Additionally, the substrate, in sense of the proportion of different substrate types, strongly affected the species richness (Figure 5). Localities represented by more types of substrate possessed the highest number of species. The number of species increased with increasing proportions of rock and shallow soil on the locality and decreased with increasing scree proportions. We also observed a higher richness on localities with a higher PDSI (Figure 6), a higher slope and with a higher standard deviation of PDSI and slope (Table 4). Their effects, however, disappeared when taking all of the other significant independent factors as covariates. The geology and topographic wetness index did not have a significant effect on species richness (Table 4). The Ellenberg indicator values suggest that only the light (L) values had a significant positive effect on species richness ($p = 0.015$; $R^2 = 0.047$), which is indicative of more dry grassland species on more open habitats. Other indicator values (for temperature, soil reaction pH and nutrient) did not significantly affect the species richness in our system ($p = 0.989$ for temperature, $p = 0.270$ for soil reaction pH and $p = 0.855$ for nutrient).

Table 4. The effect of studied factors on species richness. The effect of local habitat conditions, current and historical landscape structure on species richness and the direction of the effect (+/-). The amount of explained variance by the single independent variables with different covariates is presented; n.s. is not significant ($p > 0.05$), – not tested because previously not significant. Df error = 108 (respectively, 106 for substrate and 107 for slope and for PDSI) for all factors significant without covariates, Df error = 105 (respectively, 103 for substrate and 104 for slope and for PDSI) for all factors significant when using coordinates as covariates and Df error = 87 (respectively, 85 for substrate and 86 for slope and for PDSI) for all factors significant when using coordinates and all significant factors from the first analyses as covariates. For the abbreviation explanations, see Table 1.

				Covariates		
		D.F.	Without covariates	Coordinates (15.69%)	All significant factors (77.84%)	Direction of significance
Local habitat conditions	Substrate	3	14.15%	13.46%	3.17%	+/– *
	Substrate heterogeneity	1	39.90%	32.38%	6.51%	+
	Slope (median+max)	2	15.35%	13.31%	n.s.	+
	Slope - STD	1	12.35%	11.09%	n.s.	+
	PDSI (Dec+June)	2	35.52%	27.07%	n.s.	+
	PDSI - STD (Dec+June)	2	35.80%	26.26%	n.s.	+
	Geology	1	4.23%	n.s.	–	
	TWI	1	4.59%	n.s.	–	+
Current landsc.str.	Area 2007	1	36.03%	28.40%	1.18%	+
	Isolation 2007	1	n.s.	–	–	
Historical landscape structure	Area 1938	1	19.04%	19.37%	2.80%	+
	Area 1973	1	13.30%	8.28%	n.s.	+
	Area 1988	1	17.68%	9.92%	1.23%	+
	Area 2000	1	22.21%	16.83%	n.s.	+
	Isolation 1938	1	6.65%	5.40%	2.20%	–
	Isolation 1973	1	n.s.	–	–	
	Isolation 1988	1	n.s.	–	–	
	Isolation 2000	1	n.s.	–	–	

* (+) for rock, (–) for scree

Among factors describing the current landscape structure, only the current area of localities had significant and strong effect on the number of species. It was also the second most important overall predictor of species richness, where it alone explained 36% of the total variation in the dataset (Figure 7, Table 4). The effect of the current area was significant also after removing the effect of habitat heterogeneity (substrate, substrate heterogeneity, slope and PDSI and their standard deviations) and historical parameters of landscape structure (area in each studied periods and isolation in 1938), indicating a significant but much weaker (1.2% of explained variation) effect of the area (Table 4). In contrast to strong significant effects of the area, habitat isolation did not significantly affect the species richness (Table 4).

Among factors describing the historical landscape structure, the highest species richness was observed on localities with a larger area in 1938 (Figure 8), with a low isolation in 1938 (Figure 9) and with a larger area in 1988. These factors were significant even after removing the effects of all of the other significant factors (Table 4), suggesting that the effects of these factors are partly independent on the effect of the current area and habitat heterogeneity. Moreover, this pure effect of each of these historical factors explained even more variation in species richness than the pure effect of current habitat area (i.e., pure effect of these variables after removing the effects of all of the other significant factors from the second step of analyses). Additionally, the localities with a larger area in 1973 and 2000 exhibited a higher species richness when tested separately (Table 4).

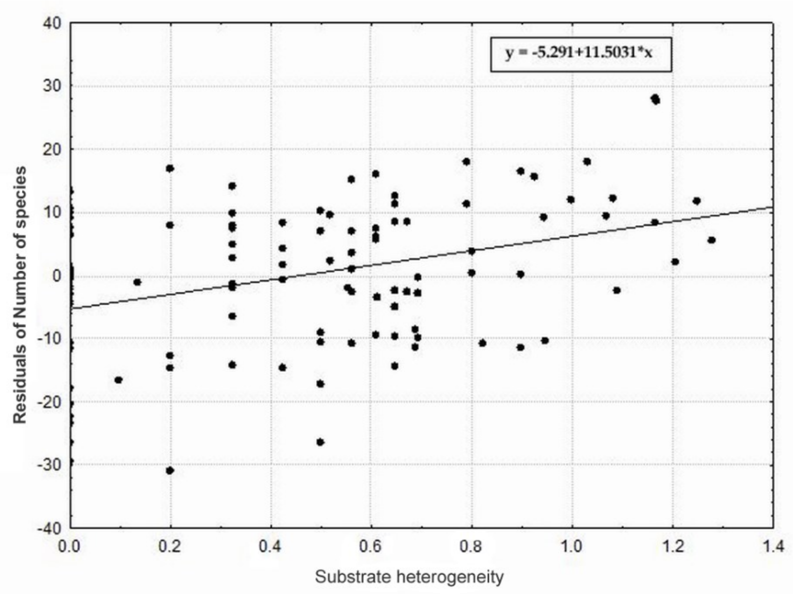


Figure 4. The effect of the substrate heterogeneity on species richness. The effect of the substrate heterogeneity at localities (Shannon diversity index of substrate types – rock, shallow soil, scree and deeper soil) on species richness (depicted as residuals of number of species with coordinates and all significant factors from the second analyses as covariates). P < 0.0001; r = 0.255.

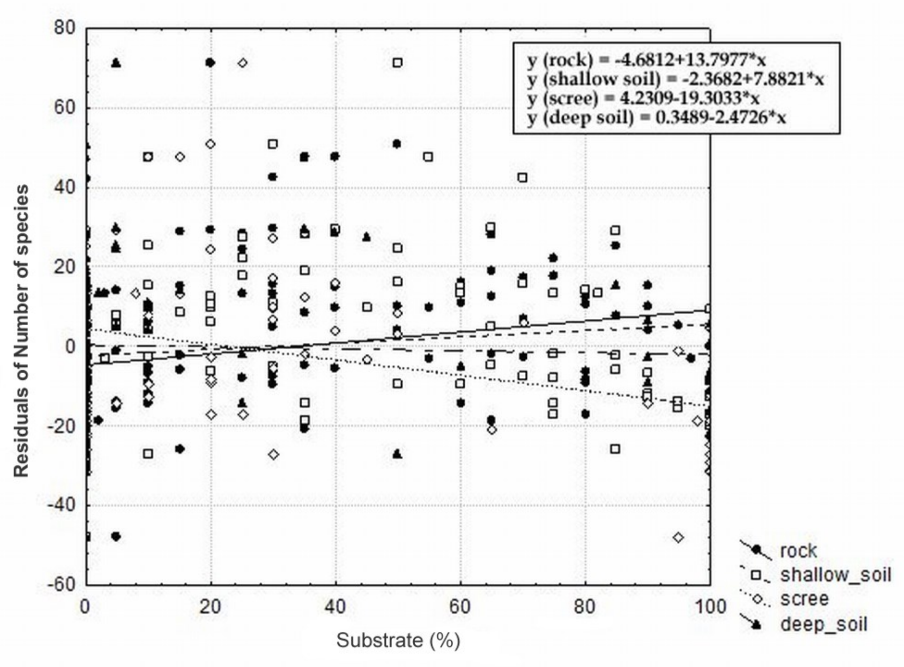


Figure 5. The effect of the substrate on species richness. The effect of the substrate in sense of the proportion of different types of substrate (rock, shallow soil, scree and deeper soil) on species richness (depicted as residuals of number of species with coordinates as covariates). P = 0.0054; r = 0.245 for rock, p = 0.154; r = 0.128 for shallow soil, p = 0.0003; r = - 0.314 for scree and p = 0.6813; r = - 0.037 for deeper soil.

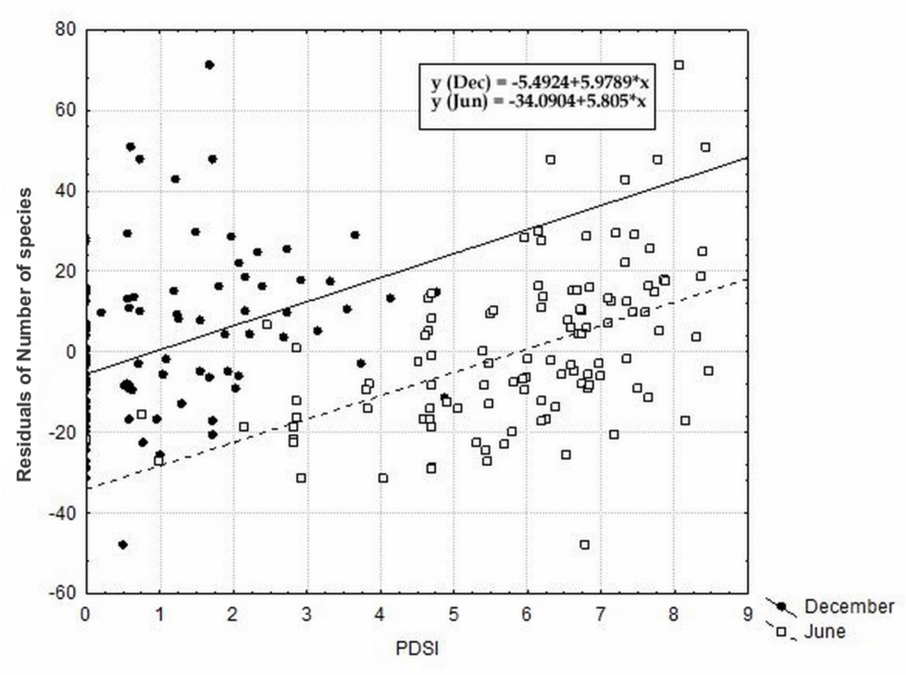


Figure 6. The effect of PDSI on species richness. The effect of potential direct solar insolation (PDSI) on December 21st and June 21st on species richness (depicted as residuals of number of species with coordinates as covariates). P < 0.0001; r = 0.360 for December and p < 0.0001; r = 0.510 for June.

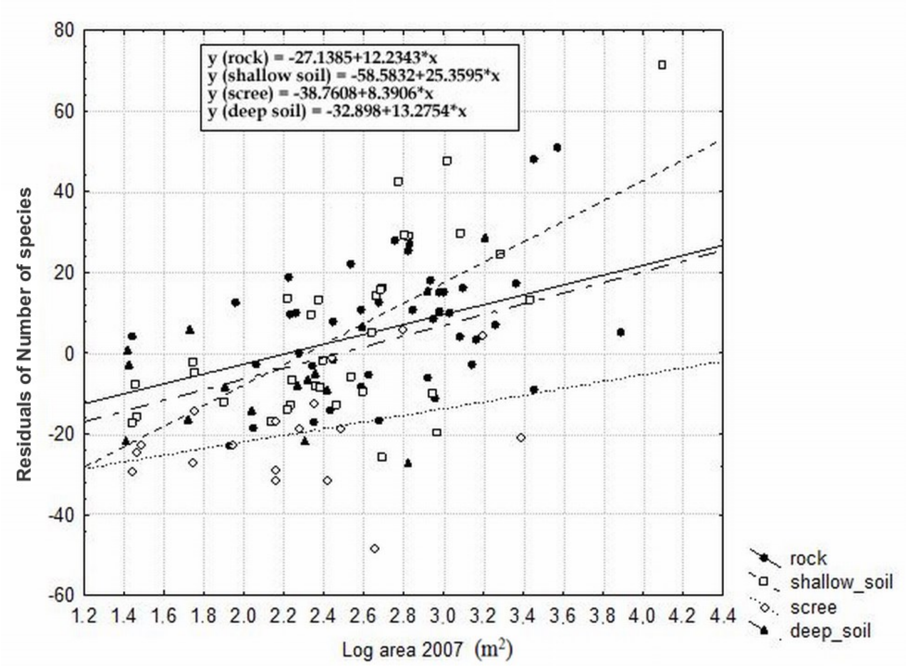


Figure 7. The effect of current area and prevailing type of substrate on species richness (depicted as residuals of number of species with coordinates as covariates). P < 0.0001; r = 0.533 for area and p = 0.0004; r = 0.367 for type of substrate.

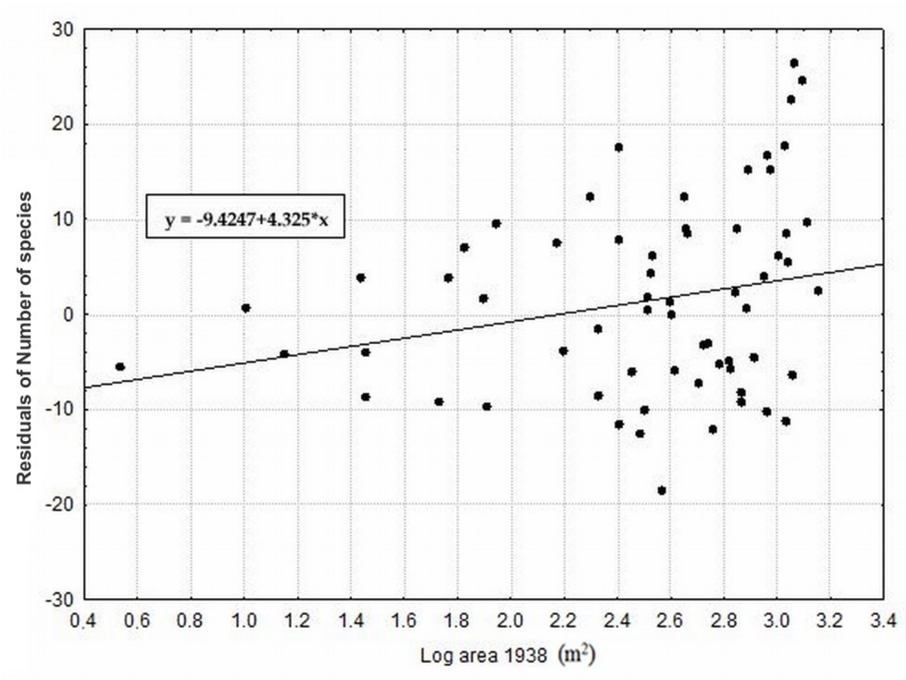


Figure 8. The effect of historical area of the locality in 1938 on species richness (depicted as residuals of number of species with coordinates and all significant factors from the second analyses as covariates). P = 0.0012; r = 0.167.

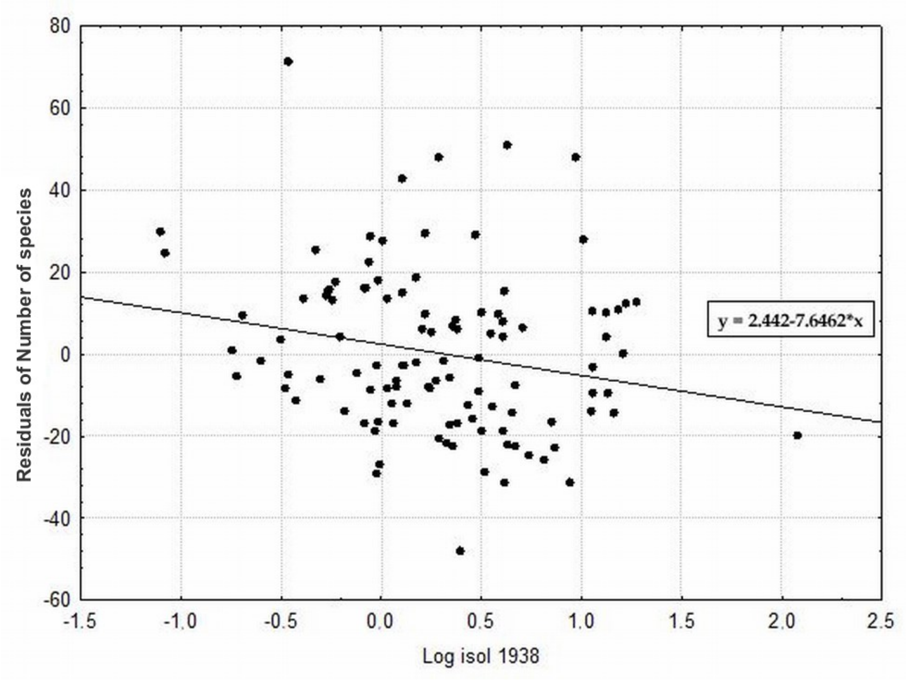


Figure 9. The effect of historical isolation in 1938 on species richness (depicted as residuals of number of species with coordinates as covariates). The outlier values for the number of species have localities with currently higher species richness regardless of their isolation in the past, and the outlier value in isolation has a locality that was much more isolated from other localities in the past and also in the present. P = 0.0325; r = - 0.232.

Altogether, the three groups of factors explained 73.9% of the species richness variance. Local habitat conditions explained 79.1% of the variation that could be explained by all of the factors together. The current landscape structure explained 48.8% of this variation, and the historical landscape structure explained 56.2% of this variation (Figure 10). The variance explained by each of the groups largely overlaps, with over 30% of the variation being attributable to at least two groups of factors.

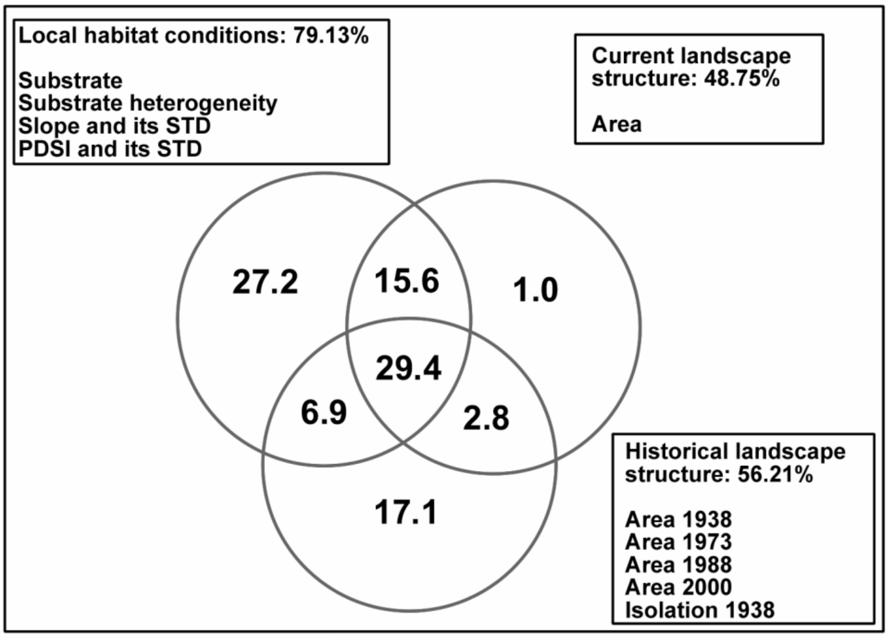


Figure 10. Variation explained by the local habitat condition, current landscape structure and historical landscape structure. For each group of factors, the significant predictors are ranked from the predictor with the highest explanatory power.

DISCUSSION

The results of this study demonstrated that the current species richness of forest openings was significantly affected by all three studied groups of variables: the local habitat conditions and the landscape structure in the present and in the past. Each of these three groups explained different but important deal of species richness variance. Local habitat conditions have the strongest effect on species richness, followed by historical landscape structure. Current landscape structure has the weakest effect. The substrate heterogeneity seems to be the most important overall factor. From historical parameters the area of habitats in 1938 was the most important and from current parameters it was the current area of habitats.

Changes in landscape structure

During the last several decades, some important changes occurred in the landscape structure of the study region, which had strong effects on the occurrence of dry grassland species at the localities. At first, from 1938 to 1988, the locality area increased by 124%. Since 1988, the area has decreased by 27%. The changes in the landscape structure of the study region were thus substantially different and the reduction of the locality area was not so dramatic in comparison with other studies on semi-natural dry grasslands performed in Belgium (Adriaens et al. 2006) or Sweden (Eriksson et al. 2002), where an area decline of approximately 90% was observed. This is because the Křivoklátsko region fulfilled a specific function in the past (local forests were used for hunting by the nobility especially in the Middle Ages). The main impact of human activity in the study region occurred much later (in the 18th and 19th centuries) and was not so intensive as in the other regions, which led to a well-preserved nature with large forest coverage (Kolbek et al. 2003). The changes in the locality area and connectivity during the studied period could also be attributed to an outbreak and subsequent gradual reduction of hoofed game (especially mouflons) in the region. The mouflons were distributed in the region after 1938, then their populations gradually increased to a peak in the 1970s and 1980s. Since then, their numbers gradually decreased (Kolbek 1996; Kolbek et al. 2003), corresponding to changes in landscape structure (Figure 3, personal observation but not tested). The occurrence of mouflons could have a strong positive effect on the persistence of forest openings because if game browsing stops completely, gradual encroachment of shrubs and thus a temporary increase and then a consequential decrease in the species richness would occur. However, the high numbers of mouflons could negatively affect the survival of dry grassland species due to strong eutrophication of some places (Petřík et al. 2011; personal observation). In our analysis the number of species was, however, not significantly affected by nutrient availability at the localities (expressed by Ellenberg indicator value for N), but it negatively responded to low light availability at the locality (expressed by an Ellenberg indicator value for L). Similarly, Turtureanu and Dengler (2012) observed an important positive effect of canopy openness (and thus light availability) on species diversity in Carpathian forest openings. Nature conservation management should thus balance both mechanisms (Petřík et al. 2011): hunting of mouflons (it was partly practiced) and removal of young trees and shrubs colonizing grassland areas (this type of management was not practiced).

Determinants of species richness

Each studied variable group (the local habitat conditions and landscape structure in the present and in the past) had a different effect on the species richness, and their relative importance also slightly differed. Similar to Adriaens et al. (2006), who explored the effects of the same three groups of variables on species richness of semi-natural grasslands, we identified a strong significant effect of the current landscape structure and local habitat conditions. However, contrary to their study, we also identified a strong significant effect of the historical landscape structure on species richness. Lindborg and Eriksson (2004) and Helm et al. (2006) also observed a significant effect of the past landscape structure but not of current factors on species diversity. Only the study performed in forests (Kolb and Diekmann 2004) observed a significant effect of all three groups of variables on species richness.

The most important overall predictor of richness of dry grassland species was the substrate heterogeneity followed by the current locality area. The number of species increased with increasing locality area and thus confirmed the positive species-area relationship (Peintinger et al. 2003; McMaster 2005; Cousins et al. 2007). A larger area per se supports more species. In agreement with Ricklefs and Lovette (1999), we also observed an indirect effect of area on species richness via increasing heterogeneity of the larger localities (according to the substrate heterogeneity and the proportion of each substrate type at the localities (i.e., "substrate")). More heterogeneous localities and localities with a prevalence of rocks and shallow soil harbored more species than localities with deeper soil because the rocks and shallow soil provided numerous different microhabitats with specific conditions and thus enabled coexistence of many dry grassland species. In contrast to our study, Dornbush and Wilsey (2010) and Oberndorfer and Lundholm (2009) observed increasing richness with increasing soil depth in a tall grass prairie and in heathlands, but they performed their studies on a plot-scale and not on a patch-scale as we did. Conversely, Adriaens et al. (2006) did not detect any effect of soil depth on species richness in semi-natural grasslands. In addition, a higher range of potential direct solar insolation and slope (standard deviation of these parameters) provided higher habitat heterogeneity and thus such localities host more species.

Species richness also increased with increasing PDSI and slope, which agrees with Adriaens et al. (2006). Localities with higher PDSI and slope values are typically occupied by more stress tolerant species, i.e., the species typical for rocks and dry grasslands (such as *Sedum acre, S. album, S. reflexum, Scleranthus perennis, Potentilla arenaria, Aurinia saxatilis*). Conversely, the localities with lower values of these factors are likely to be occupied by more competitive species (such as *Bromus sterilis, Carduus nutans, Echium vulgare, Geranium columbinum*) and many weaker competitors (such as *Arabidopsis thaliana, Alyssum alyssoides, Erophyla verna*) are thus excluded from the sites.

In contrast to Huerta-Martinez and Garcia-Moya (2004) and Chýlová and Münzbergová (2008), we did not observe an effect of geology on species richness in our study because we only investigated two extremely similar types of geology (andesites and dacites), which possesses a similar pH, and thus, relatively similar soil types developed at these substrates. This most likely also accounted for the small range of values of the soil pH (expressed by the Ellenberg indicator value for soil reaction pH) and can explain why we did not find any effect of soil pH on species richness although other studies with more heterogeneous habitat conditions found soil pH to be extremely important (Löbel et al. 2006; Merunková et al. 2012).

We also did not observe an effect of the topographic wetness index (TWI) on species richness most likely due to the small variance of TWI values, in contrast to Kopecký and Čížková (2010), who studied its effect on species composition.

Another important factor driving species richness of the habitats in fragmented landscapes could be their isolation. In contrast to strong significant effects of area, current habitat isolation did not significantly affect the species richness in the study region. Significant effects of isolation are commonly reported when studying oceanic islands due to much higher distances between islands than between habitat patches in the mainland (e.g., Brose 2001; McMaster 2005) and because the real islands are isolated for much longer and their diversity already reached equilibrium (Krauss et al. 2004; Cousins et al. 2007). In contrast to our study, many previous studies (e.g., Kolb and Diekmann 2004; Piessens et al. 2004; Tremlová and Münzbergová 2007) observed a significant effect of habitat isolation even in mainland habitats at a spatial scale similar to that studied here.

The absence of a significant effect of habitat isolation in our study system could be explained by a relatively small distance between localities, which was not enough to affect the colonization process of species or affecting only some species not adapted to long-distance dispersal (such as *Asperula cynanchica, Aurinia saxatilis, Centaurea scabiosa, Helianthemum grandiflorum* agg.*, Securigera varia*) (Krauss et al. 2004; Ozinga et al. 2005). However, well dispersed species, usually with a partly ruderal strategy (such as *Bromus sterilis, Carduus nutans, Hieracium pilosella, Senecio viscosus, Setaria viridis, Taraxacum* sect. *Erythrosperma*), may replace them and thus yield a similar number of species with different traits on localities that differ in isolation (Soons and Heil 2002; Tremlová and Münzbergová 2007). High species richness at isolated localities can also be maintained due to high species survival since the periods when the locality has been more connected, indicating an extinction debt (Kuussaari et al. 2009; Jackson and Sax 2010). Species can survive either in the aboveground vegetation (long-lived species) or in the seed bank (Piessens et al. 2004; Honnay et al. 2008; Kuussaari et al. 2009; Jackson and Sax 2010). This can explain the fact that despite no significant effect of current isolation, species richness of forest openings was significantly affected by historical isolation, specifically isolation in 1938, in the study region. According to Lindborg and Eriksson (2004), Helm et al. (2006) and Krauss et al. (2010), it is possible that in the more distant history, the effect of habitat isolation on species richness would have been much stronger. In their studies, they did not detect any effect of current landscape structure on plant species richness of semi-natural grasslands but did observe a strong effect of landscape structure in the 1950s and an even stronger effect in the 1900s and in the 1930s.

Among factors describing historical landscape structure, the locality areas was much more important in comparison with locality isolation, especially the areas in 1938 and in 1988 and partially areas in 1973 and 2000. The historical area of the localities strongly correlated with their continuity, and thus, the localities with larger areas in the past have longer uninterrupted development. In such localities, species-rich communities with many rare species well adapted to live in specific habitats may develop. These species are usually perennials that are good competitors but bad colonizers and are thus able to persist on continuous localities for a long time. Higher richness on localities with a large historical area also agrees with previous studies performed in semi-natural grasslands (e.g., Cousins and Eriksson 2002; Lindborg and Eriksson 2004; Helm et al. 2006) and in forests (e.g., Graae and Sunde 2000; Kolb and Diekmann 2004; Vellend et al. 2006; Krauss et al. 2010), documenting a delay in the species richness response to fragmentation and changes in the landscape structure. However, in contrast to the above mentioned studies, we also observed a strong effect of current area on species richness. As suggested by Öster et al. (2007), such a significant effect of current habitat area in contrast to area in the past indicates that the system is relatively in equilibrium. This may be consistent with the fact that habitats in our study area changed relatively little compared to other areas due to a higher local management stability.

Because of the relatively small study region area, and thus a small range of predictors that could be encompassed, some abiotic factors did not significantly affect the species richness; nevertheless, many of them (mainly elevation, standard deviation of elevation, topographic heterogeneity, especially shape of relief, habitat diversity, and occasionally disturbances and potential vegetation at each locality) play extremely important roles, especially at larger, regional scales. For example, Kučera (1997) and Valtr (2011) demonstrated significant effects of these various factors on species diversity on a larger scale – the entire Protected Landscape Area and Biosphere Reserve Křivoklátsko in the Czech Republic.

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SUPPORTING INFORMATION

Table S1

All species of vascular plants recorded on study localities. Included – species is included into analyses (1) or not (0), Frequency – number of localities with species presence.

	Included	**Frequency**
Acer campestre	0	8
Acinos arvensis	1	13
Agrostis capillaris	1	18
Achillea millefolium (s.str.)	1	34
Ajuga genevensis	1	86
Alliaria petiolata	0	40
Allium oleraceum	1	37
Allium senescens subsp. montanum	1	24
Alyssum alyssoides	1	2
Alyssum montanum subsp. montanum	1	8
Anemone ranunculoides	0	4
Anchusa officinalis	1	32
Antennaria dioica	1	1
Anthemis arvensis	1	1
Anthemis tinctoria	1	4
Anthericum liliago	1	2
Anthetricum ramosum	0	47
Anthoxanthum odoratum	1	7
Anthriscus sylvestris	0	7
Arabidopsis thaliana	1	41
Arabis glabra	1	19
Arabis hirsuta	1	17
Arenaria serpyllifolia agg.	1	41
Artemisia absinthium	1	37
Artemisia campesris	1	1
Asarum europaeum	0	1
Asperula cynanchica	1	30
Asplenium ruta-muraria	1	7
Asplenium septentrionale	1	52
Asplenium trichomanes agg.	1	46
Astragalus glycyphyllos	1	27
Athyrium filix-femina	0	4
Atropa bella-donna	1	1
Aurinia saxatilis	1	7
Avenella flexuosa	0	28
Ballota nigra	0	15
Barbarea vulgaris	1	17
Betonica officinalis	0	18
Brachypodium pinnatum	1	70
Brachypodium sylvaticum	0	65
Bromus sterilis	1	14
Bupleurum falcatum	0	15
Calamagrostis arundinacea	0	42
Calamagrostis epigejos	0	15

Calluna vulgaris	1	5
Campanula persicifolia	0	31
Campanula rapunculoides	1	2
Campanula rotundifolia	1	26
Campanula trachelium	0	53
Capsella bursa-pastoris	1	6
Cardamine impatiens	0	6
Cardaminopsis arenosa	0	95
Cardaria draba	1	8
Carduus acanthoides	1	25
Carduus nutans	1	12
Carex caryophyllea	1	41
Carex digitata	0	3
Carex muricata agg.	1	87
Carex ovalis	1	4
Carex pilulifera	1	8
Carex praecox	1	10
Centaurea scabiosa	1	7
Centaurea stoebe	1	7
Cerastium arvense	1	32
Cerastium brachypetalum	1	30
Cerastium holosteoides	1	15
Cerinthe minor	1	2
Circaea lutetiana	0	1
Clinopodium vulgare	1	50
Convolvulus arvensis	1	14
Corydalis cava	0	2
Cotoneaster integerrimus	1	24
Crateagus sp.	0	63
Cruciata laevipes	1	3
Cuscuta europaea	0	1
Cynoglossum officinale	1	20
Cytisus nigricans	1	7
Dactylis glomerata	1	28
Dactylis polygama	0	11
Danthonia decumbens	1	2
Dianthus carthusianorum subsp. carthusianorum	1	25
Dictamnus albus	1	1
Digitalis grandiflora	1	22
Dryopteris filix-mas	0	11
Echium vulgare	1	46
Elymus caninus	0	1
Epilobium collinum	1	1
Epilobium montanum	0	13
Erodium cicutarium	1	6
Erophila verna	1	20
Euphorbia cyparissias	1	87
Euphrasia stricta	1	1
Fallopia convolvulus	0	77
Festuca heterophylla	0	6
Festuca ovina agg.	1	90
Festuca pallens	1	16
Festuca rubra agg.	1	10

Festuca rupicola	1	23
Festuca valesiaca	1	3
Ficaria verna	0	2
Filago arvensis	1	9
Fragaria moschata	0	18
Fragaria vesca	1	13
Fragaria viridis	1	86
Fumaria schleicheri	1	3
Gagea bohemica subsp. bohemica	1	1
Gagea villosa	1	6
Galeobdolon luteum	0	3
Galeopsis angustifolia	1	83
Galeopsis ladanum	0	43
Galium album agg.	1	52
Galium aparine	0	43
Galium glaucum	1	17
Galium odoratum	0	8
Galium pumilum	1	37
Galium verum	1	26
Genista germanica	1	25
Genista tinctoria	1	25
Geranium columbinum	1	38
Geranium pusillum	1	16
Geranium pyrenaicum	1	6
Geranium robertianum	0	31
Geranium sanguineum	1	4
Geum urbanum	0	73
Glechoma hederacea	0	8
Gnaphalium sylvaticum	1	6
Hedera helix	0	2
Helianthemum grandiflorum subsp. obscurum	1	3
Hepatica nobilis	0	9
Hieracium cymosum	1	19
Hieracium laevigatum	0	4
Hieracium lachenalii	0	3
Hieracium murorum	0	40
Hieracium pilosella	1	76
Hieracium sabaudum	0	5
Hieracium schmidtii	1	25
Holosteum umbellatum	1	4
Hylotelephium maximum	1	21
Hypericum montanum	0	7
Hypericum perforatum	1	98
Chaerophyllum temulum	0	2
Chelidonium majus	0	1
Chenopodium album (s.str.)	1	3
Chenopodium polyspermum	1	2
Impatiens noli-tangere	0	5
Impatiens parviflora	0	52
Inula conyzae	1	26
Jasione montana	1	25
Jovibarba globifera subsp. globifera	1	5
Juncus conglomeratus	0	1

Juniperus communis subsp. communis	1	2
Koeleria macrantha	1	7
Lactuca serriola	1	1
Lactuca viminea	1	1
Lamium album	0	1
Lamium amplexicaule	1	8
Lamium purpureum	0	17
Lappula squarrosa	1	2
Lapsana communis	0	45
Lathyrus niger	0	5
Lathyrus pratensis	1	1
Lathyrus vernus	0	3
Leucanthemum vulgare	1	2
Lilium martagon	0	3
Linaria vulgaris	1	25
Lithospermum purpurocaeruleum	1	5
Lotus corniculatus	1	26
Luzula campestris	1	16
Luzula luzuloides	0	45
Luzula multiflora	1	13
Lychnis viscaria	1	58
Lysimachia nummularia	0	2
Malus sylvestris	1	1
Matricaria discoidea	0	1
Medicago falcata	1	3
Medicago minima	1	1
Melica nutans	0	12
Melica picta	0	1
Melica transsilvanica	1	44
Melilotus officinalis	0	1
Melittis melissophyllum	1	3
Microrrhinum minus	1	2
Moehringia trinervia	0	18
Mycelis muralis	0	14
Myosotis arvensis	1	25
Myosotis ramosissima	1	23
Myosotis sparsiflora	1	13
Myosotis stricta	1	21
Myosotis sylvatica	0	23
Omphalodes scorpioides	0	3
Onopordum acanthium	1	3
Origanum vulgare	1	64
Orobanche alba agg.	1	2
Oxalis fontana	0	1
Papaver dubium agg.	1	5
Persicaria hydropiper	0	3
Persicaria lapathifolia	0	2
Petrorhagia prolifera	1	2
Phleum phleoides	1	40
Phyteuma spicatum	0	1
Pimpinella saxifraga	1	5
Plantago lanceolata	1	3
Plantago media	1	1

Poa angustifolia	1	57
Poa annua	0	4
Poa compressa	1	16
Poa nemoralis	0	87
Poa pratensis	1	13
Polygala vulgaris	1	1
Polygonatum odoratum	0	44
Polygonum aviculare	0	29
Polypodium vulgare	0	11
Potentilla arenaria	1	15
Potentilla argentea	1	54
Potentilla inclinata	1	2
Potentilla recta	1	14
Potentilla tabernaemontani	1	60
Primula veris	1	4
Prunus spinosa	1	74
Pseudolysimachion spicatum	1	6
Pulsatilla pratensis subsp. bohemica	1	4
Pyrethrum corymbosum	0	79
Pyrus pyraster	1	10
Ranunculus auricomus agg.	0	4
Ranunculus bulbosus	1	26
Ribes alpinum	0	3
Rosa sp.	0	95
Rubus sp.	0	27
Rumex acetosa	1	5
Rumex acetosella	1	57
Salvia nemorosa	1	2
Sambucus nigra	0	50
Sanguisorba minor	1	16
Scleranthus perennis	1	29
Securigera varia	1	67
Sedum acre	1	8
Sedum album	1	36
Sedum reflexum	1	24
Sedum sexangulare	1	56
Senecio viscosus	1	38
Seseli osseum	1	10
Sesleria caerulea	1	8
Setaria viridis	1	15
Silene nutans	0	81
Silene vulgaris	1	10
Solidago virgaurea subsp. virgaurea	1	8
Sonchus arvensis	1	5
Sorbus aria	1	12
Sorbus aucuparia	0	4
Stellaria holostea	0	56
Stellaria media	0	5
Stipa pennata	1	7
Symphytum officinale	0	1
Taraxacum sect. Erythrosperma	1	39
Taraxacum sect. Ruderalia	0	40
Teucrium botrys	1	16

Teucrium chamaedrys	1	4
Thlaspi arvense	0	2
Thlaspi perfoliatum	1	5
Thymus pulegioides subsp. chamaedrys	1	88
Torilis japonica	0	29
Trifolium alpestre	1	45
Trifolium arvense	1	21
Trifolium aureum	1	4
Trifolium campestre	1	11
Trifolium dubium	1	3
Trifolium medium	1	14
Trifolium repens	0	2
Triticum aestivum	1	1
Urtica dioica	0	52
Vaccinium myrtillus	0	4
Valerianella locusta	1	31
Verbascum densiflorum	1	22
Verbascum lychnitis	1	57
Veronica arvensis	1	10
Veronica dillenii	1	49
Veronica hederifolia	0	36
Veronica chamaedrys	0	74
Veronica officinalis	0	59
Veronica prostrata	1	10
Veronica sublobata	0	2
Veronica verna	1	18
Vicia angustifolia	1	22
Vicia hirsuta	1	35
Vicia pisiformis	0	1
Vicia sepium	0	4
Vicia tenuifolia	1	2
Vicia tetrasperma	1	30
Vincetoxicum hirundinaria	0	95
Viola arvensis	1	76
Viola collina	1	1
Viola hirta	1	8
Viola odorata	1	1
Viola reichenbachiana	0	24
Viola riviniana	1	33
Viola tricolor subsp. tricolor	0	30

Table S2

The effect of studied factors on species richness of all species growing at localities (species of the xerophilous vegetation units as well as forest species). The effect of local habitat conditions, current and historical landscape structure on species richness and the direction of the effect (+/-). The amount of explained variance by the single independent variables with different covariates is presented; n.s. is not significant (p > 0.05), – not tested because previously not significant. Df error = 108 (respectively, 106 for substrate and 107 for slope and for PDSI) for all factors significant without covariates, Df error = 105 (respectively 103 for substrate and 104 for slope and for PDSI) for all factors significant when using coordinates as covariates and Df error = 87 (respectively, 85 for substrate and 86 for slope and for PDSI) for all factors significant when using coordinates and all significant factors from the first analyses as covariates. For the abbreviation explanations, see Table 1.

				Covariates		Direction of significance
		D.F.	Without covariates	Coordinates (15.69%)	All significant factors (77.84%)	
Local habitat conditions	Substrate	3	8.96%	9.70%	3.29%	+/-
	Substrate heterogeneity	1	42.35%	36.72%	8.41%	+
	Slope (median+max)	2	12.16%	13.19%	n.s.	+
	Slope - STD	1	11.90%	11.98%	n.s.	+
	PDSI (Dec+June)	2	26.03%	20.77%	n.s.	+
	PDSI - STD (Dec+June)	2	26.57%	20.33%	2.02	+
	Geology	1	n.s.	n.s.	–	
	TWI	1	n.s.	n.s.	–	
Current land.str.	Area 2007	1	33.80%	28.93%	1.63%	+
	Isolation 2007	1	n.s.	–	–	
Historical landscape structure	Area 1938	1	15.25%	13.80%	1.41%	+
	Area 1973	1	10.20%	5.58%	n.s.	+
	Area 1988	1	16.40%	9.34%	1.66%	+
	Area 2000	1	23.57%	18.73%	n.s.	+
	Isolation 1938	1	n.s.	–	–	
	Isolation 1973	1	n.s.	–	–	
	Isolation 1988	1	n.s.	–	–	
	Isolation 2000	1	n.s.	–	–	

The idea of using only species growing exclusively at studied localities and nowhere else in the study region is that we want to study how locality isolation, among other parameters, affected local species diversity. To do such an analysis it is thus absolutely crucial to use only species for which the studied localities are the only possible localities in the area. The data shown in this Supporting Information S2 are thus partly wrong, and that is another reason for showing these only in the supplementary information.

Table S3

Pair wise correlation matrix between individual variables of the local habitat conditions and the past and current landscape structure. For the abbreviation explanations, see Table 1.

		Substrate					Slope			PDSI					Geo														
		rock	shallow	scree	deep	Substr. heterog	median	max	slope-STD	Dec	June	Dec-STD	Jun-STD	TWI	andesit	area07	isol07	area38	area73	area88	area00	isol38	isol73	isol88	isol00	cont38	cont73	cont88	cont00
Substrate	rock	1.00	**-0.32**	**-0.33**	**-0.36**	**0.23**	**0.49**	**0.56**	0.09	**0.36**	**0.41**	**0.37**	**0.40**	-0.12	**-0.26**	**0.32**	0.13	-0.13	-0.12	-0.08	0.10	**0.19**	0.13	-0.09	0.16	-0.15	-0.03	0.14	0.11
	shallow_soil		1.00	**-0.45**	**-0.29**	0.15	**-0.20**	-0.18	0.05	-0.05	0.07	-0.04	0.05	-0.07	-0.08	-0.04	0.08	0.14	-0.05	0.05	-0.13	-0.10	0.12	0.13	0.06	0.01	-0.01	0.12	**-0.24**
	scree			1.00	**-0.25**	**-0.20**	0.18	0.11	-0.19	-0.14	**-0.23**	-0.15	**-0.21**	**0.20**	**0.20**	-0.05	-0.17	-0.16	**0.23**	-0.02	0.05	0.00	**-0.26**	-0.09	-0.16	-0.17	-0.02	-0.04	0.04
	deep_soil				1.00	**-0.19**	**-0.51**	**-0.53**	0.05	-0.18	**-0.27**	-0.19	**-0.27**	-0.02	0.16	**-0.24**	-0.05	0.15	-0.07	0.04	0.00	-0.10	0.00	0.04	-0.07	**0.34**	0.07	**-0.24**	0.11
	Substr. heterog					1.00	0.10	**0.23**	**0.23**	**0.29**	**0.44**	**0.30**	**0.46**	-0.08	-0.04	**0.53**	0.09	**0.21**	**0.24**	**0.19**	**0.40**	-0.12	-0.06	-0.11	0.10	0.09	**0.25**	**0.29**	**0.19**
Slope	median						1.00	**0.89**	**-0.22**	**0.39**	**0.32**	**0.35**	**0.36**	-0.10	-0.08	**0.27**	**-0.20**	-0.06	**0.25**	-0.01	0.12	0.06	-0.18	**-0.36**	-0.17	-0.08	0.14	0.13	0.01
	max							1.00	**0.20**	**0.47**	**0.43**	**0.40**	**0.48**	**-0.25**	-0.12	**0.50**	-0.15	-0.10	**0.20**	-0.02	**0.26**	0.11	-0.11	**-0.36**	-0.09	-0.15	0.11	**0.19**	0.10
	slope-STD								1.00	**0.21**	**0.28**	0.12	**0.30**	**-0.32**	-0.11	**0.49**	0.11	0.02	-0.03	0.03	**0.35**	0.06	0.12	0.00	0.14	-0.04	-0.01	0.13	**0.19**
PDSI	Dec									1.00	**0.57**	**0.95**	**0.63**	-0.13	**-0.22**	**0.58**	-0.06	**0.32**	**0.37**	0.15	**0.44**	**-0.28**	-0.12	**-0.22**	-0.04	**0.20**	**0.41**	**0.33**	**0.29**
	June										1.00	**0.59**	**0.98**	-0.11	-0.03	**0.57**	-0.02	**0.34**	**0.41**	**0.26**	**0.42**	**-0.23**	-0.03	**-0.25**	0.01	0.18	**0.41**	**0.43**	**0.27**
	Dec-STD											1.00	**0.64**	-0.14	-0.14	**0.58**	-0.09	**0.32**	**0.38**	**0.24**	**0.43**	**-0.31**	-0.13	**-0.23**	-0.06	0.18	**0.41**	**0.37**	**0.28**
	Jun-STD												1.00	-0.12	-0.05	**0.62**	-0.04	**0.35**	**0.47**	**0.24**	**0.47**	**-0.25**	-0.06	**-0.27**	-0.01	**0.21**	**0.47**	**0.44**	**0.28**
	TWI													1.00	-0.02	**-0.21**	0.06	0.18	0.17	-0.04	-0.17	**-0.21**	-0.13	0.16	0.00	0.17	0.14	-0.10	-0.09
Geo	andesites														1.00	-0.17	-0.02	0.16	0.16	**0.34**	-0.02	**-0.28**	**-0.27**	-0.16	-0.05	**0.20**	**0.20**	**0.23**	0.02
	area07															1.00	-0.03	0.18	**0.34**	**0.29**	**0.68**	-0.13	-0.05	**-0.23**	0.00	0.06	**0.30**	**0.37**	**0.45**
	isol07																1.00	0.01	**-0.22**	**-0.28**	-0.18	**0.28**	**0.57**	**0.81**	**0.96**	0.12	-0.01	-0.10	0.12
	area38																	1.00	**0.56**	**0.23**	**0.29**	**-0.69**	**-0.22**	-0.02	-0.06	**0.68**	**0.60**	0.16	**0.27**
	area73																		1.00	**0.32**	**0.43**	**-0.54**	**-0.44**	**-0.26**	**-0.25**	**0.41**	**0.70**	**0.36**	**0.36**
	area88																			1.00	**0.30**	**-0.31**	**-0.27**	**-0.41**	**-0.28**	0.16	**0.20**	**0.44**	0.14
	area00																				1.00	**-0.22**	-0.13	**-0.21**	**-0.19**	**0.19**	**0.35**	**0.35**	**0.67**
	isol38																					1.00	**0.44**	**0.24**	**0.34**	**-0.39**	**-0.51**	**-0.29**	-0.11
	isol73																						1.00	**0.54**	**0.60**	-0.04	-0.13	-0.18	0.00
	isol88																							1.00	**0.76**	0.10	-0.11	**-0.25**	0.06
	isol00																								1.00	0.04	-0.07	-0.10	0.10
	cont38																									1.00	**0.64**	0.09	**0.32**
	cont73																										1.00	**0.33**	**0.39**
	cont88																											1.00	**0.30**
	cont00																												1.00

CHAPTER 2

The effect of current and historical landscape structure and species life-history traits on species distribution in dry grassland-like forest openings

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ABSTRACT

In a fragmented landscape, the distribution of plant species is determined not only by local habitat conditions but also by the landscape structure, including its changing nature, and by the life-history traits of species present. Whereas the importance of all these factors has been investigated in a range of previous studies, our understanding of the effects of individual traits on species dependence on landscape structure is still rather limited. The aim of this study was to identify factors responsible for plant species composition in dry grassland-like forest openings and to identify the relationship between life-history traits and species distribution.

We examined the effect of current and past landscape structures, habitat continuity and habitat conditions on species composition. We tested the importance of species traits for species response to the landscape structure.

In addition to habitat conditions, species composition was determined by landscape structures in the past and at present, and the past landscape structure in each time period separately was as equally important as the present landscape structure. Perennial species that are insect or wind pollinated, flower over limited periods of time, are relatively tall, are dispersed by animals, are not ruderal, have low nutrient requirements, and maintain competitive or partly stress-tolerant strategies were found to be restricted to continuous localities existing at least since 1973 or 1988 and to localities that are currently well interconnected. In contrast, short, self-pollinated, ruderal species prevail in currently less-connected localities.

The results indicate that a knowledge of the past landscape structure is important for understanding the current species distribution and that species traits can be used as useful predictors of species responses to the past landscape structure.

Keywords Species composition; Tree-less patch; Forest matrix; Habitat fragmentation; Area; Isolation; Continuity of habitat; Dispersal traits; Persistence traits.

INTRODUCTION

At the landscape level, many species are restricted to fragmented habitats that have been reduced in area and subject to increased isolation during the last few decades or centuries (Saunders et al. 1991; Soons 2003). Small habitats usually support small populations, which can be strongly influenced by demographic and environmental stochasticity and are thus prone to extinction (Dupré & Ehrlén 2002; Münzbergová 2006; Jackson & Sax 2010). Moreover, increased isolation of the localities can limit the spread of a species and thus lead to a lower possibility of recolonization (Jacquemyn et al. 2001; Ozinga et al. 2005, 2009). Habitat fragmentation may thus lead to a reduction of species richness and changes in species composition.

The effects of habitat configurations and their changes over time on species occurrence have been well studied for different taxa, but the outcomes have been highly variable. Some studies found significant effects of current habitat area and/or connectivity on species distributions (e.g., Piessens et al. 2004, 2005; Adriaens et al. 2006; Tremlová & Münzbergová 2007; Schleicher et al. 2011). In contrast, numerous studies found species distributions unaffected by current habitat configurations (e.g., Lindborg & Eriksson 2004; Helm et al. 2006; Krauss et al. 2010).

Due to rapid landscape changes, which are typical for many European landscapes (Eriksson et al. 2002; Adriaens et al. 2006, Chýlová & Münzbergová 2008), and slow responses of species populations to these changes (Eriksson et al. 2002; Helm et al. 2006; Krauss et al. 2010; Münzbergová et al. 2013), current populations are often not in equilibrium with current landscape configurations (Münzbergová et al. 2005, Herben et al. 2006) leading to extinction debt (Kuussaari et al. 2009; Jackson & Sax 2010). On the other hand, many species may be missing from suitable habitats due to dispersal limitations (Ehrlén & Eriksson 2000; Münzbergová 2004; Münzbergová & Herben 2005; Ehrlén et al. 2006; Ozinga et al. 2009). As a result, the distributions of some species reflect historical habitat configurations rather than current habitat configurations (Helm et al. 2006; Herben et al. 2006; Lindborg 2007).

Species responses to present and past landscape structures likely depend on species-specific life-history strategies, mainly those related to dispersal and survival (Adriaens et al. 2006; Tremlová & Münzbergová 2007; Schleicher et al. 2011; Lindborg et al. 2012; Purschke et al. 2012). It has been shown that short-lived plants are more sensitive to landscape changes and respond much faster to such changes than long-lived plants. As a result, the distribution of short-lived plants is positively affected mainly by the current area and connectivity of their habitats (Lindborg 2007; Lindborg et al. 2012). In contrast, long-lived plants with clonal propagation and plants with long-lived seed banks tend to build up remnant population (Eriksson 1996) and are thus mainly affected by historical landscape structure (Lindborg 2007; Purschke et al. 2012). However, the results tend to differ between different studies and studied systems: forests (Graae & Sunde 2000; Kolb & Diekmann 2004) vs. grassland habitats (Lindborg et al. 2012; Purschke et al. 2012) vs. urban landscapes (Schleicher et al. 2011).

In contrast to the relatively numerous studies showing the importance of traits related to species survival, few similar findings have been made regarding species dispersal. Most studies that investigated dispersal traits but did not find any effects on species survival used only a single dispersal mode for each individual species (e.g., Piessens et al. 2004, 2005; Hérault & Honnay 2005; Adriaens et al. 2006; Lindborg 2007), although in reality species can disperse by multiple vectors (Ozinga et al. 2004; Adriaens et al. 2007; Vittoz & Engler 2007). Studies using multi-vector dispersal, in terms of dispersal potential on a continuous scale, are rare but show significant effects of dispersal traits on the dependence of species occurrences, either on current habitat configurations (Adriaens et al. 2007; Schleicher et al. 2011; Saar et al. 2012; Hemrová & Münzbergová 2015) or on historical habitat configurations (Purschke et al. 2012).

A useful approach for understanding species distribution in a changing landscape is to analyse the relationships between species occurrences in habitats with different configurations and life-history traits. Earlier studies, however, have not attempted to link species life-history traits directly to landscape characteristics (but see Purschke et al. 2012; Hemrová & Münzbergová 2015). Instead, they have focused only on the relationship between mean trait values (at the site level) (Lindborg 2007) or emergent groups of functionally similar species, and site or landscape descriptors (Kolb & Diekmann 2004, 2005; Adriaens et al. 2006; Lindborg et al. 2012). However, this approach cannot answer the question of how individual traits contribute to species dynamics in the landscape. In addition, many studies are focused only on species richness or diversity in relation to habitat configuration (Lindborg & Eriksson 2004; Helm et al. 2006; Cousins et al. 2007; Öster et al. 2007), but studies on plant species composition are rare and are usually carried out in forests, e.g., Kolb & Diekmann (2004); Herault & Honnay (2005); in grasslands, the only such studies are Purschke et al. (2012) and Hemrová & Münzbergová (2015). Moreover, many studies dealing with the importance of habitat configuration have not accounted for historical habitat configurations (or they have accounted only for historical land use at the habitat sites) (e.g., Dupré & Ehrlén 2002; Kolb & Diekmann 2005; Lindborg et al. 2012), which can result in the conclusion that past landscape structure is not important for current species distribution.

The aim of this study was to assess the effects of current and historical landscape structures and species life-history traits on species distribution in dry grassland-like forest openings in the forested landscape of the Křivoklátsko Biosphere Reserve, Czech Republic. In this area, the dry grassland-like forest openings are the places with the highest species richness of plants and animals and are thus places of high conservation interest. During the last century, these places experienced several changes in landscape structure (Husáková & Münzbergová 2014). To identify the determinants of plant species composition in this landscape we asked the following questions: (i) What is the importance of current and historical landscape structures for plant species composition in dry grassland-like forest openings in forest matrix? (ii) Which life-history traits explain the response of species to current and past landscape structure?

To answer these questions, we used data on the occurrences of all dry grassland species in 110 dry grassland-like forest openings. We analysed information on current landscape structure (area and isolation), past landscape structure in 1938, 1973, 1988 and 2000 (area, isolation, proportion of area of each locality that persisted at the same place as in the past – i.e., continuity of habitat), and local habitat conditions. We also assembled data on life-history traits related to seed dispersal, species life strategy and habitat requirements. Traits were used to explain the species dependence on landscape structure in different time periods.

METHODS

The study system

The study was carried out in Křivoklátsko Protected Landscape Area and Biosphere Reserve in the Czech Republic, more specifically in the Týřov Site of Community Importance (SCI) (49°58’10’’ N, 13°48’40’’ E). The study area is approximately 4 km^2 and is mainly forested. Approximately 2% of the area is occupied by dry grassland-like forest openings, with a mean area of 635 m^2 and a mean nearest distance between localities of 48 m (estimated as the distance between nearest points on the borders of neighbouring localities). From a previous study carried out in the same study region (Husáková & Münzbergová 2014), we obtained data on the distribution of 110 dry grassland-like forest openings in total, with areas ranging from 20 m^2 to 11123 m^2. These localities represented all dry grassland-like forest openings mapped in the study region, defined as open localities with less than 30% tree cover that are isolated from other localities by at least 20 m of forest. The border between the grassland and the forest was always abrupt, without a transient zone, and it was created by the trunks of trees (Husáková & Münzbergová 2014).

The plant communities of these localities are created by a specific mosaic of different xerophilous vegetation units, ranging from chasmophytic vegetation of rocks and rock crevices, vegetation of primitive soils and mobile screes, to vegetation of dry grasslands and dry herbaceous fringes (Kolbek et al. 2001). At some localities, the vegetation is also created by plants of ruderal vegetation (*Artemisietea vulgaris, Chenopodietea*) (Kolbek et al. 2001). The surrounding forests are represented by ancient dry acidophilous oak forests that have not been significantly managed due to their largely inaccessible positions (Kolbek et al. 2003). Additionally, all of our localities are not currently managed (i.e., no removal of young trees and shrubs colonizing grassland areas, mowing, grazing cattle, etc.). More information about the broader setting of the study area and management is available in Electronic Appendix S1.

Field data collection

In the field, we recorded all species of vascular plants occurring at each locality. We surveyed each locality twice per year (from April to August of 2005–2007) to include both the spring and summer plant flowering periods. Plant nomenclature followed Kubát et al. (2002).

During the field survey, we observed that some species were growing not only in the forest openings but also in the surrounding forests. To study the effects of isolation and habitat size on species composition, it was absolutely crucial to use only species for which the forest openings are the only possible habitat in the area. To exclude species occurring also in the surrounding forests from the analyses, we generated a list of species growing both in the study localities and in the surrounding forests in the study region (as in Husáková & Münzbergová 2014). In this way, we excluded 34% of the 296 species recorded at the studied localities (Electronic Appendix S2).

Landscape structure in the present and the past, and local habitat conditions

To assess historical and current landscape structures we used the data obtained in our previous study (Husáková & Münzbergová 2014). We used aerial photographs of the study region from 1938, 1973, 1988 and 2000 available from Geoportal Cenia (http://geoportal.cenia.cz). For each locality, we calculated the current area and isolation using ArcGIS (version 9.2, 2006, ESRI, Redlands, CA, USA). Due to high topographic heterogeneity, we calculated the real surface area for each locality according to a digital terrain model and orthophoto maps. The digital terrain model had a resolution of 5 × 5 m and was created using a digital contour line map with height intervals of 2 m between contour lines. Actual habitat isolation was calculated using a formula provided by Tremlová & Münzbergová (2007) (for details see Electronic Appendix S3).

Next, we calculated several variables of historical landscape structure: historical area of each current locality, its historical isolation, and the proportion of the area of each locality that persisted at the same place as in the past (i.e., continuity of locality). All historical variables were calculated for the years 1938, 1973, 1988 and 2000. All variables of historical landscape structure were, however, highly correlated (see Electronic Appendix S4). According to a preliminary analysis of the effect of each factor on species composition separately (Electronic Appendix S5), we decided to use only the proportion of area persisting at the same place in subsequent analyses because it had the highest predictive value in explaining the variation in species composition and because this factor determines what proportion of area of each current locality used to be the locality in the past. This factor thus integrates information on locality size in the past as well as its stability in space. The proportion of area persisting at a particular place was expressed as the percentage of overlap of each locality’s current area with its past area in each time period, separately.

We also calculated several variables of local habitat conditions (see Electronic Appendix S3) for use as covariates in subsequent analyses. The mean, median and range of each variable of landscape structure and local habitat conditions are presented in Electronic Appendix S6.

Life-history traits of studied species

To assess differences between studied species and their distributions related to specific variables of the landscape structure, we collected data about their life-history traits using several databases (Table 1). We also measured certain data lacking for some species using the same methods as used for the databases. We divided all the studied traits into three groups: seed dispersal traits, species life strategy traits, and species habitat requirements. Data on the longevity of seed banks were used to calculate the longevity index of a seed bank (Bekker et al. 1998). We used several categories of seed dispersal type (see Table 1), expressed as the proportion of each studied dispersal type for each species, i.e., multi-vector dispersal on a continuous scale, and analysed all these categories together. We also used data on the clonal index of each species, calculated as a sum of "classes of Lateral spread" and "classes of Number of offspring" (Klimešová & de Bello 2009). For each species, we also classified its ruderal status (see Electronic Appendix S7).

Table 1. All studied species traits and their attributes used in analyses.

Trait groups	Traits	Categories	Units	Source
Seed dispersal traits	seed dispersal type	anemochory/ autochory/ hemerochory/ chamaechory/ nautochory/ ombrochory/ endozoochory/ epizoochory/ other types of zoochory	proportions of all categories	Leda Traitbase*
	seed attached structure	awns/ pappus/ hooks/ emergences/ nothing	one of the categories	Digital seed atlas**
	terminal velocity		$m.s^{-2}$	Leda Traitbase*
	seed mass		mg	Leda Traitbase*
	seed bank longevity index		index	Leda Traitbase*
	plant height	average value	m	Kubát et al. (2002)
Species life strategy	pollination vector	insect/ wind/ self	proportions of all categories	BiolFlor database***
	plant life span	perennials/annuls/monocarp	proportions of all categories	Leda Traitbase*
	flower. period		months	Kubát et al. (2002)
	clonal index		index	CloPla database****
	CSR strategy	c/r/s/cr/cs/rs/csr	one of the categories	BiolFlor database***
	ruderal status	yes/no	one of the categories	Chytrý and Tichý (2003) *****
Ellenberg indicator values	R	value for pH		Ellenberg et al. (1991)
	N	value for nutrient		Ellenberg et al. (1991)

* Kleyer et al. (2008), ** http://seeds.eldoc.ub.rug.nl/?pLanguage=en, *** http://www2.ufz.de/biolflor/index.jsp, **** http://clopla.butbn.cas.cz, ***** For details about the other sources for delimitation of ruderal species see Electronic Appendix S7.

Statistical analyses

The statistical analyses were carried out using multivariate analysis in CANOCO for Windows (version 4.5, Ter Braak & Šmilauer 2002) and univariate analysis in Statistica (version 7.0, StatSoft, Inc. 2004, www.statsoft.com).

Because the localities are relatively close to each other, their vegetation compositions could be largely driven by their spatial relatedness. To check this, we calculated similarity in species composition (calculated as Eucleidian distance in Statistica) between all pairs of localities. We used a one-sided Mantel test in PopTools (version 2.6.9., Hood G.M. 2005, Albany) to test the relationship between similarity in species composition and geographical distance between the localities.

To take into account spatial auto-correlation among localities for the variables across the study area, we used the method of variation partitioning according to Borcard et al. (1992) and Legendre and Legendre (1998). To allow for the recognition of complex spatial trends, seven derived geographical variables were constructed by including all quadratic and cubic combinations of x and y, as suggested by Borcard et al. (1992): x, y, x^2, xy, y^2, x^3, x^2y, xy^2, y^3. We used the variables selected in the stepwise selection during the CCA analysis (Ter Braak & Šmilauer 2002) (x, y, x^2, y^2, xy) as covariates in all the subsequent tests to remove the effect of spatial relatedness of the localities. Using other stepwise selection in CCA analysis with the geographical coordinates as covariates we selected the most important abiotic variables (local habitat conditions) to use as covariates in subsequent analyses (Proportions of substrate types, Shannon diversity index of substrate types, Slope-max, PDSI-median value in June and Geology).

We then used selected coordinates and factors of local habitat conditions as covariates, and tested the effect of continuity of localities in the different time periods, along with current habitat size and isolation in one model using stepwise selection in CCA analysis. Subsequently, for each selected factor of landscape structure, we calculated the fraction of explained variation, thus expressing the pure effect of each factor after removing spatially structured variation and any other shared variation with abiotic variables (according to Borcard et al. 1992 and Økland & Eilertsen 1994).

We used CCA analysis with variation partitioning in all multivariate tests (Ter Braak & Šmilauer 2002) to get the real effects of particular factors without the effects of the other factors. Values of isolation and area were log-transformed before the analyses.

We also assessed the relative importance of the three groups of factors (current landscape structure, historical landscape structure, and local habitat conditions) for species composition; see Electronic Appendix S8 for details.

To detect which life-history traits explain species response to current and past landscape structures, we tested the effect of each individual species life-history trait on the position of the species on the first canonical axis from CCA analysis for each individual studied variable of landscape structure (significant in analysis with coordinates and selected abiotic factors as covariates) using analysis of variance for categorical and linear regression for quantitative variables in Statistica. Only species with weight greater than 5 from CCA analysis were used here, in order to remove rare species.

RESULTS

Similarity in species composition and geographical distance

The correlation between similarity in species composition and geographical distance was significant ($z = 0.07$, $p = 0.036$). This finding suggests that nearby localities are similar in terms of species composition and that geographical relationships should be taken into account when analysing the data.

The effect of studied factors on species composition

According to stepwise selection, the current isolation and the proportion of area of each locality that persisted at the same place across all studied historical periods were selected as significant after removing the effects of spatially structured and abiotic variation as covariates (Table 2).

Table 2. The effects of variables of current and historical landscape structures on species composition. The results of stepwise selection of the CCA analysis with the amount of explained variance (R^2) by the single independent variable with covariates is presented; n.s. is not significant ($p > 0.05$).

		Covariates: coordinates (11.7%) and all selected abiotic variables (13.09%)
Current	Area 2007	n.s.
	Isolation 2007	1.21%
Historical landscape structure	Proportion of area since 1938	1.26%
	Proportion of area since 1973	1.17%
	Proportion of area since 1988	1.03%
	Proportion of area since 2000	1.12%

Among the variables of the current landscape structure, the only important factor found was the isolation of localities. Isolated localities host species with ruderal strategies such as *Valerianella locusta* or *Cerastium brachypetalum* (Fig. 1). On the other hand, more connected localities host typical dry grassland and rock species, such as *Hieracium schmidtii* or *Jasione montana*.

Among factors of historical landscape structure, the most important factor was the proportion of area of each locality that persisted at the same place since 1938 or at least since 1973, 1988 or 2000. Localities with large proportions of area that persisted at the same place since 1938 (Fig. 2) are occupied by annual species with ruderal strategies, such as *Teucrium botrys* or *Geranium pusillum,* and by perennial species common in dry grassland vegetation, such as *Potentilla argentea* and *Melica transsilvanica*. Localities with large proportions of area persisting since 1973 and 1988, and partly since 2000, host many perennial species common in dry grassland vegetation that are not able to survive in forests, such as *Achillea millefolium*, *Carex caryophyllea* or *Campanula rotundifolia* or *Poa pratensis* (Fig. 2).

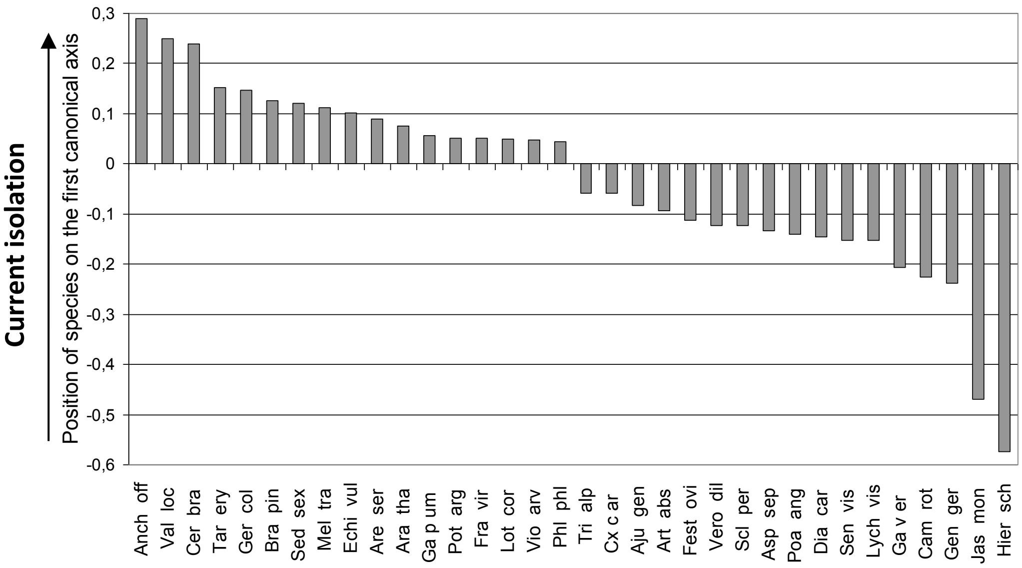

Figure 1. The effect of the current isolation of locality on species composition (CCA). The first axis explained 1.21% of variance, which is 18.06% of variance that could be explained by a single axis in an indirect analysis; p = 0.002. For definitions of the abbreviations of species names, see Electronic Appendix S2.

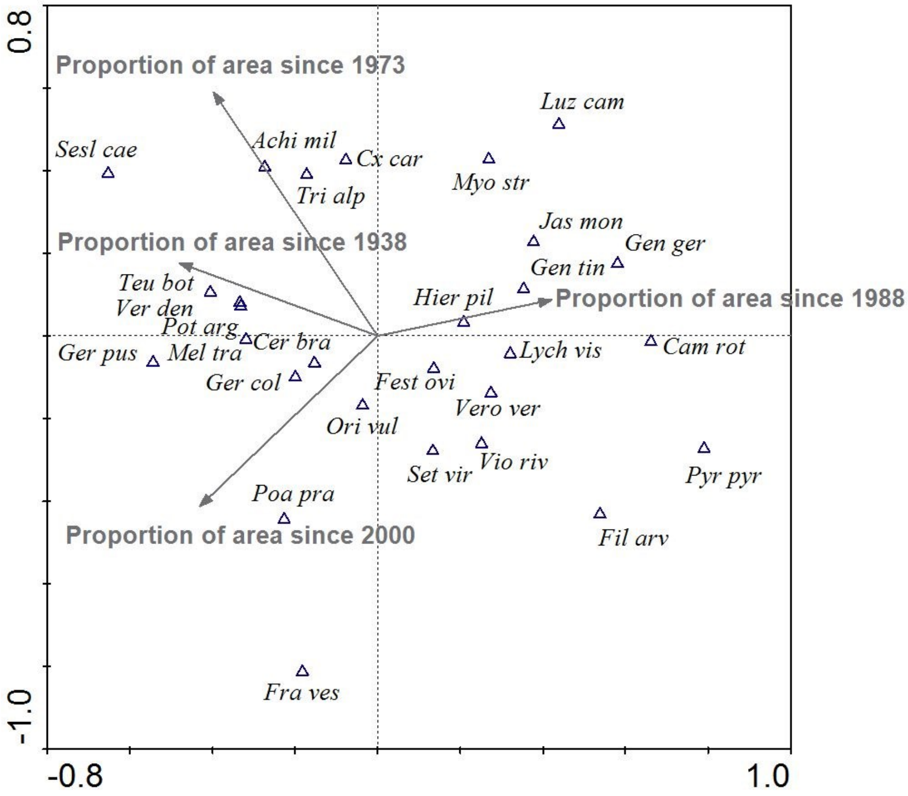

Figure 2. The effects of the proportion of area of each locality that persisted at the same place since 1938, 1973, 1988 and 2000 on species composition (CCA). These four variables together explained 4.48% of variance, which is 23.33% of variance that could be explained by the first four axes in an indirect analysis; p = 0.002. For abbreviations of species names, see Electronic Appendix S2.

The relationship between species life-history traits and species distribution

The distributions of species at localities with differences in variables of landscape structure can be explained by species life-history traits (Table 3). Specifically, currently isolated localities host species that are capable of self-pollination, prefer higher substrate pH, are of short height, and usually belong to ruderal communities. Localities with large proportions of area persisting since 1938 are occupied by not very specific species that prefer higher substrate pH and are more nutrient-demanding. On the other hand, localities with large proportions of area persisting especially since 1973 and 1988 are mostly occupied by perennial species that do not belong to ruderal communities. Moreover, species that prevail on localities with large proportions of area persisting since 1973 have rather competitive or partly stress-tolerant strategies, have seeds that are dispersed mainly endozoochorously and ombrochorly, and/or have specific structures such as awns, hooks and emergences. Localities with large proportions of area persisting since 1988 also host species that have low nutrient requirements, are relatively tall, flower for a short time, and are insect or wind pollinated. Taller species with low nutrient requirements also prevail at localities with large proportions of area persisting since 2000. For some illustrative graphs, see Electronic Appendix S9.

We performed 70 trait analyses (14 traits, 5 positions of species on the first canonical axis of variables of landscape structure from CCA analyses). The multiple replication of similar tests increases the probability that some significant values are obtained by chance (see also Dupré & Ehrlén 2002). Our results show that 19 of 70 tests were significant at least at the 5% level (compared to 4 expected by chance alone), 10 of these were significant at least at the 1% level (compared to 0.7 expected by chance alone), and 6 of these were significant at the 0.36% level (compared to 0.25 expected by chance alone). This 0.36% level represents the significance level for our results (in Table 3) using the Bonferroni correction. Because the Bonferroni correction is considered as overly conservative (Moran 2003; Garcia 2004) and many studies have not applied it for this reason (e.g., Bowman et al. 2008; McCay et al. 2009; Dittrich et al. 2014), we decided to illustrate both results, with and without this correction (see Table 3).

DISCUSSION

The results show that the current distribution of dry grassland species in the dry grassland-like forest openings has been significantly affected not only by local habitat conditions but also by landscape structures in the past and at present, and the past landscape structure in each time period separately was as important as the present landscape structure (see Electronic Appendix S8). The typical dry grassland species, i.e., long-lived species that are insect or wind pollinated, flower over limited period of time, are tall, are dispersed by animals, are not ruderal, have low nutrient requirements, and employ competitive or partly stress-tolerant strategies, are restricted to continuous localities (particularly since 1973 and 1988) and to localities that are currently well-interconnected. In contrast short, self-pollinated, ruderal species prevail in currently less-connected localities.

Table 3. Relationship between species life-history traits and habitat characteristics. Terminal velocity, seed weight, soil seed bank longevity index and clonal index were not significant in any analysis and therefore are not shown in the table. The values in the table are R^2, p-values (* p = 0.05-0.01; ** p = 0.01-0.0036; *** p < 0.0036; where the value of 0.0036 represents the significance level using the Bonferroni correction) and the direction of the effect (+/-) or the particular value is presented. n.s. is not significant (p > 0.05). The results found to be significant upon using the Bonferroni correction are depicted in bold.

	Seed dispersal traits		**Species life strategy**						**Species habitat requirements - EIV**	
Habitat characteristics	**dispersal type**	**seed attached structure**	**plant height**	**pollination vector**	**plant life span**	**flower. period**	**CSR strategy**	**ruderal**	**R**	**N**
Current isolation	n.s.	n.s.	6.34% ** –	9.21% * self	n.s.	n.s.	n.s.	3.56% * +	7.42% ** +	n.s.
Proportion of area since 1938	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**11.54%** ******* **+**	6.51% ** +
Proportion of area since 1973	24.70% * endozoo,ombro	9.96% * awns,hooks,emergences	n.s.	n.s.	**12.98%** ******* **perennials**	n.s.	**18.92%** ******* **c,csr,cs,s**	**14.30%** ******* **–**	n.s.	n.s.
Proportion of area since 1988	n.s.	n.s.	3.74% * +	**11.76%** ******* **insect, wind**	4.17% * perennials	5.12% * –	n.s.	6.87% ** –	n.s.	**14.61%** ******* **–**
Proportion of area since 2000	n.s.	n.s.	3.78% * +	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.97% * –

The effects of studied factors on species composition

The current species distribution was mostly affected by the current habitat isolation and by the proportion of area of each locality that persisted since 1938, or at least since 1973, 1988 or 2000. This could be caused by changes in the landscape structure during the last century. Specifically, there were fewer localities of dry grassland-like forest openings in the past, but they were larger and more interconnected (with the maximum occurring in 1973 and 1988) in comparison with the current landscape. Recently, we have observed a gradual reduction in the area of dry grassland-like forest openings and an increase in their isolation (Husáková & Münzbergová 2014; Electronic Appendix S10). As a consequence of the ongoing landscape fragmentation, many isolated and small localities have gradually become encroached by shrubs and more eutrophicated (Eriksson et al. 2002; Soons & Heil 2002). Our isolated localities are therefore often occupied by ruderal species (e.g., *Anchusa officinalis* or *Valerianella locusta*), which are typical especially for agricultural landscapes (Kiehl & Pfadenhauer 2007; Knappová et al. 2012). In our study system, ruderals are probably spread by local game, especially mouflons. Mouflons are quite common in the area, and visible signs of their disturbance have been found at many of the isolated localities (Petřík et al. 2011; personal observation). The isolated localities, however, also hosted several typical dry grassland species (e.g., *Melica transsilvanica*), which probably survived at the sites from the periods that featured larger areas and better connectivity of localities. However, most typical dry grassland species (such as *Hieracium schmidtii* or *Jasione montana*) were negatively affected by habitat isolation.

Similarly to Piessens et al. (2004, 2005), we also found a strong significant effect of the current habitat isolation but not of the area on species composition. However, that study did not include historical variables. The importance of historical habitat configuration has been noted in numerous previous studies (Lindborg & Eriksson 2004; Helm et al. 2006; Lindborg 2007; Chýlová & Münzbergová 2008). Many species can thus persist in the habitats due to extinction debt (Eriksson 1996; Jackson & Sax 2010) or miss there due to strong dispersal limitations (Münzbergová 2004; Ehrlén et al. 2006; Ozinga et al. 2009).

We tested three groups of variables of historical landscape structure: historical area, historical isolation, and the proportion of area of each locality that persisted at the same place since a given period. However, only the last group of variables was included in the final models. The proportion of area persisting since a given period in fact integrates two important processes: First, it determines whether the locality already existed in a particular historical period and thus informs on the continuity of locality. In addition, it determines what proportion of area of each current locality used to be the locality in the past and thus integrates information on its size in the past and its stability in space. Previous studies have shown that habitats with longer continuities have higher probabilities of being colonized due to the longer time available to species colonization (Jacquemyn et al. 2003; Hérault & Honnay 2005). In fact, they could be colonized by typical dry grassland species as well as by well-dispersed and usually short-lived species with ruderal strategies. Moreover, habitats that already encompassed large areas in the past, or at least had large proportions of area persisting at the same place, could provide better conditions for species survival and thus support larger populations, which are less influenced by demographic and environmental stochasticity and are thus less prone to extinction (Münzbergová 2006; Jackson & Sax 2010). Many typical dry grassland species (e.g., *Potentilla argentea* and *Melica transsilvanica*) could thus persist at such localities for a long time.

Importance of species life-history traits

Similarly to several previous studies (e.g., Lindborg 2007; Lindborg et al. 2012; Purschke et al. 2012), the dependence of dry grassland species distributions on present and historical habitat configurations in our study could be explained by the life-history traits of these species.

In agreement with previous studies (e.g., Groom 2001; Purves & Dushoff 2005), the isolated localities are occupied mainly by self-pollinated species that are of short height. The reduced number of pollinators and the resulting pollen limitations are the most likely explanatory factors. The short height of plant species could also be connected with their self-pollinating nature because these plants do not need to attract pollinators or expose their flowers to wind. Moreover, low stature may help them escape damage by local game, which tends to concentrate in isolated localities. We also found that currently isolated localities are significantly more often occupied by ruderal species, which usually feature good dispersal ability and fast turnover. Such species may be expected to be less affected by habitat isolation. These results are partly in accordance with Adriaens et al. (2006), who found more annual species occurring at more isolated localities due to their high seed production and fast turnover. The real isolation for some particular species could in fact be considerably higher, as not all localities may be suitable for all species (e.g., Tremlová & Münzbergová 2007), and we used only the structural isolation of localities in our tests.

Species distribution was also strongly affected by habitat continuity, represented by the proportion of area of the locality persisting at the same place since a past period. Localities persisting at the same place and having large proportions of continual area, especially since 1973 and 1988, in contrast to localities persisting since 2000, are occupied by perennial species not belonging to ruderal communities. This distribution could be explained by the fact that the habitats in our study area in 1973 and in 1988 were the largest and most connected of all the studied periods (Husáková & Münzbergová 2014). The species surviving on these localities are thus species that profited from the large area and high connectivity of the past. Similarly, Lindborg (2007) and Purschke et al. (2012) also found a significant correlation between these persistence traits and historical, but not current, habitat configuration. Contrary to our study, in other studies, long-lived species (Lindborg 2007; Purschke et al. 2012) or species with long persistence in the seed bank (Lindborg 2007) persisted in habitats that were already small and/or isolated in the past. The results of these studies show a larger time lag in species responses to habitat fragmentation compared with our study and therefore suggest a larger extinction debt (Jackson & Sax 2010). However, Purschke et al. (2012) also found that grassland age was significantly negatively correlated with plant longevity, indicating that long-lived species were mainly found in the youngest grasslands from their studied historical periods, corresponding to a 30 year timespan. These findings are partially in accordance with our results wherein long-lived species are mostly found to be growing at localities persisting at the same place for at least 30 years.

Localities with large proportion of area persisting at the same place since 1938 are also occupied by species that prefer higher substrate pH and are more nutrient-demanding, which could be explained by the fact that older localities are situated usually on places with andesite geology, characterized by higher pH than surrounding places with dacite geology, and which are also probably more nutrient rich. The localities persisting at the same place since 1973 and 1988 host competitive or partly stress-tolerant species, which have low nutrient requirements. Our findings are thus partly in contrast to (year 1938) and partly in accordance with (years 1973 and 1988) Hemrová & Münzbergová (2015), who found species with lower nutrient requirements related to old continual habitats. Similarly to Graae & Sunde (2000), we also found that the localities continuous since 1988 host species that have short flowering times and are insect or wind pollinated.

Whereas it is commonly assumed that distribution at the landscape scale is largely determined by species dispersal traits (e.g., Ehrlén & Eriksson 2000; Münzbergová 2004; Ozinga et al. 2005, 2009), most previous studies testing the effect of various species traits in fact concluded that persistence traits are better predictors of species distribution than dispersal traits (e.g., Graae & Sunde 2000; Lindborg 2007; Lindborg et al. 2012; Hemrová & Münzbergová 2015). A possible explanation for this result is that most of the studies tested the effect of only a single dispersal mode (usually anemochory, e.g., Soons et al. 2005; Diacon-Bolli et al. 2013). This explanation contrasts with the fact that most species usually disperse by multiple dispersal vectors (Ozinga et al. 2004; Adriaens et al. 2007; Vittoz & Engler 2007). We explored the importance of dispersal type (as multi-vector dispersal on a continuous scale), attachment structures, seed bank longevity, seed weight, and plant height as traits representing species dispersal ability in space and time. We found significant effects of species dispersal ability on species distribution in the landscape (similarly to Purschke et al. 2012). Most importantly, localities with large proportions of area persisting at the same place since 1973 host species with endozoochorously and ombrochorly dispersed seeds, or seeds with specific attached structures such as awns, hooks and emergences, which help them obtain dispersed epizoochorously. In addition, localities with large proportions of area persisting at the same place since 1988 and 2000 and currently well interconnected localities host taller species. Adriaens et al. (2007) has suggested that plant height may be an important trait related to epizoochorous attachment potential. Other studies, however, have suggested that plant height represents an important trait related to species ability to disperse by wind (Fakheran et al. 2010; Diacon-Bolli et al. 2013) or species competitive ability (Fakheran et al. 2010). Seed dispersal by animals is very likely in our study area, as it is known for high numbers of hoofed game, especially mouflons (Kolbek et al. 2003). These animals are known to preferably concentrate their feeding and relaxation activities at the dry grassland localities. Seed dispersal by animals is directed towards suitable habitats, and thus it is much more effective than dispersal using other vectors such as wind (Purves & Dushoff 2005; Adriaens et al. 2007).

In contrast to some other studies (e.g., Soons et al. 2005; Schleicher et al. 2011; Purschke et al. 2012, Saar et al. 2012), we did not find any relationship between species distribution and variables of seed dispersal by wind, such as terminal velocity or seed weight. This is probably because the localities are in between forests, and wind dispersal is thus less important than dispersal by animals (Vittoz & Engler 2007). In contrast to Adriaens et al. (2007), Schleicher et al. (2011) and Hemrová & Münzbergová (2015), but similarly to Purschke et al. (2012), we found only indirect relationship (via plant height) between the dispersal traits and current habitat configuration, indicating that the importance of long-distance processes has declined in the more fragmented present-day landscape and that long-term persistent species are expected to play a more important role in the future. The knowledge of past landscape structures is thus important for understanding the current species distribution, and species life-history traits can be useful predictors of species response to landscape structure and its changes over time.

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SUPPORTING INFORMATION

Appendix S1

More information about the broader setting of the study area and management.
Modified according to Husáková & Münzbergová (2014).

The study was carried out in Křivoklátsko Protected Landscape Area and Biosphere Reserve in the Czech Republic, more specifically in the Týřov Site of Community Importance (SCI). The study area is approximately 4 km^2 and is mainly forested (Fig. S1-1, 2, 3). Approximately 2% of the area is occupied by dry grassland-like forest openings (blue delimited patches in Fig. S1-1). There are also some small meadows and forest clearings in the region, but they host rather different plant species than that which could serve as source populations for (a selection of) the dry grassland species in the studied forest openings. Moreover, in the west and south-west the studied region is delimited by the Berounka River which creates a natural barrier for seed (species) dispersal.

The surrounding forests are represented by ancient dry acidophilous oak forests, which create specific mosaic with different xerophilous vegetation typical for dry grassland-like forest openings (i.e. studied localities). The occurrence of dry grassland-like forest localities in the area is given by a combination of summit and river phenomenon (Ložek 2005), by exposition, climate, geological and soil conditions and by human activities in the past.

The Křivoklátsko region fulfilled a specific function in the past (local forests were used for hunting by the nobility especially in the Middle Ages). People were thus not allowed to cut the forests, mow or keep grazing animals in the area (Kolbek et al. 2003). The main impact of human activity in the study region occurred much later (in the 18th and 19th centuries) and was not so intensive as in the other regions in our country as well as in other counties, which led to a well-preserved nature with large forest coverage. Also the distribution of dry grassland-like forest openings was thus influenced by human activities, but also not so intensively. It is for example documented, that some of dry grassland localities were occasionally used as a source of pasturage for cattle (stock) in the past (Kolbek et al. 2003). Because of their specific and badly accessible position, dry grassland-like forest openings have never been managed or influenced by human activities very much but only occasionally and extensively. Actually, none of our localities are managed at all (no removal of young trees and shrubs colonizing grassland areas, no mowing, grazing cattle, etc.) as they are occurring in the most valuables zone of Křivoklátsko Protected Landscape Area and Biosphere Reserve and should be thus without the human influences.

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Figure S1-1: Study area with all studied localities (blue delimited) in the Týřov Site of Community Importance (SCI) on current orthophoto map available from Geoportal Cenia (http://geoportal.cenia.cz).

Figure S1-2: Part of study area with Vápený hill and Týřovické rocks. Author I. Husáková.

Figure S1-3: Part of study area with hills around the Úpořský stream valley. Author I. Husáková.

Appendix S2

All species of vascular plants recorded on study localities. Abbreviations of plant species names, Included – species is included into analyses (1) or not (0), Frequency – number of localities with species presence.

	Abbreviations	Included	Frequency
Acer campestre	Ace cam	0	8
Acinos arvensis	Aci arv	1	13
Agrostis capillaris	Agr cap	1	18
Achillea millefolium (s.str.)	Achi mil	1	34
Ajuga genevensis	Aju gen	1	86
Alliaria petiolata	Alli off	0	40
Allium oleraceum	Alli ole	1	37
Allium senescens subsp. montanum	Alli mon	1	24
Alyssum alyssoides	Aly aly	1	2
Alyssum montanum subsp. montanum	Aly mon	1	8
Anemone ranunculoides	Ane ran	0	4
Anchusa officinalis	Anch off	1	32
Antennaria dioica	Ante dio	1	1
Anthemis arvensis	Anth arv	1	1
Anthemis tinctoria	Anth tin	1	4
Anthericum liliago	Ant lil	1	2
Anthetricum ramosum	Ant ram	0	47
Anthoxanthum odoratum	Ant odo	1	7
Anthriscus sylvestris	Ant syl	0	7
Arabidopsis thaliana	Ara tha	1	41
Arabis glabra	Ara gla	1	19
Arabis hirsuta	Ara hir	1	17
Arenaria serpyllifolia agg.	Are ser	1	41
Artemisia absinthium	Art abs	1	37
Artemisia campesris	Asa eur	1	1
Asarum europaeum	Asp cyn	0	1
Asperula cynanchica	Asp rumu	1	30
Asplenium ruta-muraria	Asp sep	1	7
Asplenium septentrionale	Asp tri	1	52
Asplenium trichomanes agg.	Ast gly	1	46
Astragalus glycyphyllos	Ath fil	1	27
Athyrium filix-femina	Atr bel	0	4
Atropa bella-donna	Aur sax	1	1
Aurinia saxatilis	Ave fle	1	7
Avenella flexuosa	Bal nig	0	28
Ballota nigra	Bar vul	0	15
Barbarea vulgaris	Bet off	1	17
Betonica officinalis	Bra pin	0	18
Brachypodium pinnatum	Bra syl	1	70
Brachypodium sylvaticum	Bro ste	0	65
Bromus sterilis	Bup fal	1	14
Bupleurum falcatum	Cal aru	0	15
Calamagrostis arundinacea	Cal epi	0	42
Calamagrostis epigejos	Cal vul	0	15
Calluna vulgaris	Cam per	1	5
Campanula persicifolia	Cam rap	0	31
Campanula rapunculoides	Cam rot	1	2

Campanula rotundifolia	Cam tra	1	26
Campanula trachelium	Cap bupa	0	53
Capsella bursa-pastoris	Car imp	1	6
Cardamine impatiens	Car are	0	6
Cardaminopsis arenosa	Car dra	0	95
Cardaria draba	Car aca	1	8
Carduus acanthoides	Car nut	1	25
Carduus nutans	Cx car	1	12
Carex caryophyllea	Cx dig	1	41
Carex digitata	Cx mur	0	3
Carex muricata agg.	Cx ova	1	87
Carex ovalis	Cx pil	1	4
Carex pilulifera	Cx pra	1	8
Carex praecox	Cen sca	1	10
Centaurea scabiosa	Cen sto	1	7
Centaurea stoebe	Cer arv	1	7
Cerastium arvense	Cer bra	1	32
Cerastium brachypetalum	Cer hol	1	30
Cerastium holosteoides	Cer min	1	15
Cerinthe minor	Cir lut	1	2
Circaea lutetiana	Cli vul	0	1
Clinopodium vulgare	Con arv	1	50
Convolvulus arvensis	Cor cav	1	14
Corydalis cava	Cot int	0	2
Cotoneaster integerrimus	Cra lae	1	24
Crateagus sp.	Cru lae	0	63
Cruciata laevipes	Cus eur	1	3
Cuscuta europaea	Cyn off	0	1
Cynoglossum officinale	Cyt nig	1	20
Cytisus nigricans	Dac glo	1	7
Dactylis glomerata	Dac pol	1	28
Dactylis polygama	Dan dec	0	11
Danthonia decumbens	Dia car	1	2
Dianthus carthusianorum subsp. carthusianorum	Dic alb	1	25
Dictamnus albus	Dig gra	1	1
Digitalis grandiflora	Dry dil	1	22
Dryopteris filix-mas	Dry fil	0	11
Echium vulgare	Echi vul	1	46
Elymus caninus	Ely can	0	1
Epilobium collinum	Epi col	1	1
Epilobium montanum	Epi mon	0	13
Erodium cicutarium	Ero cic	1	6
Erophila verna	Ero ver	1	20
Euphorbia cyparissias	Eup cyp	1	87
Euphrasia stricta	Eup str	1	1
Fallopia convolvulus	Fal con	0	77
Festuca heterophylla	Fest het	0	6
Festuca ovina agg.	Fest ovi	1	90
Festuca pallens	Fest pal	1	16
Festuca rubra agg.	Fest rub	1	10
Festuca rupicola	Fest rup	1	23
Festuca valesiaca	Fest val	1	3
Ficaria verna	Fic ver	0	2

Filago arvensis	Fil arv	1	9
Fragaria moschata	Fra mos	0	18
Fragaria vesca	Fra ves	1	13
Fragaria viridis	Fra vir	1	86
Fumaria schleicheri	Fum schl	1	3
Gagea bohemica subsp. bohemica	Gag boh	1	1
Gagea villosa	Gag vil	1	6
Galeobdolon luteum	Gal lut	0	3
Galeopsis angustifolia	Gal ang	1	83
Galeopsis ladanum	Gal lad	0	43
Galium album agg.	Ga alb	1	52
Galium aparine	Ga apa	0	43
Galium glaucum	Ga glau	1	17
Galium odoratum	Ga odo	0	8
Galium pumilum	Ga pum	1	37
Galium verum	Ga ver	1	26
Genista germanica	Gen ger	1	25
Genista tinctoria	Gen tin	1	25
Geranium columbinum	Ger col	1	38
Geranium pusillum	Ger pus	1	16
Geranium pyrenaicum	Ger pyr	1	6
Geranium robertianum	Ger rob	0	31
Geranium sanguineum	Ger san	1	4
Geum urbanum	Geu urb	0	73
Glechoma hederacea	Gle hed	0	8
Gnaphalium sylvaticum	Gna syl	1	6
Hedera helix	Hed hel	0	2
Helianthemum grandiflorum subsp. obscurum	Hel gra	1	3
Hepatica nobilis	Hep nob	0	9
Hieracium cymosum	Hier cym	1	19
Hieracium laevigatum	Hier lae	0	4
Hieracium lachenalii	Hier lach	0	3
Hieracium murorum	Hier mur	0	40
Hieracium pilosella	Hier pil	1	76
Hieracium sabaudum	Hier sab	0	5
Hieracium schmidtii	Hier schm	1	25
Holosteum umbellatum	Hol umb	1	4
Hylotelephium maximum	Hyl max	1	21
Hypericum montanum	Hyp mon	0	7
Hypericum perforatum	Hyp per	1	98
Chaerophyllum temulum	Chae tem	0	2
Chelidonium majus	Chel maj	0	1
Chenopodium album (s.str.)	Chen alb	1	3
Chenopodium polyspermum	Chen pol	1	2
Impatiens noli-tangere	Imp nota	0	5
Impatiens parviflora	Imp par	0	52
Inula conyzae	Inu con	1	26
Jasione montana	Jas mon	1	25
Jovibarba globifera subsp. globifera	Jov glo	1	5
Juncus conglomeratus	Jun con	0	1
Juniperus communis subsp. communis	Juni com	1	2
Koeleria macrantha	Koe mac	1	7
Lactuca serriola	Lac ser	1	1

Lactuca viminea	Lac vim	1	1
Lamium album	Lam alb	0	1
Lamium amplexicaule	Lam amp	1	8
Lamium purpureum	Lam pur	0	17
Lappula squarrosa	Lapu squ	1	2
Lapsana communis	Lap com	0	45
Lathyrus niger	Lat nig	0	5
Lathyrus pratensis	Lat pra	1	1
Lathyrus vernus	Lat ver	0	3
Leucanthemum vulgare	Leu vul	1	2
Lilium martagon	Lil mar	0	3
Linaria vulgaris	Lin vul	1	25
Lithospermum purpurocaeruleum	Lit pur	1	5
Lotus corniculatus	Lot cor	1	26
Luzula campestris	Luz cam	1	16
Luzula luzuloides	Luz luz	0	45
Luzula multiflora	Luz mul	1	13
Lychnis viscaria	Lych vis	1	58
Lysimachia nummularia	Lys num	0	2
Malus sylvestris	Mal syl	1	1
Matricaria discoidea	Mat dis	0	1
Medicago falcata	Med fal	1	3
Medicago minima	Med min	1	1
Melica nutans	Mel nut	0	12
Melica picta	Mel pic	0	1
Melica transsilvanica	Mel tra	1	44
Melilotus officinalis	Meli off	0	1
Melittis melissophyllum	Mel mel	1	3
Microrrhinum minus	Mic min	1	2
Moehringia trinervia	Moe tri	0	18
Mycelis muralis	Myc mur	0	14
Myosotis arvensis	Myo arv	1	25
Myosotis ramosissima	Myo rha	1	23
Myosotis sparsiflora	Myo spa	1	13
Myosotis stricta	Myo str	1	21
Myosotis sylvatica	Myo syl	0	23
Omphalodes scorpioides	Omp sco	0	3
Onopordum acanthium	Ono aca	1	3
Origanum vulgare	Ori vul	1	64
Orobanche alba agg.	Oro alb	1	2
Oxalis fontana	Oxa str	0	1
Papaver dubium agg.	Pap dub	1	5
Persicaria hydropiper	Poly hyd	0	3
Persicaria lapathifolia	Per lap	0	2
Petrorhagia prolifera	Pet pro	1	2
Phleum phleoides	Phl phl	1	40
Phyteuma spicatum	Phy spi	0	1
Pimpinella saxifraga	Pim sax	1	5
Plantago lanceolata	Pla lan	1	3
Plantago media	Pla med	1	1
Poa angustifolia	Poa ang	1	57
Poa annua	Poa ann	0	4
Poa compressa	Poa com	1	16

Poa nemoralis	Poa nem	0	87
Poa pratensis	Poa pra	1	13
Polygala vulgaris	Pol vul	1	1
Polygonatum odoratum	Pol odo	0	44
Polygonum aviculare	Poly avi	0	29
Polypodium vulgare	Pol vul	0	11
Potentilla arenaria	Pot are	1	15
Potentilla argentea	Pot arg	1	54
Potentilla inclinata	Pot inc	1	2
Potentilla recta	Pot rec	1	14
Potentilla tabernaemontani	Pot tab	1	60
Primula veris	Pri ver	1	4
Prunus spinosa	Pru spi	1	74
Pseudolysimachion spicatum	Pse spi	1	6
Pulsatilla pratensis subsp. bohemica	Pul pra	1	4
Pyrethrum corymbosum	Pyr cor	0	79
Pyrus pyraster	Pyr pyr	1	10
Ranunculus auricomus agg.	Ran aur	0	4
Ranunculus bulbosus	Ran bul	1	26
Ribes alpinum	Rib alp	0	3
Rosa sp.	Rosa	0	95
Rubus sp.	Rubus	0	27
Rumex acetosa	Rum ace	1	5
Rumex acetosella	Rum acla	1	57
Salvia nemorosa	Sal nem	1	2
Sambucus nigra	Sam nig	0	50
Sanguisorba minor	San min	1	16
Scleranthus perennis	Scl per	1	29
Securigera varia	Sec var	1	67
Sedum acre	Sed acr	1	8
Sedum album	Sed alb	1	36
Sedum reflexum	Sed ref	1	24
Sedum sexangulare	Sed sex	1	56
Senecio viscosus	Sen vis	1	38
Seseli osseum	Ses oss	1	10
Sesleria caerulea	Sesl cae	1	8
Setaria viridis	Set vir	1	15
Silene nutans	Sil nut	0	81
Silene vulgaris	Sil vul	1	10
Solidago virgaurea subsp. virgaurea	Sol vir	1	8
Sonchus arvensis	Son arv	1	5
Sorbus aria	Sor ari	1	12
Sorbus aucuparia	Sor auc	0	4
Stellaria holostea	Ste hol	0	56
Stellaria media	Ste med	0	5
Stipa pennata	Sti pen	1	7
Symphytum officinale	Sym off	0	1
Taraxacum sect. Erythrosperma	Tar ery	1	39
Taraxacum sect. Ruderalia	Tar rud	0	40
Teucrium botrys	Teu bot	1	16
Teucrium chamaedrys	Teu cham	1	4
Thlaspi arvense	Thl arv	0	2
Thlaspi perfoliatum	Thl per	1	5

Thymus pulegioides subsp. chamaedrys	Thy pul	1	88
Torilis japonica	Tor jap	0	29
Trifolium alpestre	Tri alp	1	45
Trifolium arvense	Tri arv	1	21
Trifolium aureum	Tri aur	1	4
Trifolium campestre	Tri cam	1	11
Trifolium dubium	Tri dub	1	3
Trifolium medium	Tri med	1	14
Trifolium repens	Tri rep	0	2
Triticum aestivum	Trit est	1	1
Urtica dioica	Urt dio	0	52
Vaccinium myrtillus	Vac myr	0	4
Valerianella locusta	Val loc	1	31
Verbascum densiflorum	Ver den	1	22
Verbascum lychnitis	Ver lych	1	57
Veronica arvensis	Vero arv	1	10
Veronica dillenii	Vero dil	1	49
Veronica hederifolia	Vero hed	0	36
Veronica chamaedrys	Vero cha	0	74
Veronica officinalis	Vero off	0	59
Veronica prostrata	Vero pro	1	10
Veronica sublobata	Vero sub	0	2
Veronica verna	Vero ver	1	18
Vicia angustifolia	Vic ang	1	22
Vicia hirsuta	Vic hir	1	35
Vicia pisiformis	Vic pis	0	1
Vicia sepium	Vic sep	0	4
Vicia tenuifolia	Vic ten	1	2
Vicia tetrasperma	Vic tet	1	30
Vincetoxicum hirundinaria	Vinc hir	0	95
Viola arvensis	Vio arv	1	76
Viola collina	Vio col	1	1
Viola hirta	Vio hir	1	8
Viola odorata	Vio odo	1	1
Viola reichenbachiana	Vio rei	0	24
Viola riviniana	Vio riv	1	33
Viola tricolor subsp. tricolor	Vio tri	0	30

Appendix S3

Calculation of habitat isolation and local habitat conditions.

Actual habitat isolation was calculated using a formula provided by Tremlová & Münzbergová (2007) and also used in Husáková & Münzbergová (2014):

$$I_i = -\sum_{j=1}^{n} \frac{S_j}{d_{ij}^{\;2}};$$

where I_i represents the isolation of the i^{th}-locality, j represents all other localities in the circuit with radius of 500 m around the i^{th}-locality, n is the total number of localities in the circuit with radius of 500 m, S_j is the area of j^{th}-locality (in m^2), and d_{ij} is the distance between locality i and all other localities j (in meters, measured as the distance between locality centres because the localities do not exhibit elongated shapes). We considered the circuit with a radius of 500 m because this distance explained the greatest variation in species composition from various tested values (100 m, 200 m, 300 m, 500 m and 1000 m). Additionally, Löbel et al. (2006) identified a circuit with a radius of 500 m as having the best fit for calculating isolation in dry grasslands. We calculated only structural isolation of localities (isolation of all dry grassland localities regardless of the suitability of each locality for each particular species, i.e., its values are the same for all species) and not functional isolation (calculated specifically for each species considering the suitability each locality for each species, i.e., its values would be specific for each species, as, e.g., in Tremlová & Münzbergová 2007) for each species separately because of difficulties with the determination of habitat suitability for historical times. We are, however, aware of the fact that the structural isolation index can be an underestimate of the real isolation that a particular species experiences or experienced in historical times. This fact should be considered when interpreting the results.

Local habitat conditions were captured by several variables. We determined the proportions of four substrate category types at each locality (Substrate) according to their soil depth (rock, shallow soil, deeper soil and scree). We also calculated the Shannon diversity index values of these proportions of substrate category types to describe habitat heterogeneity. To describe topographic heterogeneity of each locality, we calculated the median, minimum and maximum values of slope (using GIS), the potential direct solar insolation (PDSI) on the 21st day of every month from December to June (as per Herben 1987) and the diversity of these variables (standard deviation (STD)), and the topographic wetness index (TWI) (using SAGA GIS (http://www.saga-gis.org)), all derived from the digital terrain model. According to the geological map of the area (Czech Geological Institute 1997; 1:50,000), we also classified the geological conditions of each locality into two categories: andesites and dacites. Local habitat conditions were calculated for use as covariates in subsequent analyses.

References:

Czech Geological Institute 1997. Protected Landscape Area and Biosphere Reserve Křivoklátsko: geological and nature conservation map. 1:50 000.

Herben T. 1987. *Dynamika invaze Orthodontium lineare* Swaegr. [Invasion dynamics of *Orthodontium lineare* Swaegr]. Průhonice.

Löbel S., Dengler J. & Hobohm C. 2006. Species richness of vascular plants, bryophytes and lichens in dry grasslands: The effects of environment, landscape structure and competition. *Folia Geobotanica* 41: 377-393.

Appendix S4

Pair-wise correlation matrix between individual variables of the local habitat conditions and the past and current landscape structure. Type of substrate (% of rock, shallow soil, deeper soil, scree), Shannon diversity index of substrate types (S. diverz. index), slope (median, maximum, standard deviation values (STD)), insolation (PDSI - median value in December and in June, standard deviation values (STD) in December and in June), topographic wetness index (TWI), geology (Geo - andesites, dacites), Area (area), isolation (isol) and continuity (cont, i.e., proportion of area since ... (year)) in each studied period is presented. Significant correlations are in bold. According to Husáková & Münzbergová (2014).

		Substrate					Slope			PDSI					Geo														
		rock	shallow	scree	deep	S.diverz index	median	max	slope-STD	Dec	June	Dec-STD	Jun-STD	TWI	andesit	area07	isol07	area38	area73	area88	area00	isol38	isol73	isol88	isol00	cont38	cont73	cont88	cont00
Substrate	rock	1.00	**-0.32**	**-0.33**	**-0.36**	**0.23**	**0.49**	**0.56**	0.09	**0.36**	**0.41**	**0.37**	**0.40**	-0.12	**-0.26**	**0.32**	0.13	-0.13	-0.12	-0.08	0.10	**0.19**	0.13	-0.09	0.16	-0.15	-0.03	0.14	0.11
	shallow_soil		1.00	**-0.45**	**-0.29**	0.15	**-0.20**	-0.18	0.05	-0.05	0.07	-0.04	0.05	-0.07	-0.08	-0.04	0.08	0.14	-0.05	0.05	-0.13	-0.10	0.12	0.13	0.06	0.01	-0.01	0.12	**-0.24**
	scree			1.00	**-0.25**	**-0.20**	0.18	0.11	-0.19	-0.14	**-0.23**	-0.15	**-0.21**	**0.20**	**0.20**	-0.05	-0.17	-0.16	**0.23**	-0.02	0.05	0.00	**-0.26**	-0.09	-0.16	-0.17	-0.02	-0.04	0.04
	deep_soil				1.00	**-0.19**	**-0.51**	**-0.53**	0.05	-0.18	**-0.27**	-0.19	**-0.27**	-0.02	0.16	**-0.24**	-0.05	0.15	-0.07	0.04	0.00	-0.10	0.00	0.04	-0.07	**0.34**	0.07	**-0.24**	0.11
	S.diverz. index					1.00	0.10	**0.23**	**0.23**	**0.29**	**0.44**	**0.30**	**0.46**	-0.08	-0.04	**0.53**	0.09	**0.21**	**0.24**	**0.19**	**0.40**	-0.12	-0.06	-0.11	0.10	0.09	**0.25**	**0.29**	**0.19**
Slope	median						1.00	**0.89**	**-0.22**	**0.39**	**0.32**	**0.35**	**0.36**	-0.10	-0.08	**0.27**	**-0.20**	-0.06	**0.25**	-0.01	0.12	0.06	-0.18	**-0.36**	-0.17	-0.08	0.14	0.13	0.01
	max							1.00	**0.20**	**0.47**	**0.43**	**0.40**	**0.48**	**-0.25**	-0.12	**0.50**	-0.15	-0.10	**0.20**	-0.02	**0.26**	0.11	-0.11	**-0.36**	-0.09	-0.15	0.11	**0.19**	0.10
	slope-STD								1.00	**0.21**	**0.28**	0.12	**0.30**	**-0.32**	-0.11	**0.49**	0.11	0.02	-0.03	0.03	**0.35**	0.06	0.12	0.00	0.14	-0.04	-0.01	0.13	**0.19**
PDSI	Dec									1.00	**0.57**	**0.95**	**0.63**	-0.13	**-0.22**	**0.58**	-0.06	**0.32**	**0.37**	0.15	**0.44**	**-0.28**	-0.12	**-0.22**	-0.04	**0.20**	**0.41**	**0.33**	**0.29**
	June										1.00	**0.59**	**0.98**	-0.11	-0.03	**0.57**	-0.02	**0.34**	**0.41**	**0.26**	**0.42**	**-0.23**	-0.03	**-0.25**	0.01	0.18	**0.41**	**0.43**	**0.27**
	Dec-STD											1.00	**0.64**	-0.14	-0.14	**0.58**	-0.09	**0.32**	**0.38**	**0.24**	**0.43**	**-0.31**	-0.13	**-0.23**	-0.06	0.18	**0.41**	**0.37**	**0.28**
	Jun-STD												1.00	-0.12	-0.05	**0.62**	-0.04	**0.35**	**0.47**	**0.24**	**0.47**	**-0.25**	-0.06	**-0.27**	-0.01	**0.21**	**0.47**	**0.44**	**0.28**
	TWI													1.00	-0.02	**-0.21**	0.06	0.18	0.17	-0.04	-0.17	**-0.21**	-0.13	0.16	0.00	0.17	0.14	-0.10	-0.09
Geo	andesites														1.00	-0.17	-0.02	0.16	0.16	**0.34**	-0.02	**-0.28**	**-0.27**	-0.16	-0.05	**0.20**	**0.20**	**0.23**	0.02
	area07															1.00	-0.03	0.18	**0.34**	**0.29**	**0.68**	-0.13	-0.05	**-0.23**	0.00	0.06	**0.30**	**0.37**	**0.45**
	isol07																1.00	0.01	**-0.22**	**-0.28**	-0.18	**0.28**	**0.57**	**0.81**	**0.96**	0.12	-0.01	-0.10	0.12
	area38																	1.00	**0.56**	**0.23**	**0.29**	**-0.69**	**-0.22**	-0.02	-0.06	**0.68**	**0.60**	0.16	**0.27**
	area73																		1.00	**0.32**	**0.43**	**-0.54**	**-0.44**	**-0.26**	**-0.25**	**0.41**	**0.70**	**0.36**	**0.36**
	area88																			1.00	**0.30**	**-0.31**	**-0.27**	**-0.41**	**-0.28**	0.16	**0.20**	**0.44**	0.14
	area00																				1.00	**-0.22**	-0.13	**-0.21**	**-0.19**	**0.19**	**0.35**	**0.35**	**0.67**
	isol38																					1.00	**0.44**	**0.24**	**0.34**	**-0.39**	**-0.51**	**-0.29**	-0.11
	isol73																						1.00	**0.54**	**0.60**	-0.04	-0.13	-0.18	0.00
	isol88																							1.00	**0.76**	0.10	-0.11	**-0.25**	0.06
	isol00																								1.00	0.04	-0.07	-0.10	0.10
	cont38																									1.00	**0.64**	0.09	**0.32**
	cont73																										1.00	**0.33**	**0.39**
	cont88																											1.00	**0.30**
	cont00																												1.00

Appendix S5

Preliminary analysis of the effect of each factor on species composition separately.

The effect of parameters of current and historical landscape structure and local habitat conditions on species composition when tested separately. Local habitat conditions include substrate (% of rock, shallow and deep soil and scree), Shannon diversity index of substrate types, slope, PDSI and geology (i.e. significant factors when tested separately). In the first step (column) of analyses geographical coordinates were used as covariates, in second step (column) of analyses geographical coordinates and all significant factors from first step of analyses were used as covariates. The amount of explained variance by the single independent variable with different covariates is presented (see methods); n.s. is not significant (p > 0.05), – not tested because previously not significant.

		Covariates	
		Coordinates (11.70%)	All signif. factors (37.74%)
Current	Area 2007	2.02%	n.s.
	Isolation 2007	1.52%	1.08%
Historical landscape structure	Area 1938	1.79%	n.s.
	Area 1973	1.66%	n.s.
	Area 1988	1.21%	n.s.
	Area 2000	1.66%	n.s.
	Isolation 1938	1.34%	n.s.
	Isolation 1973	n.s.	–
	Isolation 1988	n.s.	–
	Isolation 2000	1.34%	n.s.
	Proportion of area since 1938	1.84%	1.03%
	Proportion of area since 1973	2.02%	0.99%
	Proportion of area since 1988	1.75%	0.99%
	Proportion of area since 2000	1.61%	n.s.
Local habitat conditions		17.93%	14.16%

Appendix S6

Means, medians, minima and maxima of the independent variables per locality.

Area = log area (m^2), Isolation = log isolation, Proportion of area since ... = proportion of area of each locality that persisted at the same place since ..., Substrate = proportion of rock, shallow soil, scree and deep soil at each locality, Shannon diversity index of substrate types, Slope – median, maximum and STD (standard deviation) values (in degrees), PDSI = insolation – median and STD (standard deviation) values in December and June, Geology – andesites or dacites (1/0), TWI = Topographic wetness index.

	Variable	Mean	Median	Min	Max
Current landscape structure	Area 2007	2.5	2.46	1.42	4.1
	Isolation 2007	0.15	0.14	-0.47	1.28
Historical landscape structure	Area 1938	1.43	1.8	0	3.16
	Area 1973	2.29	2.71	0	3.46
	Area 1988	2.08	2.5	-1.2	3.9
	Area 2000	2.56	2.63	0	3.47
	Isolation 1938	0.33	0.28	-1.1	5
	Isolation 1973	0.06	-0.03	-0.69	5
	Isolation 1988	0.07	0.06	-0.69	5
	Isolation 2000	0.15	0.15	-0.49	1.29
	Proportion of area since 1938	0.17	0	0	0.99
	Proportion of area since 1973	0.37	0.41	0	1
	Proportion of area since 1988	0.36	0.41	0	1
	Proportion of area since 2000	0.71	0.78	0	1
Local habitat conditions	Substrate - rock	0.34	0.25	0	1
	Substrate - shallow soil	0.3	0.1	0	1
	Substrate - scree	0.22	0	0	1
	Substrate - deeper soil	0.14	0	0	1
	Shannon diversity index	0.47	0.5	0	1.28
	Slope - median	27.32	28	8	41
	Slope - max	30.14	30	13	48
	Slope - STD	1.82	1.24	0	9.52
	PDSI - median - December	0.92	0.54	0	4.89
	PDSI - median - June	5.87	6.22	0	8.48
	PDSI - STD - December	0.33	0.2	0	1.22
	PDSI - STD - June	5.47	5.57	0	8.14
	Geology	0.83	1	0	1
	TWI	4.76	4.52	3.27	10.48

Appendix S7

Delimitation of ruderal species.

For each species, we decided if the species is a typical species of dry grassland habitats formed in the forest openings. The species not belonging to dry grasslands (i.e. not belonging to communities: *Asplenietea trichomanis, Thlaspietea rotundifolii, Nardo-Callunetea, Sedo-Scleranthetea, Festuco-Brometea, Trifolio-Geranietea, Quercion pubescenti-petraeae, Quercetea robori-petraeae, Dicrano-Pinion*, note that some of these communities are communities of open forests, which host many dry grassland species) were considered as "ruderal species" (i.e. species belonging to these communities: *Epilobietea angustifolii, Chenopodietea, Artemisietea vulgaris, Secalietea*; forest species also growing at localities have been already excluded, see above). This classification was done using studies on vegetation of the Czech Republic and flora of the Czech Republic (Chytrý and Tichý 2003; respectively according to Moravec et al. 1995 or to Hejný et Slavík 1988, 1990, 1992; Slavík 1995, 1997, 2000, 2004 and Chytrý et al. 2001).

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Slavík B. (ed.) 1995. *Květena České republiky 4* [Flora of the Czech Republic 4]. Academia. Praha.

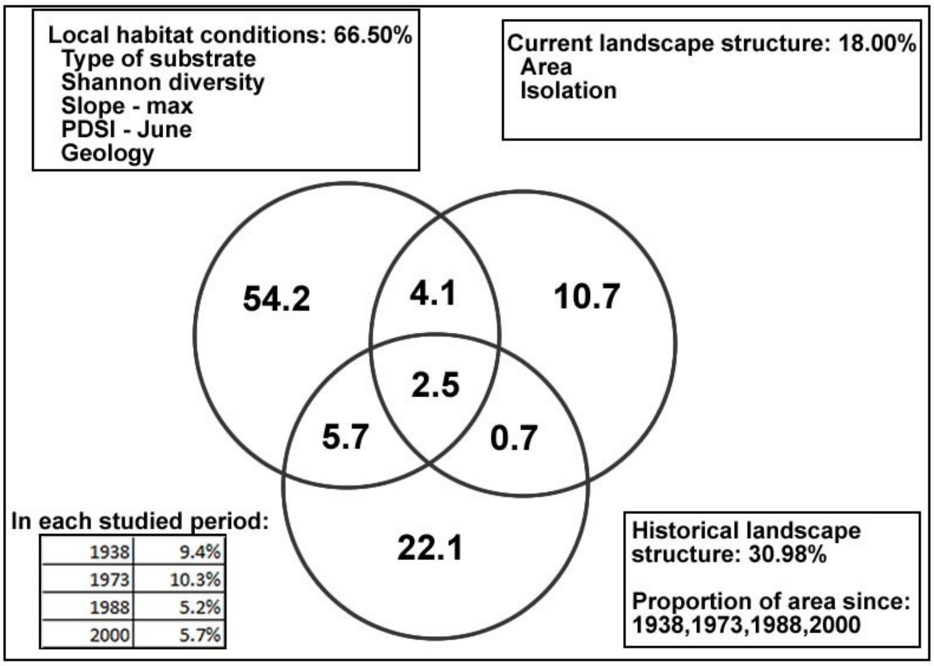
Slavík B. (ed.) 1997. *Květena České republiky 5* [Flora of the Czech Republic 5]. Academia. Praha.

Slavík B. (ed.) 2000. *Květena České republiky 6* [Flora of the Czech Republic 6]. Academia. Praha.

Slavík B. (ed.) 2004. *Květena České republiky 7* [Flora of the Czech Republic 7]. Academia. Praha.

Appendix S8

Relative importance of local habitat conditions, current landscape structure and historical landscape structure (also divided into each studied period). For each group of factors, the significant predictors are ranked from the predictor with the highest explanatory power.



Description:
To assess the relative importance of the three groups of factors – current landscape structure, historical landscape structure and local habitat conditions for species composition, we analyzed the effect of each of these three groups of factors alone. We also tested the effect of each of these groups of factors after using the other two groups of factors as covariates. Because of too many independent variables in each group we chose only those factors which were selected in stepwise selection in CCA analysis as significant when testing their independent effect on species composition using coordinates as covariates. We used the method of variation partitioning (according to Økland & Eilertsen 1994) to calculate the proportion of variance explained by each group of factors. We expressed the portion of explained variance as a relative part of totally explained variance.

Altogether, the three groups of factors – current and past landscape structure and local habitat conditions – explained 19.68% of variation in species composition. The highest proportion of the variation was explained by local habitat conditions – 66.5% of the variation that could be explained by all the factors together. Second highest important group was historical landscape structure explaining 31% of this variation and the lowest proportion was explained by current landscape structure, 18% of variation. From the historical variables, the most important were those from 1973 and also from 1938. The variance explained by each of the groups overlapped relatively little, with less than 10% of the variation being attributable to at least two groups of the factors.

References:

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Appendix S9

Some illustrative graphs for the relationship between species life-history traits and species distribution related to variables of studied localities. Depicted are those results that were significant after using the Bonferroni correction ($p < 0.0036$).

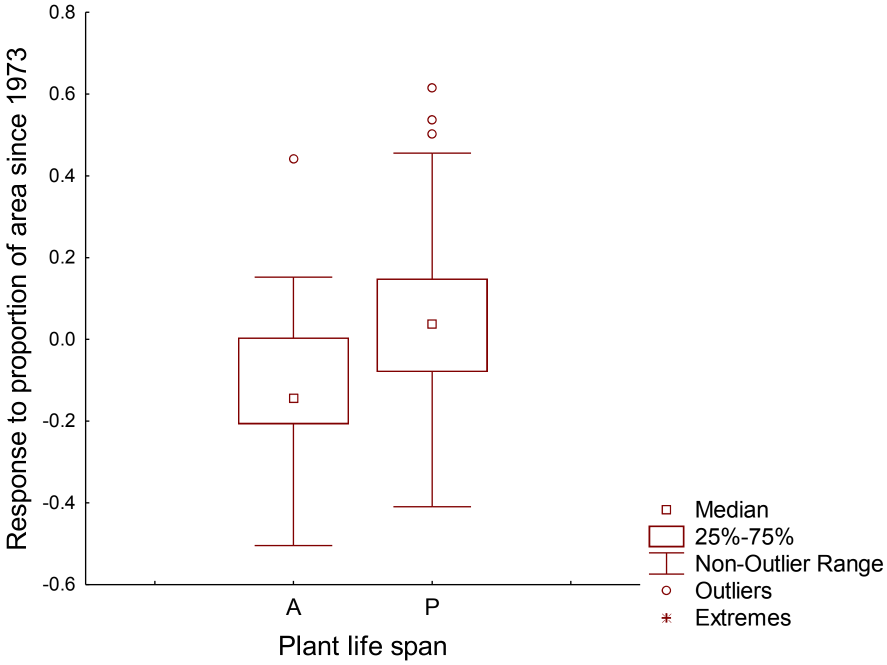

Figure S9-1: The effect of proportion of area of each locality that persisted at the same place since 1973 on the occurrence of species with specific plant life span: A – annuals, P – perennials. Response of species to proportion of area since 1973 is represented by the position of species on the first canonical axis for proportion of area since 1973. $p < 0.0001$, $R^2 = 12.98\%$ (ANOVA).

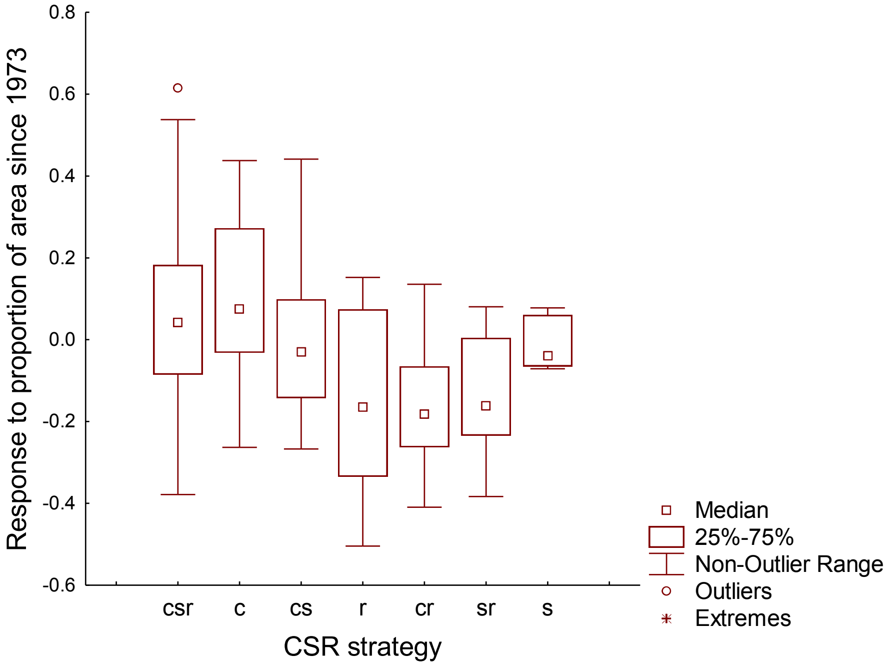

Figure S9-2: The effect of proportion of area of each locality that persisted at the same place since 1973 on the occurrence of species with specific CSR strategy (according to Grime 1979). Response of species to proportion of area since 1973 is represented by the position of species on the first canonical axis for proportion of area since 1973. $p < 0.0001$, $R^2 = 18.92\%$ (ANOVA).

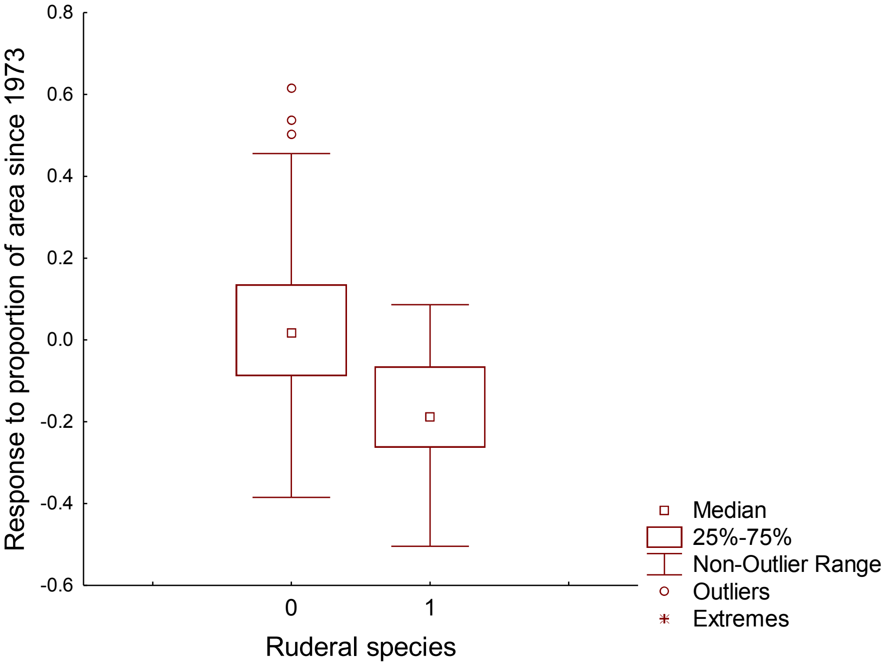

Figure S9-3: The effect of proportion of area of each locality that persisted at the same place since 1973 on the occurrence of ruderal species: 1 – ruderal species, 0 – nonruderal species. Response of species to proportion of area since 1973 is represented by the position of species on the first canonical axis for proportion of area since 1973. $p < 0.0001$, $R^2 = 14.30\%$ (ANOVA).

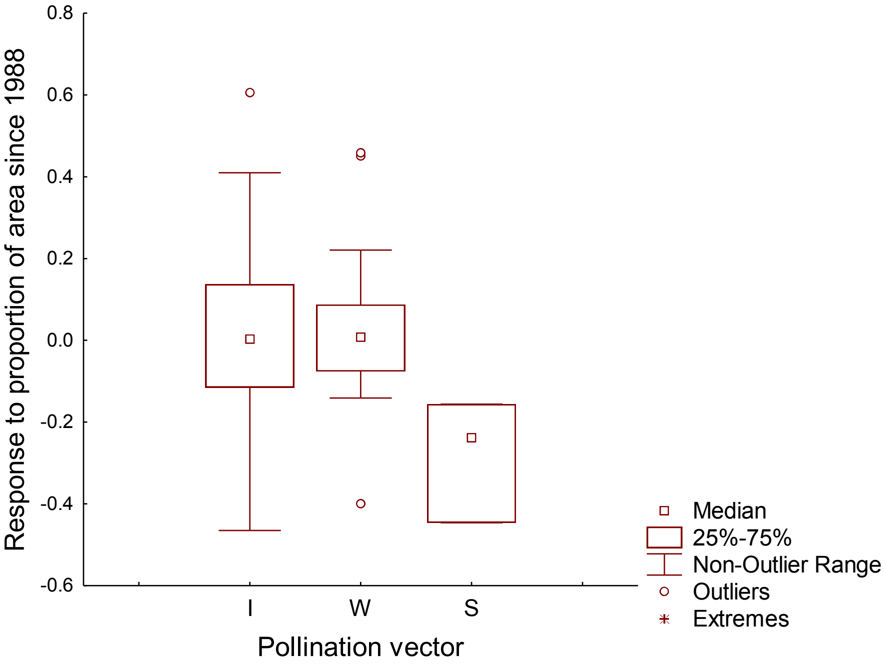

Figure S9-4: The effect of proportion of area of each locality that persisted at the same place since 1988 on the occurrence of species with specific pollination vector: I – insect, W – wind, S – self-pollination. Response of species to proportion of area since 1988 is represented by the position of species on the first canonical axis for proportion of area since 1988. $p = 0.0032$, $R^2 = 11.76\%$ (ANOVA).

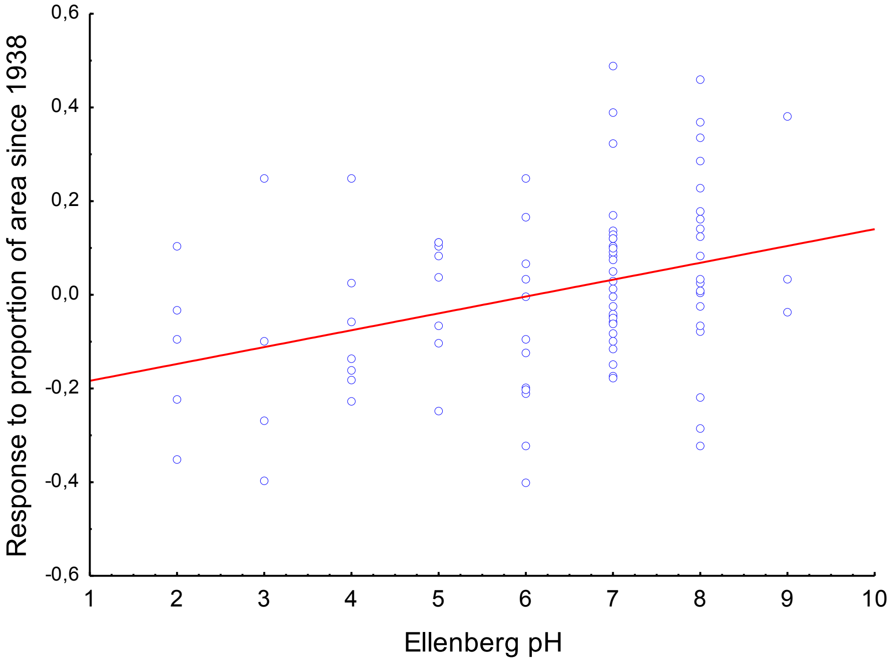

Figure S9-5: The effect of proportion of area of each locality that persisted at the same place since 1938 on the occurrence of species with specific requirements for pH of substrate (Ellenberg indicator value for pH; according to Ellenberg et al. 1991). Response of species to proportion of area since 1938 is represented by the position of species on the first canonical axis for proportion of area since 1938. $p < 0.0001$, $R^2 = 11.54\%$ (linear regression).

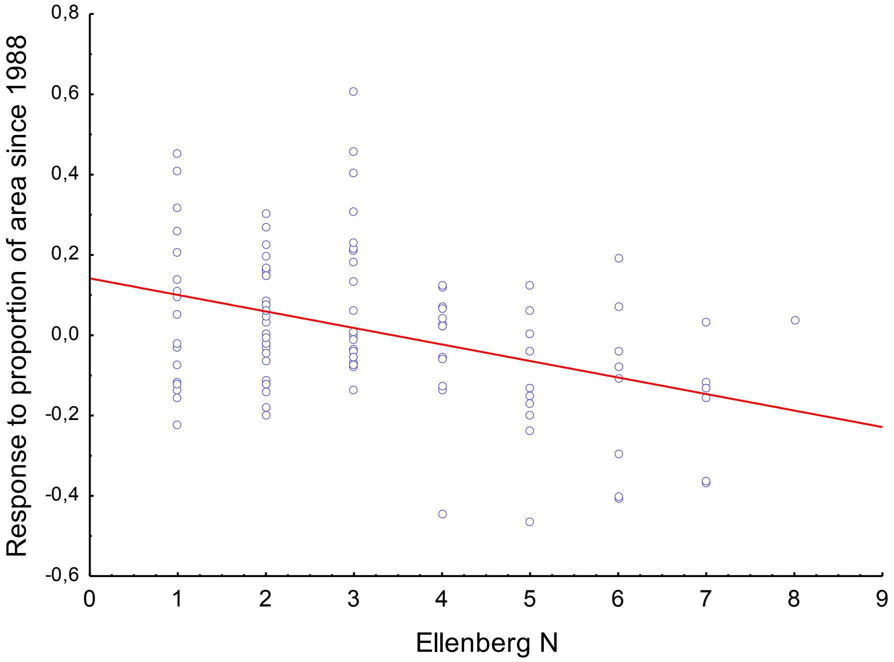

Figure S9-6: The effect of proportion of area of each locality that persisted at the same place since 1988 on the occurrence of species with specific nutrient requirements (Ellenberg indicator value for N; according to Ellenberg et al. 1991). Response of species to proportion of area since 1988 is represented by the position of species on the first canonical axis for proportion of area since 1988. $p < 0.0001$, $R^2 = 14.61\%$ (linear regression).

Appendix S10

The spatio-temporal dynamics of the studied habitats. Modified according to Husáková & Münzbergová (2014).

During the 20th century and at the beginning of the 21st century there were some important changes in the landscape structure of the study region. Specifically, in the past, there were fewer patches of dry grassland-like forest openings but they were larger and more interconnected in comparison with current situation. Number of dry grassland-like forest openings in the past ranged from 66 in 1938, via 105 in 1973, 89 in 1988 and 103 in 2000 to current number of 110 dry grassland patches in 2007. The mean area of dry grassland-like forest openings ranged from 650 m^2 in 1938, then increased via 840 m^2 in 1973 to 1080 m^2 in 1988 and subsequently decreased via 720 m^2 in 2000 to current mean area of 635 m^2 (Fig. S9-1). Today we can thus see gradual reduction in the area of dry grassland-like forest openings and increase in their isolation.

The changes in the landscape structure of the study region were thus substantially different and the reduction of the locality area was not so dramatic in comparison with other studies on semi-natural dry grasslands. The changes in the locality area and connectivity during the studied period could be probably attributed especially to an outbreak and subsequent gradual reduction of hoofed game (especially mouflons) in the region. The mouflons were distributed in the region after 1938, then their populations gradually increased to a peak in the 1970s and 1980s. Since then, their numbers gradually decreased (Kolbek 1996, Kolbek et al. 2003), corresponding to changes in landscape structure. The occurrence of mouflons could have a strong positive effect on the persistence of forest openings because if game browsing stops completely, gradual encroachment of shrubs and thus a temporary increase and then a consequential decrease in the species richness would occur. However, the high numbers of mouflons could negatively affect the survival of dry grassland species due to strong eutrophication of some places (Petřík et al. 2011). Nature conservation management should thus balance both mechanisms (Petřík et al. 2011): hunting of mouflons (it was partly practiced) and removal of young trees and shrubs colonizing grassland areas (this type of management was not practiced).

References:

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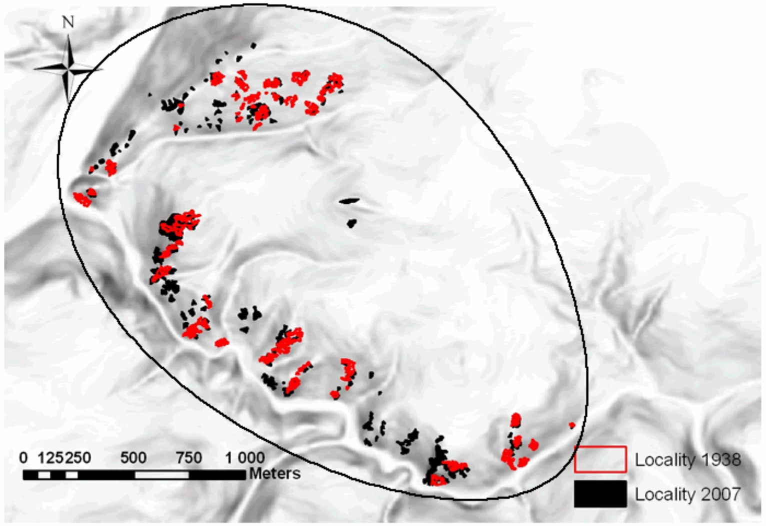
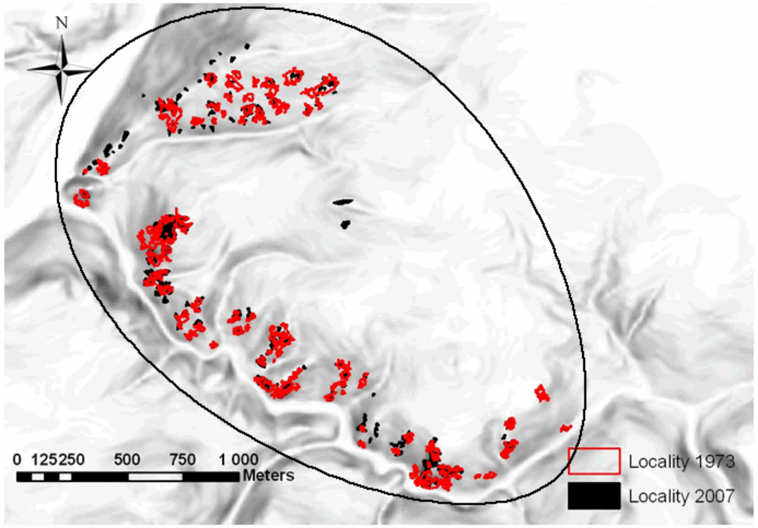
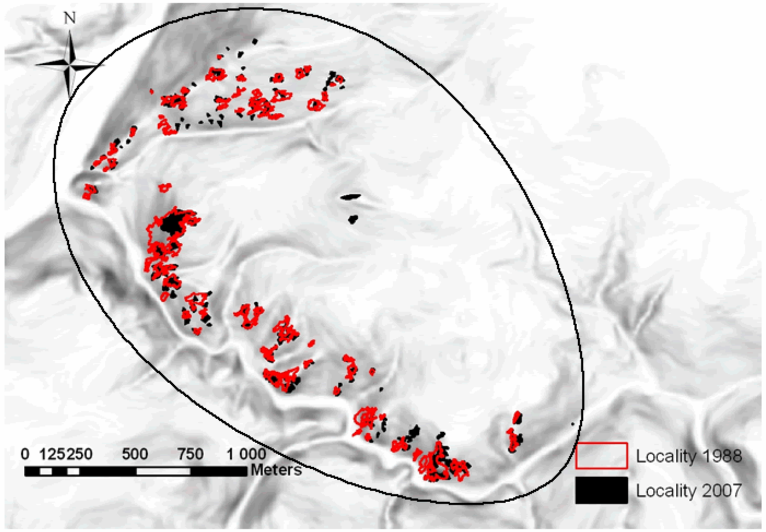
Figure S10-1: Distribution of forest openings in 1938, 1973, 1988, 2000 (red) in comparison with the current distribution – 2007 (black). The line defines the border of our study area. From Husáková & Münzbergová (2014).



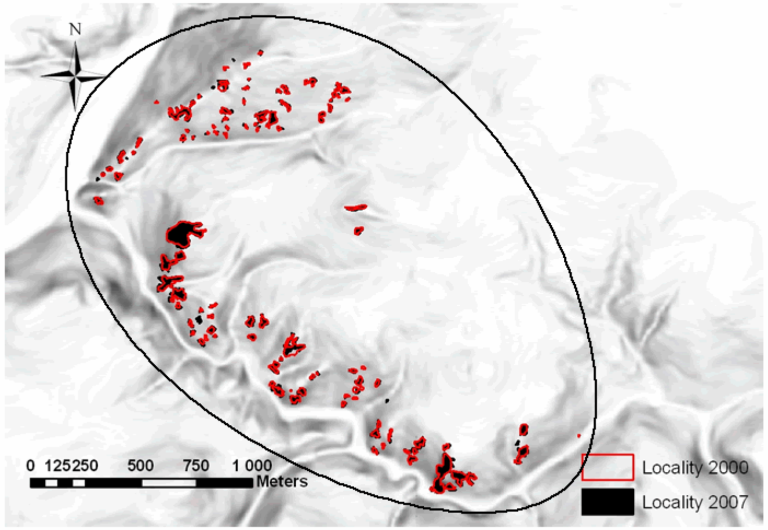
1938



1973



1988



2000

CHAPTER 3

Effects of habitat configuration and population size on performance of dry grassland species with different life-history traits

Iveta Husáková and Zuzana Münzbergová

Manuscript.

ABSTRACT

Many recent studies explored the relationships between plant performance and population size and less often also isolation. Most of these studies, however, deal only with a single species and were often done in different studied systems making their results hardly comparable. The aim of this study was thus to understand how the landscape structure and species distribution can affect plant performance of a spectrum of dry grassland species differing in their life-history traits co-occurring in the same habitat type in a fragmented landscape.

We collected data on performance of 21 dry grassland species, differing in their life-history traits and growing in populations differing in their size and connectivity. The performance related data were collected directly in the field as well as in the experimental garden where we grew plants from the seeds coming from the same populations. We tested the effect of population size and connectivity on individual performance of each species separately. Subsequently, we used species life-history traits to explain between species differences in the plant performance–landscape structure relationships.

Our results show that population size and connectivity significantly affected the performance of all the studied dry grassland species, but the effect of decreasing population size and connectivity was not always negative. Effects of population characteristics were detected not only in the field, but also in the common garden, even though they were weaker in the garden. The between species comparisons show that species more sensitive to decreasing population size and connectivity are insect-pollinated species, dispersed by wind or water, with lower terminal velocity as well as more common species. In addition, species more sensitive only to decreasing population size are long-lived species with lower SLA that are dispersed also by animals. Species more sensitive only to decreasing population connectivity are species with lighter seeds, higher SLA and lower shoot–root allocation slope at adult plants and also species with less persistent seed bank, that are not dispersed by animals.

The results indicate that species life-history traits are useful predictors of species responses to population size and connectivity. They also indicate that studies performed directly in the field likely provide stronger patterns than common garden studies. Common garden studies, are however useful to identify the long-term effects of landscape changes that are not connected to current habitat conditions and/or pollinator behaviour.

Keywords Plant performance; Fitness; Habitat fragmentation; Population size; Connectivity; Isolation; Common garden experiment; Dispersal traits; Persistence traits.

INTRODUCTION

It the landscape, many plant species are growing in fragmented habitats (Saunders et al. 1991; Soons 2003). As a consequence, many plant populations are becoming small and isolated, often due to extensive changes in land use during the past few centuries (Jacquemyn et al. 2003; Hérault and Honnay 2005; Piessens et al. 2005; Cousins et al. 2007), or natural processes (e.g. Leimu and Mutikainen 2005). Species in small populations face negative consequences of increasing level of inbreeding and loss of genetic variation within populations due to genetic drift and reduced gene flow and accumulation of deleterious mutations (Jacquemyn et al. 2002; Kolb 2005; Leimu et al. 2006; Van Geert et al. 2008). In the long term, lower level of genetic diversity may reduce the evolutionary potential of these species and thus greatly reduce their ability to respond to changing environmental conditions through adaptation and selection (Frankham 2005; Bowman et al. 2008). In a short term, genetic erosion (specifically inbreeding depression) can have immediate impact on local populations by reducing individual performance, especially their survival and reproduction (Lienert 2004; Frankham 2005). Decreasing individual performance can lead to a further decrease in population size and thus higher extinction risk of the local population (Fischer and Matthies 1998; Dupré and Ehrlén 2002; Münzbergová 2006a; Jackson and Sax 2010).

In addition to the genetic mechanisms, ecological mechanisms such as interactions with other organisms, especially pollinators and also herbivores (mainly seeds predators), play also important role for plant performance in fragmented habitats and can affect performance of plants in smaller populations (Ågren 1996; Groom 2001; Aguilar et al. 2006; Kolb 2008; Peterson et al. 2008; Dauber et al. 2010). Small and isolated populations are less attractive for pollinators, making them easily overlookable and their reproduction is thus limited by availability of pollinators as well as of compatible pollen (i.e. pollen quality and quantity), leading to a pattern referred as the Allee effect (Groom 1998, 2001; Ghazoul 2005; Jakobsson et al. 2009). Similarly, small and isolated populations may also be less attractive or available for herbivores or pathogens (Soons 2003; Groom 2001; Münzbergová 2006b), which help them to escape their herbivores, especially in case of specialized insect species (Colling and Matthies 2004). In some cases, small populations may be attacked by herbivores much more, as their populations can be already large enough to maintain viable population of herbivores but still too small to maintain populations of their natural enemies – predators and parasitoids of herbivores (Lienert 2004; McEuen and Curran 2006; Matesanz et al. 2015). Moreover, thanks to inbreeding depression and low level of genetic diversity, plants in small populations are less able to adequately respond to herbivory or regrow after being damaged (Leimu et al. 2008; Kolb 2008; Leimu and Fischer 2010).

Poor performance of plants in small and isolated populations may be caused not only by genetic deterioration and the disruption of plant–pollinator mutualisms but also by differences in habitat quality (especially in sense of specific abiotic conditions at localities) (Leimu et al. 2006), as plant reproduction is frequently resource limited (Spigler and Chang 2008). This explanation is supported by many studies that revealed the correlation between habitat degradation, population size and plant reproductive success (Adriaens et al. 2009;

Leimu 2010; Tsaliky and Diekmann 2010; Lauterbach et al. 2011; Busch and Reisch 2016; Roque et al. 2017). As most studies surveyed the species performance only in the field, it is not clear whether the differences in individual performance are really due to effects of their population size *per se*, or whether these differences can be due to differences in the actual habitat conditions at the localities. To resolve this issue, it is important to compare results from the field survey with results from common garden experiment examining plant performance in one study (Fischer and Matthies 1998). Such studies are however rare and usually done only on one or two species (e.g. Oostermeijer et al. 1994; Ouborg and Treuren 1995; Fischer and Matthies 1998; Kéry et al. 2000; Hooftman et al. 2003; Kolb 2005, 2008; Schleuning et al. 2009).

These possible dangers for small and isolated populations motivated recent studies that explored the effect of population size and less often also isolation and/or genetic diversity on plant performance measured as seed production, seed germination or plant growth (reviewed in Leimu et al. 2006; than e.g. Hensen and Wesche 2006; Kolb and Lindhorst 2006; Honnay et al. 2007; Jacquemyn et al. 2007; Peterson et al. 2008; Dostálek et al. 2010; Hornemann et al. 2012; Lauterbach et al. 2011; Putz et al. 2015; Busch and Reisch 2016; Münzbergová et al. 2018). However, the results of these studies are rather ambiguous. Many of these studies have revealed a positive relationship between population size and performance of individuals or their genetic diversity, but there are many other studies that have found no relationship (e.g. Oostermeijer et al. 1994; Ouborg and Treuren 1995; Lammi et al. 1999; Eisto et al. 2000; Leimu and Mutikainen 2005; Honnay et al. 2006; Severn et al. 2011) or even negative relationship (e.g. partly Hooftman et al. 2003 and Schleuning et al. 2009). Most of these studies, however, deal only with a single plant species, usually rare and endangered. Moreover, the different study systems can also be very heterogeneous and differ in spatial scale, environmental conditions, habitat type, age of the localities etc.

The degree to which species are affected by fragmentation, and also in which way, may vary between species and depend on their specific habitat requirements and biological attributes (Dupré and Ehrlén 2002; Kolb and Diekmann 2004, 2005). The strength and direction of the relationship between plant population size, performance and genetic variation can thus largely depend on plant species life-history traits. A lot of attention was paid mainly to pollination. It is important to take into account reproductive traits that typify the relationship with and the degree of dependence on their pollinators, i.e. whether the species are outcrossing or able of self-pollination (self-incompatible or self-compatible) and whether they are closely related to their specialized pollinators or have a larger spectrum of pollinators. It was shown that obligate and mainly outcrossing species are much more negatively affected by declining population size or connectivity (Aguilar et al. 2006; Honnay and Jacquemyn 2007), which leads to limited pollination and consequently to a reduction of genetic variation in populations (Leimu et al. 2006). On the other hand, self- or wind-pollinated species can be less susceptible to landscape fragmentation (Kolb and Lindhorst 2006). Similarly, also species with vegetative (clonal) reproduction should be less negatively affected by declining population size or connectivity as they are also not dependent on pollinators (Lienert 2004; Kolb and Lindhorst 2006).

Also, the species life span/form or longevity could affect plant performance in small populations. It was supposed that short-lived species may be more sensitive to the negative genetic consequences of small population size, because the more generations passed during a given time span than at long-lived species. Short-lived species should also be more vulnerable to the ecological consequences of small population size, such as increased pollinator limitation or increased demographic stochasticity in recruitment as they are mainly semelparous (Leimu et al. 2006). However, current studies did not support this trend (Aguilar et al. 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007). On the other hand, Angeloni et al. (2011) found that short-lived species have significantly lower level of inbreeding depression (thanks to purging effect) than long-lived species and for this reason they should be less sensitive to reduction in population size. Moreover, many short-lived species have traits that enable them to persist well also in fragmented landscape (i.e. they are often self-pollinated species with ruderal strategy, that produce large amount of small well-dispersed seeds) (Adriaens et al. 2006; Hemrová and Münzbergová 2015; Husáková and Münzbergová 2016). On contrary, long-lived species may persist at the site for a long time, but they may not perform very well there and their occurrence is thus often related to historical rather than current landscape configuration (Lindborg 2007; Purschke et al. 2012; Hemrová and Münzbergová 2015; Husáková and Münzbergová 2016). For this reason, many long-lived species may not respond to habitat fragmentation immediately (especially in their genetic variation), but with a certain time lag (Münzbergová et al. 2013; Takkis et al. 2013). Therefore, long-lived species may be more susceptible to small population size and isolation than short-lived species.

Species rarity may also affect the relationship between population size, genetic variation and performance. It is generally thought that rare and endangered species should be more negatively affected by habitat fragmentation. It was however found that also the populations of still relatively common species, usually common habitats specialists, may be equally or even more sensitive to landscape fragmentation as rare species, even when historically and naturally rare species were excluded from the analyses (partly Hooftman et al. 2003; Lienert 2004; Honnay and Jacquemyn 2007; Angeloni et al. 2011; Takkis et al. 2013), but studies on common species are still rare.

Also, many other species life-history traits could affect the relationship between population size, genetic variation and performance, especially those connected with species (seed) dispersal, but we are in fact not aware of any study, which would attempt to do this. There are however several studies, that show significant effects of dispersal traits on the dependence of species occurrences on current habitat configurations (Adriaens et al. 2007; Schleicher et al. 2011; Saar et al. 2012; Hemrová and Münzbergová 2015) or on historical habitat configurations (Purschke et al. 2012; Husáková and Münzbergová 2016). For this reason, also relationship between plant performance and population size or isolation could be affected by species dispersal traits. Also other seed parameters, such as seed weight (Weiner et al. 1997; Münzbergová and Plačková 2010), seed bank longevity (e.g. Plue et al. 2017; Münzbergová et al. 2018), or other species life strategy traits, such as CSR-strategy, specific leaf area or pattern of shoot–root allocation (Husáková et al. 2018) could affect plant performance and population size or isolation relationship.

Most of the between species comparisons were however done only as meta-analyses (Aguilar et al. 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007; Angeloni et al. 2011), which compared a large number of studies on the effect of population size and less often also isolation and/or genetic diversity on plant performance and thus included a large number of plant species. But the individual studies can be very heterogeneous, as they were often done only on single species and also in different studied systems (with different spatial scales, environmental conditions, habitat type, age of the localities etc.) making the results of such different studies hardly comparable. Studies on the effect of habitat fragmentation on performance of more than single species (usually habitat specialists) coexisting in the same habitat type (Kolb and Lindhorst 2006; Matesanz et al. 2015) and moreover studying the pattern not only in the field but also in the common garden experiments (Kéry at el. 2000; Hooftman et al. 2003), are still very rare. Moreover, even though these studies involved more than one species, they are still dealing with only two to four species and thus could not directly tested the species sensitivity to the landscape/populations characteristics against their life-history traits. Comparing higher number of carefully chosen target species within the same habitat type and landscape, which encompass a range of life forms/strategies, breeding systems, pollination and dispersal characteristics, however allow more direct comparisons between species and also extrapolating results to other species that share similar life-history characteristics (Kolb and Lindhorst 2006).

The aim of this study was to understand how the landscape structure and species distribution can affect plant performance of a spectrum of dry grassland species differing in their life-history traits co-occurring in the same habitat type in fragmented landscape. We ask the following questions: (i) What is the effect of population size and connectivity on plant performance?, (ii) How does the relationship differ between plants growing in the field and plants planted in experimental garden from seeds coming from the same original localities?, (iii) How does the relationship differ between species and can the differences be explained by species life-history traits (connected with their life span/strategy, type of pollination and seed dispersal)?

We hypothesize that plant performance will increase with population size and connectivity. The effect of population size and connectivity will be stronger in the data collected in the field than in the data from the garden. Such a difference would indicate that part of the effects is due to differences in the actual environmental conditions of the sites. Further, we hypothesize that the plant performance–population size and connectivity relationships will differ between species so that long-lived, insect-pollinated species with heavy and badly-dispersed seeds will be more negatively affected by landscape fragmentation than species with opposite life-history traits.

To answer these questions, we collected data on plant performance (reproductive and growth parameters) of 21 dry grassland species, differing in their life-history traits and growing in populations differing in their size and connectivity in the same habitat type and landscape. The performance related data were collected directly in the field as well as in the experimental garden where we planted the plants from the seeds coming from the same populations. We tested the effect of population size and connectivity on individual performance parameters of each species separately. Then we compared the results of these analyses for plants growing in the field and planted in the garden. We also assembled data on life-history traits related to pollination, seed dispersal, species abundance in the landscape and species life strategy. These traits were used to explain between species differences in the plant performance–landscape structure relationships.

METHODS

Studied species and study area

The study was carried out in Týřov Site of Community Importance (SCI) (49°58’10’’ N, 13°48’40’’ E). It is part of Křivoklátsko Protected Landscape Area and Biosphere Reserve in the Czech Republic. The region is characterised by patchy occurrence of dry grassland-like forests openings with typical dry grassland plant communities (Kolbek at al. 2001). To study the effect of the landscape structure on plant performance we selected 21 dry grassland species from a wide taxonomic range (21 genera in 12 families) strictly occurring only in these forest openings and differing in their life-history traits (Table 1). The species were selected to occur on at least 15 out of a total of 110 localities in the studied area. We also made sure to select species growing in populations differing in their size and connectivity (Appendix S1). The data on species distribution were obtained from our previous study (Husáková and Münzbergová 2016). For the purpose of this study, we were able to collect material from 12–20 (median 20) localities per species differing in their population size and connectivity (Table 1).

Data about studied species

For all the studied species and their populations, we expressed the population size as number of all flowering individuals per population.

For each species and each studied population, we collected data about plant performance directly in the field. Moreover, we also planted new plants from seeds coming from their original (maternal) localities in the field differing in their population size and connectivity in the experimental garden in the same conditions, because it enables to study the real effects of landscape structure on plant performance without confounding effects of actual habitat conditions.

Table 1. Studied species and numbers of studied populations and plants per species and per populations (median (min-max) values) in the field and in the experimental garden and numbers of Petri dishes and pots (median (min-max) values) used in experiments and seeds sown per Petri dish and per pot at each individual of each species.

Studied species	Abbrev.	Life span	Populations in the field	Studied plants per species in the field	Studied plants per population in the field	Petri dishes or pots per population in germination and garden exp.	Sown seeds per Petri dishes/pot	Studied plants per species in exp.garden	Studied plants per population in exp.garden
Acinos arvensis	Aci arv	Short-lived	17	336	20 (16-20)	10 (10-10)	40/40	170	10 (10-10)
Arabidopsis thaliana	Ara tha	Short-lived	20	402	20 (10-20)	10 (9-10)	50/100	200	10 (6-10)
Arenaria serpyllifolia agg.	Are ser	Short-lived	20	400	20 (20-20)	10 (9-10)	20/50	200	10 (10-10)
Asperula cynanchica	Asp cyn	Long-lived	18	275	17 (3-20)	10 (3-10)	30/30	170	10 (3-10)
Dianthus carthusianorum agg.	Dia car	Long-lived	17	228	16 (2-21)	10 (2-10)	20/20	138	10 (2-10)
Echium vulgare	Echi vul	Long-lived	19	333	20 (8-20)	10 (2-10)	20/20	180	10 (2-10)
Fragaria viridis	Fra vir	Long-lived	19	340	20 (8-22)	10 (3-10)	50/50	186	10 (7-10)
Geranium columbinum	Ger col	Short-lived	20	374	20 (10-21)	10 (6-10)	10/20	200	10 (10-10)
Hieracium pilosella	Hie pil	Long-lived	20	407	20 (20-22)	10 (9-10)	20/20	200	10 (9-10)
Inula conyzae	Inu con	Long-lived	15	171	8 (3-20)	8 (3-10)	50/50	121	7 (1-10)
Jasione montana	Jas mon	Short-lived	17	230	14 (3-20)	10 (2-10)	50/100	0	0
Melica transsilvanica	Mel tra	Long-lived	20	391	20 (15-21)	10 (5-10)	30/50	200	10 (9-10)
Myosotis ramosissima	Myo ram	Short-lived	20	400	20 (20-20)	10 (10-10)	20/20	200	10 (10-10)
Origanum vulgare	Ori vul	Long-lived	20	400	20 (20-20)	10 (10-10)	40/40	200	10 (7-10)
Phleum phleoides	Phl phl	Long-lived	20	400	20 (20-20)	10 (10-10)	50/50	200	10 (10-10)
Potentilla argentea	Pot arg	Long-lived	20	386	20 (10-20)	10 (9-10)	50/50	200	10 (10-10)
Scleranthus perennis	Scl per	Long-lived	12	235	20 (15-20)	10 (10-10)	20/50	120	10 (10-10)
Teucrium botrys	Teu bot	Short-lived	15	202	13 (6-20)	10 (6-10)	30/30	141	10 (6-10)
Trifolium arvense	Tri arv	Short-lived	20	391	20 (14-20)	10 (6-10)	40/40	200	10 (7-10)
Veronica dillenii	Ver dil	Short-lived	20	395	20 (15-20)	10 (9-10)	40/40	200	10 (10-10)
Vicia hirsuta	Vic hir	Short-lived	20	327	18 (5-20)	9 (2-10)	10/10	163	10 (2-10)

Plants in the field

To study plant performance directly in the field, we randomly sampled 20 flowering individuals per population of each species. In case of fewer flowering plants that could be studied in the population, we sampled the maximum number of plants possible (see Table 1 for total number of studied plants and their populations per species). For each studied plant individual, we collected data on reproductive parameters: number of flowers/fruits per plant, number of developed seeds per flower and per the whole plant, proportion of undeveloped seeds, seed weight, reproductive biomass, seed germinability and the time to 50% germination (T50) and plant height. The collection of all these data per species was carried out within one vegetation season at a time when most individuals of the species were in full maturity, i.e. the early flowering species were sampled in the spring, later flowering species in summer or autumn. Different species may have been collected in different years (2009–2011). Directly in the field we measured the plant height and counted all flowers and/or fruits per plant (incl. all ripened, non-ripened and already fallen out fruits as well as all flowers and flower buds). Several ripened fruits were sampled and subsequently we counted the number of developed and undeveloped seeds per fruit. We also expressed total number of developed seeds per plant as the number of developed seeds per flower multiplied by total number of flowers per plant. Developed seeds were then weighed (and the weight of 1 seed was determined) and prepared for sowing. In case of two species (*Arabidopsis thaliana* and *Jasione montana*) it was not possible to weigh their seeds as they were too small and light and measurement error was too high to study differences between populations. For each species (except these two mentioned above), we also expressed weight of reproductive biomass as total number of developed seeds per plant multiplied by seed weight.

Seeds from half of the individuals (i.e. usually 10) from each population of each species were used for germination experiment to determine the germinability and proportion of living seeds and the second half was used for sowing to pots in the experimental garden to study performance characteristics of cultivated individuals (see below).

The germination experiment was carried out on Petri dishes placed into the growth chamber under the regime with 12 hours photoperiod and a day/night temperature of 20/10°C and humidity of 70/50%. This regime was chosen to best characterize the conditions of seed germination in the field and to be the same for all studied species. Seeds from one individual were sown to one Petri dish. The number of seeds sown per Petri dishes differed among species, reflecting differences in seed size, but was the same within the species and ranged from 10 to 50 seeds (Table 1). Once a week, the number of germinated seeds was counted and the germinated seeds were removed from the Petri dishes. The seed was considered germinated if the radicle was visible to the naked eye. Two species had very poor germination, we thus decided to stimulate it by Gibberellic acid application. It was successful only in case of one species (*Echium vulgare*). Subsequently, we used also cold stratification and mechanical scarification at the second species (*Teucrium botrys*), which finally stimulated its germination. After the main germination phase was completed and the seeds no longer germinated for at least 3 weeks, we applied Gibberellic acid in concentration 0.02 g/100 ml distilled water to the seeds of each species to stimulate the possible germination of the remaining seeds (Kahn 1960). The timing of application of the Gibberellic acid differed between species but was the same for all the dished of the same species. The experiment was terminated after three weeks without germinating seeds after Gibberellic acid application. From the rest of ungerminated seeds we removed soft and damaged seeds. Healthy ungerminated seeds were tested for viability by tetrazolium chloride according to Cottrell (1947). Species with very small seeds could not be tested for viability by tetrazolium test (*Acinos arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Jasione montana, Myosotis ramosissima, Origanum vulgare*). In our study the parameter germinability is thus represented by proportion of all germinated and living seeds. In species with very small seeds it is represented by proportion of all germinated seeds (always done identically for all individuals within the species). We also calculated the time to reach 50% germination (T50), represented by number of days in which 50% of seed germinated out of all germinated seeds, according to Coolbear et al. (1984) modified by Farooq et al. (2005).

Plants in the experimental garden

The seeds from the second half of individuals from each population were used to establish an experiment in an experimental garden of the Institute of Botany of the Czech Academy of Sciences in Průhonice, Czech Republic (N 49°99’46’’, E 14°56’62’’).

Seeds from each individual were sown into 15 × 15 × 15 cm pots in a substrate consisting of 2 parts garden soil and 1 part sand in an experimental garden. The seeds were sown either in autumn of the year when we collected seeds (2009–2011) or in spring of the following year. We preferred autumnal sowing, but it was not possible to do it for all species as the seed preparation was time-consuming. As perennial species are more dependent on cold stratification than annuals (Grime et al. 1981, Fenner 1985), we sow all perennial species in the autumn and annual species in the early spring. The time of sowing was the same for one species. Seeds from one maternal plant were sown into one pot. The number of seeds sown per pot differed among species, but was the same within the species and ranged from 10 to 100 seeds (Table 1). Two or three weeks after most of seeds of each species started to germinate, we counted the number of seedlings and calculated the rate of seedling recruitment. Seeds of one species (*Jasione montana*) did not germinate at all. We have therefore monitored only 20 species in the garden. At the same time, only one randomly chosen individual was left in the pot to study its later growth and all other seedlings were removed from the soil. All these harvested seedlings were soaked in water to remove residual soil, divided into above- and below-ground parts, dried to constant weight at 70° C and weighed for seedlings biomass production. We refer to these as seedlings in the subsequent text.

All the plants were left to grow in the pots in the experimental garden until at least 80% individuals of each species were flowering. Seven out of 20 studied species were able to flower in the 1st year of their life and the rest in the 2nd year. One species (*Fragaria viridis*) was harvested also in the 1st year in spite of no individuals were flowering, because of its strong clonal growth, which would later make it impossible to distinguish the original studied individuals. All individuals of each species were harvested at the time when most of their fruits were mature (after 4–16 months). Firstly, we measured the height of each plant individual, counted the number of all flowers and fruits (including non-ripened, already fallen out and also flower buds) as well as collected several ripened fruits to assess seed production: number of developed and undeveloped seeds per flower (fruit) and per whole plant, seed weight and reproductive biomass. In case of non-flowering species (*Fragaria viridis*), we used the number of new (vegetative) rosettes instead of number of flowers and the new rosettes´ dry biomass weight as reproductive biomass. Then, we carefully extracted the plants from the substrate. As with the seedlings, we divided the individuals into above- and below-ground parts, washed the below-ground parts and dried to constant weight at 70°C and weighed them. We refer to these as adult plants in the subsequent text. All flowers and fruits with seeds were included into above-ground biomass of the adult plants.

Landscape structure and local habitat conditions

From our previous study (Husáková and Münzbergová 2014) we have got many parameters describing landscape structure of the studied region and additional information on local habitat conditions. Specifically, for each studied population, we used current habitat area (expressed as surface area because of high topographic heterogeneity of study region) and connectivity (i.e. opposite to isolation; expressed as a sum of area of surrounding localities in a circuit of 500 meters around each locality weighted by square of their pair wise distances and calculated according to a formula provided by Tremlová and Münzbergová (2007)). For the reason of this study, we expressed the connectivity species-specifically for each studied species and thus, we included only the localities with the occurrence of each particular species to the connectivity calculation according to knowledge of species distribution in the studied area from Husáková and Münzbergová (2016). As the habitat area was highly positively correlated with population size at each species, we used only population size and connectivity to study the effect of landscape structure on plant performance in our final analyses. Population size and connectivity were not significantly correlated ($N = 389$, $r = 0.06$, $p = 0.236$).

We also used several variables of local habitat conditions (Proportions of substrate types, Shannon diversity index of substrate types, Slope – max, Potential direct solar insolation (PDSI) – median value in June and Geology bedrock type) that were selected in our previous study (Husáková and Münzbergová 2016) as the most important for species distribution as well as geographical coordinates of the localities (x, y) to account for spatial structure of the landscape and environmental variation.

Life-history traits of the studied species

To assess how the relationship between landscape structure and various parameters of plant performance differ between different species, we collected data about their life-history traits from several databases as well as making our own measurements using the same methods used in the databases. From BiolFlor database (http://www2.ufz.de/biolflor/index.jsp) we collected data on pollination vector (insect, wind, self), breeding system ((facultative) allo-, autogamous, mixed mating) and compatibility (self-compatibility/incompatibility) and on CSR strategy. From The Leda Traitbase (Kleyer et al. 2008) we collected data on seed dispersal type, terminal velocity, plant life span and specific leaf area (SLA). We used several categories of seed dispersal type (anemochory, autochory, hydrochory and zoochory), expressed as the proportion of each studied dispersal type for each species, i.e. multi-vector dispersal on a continuous scale, and analysed all these categories together. We considered annuals and biennials as short-lived species and perennials as long-lived species. From Kubát et al. (2002) we collected data on average plant height and from CloPla database (Klimešová and de Bello 2009, http://clopla.butbn.cas.cz) clonal index of each species. We also measured the seed weight for all individuals of each species that we sampled and calculated the average seed weight per species. Moreover, we also assessed species abundance in the study region as frequency of each species in the landscape (number of localities with species occurrence). From study Husáková et al. (2018) we used data on shoot–root allocation (allometric) slopes for seedlings and for adult plants of our studied species describing pattern of allocation to above- and below-ground biomass in different life cycle stages.

Directly in the field, we also studied the soil seed bank longevity of each species (according to methodology Knevel et al. (2005)). To do this, we collected seeds of each species in one vegetation season and prepared 9 identical sets of seeds for each species. We used 100 seeds per set at most species and 50 seeds in case of three species (*Echium vulgare, Geranium columbinum, Vicia hirsuta*). Six of these sets of seeds for each species were placed into monofil bags and buried at 3 localities (i.e. two bags per species at one locality) and the last 3 sets were used as control to determine germinability in the year of seed collection (in the same way as described above). One year after burial we collected one bag of each species from each locality and tested germination of the seeds. We compared the germinability after one year and after 2 years with the control and determined a decline in germination over time (as ratio of germinability after 1 or 2 years to control). We finally used only information on seed bank longevity after 2 years of burying in analyses (hereafter referred as seed bank) as being more informative.

Statistical analysis

First, we tested the effect of population size and connectivity on individual parameters of plant performance measured in the field and in the garden for each species separately using generalised linear models with normal distribution or at some performance parameters with binomial (proportion of undeveloped seeds, germinability and recruitment) or Poisson distribution (T50). The independent variables (population size and connectivity) and also some dependent variables (flowers, developed seeds in total, reproductive biomass, below- and above-ground biomass of seedlings and adult plants and at some species also developed seeds per flower) were logarithmically transformed before analyses.

We first carried out all the tests without any covariates. We then repeated the analyses after accounting for local habitat conditions and position of each locality. To do this, we used NMDS scores derived from standardised variables of local habitat conditions and geographical coordinates (see above) calculated using NMDS analysis in R package Vegan (Oksanen et al. 2015) as covariates. As the analyses with and without covariates provided very similar results we decided to present only the results of analyses where covariates have been used (their significance was bit lower but the direction of the relationship was the same at both types of analyses). We used standardised regression coefficients to characterize the plant performance–population size or connectivity relationships for each species.

To compare the results between the garden and the field, we tested the correlation between the above obtained standardised regression coefficients from the two environments for the same performance parameters.

To identify the effect of life-history traits on plant performance–population size or connectivity relationships the values of standardised regression coefficients of each species were tested against the species life-history traits using linear regression. For each species we used one value of standardised regression coefficient for each performance parameter within the plant performance–population size or connectivity relationships (from previous analyses) and one average trait value per species. In case of pollination vector and seed dispersal type, we used several categories of dispersal mode (coded as proportion of each vector) and analysed all these categories together in one test. Thus, we had 21 points (species) in each test. All the analyses were performed in R 3.4.2 for Windows (R Development Core Team 2011).

RESULTS

The effect of population size and connectivity on individual performance

All studied species were affected by population size and connectivity at least in some performance characteristics. Much stronger effects of landscape structure on plant performance were detected for individuals growing directly in the field than for individuals planted in the experimental garden (Table 2 and 3).

Plants in the field

Population size had mostly positive effect on performance of the studied species. Fourteen out of 21 species performed better in bigger populations in the field (Table 2). These species usually flowered more, produced more developed seeds per flower and per plant, had lower proportion of undeveloped seeds, heavier seeds and more reproductive mass and they germinated better and/or faster. Other 7 species, however, performed better in smaller populations in the field. *Arenaria serpyllifolia* and *Vicia hirsuta* showed lower performance in bigger populations in all reproductive parameters. Five species (*Inula conyza, Melica transsilvanica, Myosotis ramosissima, Scleranthus perennis, Teucrium botrys*) showed lower performance in bigger populations in most, but not all, reproductive parameters. Only 6 species grew higher and other 4 species grew lower in bigger populations, while plant height was independent of population size in the other species.

Ten species performed better in the more connected populations in the field (Table 2). Other 3 species (*Arenaria serpyllifolia, Fragaria viridis, Inula conyza*) showed better performance only in one fitness parameter (germination or number of developed seeds per flower) and otherwise prospered worse in more connected populations. Eight species, however, performed worse in all reproductive parameters and 6 of these species also in growth parameters in more connected localities. Such species usually flowered less, produced less developed seeds and higher proportion of undeveloped seeds, lighter seeds and less reproductive mass and germinated worse and/or more slowly in more connected populations.

Plants in the experimental garden

In the garden, similar number of species was positively affected by population size, but fewer species were positively affected by population connectivity compared to the field.

Eleven species studied in the garden and originally coming from bigger populations performed better in most of their performance characteristics. Other four species showed higher performance in some traits and lower in other traits, when they came from bigger populations (*Echium vulgare, Geranium columbinum, Inula conyza, Phleum phleoides*).

Other four species (*Arenaria serpyllifolia, Melica transsilvanica, Origanum vulgare* and *Teucrium botrys*), however, performed worse, when coming from bigger populations (see Table 3). In case of first three species, only the parameters of adult plants (growth and reproduction) and in case of *Teucrium botrys*, only seedlings parameters were negatively affected. In *Arenaria serpyllifolia,* seedlings germination and establishment (recruitment) was even positively affected by population size. On the other hand, in three species that were otherwise affected by population size positively (*Echium vulgare, Myosotis ramosissima, Potentilla argentea*), seedlings recruitment was negatively affected by population size. Other two species (*Acinos arvensis* and *Triforium arvense*) were positively affected by population size only in recruitment and not in any other performance parameters. One species (*Vicia hirsuta*) was not affected by population size in any performance characteristics in the garden.

In the case of population connectivity, individuals of only 6 of 20 studied species performed better in the garden, when they came from more connected populations (see Table 3). Two of these species (*Myosotis ramosissima* and *Potentilla argentea*) were affected by connectivity only in seedlings recruitment and/or its growth and not as adults. On the other hand, individuals of other 10 species (*Acinos arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Asperula cynanchica, Echium vulgare, Geranium columbinum*, *Melica transsilvanica, Teucrium botrys, Veronica dillenii, Vicia hirsuta*) performed worse in the garden when they originally came from more connected populations. Then, there were 3 other species (*Dianthus carthusianorum, Scleranthus perennis* and *Triforium arvense*) that were affected partly positively and partly negatively and 1 species (*Inula conyza*) that was not affected by connectivity in any performance characteristics in the garden.

Table 2. The effect of population size and connectivity on plant performance parameters in the field conditions. Depicted are standardised regression coefficients and level of significance (*** p = 0.001-0; ** p = 0.01-0.001; * p = 0.05-0.01; x marginally significant result with p = 0.05-0.1). n.s. is not significant (p > 0.1), - not tested. Devel_1_seeds is number of developed seeds per 1 flower, dev.seeds_total is number of developed seeds per whole plant, undev.seeds is proportion of undeveloped seeds. For abbreviations of plant species see Table 1.

		height1		**flowers1**		**devel_1_ seeds1**		**undev. seeds1**		**dev.seed _ total1**		**seed. weight1**		**reprod. mass1**		**germinability1**		**T50**	
Aci	**p.size**	0.08	n.s.	0.15	**	0.02	n.s.	-0.09	n.s.	0.14	**	0.00	n.s.	0.14	**	0.02	n.s.	-0.23	n.s.
arv	**connect.**	-0.11	*	-0.19	***	0.04	n.s.	0.05	n.s.	-0.17	**	-0.15	**	-0.19	***	-0.13	***	0.12	n.s.
Asp	**p.size**	-0.20	***	-0.08	n.s.	0.13	*	-0.14	***	-0.04	n.s.	-0.05	n.s.	-0.05	n.s.	0.00	n.s.	0.05	n.s.
cyn	**connect.**	-0.08	n.s.	-0.19	***	-0.16	**	0.18	***	-0.21	***	-0.03	n.s.	-0.21	***	-0.04	n.s.	0.01	n.s.
Are	**p.size**	0.14	**	-0.01	n.s.	-0.12	*	0.08	*	-0.04	n.s.	0.07	n.s.	-0.01	n.s.	-0.04	n.s.	0.00	n.s.
ser	**connect.**	-0.28	***	-0.15	**	0.03	n.s.	-0.03	n.s.	-0.14	**	-0.17	***	-0.18	***	0.13	***	-0.13	n.s.
Ara	**p.size**	0.16	***	0.28	***	0.15	**	-0.03	n.s.	0.28	***	-	-	-	-	0.14	***	0.03	n.s.
tha	**connect.**	-0.06	n.s.	-0.14	**	-0.13	**	0.10	***	-0.15	**	-	-	-	-	-0.13	***	0.08	n.s.
Dia	**p.size**	-0.32	***	0.09	n.s.	-0.05	n.s.	-0.10	***	0.05	n.s.	0.11	x	0.08	n.s.	0.05	x	-0.11	n.s.
car	**connect.**	0.14	*	0.14	*	-0.05	n.s.	0.10	***	0.09	n.s.	0.05	n.s.	0.10	n.s.	0.20	***	-0.19	*
Echi	**p.size**	-0.04	n.s.	0.22	***	0.10	x	-0.10	x	0.23	***	0.20	***	0.25	***	0.09	x	0.02	n.s.
vul	**connect.**	0.15	**	-0.11	*	0.03	n.s.	-0.02	n.s.	-0.12	*	-0.09	n.s.	-0.13	*	0.00	n.s.	0.04	n.s.
Fra	**p.size**	0.32	***	0.11	*	0.04	n.s.	0.05	***	0.10	x	0.05	n.s.	0.11	*	0.24	***	-0.04	n.s.
vir	**connect.**	-0.01	n.s.	0.09	n.s.	-0.19	***	0.04	*	-0.11	*	0.06	n.s.	-0.10	x	0.08	**	-0.04	n.s.
Ger	**p.size**	0.16	**	0.11	*	-0.03	n.s.	0.08	n.s.	0.10	*	0.20	***	0.15	***	0.10	**	-0.23	***
col	**connect.**	-0.18	***	0.14	**	-0.03	n.s.	0.04	n.s.	0.13	**	0.00	n.s.	0.12	*	0.12	***	0.11	*
Hie	**p.size**	-0.05	n.s.	0.12	*	0.39	***	-0.36	***	0.33	***	0.28	***	0.35	***	0.30	***	0.08	n.s.
pil	**connect.**	0.04	n.s.	0.08	n.s.	0.21	***	-0.17	***	0.18	***	0.25	***	0.28	***	0.23	***	0.13	*
Inu	**p.size**	0.26	***	0.01	n.s.	-0.19	*	0.06	*	0.00	n.s.	-0.01	n.s.	-0.01	n.s.	-0.06	*	-0.16	***
con	**connect.**	-0.18	**	0.00	n.s.	0.25	***	0.06	*	0.02	n.s.	-0.04	n.s.	0.02	n.s.	-0.12	***	0.11	**
Jas	**p.size**	0.06	n.s.	0.13	*	0.38	***	-0.35	***	0.22	***	-	-	-	-	-0.01	n.s.	-0.04	n.s.
mon	**connect.**	0.24	***	0.07	n.s.	-0.10	n.s.	0.02	n.s.	0.03	n.s.	-	-	-	-	-0.22	***	0.15	***

Myo	**p.size**	-0.03	n.s.	0.03	n.s.	-0.12	*	0.12	***	0.01	n.s.	-0.22	***	-0.05	n.s.	0.08	*	-0.12	x
ram	**connect.**	0.07	n.s.	0.09	x	0.02	n.s.	-0.09	***	0.09	x	-0.01	n.s.	0.07	n.s.	0.21	***	-0.19	**
Mel	**p.size**	-0.09	x	0.26	***	-0.10	*	0.22	***	0.25	***	-0.18	***	0.21	***	-0.18	***	0.01	n.s.
tra	**connect.**	0.13	**	0.24	***	-0.17	***	0.17	***	0.22	***	-0.04	n.s.	0.20	***	-0.04	x	0.00	n.s.
Ori	**p.size**	-0.07	n.s.	0.31	***	-0.03	n.s.	0.03	n.s.	0.24	***	0.08	n.s.	0.21	***	0.09	***	-0.03	n.s.
vul	**connect.**	0.12	*	-0.08	n.s.	0.07	n.s.	-0.07	n.s.	-0.03	n.s.	0.10	*	0.03	n.s.	0.16	***	0.08	n.s.
Pot	**p.size**	-0.05	n.s.	0.16	***	-0.01	n.s.	-0.07	*	0.13	**	0.40	***	0.23	***	0.09	***	-0.01	n.s.
arg	**connect.**	-0.18	***	-0.01	n.s.	-0.06	n.s.	0.06	*	-0.04	n.s.	0.09	n.s.	0.07	n.s.	-0.01	n.s.	-0.05	n.s.
Phl	**p.size**	-0.02	n.s.	0.06	n.s.	-0.04	n.s.	-	-	0.06	n.s.	0.15	**	0.13	**	0.17	***	0.00	n.s.
phl	**connect.**	0.02	n.s.	0.17	***	0.13	**	-	-	0.17	***	-0.06	n.s.	0.11	*	-0.16	***	-0.14	x
Scl	**p.size**	-0.23	***	-0.23	***	-	-	-	-	-0.23	***	0.23	***	-0.16	*	0.04	n.s.	0.32	***
per	**connect.**	-0.08	n.s.	-0.23	***	-	-	-	-	-0.23	***	0.11	x	-0.20	**	-0.02	n.s.	-0.08	x
Tri	**p.size**	-0.20	***	0.27	***	0.26	***	-0.16	***	0.28	***	-0.20	***	0.25	***	0.03	n.s.	-0.03	*
arv	**connect.**	0.21	***	0.09	x	0.11	*	0.19	***	0.07	n.s.	0.01	n.s.	0.07	n.s.	0.13	***	0.17	***
Teu	**p.size**	-0.05	n.s.	0.00	n.s.	0.01	n.s.	-0.03	***	0.01	n.s.	-0.17	*	-0.02	n.s.	-0.10	**	-0.01	n.s.
bot	**connect.**	-0.09	n.s.	0.07	n.s.	-0.08	n.s.	0.05	***	0.05	n.s.	-0.18	**	0.00	n.s.	-0.05	n.s.	0.00	n.s.
Ver	**p.size**	0.13	**	0.13	*	0.15	**	0.03	n.s.	0.16	**	-0.01	n.s.	0.14	**	0.18	***	-0.03	n.s.
dil	**connect.**	0.28	***	0.33	***	0.09	x	0.04	n.s.	0.29	***	0.14	**	0.29	***	0.18	***	-0.06	n.s.
Vic	**p.size**	-0.09	n.s.	0.11	x	-0.13	*	0.12	*	0.04	n.s.	-0.01	n.s.	0.05	n.s.	-0.02	n.s.	0.01	n.s.
hir	**connect.**	0.14	*	0.07	n.s.	0.03	n.s.	-0.02	n.s.	0.12	x	0.00	n.s.	0.11	x	-0.15	n.s.	0.15	*

Table 3. The effect of population size and connectivity on plant performance parameters in the experimental garden conditions. Depicted are standardised regression coefficients and level of significance (*** p = 0.001-0; ** p = 0.01-0.001; * p = 0.05-0.01; x marginally significant result with p = 0.05-0.1). n.s. is not significant (p > 0.1), - not tested. Devel_1_seeds is number of developed seeds per 1 flower, dev.seeds_total is number of developed seeds per whole plant, undev.seeds is proportion of undeveloped seeds. For abbreviations of plant species see Table 1. In case of non-flowering species (*Fragaria viridis*), number of flowers is represented by the number of new (vegetative) rosettes and reproductive biomass by the new rosettes´ dry biomass weight.

		Seedlings						**Adult plants**																			
		recruitment		**log_ above1**		**log_ below1**		**height2**		**flowers2**		**dev.1_ seeds2**		**undev. seeds2**		**dev.seed_total2**		**seed. weight2**		**reprod. mass2**		**log_ above2**		**log_ below2**	
Aci	**p.size**	0.30	***	0.02	n.s.	0.08	n.s.	0.02	n.s.	0.06	n.s.	0.01	n.s.	0.08	n.s.	0.06	n.s.	0.11	n.s.	0.08	n.s.	0.03	n.s.	-0.07	n.s.
arv	**connect.**	-0.13	***	0.14	n.s.	0.16	x	0.02	n.s.	-0.12	x	-0.09	n.s.	0.09	n.s.	-0.14	x	-0.20	**	-0.18	*	-0.11	n.s.	0.06	n.s.
Asp	**p.size**	0.10	*	-0.03	n.s.	-0.01	n.s.	-0.09	n.s.	0.10	n.s.	-0.07	n.s.	0.10	***	0.11	n.s.	0.14	n.s.	0.16	x	0.15	*	0.13	x
cyn	**connect.**	-0.01	n.s.	-0.01	n.s.	0.00	n.s.	-0.04	n.s.	-0.11	n.s.	-0.12	n.s.	0.11	***	-0.16	x	-0.03	n.s.	-0.16	*	-0.09	n.s.	-0.05	n.s.
Are	**p.size**	0.17	***	0.06	n.s.	0.08	n.s.	-0.38	**	-0.44	**	-0.27	x	-0.03	n.s.	-0.22	n.s.	0.16	n.s.	-0.15	n.s.	-0.21	n.s.	-0.23	n.s.
ser	**connect.**	-0.19	***	0.06	n.s.	0.03	n.s.	0.12	n.s.	0.14	n.s.	0.09	n.s.	0.06	n.s.	0.18	n.s.	-0.03	n.s.	0.19	n.s.	0.19	n.s.	0.16	n.s.
Ara	**p.size**	0.08	**	-0.01	n.s.	0.06	n.s.	0.13	n.s.	0.13	n.s.	0.10	n.s.	-0.39	**	0.13	n.s.	0.03	n.s.	0.13	n.s.	0.08	n.s.	-0.06	n.s.
tha	**connect.**	-0.07	*	0.05	n.s.	0.04	n.s.	-0.04	n.s.	0.05	n.s.	0.02	n.s.	0.03	n.s.	0.04	n.s.	0.05	n.s.	0.04	n.s.	0.09	n.s.	0.10	n.s.
Dia	**p.size**	0.02	n.s.	-0.02	n.s.	-0.03	n.s.	-0.31	***	0.16	x	0.06	n.s.	-0.17	***	0.13	n.s.	-0.08	n.s.	0.11	n.s.	-0.09	n.s.	-0.04	n.s.
car	**connect.**	0.09	x	-0.14	n.s.	-0.12	n.s.	0.09	n.s.	0.03	n.s.	-0.14	x	0.14	**	-0.08	n.s.	0.22	**	0.00	n.s.	0.03	n.s.	0.10	n.s.
Echi	**p.size**	-0.15	*	0.10	n.s.	0.20	x	-0.01	n.s.	0.05	n.s.	-0.04	n.s.	-0.01	n.s.	0.05	n.s.	-0.02	n.s.	0.28	**	0.06	n.s.	0.04	n.s.
vul	**connect.**	-0.36	***	-0.06	n.s.	-0.14	n.s.	0.00	n.s.	0.06	n.s.	0.10	n.s.	-0.13	n.s.	0.06	n.s.	-0.27	**	-0.01	n.s.	-0.02	n.s.	-0.10	n.s.
Fra	**p.size**	0.18	***	0.00	n.s.	-0.03	n.s.	0.13	x	0.11	n.s.	-	-	-	-	-	-	-	-	0.09	n.s.	0.20	**	0.07	n.s.
vir	**connect.**	0.07	*	-0.09	n.s.	-0.06	n.s.	-0.08	n.s.	0.06	n.s.	-	-	-	-	-	-	-	-	0.07	n.s.	0.04	n.s.	0.17	*
Ger	**p.size**	0.09	*	-0.04	n.s.	-0.06	n.s.	-0.10	n.s.	-0.13	*	0.01	n.s.	-0.01	n.s.	-0.12	x	0.15	*	-0.09	n.s.	-0.12	x	-0.01	n.s.
col	**connect.**	0.09	*	0.02	n.s.	0.00	n.s.	-0.25	***	-0.25	***	0.02	n.s.	-0.02	n.s.	-0.22	***	-0.05	n.s.	-0.23	***	-0.29	***	-0.23	***
Hie	**p.size**	0.15	***	-0.03	n.s.	-0.09	n.s.	0.08	n.s.	-0.02	n.s.	-0.02	n.s.	-0.06	***	0.04	n.s.	0.15	x	0.08	n.s.	0.01	n.s.	-0.05	n.s.
pil	**connect.**	0.05	n.s.	-0.05	n.s.	-0.05	n.s.	0.15	*	0.04	n.s.	-0.06	n.s.	0.01	n.s.	0.00	n.s.	-0.02	n.s.	-0.01	n.s.	-0.09	n.s.	-0.08	n.s.
Inu	**p.size**	0.05	n.s.	-0.09	n.s.	0.07	n.s.	0.07	n.s.	0.07	n.s.	0.45	***	-0.11	n.s.	0.08	n.s.	0.20	n.s.	0.14	n.s.	-0.35	***	-0.27	**
con	**connect.**	-0.01	n.s.	-0.04	n.s.	-0.20	n.s.	0.07	n.s.	-0.01	n.s.	0.07	n.s.	0.05	n.s.	-0.02	n.s.	-0.08	n.s.	-0.04	n.s.	0.12	n.s.	-0.06	n.s.

Myo	**p.size**	-0.09	**	0.10	n.s.	0.08	n.s.	-0.01	n.s.	-0.13	n.s.	0.06	n.s.	-0.03	n.s.	-0.04	n.s.	0.04	n.s.	-0.01	n.s.	0.17	*	0.21	**
ram	**connect.**	0.11	***	-0.13	x	-0.18	*	0.02	n.s.	0.07	n.s.	-0.09	n.s.	0.11	n.s.	0.01	n.s.	-0.06	n.s.	-0.01	n.s.	-0.02	n.s.	0.03	n.s.
Mel	**p.size**	-0.03	n.s.	-0.09	n.s.	0.00	n.s.	-0.13	x	0.04	n.s.	0.01	n.s.	0.57	***	0.03	n.s.	0.00	n.s.	0.03	n.s.	-0.21	**	-0.13	x
tra	**connect.**	-0.15	***	0.00	n.s.	-0.01	n.s.	-0.16	*	-0.02	n.s.	-0.08	n.s.	0.29	***	-0.03	n.s.	0.03	n.s.	-0.01	n.s.	-0.29	***	-0.18	**
Ori	**p.size**	-0.03	n.s.	0.15	n.s.	0.04	n.s.	0.04	n.s.	-0.20	**	0.08	n.s.	-0.11	n.s.	-0.18	*	-0.03	n.s.	-0.17	*	0.07	n.s.	0.03	n.s.
vul	**connect.**	-0.02	n.s.	0.17	n.s.	0.04	n.s.	0.16	*	0.03	n.s.	0.09	n.s.	-0.11	n.s.	0.03	n.s.	0.14	x	0.06	n.s.	0.20	**	0.18	*
Pot	**p.size**	-0.09	***	-0.03	n.s.	0.16	*	0.28	***	0.23	***	-0.21	**	0.09	n.s.	0.08	n.s.	0.26	***	0.21	**	0.24	***	0.23	***
arg	**connect.**	0.20	***	0.04	n.s.	0.10	n.s.	0.04	n.s.	0.03	n.s.	-0.11	n.s.	0.07	n.s.	-0.06	n.s.	-0.07	n.s.	-0.07	n.s.	-0.03	n.s.	0.11	n.s.
Phl	**p.size**	0.08	**	-0.03	n.s.	0.09	n.s.	-0.21	**	0.12	x	0.00	n.s.	-	-	0.12	x	0.12	n.s.	0.14	x	0.07	n.s.	0.07	n.s.
phl	**connect.**	0.14	***	0.08	n.s.	0.14	n.s.	0.14	*	0.06	n.s.	0.14	x	-	-	0.06	n.s.	-0.06	n.s.	0.04	n.s.	0.10	n.s.	0.08	n.s.
Scl	**p.size**	-0.06	n.s.	0.20	*	0.17	x	0.09	n.s.	0.12	-	-	-	-	-	0.12	n.s.	0.30	***	0.20	*	0.00	n.s.	-0.10	n.s.
per	**connect.**	-0.18	***	0.30	**	0.32	***	-0.15	x	-0.06	-	-	-	-	-	-0.06	n.s.	0.16	x	0.01	n.s.	-0.16	x	0.01	n.s.
Tri	**p.size**	0.16	***	-0.04	n.s.	-0.04	n.s.	-0.05	n.s.	0.06	n.s.	0.13	n.s.	-0.05	n.s.	0.07	n.s.	0.04	n.s.	0.07	n.s.	-0.05	n.s.	-0.08	n.s.
arv	**connect.**	-0.15	**	0.10	n.s.	0.02	n.s.	0.15	n.s.	0.16	n.s.	0.18	n.s.	-0.12	**	0.18	n.s.	0.10	n.s.	0.18	n.s.	0.13	n.s.	0.10	n.s.
Teu	**p.size**	0.03	n.s.	-0.27	**	-0.18	*	0.06	n.s.	0.07	n.s.	0.06	n.s.	-0.07	n.s.	0.08	n.s.	0.01	n.s.	0.07	n.s.	0.08	n.s.	0.15	x
bot	**connect.**	-0.20	***	0.03	n.s.	-0.03	n.s.	0.16	x	-0.13	x	0.04	n.s.	-0.03	n.s.	-0.12	n.s.	-0.10	n.s.	-0.13	n.s.	-0.27	***	-0.22	**
Ver	**p.size**	0.06	*	-0.12	x	-0.11	n.s.	-0.04	n.s.	-0.02	n.s.	0.06	n.s.	-0.12	*	0.00	n.s.	-0.08	n.s.	-0.03	n.s.	0.07	n.s.	-0.13	x
dil	**connect.**	0.02	n.s.	0.07	n.s.	-0.01	n.s.	0.01	n.s.	0.05	n.s.	0.12	n.s.	0.17	**	0.12	n.s.	-0.09	n.s.	0.06	n.s.	0.08	n.s.	-0.18	**
Vic	**p.size**	0.06	n.s.	0.03	n.s.	-0.02	n.s.	0.06	n.s.	0.04	n.s.	0.19	n.s.	-0.22	n.s.	0.16	n.s.	0.21	n.s.	0.19	n.s.	0.15	x	0.13	x
hir	**connect.**	-0.10	*	0.08	n.s.	0.04	n.s.	0.10	n.s.	0.01	n.s.	-0.24	n.s.	0.35	n.s.	-0.30	n.s.	-0.23	n.s.	-0.33	n.s.	0.01	n.s.	-0.01	n.s.

Comparison of plant performance–population size or connectivity relationships in the field and in the experimental garden

Nine species were positively affected by population size both in the garden and in the field. Two species were negatively affected by population size in both environments (see Table 4). There are, however, 3 species (*Inula conyza, Myosotis ramosissima, Scleranthus perennis*) that performed worse in bigger populations in the field and better in the garden. Contrary, other 5 species (*Echium vulgare, Geranium columbinum, Melica transsilvanica, Origanum vulgare, Phleum phleoides*) performed better in bigger populations in the field but worse in the garden.

Four species were positively affected by population connectivity and other 6 species were negatively affected by connectivity in both environments (see Table 4). Other 6 species (*Geranium columbinum, Melica transsilvanica, Veronica dillenii, Vicia hirsuta* and partly *Dianthus carthusianorum,* and *Triforium arvense*) performed better in more connected populations in the field but worse in the garden. Other 3 species (*Fragaria viridis, Potentilla argentea, Scleranthus perennis*) performed worse in more connected populations in the field but better in the garden.

Table 4. Comparison of general effect of population size and connectivity on plant performance in the field and in the garden (conclusion the results from table 2 and 3).

	pop.size–performance relationships		pop.connectivity–performance relationships	
	FIELD	**GARDEN**	**FIELD**	**GARDEN**
Aci arv	++	+	--	--
Asp cyn	+	+	--	-
Are ser	-	-	-	-
Ara tha	++	+	--	-
Dia car	+	+	**++**	**+/-**
Echi vul	**++**	**+/-**	-	-
Fra vir	++	+	**-**	**+**
Ger col	**++**	**+/-**	**+**	**-**
Hie pil	++	+	++	+
Inu con	**-**	**+/-**	-	0
Myo ram	**-**	**+**	++	+
Mel tra	**+/-**	**-**	**+**	**--**
Ori vul	**++**	**--**	++	++
Pot arg	++	+	**-**	**+**
Phl phl	**++**	**+/-**	++	+
Scl per	**--**	**++**	**--**	**+/-**
Tri arv	+	+	**+**	**+/-**
Teu bot	-	-	-	--
Ver dil	++	++	**++**	**-**
Vic hir	-	0	**+/-**	**-**

Table 4 legend: ++ indicates positive relationship in at least 3 performance parameters, + is positive relationship in 1–2 performance parameters, +/- is positive relationship in some performance parameters and negative in others, - is negative relationship in 1–2 performance parameters, -- is negative relationship in at least 3 performance parameters, 0 is no significant relationship. For abbreviations of plant species see Table 1. Dark grey rows represent the decrease in population size or connectivity–performance relationship and light grey rows represent increase in this relationship of plants in the common garden compared to the field.

There were no significant correlations between standardised regression coefficients describing the relationship between population size or connectivity and the same performance parameters of each species measured in the field and in the experimental garden (see Appendix S2).

The effect of life-history traits on plant performance–population size or connectivity relationships

Insect-pollinated species produced lower proportion of undeveloped seeds with longer germination time when coming from bigger populations (Table 5A). Species, whose seeds are dispersed by zoochory and/or hydrochory, were higher in bigger populations in the field than species dispersed by anemochory and autochory. Similarly, species dispersing by zoochory and anemochory flowered more and produced more developed seeds in total (Fig. 1) and species dispersing by zoochory and autochory also allocated more to below-ground biomass in the garden when coming from bigger populations. Smaller proportion of undeveloped seeds in bigger populations in the field was also produced by species with lower terminal velocity. But contrary, species with lower average plant height germinated better and also established more viable seedlings than higher species when coming from bigger populations. Seed weight and also seed bank longevity had however no effect on plant performance–population size relationship. Also species that were more common in the studied landscape allocated more biomass to reproduction and their seeds germinated better in bigger populations in the field. Moreover, long-lived species were more positively affected by population size than short-lived species as they produced heavier seeds in bigger populations in the field (Fig. 2). Species with smaller SLA, i.e. with thicker leaves, allocated more biomass to reproduction in the garden when coming from bigger populations (Fig. 3).

Species from more connected populations (Table 5B) produced more flowers in the garden in case they are insect- or wind-pollinated species compared to those able of self-pollination. There was however no effect of breeding system or type of compatibility (self-compatibility or incompatibility) on plant performance–population connectivity relationship. Furthermore, species that are dispersed by anemochory or hydrochory produced more developed seeds and allocated more biomass to reproduction in the garden when coming from more connected populations compared to species dispersed by zoochory and autochory (Fig. 4). This is supported also by the fact that species with lower terminal velocity produced more developed seeds per flower in the field and also more developed seed per plant and more reproductive biomass in the garden when coming from more connected populations. Also species with lighter seeds flowered more, produced more developed seeds in total and allocated more biomass to reproduction as well as to above- and below-ground organs in the garden when coming from more connected populations. Species with less persistent seed bank (in sense of at least two years) germinated better and subsequently established more viable seedlings (Fig. 5) that were however smaller in above-ground biomass when coming from more connected populations in the field. Furthermore, species that were more common in the studied landscape produced heavier seeds and lower proportion of undeveloped seeds in more connected populations in the field. Also species with higher SLA performed better and allocated more biomass to reproduction in more connected populations in the field. Species with lower shoot–root allocation (allometric) slopes in the adult age (i.e. allocated proportionally more to below- compared to above-ground biomass with increasing size) were higher in more connected populations in the field.

Table 5. The effect of life-history traits on **(A) plant performance–population size** and **(B) plant performance–population connectivity relationships**. CSR-strategy, clonal index, breeding system and compatibility (self-compatibility/incompatibility) were not significant in any analysis and therefore are not shown in the table. The values in the table are R^2, level of significance (** p = 0.01-0.001; * p = 0.05-0.01; x marginally significant result with p = 0.05-0.1) and the direction of the effect (+/-) or the particular values in categorical variables. n.s. is not significant ($p > 0.1$). Devel_1_seeds is number of developed seeds per 1 flower, dev.seeds_total is number of developed seeds per whole plant, undev.seeds is proportion of undeveloped seeds. The performance parameters marked by 1 are for plants measured in the field and by 2 are for plants planted and measured in experimental garden, log_above-S and below-S are the seedlings′ biomass weights and log_above-A and below-A are the adult plants′ biomass weights in the garden. Allocation slope-S and -A represent shoot–root allocation (allometric) slopes for seedlings (S) and adult plants (A). Significant results ($p < 0.05$) are in dark grey and marginally significant results ($p < 0.1$) are in light grey.

A. Plant performance–population size relationships

		Pollination	Seed dispersal traits					Species abundance	Species life strategy			
	Pop. size	**pollination vector**	**dispersal type**	**terminal velocity**	**log seed weight**	**plant height**	**seed bank**	**freq. in landscape**	**plant life span**	**SLA**	**allocation slope-S**	**allocation slope-A**
Field	**height1**	n.s.	**0.375** * **zoo,hydro> anemo,auto**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	flowers1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	devel_1_seeds1	n.s.	n.s.	*0.173 x -*	n.s.	*0.162 x -*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	undev.seeds1	n.s.	n.s.	**0.239** * **+**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	dev.seed_total1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	seed_weight1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.170 x +*	**0.217** * **long>short-lived**	n.s.	n.s.	n.s.
	reprod.mass1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.254** * **+**	n.s.	n.s.	n.s.	n.s.
	germinability1	n.s.	n.s.	n.s.	n.s.	**0.188** * **-**	n.s.	**0.432** ** **+**	n.s.	n.s.	n.s.	*0.157 x -*
	T50	**0.364** * **insect > wind**	n.s.	n.s.	n.s.	n.s.	*0.174 x +*	n.s.	n.s.	n.s.	n.s.	n.s.
Garden – adult plants and seedlings	**recruitment**	n.s.	n.s.	n.s.	n.s.	**0.209** * **-**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	log_above-S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	log_below-S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	height2	n.s.	n.s.	n.s.	n.s.	n.s.	*0.150 x +*	n.s.	n.s.	n.s.	n.s.	n.s.
	flowers2	n.s.	**0.457** * **zoo,anem>hydr**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.180 x -*	n.s.	n.s.
	devel_1_seeds2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	undev.seeds2	**0.638** ** **wind > insect**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	dev.seed_total2	n.s.	**0.428** * **zoo,anem>hydr**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.205 x -*	n.s.	n.s.
	seed_weight2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	reprod.mass2	n.s.	n.s.	n.s.	*0.149 x +*	n.s.	n.s.	n.s.	*0.144 x* *long>short-lived*	**0.402** ** **-**	n.s.	n.s.
	log_above-A	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	log_below-A	n.s.	**0.472** * **zoo,auto>anemo,hydro**	*0.191 x +*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

B. Plant performance–population connectivity relationships

		Pollination	Seed dispersal traits					Species abundance	Species life strategy			
	Pop. connectivity	pollination vector	dispersal type	terminal velocity	log seed weight	plant height	seed bank	freq. in landscape	plant life span	SLA	allocation slope-S	allocation slope-A
Field	**height1**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.171* x +	n.s.	**0.207** * -
	flowers1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	devel_1_seeds1	n.s.	n.s.	**0.215** * -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	undev.seeds1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.217** * -	n.s.	n.s.	n.s.	n.s.
	dev.seed_total1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.148* x +	n.s.	*0.144* x -
	seed_weight1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.322** * +	n.s.	n.s.	*0.175* x -	n.s.
	reprod.mass1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.261** * +	n.s.	n.s.
	germinability1	n.s.	n.s.	n.s.	n.s.	*0.139* x -	n.s.	*0.160* x +	n.s.	n.s.	*0.174* x -	n.s.
	T50	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.191* x +	n.s.
Garden – adult plants and seedlings	**recruitment**	n.s.	n.s.	*0.144* x -	n.s.	n.s.	**0.207** * -	n.s.	n.s.	n.s.	n.s.	n.s.
	log_above-S	n.s.	n.s.	n.s.	n.s.	n.s.	**0.259** * +	n.s.	n.s.	n.s.	n.s.	n.s.
	log_below-S	n.s.	n.s.	n.s.	n.s.	n.s.	*0.158* x +	n.s.	n.s.	n.s.	n.s.	n.s.
	height2	*0.325* x *insect>self, wind*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	flowers2	**0.385** * **insect,wind > self**	*0.363* x *anemo,hydro> auto,zoo*	n.s.	**0.284** * -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	devel_1_seeds2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	undev.seeds2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	dev.seed_total2	n.s.	**0.506** * **anemo,hydro>zoo, auto**	**0.242** * -	**0.447** ** -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	seed_weight2	n.s.	*0.356* x *anemo>zoo*	*0.162* x -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.205* x -	n.s.
	reprod.mass2	n.s.	**0.439** * **anemo,hydro>zoo, auto**	**0.205** * -	**0.415** ** -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	log_above-A	n.s.	*0.373* x *zoo,hydro>auto*	*0.155* x -	**0.309** * -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	log_below-A	n.s.	n.s.	n.s.	**0.208** * -	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

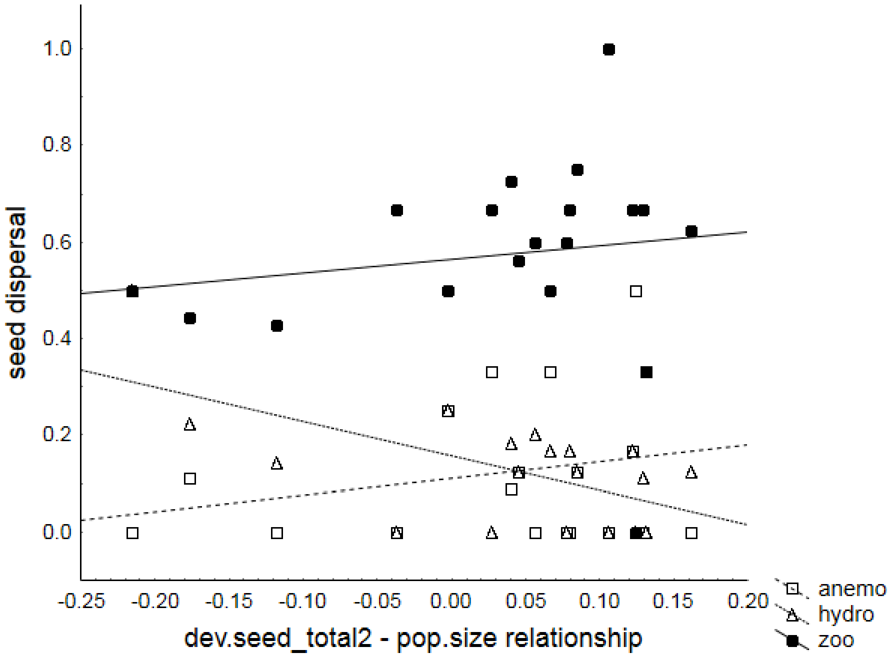


Figure 1. Effect of seed dispersal type (proportion of seeds dispersed by a given mode, y axis) on the relationship between number of developed seeds per plant measured in the garden and population size (x axis) of different species. The seed dispersal type was tested as multi-vector dispersal on a continuous scale, where all the categories were analysed together. For the whole model: P = 0.034, R^2 = 0.428.

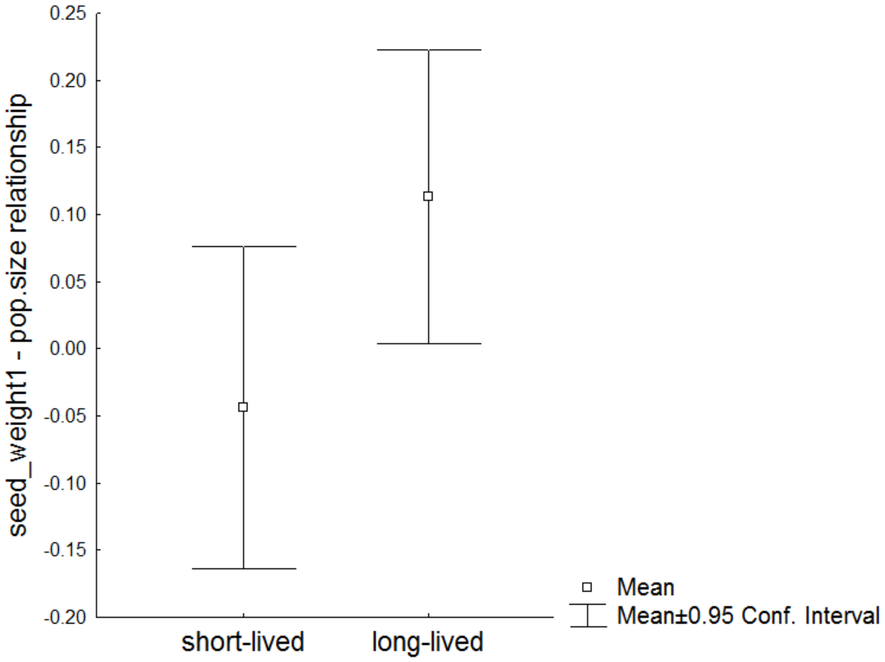


Figure 2. The relationship between seed weight of plants growing in the field and population size of short-lived and long-lived species. P = 0.045, R^2 = 0.217.

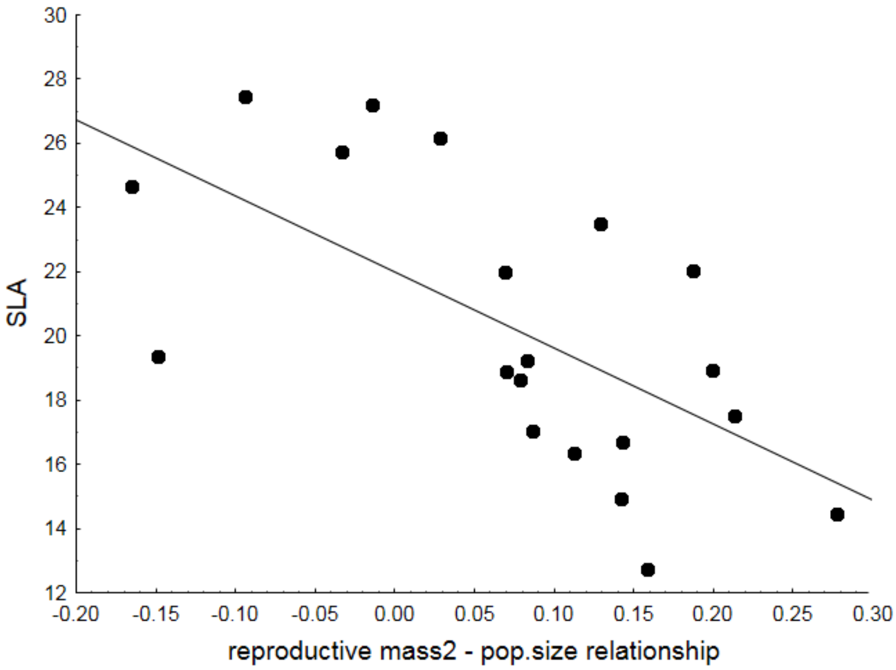


Figure 3. Effect of SLA (y axis) on the relationship between reproductive biomass measured in the garden and population size (x axis) of different species. P = 0.003, R^2 = 0.402.

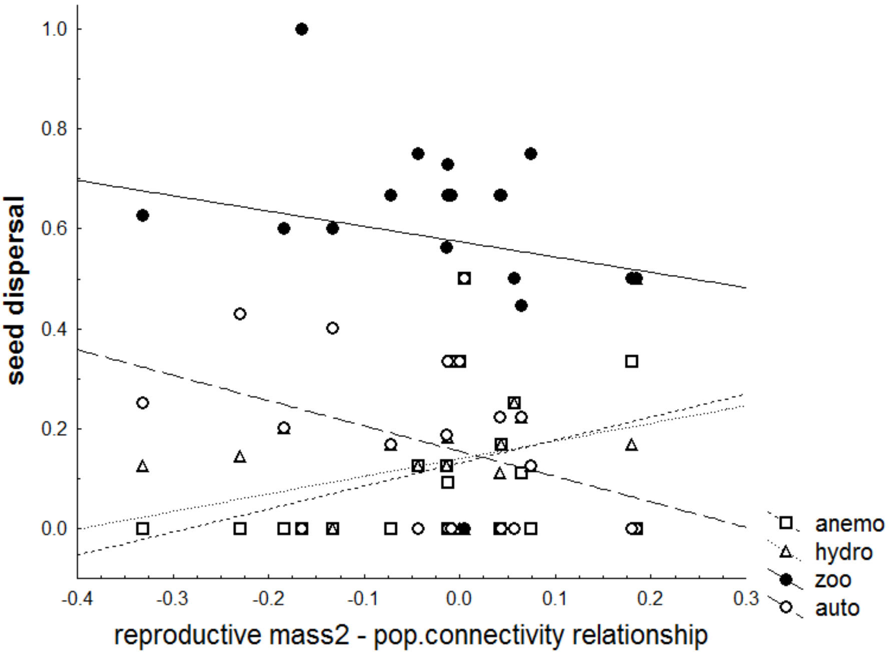


Figure 4. Effect of seed dispersal type (proportion of seeds dispersed by a given mode, y axis) on the relationship between reproductive biomass measured in the garden and population connectivity (x axis) of different species. The seed dispersal type was tested as multi-vector dispersal on a continuous scale, where all the categories were analysed together. For the whole model: P = 0.023, R^2 = 0.439.

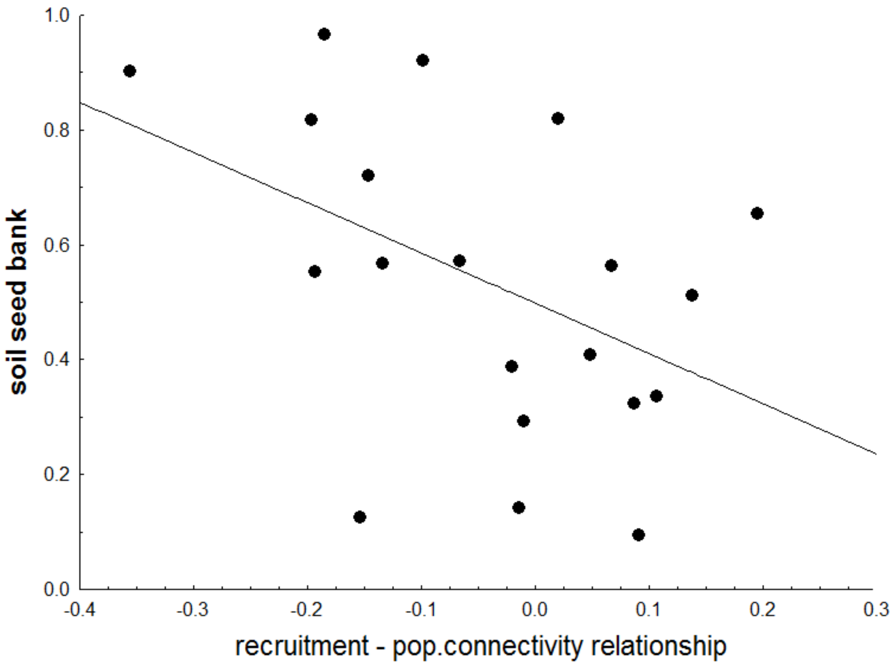


Figure 5. Effect of soil seed bank longevity (y axis) on the relationship between seedlings recruitment and population connectivity (x axis) of different species. P = 0.044, R^2 = 0.207.

DISCUSSION

Our results show that population size and connectivity significantly affected the performance of all the studied dry grassland species. Contrary to our expectation, the effects of decreasing population size and connectivity were not always negative, but at some species they were even positive. These effects were detected not only in the field, but also in the common garden environment, though they were weaker in the garden. This indicates that the differences in plant performance of most dry grassland species are not only due to differences in the actual habitat conditions (incl. biotic interactions) at the localities but also due to effects of their population size and connectivity *per se*. The between species comparisons identified range of species traits determining species sensitivity to changes in population size and connectivity. While the sets of traits determining species response to population size and connectivity are largely overlapping, still we also detected traits indicating response to one or the other factor only.

The effect of population size and connectivity on individual performance

We found that more than half of studied dry grassland species performed better in bigger and/or more connected populations. This finding is in agreement with our expectation as well as with many previous studies that also found positive effect of population size (e.g. Fischer and Matthies 1998; Kéry et al. 2000; Mavraganis and Eckert 2001; Jacquemyn et al. 2002, 2007; Kolb 2005; Adrieans et al. 2009; Tsaliky and Diekmann2010; Hornemann et al. 2012; Lauterbach et al. 2012; Putz et al. 2015; Busch and Reisch 2016; Münzbergová et al.

2018) and/or negative effect of population isolation (partly Schleuning et al. 2009; Matesanz et al. 2015) on plant performance. Even though the population size and connectivity were not significantly positively correlated, similar species performed better in bigger and more connected populations and *vice versa*, but there were also some species that performed better in bigger and more isolated populations or in smaller and better connected ones. The species most negatively affected by small population size and decreasing connectivity directly in the field were seven species with very different life span/form, type of pollination or breeding/compatibility system (*Dianthus carthusianorum agg., Geranium columbinum, Hieracium pilosella, Origanum vulgare, Phleum phleoides, Triforium arvense, Veronica dillenii*).

Lower performance of plants in small and isolated habitats may be caused by pollen and pollinator limitation and/or low level of genetic variation and inbreeding depression (Groom 1998, 2001; Hooftman et al. 2003; Kolb 2005). Also changed abiotic and biotic conditions at some localities (e.g. encroaching by shrubs and eutrophication, as a consequence of the ongoing landscape fragmentation (Eriksson et al. 2002; Soons and Heil 2002)) can reduce their growth and reproduction. While we attempted to account for differences in local abiotic conditions using covariates, it is likely that we were not able to capture all possible differences.

Despite the prevailing negative effect of reduced population size and connectivity, we also found opposite trends in several species. While such opposite trends are rare, they have been previously detected e.g. by Luijten et al. (2000); Hooftman et al. (2003); Kolb and Lindhorst (2006); Schleuning et al. (2009); Putz et al. (2015). The possible explanation of this pattern is that small and isolated populations have better specific abiotic or biotic conditions (even though we accounted for local abiotic conditions and position of each locality). For example, plants in these small and isolated populations may be less attacked by herbivores or pathogens (Soons 2003; Groom 2001; Münzbergová 2006b) and thus the seed production and proportion of developed seeds is not reduced by seed predators.

Other possible explanation is that these species can persist in small and isolated populations from the past, when their localities were larger and more inter-connected. This explanation is possible for long-lived perennial species (in our system *Scleranthus perennis* and *Inula conyza*) as old established individuals may still reflect a historical situation with different habitat configuration. Reduced size or increased isolation of these populations may support higher survival of heterozygotes due to a higher fitness of heterozygotes and/or a lower fitness of homozygotes (inbreeding depression) (Van Geert et al. 2008).

Also for this reason, it is important to realise common garden experiments that enable not only indicate whether a part of variation in plant performance is due to differences in the actual environmental (abiotic as well as biotic) conditions at localities (Fischer and Matthies 1998), but also enable to study new individuals, i.e. those, with possibly already reduced genetic variation and thus those individuals really responding to current populations and landscape configuration (Van Geert et al. 2008).

In comparison with the field results, we found fewer significant effects of population size and connectivity on plant performance in the common garden environment, but still most species were significantly affected by the population size and/or connectivity of their parent's plants at least in some performance parameters. These results demonstrate that not only actual abiotic and biotic conditions (incl. Allee effect) at localities but also other processes can significantly affect performance of dry grassland species.

Species showing negative response to reduced population size and/or connectivity often show such a response in both the field and the common garden. This indicates that these species suffer from negative consequences of inbreeding and loss of genetic variation within populations due to genetic drift and reduced gene flow and accumulation of deleterious mutations (Kéry et al. 2000; Jacquemyn et al. 2002; Hensen et al. 2005; Hensen and Oberprieler 2005; Kolb 2005; Leimu et al. 2006; Van Geert et al. 2008; Lauterbach et al. 2012; Dostálek et al. 2010).

The agreement between patterns detected in the field and in the common garden can however be also due to trans-generational effects (Latzel 2015). In this case, the offspring phenotype is influenced by a parental phenotype independently of the genes that the parents pass to the offspring. Environmental maternal effects mediated by seed size (i.e. the seed size is influenced by the environment in which the mother plant was growing (Roach and Wulff 1987)) can play a particularly significant role during early stages of plant development, with decreased relevance during maturity (Weiner et al. 1997; Latzel 2015). These environmental maternal effects can probably explain the high number of significant relationships between population size or connectivity and seedling recruitment in the common garden environment. Especially at the species that were influenced by population size or connectivity only at seedling recruitment and/or seedling growth, these environmental maternal effects are the most probable explanation of differences between individual performances. The importance of environmental maternal effects for plant performance has also been demonstrated in some previous studies (Oostermeijer et al. 1994; Weiner et al. 1997; Münzbergová and Plačková 2010). Moreover, epigenetic trans-generational effects can alter phenotypes for the entire lives of individuals and even for several generations (Latzel 2015). To control for trans-generational effects, at least two generations of plants would be needed to grow in a common garden environment prior to the main experiment (Latzel 2015). This control however is not possible in our case, as can mask some other factors determining between populations differences (e.g. due to limited pollination only within the plants in experiment).

At some other species we however found negative effects of population size and/or their connectivity in both environments, which indicate that, except possible maternal and epigenetic effects described above (for *Teucrium botrys* in relation to population size and for *Arabidopsis thaliana* and *Arenaria serpyllifolia* in relation to connectivity), these species can really perform better in small (*Arenaria serpyllifolia*) or isolated (*Acinos arvensis, Asperula cynanchica, Echium vulgare, Teucrium botrys*) populations and that this pattern is not only due to local abiotic and biotic conditions, but rather by the population size or connectivity *per se*. Similar pattern was found also in several other studies (Luijten et al. 2000; Hooftmann et al. 2003; Kolb and Lindhorst 2006; Schleuning et al. 2009). The possible explanation is that differences in performance between plants from different populations are more likely to be caused by differential selection pressures between populations, than by genetic drift. In small and isolated populations, the number and diversity of pollinating insects may decrease, which in insect-pollinated species, may result in pollen limitation, and consequently reduced reproduction (Ågren 1996). Therefore, plants, which produce more flowers and thereby attract more pollinators, may be selected for (Hooftmann et al. 2003). Selection can also favor the survival of heterozygotes in small populations which, in result, increases the plants fitness/performance in small populations (Luijten et al. 2000). Contrary, lower performance of plants in large populations could be due to increased inbreeding because of pollination between closely related neighboring plants in dense and large populations (Schleuning et al. 2009).

On the other hand, other two species performed better in small (*Vicia hirsuta*) or isolated populations (*Inula conyza*) only in the field, but this effect disappeared when growing in the garden. This indicate, that their performance is mostly driven by specific factors of the local environmental (abiotic and biotic) conditions at localities including for example lower level of herbivory, especially seed predators (Groom 2001), and/or higher level of resources (nutrients) (Knight et al. 2005; Červenková and Münzbergová 2014) which allow them to produce more developed and/or lower proportion of undeveloped seeds in smaller or more isolated populations compared to bigger and more inter-connected ones, but these species are otherwise not affected by population size or connectivity.

At several other species, we also found that they performed better or worse in bigger and/or more inter-connected populations in the field, but they showed the opposite pattern in the garden. In such cases, local abiotic and biotic conditions at localities and thus disadvantages and advantages of small and isolated populations, such as e.g. Allee effect, changed level of herbivory, resource availability etc., overlaps the genetic consequences (either negative or positive) or possible maternal as well as epigenetic trans-generational effects of small and isolated populations in the field, that can however been expressed at the plants grown in the common garden environment, coming from different populations in the field. Such pattern thus demonstrates the huge importance of local conditions at localities for plant performance in different populations.

The effect of life-history traits on plant performance–population size or connectivity relationships

According to our expectation we found big differences in plant performance–population size or connectivity relationships between studied dry grassland species and the strength and direction of these relationships largely depend on species life-history traits. In agreement with previous studies (Lienert 2004; Aguilar et al. 2006; Kolb and Lindhorst 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007), we found relatively high importance of pollination mode showing that insect-pollinated species were more strongly positively affected by population size and connectivity, whereas species able of self-pollination were less negatively affected by population isolation. These findings thus show that insect-pollinated species, i.e. species more dependent on their pollinators, are more susceptible to small population size and connectivity because of possible Alee effect and, subsequently, pollen and pollinator limitation in small and isolated populations (Ågren 1996; Groom 1998; Kolb 2005, 2008; Peterson et al. 2008; Dauber et al. 2010). Contrary to the previous studies, we however found no effect of breeding system or type of compatibility (self-compatibility or incompatibility) on plant performance–population size or connectivity relationship. In contrast to some previous studies (Lienert 2004; Kolb and Lindhorst 2006) but in agreement with other study (Honnay and Jacquemyn 2007), also clonality and thus the ability of vegetative reproduction did not have any effect on plant performance of our studied species.

In line with our expectation and study of Angeloni et al. (2011) but in contrast to other previous studies, that did not find any effects (Aguilar et al. 2006; Leimu et al. 2006; Honnay and Jacquemyn 2007), we found that long-lived species and species with lower SLA (i.e. slow-growing species; Hemrová and Münzbergová 2015) are more susceptible to decreasing population size in comparison to short-lived species with higher SLA. Such pattern can be explained by the fact that longer-lived slow-growing species may be more sensitive to higher pollen and pollinator limitation (as they are mainly outcrossing) and thus may suffer stronger inbreeding depression. In addition, they may also be more sensitive to lower habitat quality in smaller populations. In contrast, slow-growing species (i.e. with lower SLA) and species with higher shoot–root allocation slope at adult plants, allocating proportionally more to above- than to below-ground biomass with increasing size, which is typical for later flowering species with lower SLA (Husáková et al. 2018) are less sensitive to habitat isolation than faster-growing species. This indicates that reduction in population size represent an immediate danger for long-lived species likely due to reduced pollinator availability and more intense inbreeding depression. In contrast, the effects of habitat isolation are slower and the long-lived species may respond to it just with a certain time lag (Münzbergová et al. 2013; Takkis et al. 2013).

Seed dispersal characteristics turned out to be the best predictors of the plant performance in relation to different population size and connectivity, though their effects have not been studied previously. Especially, species that are dispersed by animals and also by wind or water and/or have lower terminal velocity (i.e. adaptation to long-term dispersal by wind) are more sensitive to decreasing population size. Species dispersed by animals are, however, also less sensitive to decreasing population connectivity. As the study area is known with the high concentration of hoofed game, especially mouflons (Kolbek et al. 2003), which preferably concentrate their feeding and relaxation activities just at dry grassland localities, they thus can support effective seed dispersal directly towards suitable, even isolated, habitats (Husáková and Münzbergová 2016) and thus also ensure sufficient gene flow between populations. In addition, also species with no specific dispersal adaptations are less negatively affected by decreasing connectivity and performed better in more isolated populations. Such species can have some mechanisms enabling them the long-term persistence, such as for example clonality or ability of self-pollination and self-compatibility, as was shown by

Lienert (2004) or Kolb and Lindhorst (2006). Moreover, species performing better in more isolated populations have usually heavier seeds and higher terminal velocity and thus they are not adapted to wind dispersal but rather for persisting at the site. Also species with more persistent seed bank performed better in more isolated populations compared to species with no or transient seed bank, which can serve as reservoir of the genetic diversity of the populations and may at least partly contribute to genetic extinction debt of the populations as recently demonstrated Plue et al. (2017) and Münzbergová et al. (2018).

In accordance with few previous studies (partly Hooftman et al. 2003; Lienert 2004; Honnay and Jacquemyn 2007; Angeloni et al. 2011; Takkis et al. 2013), we also found that more common species within our study area were more sensitive to declining population size and connectivity, while rare species were less negatively affected by habitat fragmentation. This suggests that some species may be able to adapt to live in small and isolated populations.

In summary, our results thus demonstrate, that performance of common habitat specialist can be strongly affected by habitat fragmentation and that the strength and direction of these relationships largely depend on species life-history traits, mostly those connected with their seed dispersal and pollination and less importantly also with their longevity.

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SUPPORTING INFORMATION

Appendix S1. Mean, minimal and maximal value of population size and connectivity for studied species. Population size is represented by number of all flowering individuals per population and locality. Population connectivity is represented by opposite values of isolation index, where higher value of population connectivity express more connected population and provides information about how large and distant populations surround the target population for which the isolation index was calculated.

	Population size			Population connectivity		
Studied species	**mean**	**min**	**max**	**mean**	**min**	**max**
Acinos arvensis	135	21	493	0.3	0.02	1.09
Arabidopsis thaliana	122	3	1020	0.32	0.04	0.79
Arenaria serpyllifolia agg.	11516	140	69025	0.33	0.06	0.68
Asperula cynanchica	7321	12	87850	0.47	0.05	1.41
Dianthus carthusianorum agg.	338	2	5075	0.49	0.01	1.55
Echium vulgare	372	8	4598	0.35	0.06	0.82
Fragaria viridis	6518	426	39600	0.62	0.27	1
Geranium columbinum	1720	45	8580	0.38	0.09	0.95
Hieracium pilosella	686	84	2440	0.67	0.09	2.18
Inula conyzae	17	3	94	0.33	0.11	0.78
Jasione montana	29	4	147	0.77	0.07	2.78
Melica transsilvanica	4558	70	47059	0.31	0.03	0.93
Myosotis ramosissima	114	15	320	0.25	0.05	0.48
Origanum vulgare	3117	146	10638	0.55	0.14	1.3
Phleum phleoides	148	16	525	0.29	0	0.56
Potentilla argentea	883	59	6272	0.37	0.07	0.97
Scleranthus perennis	3611	50	24000	0.44	0.05	2.07
Teucrium botrys	433	17	6286	0.23	0.02	0.74
Trifolium arvense	27	6	97	0.31	0	0.89
Veronica dillenii	509	28	1500	0.42	0.07	0.99
Vicia hirsuta	143	12	1232	0.32	0.08	0.87

Appendix S2. Pairwise correlation matrix showing correlations between the relationships of population size or connectivity and the same performance parameters of each species (standardised regression coefficients) measured in the field and in the experimental garden. Devel_1_seeds is number of developed seeds per 1 flower, dev.seeds_total is number of developed seeds per whole plant, undev.seeds is proportion of undeveloped seeds. The values shown are the correlation coefficients. Values in italics and grey rows are marginally significant ($0.05 < p \leq 0.1$).

Field – garden	**Pop. size**	**Pop. connectivity**
height	0.197	0.181
flowers	-0.006	0.112
devel_1_seeds	-0.160	*0.409*
undev.seeds	0.246	0.130
dev.seed_total	-0.108	0.048
seed_weight	*0.441*	*0.414*
reprod.mass	-0.047	0.015

CHAPTER 4

Species traits and shoot–root biomass allocation in 20 dry-grassland species

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ABSTRACT

A plant has a limited amount of resources at any time and it allocates them to different structures. In spite of the large number of previous studies on allocation patterns within single species, knowledge of general patterns in species allocation is still very limited. This is because each study was done in different conditions using different methodology, making generalization difficult. We investigate intraspecific above- vs. below-ground biomass allocation among individuals across a spectrum of dry-grassland plant species at two different developmental stages and ask whether allocation is age- and species-specific, and whether differences among species can be explained by their life-history traits and phylogeny.

We collected data on above- and below-ground biomass of seedlings and adult plants of 20 species from a common garden experiment. We analysed data on shoot–root biomass allocation allometrically and studied the relationship between the allometric exponents (slopes on log-log scale), species life-history traits and phylogenetic distances.

We found isometric as well as allometric patterns of biomass allocation in the studied species. Seedlings and adult individuals of more than half of the species differed in their above- vs. below-ground biomass allometric exponents. Seedlings and adult individuals of the remaining species differed in their allometric coefficients (intercepts). Annual species generally allocated proportionally more to above- than below-ground biomass as seedlings than as adults, whereas perennial species showed the opposite pattern. Plant life-history traits, such as plant life span, age of first flowering, month in which the species begin flowering, and specific leaf area (SLA) were much more important in explaining differences in shoot–root allometry among species than were phylogenetic relationships. This suggests that allocation patterns vary greatly among closely related species but can be predicted based on species life-history traits.

Keywords Above-ground biomass; Below-ground biomass; Allometry; Isometry; Seedling; Adult plant; Life-history traits; Phylogenetic relatedness; Root–shoot ratio.

INTRODUCTION

Each plant has a given amount of resources at any time and it allocates them to different functions and structures. Different allocation patterns reflect different strategies that are the results of selection pressures and constraints (Weiner 2004; Weiner et al. 2009a). Similarly, changes in allocation during ontogeny reflect the changing priorities of an organism during its development.

Many studies have emphasized the variability in biomass partitioning observed across plants. These studies fall primarily within the optimal partitioning theory, also called the balanced-growth hypothesis, which suggests that plants should allocate biomass to the organ that acquires the most limiting resource (Bloom et al. 1985; Shipley and Meziane 2002; McCarthy and Enquist 2007).

Alternatively, variation in biomass allocation among plants can be driven also by differences in plant size (Coleman et al. 1994; McConnaughay and Coleman 1999; Müller et al. 2000; Reich 2002; Weiner 2004; Weiner et al. 2009a, b). Many morphological and physiological changes, including biomass allocation patterns, occur under the normal course of growth and development (Coleman et al. 1994; McConnaughay and Coleman 1999; Weiner 2004), a phenomenon sometimes referred to as ontogenetic drift (Evans 1972; Gedroc et al. 1996). For this reason, it is difficult to distinguish between variation in biomass allocation patterns due to resource limitations in the environment ("true" plasticity) from size-dependent allometric patterns ("apparent" plasticity; McConnaughay and Coleman 1999; Weiner 2004; McCarthy and Enquist 2007).

Another approach to explaining variability in biomass partitioning in relationship to plant size is allometric biomass partitioning theory (Niklas and Enquist 2002; Niklas 2006; Cheng and Niklas 2007). This theory predicts that plants must allocate biomass proportionally among plant parts (such as leaves, stem and roots) and examines how biomass allocation pattern among species changes with plant size according to allometric relationship which can be described by the allometric equation characterized by an allometric (scaling) exponent (slope of log-log relationship between mass of plant parts) and the allometric coefficient (intercept or "elevation" of the relationship). Under ideal conditions, allometric biomass partitioning theory often predicts that the scaling exponent is equal to one, which means that above- and below-ground biomass scale isometrically, independently of the plant size. Isometric scaling relationships have been documented in numerous studies at various scales – from individual level (Niklas and Enquist 2002; Niklas 2006; Cheng and Niklas 2007; Zhou et al. 2014; Cheng et al. 2015) to community level (Enquist and Niklas 2002; Yang et al. 2009, 2010; Cheng et al. 2015). Many other studies, however, revealed scaling exponent different from one, indicating an allometric (i.e. non-isometric) relationship between below- and above-ground biomass – at the individual (Müller et al. 2000; Weiner et al. 2009b; Wang et al. 2010; Li et al. 2013; Pan et al. 2013; Janeček et al. 2014; Lohier et al. 2014; Zhou et al. 2014) as well as community level (Wu et al. 2013; Wang et al. 2014a). Because allocation patterns changes with plant size, allocation should be analysed and interpreted allometrically (Weiner 2004; Weiner et al. 2009a,b) and not as ratios (e.g. root–shoot ratio) or proportions, which is the traditional and commonly used approach, in which allocation is assumed to be size independent (Mokany et al. 2006).

Biomass allocation patterns, including reproductive allocation, were traditionally studied using manipulative experiments studying the effect of two or more resource levels on biomass allocation of one or a few species, usually annual weeds or crops (e.g. Mao et al. 2012; Xie et al. 2012; Qin et al. 2013; Wang et al. 2014b) and only rarely for several species (Müller et al. 2000; Shipley and Meziane 2002). Most of the data have been collected at one point in time or at the same plant age (McConnaughay and Coleman 1999). Such studies did not usually take plant size into account in interpreting the differences in allocation patterns at different resource levels. More recent studies, however, have considered plant size and demonstrated the importance of ontogenetic drift, sometimes showing no or only small but significant direct effects of resources or other environmental factors on allometric patterns (Gedroc et al. 1996; Müller et al. 2000; Janeček et al. 2014; Lohier et al. 2014).

Biomass allocation patterns could also vary with plant life-history strategy, but studies comparing species of different strategies are rare. For annual plants, it was found that the proportion of allocation to roots declines during growth and development (Coleman et al. 1994; Gedroc et al. 1996; McConnaughay and Coleman 1999). The direction of preferential allocation to roots or shoots over the course of ontogeny is more complicated and less consistent among perennial plants. The proportion of allocation to roots increased during ontogeny in some perennial species (e.g. *Leontodon hispidus* – Niinemets 2004) whereas it decreased in others (e.g. *Plantago lanceolata* – Janeček et al. 2014). Meta-analyses by Poorter et al. (2012) compared a large number of species from a wide range of published experiments in the literature (especially focused on the effect of resource levels on allocation of species) and found that most herbaceous species show a trend towards a decreased root allocation and an increased shoot allocation with size, but there was no evidence for differences in allocation patterns between perennials and annuals. Their findings were based on a range of different studies performed with different methods. This variation could mask individual species differences. It was also found that perennials exhibited "apparent" plasticity in relation to different resource levels and did not change their allocation strategy whereas annuals did, thus exhibiting "true" plasticity (Mao et al. 2012). Similarly, Zhou et al. (2014) found isometric allocation or evidence for the optimal partitioning theory in annuals but allometric allocation in ephemeral perennials. These two studies (Mao et al. 2012; Zhou et al. 2014) included very few (1–3) species within each category. For allocation to reproduction, a review of studies on more than 50 different species found isometric allocation in many short-lived and clonal plants, but allometric allocation by longer-lived plants (Weiner et al. 2009a).

Differences in biomass allocation patterns of species may also depend on many other life-history traits, such as growth form, Grime's CSR ecological strategy (competitors/stress-tolerators/ruderals), seed size, age of first flowering, clonality, stem height, or leaf traits such as specific leaf area (SLA), leaf thickness or leaf longevity, but almost nothing is known about these effects. An exception is the study based on a global dataset, which showed the importance of growth form for allocation into above-ground organs and positive relationship of leaf mass with leaf life span and negative relationship with SLA and maximum photosynthetic rate (McCarthy et al. 2007). Another study (Fortunel et al. 2009) found that early successional species allocated proportionally more biomass into reproduction than later successional species, consistent with high colonization abilities of early successional species and high competitive ability of later successional species. Seed size can also be important for seedling biomass allocation as was demonstrated within a single species (Chacon et al. 1998). But it is not clear if this relationship applies to other species or across species.

It could be expected that more closely related species will show more similar allocation patterns than distantly related species. Phylogeny was the strongest predictor of biomass allocation pattern in a global dataset at higher taxonomic levels (i.e. between clades and families) and across a wide spectrum of plant sizes (McCarthy et al. 2007; McCarthy and Enquist 2007). Nothing is, however, known on the importance of phylogenetic relatedness for biomass allocation patterns at lower taxonomic levels – i.e. between different species from the same habitat and vegetation type.

In spite of the relatively high number of studies carried out on biomass allocation, most of them have focussed on the effect of various resource levels on allocation pattern of one or a few species. The published studies used very different methodologies, making comparisons among different life-history traits and phylogenetic relatedness of different species coming from the same vegetation type difficult. Moreover, there are three fundamentally different kinds of allometric relationships, which address very different questions, but have been conflated throughout much of the literature: (1) Broad interspecific, (2) Static intraspecific among individuals within a species, (3) Allometric growth of individuals (Weiner et al. 2009a). Mixing of these three types of comparisons further complicates any comparative studies.

The aim of this study was to investigate the pattern of above- versus below-ground biomass allocation across a spectrum of dry-grassland plant species within one community. By comparing data from each species separately and comparing only the resulting coefficients among species, we investigate static intraspecific allometry in our study. We ask the following questions: (i) How does allocation to above- vs. below-ground biomass differ (a) between seedlings and adult plants within and (b) among species? (ii) Can we explain the between species differences in allocation pattern in terms of species life-history traits? (iii) Do closely related species within the same community show more similar allometric relationships than less related species? We hypothesize that allocation of biomass into different structures changes during ontogeny, that allocation will vary among different life spans/forms, and that closely related species will have similar allocation patterns because of their shared evolutionary history.

To answer these questions, we set up a common garden experiment on above- and below-ground biomass of seedlings and adult plants of 20 dry-grassland species from the same community, including species from different families and with different life-history traits. We tested whether the slope of the relationship between allocation into above- and below-ground biomass of each species and each age category is significantly different from one (isometry) and whether the allocation slopes differ between seedlings and adult plants of each species. Then we used the values of allocation slopes and tested them against species life-history traits and phylogenetic distances.

MATERIALS AND METHODS

Study species and data collection

Twenty herbaceous dry-grassland plant species from a wide taxonomic range (20 genera in 11 families) and differing in life-history traits were selected to study the general pattern of biomass allocation above- vs. below-ground (Table 1). We collected seeds of all these species in the field from natural dry-grassland plant communities in forest openings in the Protected Landscape Area and Biosphere Reserve Křivoklátsko in the Czech Republic. We sampled 120–200 individuals of each species (for details see Table 1) occurring at 12–20 localities to obtain a sufficient number of seeds. In autumn of the year when we collected seeds (2009–2011) or in spring of the following year (depending on whether the seeds require cold stratification), we sowed a given number of seeds from each individual into 15 × 15 × 15 cm pots in a substrate consisting of 2 parts garden soil to 1 part sand in an experimental garden. The number of seeds per pot differed among species, reflecting differences in seed size (Table 1).

Two or three weeks after most of seeds of each species started to germinate, we counted the number of individuals ("seedlings") and removed them from the soil. We left one randomly-chosen individual in each pot to study its later growth. Because all individuals were very small at this time, there was no competition among them. Due to the small size of individuals and their root systems as well as the sandy substrate, it was easy to extract the whole root system of each plant from the soil. All harvested seedlings were washed in water to remove residual soil, divided into above- and below-ground parts, dried to constant weight at 70° C and weighed. We refer to these as "seedlings".

The remaining plants were left to grow in the pots in the experimental garden until most of their fruits were mature (4–16 months) and then were harvested. The plants were carefully extracted from the substrate and we were able to extract almost all the root biomass for each individual. The plants did not experience competition as there was only one plant per pot. As with the seedlings, we divided the individuals into above- and below-ground parts, washed the below-ground parts and dried to constant weight at 70°C and weighed them. In the following text we refer to these as "adult plants". All flowers and fruits with seeds were included in the above-ground biomass of adult plants.

Table 1. Studied species.

Studied species	Abbrev.	Life span	No. of sampled individuals and pots in the exp.	No. of sown seeds per pot
Acinos arvensis	Aci arv	annual	170	40
Arabidopsis thaliana	Ara tha	annual	200	100
Arenaria serpyllifolia agg.	Are ser	annual	200	50
Asperula cynanchica	Asp cyn	perennial	170	30
Dianthus carthusianorum agg.	Dia car	perennial	138	20
Echium vulgare	Echi vul	perennial	180	20
Fragaria viridis	Fra vir	perennial	186	20
Geranium columbinum	Ger col	annual	200	20
Hieracium pilosella	Hie pil	perennial	200	20
Inula conyzae	Inu con	perennial	121	50
Melica transsilvanica	Mel tra	perennial	200	50
Myosotis ramosissima	Myo rha	annual	200	20
Origanum vulgare	Ori vul	perennial	200	40
Phleum phleoides	Phl phl	perennial	200	50
Potentilla argentea	Pot arg	perennial	200	50
Scleranthus perennis	Scl per	perennial	120	50
Teucrium botrys	Teu bot	annual	141	30
Trifolium arvense	Tri arv	annual	200	40
Veronica dillenii	Ver dil	annual	200	40
Vicia hirsuta	Vic hir	annual	163	10

Life-history traits and phylogeny of the studied species

To interpret differences among the species in their allocation to above- vs. below-ground biomass, we collected data about their life-history traits from several databases as well as making our own measurements using the same methods used in the databases. We measured the seed weight for all individuals of each species that we sampled and calculated the average seed weight per species. We used the data from the experiment to determine if individuals of each species were able to flower in their first season (i.e., if most of individuals of a species flowered in the first year). We collected data on plant life span and specific leaf area (SLA) from the Leda Trait base (Kleyer et al. 2008); plant height and month in which each species usually starts to flower (which corresponded to our observations) from Kubát et al. (2002); clonality of each species from CloPla database (Klimešová and de Bello 2009, http://clopla.butbn.cas.cz), and CSR strategy and leaf persistence from BiolFlor database (http://www2.ufz.de/billfold/index.jsp).

To assess phylogenetic distance between the species we used the Daphne database (Durka and Michalski 2012, http://www.esajournals.org/doi/abs/10.1890/12-0743.1) and calculated matrix of pairwise phylogenetic distances between studied species using R software – Ape package (Paradis et al. 2004).

Statistical analysis

Analysis of above- versus below-ground biomass allocation

Since there are no dependent and independent variables in allometric analyses and both variables have errors, the analyses of the above- vs. below-ground biomass relationships were performed using the standardized major axis (SMA) regression on log–log-transformed data using SMATR package (version 3.4-3; http://bio.mq.edu.au/ecology/SMATR/) in R (version 3.0.2; https://www.r-project.org/). SMA is appropriate when the purpose is to estimate the linear relationship between two variables, especially when the slope of the relationship is of primary interest (Warton et al. 2012).

To ask whether the allocation patterns of the studied species are species and age specific, we first tested the effect of each factor separately using SMA. Because both factors (species and age) were important for allocation patterns, we used ANCOVA with type III sum of squares (S-plus 4.6, MathSoft Inc. 1999, Washington, US) to test the effect of both factors as well as their interactions on allocation patterns (here expressed as log above-ground biomass ~ log below-ground biomass + species + age + species×age) and identify an effect of each particular factor without the effect of all the other factors. We used this analysis because it is not possible to use SMA to test the effect of multiple factors in one model.

We then asked if there is a significant relationship between allocation to above- and below-ground biomass of each species for each age category separately and if the slope of this relationship is significantly different from one (i.e. if the allocation pattern is allometric or isometric; Table 2). Tests of heterogeneity in slopes among seedlings and adult plants of each species were then performed separately (Warton et al. 2006). If there was no significant difference in slopes, we tested for difference in intercept (elevation on y-axis; Table 2), which is meaningful only when the allometric slopes are considered to be equal (Warton et al. 2006, 2012; Li et al. 2013). In addition, we also compared the values of biomass allocation for all seedlings and adult plants together to find out if there is any general allocation pattern for seedlings and adult plants (Table 2).

The effect of life-history traits on the allocation patterns of species

To identify the effect of life-history traits on allocation patterns of species at seedlings and adult plants the values of allocation slope of each species were tested against the species life-history traits in one model using stepwise linear regression, as well as separately using analysis of variance (ANOVA) or linear regression (S-plus 4.6). For each species we used one value of slope for seedlings and one value for adult plants (from previous analysis) and one average trait value. Thus, we had 20 points (species) in each test.

The effect of phylogeny on allocation pattern

For testing the effect of phylogeny on the allocation pattern of species separately for seedlings and adult plants, we calculated phylogenetic signal using Ape and Picante packages in R (Paradis et al. 2004 and Kembel et al. 2010) and used Blomberg K to express the effect of phylogenetic affinity (as the matrix of pairwise phylogenetic distances between species, expressed according to Daphne database; Durka and Michalski 2012) on the values of allocation slope of seedlings and adult plants of the studied species. We also calculated Pagel´s lambda with the "Crunch" and "Brunch" function in Caper package in R (Orme et al. 2013) to look for a phylogenetic signal in our data. Since the Pagel´s lambda as well as the Blomberg K revealed no phylogenetic signal in our data (see below), we did not perform phylogenetically corrected ("pgls") tests.

RESULTS

The effect of species and age

We found strong and significant effects of species and age as well as a species-age interaction on allocation pattern when all the other factors were included as covariates ($F = 98.16$, $p < 0.001$ for species, $F = 1114.09$, $p < 0.001$ for age, $F = 106.29$, $p < 0.001$ for species×age), indicating that allocation pattern at dry-grassland plants is species and age specific.

Allocation pattern into above- and below-ground biomass at seedlings and adult plants

Ten of the 20 species showed isometric (size independent) biomass allocation to shoots versus roots as seedlings, while the other ten species showed allometric allocation patterns, i.e. the allometric exponent, estimated from the log above- versus log below-ground biomass, was significantly different from one (Table 2A). Within the latter group, a slope lower than one indicates that larger individuals of these species have relatively more roots than shoots compared to smaller individuals (Table 2A and Fig. 1A, B). Overall, seedlings of all of these species had allometric allocation patterns for above- vs. below-ground biomass with a slope significantly lower than one, indicating that larger individuals generally have higher root–shoot ratio than smaller individuals (Table 2A).

For adult plants, 8 of the 20 species' allocation patterns were not significantly different from isometric, while allocation patterns of the other 12 species were significantly different from isometric (Table 2B). Five of these 12 species had allometric slopes larger than one, which indicates that larger individuals have more above- compared to below-ground biomass than smaller individuals (Table 2B and Fig. 1A, B), whereas seven species had slopes less than one. Overall, adult individuals of all of these species had an above- vs. below-ground allometric allocation slope significantly lower than one, indicating that larger individuals generally have a higher root–shoot ratio than smaller individuals (Table 2B).

Comparison of allocation patterns of seedlings and adult plants

Twelve species' seedling–adult pairs differed significantly in their allocation slopes (Table 2C). Also, overall, seedlings of all species taken together significantly differed from adult individuals in their allocation patterns, with seedlings having higher allometric slopes

Table 2. Above- versus below-ground allometric relationships for seedlings and adult plants. A and B: the relationship between log above- and log below-ground biomass for seedlings (A) and adult plants (B). Depicted are R^2-values of the above- vs. below-ground relationship (significant in all cases, $p < 0.0001$) and allocation slopes of these relationships for each species. P-values, F stat. and d.f. are parameters of tests whether the allocation slopes are significantly different from 1, i.e., whether the relationship is allometric. Bold values are significantly different from 1. **C and D**: comparisons of allocation patterns of seedlings and adult plants of each species. **C**: test if there is significant difference between allocation slopes of seedlings and adult plants. In case of no significant difference, the results of tests for difference in intercept (**D**) are depicted, assuming the lines are parallel. For all these tests, p-values and statistical value (LR = the Bartlett-corrected likelihood ratio statistic in C and Wald statistic in D) are shown. Bold values are significant ($p < 0.05$). Direction of change in C means change between slopes of seedlings (S) and adult (A) plants and in D shift in intercept between seedlings (S) and adult (A) plants. For abbreviations of plant species see Table 1, "all": all species tested together.

	A. Seedlings: above- and below-ground allometry					**B. Adult plants: above- and below-ground allometry**					**C. Difference in slope of seedlings vs. adult plants**			**D. Difference in intercept**		
species	**slope**	**R^2**	**p-value**	**F stat.**	**d.f.**	**slope**	**R^2**	**p-value**	**F stat.**	**d.f.**	**p-value**	**LR**	**Direction of change**	**p-value**	**Wald**	**Direction**
Aci arv	1.058	0.734	0.21	1.59	131	**0.851**	0.406	**0.007**	7.421	168	**0.004**	8.435	**S>A**			
Ara tha	0.998	0.506	0.977	0.001	77	1.087	0.605	0.2	1.665	95	0.408	0.686	S<A	**< 0.001**	29.809	**S<A**
Are ser	0.95	0.802	0.137	2.238	170	1	0.753	0.998	0	46	0.531	0.392	S<A	0.07	3.278	
Asp cyn	**0.835**	0.818	**< 0.001**	21.218	118	0.904	0.396	0.102	2.705	161	0.277	1.183	S<A	**0.010**	6.717	**S<A**
Dia car	**0.713**	0.773	**< 0.001**	44.124	84	1.071	0.644	0.189	1.74	131	**< 0.001**	28.774	**S<A**			
Echi vul	0.948	0.709	0.41	0.687	71	**1.136**	0.769	**0.01**	6.815	96	**0.027**	4.921	**S<A**			
Fra vir	0.985	0.845	0.671	0.182	129	0.984	0.567	0.735	0.115	180	0.976	0.001	S=A	**< 0.001**	12.609	**S>A**
Ger col	1.027	0.797	0.458	0.553	162	**0.843**	0.387	**0.002**	9.457	198	**0.003**	8.79	**S>A**			
Hie pil	**0.89**	0.713	**0.018**	5.75	121	**0.751**	0.492	**< 0.001**	30.629	184	**0.018**	5.603	**S>A**			
Inu con	0.952	0.661	0.534	0.392	54	**1.694**	0.3	**< 0.001**	44.402	102	**< 0.001**	23.738	**S<A**			
Mel tra	**0.736**	0.387	**< 0.001**	24.768	157	**0.846**	0.627	**< 0.001**	13.856	184	0.071	3.254	S<A	**< 0.001**	12.621	**S<A**
Myo rha	0.896	0.545	0.143	2.187	82	0.922	0.305	0.179	1.818	191	0.765	0.089	S<A	**< 0.001**	87.419	**S<A**
Ori vul	**0.687**	0.393	**< 0.001**	17.013	70	0.97	0.545	0.528	0.399	194	**0.001**	10.412	**S<A**			
Phl phl	0.965	0.615	0.546	0.367	112	**0.866**	0.392	**0.013**	6.312	183	0.187	1.739	S>A	**0.005**	8.052	**S<A**
Pot arg	**0.89**	0.574	**0.014**	6.204	194	**1.211**	0.651	**< 0.001**	21.12	198	**< 0.001**	23.403	**S<A**			
Scl per	0.942	0.84	0.165	1.962	88	1.106	0.578	0.093	2.867	118	**0.030**	4.726	**S<A**			
Teu bot	**1.134**	0.854	**< 0.001**	13.644	126	**1.389**	0.692	**< 0.001**	29.421	81	**0.005**	8.056	**S<A**			
Tri arv	**1.167**	0.848	**0.011**	7.12	45	**1.125**	0.925	**0.001**	12.266	66	0.588	0.294	S>A	**0.001**	10.131	**S<A**
Ver dil	**0.772**	0.375	**< 0.001**	21.216	194	**0.422**	0.332	**< 0.001**	93.758	66	**< 0.001**	25.474	**S>A**			
Vic hir	**1.158**	0.765	**0.02**	5.736	62	**0.799**	0.435	**0.002**	9.786	108	**< 0.001**	14.706	**S>A**			
all	**0.881**	0.77	**< 0.001**	158.89	2285	**0.802**	0.752	**< 0.001**	557.38	2788	**< 0.001**	47.052	**S>A**			

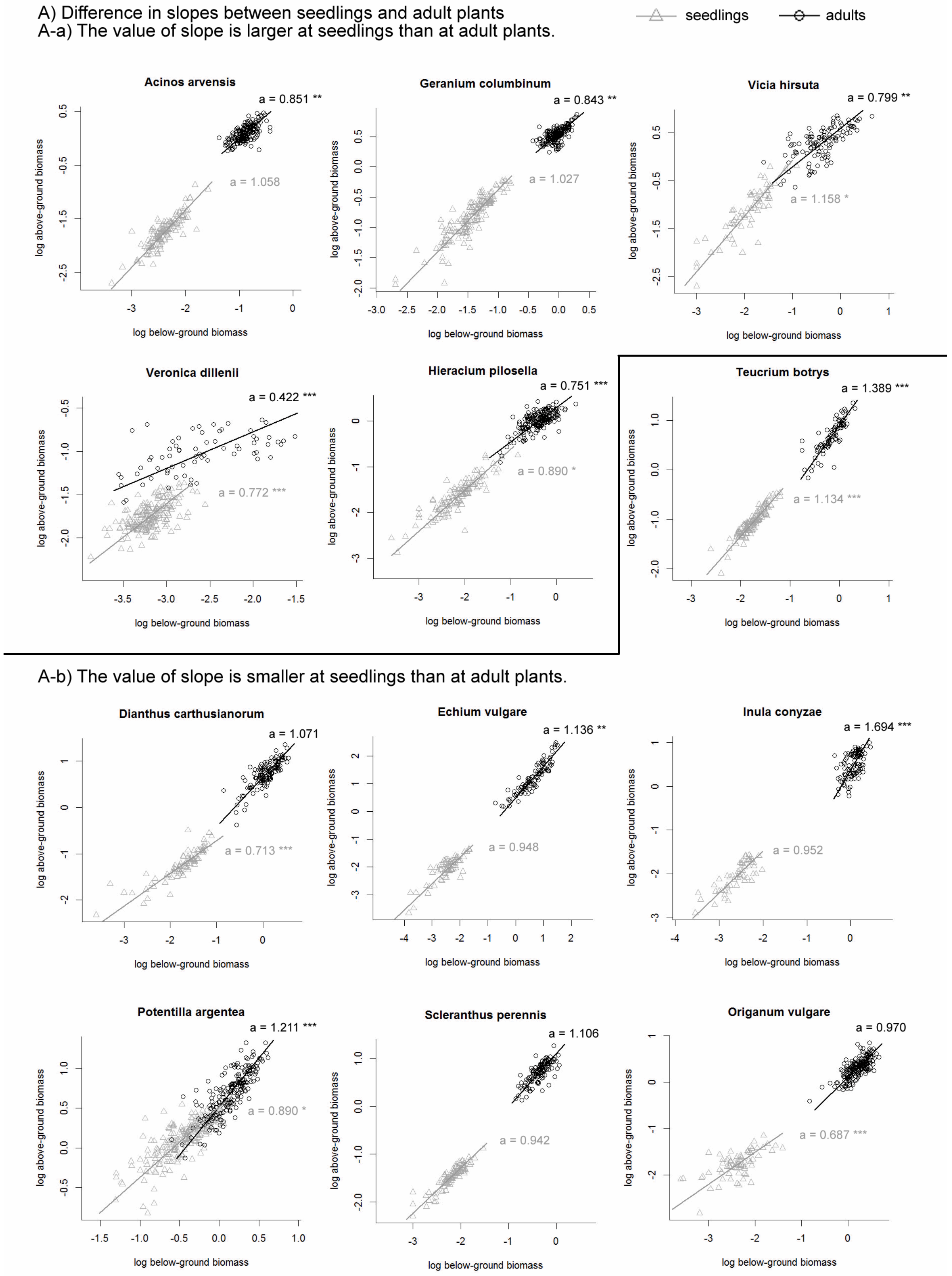

Figure 1: *part A (description below).*

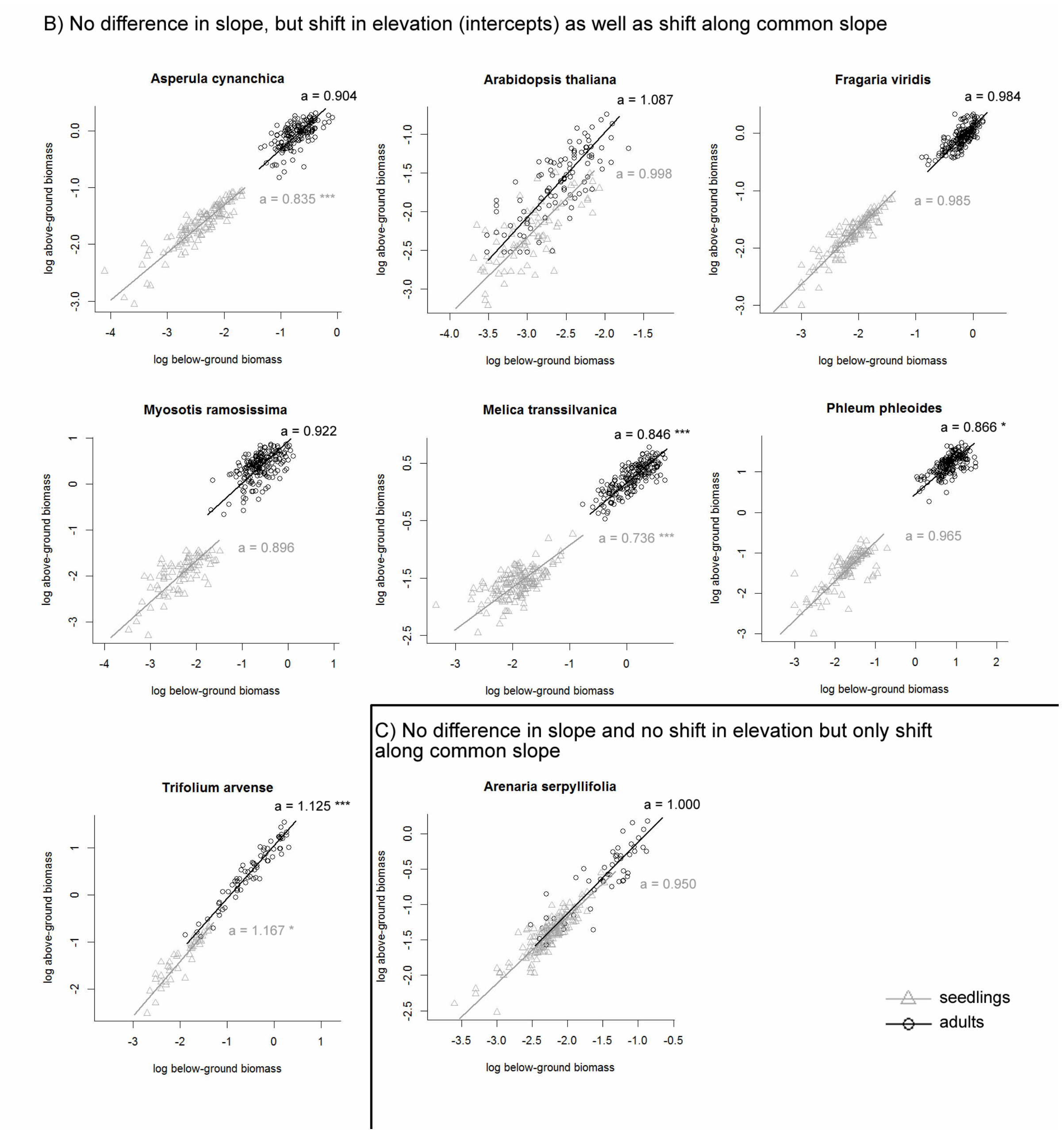

Figure 1: Comparison of seedlings and adult plants allocation patterns.

A: Shoot–root allometric slopes differ between seedlings and adult plants. A-a) The value of slope is significantly larger at seedlings than at adult plants of these species. A-b) The value of slope is significantly smaller at seedlings than at adult plants of these species.

B and C: Shoot–root allocation slopes do not differ between seedlings and adult plants, but there are B) shifts in elevation (intercepts) as well as shifts along common slope or C) only shift along common slope.

Grey triangles and lines are for seedlings, black circles and lines are for adult plants. Depicted are the values of allocation slopes (a), which were significant at probability levels of $p < 0.0001$ in all cases. *, ** and *** indicate whether the allocation slopes are significantly different from 1, i.e., whether the relationship is allometric, at probability levels of 0.05, 0.01 and 0.001, respectively.

than adults (Table 2C). There was shift in the value of slope from greater than one to less than one with increasing age in some annual species (Table 2C and Fig. 1A-a). On the other hand, there was shift from slope smaller than one to larger than one in late flowering (i.e. in the second season) perennial species (Table 2C and Fig. 1A-b).

We found a significant decline in the allometric slope between seedlings and adult plants for one annual (*Veronica dillenii*) and one perennial clonal species (*Hieracium pilosella*; Fig. 1A-a) but the slopes were still less than one in all cases. Larger individuals have more roots compared to shoots than smaller individuals, and this tendency is stronger for adults than for seedlings. On the other hand, one perennial clonal species (*Origanum vulgare*) and one annual species (*Teucrium botrys*) significantly increased their slopes from seedlings to adult plants but the slopes remained lower than one and higher than one, respectively (Fig. 1A-b).

The other eight seedling–adult species pairs did not differ in their above- vs. below-ground allometric slopes but there was a shift in elevation (i.e. y-intercept; Table 2D and Fig. 1B) or no difference between allocation patterns of seedlings vs. adults (*Arenaria serpyllifolia*; Fig. 1C).

The effect of life-history traits

Allometric patterns varied significantly with life-history traits (Table 3). Seedlings of annual species allocated proportionally more to above- compared to below-ground biomass with increasing size (Fig. 2). On the other hand, seedlings of perennials (Fig. 2) allocated proportionally more into below- compared to above-ground biomass with increasing size. We also found a marginally significant positive effect of seed weight on seedlings allocation preferences, indicating that species with heavier seeds allocated proportionally more in above- and less in below-ground biomass with increasing size than species with lighter seeds. There was a marginally significant effect of leaf persistence on the allocation pattern of seedlings. Plants with persistent green or overwintering green leaves tended to allocate proportionally more into above- compared to below-ground biomass with increasing size than did plants with deciduous green leaves. SLA was selected by stepwise regression as the second most important trait related to differences in allocation for seedlings after life span.

We did not find a significant difference in allocation pattern between annuals vs. perennials at adult plants. But species with higher allometric slopes in the adult stage (i.e. allocated proportionally more to above- compared to below-ground biomass with increasing size) usually did not flower in the first year of life (Fig. 3) and/or started flowering later in the growth season (usually from June and July; Fig. 4). Adult plants with lower SLA (Fig. 5) allocated proportionally more to above- compared to below-ground biomass with increasing size.

Table 3. The effect of species traits on the average value of shoot–root allometric slope of each species.
Shoot–root allometric slope for seedlings and for adult plants. Average values of species life-history traits: log seed weight (mg), life span (A = annuals, P = perennials), CSR strategy, flowering in 1st year of species life (yes, no), flowering from which month, clonal growth (yes, no), average plant height (m), SLA (mm^2/mg), leaf persistence (p = persistent green leaves, w = overwintering green leaves, s = summer green leaves). Results of stepwise regression (* indicates selected trait) and linear regression or ANOVA with the R^2 and p-values and the direction of the effect (+/-) or the particular value are presented. Bold values are significant ($p < 0.05$).

	Shoot–root allometric slope for seedlings				Shoot–root allometric slope for adult plants			
	selected in stepwise regression	**p**	**R^2**	**effect**	**selected in stepwise regression**	**p**	**R^2**	**effect**
log seed weight	*	0.091	0.151	+		0.850	-	
life span	*	**0.012**	**0.304**	A>P		0.353	-	
CSR strategy		0.501	-			0.619	-	
flowering in 1st year		0.737	-			**0.049**	**0.198**	no>yes
flowering from (month)		0.893	-		*	**0.004**	**0.375**	later flowering
clonal		0.171	-			0.833	-	
plant height		0.538	-			0.288	-	
SLA	*	0.774	-			**0.044**	**0.207**	-
leaf persistence		0.081	0.256	w>p>s		0.376	-	

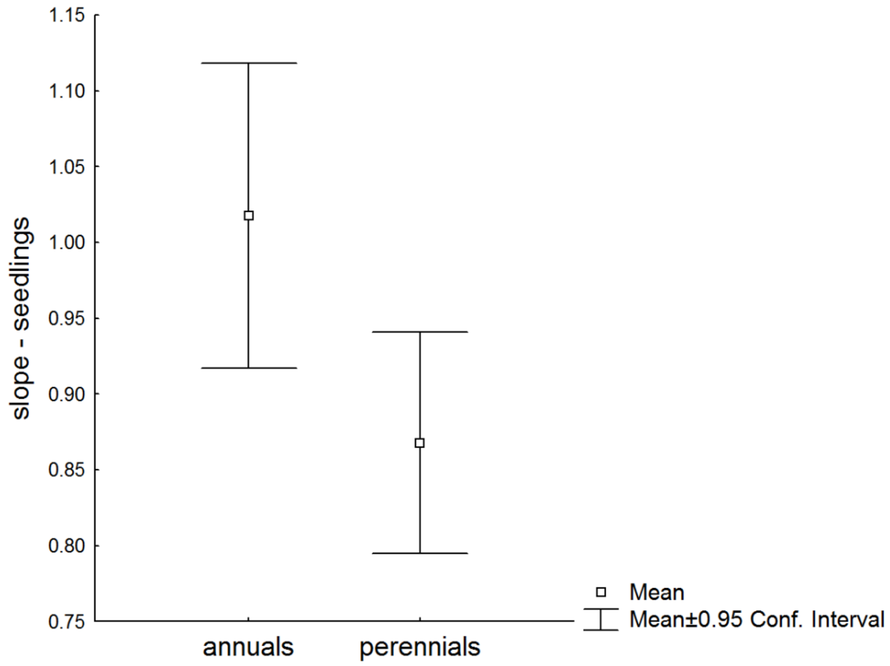

Figure 2: Shoot–root allometric slope of seedlings. P = 0.012, R^2 = 0.304.

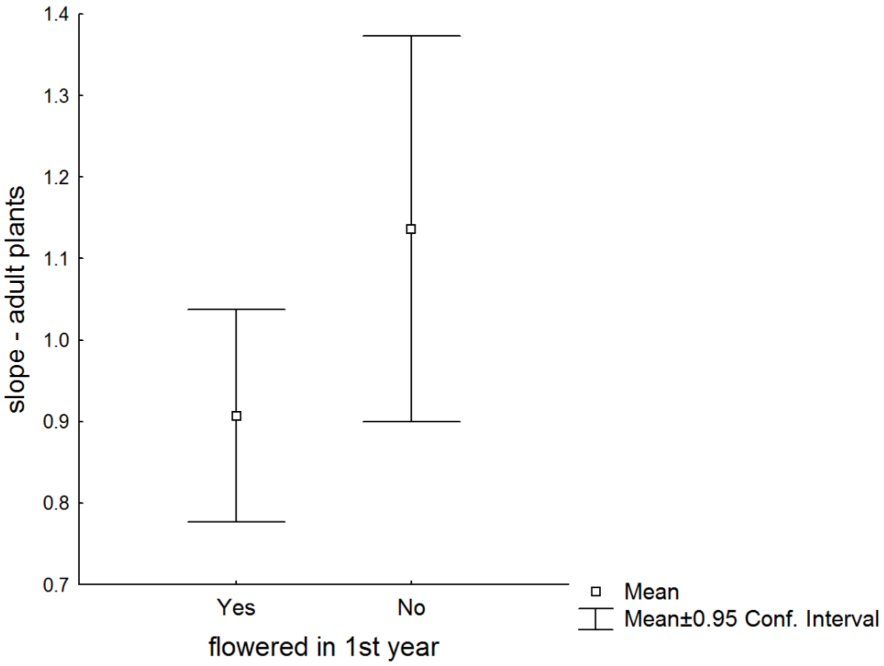

Figure 3: Shoot–root allometric slope of species that flowered in 1st year of their lives and those that did not. All adult plants of each species were harvested at the time of fruiting and maturating of most of their fruits. P = 0.049, R^2 = 0.198.

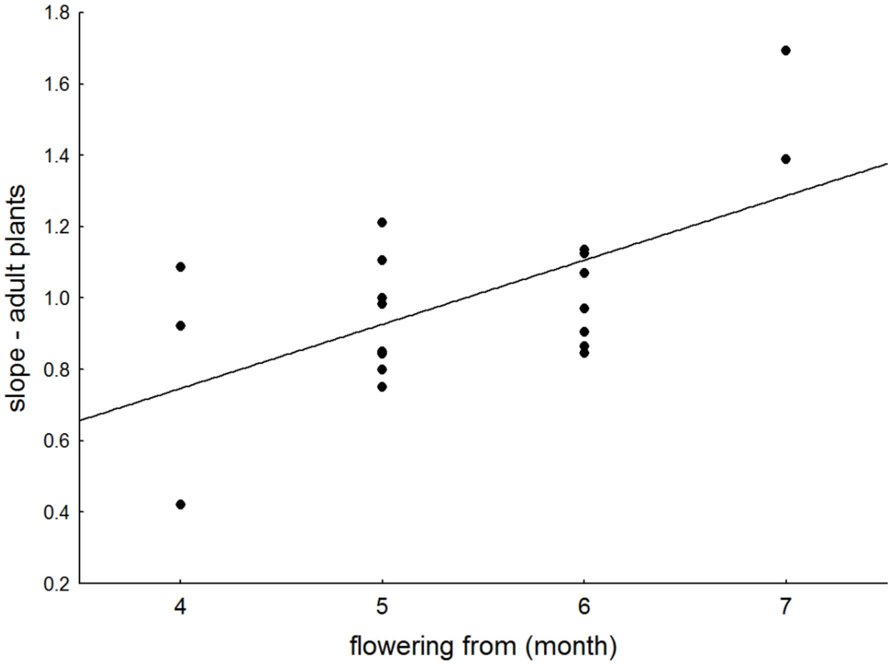

Figure 4: Relationship between shoot–root allometric slope and month in which plants of each species start flowering within the season (4 = April, 5 = May, 6 = June, 7 = July). P = 0.004, $R^2 = 0.375$.

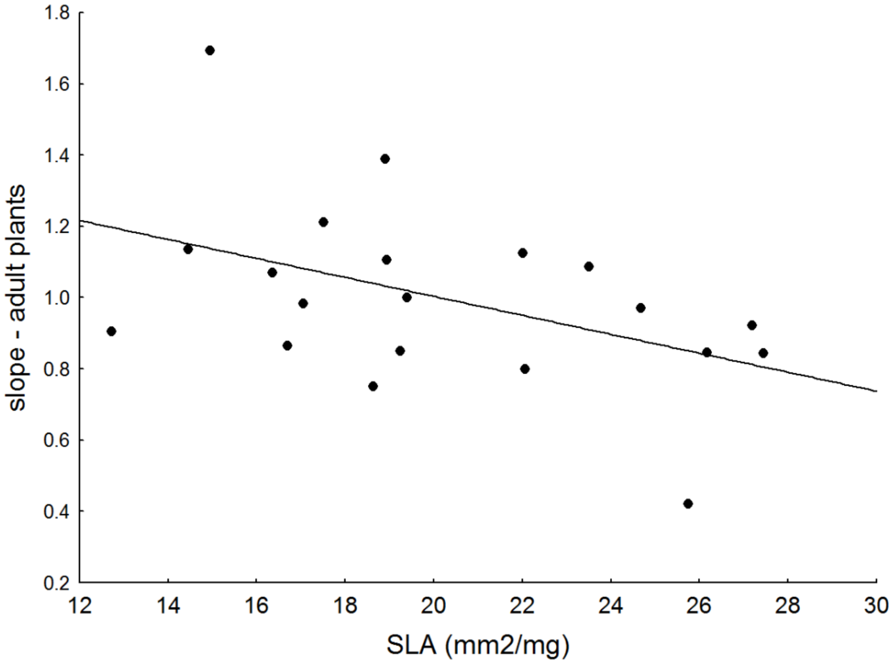

Figure 5: Relationship between shoot–root allometric slope of adult plants and specific leaf area (SLA). P = 0.044, $R^2 = 0.207$.

The effect of phylogeny

We found no evidence for the effect of phylogeny on seedling or adult plants allocation patterns (for seedlings: Blomberg K = 0.434, p = 0.659; for adult plants: Blomberg K = 0.384, p = 0.850). Species that were closely related did not have similar allometric allocation patterns.

DISCUSSION

Overall allocation pattern of all species together

Generally, the results showed that allocation to above- vs. below-ground organs differed between seedlings and adult plants when analysed across all the 20 dry-grassland species. This is in agreement with our hypothesis and several previous studies (Coleman et al. 1994; McConnaughay and Coleman 1999; Weiner 2004) suggesting that ontogenetic drift changes allocation (Evans 1972; Gedroc et al. 1996). Consistent with other studies (Müller et al. 2000; Weiner et al. 2009b; Wang et al. 2010; Li et al. 2013; Pan et al. 2013; Janeček et al. 2014; Lohier et al. 2014; Zhou et al. 2014), we also found that root–shoot ratio changes with plant size for both seedlings and adult plants. In contrast to some previous studies, however, we generally found increasing root–shoot ratio with increasing plant size in the analyses across species and this pattern was stronger for adult plants than for seedlings. A similar pattern was observed by Shipley and Meziane (2002), who found a preferential allocation to roots during plant ontogeny in a dataset of 22 herbaceous plant species. In contrast to our study, their experiment was very short: only 35 days post germination, and thus their plants were very young even at time of the last harvest. As Shipley and Meziane (2002) state, a possible explanation for the observed allocation patterns is that the rate of nutrient uptake decreases as roots became older and larger. Moreover, during the growing season or over the plant's life, the amount of resources available, especially in pots, becomes depleted, which may result in higher demands on roots. An alternative explanation could be that studied species are typical dry-grassland species, originating from dry, rocky localities where water and nutrients are limiting factors. For this reason, large plants need to have proportionally much more roots to acquire sufficient amount of water and nutrients than small plants. The maternal environment could affect root allocation in the plants in our experiment in spite of the fact that the studied plants were regularly watered and grew in nutrient rich soil (Weiner et al. 1997). This is consistent with a previous study in which plants transplanted from a low-nutrient to high-nutrient environment did not change their shoot vs. root allometries (Gedroc et al. 1996).

Allocation patterns of seedlings and adult plants of individual species

We found that patterns of biomass allocation differed among species and between developmental stages when analysing data on each species and stage separately. Our results are in contrast to the expectation of isometric allocation at annuals and allometric allocation of perennials (Mao et al. 2012; Zhou et al. 2014). We found allometric as well as isometric patterns of allocation in both groups of plants. Specifically, seedlings of annual species with short life cycles and ruderal strategies had allometric slopes greater than one, indicating that larger individuals have relatively more shoots compared to roots than smaller individual, whereas young perennials usually had slopes lower than one. For adults, allometric slopes greater than one were observed in perennials, possibly because of higher allocation to flowers and fruits by larger individuals. On the other hand, adult annuals, one clonal perennial and grasses had slopes lower than one, indicating that larger individuals had more roots compared to shoots than did smaller individuals. For the one clonal perennial (*Hieracium pilosella*) with an allometric slope less than one, the slope could be a consequence of its growth form: larger individuals consist of a main rosette plus newly originated secondary rosettes, each of which has its own roots. For grasses, the preferential allocation into roots than to shoots with increasing size could be related to their mode of persistence and life form. A higher root–shoot ratio for grassland vegetation than for other vegetation types (e.g. understory plants or canopy trees in subtropical forests) has been reported in previous studies (Mokany et al. 2006; Cheng et al. 2015).

Comparison of allocation patterns of seedlings vs. adults

Intraspecific comparisons of allocation patterns of seedlings and adult plants of each species indicated that nearly all species had different allometric patterns at the two developmental stages. Seedlings and adults of more than half of the species differed significantly in their allometric slopes, the remaining species differed only in their proportion of above- and below-ground biomass with size, but in a constant way.

Comparing allocation patterns at these two stages among species, we found that annual species generally decreased their allocation slopes from seedlings to adult individuals whereas perennial species flowering in the second year or later usually increased in their allocation slopes with the age. There were, however, few species that deviated strongly from this pattern.

Our results are partly in accordance with previous studies (Coleman et al. 1994; Gedroc et al. 1996; McConnaughay and Coleman 1999; Müller et al. 2000; Weiner 2004; Poorter et al. 2012; Janeček et al. 2014), which found that most herbaceous species show a trend towards decreased root allocation and an increased shoot allocation with size during development. Some of our findings, however, are not consistent with these previous studies, especially those for annuals, where we found changes in allocation pattern between seedlings and adult plants opposite to most previous studies (Coleman et al. 1994; Gedroc et al. 1996; McConnaughay and Coleman 1999). These differences could be due to the short life cycle of some annuals that grew rapidly when they were young, but later, during flowering and maturing fruits and seeds, needed more water and nutrients to sustain such large number of fruits and seeds, so large adults allocated proportionally more to roots than shoots than did small adults.

The differences in our results from previous studies could be also due to the different ages and condition of the harvested plants. All our seedlings were harvested two or three weeks after most of the seeds of that species started to germinate. Many previous studies investigated species allocation earlier, so our seedlings could be older than those in some previous studies (e.g. Gedroc et al. 1996; McConnaughay and Coleman 1999; Shipley and Meziane 2002; Weiner 2004). Also, we considered all seedlings to be at the same developmental stage, but they may not have been. Seedlings of some annuals may have already lived a quarter of their lifespan, whereas some perennials would be just establishing in this time. Adults of each species were harvested at the time of maturation of most of their fruits and seeds, so it is reasonable to assume that they were all at a similar developmental stage, although the length of their growth periods varied among the species. At maturation, annuals and perennials may look similar but they behaved differently: annuals finish their life cycle after fruiting and seeding, while perennials store resources for the next season. This could be why our results are different from those of Niinemets (2004), who studied allocation patterns over the whole life cycle of one perennial species, and found increasing allocation to roots during ontogeny.

The effect of life-history traits and phylogeny on intraspecific allocation patterns

Intra- and interspecific biomass allocation pattern can be very different, so it is important not to conflate them (Weiner et al. 2009a). To study interspecific differences in allocation patterns we used values of intraspecific allocation slopes for each species and we tested how they depend on various species life-history traits and phylogeny.

In our study, seedlings of annuals allocated proportionally more to above- than below-ground biomass with increasing size compared to seedlings of perennials. McCarthy et al. (2007) also found important effects of growth form (evergreen trees, deciduous trees, shrubs, forbs and graminoids) for allocation to above-ground organs, but allocation to roots was not influenced by growth form. Contrary to our hypothesis, there was no effect of life span on shoot–root allometry of adult plants. The most important factors affecting adult allocation pattern were the age of first flowering and the month in which the species start to flower. Species that did not flower in the first year of life or started flowering late in the growing season, allocated proportionally more to above- compared to below-ground biomass with increasing size and thus had a higher root–shoot allometric slope as adults. This could be due to the rate of growth and development of the species within the growing season.

Our results also indicate that species with lower SLA allocated proportionally more to above- compared to below-ground biomass with increasing size as adults. Also, species with higher allometric slopes and thus higher allocation to above-ground biomass as seedlings tended to have marginally significantly more persistent leaves than species with higher allocation to roots. These results are consistent with those of McCarthy et al. (2007) who also showed higher allocation to shoots in species with longer-lived leaves and smaller SLA. There was a marginally significant positive effect of seed weight on preferential shoot allocation of seedlings, as observed by Chacon et al. (1998). Our results show this over a wide spectrum of species differing in seed weight. We also tested the effect of other species life-history traits on allocation slopes: CSR strategy, plant height and clonality, but none of these were significant. Species habitat requirements may play an important role in biomass allocation, but all our studied species come from the same habitat type, so there was very little variation in habitat requirements. Other factors, such as mycorrhiza, root or shoot herbivory, can have important effects on biomass allocation but they are outside of the scope of our study.

Despite the importance of species life-history traits for allocation patterns and in contrast to our hypothesis, we did not find any effect of phylogenetic relatedness of species on the allocation patterns. These results are not consistent with previous studies (McCarthy et al. 2007; McCarthy and Enquist 2007) that found phylogeny to be the strongest predictor of biomass allocation. These studies were, however, carried out within a global dataset at higher taxonomic levels and across a wide spectrum of plant sizes. Our results thus demonstrate that phylogenetic relatedness of species at a lower taxonomic level – i.e. between different species from the same habitat and vegetation type, may not be important for explaining the variation in biomass allocation patterns, and that other factors, such as life-history traits, could thus play much more important role. This could be because biomass allocation patterns are evolutionarily labile and can evolve quickly (Weiner 2004).

CONCLUSION

Our results demonstrate the importance of changes in biomass allocation to above- vs. below-ground organs between seedlings and adult plants at both intraspecific and interspecific level. The results are consistent with allometric biomass partitioning theory, as we found isometric as well as allometric patterns of biomass allocation in dry-grassland species. In general, seedlings as well as adult plants of the studied dry-grassland species showed allometric allocation patterns with root–shoot ratio increasing with increasing size, and this tendency was stronger for adult plants than for seedlings. Annual species usually decreased their shoot–root allometric slopes from young to adult individuals, whereas perennial species flowering in the second year or later usually increased their shoot–root slopes from seedlings to adults. Differences in species' life span had the strongest effect on intraspecific biomass allocation patterns in seedlings. For adult plants, the most important traits affecting intraspecific allocation patterns were the age of first flowering and month in which the species usually start to flower. Leaf traits, such as SLA, also had important effects on biomass allocation. Our results confirm that biomass allocation pattern is species- and age-specific and that plant life-history traits are much more important in explaining allocation pattern among species than is phylogenetic relatedness among species within a habitat.

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Contribution of Iveta Husáková to the papers with co-authors included in the Ph.D. thesis:

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