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Diſſertation

Vliv biotických interakcí na populační biologii lučních rostlin

The role of biotic interactions in population biology of meadow plants

Einfluſſ der biotiſchen Interaktionen auf Populationſbiologie der Wieſenpflanzen

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Prohlášení

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Declaration

Hereby I declare that I did the present doctoral dissertation by myself only with the use of sources cited. The detailed papers that form the part of dissertation were co-authored and author contributions of individual authors are stated in the proper section. I have not used any part of this dissertation for claiming any other academic title.

Beſtätigung

Hierbei beſtätige ich, daſſ ich dieſe Diſſertation ſelbſt nur mit Anwendung der zitierten Quellen aufgearbeitet habe. Die detaillierte Studien, die bilden ein Teil dieſer Arbeit, wurden in Zuſammenarbeit mit anderen Autoren durchgeführt und ihren Anteil auf ihnen iſt in dem angehörigen Kapitel beſchrieben. Kein Teil dieſer Diſſertation wurde zur Erwerbung einen anderen akademiſchen Titel benützt.

Ve Svatém Janu t. Krsovicích, 15. listopadu, L.P. 2014. Zdeněk Janovský

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Summary

In present thesis, I treat the topic of impacts of plant-animal interactions, namely herbivory and pollination, on plant life cycle and lifetime fitness. First, I identify the components of the impact of plant-animal interactions: i) interaction frequency; ii) per-interaction effect; iii) sensitivity of the plant's life cycle to the changes in vital rate impacted by the animals. Furthermore, I also classify other causes changing the outcome of a plant-animal interaction into two categories: i) plant's traits; ii) plant's environment. A review of extant literature on the topic revealed that especially the role of plant's environment in changing the outcome of plant-animal interactions is largely understudied and I attempt to reduce this gap in knowledge in the five detailed studies encompassed in this thesis.

The detailed studies focus on a model system of Central European wet grasslands and especially on three species typical to it: *Succisa pratensis*, *Achillea millefolium* and *A. ptarmica*. The first two studies examine the effects of environment on frequency of plant-animal interactions. The next two studies are more integrative, one focusing on the impacts of different herbivore groups on the complete life cycle and the other on interaction of herbivory and pollination on plant lifetime fitness. The fifth detailed study focuses on factors at landscape scale influencing the frequency of pollination interaction and reproductive success of common wet grassland plant species.

The studied components of plant's environment affected mainly the interaction frequency in case of pollinators, while in case of herbivores the effect of plant's environment affected more the per-interaction effect. We also bring evidence of considerable effects of plant's environment on sensitivity of plant's life cycle to herbivory. The detailed studies brought also some interesting results, which are more specific to study systems examined: i) Herbivores conferred long-term fitness advantage to plants distributing the same reproductive effort into more flowering events suggesting potential of plant-animal interactions to shape plant life history strategies; ii) Generalist and specialist invertebrate folivores were influenced in their occurrence by surrounding vegetation to a similar degree; iii) Pollinator assemblages of the same plant species can differ to a high degree at the scale of tens of metres within one population; iv) Management induced changes in reproductive timing can cause a temporal mismatch of a plant and its pollinators, whose effect is an order of magnitude greater than effect of site quality, isolation or attractiveness to pollinators; v) The interactions of herbivores and surrounding vegetation cause non-systematic changes in sensitivity of life cycle transitions opening thus numerous possibilities for higher order interactions with other components of plant's environment.

Keywords

plant-animal interactions, pollination, herbivory, plant-pollinator-environment interaction, plant-herbivore-environment interaction, Succisa pratensis*,* Achillea millefolium agg.*,* Achillea ptarmica*,* Euphydryas aurinia*, flowering strategies, integral projection models, Syrphidae*

Shrnutí

V předkládané práci se zabývám dopady herbivorie a opylování na životní cyklus rostlin a jejich celoživotní biologickou zdatnost. Nejprve se pokouším rozdělit dopad herbivorie a opylování do jednotlivých složek: 1) četnost výskytu interakce; 2) dopadu interakce pro rostlinu za jednotku výskytu interakce; a 3) sensitivity životního cyklu rostliny ke změnám životních funkcí rostliny způsobených interakcí s živočichy. Dále se zabývám faktory, jež mohou pozměnit dopady interakci rostlin a živočichů pro rostliny, a dělím je do dvou kategorií: 1) vlastností rostlinných jedinců; a 2) vlastností okolí rostliny. Rozbor dosavadních prací na dané téma poukázal na nedostatek studií věnujících se vlivu vlastností okolí rostliny na dopady jejich interakcí s živočichy ve srovnání s pracemi věnujícími se vlivu vlastností rostlinných jedinců. Tuto mezeru v našich znalostech se snažím alespoň zčásti vyplnit pěti podrobnými studiemi zahrnutými do této práce.

Detailní studie se soustředí na modelový systém středoevropských vlhkých luk, a to zejména na tři pro ně typické druhy: čertkus luční, řebříček obecný a řebříček bertrám. První dvě podrobné studie zkoumají vlivy okolí rostliny na četnost výskytu interakcí rostlin a živočichů. Následující dvě studie se věnují vlivům okolí rostliny na více složek dopadů interakce rostlin a živočichů, a to třetí studie na vliv herbivorů na celý životní cyklus rostliny a čtvrtá na vliv herbivorů a opylovačů na jejich celoživotní zdatnost. Poslední z podrobných studií se soustředí na krajinné faktory ovlivňující četnost opylování a úspěšnost rozmnožování běžných druhů rostlin vlhkých luk.

Zkoumané vlastnosti okolí rostliny ovlivňovaly v případě opylovačů především četnost výskytu interakce, naopak v případě herbivorů ovlivňovaly spíše vlastní dopad dané interakce za jednotu výskytu. Dále se nám podařilo doložit výrazné vlivy vlastností okolí rostliny na sensitivitu jejího životního cyklu vůči herbivorii. Podrobné studie rovněž přinesly některé zajímavé výsledky partikulárnější povahy: 1) Herbivorie zvýhodňovala z dlouhodobého hlediska rostliny, které rozložily stejné reprodukční úsilí v čase a kvetly vícekrát; 2) Četnost nespecialisovaných i specialisovaných bezobratlých herbivorů listových růžic závisela na okolní vegetaci stejnou měrou; 3) Společenstva opylovačů stejného rostlinného druhu se mohou výrazně lišit i na pár desítkách metrů v rámci jedné populace; 4) Změny v načasování kvetení způsobené lidským hospodařením mohou způsobit minutí se kvetení daného rostlinného druhu a výskytu jeho opylovačů, kterýžto efekt je o řád větší než efekty kvality či prostorové isolace lokality či její atraktivity pro opylovače; 5) Interakce působení herbivorů na rostliny a okolní vegetace způsobují těžko předvídatelné změny sensitivity životního cyklu rostliny k jednotlivým dílčím životním pochodům, umožňujíce tak vznik interakcím vyššího řádu s dalšími vlastnostmi okolí.

Klíčová slova

interakce rostlin a živočichů, herbivorie, interakce rostlina-opylovač-okolí, interakce rostlina-herbivor-okolí, Succisa pratensis*,* Achillea millefolium agg.*,* Achillea ptarmica*,* Euphydryas aurinia*, strategie kvetení, integrální projekční modely*

Zusammenfassung

Diese Dissertation beschäftigt sich mit den Einflüssen von Herbivorie und Bestäubung auf Pflanzenlebenszyklus und lebenslange biologische Tüchtigkeit. Zuerst wird die Wirkungen der Herbivorie und Bestäubung in folgende einzelne Komponente zu gliedern versucht: 1) Häufigkeit des Vorkommens der Interaktion; 2) Wirkung der Interaktion auf den Pflanzenindividuum pro Einheit; und 3) Sensitivität des Lebenszyklus zu Änderungen der Lebensfunktion betroffen von der Interaktion mit Tieren. Weiter werden die Pflanzen-Tier-Interaktion modifizierende Faktore behandelt und zwar sind sie in zwei Gruppen geteilt: 1) Eigenschaften der Pflanzenindividuen; und 2) Eigenschaften der Umwelt der Pflanze. Analyse der existierenden Arbeiten, die sich mit diesem Thema beschäftigen, hat den Mangel der Studien über den Einfluss der Pflanzenumwelt im Vergleich zur Studien über den Einfluss der Pflanzeneigenschaften entdeckt. Die fünf ausführlichere Studien auf das Thema, die sind in die Dissertation eingeschlossen, versuchen die anwesende Lücke unsers Wissens ein bisschen auszufüllen.

Die ausführliche Studien widmen sich auf einen Modelsystem von mitteleuropäischen Feuchtwiesen, besonders auf drei für sie typischen Arten: Gewöhnlicher Teufelsabbiss, Gemeine Schafgarbe und Bertram-Schafgarbe. Die ersten zwei Studien untersuchen die Einflüsse der Pflanzenumwelt auf die Häufigkeit des Vorkommens der Pflanzen-Tier-Interaktionen. Die folgenden zwei Studien beschäftigen sich mit Einflüssen der Pflanzenumwelt auf mehrere Komponente der Auswirkungen von Pflanzen-Tier-Interaktionen, und zwar die dritte auf Auswirkung von Herbivoren auf den ganzen Pflanzenlebenszyklus, wohingegen die vierte Studie auf den Einfluss von Herbivoren und Bestäubern auf die lebenslange biologische Tüchtigkeit der Pflanze. Die letzte der ausführlichen Studien konzentriert sich auf Faktoren auf dem Landschaftsniveau, die die Quantität der Bestaubung und Vermehrenserfolg der häufigen Pflanzenarten der Feuchtwiesen beeinflussen.

Die untersuchte Pflanzenumwelteigenschaften beeinflussten im Falle der Bestäuber besonders die Häufigkeit der Interaktion, trotz dessen beeinflussten sie eher die pro Einheit Auswirkungen der Interaktion im Falle der Herbivoren. Weiter ist es uns die wichtigen Einflüsse der Pflanzenumwelt auf die Sensitivität ihres Lebenszyklus zur Herbivorie zu dokumentieren gelungen. Die ausführliche Studien brachten auch einige interessante Ergebnisse, die partikulärere sind: 1) Herbivorie bevorteilte aus langfristigem Sichtspunkt die Pflanzenindividuen, die die gleiche Vermehrungsinvestition in mehrere Blühensbegebenheiten teilten; 2) Häufigkeit von beiden unspezialisierten und spezialisierten wirbellosen Blattrosettenfresser wurde zum selben Grad von umgebender Vegetation abhängig worden; 3) Bestäubergesllschaften von einer Pflanzenart können sich deutlich auf einige Zehnten Meter binnen einer Population unterschieden; 4) Die menschenwirtschaftsverursachte Änderungen in Blühenzeiten können die zeitliche Meidung von Blühen bestimmter Pflanzenart und Vorkommen ihrer Bestäuber verursachen, wessen Effekt eine Ordnung höher als Effekte der Habitatqulität und räumliche Isolation der Populationen sein kann; 5) Die Interaktion zwischen Wirkungen der Hebivoren und der Pflanzenumwelt verursachen schwer vorhersagbare Änderungen in Pflanzenlebenszyklussensitivität zur einzelne Lebensfunktionen und deshalb ermöglichen sie Interaktionen höherer Ordnung mit anderen Pflanzenumwelteigenschaften.

Schlüsselwörter:
Pflanzen-Tier-Interaktionen, Herbivorie, Pflanzen-Bestäuber-Pflanzenumwelt-Interaktionen, Pflanzen-Herbivore-Pflanzenumwelt-Interkationen, Succisa pratensis*,* Achillea millefolium agg.*,* Achillea ptarmica*,* Euphydryas aurinia*, Blühenstrategie, Integralprojektionsmodel*

Author contributions

This thesis consists of general introduction and synthesis, one published paper and four submitted papers for publication. All papers are co-authored. The nature of the contributions by the different authors is outlined below.

Paper I – Surrounding vegetation mediates occurrence of both generalist and specialist invertebrate folivores, but not occurrence of vertebrate herbivores and seed predators; **Janovský, Z.**, Janovská, M., Weiser, M., Horčičková, E., Říhová, D., Münzbergová, Z.; Manuscript.

Zdeněk Janovský – experimental design, field work, statistical analysis, writing, editing
Marie Janovská – field work, data management, editing
Martin Weiser – field work, statistical analysis, editing
Eva Horčičková – field work, editing
Dagmar Říhová – examining seed production and seed predation, editing
Zuzana Münzbergová – experimantal design, editing

Paper II – Your neighbour's pollinator does not have to be your pollinator: Examining spatial homogeneity of pollination; Janovský, Z., Mikát, M., Hadrava, J., Horčičková, E., Kmecová, K., Požárová, D., Smyčka, J., Herben, T.; PLoS ONE, Vol. 8, Issue 10, October 2013; e77361.

Zdeněk Janovský – experimental design, field work (botanical part), statistical analysis, writing, editing
Michael Mikát – experimental design (zoological part), field work (zoological part), editing
Jiří Hadrava – experimental design (zoological part), field work (zoological part), editing
Eva Horčičková – field work (botanical part), editing
Kateřina Kmecová – field work (botanical part), editing
Doubravka Požárová – field work (botanical part), editing
Jan Smyčka – field work (botanical part), editing
Tomáš Herben – statistical analysis, writing, editing

Paper III – The interaction of herbivory and plant's environment results in complex changes in plant life-cycle, not only in changes in population growth rates; Janovský, Z., Říhová, D.; Manuscript.

Zdeněk Janovský – experimental design, field work, statistical analysis, writing, editing
Dagmar Říhová – examining seed production and seed predation, editing

Paper IV – Opposing selective pressures of pollinators and seed predators cause to plants distribute their reproductive effort over time; Janovský, Z., Pavlíková, A., Říhová, D., Herben, T.; Manuscript.

Zdeněk Janovský – experimental design, field work (plant demography, pollinator observations, harvesting), statistical analysis, writing, editing
Anežka Pavlíková – field work (pollinator observations, harvesting), editing
Dagmar Říhová – field work (pollinator observations, harvesting), examining seed production and seed predation, editing
Tomáš Herben – statistical analysis, editing

Paper V – Reproduction timing and pollinator abundance, but not site quality, drive reproductive success of two common Achillea species in an agricultural landscape; Janovský, Z., Tomšová, P., Jersáková, J., Herben, T.; Manuscript.

Zdeněk Janovský – experimental design, field work (plot setup, plot attractiveness assessment, pollinator censuses), statistical analysis, writing, editing
Pavla Tomšová – field work (plot setup, plot attractiveness assessment, pollinator censuses, harvesting), laboratory work (seed production assessment, germination tests, flow cytometry of seedlings), editing
Jana Jersáková – experimental design, writing, editing
Tomáš Herben – statistical analysis, writing, editing

Acknowledgement

1 Synthesis of detailed studies

1.1 Introduction

The attention of vegetation ecologists was attracted by the relationship of plants and their population dynamics with abiotic conditions for most of the 20th century. In the last two decades, more attention has been paid also to plant-animal interactions and a growing body of evidence indicates that in many systems they constitute the main driver of the dynamics of plant populations. This is especially true for the two perhaps commonest and most studied plant-animal interactions, herbivory (including seed predation) and pollination.

The ways how herbivory and pollination affect plant individuals differ in many aspects. Pollinators directly affect only plant reproduction, although they may also indirectly affect the reserves available to plants in the next season (Miller *et al.*, 2012, Ehrlén and van Groenendael, 2001, Sletvold and Ågren, 2011a). Successful pollination is necessary (to variable extent) for plant reproduction, but it is not the only condition and other factors, such as plant size are generally of greater importance (e.g. Childs *et al.*, 2004, Sletvold, 2002, Williams, 2009). The effect of pollinators can be mainly only positive, although ineffective pollinators can affect plants negatively by decreasing male fitness component (de Jager and Ellis, 2014, Larsson, 2005, Ibanez *et al.*, 2009), clogging stigmas with incompatible pollen (Scribailo and Barrett, 1994, Jakobsson *et al.*, 2008) or depraving the legitimate pollinators of rewards and decreasing plant's attractiveness to them (Sowig, 1989, Brody *et al.*, 2008).

Herbivores, on the other hand, affect one or more vital rates by removing plant tissues. The loss of tissues leads relatively consistently to negative impacts on plant individual fitness, with the only exception being herbivore-induced overcompensation of seed production in meristem-limited semelparous species (Hendrix, 1979, Lennartsson *et al.*, 1997, Rautio *et al.*, 2005). There are also important differences in impacts on vital rates among various herbivore groups with the main distinction running between predispersal and postdispersal seed predators on one hand and folivores, stalk grazers and below-ground herbivores on the other, since the first ones do not affect the mother plant in any other way than its reproduction. Thus the effect of seed predators is lesser in plant species with long-lived individuals (either as seeds in annuals or as large individuals in iteroparous perennials) compared to short-lived species with transient seed banks (Louda and Potvin, 1995, Maron and Crone, 2006). In some cases, the different developmental stadia of the same organism can act as pollinators and herbivores and affect plants in complex ways, as is for example the case of meadow plant *Trollius europaeus* and *Chiastochaeta* flies (Jaeger et al., 2001).

Effects of herbivores and pollinators on individual plant fitness do not necessarily imply their effect on population structure and growth rate of plant populations. Effects of herbivores and pollinators on plant populations range from the considerable ones (Maron and Crone, 2006, von Euler *et al.*, 2014, Törang *et al.*, 2008, Lennartsson, 2002) to negligible albeit significant ones (Münzbergová, 2005, Fröborg and Eriksson, 2003, Ehrlén, 1996). The effect of herbivores/pollinators on population characteristics is the combination of their actual effect on vital rates of plant individuals (hereafter per-interaction effect; for definition see also Sánchez-Lafuente et al., 2012), frequency of occurrence of this interaction (hereafter interaction frequency) and sensitivity of population characteristics to the affected vital rates (Kolb *et al.*, 2007, e.g. Münzbergová, 2005). For example, a relatively strong effect of seed predators on seed production can be almost cancelled out by high mortality of seedlings (Fröborg and Eriksson, 2003), which makes the establishment of new individuals a more or less stochastic process.

Herbivore/pollinator occurrence, their effects on vital rates of individuals and sensitivity of population characteristics to these vital rates are influenced also by other factors than herbivores and pollinators. These can be essentially divided into within-population variation in plant individual's traits (i.e. internal influences; Fig. 1 – 1 and 2) and characteristics of its environment (i.e. external influences; Fig. 1 – 3, 4, 6). These factors may either directly affect the same vital rate by changing the pollinator/herbivore occurrence (Fig. 1 – 1 and 4) or impact on plant vital rates (2 and 3) or they may affect a different stage of the plant's life cycle and thus change the sensitivity of the life-cycle stage directly affected by a given plant-animal interaction (Fig. 1 – 6). Finally, the individual plant-animal interactions (e.g. pollination and predispersal seed predation) can also interact with each other yielding non-additive combinations of their isolated effects (Fig. 1 – 5).

All these listed mechanisms contribute in their fashion to modulating the impact of a given plant-animal interaction on plant's life-time fitness. While measuring the changes in per-interaction effects of plant-animal interactions (Fig. 1 – 2 and 3) on a particular vital rate is relatively easily tractable, measuring the changes in impact on a particular vital rate due to changed frequency of a plant-animal interaction (Fig. 1 – 1 and 4) or due to changes of sensitivity to a given vital rate (Fig. 1 – 5 and 6) is almost impossible to conduct on plant individuals. However any of these groups cannot be isolated for study from the rest, since the actual effect of plant-animal interaction on plant life-time fitness is their combination and we cannot rule out, that any of these processes does not introduce non-linearities into the relationship. Indeed, there is already limited evidence that this can happen (e.g. Vanhoenacker et al., 2013). Necessity to monitor all these groups of effects modulating outcome of plant-animal interactions is typically solved by monitoring whole populations (e.g. Ehrlén, 2002) and extrapolating the observed patterns to plant individuals, while assuming transitivity between individuals, years and life-cycle stages. Our possibilities to verify these assumptions are at the current state of knowledge rather limited, since our knowledge of occurrence and intensity of the listed mechanisms is scanty as well.

In following sections of introduction I would like to review available knowledge on mechanisms modulating effect of plant-animal interactions on life-time fitness as well as outline in what directions the present theses can help fill in the extant gaps. First, I try to summarise extant knowledge on the role of intrapopulation trait variation in direct shaping the outcome of plant-animal interaction (1 and 2). Then I review available information on the modulating effect of plant's environment (3 and 4) and on interferences and synergies between different plant-animal interactions (5). Finally, I present the several existing studies indicating the impact of plant-animal interactions depends also on other factors influencing different life-history transitions (6).

1.1.1 Intrapopulation trait variation and plant-animal interactions (components 1 and 2)

The relationship between individual trait variation and interaction frequency (both of herbivory and pollination) has been mainly studied on plant/inflorescence size, while other traits have been especially in case of herbivores rather neglected. Pollinators seemingly visit more frequently large conspicuous inflorescences although these may pay for it by longer pollinator visits and higher rates of self-pollination (Sánchez-Lafuente et al., 2012). However, the relationships between visitation and inflorescence size mainly disappear or revert once pollinator occurrence is expressed not per plant but per unit inflorescence (Thomson, 1988, Brody and Mitchell, 1997, Klinkhamer and de Jong, 1990, Parachnowitsch and Caruso, 2008, Cariveau *et al.*, 2004), leaving thus only limited possibilities for interactions of pollinator occurrence with inflorescence traits other than size (e.g. conspicuousness, spur length or individual flower size; Sletvold and Ågren, 2011b, Brunet and Sweet, 2006, Hansen and Totland, 2006, Törang *et al.*, 2008) and rewards (e.g. Thomson, 1988). However it is necessary to note, that in more detailed studies, more intricate density-dependent effects of inflorescence size may be sometimes detectable (Grindeland et al., 2005).

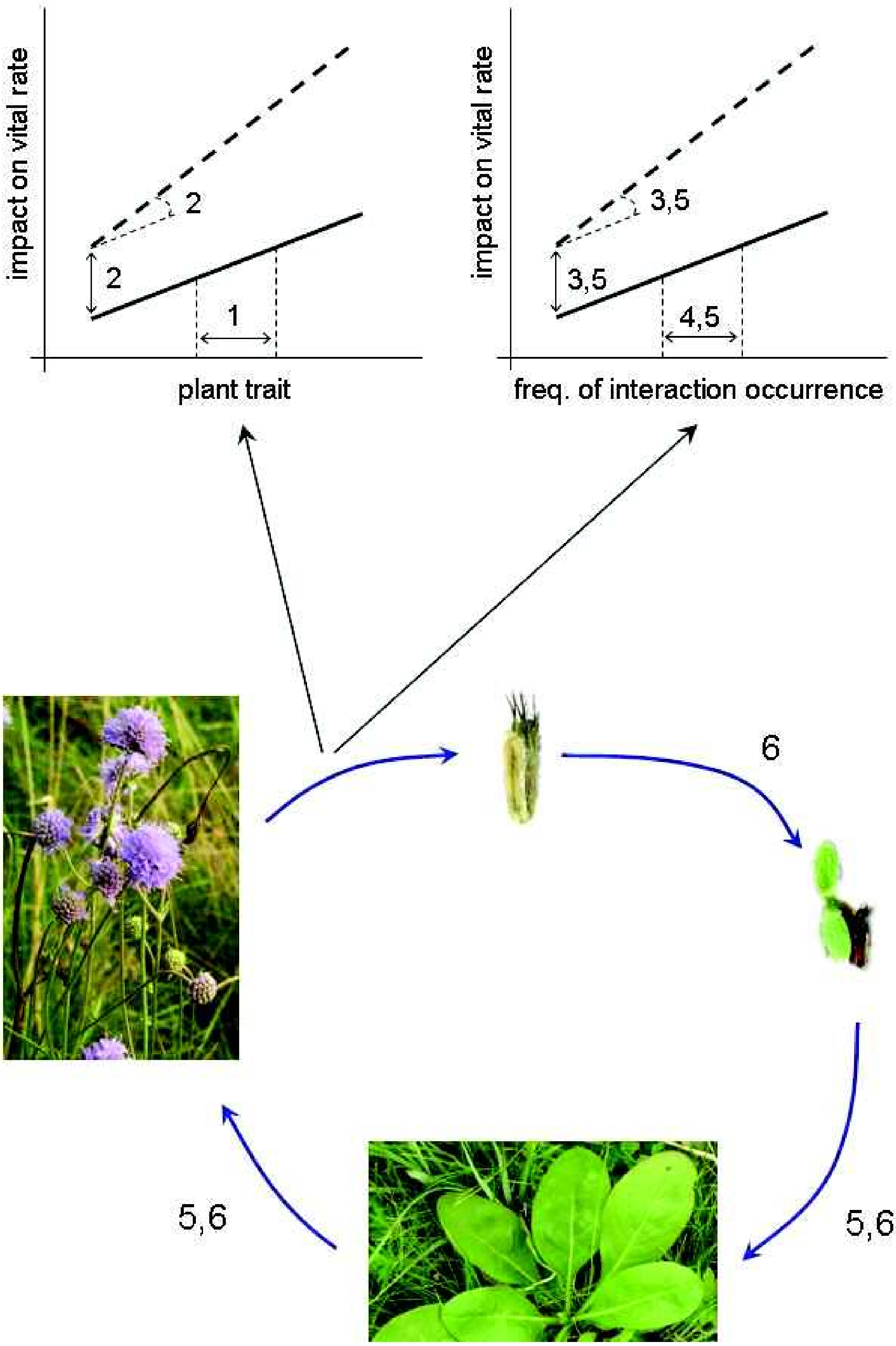


Fig. 1: Life cycle scheme of the main focal species *Succisa pratensis* with a scheme depicting possible ways how plant traits and plant's environment can modulate the impact of plant-animal interactions on plant individual's life-time fitness and population

dynamics; 1 – effect of plant traits on interaction frequency of occurrence; 2 – effect of plant traits on interaction intensity; 3 – effect of plant's environment on interaction intensity; 4 – effect of plant' environment on interaction frequency of occurrence; 5 – interacting effect of two different plant-animal interactions; 6 – interacting effect of plant's environment on different life-cycle stages than the one directly affected by plant-animal interaction

Knowledge of interactions of herbivore occurrence with plant size (information on other plant traits lacks) largely differs among herbivore functional groups. A relatively general relationship emerges in case of predispersal seed predators, which prefer to attack plants with larger inflorescences (Brody and Mitchell, 1997, KolbEhrlén *et al.*, 2007). Far less information is disposable concerning other herbivore functional groups, but the folivores (Ehrlén, 1995, Piqueras, 1999), below-ground herbivores (Prins et al., 1992), florivores (Sletvold and Grindeland, 2008) and stalk grazers (Gómez, 2005, Ehrlén and Münzbergová, 2009) seem to follow a similar pattern suggesting that herbivores tend generally to affect plant individuals more differentially than pollinators.

Herbivore effects can differ with plant size in both directions. They may affect larger plants more as is frequently the case of seed predators (Leimu *et al.*, 2002, KolbEhrlén *et al.*, 2007, Brody and Mitchell, 1997) and in documented cases also stalk grazers (Ehrlén, 1995, Paige and Whitham, 1987) and folivores (Piqueras, 1999) or their effect may be of the same or lower absolute size leading to decrease of herbivore effect with plant size (e.g. Alonso and Herrera, 1996, Ollerton and Lack, 1998). In general, it can be concluded that both herbivore occurrence and impact on individual plant fitness interact more frequently with plant size leading to differential impacts on individuals. Similar effects of pollinators are less common and typically involve some special floral traits under selection other than plant (or inflorescence) size. The potential of plant-trait interactions of herbivores and pollinators alone for differential impacts on plant population dynamics and individual life-time fitness is rather limited. Among the so far reported evidence, there are only slight suggestions involving the change of pollinator behaviour with inflorescence size and density leading to differential crosspollination frequencies and presumable subsequent offspring quality (Grindeland *et al.*, 2005, Klinkhamer and de Jong, 1990, Nattero *et al.*, 2011).

1.1.2 The role of plant's environment in modulating plant-animal interactions (components 3 and 4)

The effects of plant's environment on pollinator/herbivore per-interaction effect on plant vital rates represents an interaction of pollinator/herbivore, plant's environment and focal plant individual's traits (e.g. an environment allows the plant to grow larger stalks and thus the effect of stalk grazers usually grazing whole stalks also grows bigger), whereas the effects of plant's environment pollinator/herbivore interaction frequency represent only a second-order interaction between plant's environment and pollinator/herbivore with plant playing more or less a passive role. There is also a substantial difference in mechanisms of varying per-interaction effect between pollinators and herbivores.

Per-interaction effects of pollinators can vary with varying pollinator pollen load (and its quality) as well as probability of transporting pollen to a proper partner (see e.g. Larsson, 2005). An important determinant of per visit pollination effectiveness of a given plant individual is the composition and spatial structure of surrounding conspecific and heterospecific individuals (Chittka *et al.*, 1999, Kunin, 1993, but see on the other hand Ellis and Johnson, 2012, Nattero *et al.*, 2011, Schuett and Vamosi, 2010), which impact both pollen load and pollen carryover to legitimate acceptors. Pollen quality can be altered by these factors too (Liao et al., 2011), especially in combination with individual plant characteristics (Cosacov *et al.*, 2008, Grindeland *et al.*, 2005, Klinkhamer and de Jong, 1990). Finally, change in per-interaction pollen load can be also a result of effects of plant's surroundings on pollinator assemblage composition (Lázaro et al., 2009) in systems featuring pollinators with pollinators of different carryover capacity (e.g. Larsson, 2005, Brunet and Sweet, 2006). Nevertheless, differential per-interaction effects of pollinators on plant individual fitness can be mostly seen only as weak compared to effects of pollinator abundances. This is also due to the fact that in most plant species the relationship of fitness gain and pollen receipt is decelerating (Campbell, 1986, Cane and Schiffhauer, 2003, Niesenbaum, 1999, Silander and Primack, 1978) making more important the distinction between no and at least some pollen received. The most important features of plant's surroundings interacting with pollinator abundance experienced by individual plants are the numbers of flowering conspecifics and heterospecifics in plant's neighbourhood. Although both factors are frequently reported to positively affect pollinator presence (e.g. Hegland and Boeke, 2006, Ebeling *et al.*, 2008, HeglandGrytnes *et al.*, 2009, Liao *et al.*, 2011), the pollinator densities experienced by individual plants either do not change (Bosch and Waser, 1999, Kirchner *et al.*, 2005, Hegland and Totland, 2012) or even decline (Kunin, 1997), but some evidence of per plant positive density effects also exists (Liao et al., 2011). Moreover, increasing abundance of flowering conspecifics can also lead to increased attraction of nectar robbers (Sowig, 1989, Jennersten and Nilsson, 1993). Still some positive effects of surrounding conspecifics and (mostly) also heterospecifics on plant reproduction (Hegland and Totland, 2012) and pollen limitation alleviation (Jakobsson et al., 2009) were detected, but the increases in plant seed production should be treated with caution, due to a detected trade-off between number of offspring produced and their vigour (Hegland and Totland, 2008).

On the other hand, the potential for three-way interactions of herbivores, plant's environment and plant individuals is much greater. Differential impacts of herbivores on plants can be a result of different plant sizes in different environments, while the effect of herbivores remains constant, e.g. stalk grazers always removing the whole stalk (Gómez, 2005, Ehrlén and Münzbergová, 2009). The substantial difference from interactions of herbivory with plant size described in previous sections lies in the causal relationship between plant size and its environment, which is consistent across populations, years etc. (see e.g. Vanhoenacker et al., 2009). A more intricate way of interactions of herbivory and plant's environment lies in changing the outcome of other processes such as competition by herbivores. According to the expectation, impacts of herbivory on plant individuals of the same species are more pronounced in more competitive (Kim *et al.*, 2013, Rand, 2003, Russell and Spencer, 2010), and also more productive (Bonser and Reader, 1995), environments, although cases of no interaction also occur (Suwa *et al.*, 2010, Rees and Brown, 1992). Finally, surrounding environment can also limit plant's growth and allocation in reproduction and thus limit possible impact of herbivores on the plant, with plant's environment possibly encompassing abiotic conditions (Knochel and Seastedt, 2010) but also e.g. infestation by pathogens (Barker, 2008, Swope and Parker, 2010).

Once we move to effects of plant's environment on interaction frequency, i.e. effects of plant's environment on pollinator and herbivore occurrence on plants, the herbivores are also more prone to such effects than pollinators, since they (with exception of large vertebrates) are often more tightly linked to a given site than the more mobile pollinators and thus their vital rates are more influenced by local environmental characteristics (e.g. they need to find shelter, breeding sites etc. in neighbourhood). Despite these premisses, the body of evidence of effects of plant's environment on plants through herbivore occurrence is not too extensive and comprises both works confirming such influence (Loaiza *et al.*, 2011, Sullivan and Howe, 2011, Miller *et al.*, 2009, Reader, 1992, Förare and Engqvist, 1996, Kolb *et al.*, 2007) as well as those that have found no or only temporally inconsistent effects (von Euler *et al.*, 2014, Rose *et al.*, 2011). It is difficult to draw any summarising conclusions out of this evidence, since the existing studies vary in two fundamental features: i) herbivore group studied; ii) ranges (and types) of environmental gradients examined. Advancing of our understanding in this area would thus require more studies focusing either on more herbivore groups across the same gradient or one herbivore group across different gradients. The large vertebrate herbivores represent a different case, since they operate at rather different spatial scales than a single herb individual. Thus the effects of plant's environment on occurrence of large vertebrate herbivory can in fact be the result of large vertebrate choice at the patch level, for example due to presence of unpalatable neighbours (Baraza et al., 2006) or shrubs limiting herbivore movement (Gómez, 2005). A relatively understudied effect of plant's environment on herbivore occurrence is the impact of habitat fragmentation and spatial configuration of biotopes (but see Zabel and Tscharntke, 1998, Hůla *et al.*, 2004, Gutiérrez *et al.*, 2001). These mechanisms are more focused in pollination studies, where the results have more important implications for current nature conservation.

The examination of effects of plant's environment on plants mediated by changes in overall pollinator abundances has so far concentrated on large scale (hundreds to thousands metres) studies aimed primarily at detecting effects of habitat fragmentation and on impacts of climate change on plant-pollinator interactions. The effect of habitat fragmentation is relatively well documented in terms of reduced pollinator occurrence and pollen limitation of plants (see Hadley and Betts, 2012 for review). The proximate mechanisms of overall decreases of pollinator abundance in plant populations involve distance of target plant populations to larger semi-natural fragments hosting pollinators (Steffan-Dewenter and Tscharntke, 1999, Jakobsson and Ågren, 2014, Kohler *et al.*, 2008, Jauker *et al.*, 2009, Öckinger and Smith, 2007) as well as the actual configuration of surrounding biotopes (Dyer et al., 2012), especially the linear biotopes some of which constitute corridors (van Geert *et al.*, 2010, Cranmer *et al.*, 2012) and others obstacles (Bhattacharya *et al.*, 2003, Lövei *et al.*, 1998). The temporal dimension of plant's environment, i.e. effects of environment temporal synchrony of plant's flowering and its pollinators' emergence times, has been also studied to some degree. Such work was done especially in connection with global warming indicating shifts in both plant flowering and pollinator emergence phenology but failed to provide evidence of warming-induced plant-pollinator mismatches (Bartomeus *et al.*, 2011, e.g. HeglandNielsen *et al.*, 2009, Forrest *et al.*, 2010, Iler *et al.*, 2013). On the other hand, much less attention has been so far paid to two other areas of possibly important indirect influences of plant's environment mediated by overall pollinator abundances: i) temporal plant-pollinator mismatches caused by other sources of variation than climate, e.g. hay cut timing (but see Noordijk et al., 2009); ii) variation in pollinator composition due to plant's environment (e.g. vegetation structure, co-flowering species) at intermediate scales of tens to few hundred metres, i.e. within the same habitat fragment but among subpopulations of the focal plant species (but see Jakobsson et al., 2009).

1.1.3 Interferences and synergies between different plant-animal interactions (component 5)

Compared to interaction of plant's environment with plant-animal interactions, mutual interactive influences of two (or more) plant-animal interactions on plant individuals are relatively well-documented. Generally, we can distinguish the *a priori* antagonistic interactions of herbivores and pollinators and additive or non-additive effects of two (or more) herbivores sharing the same host plant.

The most commonly studied systems featuring herbivores and pollinators are those focusing on pollination and predispersal seed predation. In most cases, such systems result in the negative effect of seed predators cancelling out any beneficial effects of pollinators (Parachnowitsch and Caruso, 2008, Herrera, 2000, Herrera *et al.*, 2002, Lay *et al.*, 2011, Brody and Mitchell, 1997) although pollinators sometimes still contribute to a lesser degree (Lundin *et al.*, 2013, Swope and Parker, 2010) and this pattern may be dependent on overall levels of seed predation and pollination with different results under low pollinator abundances (Vanhoenacker et al., 2013). The importance of pollinators seems to increase also in plant species highly dependent on successful pollination (Montgomery, 2009), such as orchids (Sletvold et al., 2010). Stalk grazers and folivores usually do not directly interact in their effects with pollinators (Morris et al., 2007), but sometimes they interact indirectly through shifts in plant phenology (Sharaf and Price, 2004, Gómez, 2005, Rautio *et al.*, 2005). Herbivores other than seed predators can also interact with pollinators by decreasing the plant's attractiveness and thus its pollinator visitation rates (Lehtilä and Strauss, 1997, Lay *et al.*, 2011, Kessler *et al.*, 2011) or by decreasing the amount of resources available for setting seeds (Juenger and Bergelson, 1997, Barber *et al.*, 2011). In some cases, the interaction of herbivores and pollinators may also be involved in maintaining co-existence of different plant phenotypic forms (Asikainen and Mutikainen, 2005, de Jager and Ellis, 2014, Collin and Shykoff, 2010).

The plethora of studies examining the effects of two and more herbivores on individual plant performance (originating mainly from biocontrol, agricultural pest studies) has been summarised by Morris et al. (2007). Their metaanalysis did not find any significant non-additive trend in the outcome of interaction of two herbivores. Although there are studies reporting less-than-additive outcomes of the two interactions (of more recent works e.g. Brody *et al.*, 2008, Tack *et al.*, 2009), the majority of studies reports additive effects (of more recent works e.g. Irwin and Brody, 2011, Barber *et al.*, 2011). Less than additive outcomes can be expected under conditions of competition of herbivores for the common resource (Tack et al., 2009) or under conditions of one herbivore directly influencing availability of resource for the other one (Irwin and Brody, 2011, Blossey and Hunt-Joshi, 2003, Knochel and Seastedt, 2010).

Population-level effects of "cross-talk" between different plant-animal interactions have been rather understudied so far. There are two studies examining effects of more than herbivore on population dynamics (Leimu and Lehtilä, 2006, Rose *et al.*, 2005), but they do not explicitly consider they interaction. However, the mere combination of the isolated herbivore effects can result in non-linear effects on plant population dynamics (Leimu and Lehtilä, 2006). As far as I know, only one study explicitly addressed impacts of interactions with pollinators and herbivores on plant population dynamics, but it found neither any effect of pollinators nor of their interaction with herbivory (García and Ehrlén, 2002). However this may not be the prevailing case, since the interactions of herbivores and pollinators can shift genetic structure and associated trait composition in plant populations already in several seasons (Ågren et al., 2013). Such results emphasize the need for closer study of "cross-talk" of plant-animal interactions, since in field conditions essentially no such interaction occurs on itself (perhaps with exception of the very extreme habitats).

1.1.4 Changes in sensitivity of plant populations to effects of herbivores and pollinators (component 6)

Sensitivity of individual's life-time fitness or of population characteristics to plant animal interactions can change with impacts of other factors on different life-cycle stages. Thus although the per-interaction effect on the given vital rate as well as interaction frequency do not change, the same plant-animal interaction can have different impacts on individuals/populations in different environments. These changes in sensitivity are largely understudied (even compared to the number of studies of direct effects of plant's environment on these interactions), since such data require information on a large number of species' populations in all possible stages (i.e. growing, stable and declining).

Oostermeijer et al. (1996) demonstrated on data from several populations × seasons that elasticities (i.e. relative contributions to observed asymptotic population growth rate) of individual plant vital rates vary systematically with population growth rates. While importance of fecundity and growth increased with increasing population growth rates, the opposite was true for survival and shrinkage of individuals which were most important in declining populations. The effects of interactions with herbivores and pollinators on plant vital rates can thus impact population growth rates to a different degree (e.g. the effect of seed predators on plant seed production may be only marginal in declining populations, which are safe-site limited instead of seed limited growing populations).

There are at least two other pieces of evidence suggesting a link of this variation in plant-animal interaction impact with changing plant's environment. Populations of *Primula veris* along a successional gradient of canopy shading were differentially susceptible to seed predation (in terms of matrix sensitivities; Kolb *et al.*, 2007) and this was mainly (but not exclusively) due to changing overall ability of plants to set seeds and of seedlings to establish (Lehtilä et al., 2006). A similar pattern was observed in case of related *P. farinosa*, where this ability of plants to set seeds (and connected changes in susceptibility of population growth rate to seed predation) could be in some cases linked to site productivity (von Euler et al., 2014). However, in this particular system, also an indirect interaction of site productivity and seed predators as mediated by plant inflorescence size was detected in some years (Vanhoenacker et al., 2009). This only further documents the necessity of concurrent study of both plant-animal interaction and plant's environment on plant population dynamics, since the conditionally differential outcomes of plant animal interactions may be commoner than expected (see also e.g. Ibanez *et al.*, 2009, Ågren *et al.*, 2008).

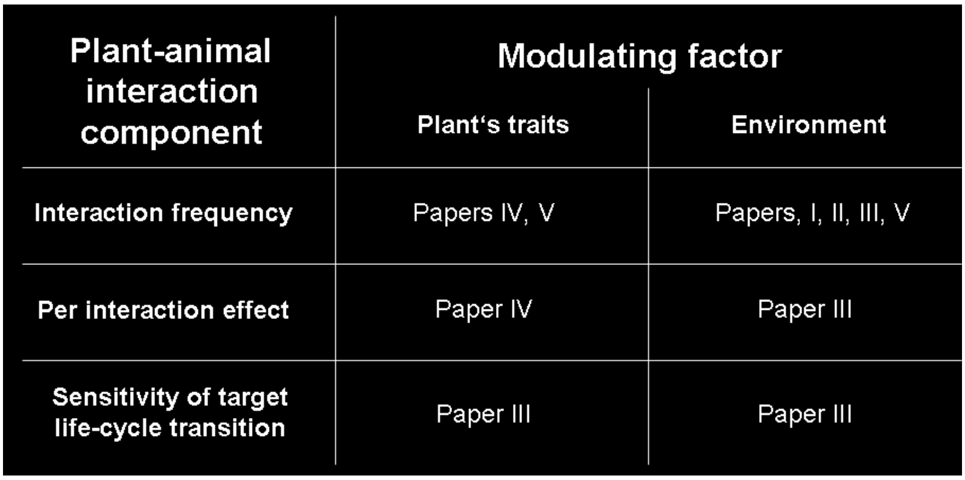
1.2 Aims of the thesis

Our understanding of plant-animal interactions has advanced in exploring the per-interaction effects of plant-animal interactions on vital rates of plant individuals. There is also relatively good knowledge on interaction of per-interaction effects of plant-animal interactions with plant's surrounding environment. On the contrary, we have not explored much the impacts of plant-animal interactions at the individual life-time fitness/population level, which can non-trivially translate the per-interaction effects on a particular vital rate to the whole life cycle. Furthermore, we still lack information on existence and importance of environmental drivers of plant-animal interactions at intermediate spatial scales (from subpopulations to landscapes, i.e. units of square kilometres). The present thesis aims to help to fill in these gaps by studying structure of plant-animal interactions in grassland ecosystems of Central Europe.

Specifically, I focus first on documenting the extent of effects of variation in plant's environment on occurrence of herbivores (Paper I) and pollinators (Paper II). Then we assess impact of different herbivores on plant population dynamics under the range of environments (Paper III). Further we focus in detail on interaction of herbivores and pollinators that shapes plant flowering strategies and consequently life-time fitness (Paper IV). And finally in Paper V, we identify the interaction between pollinators, reproductive success of common plant species and site characteristics and grassland management at landscape scale.

In terms of components of effects of individual plant-animal interactions (see Fig. 1), we focused on adding the lacking information on the effects of interaction frequency in relation with environment for both herbivores and pollinators and on comparing their relative importance (Table 1). The Papers III and IV then aim to synthesise over more components of effects of plant-animal interactions. While Paper III encompasses effects of plant's environment on all components of plant-animal interaction including the changes in sensitivity of life-cycle transitions, Paper IV is based more on the individual scale and addresses the complex effects of plant-animal interactions on a longer time scale including their mutual interference (but not including the possibly changing sensitivity of life-cycle transition).

Table 1: Summarisation of the plant-animal interaction components addressed by individual studies included into present thesis



Plant-animal interaction component	Modulating factor	
	Plant's traits	Environment
Interaction frequency	Papers IV, V	Papers, I, II, III, V
Per interaction effect	Paper IV	Paper III
Sensitivity of target life-cycle transition	Paper III	Paper III

1.3 Studied systems

I chose as my study system long-lived perennial plants occurring in semi-natural species-rich wet meadows of Central Europe. I focus in more detail on a once common but declining flagship species of these meadows, *Succisa pratensis*, its herbivores (generalist and specialist invertebrate folivores, vertebrate folivores, stalk grazers and predispersal seed predators; Fig. 2; Papers I, III and IV) and its hoverfly pollinators (Fig. 3; Paper IV). For documenting interactions of pollinators and landscape management, I chose as my study system the two more widespread long-lived perennials co-occuring with *S. pratensis*, *Achillea ptarmica* and *A. millefolium* (Fig. 3; Paper V). These three species species also share hoverflies as their main pollinators. Finally, Paper II focused on 8 dominant species at a single wet to moderately wet meadow (these species included also *S. pratensis*).

All five studies have been conducted between 2010 and 2013 in two study areas in the Czech Republic. The first study area lies in PLA Slavkovský les around the Ovesné Kladruby village (N 49.9528839, E 12.7785539, WGS 1984, region of Karlovy Vary; involved Papers I, III). Studies underlying the remaining papers were conducted in Central Bohemia, region of Kutná Hora at neighbouring meadows K Handrkovu and Na nové kopanině (N 49.8466564, E 15.1498422; Papers II, IV) or in a broader landscape around them (Paper V).

Fig. 2: *Succisa pratensis*, the main study species (A, see Papers I, III, IV and partly II) and damge caused by its herbivores; B – damage caused by vertebrate folivores (mainly roe deer and cattle); C, D – outcomes of generalist invertebrate folivory, 2.5 cm wide plastic tag used for permanent marking can be seen in section D; E – caterpillars of specialised butterfly *Euphydryas aurinia*; F – stalks grazed by large vertebrates (again mainly roe deer and cattle); G – a seed consumed by seed predators, presumably larvae of Tortricidae butterflies.

1.4 Synopsis of employed methods

The core of the studies in Papers I, III, IV relies in monitoring individually tagged plant individuals for two (Papers I and III) or five years (Paper IV). The vital rates of tagged plants (i.e. survival, growth, reproduction) were measured and presence of damage by particular herbivore groups (at semi-quantitative scale) was recorded. Pollinator activity was monitored at the same 12 permanent plots as the plants monitored for vital rates and herbivory in replicated censuses with actual weather conditions and portion of *S. pratensis* florets open recorded, however we use only standardised sums (per unit inflorescence and unit time) of pollinator visitation in Paper IV. Plant's environment in Papers I and III was characterised by dividing the 27 populations into subpopulation patches and by characterising these patches by means of phytosociological relevés. Our design thus allowed us to assess the effects of plant's environment on herbivore occurrence only at the population and subpopulation/patch level.

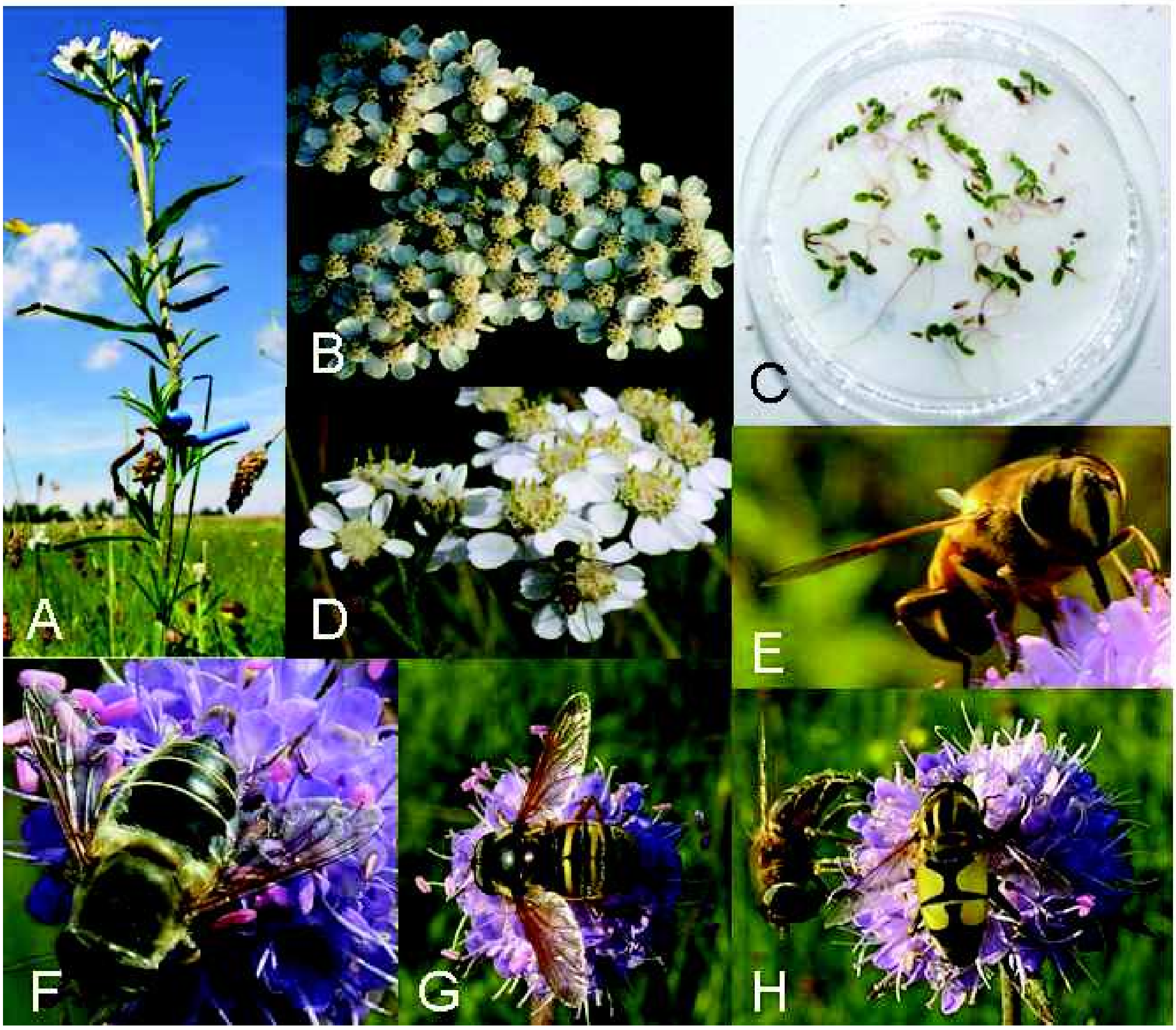


Fig. 3: A – *Achillea ptarmica* individual studied for seed production with tag (Paper V); B – *Achillea millefolium* agg. (the second study species of Paper V); C – Petri dish with germinated *Achillea* seedlings (reproductive success was measured in Paper V both as seed production and seed germination rate); D – flowering *A. ptarmica* pollinated by hoverfly *Sphaerophoria scripta* agg.; E – hoverfly *Eristalis tenax*, responsible for ca 45% of pollinator visits of *S. pratensis* in Paper IV; F – *E. interruptus*, responsible for ca 26% of pollinator visits of *S. pratensis* in Paper IV; G – a relatively rare hoverfly species *Sericomyia silentis*, which specialises in the study are almost solely on *S. pratensis*; H – *Helophilus* cf. *trivittatus*, *Helophilus* spp. form the third important hoverfly group pollinating *S. pratensis* with ca 16% of visits in Paper IV.

On the contrary, data for studies reported in Papers II and V were conducted at plots within one season. I used a regular grid of 4×4 m plots covering whole meadow K Handrkovu, where all plant flowering stalks were recorded (quantitatively or semi-quantitatively depending on species). The plots were then again censused for pollinators at repeated visits, where all insects contacting plant reproductive structures (i.e. pollinators in our definition) at the moment of encounter were recorded. For sake of robustness of obtained data, we used only data for eight dominant plant species with more than 200 recorded pollinators. The landscape-scale study featuring the *Achillea* species (Paper V) had the most complicated the design featuring two species at two types of habitats (semi-natural meadows and surrogate verge sites) in three time intervals (beginning, peak and end of the flowering period). The plots were assigned to *Achillea* populations at spatially stratified random design. At each plot, amount of floral resources was assessed in 4×4 m (meadows) or 4×2 m plots (verges) in the same way as in Paper II, but also the amount of floral resources was assessed (on a logarithmic scale) in 15 m circular surroundings of the plot as well as the population sizes of focal *Achillea* species. Within each plot 5 five actively individuals that flowered (defined as possessing pollen presenting florets) at the start of the focal 5 day monitoring time period were tagged and later after seed ripening harvested for determination of seed set (Fig. 3). The insects were censused at least five times during the 5 day monitoring time period.

The methodological apparatus of data analyses consisted mainly of linear and generalised linear models applied both to basic (Papers I and IV), and more structured designs (Papers III and V). Datasets on pollinator composition (Paper II) were by their nature multivariate and therefore they were analysed by means of redundancy analysis (RDA). In Paper III examining the effects of herbivores on plant population dynamics, I assembled from the regressions of vital rates an integral projection model (Easterling et al., 2000; hereafter IPM) and analysed the resulting IPM matrices by standardised population biology tools (Caswell, 2001). We aimed at disentangling the main effects of plant's environment (as represented by surrounding vegetation) on vital rates, the main effects of interaction frequency of herbivory and its per-interaction effect as well as their interactions with plant's environment and changes in sensitivity of life-cycle transitions in different environments. This we achieved by considering different parameterisations of the obtained IPMs.

The study of interactions of pollinators and plant's environment in Paper V contained two different groups of predictors of plant reproductive success. The predictors describing plant's environment (i.e. timing of flowering, meadow/verge sites, site attractiveness, local *Achillea* population) could influence besides the *Achillea* reproductive success (i.e. seed production and germinability) also other predictors possibly affecting *Achillea* reproductive success (i.e. plant size, reproductive investment, pollinator densities). These two different groups of predictors (corresponding to endogeneous and exogeneous variables in path-analysis terminology) were reflected by the ordering of factors in individual linear regressions applied to obtained data. Combining the results of these regressions and amounts of explained variability allowed us to separate again the direct effects of environment, pollinators, their interaction and indirect effects.

1.5 Main results of the individual studies

1.5.1 *Paper I –Folivores tend to be affected more than other herbivore groups by vegetation type a plant grows in*

We assessed the effects of surrounding vegetation on five herbivore groups in 27 populations divided into 103 subpopulations at both these levels. All three folivore functional groups (generalist invertebrates, larvae of specialist butterfly *Euphydryas aurinia* and large vertebrates) turned out to be affected by the vegetation type *S. pratensis* grew in either at the subpopulation scale (large vertebrates) or at both studied scales (generalist invertebrates, *E. aurinia*), but the effect of vegetation types was highly relevant only for *E. aurinia* and generalist invertebrates. On the other hand, seed predators responded only to focal plant population characteristics (e.g. flower head density) and stalk grazing by large vertebrates was not influenced by any of the predictors considered.

Absence of response to vegetation types in seed predators and stalk grazers can be explained by their high mobility, low impact of surrounding vegetation on size and detectability of their target structures and selectivity with respect to individual plants (data not shown). The same large herbivores, i.e. the roe deer, which cause stalk grazing, cause vertebrate folivory, but this we explain as a side product of their selectivity to vegetation, they graze at, since the leaves of *S. pratensis* protected by a suite of alkaloids and iridoids (Torssell, 1963, Jensen, 1992) represent a very unlikely first-choice fodder. On the contrary, the generalist invertebrate folivores and specialist *E. aurinia* directly interact with the surrounding vegetation and thus its large effect on their occurrence needs to be taken into account when assessing their impacts on *S. pratensis* population dynamics.

1.5.2 Paper II – Plant pollinator spectra as a function of plant's surroundings

All examined plant species showed pronounced differences in their pollinator composition (at the level of functional groups) at the scale of few tens of metres. In four out of eight species tested (and fifth marginally significantly), pollinator densities and composition responded to conspecific flowering stalk density. Pollinator composition and partly densities of one species were also influenced by the overall composition of flowering species. Where detected, the effects of increasing abundance of flowering conspecifics negatively affected overall pollinator densities with the strongest effect on the most dominant pollinator groups.

We attempted to explain this by saturation of pollinators with increasing flower supply, which is also supposed to occur at lower focal plant densities for dominant pollinator groups and thus the proportion of visits undertaken by dominant pollinators declines with increasing focal plant density. In general, we were able to demonstrate that effects of plant's environment can influence considerably its pollinator densities and composition even in relatively homogeneous conditions of a single meadow.

1.5.3 Paper III – The effect of interaction of herbivores and plant's environment on plant population dynamics is comparable to their main effects

We expressed the impacts of herbivores, plant's environment (here vegetation type *S. pratensis* individuals grow in) and their interaction on *S. pratensis* life cycle and population dynamics by differences in projected deterministic asymptotic growth rates λ. Using this measure, which is one of few utilisable measures of impact on life cycle in iteroparous perennials, indicated that plant's environment was the most important in determining population growth rate (mean $|\Delta\lambda| = 0.113$), but the main effect of herbivory (mean $|\Delta\lambda| = 0.073$) and interaction effect of herbivory and plant's environment were of the same order of magnitude. The component of the interaction term corresponding to per-interaction effect + effects of changes in sensitivity of given vital rates (mean $|\Delta\lambda| = 0.047$) was much more important than the effects of plant's environment on interaction frequency (i.e. herbivore occurrence; mean $|\Delta\lambda| = 0.0092$). Low importance of interaction frequency component can be also (partly) explained by the fact that only generalist invertebrates (out of the herbivore groups affecting substantially plant vital rates) react in their occurrence to vegetation types (Paper I) and their contribution to overall effect of herbivory on population growth was less important than in stalk grazers and seed predators.

The interaction of herbivory and plant's environment led to non-trivial changes in elasticities of different transitions in IPM. Herbivory resulted in a general trend of the decrease of importance of reproduction and seedling transitions in *S. pratensis* life cycle. However, the changes of elasticities in established plants were vegetation-type specific, both in terms of plant size (in some vegetation types smaller plants gained consistently on importance, whereas in others larger plants gained on importance) and in terms of vital rates (in most vegetation types importance of stasis and shrinkage increased at the expense of growth). Such changes have potential to change substantially (and differentially with respect to vegetation types) susceptibility of *S. pratensis* populations to other drivers of its population dynamics (e.g. pollinators).

1.5.4 Paper IV – Plant flowering strategy is shaped by opposing selection pressures by pollinators and seed predators

Both pollinators and seed predators exerted selection pressures on *S. pratensis*, which turned into differences in one-year plant fitness. The effect of seed predators was approximately 2.5 times greater than that of pollinators. We considered two contrasting flowering strategies, when assessing long-term four-year individual plant fitness. On one hand, we took plants that flowered only once during the whole period and on the other plants that flowered at least twice and this we corrected for reproduction investment (i.e. summed number of florets produced). Plants flowering more frequently had advantage against those, which allocated the same reproductive investment only into one flowering event. Their advantage increased with total reproductive investment from a negligible one to approximately 25% higher four-year seed production across the region of coexistence of the two strategies.

The advantage of plants flowering more frequently disappeared once we assumed no seed predation (i.e. counting all predated seeds as healthy developed seeds). This result corresponds to detected preferences of the two animal groups. While pollinators (expressed as visitation per unit inflorescence and time) did not respond to inflorescence size (i.e. reproductive investment), seed predators (expressed as # predated seeds per unit inflorescence size) did. Pollinators responded more to stalk heights, which are not much correlated with total inflorescence height and perhaps constitute a second, less important axis of variation in *S. pratensis* reproductive allocation. In general, our results support a view that under common field conditions seed predators are more important than pollinators and their preferences for larger inflorescences result in a notable fitness advantage of plants flowering more frequently (compared to those flowering less frequently with the same reproductive investment).

1.5.5 Paper V –Timing of flowering indirectly affects plant reproductive success through changes in pollinator densities

Pollinator densities turned out to be a dominant factor determining reproductive success of both *Achillea* species and in case of *A. millefolium* they were even the only factor directly influencing seed production. Hoverfly densities markedly decreased with advancing flowering season of *Achillea* (from median catch per census of ca 11 hoverflies at the beginning of flowering season to ca 5 at peak and to ca 1 at the end of flowering season). The effects of other factors on hoverfly densities such as plot attractiveness or sizes of *Achillea* populations were an order of magnitude weaker. Plant's environment never directly influenced reproductive success of *Achillea* and its effects only indirect mediated by hoverfly densities. However, our experimental design did not allow us to test for interactions between pollinators and plant's environment.

Flowering season of *Achillea* species is besides other influences determined also by management practices, mainly timing of the hay cut in semi-natural meadows. It causes the shift in flowering of meadow populations, which is pronounced especially in *A. ptarmica*. This shift results not only in lower experienced hoverfly densities due to seasonal decline in hoverfly abundances but also in loss of total seed production in part of the meadow populations due to the second hay cut (they do not manage to get the seeds ripe before hay cut). Thus the plant's environment has a rather overriding effect over the plant-animal interaction, although there was still some variation in reproductive success influenced by other drivers of pollinator abundances (such as the difference between semi-natural and surrogate verge sites).

1.6 Discussion of thesis results

1.6.1 Effects of plant's environment on interaction frequency

Both papers aimed at understanding the extent of influences of plant's environment on frequency of occurrence of plant-animal interactions indicated such effects occur (Paper I and II). While this seems to be a general pattern (in studied system) in case of pollinators (all studied plant species with sufficient data), we found significant effects of plant's environment (other than those mediated by focal plant traits) only in foliage feeding groups of herbivores. Closer examination reveals also the differences between the two groups in mechanisms behind the observed pattern.

Dependence of pollinator density and composition on conspecific flower density is congruent with most of the existing literature, i.e. general decrease of per plant visitation rates (Kunin, 1997, Bosch and Waser, 1999, Hegland and Totland, 2012, Kirchner *et al.*, 2005) as well as more diverse pollinator composition in high abundance patches (Lázaro et al., 2009). However these data came either from distinctly spatially separated sites or from one site observed at different times of year and thus the observed pattern could not be unambiguously attributed to pollinator local choices. In our study (Paper II), pollinators could freely move across the relatively small study meadow and therefore the relative influence of factors other than pollinator preferences on their spatial distribution was much smaller.

Spatially inhomogeneous structuring of pollinator communities at medium spatial scales (tens of metres) could have impacts on plant population dynamics in two ways: 1) decreasing reproductive success of individuals in dense patches; 2) promoting assortative mating; both of which would deserve further study. There is already some supporting evidence for the first conjecture, namely that the species with most pollinator visits in a similar system (some of which were also among our study species) were pollen limited (Hegland and Totland, 2008, but see also Jakobsson *et al.*, 2009 for contrasting evidence in one species). Assortative mating of individuals could cause problems especially in small populations and in self-incompatible species (Young et al., 2012) further decreasing the effective population size, which may be the case even for relatively common species in the study area (Paper V).

The observed pattern of more visits per plant in plots with lower conspecific densities could be also caused by negative correlation between plant attractiveness (based on individual traits) and conspecific patch density. Indeed in data for Paper III, we found that less dense patches contained on average greater individuals (data not shown), but we believe this was not the problem in Paper II, since conspecific densities in all studied plots varied an order of magnitude less compared to conspecific patch densities studied in Paper III. Unfortunately, this possible explanation for patterns of pollinator densities in patches of various densities has not been considered in studies of plant-individual visitation rates

(Grindeland *et al.*, 2005, Klinkhamer and de Jong, 1990) and thus we cannot unambiguously ascribe the effects conspecific plant density to one of these factors (although the effect of density per se is much more probable).

On the other hand, herbivores form both from the taxonomical and ecological viewpoint a much more diverse group interacting with plants. The conducted comparison of five herbivore groups across the same (relatively mild) gradient (Paper I) allowed us to shift from asking questions whether a particular herbivore group responds to a certain feature of plant's environment to asking questions about relative importance of plant's environment for occurrence among different herbivore functional groups. In general, evidence accumulates that herbivores constitute a much more heterogeneous group and have to be treated separately when considering their interactions with plant's environment.

Specifically, our results from a relatively mild environmental gradient support an already existing notion that seed predators do not respond to plant's environment (von Euler *et al.*, 2014, Rose *et al.*, 2011) unless we consider really strong gradients of microclimatic conditions (Kolb *et al.*, 2007, Miller *et al.*, 2009). An unexpected result of Paper I was the comparable degree of dependence on surrounding vegetation of both generalist invertebrate folivores and specialist butterfly *Euphydryas aurinia*, known for its specific habitat needs (Konvička *et al.*, 2003, Porter, 1982, Anthes *et al.*, 2003, Fowles and Smith, 2006). Such result suggests that once we control difference in diet breadths (in our case by focusing on herbivores of *Succisa pratensis*) we could expect similar sensitivity to environmental conditions of both specialist and generalist herbivores. More precisely known and seemingly more stringent environmental preferences of specialists could be only a result of lack of studies of generalists at comparable environmental gradients (but see Loaiza et al., 2011). One of the reason for this lack of studies of generalist herbivores may be also bad identifyability of damage caused by generalist herbivores, which means in fact that often more than one generalist herbivore are studied at once.

1.6.2 Consistence of effects of plant's environment on interaction frequency

The importance of effects of plant's environment on interaction frequency is sometimes is sometimes very contrasting. Paper V documents influences of plant's environment on interaction frequency as their most important driver, whereas the study of pollinator visitation coupling the Paper IV (Pavlíková, A. et al., unpublished data) detected only a much weaker effect of plant's environment on pollinator visitation with the effect of plant traits being more important. A possible explanation to this pattern may be the spatial and temporal scale of both experiments (Paper V was done at landscape scale within two months, while Paper IV was done at the scale of metres within days). Measured environmental characteristics also overlapped only partially. Such contradictory results indicate that factors determining the nature of plant-animal interactions change with scales. Results of this thesis allow formulating a relatively bold but not yet testable hypothesis that pollinators with their loose relationship to target plants would be much more prone to these changes in factors determining their occurrence (with increasing temporal and spatial scale the scale of operation of these determinants would increase also) , while the less mobile the herbivores shall be the more their occurrence could be predicted based upon the knowledge of local conditions.

1.6.3 Impacts of biotic interactions on plant population dynamics

Once we move from herbivore occurrence to their per-interaction effect, a different picture of role of plant's environment in outcome of plant-herbivore interactions emerges. In our study systems, direct effects of environment, its interaction with per-interaction effect of herbivores and environment-induced changes to sensitivity of life cycle to vital rate affected by herbivore distinctly prevail over effects of environment on herbivore interaction frequency (Paper III). However, a longer study would be necessary to establish such conclusion on a firmer basis, but still the common presence of interactions of per-interaction effects of herbivory with plant's environment suggests that such may be at least of the same importance as effects of plant's environment on herbivore interaction frequency.

Another contribution of Paper III lies in an attempt to partition out the individual components of impact of plant-herbivore interactions on plant population dynamics (i.e. interaction frequency, per-interaction effect and sensitivity of life-time fitness and population dynamics to a given life-cycle transition). We hope this partition can help bridge the gap between unstructured measures of herbivore impact on plant population growth rates, i.e. $\Delta\lambda$ (e.g. Ehrlén, 2003, Miller *et al.*, 2009, Kauffman and Maron, 2006, Münzbergová, 2005), evidence for changes in sensitivity to different plant vital rates across different local conditions (Oostermeijer *et al.*, 1996, Kolb *et al.*, 2007, von Euler *et al.*, 2014) and effects of plant environment on interaction frequency (Loaiza *et al.*, 2011, Förare and Engqvist, 1996, Östergård and Ehrlén, 2005, Kolb *et al.*, 2007). We suggest that such approach can also explain part of the contradictory results often obtained when studying plant-animal interactions (see e.g. Knochel and Seastedt, 2010). However, an important disadvantage of our approach is the necessity of study of more populations across multiple years.

The detected changes in elasticity of different vital rates due to herbivory constitute another important outcome of Paper III. The structure of these changes had some common features, but the vegetation-type-specific changes were important as well. These changes in elasticities thus alter considerably the susceptibility of studied plant populations to other environmental factors (e.g. cessation of management). This is, up to our knowledge, the first evidence that plant-animal interactions themselves alter sensitivity of plant populations to other factors. Herbivory-induced changes in elasticities should be examined with the same attention as the changes in population growth rates λ, since they can provide us information on the vital rates important to the species under a broader range of conditions than the actual ones (cf. Lehtilä et al., 2006).

Effects of pollinators on plant population dynamics seem to be generally much more shaped by effects of plant's environment on interaction frequency. Their high mobility and predominantly low dependence on particular plant species (i.e. generalisation; Lázaro *et al.*, 2008, Olesen *et al.*, 2008, Memmott, 1999) enables them to optimise their choice of feeding patches (e.g. Hegland and Boeke, 2006, Hatfield and LeBuhn, 2007, Kleijn and van Langevelde, 2006, Pontin *et al.*, 2006) and thus reflect plant's environment more than e.g. the specialist or less mobile herbivore groups (in this respect, large vertebrate herbivores are the only herbivore group, which is in a relatively similar situation as pollinators). Furthermore, insect pollinator activity is also more prone to climatic and microclimatic variation than that of herbivores, because (at least) three conditions for successful pollination need to be met synchronously and all of them are climate/microclimate-dependent: i) right timing of pollinator development; ii) weather conditions favourable for pollinator activity; iii) right timing of flowering of pollinated plants. In this light, the results of Paper V add to existing evidence of importance of the temporal component and its dependence on landscape management.

1.6.4 The role of biotic interactions in selection of plant flowering strategies

Seed predators exerted stronger influence on *S. pratensis* reproductive success than pollinators (Paper IV) and no interaction was found among the effects of these two groups, which is congruent with the main body of literature on this topic (Parachnowitsch and

Caruso, 2008, Herrera, 2000, Herrera *et al.*, 2002, Brody and Mitchell, 1997, Lay *et al.*, 2011). Increase of seed predation intensity with inflorescence size combined with lack of this relationship in case of pollinators sets basis for non-linear combinations of selection pressures conveyed by these two groups and non-linear returns of reproductive investment dependent on actual abundance of pollinators and seed predators (Vanhoenacker et al., 2013). It is this non-linearity of seed predator selection pressure, which is likely to be the cause of long-term advantage of more frequently flowering plant individuals.

Over a broader scope, our results suggest that plant-animal interactions (in our case predispersal seed predation) may thus be the important driver behind differentiation of flowering strategies in long-lived iteroparous plant species. In fact, a similar process was observed in case of normally semelparous species *Cynoglossum officinale*, where partial release from biotic interactions enabled switch to iteropary (Williams, 2009, Maron *et al.*, 2010). Plant-animal interactions could thus as well be part of an explanation to recently discovered trade-offs between plant life-expectancy and intensity of reproduction at among-species level (Mbeau-Ache and Franco, 2013).

1.6.5 Relative importance of plant-animal interaction components

When considering the general framework delimiting the components of plant-animal interactions outlined in the introduction (Fig. 1), this thesis is rather a set of case studies gathering evidence for future evaluation of relative importances of these components. So far, only several preliminary and partial conclusions can be drawn (for detailed argumentations see above). The effects of interaction frequencies seem to be more important than per-interaction effects in case of pollinators, while in case of herbivores, the relative importance of interaction frequency and per-interaction effects seems to depend on particular herbivore group, although interaction frequency seems to be generally of lesser importance compared to per-interaction effect. Of relative importance of changes in sensitivity of the life cycle transitions, we cannot draw almost any conclusions. The works of Oostermeijer et al. (1996) suggest that most prone to changes in sensitivity should be effects of pollinators and those herbivore groups, which affect directly plant reproduction. In general it could be concluded that all considered mechanisms determining the impact of plant-animal interactions on plant lifetime fitness need to be taken into account.

1.7 Conclusions

We found contrasting patterns in effects of plant's environment on occurrence of both pollinators and herbivores. While such effects seemed to be quite general in pollination, only those groups of herbivores most tightly linked to given plant individuals showed a distribution reflecting the characteristics of plant's environment. These patterns translated also into the relative importance of effects of plant's environment on plant population dynamics mediated by changes in frequency of a plant-animal interaction. Effects on interaction frequency were dominant when studying the role of pollination in reproductive success, whereas they were weaker compared to the direct interaction of effect of herbivores with plant's environment. Herbivores exerted a similarly important influence as plant's environment (or more exactly surrounding vegetation as one of its major components) over plant population dynamics. Importantly, we were able to document for the first time changes in elasticities of vital rates following the effects of interaction of herbivory and plant's environment, which provide a basis for higher order interactions of herbivory, surrounding vegetation and other components of plant's environment. The profound effects of herbivores on plant life-history manifested themselves also in the form of fitness advantage of *S. pratensis* individuals flowering more frequently (when controlled for total reproductive investment). Advantage of the "flowering more frequently" strategy was mainly caused by consistent non-linear selection pressure of seed predators. Summed together, the results of the thesis underscore the importance of study of plant-animal interactions for understanding the life-histories of iteroparous perennial plants. Now, the comparative studies involving many species are necessary in order to find and test the general rules underlying the immense variation in the outcomes of plant-animal interactions. I hope this thesis helped a bit in suggesting on what phenomena to concentrate and how to interpret the obtained results.

1.8 References

Ågren, J., Ehrlén, J. & Solbreck, C. (2008) Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology,* **96,** 334-345.

Ågren, J., Hellström, F., Toräng, P. & Ehrlén, J. (2013) Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. *Proceedings of the National Academy of Sciences,* **110,** 18202-18207.

Alonso, C. & Herrera, C.M. (1996) Variation in herbivory within and among plants of *Daphne laureola* (*Thymelaeaceae*): Correlation with plant size and architecture. *Journal of Ecology,* **84,** 495-502.

Anthes, N., Fartmann, T., Hermann, G. & Kaule, G. (2003) Combining larval habitat quality and metapopulation structure: The key for successful management of pre-alpine *Euphydryas aurinia* colonies. *Journal of Insect Conservation,* **7,** 175-185.

Asikainen, E. & Mutikainen, P. (2005) Preferences of pollinators and herbivores in gynodioecious *Geranium sylvaticum*. *Annals of Botany,* **95,** 879-886.

Baraza, E., Zamora, R. & Hodar, J.A. (2006) Conditional outcomes in plant-herbivore interactions: neighbours matter. *Oikos,* **113,** 148-156.

Barber, N.A., Adler, L.S. & Bernardo, H.L. (2011) Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. *Oecologia,* **165,** 377-386.

Barker, G.M. (2008) Mollusc herbivory influenced by endophytic clavicipitaceous fungal infections in grasses. *Annals of Applied Biology,* **153,** 381-393.

Bartomeus, I., Ascher, J.S., Wagner, D., Danforth, B.N., Colla, S., Kornbluth, S. & Winfree, R. (2011) Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences of the United States of America,* **108,** 20645-20649.

Bhattacharya, M., Primack, R.B. & Gerwein, J. (2003) Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? *Biological Conservation,* **109,** 37-45.

Blossey, B. & Hunt-Joshi, T.R. (2003) Belowground herbivory by insects: Influence on plants and aboveground herbivores. *Annual Review of Entomology,* **48,** 521-547.

Bonser, S.P. & Reader, R.J. (1995) Plant competition and herbivory in relation to vegetation biomass. *Ecology,* **76,** 2176-2183.

Bosch, M. & Waser, N.M. (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (*Ranunculaceae*). *American Journal of Botany,* **86,** 871-879.

Brody, A.K., Irwin, R.E., McCutcheon, M.L. & Parsons, E.C. (2008) Interactions between nectar robbers and seed predators mediated by a shared host plant, *Ipomopsis aggregata*. *Oecologia,* **155,** 75-84.

Brody, A.K. & Mitchell, R.J. (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia,* **110,** 86-93.

Brunet, J. & Sweet, H.R. (2006) Impact of insect pollinator group and floral display size on outcrossing rate. *Evolution,* **60,** 234-246.

Campbell, D.R. (1986) Predicting plant reproductive success from models of competition for pollination. *Oikos,* **47,** 257-266.

Cane, J.H. & Schiffhauer, D. (2003) Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon Ericaceae*). *American Journal of Botany,* **90,** 1425-1432.

Cariveau, D., Irwin, R.E., Brody, A.K., Garcia-Mayeya, L.S. & von der Ohe, A. (2004) Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos,* **104,** 15-26.

Caswell, H. (2001) *Matrix population model: construction, analysis, and interpretation.* Sinauer Associates, Inc. Publishers, Sunderland, MA, USA.

Collin, C.L. & Shykoff, J.A. (2010) Flowering phenology and female fitness: impact of a pre-dispersal seed predator on a sexually polymorphic species. *Plant Ecology,* **206,** 1-13.

Cosacov, A., Nattero, J. & Cocucci, A.A. (2008) Variation of pollinator assemblages and pollen limitation in a locally specialized system: The oil-producing *Nierembergia linariifolia* (Solanaceae). *Annals of Botany,* **102,** 723-734.

Cranmer, L., McCollin, D. & Ollerton, J. (2012) Landscape structure influences pollinator movements and directly affects plant reproductive success. *Oikos,* **121,** 562-568.

de Jager, M.L. & Ellis, A.G. (2014) Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects. *Annals of Botany,* **113,** 213-222.

Dyer, R.J., Chan, D.M., Gardiakos, V.A. & Meadows, C.A. (2012) Pollination graphs: quantifying pollen pool covariance networks and the influence of intervening landscape on genetic connectivity in the North American understory tree, Cornus florida L. *Landscape Ecology,* **27,** 239-251.

Easterling, M.R., Ellner, S.P. & Dixon, P.M. (2000) Size-specific sensitivity: Applying a new structured population model. *Ecology,* **81,** 694-708.

Ebeling, A., Klein, A.M., Schumacher, J., Weisser, W.W. & Tscharntke, T. (2008) How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos,* **117,** 1808-1815.

Ehrlén, J. (1995) Demography of the perennial herb *Lathyrus vernus* - 1. Herbivory and individual performance. *Journal of Ecology,* **83,** 287-295.

Ehrlén, J. (1996) Spatiotemporal variation in predispersal seed predation intensity. *Oecologia,* **108,** 708-713.

Ehrlén, J. (2002) Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). *Perspectives in Plant Ecology, Evolution and Systematics,* **5,** 145-163.

Ehrlén, J. (2003) Fitness components versus total demographic effects: Evaluating herbivore impacts on a perennial herb. *American Naturalist,* **162,** 796-810.

Ehrlén, J. & Münzbergová, Z. (2009) Timing of flowering: Opposed selection on different fitness components and trait covariation. *American Naturalist,* **173,** 819-830.

Ehrlén, J. & van Groenendael, J. (2001) Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. *Journal of Ecology,* **89,** 237-246.

Ellis, A.G. & Johnson, S.D. (2012) Lack of floral constancy by bee fly pollinators: implications for ethological isolation in an African daisy. *Behavioral Ecology,* **23,** 729-734.

Förare, J. & Engqvist, L. (1996) Suboptimal patch and plant choice by an ovipositing monophagous moth - An insurance against bad weather? *Oikos,* **77,** 301-308.

Forrest, J., Inouye, D.W. & Thomson, J.D. (2010) Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? *Ecology,* **91,** 431-440.

Fowles, A.P. & Smith, R.G. (2006) Mapping the habitat quality of patch networks for the marsh fritillary *Euphydryas aurinia* (Rottemburg, 1775) (Lepidoptera, Nymphalidae) in Wales. *Journal of Insect Conservation,* **10,** 161-177.

Fröborg, H. & Eriksson, O. (2003) Predispersal seed predation and population dynamics in the perennial understorey herb *Actaea spicata*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **81,** 1058-1069.

García, M.B. & Ehrlén, J. (2002) Reproductive effort and herbivory timing in a perennial herb: Fitness components at the individual and population levels. *American Journal of Botany,* **89,** 1295-1302.

Gómez, J.M. (2005) Long-term effects of ungulates on performance, abundance, and spatial distribution of two montane herbs. *Ecological Monographs,* **75,** 231-258.

Grindeland, J.M., Sletvold, N. & Ims, R.A. (2005) Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Functional Ecology,* **19,** 383-390.

Gutiérrez, D., León-Cortés, J.L., Menéndez, R., Wilson, R.J., Cowley, M.J.R. & Thomas, C.D. (2001) Metapopulations of four lepidopteran herbivores on a single host plant, *Lotus corniculatus*. *Ecology,* **82,** 1371-1386.

Hadley, A.S. & Betts, M.G. (2012) The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. *Biological Reviews,* **87,** 526-544.

Hansen, V.I. & Totland, Ø. (2006) Pollinator visitation, pollen limitation, and selection on flower size through female function in contrasting habitats within a population of *Campanula persicifolia*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **84,** 412-420.

Hatfield, R.G. & LeBuhn, G. (2007) Patch and landscape factors shape community assemblage of bumble bees, *Bombus* spp. (Hymenoptera : Apidae), in montane meadows. *Biological Conservation,* **139,** 150-158.

Hegland, S.J. & Boeke, L. (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology,* **31,** 532-538.

Hegland, S.J., Grytnes, J.-A. & Totland, Ø. (2009) The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research,* **24,** 929-936.

Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.L. & Totland, Ø. (2009) How does climate warming affect plant-pollinator interactions? *Ecology Letters,* **12,** 184-195.

Hegland, S.J. & Totland, Ø. (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos,* **117,** 883-891.

Hegland, S.J. & Totland, Ø. (2012) Interactions for pollinator visitation and their consequences for reproduction in a plant community. *Acta Oecologica-International Journal of Ecology,* **43,** 95-103.

Hendrix, S.D. (1979) Compensatory reproduction in a biennial herb following insect defloration. *Oecologia,* **42,** 107-118.

Herrera, C.M. (2000) Measuring the effects of pollinators and herbivores: Evidence for non-additivity in a perennial herb. *Ecology,* **81,** 2170-2176.

Herrera, C.M., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M., García, M.B., Guitián, J. & Manzaneda, A.J. (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 16823-16828.

Hůla, V., Konvička, M., Pavlička, A. & Fric, Z. (2004) Marsh Fritillary (*Euphydryas aurinia*) in the Czech Republic: monitoring, metapopulation structure, and conservation of an endangered butterfly. *Entomologica Fennica,* **15,** 231-241.

Childs, D.Z., Rees, M., Rose, K.E., Grubb, P.J. & Ellner, S.P. (2004) Evolution of size-dependent flowering in a variable environment: construction and analysis of a stochastic integral projection model. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **271,** 425-434.

Chittka, L., Thomson, J.D. & Waser, N.M. (1999) Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften,* **86,** 361-377.

Ibanez, S., Dujardin, G. & Després, L. (2009) Stability of floral specialization in Trollius europaeus in contrasting ecological environments. *Journal of Evolutionary Biology,* **22,** 1183-1192.

Iler, A.M., Høye, T.T., Inouye, D.W. & Schmidt, N.M. (2013) Nonlinear flowering responses to climate: are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B-Biological Sciences,* **368**.

Irwin, R.E. & Brody, A.K. (2011) Additive effects of herbivory, nectar robbing and seed predation on male and female fitness estimates of the host plant *Ipomopsis aggregata*. *Oecologia,* **166,** 681-692.

Jaeger, N., Pompanon, F. & Després, L. (2001) Variation in predation costs with Chiastocheta egg number on Trollius europaeus: how many seeds to pay for pollination? *Ecological Entomology,* **26,** 56-62.

Jakobsson, A. & Ågren, J. (2014) Distance to semi-natural grassland influences seed production of insect-pollinated herbs. *Oecologia,* **175,** 199-208.

Jakobsson, A., Lázaro, A. & Totland, Ø. (2009) Relationships between the floral neighborhood and individual pollen limitation in two self-incompatible herbs. *Oecologia,* **160,** 707-719.

Jakobsson, A., Padrón, B. & Traveset, A. (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives - A study of pollinator behaviour and reproduction success. *Biological Conservation,* **141,** 136-145.

Jauker, F., Diekötter, T., Schwarzbach, F. & Wolters, V. (2009) Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. *Landscape Ecology,* **24,** 547-555.

Jennersten, O. & Nilsson, S.G. (1993) Insect flower visitation frequency and seed production in relation to patch size of *Viscaria vulgaris* (Caryophyllaceae). *Oikos,* **68,** 283-292.

Jensen, S.R. (1992) Systematic implications of the distribution of iridoids and other chemical compounds in the Loganiceae and other families of the Asteridae. *Annals of the Missouri Botanical Garden,* **79,** 284-302.

Juenger, T. & Bergelson, J. (1997) Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology,* **78,** 1684-1695.

Kauffman, M.J. & Maron, J.L. (2006) Consumers limit the abundance and dynamics of a perennial shrub with a seed bank. *American Naturalist,* **168,** 454-470.

Kessler, A., Halitschke, R. & Poveda, K. (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. *Ecology,* **92,** 1769-1780.

Kim, T.N., Underwood, N. & Inouye, B.D. (2013) Insect herbivores change the outcome of plant competition through both inter- and intraspecific processes. *Ecology,* **94,** 1753-1763.

Kirchner, F., Luijten, S.H., Imbert, E., Riba, M., Mayol, M., Gonzalez-Martinez, S.C., Mignot, A. & Colas, B. (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos,* **111,** 130-142.

Kleijn, D. & van Langevelde, F. (2006) Interacting effects of landscape context and habitat quality on flower visiting insects in agricultural landscapes. *Basic and Applied Ecology,* **7,** 201-214.

Klinkhamer, P.G.L. & de Jong, T.J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos,* **57,** 399-405.

Knochel, D.G. & Seastedt, T.R. (2010) Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. *Ecological Applications,* **20,** 1903-1912.

Kohler, F., Verhulst, J., van Klink, R. & Kleijn, D. (2008) At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes? *Journal of Applied Ecology,* **45,** 753-762.

Kolb, A., Ehrlén, J. & Eriksson, O. (2007) Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution and Systematics,* **9,** 79-100.

Kolb, A., Leimu, R. & Ehrlén, J. (2007) Environmental context influences the outcome of a plant-seed predator interaction. *Oikos,* **116,** 864-872.

Konvička, M., Hula, V. & Fric, Z. (2003) Habitat of pre-hibernating larvae of the endangered butterfly *Euphydryas aurinia* (Lepidoptera : Nymphalidae): What can be learned from vegetation composition and architecture? *European Journal of Entomology,* **100,** 313-322.

Kunin, W.E. (1993) Sex and the single mustard - Population density and pollinator behavior effects on seed set. *Ecology,* **74,** 2145-2160.

Kunin, W.E. (1997) Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology,* **85,** 225-234.

Larsson, M. (2005) Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (*Dipsacaceae*). *Oecologia,* **146,** 394-403.

Lay, C.R., Linhart, Y.B. & Diggle, P.K. (2011) The good, the bad and the flexible: plant interactions with pollinators and herbivores over space and time are moderated by plant compensatory responses. *Annals of Botany,* **108,** 749-763.

Lázaro, A., Hegland, S.J. & Totland, Ø. (2008) The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia,* **157,** 249-257.

Lázaro, A., Lundgren, R. & Totland, Ø. (2009) Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos,* **118,** 691-702.

Lehtilä, K. & Strauss, S.Y. (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia,* **111,** 396-403.

Lehtilä, K., Syrjänen, K., Leimu, R., García, M.B. & Ehrlén, J. (2006) Habitat change and demography of *Primula veris*: Identification of management targets. *Conservation Biology,* **20,** 833-843.

Leimu, R. & Lehtilä, K. (2006) Effects of two types of herbivores on the population dynamics of a perennial herb. *Basic and Applied Ecology,* **7,** 224-235.

Leimu, R., Syrjänen, K., Ehrlén, J. & Lehtilä, K. (2002) Pre-dispersal seed predation in *Primula veris*: among-population variation in damage intensity and selection on flower number. *Oecologia,* **133,** 510-516.

Lennartsson, T. (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology,* **83,** 3060-3072.

Lennartsson, T., Tuomi, J. & Nilsson, P. (1997) Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (*Gentianaceae*). *American Naturalist,* **149,** 1147-1155.

Liao, K., Gituru, R.W., Guo, Y.H. & Wang, Q.F. (2011) The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (*Orobanchaceae*) through variation in bumble-bee foraging behaviour. *Annals of Botany,* **108,** 877-884.

Loaiza, V., Jonas, J.L. & Joern, A. (2011) Grasshoppers (Orthoptera: Acrididae) select vegetation patches in local-scale responses to foliar nitrogen but not phosphorus in native grassland. *Insect Science,* **18,** 533-540.

Louda, S.M. & Potvin, M.A. (1995) Effect of inflorescence feeding insects on the demogrpahy and lifetime fitness of a native plant. *Ecology,* **76,** 229-245.

Lövei, G.L., Macleod, A. & Hickman, J.M. (1998) Dispersal and effects of barriers on the movement of the New Zealand hover fly *Melanostoma fasciatum* (Dipt., Syrphidae) on cultivated land. *Journal of Applied Entomology,* **122,** 115-120.

Lundin, O., Smith, H.G., Rundlöf, M. & Bommarco, R. (2013) When ecosystem services interact: crop pollination benefits depend on the level of pest control. *Proceedings of the Royal Society B-Biological Sciences,* **280**.

Maron, J.L. & Crone, E. (2006) Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 2575-2584.

Maron, J.L., Horvitz, C.C. & Williams, J.L. (2010) Using experiments, demography and population models to estimate interaction strength based on transient and asymptotic dynamics. *Journal of Ecology,* **98,** 290-301.

Mbeau-Ache, C. & Franco, M. (2013) The time distribution of reproductive value measures the pace of life. *Journal of Ecology,* **101,** 1273-1280.

Memmott, J. (1999) The structure of a plant-pollinator food web. *Ecology Letters,* **2,** 276-280.

Miller, T.E.X., Louda, S.M., Rose, K.A. & Eckberg, J.O. (2009) Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs,* **79,** 155-172.

Miller, T.E.X., Williams, J.L., Jongejans, E., Brys, R. & Jacquemyn, H. (2012) Evolutionary demography of iteroparous plants: incorporating non-lethal costs of reproduction into integral projection models. *Proceedings of the Royal Society B-Biological Sciences,* **279,** 2831-2840.

Montgomery, B.R. (2009) Do pollen carryover and pollinator constancy mitigate effects of competition for pollination? *Oikos,* **118,** 1084-1092.

Morris, W.F., Hufbauer, R.A., Agrawal, A.A., Bever, J.D., Borowicz, V.A., Gilbert, G.S., Maron, J.L., Mitchell, C.E., Parker, I.M., Power, A.G., Torchin, M.E. & Vazquez, D.P. (2007) Direct and interactive effects of enemies and mutualists on plant performance: A meta-analysis. *Ecology,* **88,** 1021-1029.

Münzbergová, Z. (2005) Determinants of species rarity: Population growth rates of species sharing the same habitat. *American Journal of Botany,* **92,** 1987-1994.

Nattero, J., Malerba, R., Medel, R. & Cocucci, A. (2011) Factors affecting pollinator movement and plant fitness in a specialized pollination system. *Plant Systematics and Evolution,* **296,** 77-85.

Niesenbaum, R.A. (1999) The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (*Nyctaginaceae*). *American Journal of Botany,* **86,** 261-268.

Noordijk, J., Delille, K., Schaffers, A.P. & Sýkora, K.V. (2009) Optimizing grassland management for flower-visiting insects in roadside verges. *Biological Conservation,* **142,** 2097-2103.

Öckinger, E. & Smith, H.G. (2007) Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of Applied Ecology,* **44,** 50-59.

Olesen, J.M., Bascompte, J., Elberling, H. & Jordano, P. (2008) Temporal dynamics in a pollination network. *Ecology,* **89,** 1573-1582.

Ollerton, J. & Lack, A. (1998) Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus* (Fabaceae). *Plant Ecology,* **139,** 35-47.

Oostermeijer, J.G.B., Brugman, M.L., DeBoer, E.R. & DenNijs, H.C.M. (1996) Temporal and spatial variation in the demography of Gentiana pneumonanthe, a rare perennial herb. *Journal of Ecology,* **84,** 153-166.

Östergård, H. & Ehrlén, J. (2005) Among population variation in specialist and generalist seed predation - the importance of host plant distribution, alternative hosts and environmental variation. *Oikos,* **111,** 39-46.

Paige, K.N. & Whitham, T.G. (1987) Overcompensation in response to mammalian herbivory - the advantage of being eaten. *American Naturalist,* **129,** 407-416.

Parachnowitsch, A.L. & Caruso, C.M. (2008) Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. *Ecology,* **89,** 1802-1810.

Piqueras, J. (1999) Herbivory and ramet performance in the clonal herb *Trientalis europaea* L. *Journal of Ecology,* **87,** 450-460.

Pontin, D.R., Wade, M.R., Kehrli, P. & Wratten, S.D. (2006) Attractiveness of single and multiple species flower patches to beneficial insects in agroecosystems. *Annals of Applied Biology,* **148,** 39-47.

Porter, K. (1982) Basking behaviour in larvae of the butterfly *Euphydryas aurinia*. *Oikos,* **38,** 308-312.

Prins, A.H., Nell, H.W. & Klinkhamer, P.G.L. (1992) Size-dependent root herbivory on *Cynoglossum officinale*. *Oikos,* **65,** 409-413.

Rand, T.A. (2003) Herbivore-mediated apparent competition between two salt marsh forbs. *Ecology,* **84,** 1517-1526.

Rautio, P., Huhta, A.P., Piippo, S., Tuomi, J., Juenger, T., Saari, M. & Aspi, J. (2005) Overcompensation and adaptive plasticity of apical dominance in Erysimum strictum (Brassicaceae) in response to simulated browsing and resource availability. *Oikos,* **111,** 179-191.

Reader, R.J. (1992) Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture. *Oikos,* **65,** 414-418.

Rees, M. & Brown, V.K. (1992) Interactions between invertebrate herbivores and plant competition. *Journal of Ecology,* **80,** 353-360.

Rose, K.E., Louda, S.M. & Rees, M. (2005) Demographic and evolutionary impacts of native and invasive insect herbivores on *Cirsium canescens*. *Ecology,* **86,** 453-465.

Rose, K.E., Russell, F.L. & Louda, S.M. (2011) Integral projection model of insect herbivore effects on *Cirsium altissimum* populations along productivity gradients. *Ecosphere,* **2**.

Russell, F.L. & Spencer, M.N. (2010) Combined effects of folivory and neighbor plants on Cirsium altissimum (tall thistle) rosette performance. *Plant Ecology,* **208,** 35-46.

Sánchez-Lafuente, A.M., Rodríguez-Gironés, M.A. & Parra, R. (2012) Interaction frequency and per-interaction effects as predictors of total effects in plant-pollinator mutualisms: a case study with the self-incompatible herb *Linaria lilacina*. *Oecologia,* **168,** 153-165.

Scribailo, R.W. & Barrett, S.C.H. (1994) Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (*Pontederiaceae*). *Sexual Plant Reproduction,* **7,** 273-281.

Sharaf, K.E. & Price, M.V. (2004) Does pollination limit tolerance to browsing in *Ipomopsis aggregata*? *Oecologia,* **138,** 396-404.

Schuett, E.M. & Vamosi, J.C. (2010) Phylogenetic community context influences pollen delivery to *Allium cernuum*. *Evolutionary Biology,* **37,** 19-28.

Silander, J.A. & Primack, R.B. (1978) Pollination intensity and seed set in Evening Primrose (*Oenothera fruticosa*). *American Midland Naturalist,* **100,** 213-216.

Sletvold, N. (2002) Effects of plant size on reproductive output and offspring performance in the facultative biennial *Digitalis purpurea*. *Journal of Ecology,* **90,** 958-966.

Sletvold, N. & Ågren, J. (2011a) Among-population variation in costs of reproduction in the long-lived orchid *Gymnadenia conopsea*: an experimental study. *Oecologia,* **167,** 461-468.

Sletvold, N. & Ågren, J. (2011b) Nonadditive effects of floral display and spur length on reproductive success in a deceptive orchid. *Ecology,* **92,** 2167-2174.

Sletvold, N. & Grindeland, J.M. (2008) Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. *Acta Oecologica-International Journal of Ecology,* **34,** 21-25.

Sletvold, N., Grindeland, J.M. & Ågren, J. (2010) Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist,* **188,** 385-392.

Sowig, P. (1989) Effects of flowering plants patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera, Apidae). *Oecologia,* **78,** 550-558.

Steffan-Dewenter, I. & Tscharntke, T. (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia,* **121,** 432-440.

Sullivan, A.T. & Howe, H.F. (2011) Response of two prairie forbs to repeated vole herbivory. *Oecologia,* **165,** 1007-1015.

Suwa, T., Louda, S.M. & Russell, F.L. (2010) No interaction between competition and herbivory in limiting introduced *Cirsium vulgare* rosette growth and reproduction. *Oecologia,* **162,** 91-102.

Swope, S.M. & Parker, I.M. (2010) Trait-mediated interactions and lifetime fitness of the invasive plant *Centaurea solstitialis*. *Ecology,* **91,** 2284-2293.

Tack, A.J.M., Ovaskainen, O., Harrison, P.J. & Roslin, T. (2009) Competition as a structuring force in leaf miner communities. *Oikos,* **118,** 809-818.

Thomson, J.D. (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evolutionary Ecology,* **2,** 65-76.

Törang, P., Ehrlén, J. & Ågren, J. (2008) Mutualists and antagonists mediate frequency-dependent selection on floral display. *Ecology,* **89,** 1564-1572.

Torssell, K. (1963) Inhaltsstofe von *Scabiosa succisa* L. *Arkiv for Kemi,* **21,** 93-96.

van Geert, A., van Rossum, F. & Triest, L. (2010) Do linear landscape elements in farmland act as biological corridors for pollen dispersal? *Journal of Ecology,* **98,** 178-187.

Vanhoenacker, D., Ågren, J. & Ehrlén, J. (2009) Spatial variability in seed predation in *Primula farinosa*: local population legacy versus patch selection. *Oecologia,* **160,** 77-86.

Vanhoenacker, D., Ågren, J. & Ehrlén, J. (2013) Non-linear relationship between intensity of plant-animal interactions and selection strength. *Ecology Letters,* **16,** 198-205.

von Euler, T., Ågren, J. & Ehrlén, J. (2014) Environmental context influences both the intensity of seed predation and plant demographic sensitivity to attack. *Ecology,* **95,** 495-504.

Williams, J.L. (2009) Flowering life-history strategies differ between the native and introduced ranges of a monocarpic perennial. *American Naturalist,* **174,** 660-672.

Young, A.G., Broadhurst, L.M. & Thrall, P.H. (2012) Non-additive effects of pollen limitation and self-incompatibility reduce plant reproductive success and population viability. *Annals of Botany,* **109,** 643-653.

Zabel, J. & Tscharntke, T. (1998) Does fragmentation of *Urtica* habitats affect phytophagous and predatory insects differentially? *Oecologia,* **116,** 419-425.

2 Paper I

Surrounding vegetation mediates occurrence of both generalist and specialist invertebrate folivores, but not occurrence of vertebrate herbivores and seed predators

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[*] **Author contributions:**
ZJ and ZM designed the study. ZJ, MJ, MW, EH conducted the fieldwork. DŘ analysed the samples for seed production and predispersal seed predation. ZJ performed the statistical analyses and wrote the manuscript. All authors contributed to editation of the text.

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2.1 Abstract

The overall impact of herbivores on plant population dynamics is determined by combination of herbivore impact on individuals and frequency of herbivore occurrence within the plant population. While the first component is well-explored, the second is much less-studied and may depend at subpopulation and population scales both on intraspecific trait variation and on local environmental conditions. In present study, we focus on five herbivore functional groups of a wet meadow perennial, *Succisa pratensis*, and assess their occurrence at subpopulation and population level with respect to *S. pratensis* (sub)population characteristics and surrounding vegetation (regarded as a proxy of local biotic and abiotic conditions).

Occurrence of both invertebrate and vertebrate folivores depended on vegetation at the subpopulation scale (i.e. metres), but vegetation was important at the population scale only in case of the two invertebrate folivore groups. Contrary to our expectations, generalist and specialist invertebrate folivores did not differ in magnitude of their dependence on vegetation. On the other hand, occurrence of stalk grazers did not vary with any of the tested factors and predispersal seed predators responded only to plant population characteristics, but not to vegetation.

Our results demonstrate that two important herbivore groups responded substantially to a relatively finely delimited gradient of local conditions even at the spatial scale of metres. Such spatial heterogeneity could give rise to a mosaic of different subpopulations within the plant population each with a different dynamics. This would in turn substantially change the target plant species' long-term persistence prospects in landscape.

Keywords:
Succisa pratensis, *Euphydryas aurinia*, plant-animal interaction, spatial variation, predispersal seed predation, specialist herbivore, generalist herbivore

2.2 Introduction

Herbivores can be one of the important drivers of plant population dynamics (e.g. Louda and Potvin, 1995, Maron and Crone, 2006, Brody et al., 2007). The spectrum of herbivores the plants face is typically very broad (e.g. Ehrlén, 1995, Irwin and Brody, 2011, Rose et al., 2011) and correspondingly broad is the range of plant vital rates the herbivores directly affect (survival, growth, reproduction, germination, clonal growth). The overall impact of herbivores on plant population dynamics is determined by combination of herbivore impact on plant individuals and frequency of herbivore occurrence within the plant population. While the first component can be studied both under experimental (e.g. Rautio et al., 2005, Ågren et al., 2013) and *in situ* conditions (e.g. Kolb *et al.*, 2007, Ehrlén and Münzbergová, 2009), the second generally less-studied component can be studied only in the field.

Herbivore occurrence is often mediated by target plant's traits; mainly size (Brody, 1997, Piqueras, 1999, Ehrlén and Münzbergová, 2009). Additional biotic and abiotic characteristics (such as microclimate, topography or identity of co-occurring plant species) of the site can be important drivers of herbivore occurrence too (Miller *et al.*, 2009, Reader, 1992, Kolb *et al.*, 2007, Rand, 1999, Münzbergová and Skuhrovec, 2013). Herbivores even sometimes respond to relatively subtle differences in environmental conditions at sub-site or sub-population level, such as amounts of available foliar nutrients (Loaiza et al., 2011) or differences in host plant density and presence of alternative hosts (Östergård and Ehrlén, 2005). On one hand, there is evidence that effect of micro-site abiotic conditions can outweigh the effect of plant individual traits (Förare and Engqvist, 1996), but on the other some studies from relatively strong abiotic and biotic gradients report no effect of habitat conditions on herbivore occurrence or an effect inconsistent in time (Rose et al., 2011, von Euler et al., 2014).

Pattern of herbivore occurrence is a result of three selection steps, all of which can be potentially influenced by biotic and abiotic site characteristics (but also population spatial configuration, see e.g. Zabel and Tscharntke, 1998). Before attacking a particular plant, the herbivore needs to encounter and select a population within the landscape and subsequently it needs to choose a particular patch or subpopulation within this population. Only after that, it is able to decide among the plant individuals based on their individual traits. Characteristics of the focal plant population or of its individuals are likely to affect only the second and third step of the plant selection process, since herbivores are rarely able to perceive the suitability of focal plant population before encountering it. Herbivore's choice of patch may be driven also by other factors besides its attractiveness from the food consumption viewpoint. Surrounding vegetation can affect for example herbivore plant detection probability (Ågren et al., 2006) or availability of larval habitats (Sjödin et al., 2008). The actual herbivore choices are likely to differ between individual herbivore groups, because of their different perceptual abilities and mobility (vertebrates/invertebrates) as well as because of their different degree of dependence on focal plant species (specialists/generalists).

In present study, we examined factors influencing occurrence of five herbivore groups at population and subpopulation level (at the scale of metres) in a plant species *Succisa pratensis* with well-described ecology (Mildén et al., 2007, Pauli et al., 2002, Herben et al., 2006, Vergeer et al., 2003), which possesses a wide range of herbivores including the Europe-wide protected specialist butterfly *Euphydryas aurinia*. We focused on the role of surrounding vegetation as a proxy of both structure of the habitat and its abiotic conditions. At subpopulation level, we also studied the effect of population characteristics of the focal species and their interplay with surrounding vegetation. We hypothesised that occurrence of damage by invertebrate herbivores will be better predicted by surrounding vegetation (taken as a proxy of local biotic and abiotic conditions) and population characteristics (mean size, population density, proportion of flowering individuals) than the occurrence of damage caused by vertebrates. Additionally, we hypothesised that the specialist (*E. aurinia*) discriminates more among plant (sub)population characteristics. We realised this aim by means of a two-year demographic study on 27 populations with ca 2700 individuals divided into 102 subpopulations.

2.3 Materials and methods

2.3.1 Study system

Succisa pratensis Moench (*Dipsacaceae*) is a typical wet meadow iteroparous perennial with a ground leaf rosette (Adams, 1955). It occurs in nutrient poor habitats varying in pH and humidity with a distribution from northern Spain to central Siberia (Adams, 1955, Meusel and Jäger, 1992). One to several stalks bear dichasia of flowerheads consisting of light-blue florets, which can produce only one seed each (Adams, 1955). The species flowers in late summer (mid-August to late-September). The leaves are defended by alkaloid gentianin and iridoids (Jensen, 1992, Torssell, 1963). Observed plant damage could be attributed to five herbivore functional groups (see Supplementary material Figs. 1 to 5): (i) generalist invertebrate folivores (caterpillars of Noctuidae moths, snails etc.; hereafter invertebrate folivores); (ii) a specialist invertebrate folivore (caterpillars of Europe-wide protected *Euphydryas aurinia*); (iii) generalist vertebrate folivores (mainly roe deer and cows; hereafter vertebrate folivores); (iv) vertebrate stalk grazers (most likely the same species as in iii); (v) seed predators (larvae of micro-moths, presumably of Tortricidae family).

The studied populations are located in an area of approximately 3.5×5 km within the Protected landscape area Slavkovský les (Western Bohemia, Czech Republic), an upland region with altitude varying between 700 and 800 m a.s.l. and mild suboceanic climate (the annual average temperature 7°C, annual precipitation ca 800 mm ; Tolasz, 2007). *S. pratensis* is still quite widely spread in the area and occurs both in meadows (also abandoned ones) and pastures in a wide range of conditions (see Supplementary material Table 2).

2.3.2 Study design

We chose 27 populations of *S. pratensis* within the study area so that they covered the whole range of represented vegetation types as well as possible. At each site, a transect was delimited and all plants found were individually tagged and followed through 2010 and 2011. We tagged individually at least 80 non-seedling individuals at each population during its flowering. For each individual, we recorded its position with respect to transect, size-related traits and occurrence of herbivory. Within each transect, four 1×1 m vegetation samples were placed in random design stratified along the transect main axis (the exceptions are described in Supplementary material Table 1). A subpopulation was then delimited as the set of measured plants being closer to a given vegetation sample than to any other. We consider surrounding vegetation rather as an unstructured proxy of complex local biotic and abiotic conditions.

2.3.3 Measures of plant characteristics and herbivory magnitude

We measured characteristics connected to biomass, reproductive effort, traces of herbivore occurrence on each plant individual. In order to obtain the non-destructive estimate of plant biomass we combined information on no. of leaves, width of the largest leaf and its length according to Eq. 1. The square-root transform of this measure is a reasonable approximate of total (above+belowground) plant vegetative biomass (R^2 = 0.799, n = 80; Janovský, Z., unpublished data). The biological interpretation of this measure is the upper bound estimate of leaf area of the individual. Plant reproductive effort was measured as no. of stalks and no. of inflorescences produced.

$$\#\, leaves \times \pi \times (width/2) \times (length/2) \qquad \text{Eq. 1}$$

Herbivore occurrence was inferred from damage detectable on plant individuals. In the three common herbivore functional groups, i.e. invertebrate folivores, stalk grazers and seed predators, we measured the magnitude of herbivory, while in the two rarer herbivore groups, i.e. *E. aurinia* and vertebrate folivores, we recorded only whether the given plant individual was affected. The magnitude of invertebrate damage was assessed as percentage loss of foliage at five-grade semiquantitative scale (0–5%, 5–15%, 15–25%, 25–50%, 50–100%) multiplied by plant vegetative size (see above; again a square-root transform of this measure was used due to a severely right-skewed distribution of the original variable). Severity of stalk grazing was expressed as relative proportion of stubs of grazed stalks out of total stalks produced. Finally, magnitude of seed predation was expressed as ratio of predated seeds out of total developed seeds (i.e. predated + healthy seeds). Seed predation was measured only in 2011.

2.3.4 Classification of surrounding vegetation into types

We considered several approaches how to summarise information on surrounding vegetation: detrended correspondence analysis (DCA), multivariate regression trees (De'Ath, 2002) and classical Twinspan analysis (Hill, 1979, as modified by Roleček et al., 2009) for classification of vegetation samples defining the subpopulations. BIC-informed (Schwarz, 1978) selection of the most suitable classification, i.e. explaining most variation in several subpopulation characteristics, has indicated the Twinspan classification with eight clusters as the most informative one (for details on the parameters of analysis and selection procedure see Supplementary material Appendix 3; synoptic tables of vegetation composition of individual clusters are listed in Supplementary material Appendix 4). We assigned each vegetation sample (i.e. subpopulation) identity of corresponding cluster (hereafter vegetation type) and use it in further analyses as categorical predictor with eight levels.

2.3.5 Calculation of subpopulation- and population-level variables

We calculated subpopulation level means of all listed variables measured at individual level (i.e. plant size parameters and herbivore damage; where necessary means were weighted, e.g. stalk grazing, see above). We also computed estimates of density per square metre (for details of the computation see Supplementary material Table 1): (i) of all plants (used in analyses for all folivores); (ii) of flowering stalks (used for stalk grazers); (iii) of inflorescences (used for seed predators). We refer to them hereafter as density, meaning always the one appropriate to a given herbivore group. We calculated the population level characteristics in a similar manner. Since the analysis at population level was aimed at testing the effect of vegetation types, we had to assign each population only to one vegetation type in order to avoid pseudoreplication. We chose the type that majority of its subpopulations belonged to (in cases of ties we decided for the type, where majority of plants grew). Population-level means of variables were then computed only from plants belonging to subpopulations of the assigned vegetation type. For all calculations, we pooled data from 2010 and 2011 (i.e. always calculated one mean for individuals from 2010 and 2011 together), since we were interested in deterministic effects of population characteristics and vegetation types rather than year-to-year fluctuations due to weather.

2.3.6 Data analysis

For each herbivore functional group, we conducted two analyses: (i) analysis at subpopulation level aimed at both plant subpopulation characteristics and vegetation types; (ii) analysis at population level aimed only at testing the effect of vegetation types due to available number of populations (i.e. degrees of freedom). In all cases, we applied linear regression with weights set to cases in order to reflect different number of independent observations the information in each (sub)population was based on (no. of plants in case of all folivores, no. of flowering stalks in case of stalk grazers and no. of inflorescences sampled in case of seed predation). We checked the assumptions of linear regression by inspecting diagnostic plots. We also visually checked for possible spatial autocorrelation of the population level analysis by plotting the regression residuals into the map (no clustering or any other patterns were observed). All computations were undertaken in R 3.0.3 statistical environment (R Core Development Team, available at www.r-project.org).

The subpopulation-level analyses aimed at identifying the factors influencing herbivore occurrence with respect to subpopulations within the population, i.e. after entering population identity as a covariate. Therefore we considered as predictors plant traits, density, vegetation types and all their second level interactions. Plant traits reflected plant's size as perceived by each herbivore group (all folivores: mean plant vegetative size and proportion of individuals flowering; stalk grazers: mean flowering plant vegetative size and mean no. of stalks per plant; seed predators: mean vegetative size of individuals sampled for seed predation and mean no. of inflorescences per sampled individual). Population-level analyses included as the only predictor vegetation type.

2.4 Results

2.4.1 Classification of vegetation into types

Based upon the BIC-informed model selection (for details see Supplementary material Appendix 3), the Twinspan classification into 8 distinct types turned out to be most informative followed by a classification based on the 1st axis of DCA (ΔBIC = 27.4) and

Table 1: Results of linear models of herbivory intensity at the level of subpopulations; different residual and population identity degrees of freedom (column Df) correspond to inv. folivores + E. aurinia + vertebrate folivores, stalk grazers, seed predators respectively; please note that the main effect of vegetation has 6 df instead of 7 due to effect of cluster 1 being integrated out by site identity (vegetation belonging to it occurs only at two sites and no other vegetation type has been recorded there)

Predictor	Df	Invertebrate folivores			Euphydryas aurinia			Vertebrate folivores			Stalk grazers			Seed predators		
		Effect	R^2	P-value	Effect	R^2	P-value	Effect	R^2	P-value	Effect	R^2	P-value	Effect	R^2	P-value
population identity	26/26/22		87.6%	<0.001		61.4%	<0.001		66.8%	<0.001		68.1%	<0.001		67.0%	<0.001
# stalks	1		not tested			not tested			not tested			n.s.			not tested	
# flower heads	1		not tested			not tested			not tested			not tested			n.s.	
size	1	+	3.7%	<0.001		n.s.			n.s.			n.s.		+	2.7%	0.011
% flowering	1		n.s.			n.s.		-	3.7%	<0.001		not tested			not tested	
density	1		n.s.			n.s.			n.s.			n.s.			n.s.	
veg. type	6		1.4%	0.033		6.1%	0.009		n.s.			n.s.			n.s.	
size × % flowering	1		n.s.			n.s.		+	1.2%	0.030		not tested			not tested	
size × veg. type	7		2.2%	0.005		5.2%	0.036		9.2%	<0.001		n.s.			n.s.	
% flowering × veg. type	7		n.s.			8.6%	0.002		4.0%	0.037		not tested			not tested	
# fl. heads × density	1		not tested			not tested			not tested			not tested		+	6.9%	<0.001
size × density	1		n.s.			n.s.			n.s.			n.s.		-	5.0%	0.001
residuals	42/41/18		3.8%			12.8%			9.7%			18.7%			18.7%	

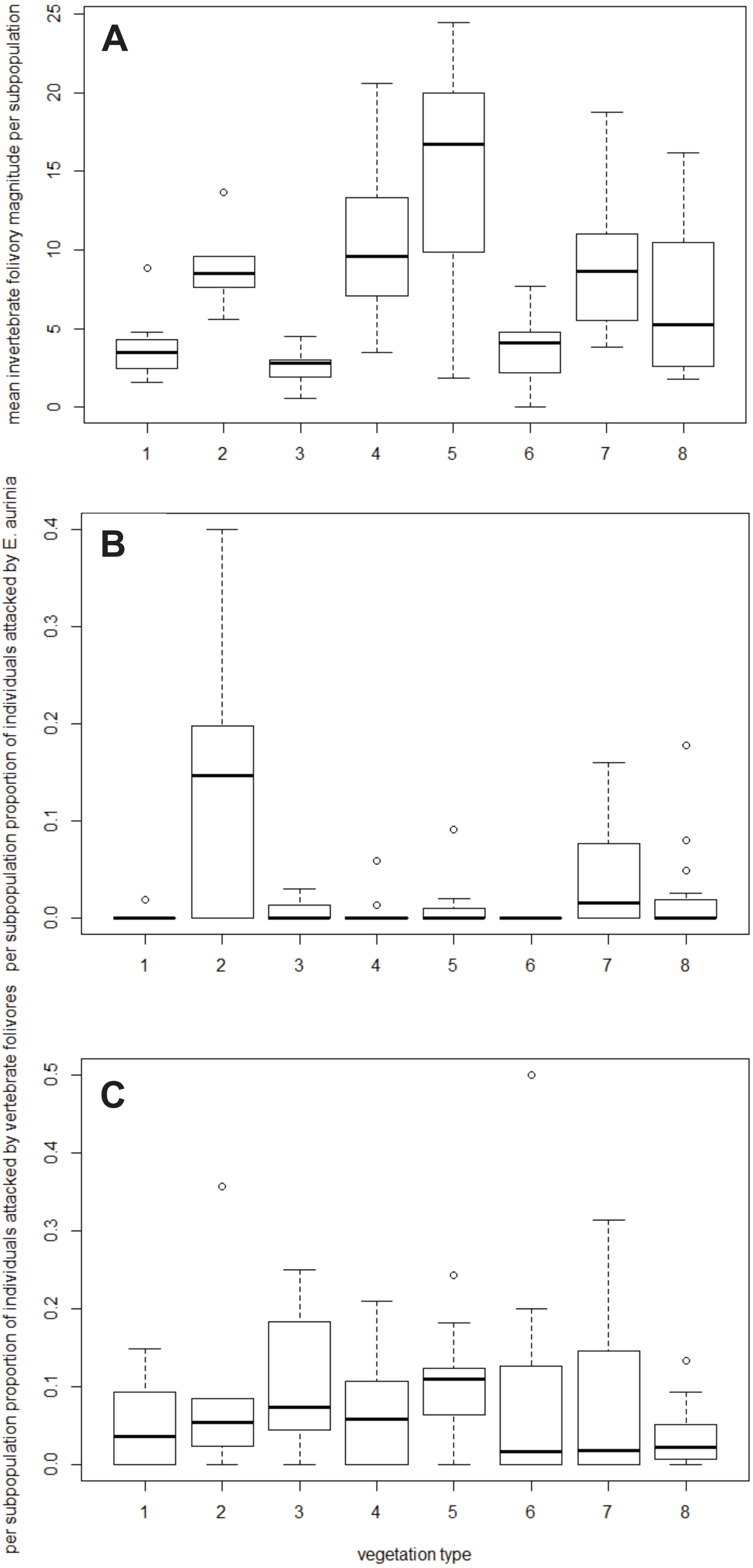
A
mean invertebrate folivory magnitude per subpopulation
0
5
10
15
20
25
1
2
3
4
5
6
7
8
B
per subpopulation proportion of individuals attacked by E. aurinia
0.0
0.1
0.2
0.3
0.4
1
2
3
4
5
6
7
8
C
per subpopulation proportion of individuals attacked by vertebrate folivores
0.0
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
6
7
8
vegetation type

Fig. 1: Boxplots of dependence of herbivory intensity on vegetation types: (A) intensity of herbivory by invertebrate generalist folivores; (B) proportion of plants attacked by *E. aurinia*; (C) proportion of plants grazed by vertebrate folivores; (see Supplementary material Appendices 3 and 4 for more details on delimitation of vegetation types).

Twinspan classification into 2 distinct types (ΔBIC = 33.4). Types 1–3 represent medium wet stands with nutrient levels decreasing from 1 to 3 (for details on species composition see Supplementary material Appendix 4). Type 4 represents sedge-dominated stands at mire edges. Types 5 and 6 consist of wet meadows on neutral soils (compared to the rest on acidic soils) with moderate nutrient levels. Type 6 differs by being at more disturbed sites at extant or recently abandoned pastures. Types 7 and 8 encompass wet acidic meadows with Type 7 containing the less wet stands often dominated by competitive grasses such as *Deschampsia caespitosa* L.

2.4.2 Analyses at subpopulation level

At subpopulation level, only invertebrate and vertebrate folivores and seed predators made their choices according to local plant characteristics (Table 1). Folivores and seed predators focused on larger plants, yet the occurrence of seed predators was highly dependent on densities of their food source, i.e. inflorescences. Relative damage by vertebrate folivores decreases in subpopulations with higher concentration of flowering plants. The preferences of all folivores, i.e. invertebrate, vertebrate and *E. aurinia*, to plant characteristics were to large extent dependent on interactions with vegetation type. Invertebrate folivore and *E. aurinia* occurrences varied systematically with vegetation type (Fig. 1), which was more pronounced in *E. aurinia*.

2.4.3 Analyses at population level

At population level, only invertebrate folivores and *E. aurinia* showed relatively strong links to vegetation types (Table 2). However, it is also important to bear in mind, that our dataset had power to discover only relatively strong relationships (a model with 8 degrees of freedom and 27 degrees of freedom in total). Nevertheless, results of population level analyses were congruent with the results found on the subpopulation level contributing to an overall pattern that folivores are more influenced by surrounding vegetation, compared to stalk grazers and seed predators focusing on plant parts higher above ground, which are far less influenced by the surrounding vegetation (Table 3).

Table 2: Summary of linear models of herbivore occurrence at population level regressed against vegetation type; res. df in all cases 19 with exception of seed predation (14); df of vegetation types 7 in all cases

Herbivore group	R^2	P-value
Invertebrate folivores	48.5%	0.049
Euphydryas aurinia	70.7%	<0.001
Vertebrate folivores	-	n.s.
Stalk grazers	-	n.s.
Seed predators	-	n.s.

Table 3: Summarisation of sources of variation in herbivore occurrence for subpopulation and population level

Source of variation	Inv. folivores	*E. aurinia*	Vert. folivores	Stalk grazers	Seed predators
Summed effect of plant characteristics at subpopulation level	3.7%	0%	4.9%	0%	14.6%
Main effect of veg. type at subpop. level	1.4%	6.1%	0%	0%	0%
Interaction of veg. type x plant characteristics	2.2%	13.7%	13.2%	0%	0%
Amount of among-subpopulation variation explained by population identity	87.6%	61.4%	66.8%	68.1%	67.0%
Main effect of veg. type at population level	48.5%	70.7%	0%	0%	0%

2.5 Discussion

Our results allowed us to compare for the first time the degree of dependence on surrounding vegetation for all main herbivore functional groups (with exception of below-ground herbivores) within the same system, i.e. at the same range of abiotic and biotic conditions. Three out of five herbivore functional groups damaged differently the target plant species in different vegetation types at the level of subpopulations within one population (and spatial scale of metres). The differences among the vegetation types in preference for *S. pratensis* were found despite a relatively fine delimitation of vegetation types. Furthermore, the occurrence of generalist folivores and specialist *E. aurinia* was directly influenced by vegetation type both at subpopulation and population level. Thus our data show that different subpopulations (at the scale of metres) of one population may experience systematically different frequency of biotic interactions driven by slight changes in local biotic and abiotic conditions as reflected by vegetation composition. Additionally, interaction frequency in two cases varied substantially also among populations according to their vegetation composition, indicating a potential of these two herbivore groups to shape the realised niche of *S. pratensis*.

2.5.1 The higher the food, the less care for surrounding vegetation

Only those herbivore groups feeding on leaf rosettes i.e. directly coming into contact with surrounding vegetation, responded to it. This finding is essentially in line with other published studies, indicating that seed predation levels do not differ across vegetation types (Rose et al., 2011, von Euler et al., 2014 in two out of three years), while magnitude of folivory changes with surrounding vegetation (Loaiza et al., 2011).

We can only hypothesize about the explanation for the observed pattern. However, some mechanisms are very likely based on herbivore perceptual abilities and life history. Insect seed predators developing within the inflorescences are likely to reflect mainly plant individual traits and resource concentration (influencing detectability and long-term persistence probability) on (sub)population levels (see Table 1 and Östergård and Ehrlén, 2005, Östergård et al., 2009), since inflorescences of most plants are placed above the foliage of surrounding vegetation. On the other hand, invertebrate folivores feed on plants in most cases externally and thus they need favourable microclimate, hiding places etc. and therefore are likely to reflect even relatively finely described differences in vegetation composition and abiotic conditions. Another possible mechanism influencing these generalists can be presence of other preferred species nearby (Rand, 1999). Differing responses of stalk grazers and vertebrate folivores cannot be explained in a similarly intuitive way, since the animals causing the observed damage largely overlap. When grazing stalks, roe deer tend pick up individual plants within a subpopulation based upon their properties (Ehrlén, 1997, Ågren et al., 2013, Janovský, Z., unpublished data) and being highly mobile, they are able to locate them anywhere in the population. On the other hand, we hypothesize that vertebrate grazing of leaf rosettes happens more or less accidentally within the subpopulations, where roe deer tend to graze for grass, since the leaves heavily protected with alkaloids and other secondary metabolites (Torssell, 1963, Jensen, 1992) are not a likely first-choice target. Thus we interpret the dependence of generalist vertebrate grazing on surrounding vegetation rather as a reflection of their preference of other plants to graze on.

According to our expectations, the larvae of specialist butterfly *E. aurinia* showed highest degree of preference out of all herbivore groups with respect to plant characteristics at the subpopulation level as well as with respect to vegetation type at both subpopulation and population level. It is also worth noting that reported preference of *E. aurinia* for vegetation with cushion grasses such as *Nardus stricta* (Konvička et al., 2003) holds true for our dataset only partly (Fig. 1B, veg. types 2 and 7), while wetter (veg. type 8) and most nutrient poor vegetation with cushion grasses (veg. type 3) host much lower densities of *E. aurinia*, which are comparable to vegetation without cushion grasses (veg. type 5).

2.5.2 Implications for plant population dynamics

The levels of herbivory differed for two herbivore groups among the subpopulations within the same population. This does not necessarily mean that populations of *S. pratensis* are structured into subpopulations with different demographic dynamics, because we did not measure impacts of herbivores on individual plant fitness. Nevertheless, herbivores (all groups with exception of vertebrate folivory) affect *S. pratensis* individual fitness (Janovský, Z., unpublished data), which makes the question of impacts of spatial inhomogeneity in herbivore occurrence on plant populations relevant.

Most currently used models of plant population dynamics rely on an implicit assumption that the probability of herbivore occurrence is spatially homogeneous across all individuals within a plant population (see e.g. Dahlgren and Ehrlén, 2009, Münzbergová, 2005, Maron and Crone, 2006), which actually makes them feasible to parameterise. Violating this assumption could lead to different predictions of local population dynamics due to variability of growth rates among subpopulations. For example, if the overall population would be predicted to decline, variation of favourability of conditions among subpopulations could lead to establishment of "source" and "sink" subpopulations, increasing thus the chance of long-term persistence of population, but also its vulnerability for example to disturbance, compared to an unstructured population. This effect would be even more pronounced if the impacts of herbivory were nonlinear, since commonly utilised demographic models assume all individuals to be affected with the same probability. Similar spatial structuring of herbivory and population dynamics has been observed in Mediterranean shrubby ecosystems grazed by large ungulates herbivores (e.g. Gómez, 2005, Baraza et al., 2006). Our results indicate spatial structuring of herbivory may be a common phenomenon even in relatively spatially unstructured ecosystems such as grasslands. Moreover, this seems to be an important issue especially for systems featuring foliar feeding invertebrates (Table 3).

Spatial structuring of herbivore occurrence at the population level can have similar implications for landscape dynamics of plant populations, since different levels of herbivory would lead for example to different seed production, i.e. colonisation potential, of the populations. Moreover under such circumstances, herbivores interacting with vegetation composition would become one of the factors shaping the realised niche of a given plant species (and thus amount and suitability of sites within landscape), a role typically not assumed in models of landscape dynamics (see e.g. Herben et al., 2006, Verheyen et al., 2004, Valdés and García, 2011). Further understanding of the correlation structure of the drivers of plant population dynamics seems to be a necessary step for defining the axes of the niche space of plant species and understanding the mechanisms underlying plant population dynamics.[*]

2.6 References

Adams, A. W. (1955) *Succisa pratensis* Moench (*Scabiosa succisa* L.). *Journal of Ecology,* **43,** 709-718.

Ågren, J., Fortunel, C. & Ehrlén, J. (2006) Selection on floral display in insect-pollinated *Primula farinosa*: effects of vegetation height and litter accumulation. *Oecologia,* **150,** 225-232.

Ågren, J., Hellström, F., Toräng, P. & Ehrlén, J. (2013) Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. *Proceedings of the National Academy of Sciences,* **110,** 18202-18207.

Baraza, E., Zamora, R. & Hodar, J. A. (2006) Conditional outcomes in plant-herbivore interactions: neighbours matter. *Oikos,* **113,** 148-156.

Brody, A. K. (1997) Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology,* **78,** 1624-1631.

Brody, A. K., Price, M. V. & Waser, N. M. (2007) Life-history consequences of vegetative damage in scarlet gilia, a monocarpic plant. *Oikos,* **116,** 975-985.

Dahlgren, J. P. & Ehrlén, J. (2009) Linking environmental variation to population dynamics of a forest herb. *Journal of Ecology,* **97,** 666-674.

De'Ath, G. (2002) Multivariate regression trees: a new technique for modeling species-environment relationships. *Ecology,* **83,** 1105-1117.

Ehrlén, J. (1995) Demography of the perennial herb *Lathyrus vernus* - 1. Herbivory and individual performance. *Journal of Ecology,* **83,** 287-295.

Ehrlén, J. (1997) Risk of grazing and flower number in a perennial plant. *Oikos,* **80,** 428-434.

Ehrlén, J. & Münzbergová, Z. (2009) Timing of flowering: Opposed selection on different fitness components and trait covariation. *American Naturalist,* **173,** 819-830.

Förare, J. & Engqvist, L. (1996) Suboptimal patch and plant choice by an ovipositing monophagous moth - An insurance against bad weather? *Oikos,* **77,** 301-308.

Gómez, J. M. (2005) Long-term effects of ungulates on performance, abundance, and spatial distribution of two montane herbs. *Ecological Monographs,* **75,** 231-258.

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

Hill, M. O. (1979) TWINSPAN - A FORTRAN Program for Arranging Multivariate Data in an Ordered Two-Way Table by Classification of the Individuals and Attributes. Cornell University, Ithaca, NY.

Irwin, R. E. & Brody, A. K. (2011) Additive effects of herbivory, nectar robbing and seed predation on male and female fitness estimates of the host plant *Ipomopsis aggregata*. *Oecologia,* **166,** 681-692.

Jensen, S. R. (1992) Systematic implications of the distribution of iridoids and other chemical compounds in the Loganiceae and other families of the Asteridae. *Annals of the Missouri Botanical Garden,* **79,** 284-302.

Kolb, A., Leimu, R. & Ehrlén, J. (2007) Environmental context influences the outcome of a plant-seed predator interaction. *Oikos,* **116,** 864-872.

Konvička, M., Hula, V. & Fric, Z. (2003) Habitat of pre-hibernating larvae of the endangered butterfly *Euphydryas aurinia* (Lepidoptera : Nymphalidae): What can be learned from vegetation composition and architecture? *European Journal of Entomology,* **100,** 313-322.

Loaiza, V., Jonas, J. L. & Joern, A. (2011) Grasshoppers (Orthoptera: Acrididae) select vegetation patches in local-scale responses to foliar nitrogen but not phosphorus in native grassland. *Insect Science,* **18,** 533-540.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Louda, S. M. & Potvin, M. A. (1995) Effect of inflorescence feeding insects on the demogrpahy and lifetime fitness of a native plant. *Ecology,* **76,** 229-245.

Maron, J. L. & Crone, E. (2006) Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 2575-2584.

Meusel, H. & Jäger, E. J. (1992) *Vergleichende Chorologie der zentraleuropäischen Flora, Band III.* Gustav Fischer Verlag, Jena, Germany.

Mildén, M., Cousins, S. A. O. & Eriksson, O. (2007) The distribution of four grassland plant species in relation to landscape history in a Swedish rural area. *Annales Botanici Fennici,* **44,** 416-426.

Miller, T. E. X., Louda, S. M., Rose, K. A. & Eckberg, J. O. (2009) Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs,* **79,** 155-172.

Münzbergová, Z. (2005) Determinants of species rarity: Population growth rates of species sharing the same habitat. *American Journal of Botany,* **92,** 1987-1994.

Münzbergová, Z. & Skuhrovec, J. (2013) Effect of habitat conditions and plant traits on leaf damage in the Carduoideae subfamily. *Plos One,* **8**.

Östergård, H. & Ehrlén, J. (2005) Among population variation in specialist and generalist seed predation - the importance of host plant distribution, alternative hosts and environmental variation. *Oikos,* **111,** 39-46.

Östergård, H., Hambäck, P. A. & Ehrlén, J. (2009) Responses of a specialist and a generalist seed predator to variation in their common resource. *Oikos,* **118,** 1471-1476.

Pauli, D., Peintinger, M. & Schmid, B. (2002) Nutrient enrichment in calcareous fens: Effects on plant species and community structure. *Basic and Applied Ecology,* **3,** 255-266.

Piqueras, J. (1999) Herbivory and ramet performance in the clonal herb *Trientalis europaea* L. *Journal of Ecology,* **87,** 450-460.

Rand, T. A. (1999) Effects of environmental context on the susceptibility of Atriplex patula to attack by herbivorous beetles. *Oecologia,* **121,** 39-46.

Rautio, P., Huhta, A. P., Piippo, S., Tuomi, J., Juenger, T., Saari, M. & Aspi, J. (2005) Overcompensation and adaptive plasticity of apical dominance in Erysimum strictum (Brassicaceae) in response to simulated browsing and resource availability. *Oikos,* **111,** 179-191.

Reader, R. J. (1992) Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture. *Oikos,* **65,** 414-418.

Roleček, J., Tichý, L., Zelený, D. & Chytrý, M. (2009) Modified TWINSPAN classification in which the hierarchy respects cluster heterogeneity. *Journal of Vegetation Science,* **20,** 596-602.

Rose, K. E., Russell, F. L. & Louda, S. M. (2011) Integral projection model of insect herbivore effects on *Cirsium altissimum* populations along productivity gradients. *Ecosphere,* **2**.

Schwarz, G. (1978) Estimating the Dimension of a Model. *The Annals of Statistics,* **6,** 461-464.

Sjödin, N. E., Bengtsson, J. & Ekbom, B. (2008) The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology,* **45,** 763-772.

Tolasz, R. (2007) Atlas podnebí Česka [Climatic atlas of the Czech Republic]. pp. 255. Český hydrometeorologický ústav, Praha.

Torssell, K. (1963) Inhaltsstofe von *Scabiosa succisa* L. *Arkiv for Kemi,* **21,** 93-96.

Valdés, A. & García, D. (2011) Direct and indirect effects of landscape change on the reproduction of a temperate perennial herb. *Journal of Applied Ecology,* **48,** 1422-1431.

Vergeer, P., Rengelink, R., Copal, A. & Ouborg, N. J. (2003) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology,* **91,** 18-26.

Verheyen, K., Vellend, M., Van Calster, H., Peterken, G. & Hermy, M. (2004) Metapopulation dynamics in changing landscapes: A new spatially realistic model for forest plants. *Ecology,* **85,** 3302-3312.

von Euler, T., Ågren, J. & Ehrlén, J. (2014) Environmental context influences both the intensity of seed predation and plant demographic sensitivity to attack. *Ecology,* **95,** 495-504.

Zabel, J. & Tscharntke, T. (1998) Does fragmentation of *Urtica* habitats affect phytophagous and predatory insects differentially? *Oecologia,* **116,** 419-425.

3 Paper II

Conspecific and heterospecific plant densities at small-scale can drive plant-pollinator interactions

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3.1 Abstract

Generalist pollinators are important in many habitats, but little research has been done on small-scale spatial variation in interactions between them and the plants that they visit. Here, using a spatially explicit approach, we examined whether multiple species of flowering plants occurring within a single meadow showed spatial structure in their generalist pollinator assemblages.

We report the results for eight plant species for which at least 200 individual visits were recorded. We found that for all of these species, the proportions of their general pollinator assemblages accounted for by particular functional groups showed spatial heterogeneity at the scale of tens of metres. This heterogeneity was connected either with no or only subtle changes of vegetation and flowering species composition. In five of these species, differences in conspecific plant density influenced the pollinator communities (with greater dominance of main pollinators at low-conspecific plant densities). The density of heterospecific plant individuals influenced the pollinator spectrum in one case.

Our results indicate that the picture of plant-pollinator interactions provided by averaging data within large plots may be misleading and that within-site spatial heterogeneity should be accounted for in terms of sampling effort allocation and analysis. Moreover, spatially structured plant-pollinator interactions may have important ecological and evolutionary consequences, especially for plant population biology.

Keywords:
pollination networks; plant-pollinator interactions; spatial patterns; hoverflies; honeybee; bumblebees; Syrphidae; *Apis mellifera; Bombus; Centaurea; Angelica; Succisa; Hypericum; Sanguisorba; Ranunculus; Selinum; Trifolium*

3.2 Introduction

Generalist pollinators constitute a major proportion of pollinators (both in terms of species and individuals) in many ecosystems (Lázaro et al., 2008, Memmott, 1999, Olesen et al., 2008). Additionally, they are involved in the responses of plant-pollinator interactions to ecosystem changes such as the spread of invasive plants (Bartomeus et al., 2010) and ecosystem restoration (Forup et al., 2008). Moreover, the diversity of their visited plant species (i.e. "degree of generalism") directly influences some key pollinator network characteristics including network asymmetry (Bastolla et al., 2009) and the number of cross-links among the network modules (Olesen et al., 2007).

Although generalist pollinator species are characterised by pollinating multiple species of plants, not only can there be specialization among individuals due to flower constancy (e.g. Waser, 1986), but the predominant species pollinated by a given generalist species can vary spatially and temporally. Indeed, considerable evidence has accumulated of temporal variation, which commonly is caused by year-to-year or within-season turnover in the spectrum of flowering plant species (Petanidou et al., 2008, Olesen et al., 2008). However, our knowledge of spatial variability in plant-pollinator interactions involving generalist pollinators is much more scant, and is primarily based on comparisons either at continental scales (Olesen and Jordano, 2002) or among localities several kilometres apart (Forup et al., 2008). Thus, the ecological effects of small-scale differences in plant and pollinator spatial distributions as well as species compositions have largely escaped field investigation, despite the predicted importance of such variation in mutualist networks (Morales and Vázquez, 2008). Therefore, we largely lack empirical data on spatial heterogeneity of plant-pollinator interactions at scales ecologically meaningful to pollinator individuals (but see Herrera, 2005).

Multiple phenomena can yield small-scale spatial inhomogeneities in plant-pollinator interactions. Firstly, spatial distribution of plants tends to be aggregated at the scales ranging from tens of centimetres to tens of metres (Greig-Smith, 1983). Secondly, foraging ranges of insect pollinators vary from a few hundred metres to a few kilometres (Greenleaf et al., 2007) and their nest densities may be low (e.g. Goulson, 2010), contributing heterogeneity in local pollinator distribution. The resulting heterogeneity within sites can translate into differential pollinator visitation and affect both plant and pollinator fitness. For example, reproductive success of individual plants is known to be affected both by neighbourhood floral composition and among-site differences in pollinator composition (e.g. Jakobsson et al., 2009, Schuett and Vamosi, 2010, Brunet and Sweet, 2006, Larsson, 2005). Similarly, pollinator fecundity and survival can be affected by local environmental heterogeneity (Williams and Kremen, 2007, Westphal et al., 2006). Similarly, spatial differences in plant-pollinator interactions are a factor influencing evolution of floral attraction of generalists versus specialists (Waser et al., 1996).

The lack of consideration of small-scale spatial structure in plant-pollinator interactions is evident in typical plant-pollinator (especially network) studies, which record plant-pollinator assemblages using sizeable plots (usually with dimensions of several tens of metres). Such approach implicitly assumes spatial homogeneity of plant-pollinator interactions within the plot. This means that the plot-level aggregated pollinator spectrum (recorded species proportions in a pollinator assemblage of a given plant species) represents the pollinator spectrum of each included individual of the species. Moreover, small-scale spatial heterogeneity in plant-pollinator interactions might at least in part underlie the influence that plot size has on the number of interactions discovered per unit of sampling effort (see Gibson et al., 2011).

Here, we examine spatial homogeneity of pollinator spectra at a moderately sized mesophytic meadow (largest dimension ca. 260 m) with relatively homogeneous flowering plant composition. It contains minimum obstacles to pollinator movement (presumably allowing pollinators to move according to their preferences). We ask whether plant spatial distribution and consequent variability in small-scale spatial assemblages of plants influence spatial homogeneity of pollinator visitation. In our study, we used a spatially explicit sampling design and quantified both pollinator and flowering plant abundances. Specifically, we ask these questions:

1) Is the pollination network spatially homogeneous at the scale of several tens of meters? I.e. do individuals of the same plant species experience similar pollinator assemblages at different positions within a meadow?
2) How does the local abundance of conspecific plants and highly visited heterospecifics influence the pollinator assemblages of given species?

3.3 Materials and methods

3.3.1 *Ethics statement*

The study did not involve any endangered or protected insect species and complied with the current laws of the Czech Republic. No permissions for this kind of research were necessary.

3.3.2 *Study site*

The study was conducted at the K Handrkovu meadow near Vernýřov village, Central Bohemia, Czech Republic (N 49.8466, E 15.1498; WGS 1984). The area of the meadow is 4.5 ha, including unmown verges (ca 0.3 ha). The local climate is moderately sub-oceanic (annual mean temperature around 8°C and annual precipitation around 650 mm; Tolasz, 2007). The vegetation of the meadow could be classified as E3.4 - Moist or wet eutrophic and mesotrophic grassland in the EUNIS classification. There are two peaks of flowering at the meadow (both in terms of diversity and abundance). The first one occurs in May before the first hay cut (beginning of June) and the second one in August before the second hay cut (mid-September).

3.3.3 Study design

We used a grid of 93 points spaced 20 m apart, roughly covering the entire meadow, to delineate 93 plots, each centred on one of the grid points (Fig. 1). Each plot measured 4 x 4 m and was used for censuses of both flowering plants and pollinators. Additionally, 10 plots of size 2 x 8 m were delineated in the main adjacent linear unmown meadow verges (Fig. 1), since they potentially share insect pollinators with the meadow; these were also used for the censuses. Both plant and pollinator censuses were performed between the 20th and 26th of August 2011, during the second peak flowering period.

Each time a pollinator census was conducted for a given plot, we would record all pollinators visiting insect-pollinated plant species at the time we reached the plot. For the purpose of this study, we assume all flower visitors that were observed to touch the plant's reproductive structures to be pollinators. We are aware that mechanistic evidence for pollination would be necessary to classify the visitors unambiguously. However, most recorded visitors were already found to function as pollinators by other studies. Each pollinator individual was recorded only once, along with the species identity of the visited plant. All meadow plots were censused for pollinators approximately 20 times (range 19-25) and all verge plots approximately 10 times (range 10-11), with observations randomized with respect to date and time of day. Censuses were conducted between 7 and 19 o'clock at weather favourable to insect activity. The pollinators were identified to the lowest taxonomic level possible in the field, after catching the individuals carefully in insect nets. Voucher specimens for morphospecies were collected for Syrphidae and Hymenoptera in order to confirm their identification later. The voucher specimens were deposited at the Dept. of Zoology, Faculty of Science, Charles University in Prague. For the purposes of the presently described study, the observed pollinators were categorized in 12 functional groups: honeybee (*Apis mellifera*), solitary bees, bumblebees (*Bombus* spp.), hoverflies (Syrphidae), true flies (Muscidae), flesh flies (Sarcophagidae), blowflies (Calliphoridae), tachinid flies (Tachinidae), other Diptera, other Hymenoptera, beetles (Coleoptera), and butterflies (Lepidoptera).

A plant census was done once at each plot during the study period. For 17 plant species (chosen based upon previous research at the site showing them to be attractive to hoverflies), the numbers of flowering stalks were counted (see Appendix 1, Tables 1, 2 and Appendix 6, Fig. 8 for complete list). For the remaining flowering species, abundances were assessed semi-quantitatively by recording the presence/absence of their flowering stalks within a lattice of 64 subplots superimposed over each plot (subplot size 0.5 x 0.5 m). *Hypericum maculatum* and *H. perforatum* were not distinguished, since they often were interspersed and are indistinguishable without close examination. Here, we report our plant-pollinator interaction results only for those plant species (eight) for which we recorded at least 200 individual visits by pollinators.

Flowering vegetation composition of the plots was summarised by means of detrended correspondence analysis (DCA) in order to identify the main gradients in flowering species composition. We used the sample scores of the plots on the first two ordination axes in further analyses. The first ordination axis explained 14.9% of variability in flowering species composition, corresponding to the moisture gradient (drier towards positive values). The second axis explained 7.7% of variability and could be interpreted as a nutrient or meadow/verge gradient (more nutrients and verge character towards positive values; for details see Appendix 2, Figs. 1 and Fig. 2).

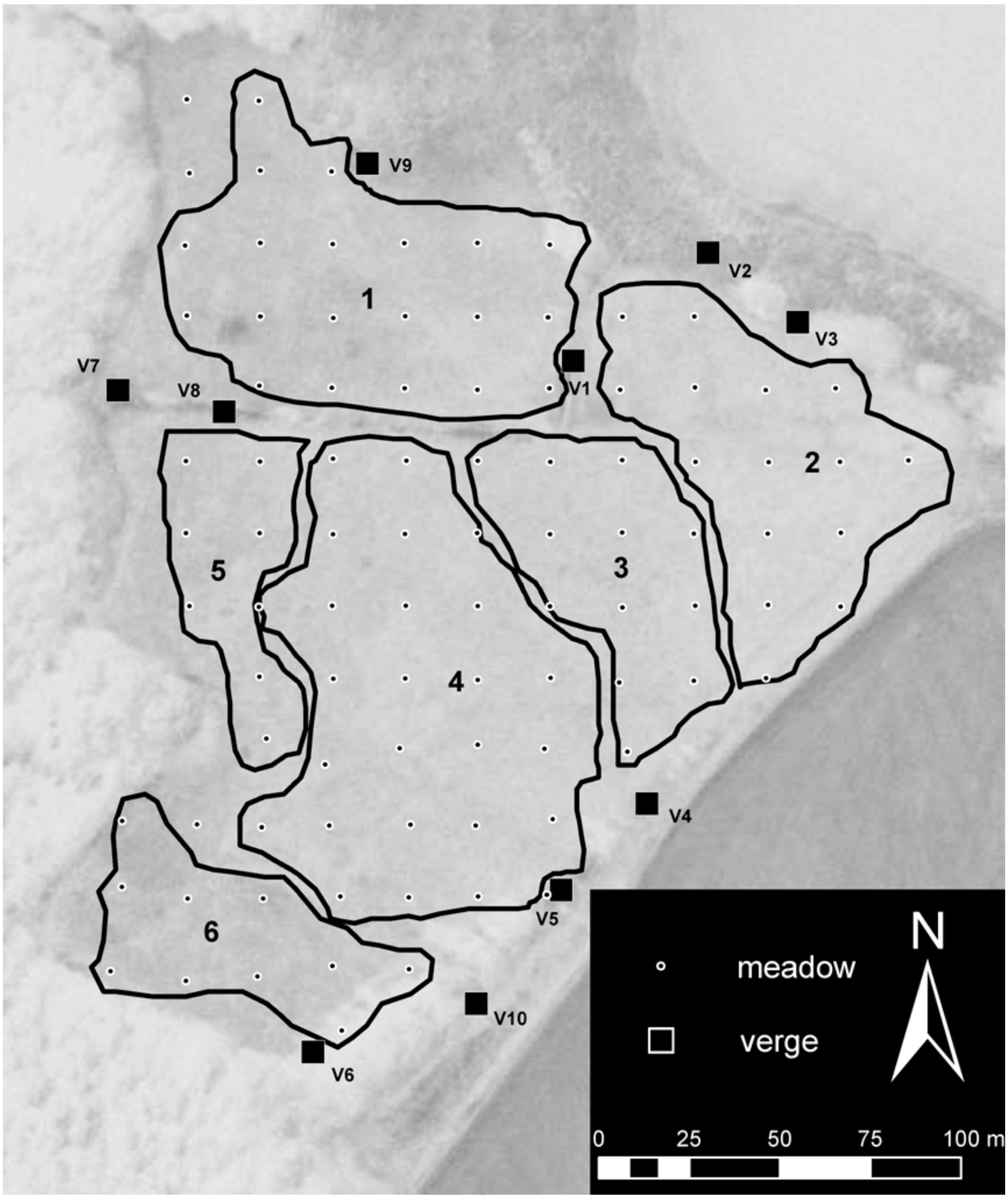

Fig. 1: Delimitation of the meadow sectors according to plant community composition; Sectors: 1 – wet, nutrient-poor stands; 2 – mesic to intermittently wet, nutrient-rich stands; 3 – intermittently wet, nutrient moderately rich stands; 4 – mesic, nutrient-rich stands; 5 – moderately wet, nutrient moderately rich stands; 6 – very wet, nutrient moderately rich stand; (for information on flowering plant composition please refer to Appendix 1, Table 4). Aerial photograph credit: Czech Office for Surveying, Mapping and Cadastre.

For use in addressing Question 1 (degree of spatial homogeneity of pollinator networks), we divided the meadow into several spatially contiguous sectors based upon vegetation similarity (Fig. 1). The delimitation of sectors was done on basis of expert knowledge (Z. Janovský) and took into account all species occurring at the meadow (including grasses and non-flowering species and following the local fine-scale classification Chytrý, 2007). The verge plots were treated individually, with the exception of neighbouring verges nos. 2 and 3, which had very similar vegetation and conditions. In the case of *T. hybridum*, we delimited the sectors at a coarser scale than for other species due to low numbers of visits in the wetter sectors of the meadow, resulting in only two sectors, one in the wetter part of the meadow and the other in the drier part (for details see Appendices 3 and 4, Figs 3 and 4). When addressing Question 1, the pollinator records for each of the 8 focal plant species were summed across all the plots in each sector.

To address Question 2 (effects of conspecific and heterospecific neighbour abundances), for each of the eight plant species on which we are focusing here, we only used data from plots from which at least five pollinator individuals were recorded for that species. We chose this arbitrary threshold in order to obtain reasonable estimates of pollinator composition (and density) suitable for further analysis. For each focal plant species, due to the varied observation effort at different plots, the pollinator functional group counts were standardized by dividing them by the product of the number of flowering stalks of that species and the number of plot pollinator censuses. Our data therefore represent pollinator functional group densities per flowering stalk and census and we further refer to them as pollinator densities. Densities defined in this way, in contrast to simple per-plot densities, have a straightforward interpretation in terms of potential effects on plant reproduction.

All multivariate analyses were conducted in CANOCO for Windows 4.56 (ter Braak and Šmilauer, 2002).

3.3.4 Data analysis

For Question 1, differences among the pollinator spectra (i.e. in terms of proportions of individual pollinators accounted for by the pollinator functional groups) of the meadow sectors were evaluated for significance using the χ^2-test. Data on *S. carvifolia* were not included in this analysis, because it occurred in only one meadow sector. The pollinator groups with low occurrence (i.e. yielding expected values lower than five) were always merged into a category designated as “other” so that the pollinator spectrum matrices met the χ^2-test assumptions. For *S. officinalis*, this did not suffice to meet the criterion, and therefore for this species the “other” pollinator functional group was not included in the analysis. All computations were done in the R 2.12.0 statistical environment (R Foundation for Statistical Computing, Vienna, http://www.R-project.org/).

For Question 2, the data were analysed by multivariate analyses, namely redundancy analysis (RDA), which is a multivariate extension of multiple regression (Legendre and Legendre, 2003). Similarly to the analysis for Question 1, rare pollinator groups were placed in the “other” category. For each of the eight plant species for which we had sufficient data, we included the following as predictors: (i) conspecific log-abundances (ii) heterospecific log-abundances of the other focal plant species and (iii) the sample scores along the first two DCA axes for overall flowering plant composition (for details see Appendix 2, Figs 1 and 2, for complete lists of predictors [seven to eight per species] considered in forward selection in analyses see Appendix 1, Table 3). Log-abundances of heterospecifics were included for species that occurred in at least 3 of the plots of the given focal species. In the cases of *S. pratensis* and *A. sylvestris,* the plot type (meadow/verge) was also used as a predictor. RDA was used based upon the preliminary DCA analyses, which indicated relative monotony of pollinator functional group responses along the first ordination axis (in all cases gradient length between 1 and 2 S.D.; Lepš and Šmilauer, 2003).

The predictors were tested by means of forward selection and subsequent permutation tests (4999 permutations in each run). In each step, a predictor was tested that explained the most variability. If the first tested predictor was not significant ($\alpha<0.05$), then the predictor with the second highest explained variability was tested, and so on. If a predictor was significant, we would include it in the model and continue again with testing the predictor with the highest explained variability. The selection ended when no more predictors were found significant.

3.4 Results

The pollinator spectra of all species included in the test of Question 1 were spatially heterogeneous (Table 1). In the case of *S. officinalis*, one of the functional groups (blow flies – Calliphoridae) was almost completely absent from one of the meadow sectors, constituting a qualitative difference among sectors. For four plant species (*A. sylvestris*, *Hypericum* spp., *R. acris,* and *T. hybridum*), the most abundant pollinator functional group showed substantial differences (16% to 50% change) in the proportions of the pollinator spectrum for which it accounted (Fig. 2 and Appendix 3, Fig. 3).

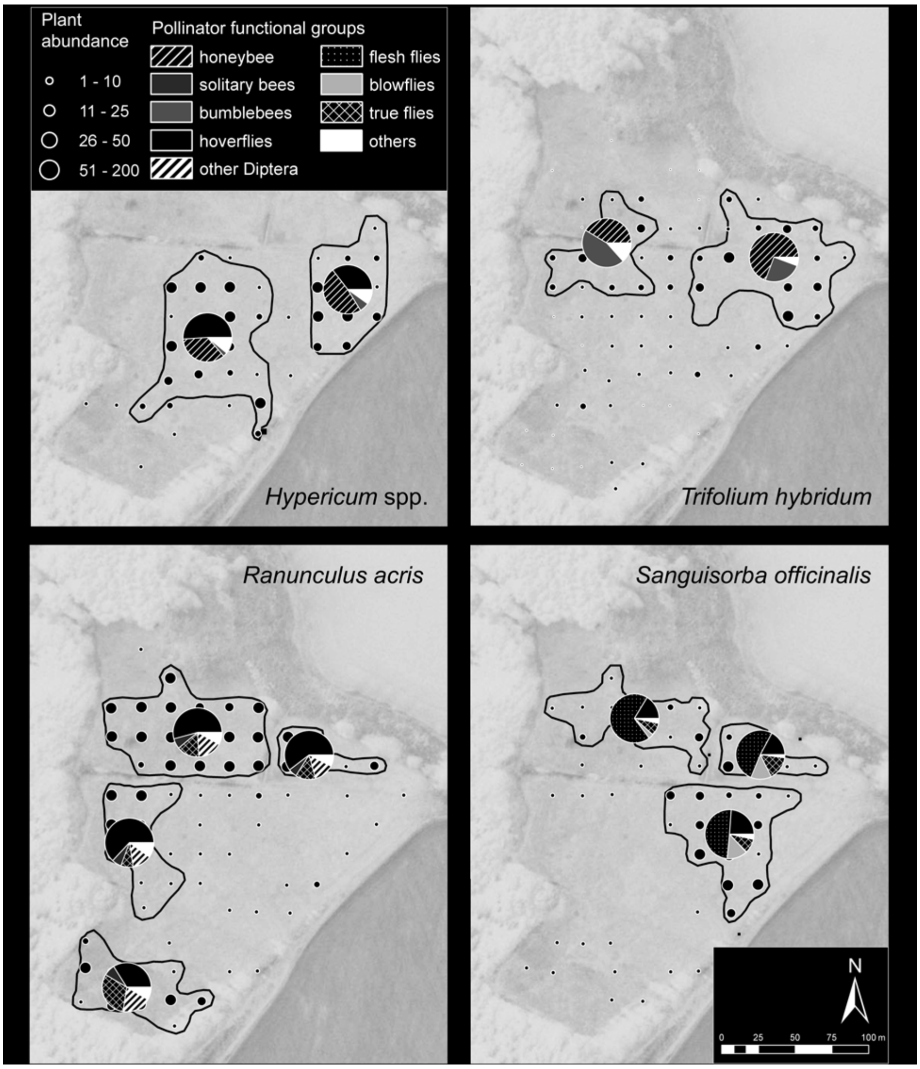

Fig. 2: Maps of recorded pollinator spectra for different meadow sectors with more than 50 pollinators recorded for four of the focal plant species. Flowering stalk abundances depicted by size of the dots; please note the different, semi-quantitative scale for *Trifolium hybridum* (0-64 subplots occupied). Please note that in each case the category “others” comprises different pollinator groups (see Materials and Methods for explanation). For more detailed information on pollinator abundances and spectra please refer to Appendices 3 and 4, Figs. 3 and 4.

Table 1: Summary of occurrence, pollinator spectra, and their differences for the eight most visited plant species; degrees of freedom and P-values are from the χ^2-tests of homogeneity of pollinator assemblages of the given plant species among different meadow sectors; (* Please note that in the case of *T. hybridum,* the no. of flowering stalks corresponds to the number of occupied subplots, for details see Materials and Methods and Appendix 1, Tables 1, 2.)

Plant	**No. of occupied plots**	**No. of flowering stalks**	**No. of recorded pollinators**	**Main pollinator groups**	**No. of sectors**	**Df**	**P-value**	**Main difference**
Angelica sylvestris	9	100	1281	other Diptera (60%), other Hymenoptera (26%), other (14%)	7	12	<0.001	Varying proportions of other Diptera (29-79%) and other Hymenoptera (13-41%)
Centaurea jacea	62	1707	926	honeybee (63%), bumblebees (28%), other (9%)	2	2	0.005	Two-fold difference in bumblebee proportion (15% to 29%)
Hypericum spp.	41	1732	291	hoverflies (43%), honeybee (42%), bumblebees (4%), other (11%)	2	3	0.028	Change of dominance between hoverflies and honeybee (51% to 35% and 35% to 48% resp)
Ranunculus acris	68	2776	514	hoverflies (52%), true flies (16%), other Diptera (16%), other (16%)	4	9	<0.001	One sector co-dominated by true flies (30%) and hoverflies (32%) instead of hoverflies alone
Sanguisorba officinalis	52	888	526	flesh flies (52%), hoverflies (20%), blowflies (13%), true flies (12%), other(3%)	3	6	0.019	Near-absence of blowflies in the wettest sector, higher dominance of flesh flies there (69%)
Selinum carvifolia	24	285	355	other Diptera (36%), hoverflies (24%), other Hymenoptera (18%), true flies (10%), other (12%)	1	-	-	-
Succisa pratensis	17	203	414	hoverflies (84%), other (16%)	3	2	0.002	Higher proportion of other pollinators in one verge plot (32%)
Trifolium hybridum	66	902*	327	honeybee (60%), bumblebees (31%), other (9%)	2	2	<0.001	Bumblebees increase from 25% in the drier sector to 45% in the wetter one

The multivariate analyses identified significant correlations of pollinator densities with at least one measure of vegetation composition in five of the eight species tested (Table 2). Pollinator spectra of four species (*C. jacea*, *Hypericum* spp., *R. acris*, *S. officinalis*) and marginally one other (*S. carvifolia*) were affected by abundance of conspecifics (Figs. 3 and 4, Appendix 5, Fig. 5). Only in the cases of *R. acris* and *T. hybridum* were the pollinator spectra influenced by abundances of other plant species (*S. officinalis* and *R. acris*, respectively).

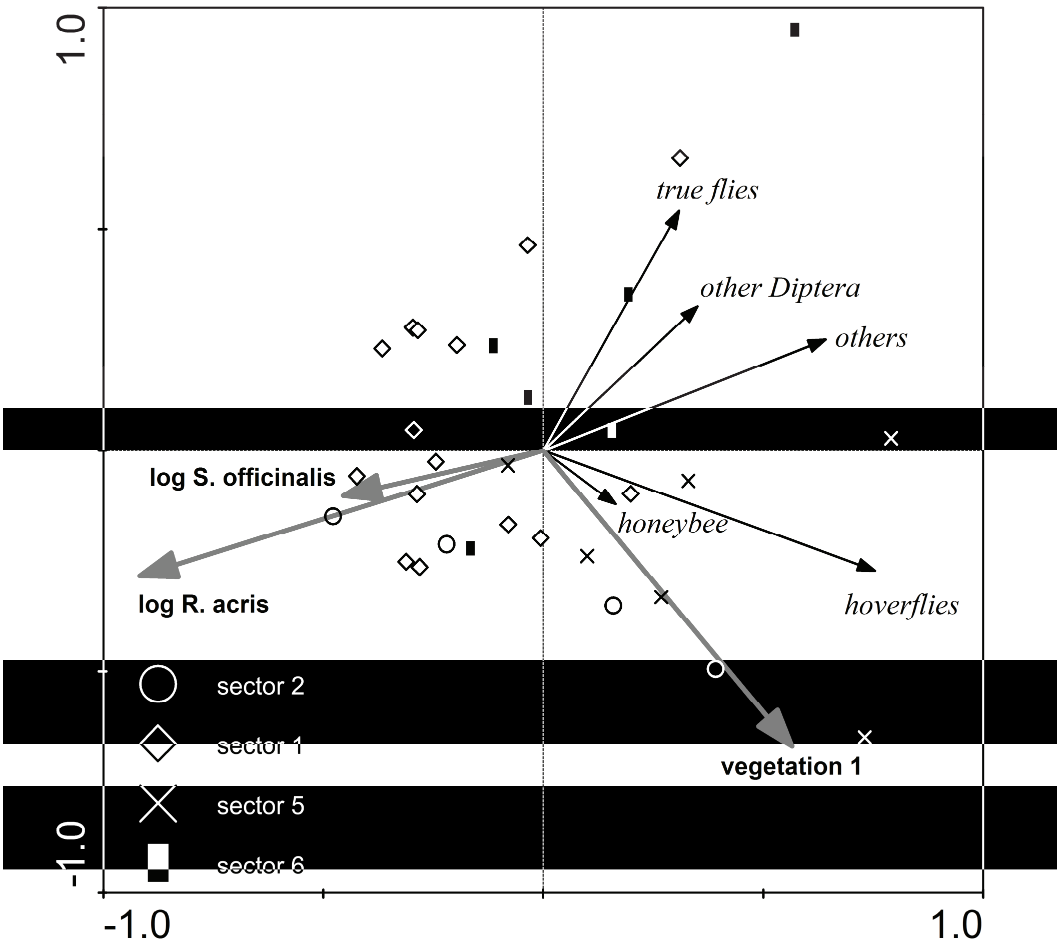

Fig. 3: Ordination diagram of RDA of pollinator densities on *Ranunculus acris*; Environmental variables included in the final model based on the forward selectin were: logarithm of flowering stalk abundance of *Ranunculus acris* (log R. acris), logarithm of flowering stalk abundance of *Sanguisorba oficinalis* (log S. officinalis), and 1st axis of DCA of flowering plant composition (vegetation 1); plots were categorized according to the sector in which they were located(see Fig. 1 for definition of sectors); 1st ordination axis explains 34.7% of total variability in pollinator density, 2nd axis explains 15.2%.

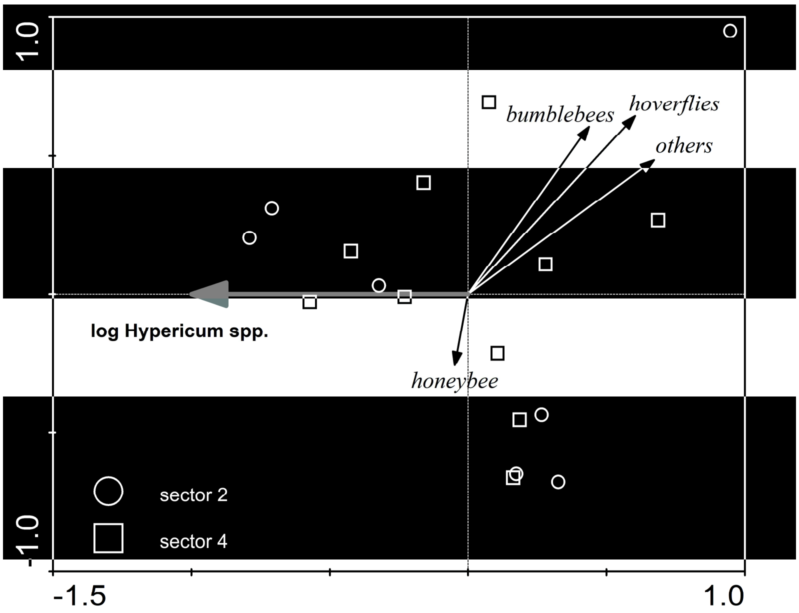

Fig. 4: Ordination diagram of RDA analysis of pollinator densities on *Hypericum* spp.; Environmental variables included in the final model, based on forward selection were: logarithm of flowering stalk abundance of *Hypericum* spp. (Hypericum (log); plots were categorized according to the sector in which they were located (see Fig. 1 for definition of sectors); 1st ordination axis (canonical) explains 27.5% of total variability in pollinator density, 2nd axis (non-canonical) explains 30.2%.

Table 2: Results of the multivariate analyses (RDA with forward selection) of interdependence between plant pollinator densities at each given plot; significant variables ($p<0.05$) given in bold, marginally significant ($p<0.1$) given in regular font, "-" denotes variable not included in forward selection (for details see Materials and Methods), variability explained – sum of varibiality in pollinator spectra explained by significant terms.

Plant	**No. of plots with >4 pollinators recorded**	**Meadow/ verge**	**DCA of all flowering plants** 1st axis	2nd axis	**Plant species abundances selected**	**Variation explained**
Angelica sylvestris	9	n.s.	n.s.	n.s.	n.s.	
Centaurea jacea	30	-	n.s.	n.s.	**Centaurea (0.0064)**; Hypericum (0.0650)	21.9%
Hypericum spp.	17	-	n.s.	n.s.	**Hypericum (0.0014)**	27.5%
Ranunculus acris	31	-	**0.0048**	n.s.	**Ranunculus (0.0002);** **Sanguisorba (0.0214)**	49.9%
Sanguisorba officinalis	19	-	n.s.	0.0964	**Sanguisorba (0.0008)**	46.0%
Selinum carvifolia	10	-	n.s.	n.s.	Selinum (0.0544)	
Succisa pratensis	12	n.s.	n.s.	n.s.	n.s.	
Trifolium hybridum	17	-	n.s.	n.s.	**Ranunculus (0.0418)**	18.7%

3.5 Discussion

We demonstrate for a relatively large dataset (4634 visits for 8 plant species) that plant-pollinator interactions are spatially heterogeneous at the spatial scale of tens of metres. This was true despite the meadow's moderate size (well within most foraging ranges), isolation (i.e. the detected heterogeneity could not be a reflection of plant or pollinator distributions outside the site) and, importantly, rather homogeneous distribution of all major entomophilous plants over it. In all seven plant species that we had sufficient data to test for the spatial homogeneity of the pollination network, we found different pollinator spectra in different parts of the meadow. The pollinator spectra were influenced both by conspecific densities in the plot and by the densities of other flowering plant species there. The effect of conspecific densities was predominant.

Possible explanations for the observed spatial turnover in plant pollinator interactions include: (i) the interplay between density of a given plant and the per-plant densities of its pollinators; (ii) influence of heterospecific plant densities on pollinators of the constituent plant species (resulting in facilitation or competition); (iii) heterogeneity in pollinator spatial distribution due to abiotic factors or pollinator autecology. Our data can directly address only the first and second possible explanations, but also suggest processes possibly underlying the third explanation.

3.5.1 The influence of conspecific plant density on pollinator composition and densities

Our results show quite clearly the dependence of the pollinator spectrum of a given plant species on its own density. This was found in four of eight studied species (those with the largest datasets) while in a fifth species, *S. carvifolia*, the same trend was marginally significant. In general, if there was an effect of conspecific density (see Figs. 3 and 4 and Suppplementary files S2, Figs. S5, S6, S7) on pollinator abundance, it was negative. For each focal plant, its most abundant pollinator group always decreased with increasing conspecific density. This decrease was usually stronger compared to other pollinator groups. Thus, dominance of the most abundant pollinator group was stronger in plots with low conspecific density, while high conspecific density plots hosted more diverse pollinator spectra. A similar pattern in diversity of pollinator spectra was observed Lázaro et al. (2009). However, they could not attribute it unambiguously to either focal plant density or within-season turnover in plant and pollinator densities (as their data covered the whole flowering season of the species and were pseudoreplicated in time). A possible explanation may be that virtually all available individuals of the dominant pollinator may be attracted to the patch already at lower abundances of the target plant species, while the less common pollinators may be attracted more to the target plant species only at its higher abundances.

Further, the overall increase in pollinator abundances did not match the increase of target plant abundances, leading to decreases in pollinator densities and possible increases in intra-specific competition for pollinators. The results of previous studies have been mixed, with some reporting positive associations between target plant densities and pollinator visitation rates (Jakobsson et al., 2009, Hegland and Boeke, 2006) and some finding no association (Bosch and Waser, 1999, Klinkhamer and de Jong, 1990) or even a negative association (Kunin, 1997). Since many of the studies reporting positive effects did not use a visitation rate standardized per individual plant, and the slopes of their visitation plotted against plant density were often lower than one, we believe that the occurrence of positive effects has been overstated. Thus, we suggest that pollinator saturation may be a commoner phenomenon than previously thought.

Nonetheless, it is less clear whether the observed decrease of pollinator densities and increase of diversity of pollinator spectra in high conspecific density plots effectively translates into decrease in plant reproductive output per unit reproductive effort. Indeed, studies showing no or positive effect of conspecific density on fitness prevail (Kirchner et al., 2005, Schuett and Vamosi, 2010, Jakobsson et al., 2009, Bosch and Waser, 2001). These outcomes could be explained either by the fact that even the recorded "low" pollinator densities did not cause pollen limitation, or other properties of high conspecific density stands outweighed the negative effects of lower pollinator densities. On the other hand, in systems including pollinators of very different effectiveness (carryover capacity; sensu Larsson, 2005), differences in pollinator spectrum composition probably translate into differences in reproductive success (Brunet and Sweet, 2006). This is not the case for most of our eight focal species, since the three most common pollinator groups in our system– honeybee, bumblebees, and hoverflies – have similar effectiveness (Rader et al., 2009). However, it might play a role for *R. acris* and *S. officinalis*, which are visited both by furry dipterans (most hoverflies) and non-furry dipterans, which are reported to have much lower carryover capacity (i.e. effectiveness; Larsson, 2005).

3.5.2 The influence of abundances of heterospecific flowering plants

The effects of heterospecific densities on pollinator spectra were detected only in two species (*R. acris* and *T. hybridum*). In the case of *R. acris*, the density of a neighbour species, *S. officinalis,* affected the pollinator spectrum in the same way as conspecific density, i.e. decreased per flower stalk densities of all pollinator groups. Hoverflies and true flies, the key pollinators of *R. acris*, also visit *S. officinalis*, but the relationship between these two plants and their pollinators is asymmetric in that flesh flies (the main pollinators of *S. officinalis*) scarcely visit *R. acris*. This contrasts with the predominantly positive interspecific interactions among plant species found by Hegland et al. (2009) in a similar system in southern Norway. This difference in findings may have been caused by different overall flower densities, with negative interactions starting to outweigh the interspecific facilitation only at high floral densities (e.g. Ghazoul, 2006). Mutually negative relationships have also been reported from systems involving closely related species with similar floral displays (e.g. Schuett and Vamosi, 2010, Kunin, 1993), which, however, was not the case here.

The moisture gradient in overall floral composition (1st axis of vegetation DCA) was correlated only with hoverfly abundances on *R. acris* (positively towards drier areas). This outcome may reflect two trends in our data: (i) decreasing overall floral dominance of *R. acris* and (ii) the presence of most other hoverfly-sharing plant species only in wetter parts of the meadow. Unlike in wetter parts, the generalist pollinators not preferring *R. acris* would not need to visit it in drier parts of the meadow with abundant preferred plant species. On the other hand, hoverflies visiting *R. acris* have to concentrate on it in drier meadow parts where they lack alternative visited species (with the exception of *S. officinalis*).

We suggest that the effect of *R. acris* densities on pollinators of *Trifolium hybridum* is an artefact, with *R. acris* only being better than the 1st DCA axis as a surrogate for moisture gradient. In general, we suggest that the test power of our relatively large dataset is still quite low for revealing effects of heterospecific plant abundances (unlike for conspecific abundances).

3.5.3 Other possible causes of heterogeneity in pollinator spatial distributions

Although we assume, based upon the pollinator foraging distances, that pollinators can reach all their preferred plots and plant species within our study meadow, it is uncertain whether they really do. Optimal foraging theory (Charnov, 1976) predicts that preference for a certain host plant should be a combination of its profitability (e.g. net of energy gain from nectar) and its distance (i.e. decrease in encounter rate). Thus, despite the presence of preferred sources, the proportion of pollinators visiting suboptimal but nearer sources should increase with increasing mismatch between the breeding/emerging sites of pollinators and the locations of their preferred plant sources. This could be especially true in the case of Hymenoptera, which must return repeatedly to their nests. Additionally, various phenomena could cause pollinators to avoid foraging in some areas, e.g. for bumblebees, the immediate vicinity of their nest (Goulson, 2010, Osborne et al., 1999). All these factors are likely to influence encounter probabilities and mobility, the key factors structuring mutualist networks (see (Morales and Vázquez, 2008)). Direct competition among pollinators (e.g. (Sowig, 1989, Forup and Memmott, 2005)) could also influence spatial distribution of pollinator densities. If floral resources were limiting, we would not observe a relationship between conspecific plant densities and pollinator densities per plant, because the floral resource would be saturated with pollinators. Since we observed a decrease in pollinator densities per plant with increasing conspecific plant densities, direct competition does not seem to affect considerably our system.

Abiotic factors, particularly shading, might influence pollinator spatial distribution in our system. The bordering forest shades some of the plots, yielding differences in light period of up to three hours. Most pollinators ceded to visit shaded plots, but bumblebees continued to visit them, it might be due to their larger size and partial thermoregulation (Goulson, 2010). Plot wetness could also affect the pollinator spectra, with rising proportions of true flies and flesh flies in the spectra of *R. acris* and *S. officinalis* in wetter areas (possibly due to nearness of emergence sites).

3.5.4 Implications for interpreting plant-pollinator interactions

Our results indicating strong spatial heterogeneity of plant-pollinator interactions have two main implications, each explored below: (i) plot size and sampling effort allocation in plant-pollinator studies needs to take this heterogeneity into account; (ii) spatial structure of sampling effort may potentially change the probability of detection of modules in pollination networks (sensu Olesen et al., 2007) (both probabilities of false negatives and false positives).

We found significant spatial effects on pollinator spectra at the scales of tens of metres, which suggests that results obtained by averaging data from large plots or transects (commonly measuring even 100 metres) do not provide a reliable representation of pollinator spectra experienced by individual plants. Sampling plot heterogeneity increases the probability of discovering a particular plant-pollinator interaction and thus decreases the probability of falsely designating species as specialists (see (Dorado et al., 2011)). However, it can also create what we would term “false generalists”, since the term generalist may both apply to a species, where also the individuals behave as generalists, or to a “false generalist” species, whose individuals actually visit narrower but differing spectra of plants (or they even act as specialists). While there is no difference among such species from viewpoint of pollinator ecology, the plants perceive the second species as more specialised with corresponding benefits for pollination. Large heterogeneous plots increase probability of including areas, where the pollinator individuals actually visit narrower plant spectra.

Moreover, the cumulative pollinator spectra (i.e. from all individuals of a pollinator across an entire study site) recorded will be influenced by the degree to which the spatial distribution of sampling corresponds to the heterogeneity of these interactions. Gibson et al. (2011) recommended even sampling effort allocation with respect to overall plant abundances. Based upon our results, we suggest extending this recommendation to even sampling of the whole range of conspecific plant densities at the site, or better yet to divide the site into subplots of pollinator-meaningful size and then sample them evenly. It is an open question how small-scale spatial heterogeneity in plant-pollinator interactions translates into higher order pollination network properties. However, increased spatial heterogeneity in plant-pollinator interactions requires greater sampling to reliably describe the plant-pollinator interactions. This makes more pronounced the common problem of undersampling of pollinator networks (cf. (Chacoff et al., 2012)) known to affect higher order network properties (Blüthgen, 2010, Vázquez et al., 2009).

Modules in plant-pollinator networks have been proposed as possible co-evolutionary units, where selection could act on both plants and pollinators (Olesen et al., 2007). This would require the modules to be stable both in space and time and relatively isolated in terms of gene flow. Moreover, the observed spatial heterogeneity in pollinator visitation can possibly have significant effects on probabilities of delimitation of network modules, depending on the sampled part of the meadow. For example, blow flies (Calliphoridae) were, in one of our meadow sectors, at most only an accessory pollinator group of *S. officinalis*, whereas in the rest of the meadow they were the third most common visitors to this species, only rarely visiting other plants. Thus, the chances of delimiting a pollination-network module around *S. officinalis* differed greatly over the scale of only tens of metres.

Taken together, our results imply that the influences of local context (i.e. conspecific and heterospecific flowering plant densities) are not only detectable in plant-pollinator networks, but also exert relatively strong influence on their structure. Therefore the plant-pollinator networks should consider more the spatial aspect of their sampling structure.[4]

3.6 References

Bartomeus, I., Vila, M. & Steffan-Dewenter, I. (2010) Combined effects of *Impatiens glandulifera* invasion and landscape structure on native plant pollination. *Journal of Ecology,* **98,** 440-450.

Bastolla, U., Fortuna, M. A., Pascual-García, A., Ferrera, A., Luque, B. & Bascompte, J. (2009) The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature,* **458,** 1018-U91.

Blüthgen, N. (2010) Why network analysis is often disconnected from community ecology: A critique and an ecologist's guide. *Basic and Applied Ecology,* **11,** 185-195.

Bosch, M. & Waser, N. M. (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (*Ranunculaceae*). *American Journal of Botany,* **86,** 871-879.

Bosch, M. & Waser, N. M. (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia,* **126,** 76-83.

Brunet, J. & Sweet, H. R. (2006) Impact of insect pollinator group and floral display size on outcrossing rate. *Evolution,* **60,** 234-246.

Dorado, J., Vazquez, D. P., Stevani, E. L. & Chacoff, N. P. (2011) Rareness and specialization in plant-pollinator networks. *Ecology,* **92,** 19-25.

Forup, M. L., Henson, K. S. E., Craze, P. G. & Memmott, J. (2008) The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. *Journal of Applied Ecology,* **45,** 742-752.

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[4] **Acknowledgements**
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Forup, M. L. & Memmott, J. (2005) The relationship between the abundances of bumblebees and honeybees in a native habitat. *Ecological Entomology,* **30,** 47-57.

Ghazoul, J. (2006) Floral diversity and the facilitation of pollination. *Journal of Ecology,* **94,** 295-304.

Gibson, R. H., Knott, B., Eberlein, T. & Memmott, J. (2011) Sampling method influences the structure of plant-pollinator networks. *Oikos,* **120,** 822-831.

Goulson, D. (2010) *Bumblebees - Behaviour, ecology, and conservation.* Oxford University Press, Oxford.

Greenleaf, S. S., Williams, N. M., Winfree, R. & Kremen, C. (2007) Bee foraging ranges and their relationship to body size. *Oecologia,* **153,** 589-596.

Greig-Smith, P. (1983) *Quantitative plant ecology.* Blackwell Scientific, Oxford.

Hegland, S. J. & Boeke, L. (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology,* **31,** 532-538.

Hegland, S. J., Grytnes, J.-A. & Totland, Ø. (2009) The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research,* **24,** 929-936.

Herrera, C. M. (2005) Plant generalization on pollinators: Species property or local phenomenon? *American Journal of Botany,* **92,** 13-20.

Chacoff, N. P., Vazquez, D. P., Lomascolo, S. B., Stevani, E. L., Dorado, J. & Padron, B. (2012) Evaluating sampling completeness in a desert plant-pollinator network. *Journal of Animal Ecology,* **81,** 190-200.

Charnov, E. L. (1976) Optimal foraging, marginal value theorem. *Theoretical Population Biology,* **9,** 129-136.

Chytrý, M. (2007) Vegetace České republiky. 1. Travinná a keříčková veegtace [Vegetation of the Czech Republic. Vol. 1 - Grassland and heathland vegetation]. pp. 528. Academia, Praha, Česká republika.

Jakobsson, A., Lázaro, A. & Totland, Ø. (2009) Relationships between the floral neighborhood and individual pollen limitation in two self-incompatible herbs. *Oecologia,* **160,** 707-719.

Kirchner, F., Luijten, S. H., Imbert, E., Riba, M., Mayol, M., Gonzalez-Martinez, S. C., Mignot, A. & Colas, B. (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos,* **111,** 130-142.

Klinkhamer, P. G. L. & de Jong, T. J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos,* **57,** 399-405.

Kunin, W. E. (1993) Sex and the single mustard - Population density and pollinator behavior effects on seed set. *Ecology,* **74,** 2145-2160.

Kunin, W. E. (1997) Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology,* **85,** 225-234.

Larsson, M. (2005) Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (*Dipsacaceae*). *Oecologia,* **146,** 394-403.

Lázaro, A., Hegland, S. J. & Totland, Ø. (2008) The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia,* **157,** 249-257.

Lázaro, A., Lundgren, R. & Totland, Ø. (2009) Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos,* **118,** 691-702.

Legendre, P. & Legendre, L. (2003) *Numerical Ecology.* Elsevier, Amsterdam, Netherlands.

Lepš, J. & Šmilauer, P. (2003) *Multivariate analysis of ecological data using CANOCO.* Cambridge University Press, Cambridge.

Memmott, J. (1999) The structure of a plant-pollinator food web. *Ecology Letters,* **2,** 276-280.

Morales, J. M. & Vázquez, D. P. (2008) The effect of space in plant-animal mutualistic networks: insights from a simulation study. *Oikos,* **117,** 1362-1370.

Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. (2007) The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 19891-19896.

Olesen, J. M., Bascompte, J., Elberling, H. & Jordano, P. (2008) Temporal dynamics in a pollination network. *Ecology,* **89,** 1573-1582.

Olesen, J. M. & Jordano, P. (2002) Geographic patterns in plant-pollinator mutualistic networks. *Ecology,* **83,** 2416-2424.

Osborne, J. L., Clark, S. J., Morris, R. J., Williams, I. H., Riley, J. R., Smith, A. D., Reynolds, D. R. & Edwards, A. S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology,* **36,** 519-533.

Petanidou, T., Kallimanis, A. S., Tzanopoulos, J., Sgardelis, S. P. & Pantis, J. D. (2008) Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters,* **11,** 564-575.

Rader, R., Howlett, B. G., Cunningham, S. A., Westcott, D. A., Newstrom-Lloyd, L. E., Walker, M. K., Teulon, D. A. J. & Edwards, W. (2009) Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology,* **46,** 1080-1087.

Schuett, E. M. & Vamosi, J. C. (2010) Phylogenetic community context influences pollen delivery to *Allium cernuum*. *Evolutionary Biology,* **37,** 19-28.

Sowig, P. (1989) Effects of flowering plants patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera, Apidae). *Oecologia,* **78,** 550-558.

ter Braak, C. J. F. & Šmilauer, P. (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca.

Tolasz, R. (2007) Atlas podnebí Česka [Climatic atlas of the Czech Republic]. pp. 255. Český hydrometeorologický ústav, Praha.

Vázquez, D. P., Blüthgen, N., Cagnolo, L. & Chacoff, N. P. (2009) Uniting pattern and process in plant-animal mutualistic networks: a review. *Annals of Botany,* **103,** 1445-1457.

Waser, N. M. (1986) Flower constancy - Definition, cause, and measurement. *American Naturalist,* **127,** 593-603.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalization in pollination systems, and why it matters. *Ecology,* **77,** 1043-1060.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2006) Foraging trip duration of bumblebees in relation to landscape-wide resource availability. *Ecological Entomology,* **31,** 389-394.

Williams, N. M. & Kremen, C. (2007) Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological Applications,* **17,** 910-921.

4 Paper III

The interaction of herbivory and plant's environment results in complex changes in plant's life-cycle, not only in changes in population growth rates

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4.1 Abstract

Herbivores affect plant population dynamics by influencing one or more plant vital rates. In a similar way, plant's surrounding environment can affect plant population dynamics as well as occurrence and behaviour of herbivores. This opens a multitude of only little-explored possibilities for interactions of herbivores and plant's surrounding environment.

Here, we focus on interactions of five herbivore groups and of surrounding vegetation (taken as a proxy of local biotic and abiotic conditions) in their effects on population dynamics of a perennial herb *Succisa pratensis* by means of an individual-based demographic study. We analyse a set of integral projection models focusing on effects of vegetation × herbivory interaction both on population growth rate λ and elasticity of vital rates.

The main effects of vegetation and herbivory and their interaction were of the same order of magnitude and all capable to influence *S. pratensis* populations considerably. Main effect of herbivores induced systematic changes in elasticities corresponding to vital rates, decreasing the importance of reproduction and growth at the expense of stasis. This pattern was partly masked by vegetation × herbivory interaction, which varied the importance of individual size categories and the extent of growth suppression at the expense of stasis.

Important vegetation × herbivory interactions emphasize the necessity to study effects of herbivory always in the context of plant's environment. Moreover, the non-trivial changes in elasticities indicate the possibility of existence of higher order interactions of herbivory and vegetation with other components of plant's environment.

Keywords:
Succisa pratensis, *Euphydryas aurinia*, plant-herbivore interaction, roe deer, predispersal seed predation, plant-environment interaction, integral projection model, elasticity, population dynamics

4.2 Introduction

Herbivores can influence population dynamics at different stages of plant life-cycle. Some herbivore functional groups, such as predispersal seed predators, impact only one plant vital function without having influence on the others (Louda and Potvin, 1995, von Euler et al., 2014, Miller et al., 2009), whereas other groups, such as folivores, have more complex effects on individual's life-cycle (García and Ehrlén, 2002, Ehrlén, 2002, Palmisano and Fox, 1997). However, all herbivore groups have in common that their direct effects on plants (with exception of some monocarps, Rautio et al., 2005, Hendrix, 1979) are negative due to loss of plant tissues and/or propagules. Overall herbivore effect on a particular vital rate consists of two components – frequency of occurrence of a given type of herbivory and its per-interaction effect. When considering the impact on the whole life cycle or population dynamics and not only the target vital rate a third component emerges: sensitivity of life-cycle or population characteristics to changes in the vital rates impacted by herbivores. The sensitivities of vital rate in plant populations vary considerably among species (e.g. Silvertown et al., 1992), but also among populations of a particular species (Oostermeijer et al., 1996).

All components of herbivore effect on plant life cycle and populations can be also affected by interaction with plant's surrounding environment. The effects of plant's environment on herbivore occurrence are relatively well-documented for abiotic gradients (e.g. Baraza et al., 2006, Förare and Engqvist, 1996, Miller et al., 2009), but evidence from less-structured ecosystems such as grasslands and less steep gradients is also accumulating (Loaiza et al., 2011; Paper I). With respect to herbivore groups, the foliar feeding invertebrates seem to be affected more by plant's local environment (Loaiza et al., 2011; Paper I) than large vertebrates (Hegland et al., 2010) and predispersal seed predators (Rose et al., 2011, von Euler et al., 2014).

Changes of herbivore per-interaction effect due to plant's environment may be either mediated by changed extent of damage or they may be a result of changed plant's ability to cope with the damage caused. The changes in damage extent may be either due to a systematic change in plant size with environmental conditions (a common case in stalk grazers; Gómez, 2005, Ehrlén and Münzbergová, 2009) or due to systematic increase in herbivore damage with plant size (e.g. Vanhoenacker et al., 2009). The changes to plant's ability to cope with herbivory due to its environment may both amplify the effect of herbivory (Bonser and Reader, 1995, Knochel and Seastedt, 2010, Eckberg et al., 2014) or they may counteract it (von Euler et al., 2014).

On the other hand, the interactions of plant's environment with sensitivity of population characteristics to vital rates affected by herbivores have not been much explored so far. Up to our knowledge, this was studied only in two systems, both focusing a perennial herb and predispersal seed predators on a relatively steep abiotic gradient (Kolb *et al.*, 2007, von Euler *et al.*, 2014). It remains a matter of question how strong the effect of environment on sensitivity is in other herbivore groups and less steep gradients.

The discussed components of effects of herbivory affect both plant individuals and their populations. Unfortunately, it is not feasible to measure the impact on lifetime fitness in iteroparous perennials. However, this problem can be circumvented by the use of population characteristics (e.g. Maron and Crone, 2006, Ehrlén, 2002, Kolb *et al.*, 2007), which can be used under certain assumptions also for inferring individual lifetime fitness (e.g. Ehrlén, 2002). An important advantage of population-level approach is that the impacts of herbivores on different vital rates can be integrated into one biologically meaningful measure (deterministic population growth rate λ) and sensitivity of life cycle to individual vital rate changes can be directly measured and compared between each other (by means of elasticities; Caswell, 2001). On the other hand, population-level metrics do not allow to directly isolate the effects of herbivores, environment and their interactions, since the affected individual vital rates combine non-linearly into λ and elasticties. In present study, we attempt to partially bridge this gap by constructing integral projection models (hereafter IPM; Easterling et al., 2000) from the same dataset with different parameterisations. By comparing the obtained set of IPMs, we attempt to elucidate the main effects of both herbivory and plant's environment and their interaction, which we further divide into two components: i) effects of environment on herbivore occurrence; and ii) effects of environment both on herbivore per-interaction effect and sensitivities of affected vital rates.

Our main aim is to assess the effect of herbivores, surrounding vegetation (regarded as a proxy of plant's environment) and their interaction on population dynamics of *Succisa pratensis*, a declining long-lived wet meadow perennial. We were interested in joint effects of five aboveground herbivore functional groups on population growth rates (i.e. impacts on the whole life-cycle) and also in specific changes of contribution of different size categories and vital rates to population growth rate (i.e. elasticities). We examine their effects by means of a two-year demographic study on ca 2700 individuals across 27 populations and 8 vegetation types.

4.3 Materials and methods

4.3.1 Study system

Succisa pratensis Moench (*Dipsacaceae*) is a typical wet meadow iteroparous perennial with a ground leaf rosette and flowering stalks bearing dichasia of flowerheads. It occurs in a relatively wide range of grasslands varying in humidity, pH and nutrients (Adams, 1955). The species is long-lived (Hooftman et al., 2003) and forms only a transient seed bank (Jensen, 2004). Observed plant damage could be attributed to five herbivore functional groups (see Appendix 1): (i) generalist invertebrate folivores (caterpillars of Noctuidae moths, molluscs etc.; hereafter invertebrate folivores); (ii) a specialist invertebrate folivore (caterpillars of Europe-wide protected *Euphydryas aurinia*); (iii) generalist vertebrate folivores (mainly roe deer and cows; hereafter vertebrate folivores); (iv) vertebrate stalk grazers; (v) predispersal seed predators (larvae of micro-moths, presumably of Tortricidae family).

The studied populations are located in the Protected Landscape Area Slavkovský les (Karlovarský region, Czech Republic) in an upland region with altitude varying between 700 and 800 m a.s.l. and mild suboceanic climate. Species particular characteristics as well as the study area are described more in detail in (Paper I).

4.3.2 Study design

We chose 27 populations of *S. pratensis* covering the whole range of vegetation types it occurs in the study area in as best as possible. At each site, a transect through the population was delimited and at least 80 found non-seedling individuals (and all seedlings found) were individually tagged and followed through 2010 and 2011. For each individual, we recorded its position, survival, size-related traits, reproduction-related traits and damage caused by herbivores (Table 1) during the flowering time (late August to early September). Additionally, terminal flower head was collected from all tagged flowering plants and examined for seed production and seed predation in 2011. The transects were divided along the main transect axis into 4 subpopulations of approximately same area and one randomly placed 1×1 m vegetation sample was recorded within each subpopulation and classified by Twinspan method into one of eight vegetation types (Paper I). The study design is described in detail in (Paper I). In this article, we focus on reporting the effects of herbivores on plant population dynamics.

4.3.3 Seedling establishment data

Seedling establishment data were collected in a separate experiment (Z. Janovský, unpublished data), which was conducted on a partially overlapping set of sites from present study (Appendix 2) from 2008 to 2009. Four to ten pairs of 30 × 30 cm plots were established at each site (137 pairs in total). In each pair, one plot served as natural control and the other received additional 100 *S. pratensis* seeds in September 2008. In August 2009 all plots were searched for seedlings and seedlings were measured according to the same protocol as other individuals. Plots were classified into vegetation types based on the results of classification of the 1×1 m vegetation samples (see above). Rates of seedling establishment were analysed by means of generalised least square regression of log('seed addition plot' – 'control plot') variable with power function for variance and vegetation type as the only predictor.

Since we lacked direct data for seedling establishment in 2011 and observed *S. pratensis* germination rates reached its maximum in 2009 (Z. Janovský, unpublished data), we wanted to standardise 2009 establishment rates to average germination rates in the study area. For this purpose, we utilised 4-year data (2006–2009) on seedling establishment from a subset of four sites (Appendix 2) and calculated a ratio of mean seedling establishment at these 4 sites in 2009 to overall mean. Consequently, we divided all results from 2009 by the obtained coefficient 1.512 before entering into integral projection model.

Table 1: Measures of plant characteristics and herbivory magnitude; variables in bold were used as variables in vital rate regressions (Table 3)

Variable	Measurement
width	width of the largest leaf
length	length of the largest leaf
leaves	# leaves
size	$\sqrt{leaves \times \pi \times \left(\frac{length}{2}\right) \times \left(\frac{width}{2}\right)}$
flowering	**vegetative/flowering (0/1)**
# stalks*	# flowering stalks produced
flower heads*	**# flower heads produced**
relative invertebrate folivory	semiquantitative assesment of % foliage lost (0-5%, 5-15%, 15-25%, 25-50%, 50-100%)
invertebrates	**relative invertebrate folivory × vegetative size**
vertebrate folivory	**present/absent**
E. aurinia	**present/absent**
stalk grazing*	**# stalks grazed/# stalks**
florets†*	**# florets in terminal flower head (i.e. maximum number of seeds, which can be produced)**
floret correction coefficient¶*	**ratio of number of florets in terminal flower head to mean number of florets of an individual**
seed production†*	**# well-developed (non-aborted non-predated) seeds in terminal flowerhead/florets**
seed predation†*	**# predated seeds in terminal flowerhead**
seedling establishment‡	**number of seedlings surviving first summer per 1 seed**
seedling size‡	**size (see above) of seedlings**

* Variable is defined only for flowering individuals.
† Variable was measured only in 2011 see text for details.
¶ Variable was measured in calibration dataset assembled from (Paper IV).
‡ Variable was measured in seedling establishment experiment.

4.3.4 IPM modelling strategy

We partitioned out the components corresponding to effects of herbivory, surrounding vegetation and their interaction by means of two sets vital rate regressions and three possible parameterisations (Table 2; for regression details see Appendix 3). We constructed IPMs both including effects of vegetation types (for each vegetation type one IPM, i.e. 8 IPMs, hereafter vegetation-yes IPM) and not-including them (1 IPM for all vegetation types, hereafter vegetation-no IPM). These two sets of IPM equation coefficients were then parameterised with three herbivory parameterisations: i) a null-herbivory parameterisation (hereafter no-herbivory), effectively constituting either a null model in case of vegetation-no IPM or a model containing only main effect of vegetation in case of vegetation-yes IPM; ii) a parameterisation with herbivory-plant size relationship independent of vegetation types (hereafter general herbivory) corresponding to main effect of herbivory in case of vegetation-no IPM and interaction vegetation × herbivory in case of vegetation-yes IPM (only components corresponding to per-interaction effects and differences in sensitivity to changes in vital rates); iii) a parameterisation with vegetation-type-specific herbivory-plant size relationship (hereafter vegetation-specific herbivory), which was done only for vegetation-yes IPM and corresponds to components of interaction vegetation × herbivory influencing the interaction frequency (i.e. herbivore occurrence). The difference between the general herbivory and vegetation-specific herbivory parameterisations consisted only of vegetation specific invertebrate folivore occurrence, since other herbivore groups did not show any differences in occurrence with respect to vegetation types (Paper I; for details of herbivory-plant size relationship construction see Appendix 4).

Table 2: Examined combinations of IPM formulation (vegetation-yes/vegetation-no) and herbivore parameterisations

Herbivory parameterisation	**Vegetation types**	
	not included into IPM	**included into IPM**
no herbivory	"null model" only effects of plant characteristics	"main effect" of vegetation type, no effect of herbivory
general herbivory; herbivore-plant size relationship independent of vegetation type	"main effect" of herbivory, no effect of veg. type	"main effects" + interaction herbivory × veg. type (sensitivity and per-interaction effect components)
vegetation-specific herbivory; vegetation-specific herbivory-plant size relationship	---	both "main effects" and interaction herbivory×veg. type (all components)

4.3.5 Vital rate regressions

We utilised logistic regression for determining survival and flowering probability, OLS regression for flower heads and florets, generalised least square regression with power function for variance for growth and for seedling size and generalised linear model of quasibinomial family for seed production (Appendix 3). Only significant factors explaining more than 0.5% of variability (R^2 for OLS regressions and McFadden's pseudo-R^2 for logistic regression) were included into the final equations for IPM (Table 3; for coefficients of all IPMs see Appendix 5). We found in preliminary analysis that size in time (t) is approximately four times better predictor of flowering probability in time (t+1) than size(t+1), therefore we used size(t) in this vital rate regression. Since we sampled in present study only terminal flower heads, which consist of more florets than laterals (Adams, 1955), we had to recalculate the predicted number of florets from terminal to average flower head. This we contrived by introducing a floret correction coefficient obtained from a different dataset, where florets were counted on all flower heads (Paper IV). We modelled the coefficient by means of OLS regression assuming a hyperbolic relationship between the ratio and no. of flower heads (for details on regression and dataset see Appendix 6).

4.3.6 IPM assembly and analysis

The integral projection model contained only the size-structured stages, since no seed bank is being formed and seedlings were already of similar size as small vegetative individuals at least at some sites (data not shown). Thus the whole IPM kernel consisted of growth matrix G describing the transitions from size(t) to size(t+1), survival vector S and fecundity matrix F. The process, terminology and coding of IPM assembly basically followed Appendices of Merow et al. (2014), but we had to accommodate them for the use of size(t) as size-based predictor of flowering in time t+1 (see above). This was achieved by matrix multiplication of the vector of flowering probabilities predicted with size(t) by G. We limited predictions of seed production above 95% quantile of size(t+1) to the value predicted at this quantile, since above this threshold the vital rate regressions provided a poor fit due to the lack of data. The plant size limits over which the IPM kernels were integrated were set to 1 (corresponding to the smallest measured seedlings) and to maximum observed plant size in a given vegetation type multiplied by 1.1. The IPM kernel was discretised into 100 evenly spaced size categories and possible evictions (for discussion of the problem see Williams et al., 2012) were treated by standardising the columns of *G* matrix and probability densities of seedling size distribution (before multiplication by fecundity vector) to 1 prior to any other use.

We computed deterministic population growth rates (hereafter λ) and matrix elasticities for all constructed IPMs. We express the effects of herbivores, vegetation type and their interactions in terms of Δλ and Δelasticity matrices, which we constructed by subtracting the elasticity matrix (discretised IPM kernel) of vegetation-no herbivory-no IPM from elasticity matrix of general-herbivory vegetation-no IPM and by subtracting the elasticity matrices of vegetation-yes no-herbivory IPMs from elasticity matrices of vegetation-yes vegetation-specific IPMs. While Δλ describes the net effect of a given factor on *S. pratensis* life cycle, Δelasticity matrices indicate changes in elasticity (i.e. standardised sensitivity) of vital rates due to herbivory. All computations were undertaken in R 3.0.1 statistical environment (available at www.r-project.org) using besides standard installation package popbio 2.4 (Stubben and Milligan, 2007) for calculation of elasticities.

4.4 Results

The general structure of all constructed IPMs was relatively similar with hump-shaped relationship between size(t) and size(t+1) and relatively size independent (above certain threshold) reproduction (Appendix 7). High mortality at one of the four populations belonging to vegetation type 5 (population WE2, the likely cause was wild boar disturbance) was the main cause of unintuitive effects of herbivory on λ, since this was the only population within the vegetation type with low herbivory (data not shown). This non-causal correlation with plant mortality partly (in terms of λ) obscured direct effects herbivory. We decided not to leave out the population from the model, since the effects of herbivores on elasticities are not obscured by the absolute value of λ. However when computing mean Δλ, we leave out vegetation type 5 in all applicable cases.

We found large differences in λ among vegetation types, when compared to vegetation-no no-herbivory model mean |Δλ| = 0.113, (Fig. 1). The effects of herbivory on λ were a bit weaker than effects of vegetation types (mean |Δλ| = 0.073) and were consistently negative (with the exception of vegetation type 5). The per-interaction effect and sensitivity to vital rates components of vegetation × herbivory interaction generated only half as large differences in λ (mean |Δλ| = 0.047) than the main effects of herbivory and vegetation types. The difference between general-herbivory and vegetation-specific herbivory parameterisations, i.e. the herbivore occurrence component of vegetation × herbivory interaction, was only negligible (mean |Δλ| = 0.0092).

Herbivory in terms of elasticities consistently increased the importance of stasis (i.e. survival and shrinkage) on the expense of growth and to lesser degree (in terms of absolute change) also of reproduction (Fig. 2; Appendix 9, Fig. S15). However, the magnitude of change differed considerably among vegetation types. Vegetation-type-specific changes in elasticities under herbivory could be observed also when comparing the size classes (Fig. 3). Generally two types of changes of contribution of individual size classes to λ could be observed: i) increased contribution of medium and large plants (veg. types 1,5,7,8); ii) increased contribution of small and medium plants (veg. types 2,3,4,6). The second pattern was also the one imposed by herbivores, when the effects of surrounding vegetation were not taken into account (Appendix 9, Figs S16 and S17). Importance of seedlings decreased when accounting for herbivory under both vegetation-no and vegetation-yes models (in all vegetation types). In some vegetation types (1 and 8), the changes in elasticities were mainly driven by decrease in seedling and small individuals, which was approximately evenly distributed in increase in all other transitions (Fig. 4). On the contrary, the same decrease was compensated by a more or less targeted increase in other regions of the kernel in the remaining vegetation types.

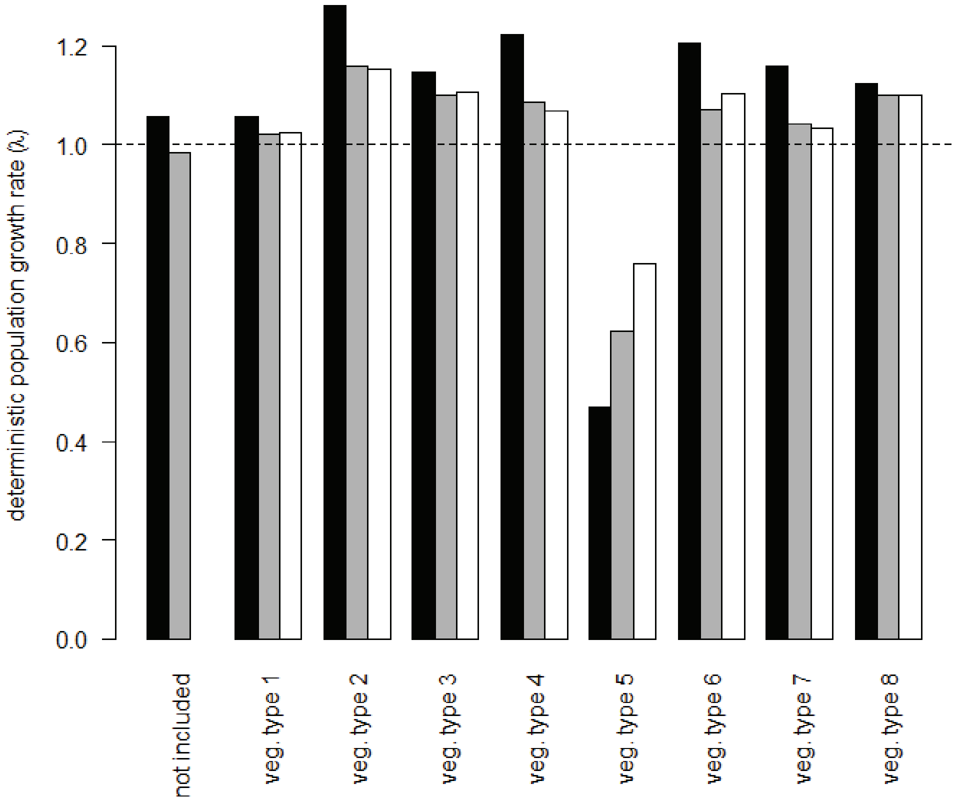

Fig. 1: Deterministic population growth rates derived from vegetation-no IPMs (not-included category) and vegetation-yes IPMs; black bars – no-herbivory parameterisation, grey – general-herbivory parameterisation, white – vegetation-specific herbivory

4.5 Discussion

We found that both herbivory and plant's environment (as expressed by vegetation type) had important effects on population growth rates as well as their interaction. The effects of vegetation × herbivory interaction were mainly attributable to changes of per-interaction effect of herbivory and changes in sensitivity of λ to vital rates affected by herbivory, whereas the contribution of changes in interaction frequency due to vegetation was only negligible. This was mainly due to the fact that only one considered herbivore group (generalist invertebrate folivores) contributed to effect of interaction frequency (Paper I). Herbivory generally increased importance of stasis on the expense of growth and reproduction regardless of vegetation types. However its effects on elasticities of different size categories were strongly dependent on vegetation type both in terms of direction and intensity. Thus despite some general effects of herbivory, our results indicate the necessity of evaluating the effects of herbivory across the whole range of environmental conditions a plant species occurs in.

Table 3: Summary of regression equations used for IPM construction; please note that parameterisation corresponds to models not including vegetation types among predictors (see Appendix 5for all coefficients)

Vital rate	Response variable	Equation	Significant terms containing vegetation types	Not-included significant terms
Mortality	logit(mortality(t→t+1))	0.43 + 0.14*size(t) - 0.0023*size(t)^2 - 0.028*invertebrates(t)	veg. type, size(t):veg. type, invertebrates:veg. type	flowering(t):*E. aurinia*
Growth	sqrt(size(t+1))	mean = 1.96 + 0.16*size(t) - 0.0017*size(t)^2 - 0.0061*inv.(t) sd = 0.32*mean^0.56	veg. type, size(t):veg. type, invertebrates:veg. type	size(t):vertebrates,(t) size(t):*E. aurinia*(t)
Reproduction - seed production	logit(flowering(t+1))	-4.26 + 0.26*size(t) - 0.0024*size(t)^2 - 0.032*invertebrates(t)	veg. type	*E. aurinia*(t)
	log(flower head(t+1)+1)	1.11 - 1.49*stalk grazing(t+1) + 0.041*size(t+1) - 0.000086*size(t+1)^2	veg. type, stalk grazing:veg. type, size(t+1):veg. type, size(t+1)^2:veg. type	-
	florets(t+1)	24.91 + 6.98*flower head + 3.46*size(t+1) - 0.035*size(t+1)^2 - 0.33*flower head*size(t+1) + 0.0041*flower head*size(t+1)^2	veg. type	-
	floret correction coefficient	0.51+0.49*(1/sqrt(flower head(t+1)))	not tested	not applicable
	logit(seed production(t+1))	-0.2 - 2.43*seed predation + 0.0052*florets	-	-
Reproduction - seedling establishment	seedling establishment(t+1)	0.0053	veg. type	-
	seedling size(t+1)	mean = 3.06 sd = 1.24; the formula for individual vegetation types: sd = 0.27*mean^1.29	veg. type	-

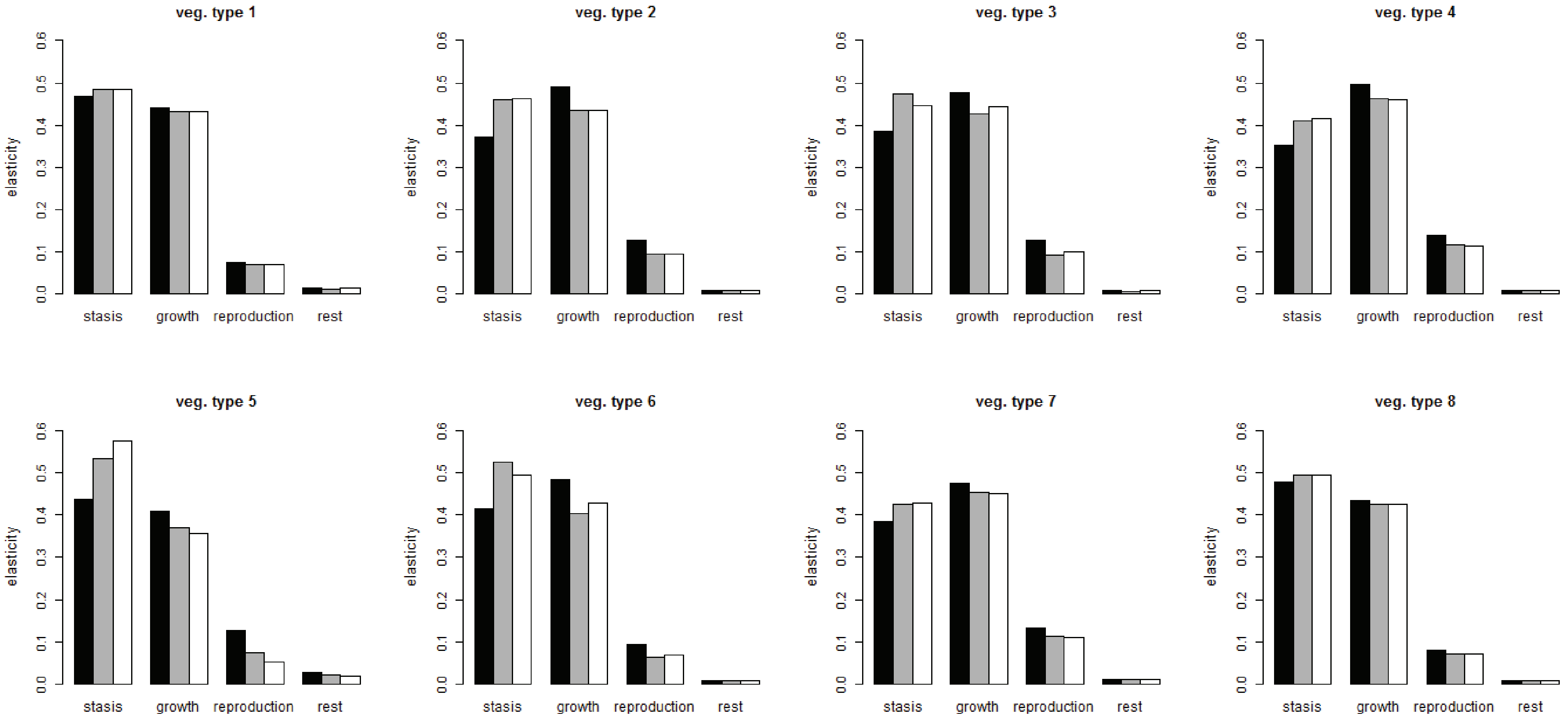

Fig. 2: Comparison of elasticities of vital rates for vegetation-yes IPMs; black bars – no-herbivory parameterisation, grey – general-herbivory parameterisation, white – vegetation-specific herbivory; growth was defined as increase in size greater than 20%; reproduction as all transitions to sizes smaller than 98% quantile of Normal distribution of seedling sizes; rest denotes elasticities of transitions with value lower than 0.001 (i.e. less than 0.1% plants entering from a given size the given new size)

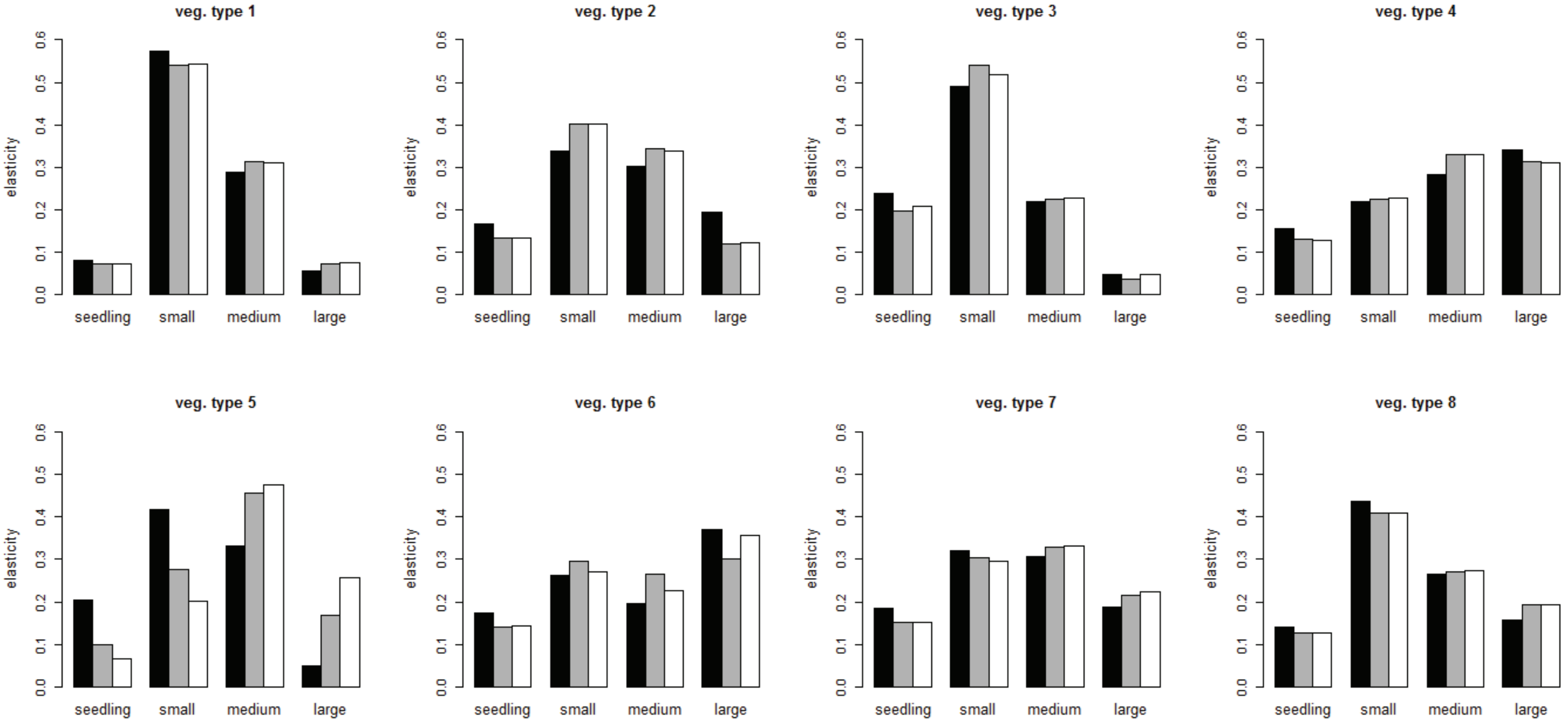

Fig. 3: Comparison of elasticities of size categories (see Materials and Methods section for delimitation) for vegetation-yes IPMs; black bars – no-herbivory parameterisation, grey – general-herbivory parameterisation, white – vegetation-specific herbivory; The size classes are defined in terms of size(t) as follows: 1) seedlings (al individuals smaller than 98% quantile of seedling size, hereafter q98); 2) small individuals (q98 < size(t) < q98 + 10); 3) medium individuals (q98 + 10 < size(t) < q98 + 20); 4) large individuals (size(t) > q98 + 20). For the observed proportion of individuals within each size category, see Appendix 8.

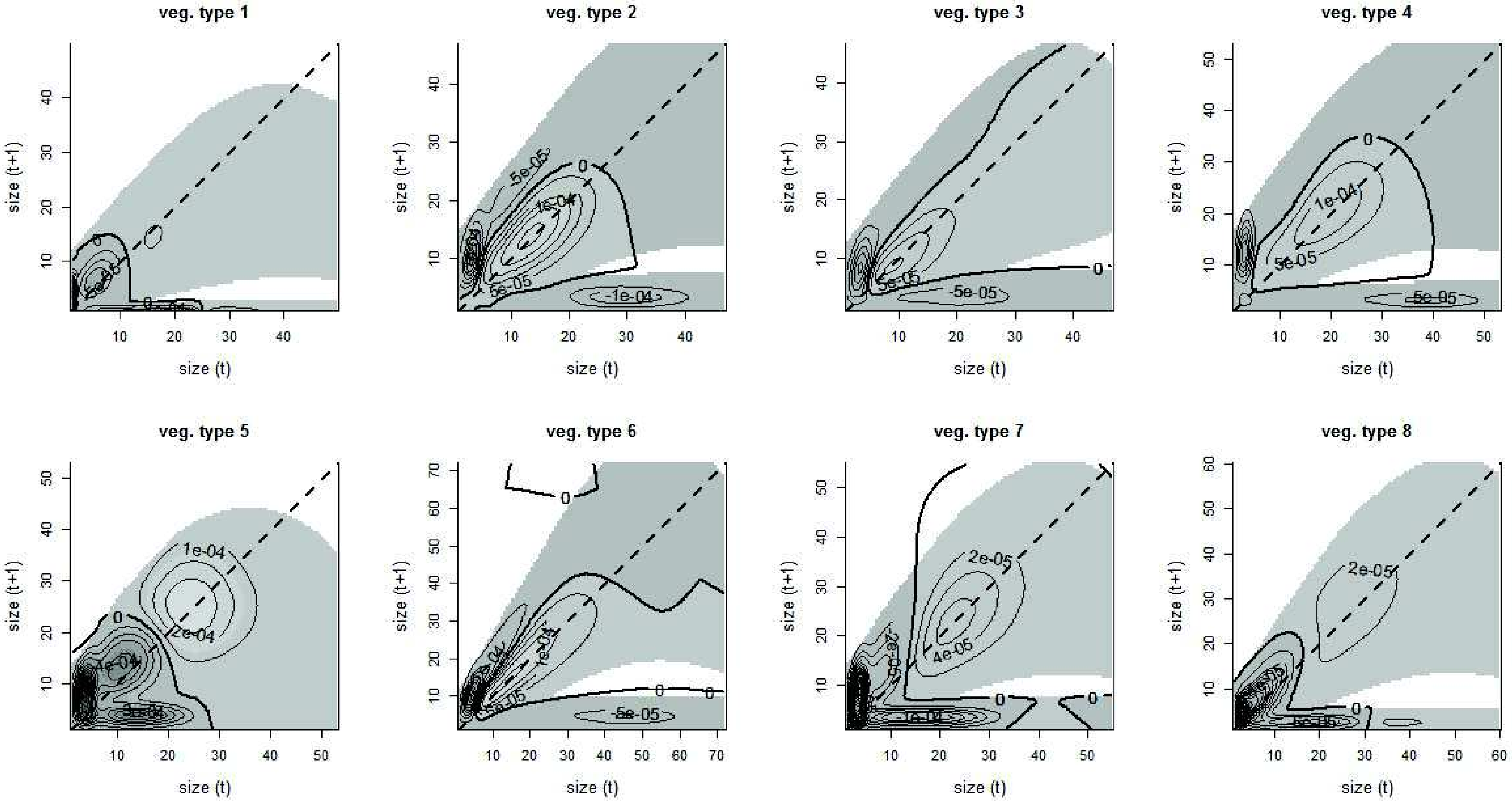

Fig. 4: Differences in elasticities between vegetation-yes vegetation-specific herbivory IPMs and vegetation-yes no-herbivory IPMs; positive differences indicate increase in importance of a given transition under the vegetation-specific herbivory; grey-scale denotes area with transition values greater than 0.001 (i.e. more than 0.1% plants from a given size enter this new size) or belonging to reproduction section of IPM

4.5.1 Is herbivory capable of influencing population sizes of long-lived perennials?

The detected reductions in λ due to herbivory around |Δλ| = 0.07 represent a fairly strong influence on populations, if we consider that λ of *S. pratensis* are rarely higher than 1.3 approximately half of the site × year combinations had λ between 0.9 and 1.1 (Jongejans and de Kroon, 2005, Mildén et al., 2006). Similar magnitudes of herbivore effects were found also in other long-lived perennials with low variation in λ (Ehrlén, 2003, Miller et al., 2009, von Euler et al., 2014). However, the relatively small differences can accrete over years to substantial differences in population sizes due to low year-to-year variation in overall λ (Kalisz et al., 2014), but only if herbivory exerts the dominant and consistent influence on the population (Kolb, 2012). It is also notable, that the observed differences in λ were comparable to the effect of plant's environment, suggesting that herbivores could shape *S. pratensis* niche to a similar degree, provided the observed differences in λ proved to be long-term (but see von Euler et al., 2014).

4.5.2 Relative importance of components of vegetation × herbivory interaction

The vegetation × herbivory interaction turned out to have only ca. one third weaker effect on λ than the main effect of herbivory. Such results as well as other similar studies (Hegland *et al.*, 2010, Kauffman and Maron, 2006, Kolb *et al.*, 2007) would suggest that this phenomenon should deserve more attention. In this light, it would be important to partition out the vegetation × herbivory interaction components corresponding to changes in per-interaction effect of herbivores and to sensitivity of life cycle to the particular vital rate affected by herbivores, since the two components are likely to exhibit different behaviour. While effects of plant's environment on per-interaction effect of herbivores are likely to depend on herbivore and plant species identity, the changes in sensitivity of life cycle to herbivore impacts may have both systematic and study-system-specific components.

Oostermeijer et al. (1996) described a systematic intraspecific decrease in elasticities of reproduction with decreasing λ (similar but not tested relationship was also reported by Kolb *et al.*, 2007). Such decrease can be expected in our data too, since herbivores affecting the reproduction turned out to be the most important ones (data not shown, but see vital rate regression details in Appendix 3) and *S. pratensis* possesses a similar life history as the species studied in above mentioned works. A systematic decrease in elasticity of reproduction with decreasing λ is thus likely to turn into smaller effect of herbivory (such relationship could be indeed observed when plotting Δλ against λ under no-herbivory, but cannot be tested due to n=7; data not shown).

On the other hand, Oostermeijer et al. (1996) documented increasing elasticity of survival with decreasing λ. Corresponding increase of impact of folivores on λ with decreasing overall value of λ could be observed in a similar study system (Hegland et al., 2010). The relatively low Δλ of the herbivore occurrence component of vegetation × herbivory interaction can well fit into the same scheme. This component is attributable solely to changes in occurrence of invertebrate folivores, which affect mainly growth and survival (Appendix 3) and thus their small effect can be expected, since 2011 was particularly a very good year for *S. pratensis* with high survival and growth (Z. Janovský, unpublished data). We expect the importance of folivores and thus herbivore occurrence component of vegetation × herbivory interaction to increase in less favourable years also due to the fact that there are large differences in folivore occurrence among vegetation types (Appendix 4). These differences are comparable to systems featuring strong abiotic gradient and strong herbivore effects on plant population dynamics (e.g. Kolb *et al.*, 2007).

4.5.3 Changes in distribution of elasticites due to herbivory

Changes in distribution of elasticities could be divided into the changes common to all vegetation types and those unique to a given vegetation type or their subset. Herbivory caused a common decrease in the importance of reproduction and seedling growth (Figs 2 and 4 and Appendix 9, Figs S15 and S17), which can be attributed mainly to stalk grazers and seed predators (see above). Another common feature was the general increase of elasticities attributable to stasis (i.e. survival and shrinkage), which can be linked with decreasing λ (Oostermeijer et al., 1996), but also with increasing environmental stress coupled with decrease in λ (Lehtilä et al., 2006).

Contrary to changes of elasticities according to vital rates, the changes in elasticities according to size categories did not show any particular pattern. The structure of changes in elasticities did not differ among vegetation types only in terms of size categories but also in terms of vital rates, i.e. if the increase in importance for determining λ encompassed both stasis and growth or only stasis (Fig. 4). We suggest that these irregular changes are mainly the result of vegetation × herbivory interaction, since including main effect of vegetation types into IPMs did not change the distribution of elasticities with respect to plant size (Appendix 8, Fig. S14). Vegetation-specific changes in elasticities due to herbivores provide also the means for higher order interactions of herbivory with other components of plant's environment not described by vegetation types.

From the nature conservation viewpoint, this also means that populations of *S. pratensis* would respond differently to conservation management due to herbivory. This is imposed on them, since *S. pratensis* is one of the key hosts of *Euphydryas aurinia*, a Europe-wide endangered butterfly. A common conservation management of *S. pratensis* populations is mowing, which impacts large individuals the most. Herbivores in some vegetation types (e.g. the most frequent vegetation types 7 and 8) cause shift of elasticties from small individuals to large ones, which would make the mown populations more vulnerable. This only further emphasizes the necessity of studying impacts of herbivory on plant populations always in a particular relevant environmental context.

4.6 Conclusions

We demonstrated that the main effect of herbivory can be of similar magnitude as the main effect of plant's environment (as captured by the surrounding vegetation type). Further, the vegetation × herbivory interaction type was of comparable magnitude as the main effects and of magnitude capable of long-term effects on *S. pratensis* population dynamics. The decomposition of vegetation × herbivory interaction into herbivore occurrence component on one hand and per-interaction effect and sensitivity to impacted vital rate on the other has indicated greater importance of the second component and evidence from other studies suggest that the important determinant might be the systematic relationships between plant's environment, elasticity of the life cycle to impacted vital rates and population growth rate λ. The vegetation × herbivory interaction also led to vegetation-type-specific changes in elasticities associated with individual life-cycle transitions. Such changes in elasticities set a basis for higher order interactions of vegetation, herbivory and other components of plant's environment.[*]

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4.7 References

Adams, A. W. (1955) *Succisa pratensis* Moench (*Scabiosa succisa* L.). *Journal of Ecology,* **43,** 709-718.

Baraza, E., Zamora, R. & Hodar, J. A. (2006) Conditional outcomes in plant-herbivore interactions: neighbours matter. *Oikos,* **113,** 148-156.

Bonser, S. P. & Reader, R. J. (1995) Plant competition and herbivory in relation to vegetation biomass. *Ecology,* **76,** 2176-2183.

Caswell, H. (2001) *Matrix population model: construction, analysis, and interpretation.* Sinauer Associates, Inc. Publishers, Sunderland, MA, USA.

Easterling, M. R., Ellner, S. P. & Dixon, P. M. (2000) Size-specific sensitivity: Applying a new structured population model. *Ecology,* **81,** 694-708.

Eckberg, J. O., Tenhumberg, B. & Louda, S. M. (2014) Native insect herbivory limits population growth rate of a non-native thistle. *Oecologia,* **175,** 129-138.

Ehrlén, J. (2002) Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). *Perspectives in Plant Ecology, Evolution and Systematics,* **5,** 145-163.

Ehrlén, J. (2003) Fitness components versus total demographic effects: Evaluating herbivore impacts on a perennial herb. *American Naturalist,* **162,** 796-810.

Ehrlén, J. & Münzbergová, Z. (2009) Timing of flowering: Opposed selection on different fitness components and trait covariation. *American Naturalist,* **173,** 819-830.

Förare, J. & Engqvist, L. (1996) Suboptimal patch and plant choice by an ovipositing monophagous moth - An insurance against bad weather? *Oikos,* **77,** 301-308.

García, M. B. & Ehrlén, J. (2002) Reproductive effort and herbivory timing in a perennial herb: Fitness components at the individual and population levels. *American Journal of Botany,* **89,** 1295-1302.

Gómez, J. M. (2005) Long-term effects of ungulates on performance, abundance, and spatial distribution of two montane herbs. *Ecological Monographs,* **75,** 231-258.

Hegland, S. J., Jongejans, E. & Rydgren, K. (2010) Investigating the interaction between ungulate grazing and resource effects on Vaccinium myrtillus populations with integral projection models. *Oecologia,* **163,** 695-706.

Hendrix, S. D. (1979) Compensatory reproduction in a biennial herb following insect defloration. *Oecologia,* **42,** 107-118.

Hooftman, D. A. P., van Kleunen, M. & Diemer, M. (2003) Effects of habitat fragmentation on the fitness of two common wetland species, *Carex davalliana* and *Succisa pratensis*. *Oecologia,* **134,** 350-359.

Jensen, K. (2004) Dormancy patterns, germination ecology, and seed-bank types of twenty temperate fen grassland species. *Wetlands,* **24,** 152-166.

Jongejans, E. & de Kroon, H. (2005) Space versus time variation in the population dynamics of three co-occurring perennial herbs. *Journal of Ecology,* **93,** 681-692.

Kalisz, S., Spigler, R. B. & Horvitz, C. C. (2014) In a long-term experimental demography study, excluding ungulates reversed invader's explosive population growth rate and restored natives. *Proceedings of the National Academy of Sciences of the United States of America,* **111,** 4501-4506.

Kauffman, M. J. & Maron, J. L. (2006) Consumers limit the abundance and dynamics of a perennial shrub with a seed bank. *American Naturalist,* **168,** 454-470.

Knochel, D. G. & Seastedt, T. R. (2010) Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. *Ecological Applications,* **20,** 1903-1912.

Kolb, A. (2012) Differential effects of herbivory and pathogen infestation on plant population dynamics. *Plant Ecology,* **213,** 315-326.

Kolb, A., Leimu, R. & Ehrlén, J. (2007) Environmental context influences the outcome of a plant-seed predator interaction. *Oikos,* **116,** 864-872.

Lehtilä, K., Syrjänen, K., Leimu, R., García, M. B. & Ehrlén, J. (2006) Habitat change and demography of *Primula veris*: Identification of management targets. *Conservation Biology,* **20,** 833-843.

Loaiza, V., Jonas, J. L. & Joern, A. (2011) Grasshoppers (Orthoptera: Acrididae) select vegetation patches in local-scale responses to foliar nitrogen but not phosphorus in native grassland. *Insect Science,* **18,** 533-540.

Louda, S. M. & Potvin, M. A. (1995) Effect of inflorescence feeding insects on the demogrpahy and lifetime fitness of a native plant. *Ecology,* **76,** 229-245.

Maron, J. L. & Crone, E. (2006) Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 2575-2584.

Merow, C., Dahlgren, J. P., Metcalf, C. J. E., Childs, D. Z., Evans, M. E. K., Jongejans, E., Record, S., Rees, M., Salguero-Gómez, R. & McMahon, S. M. (2014) Advancing population ecology with integral projection models: a practical guide. *Methods in Ecology and Evolution,* **5,** 99-110.

Mildén, M., Münzbergová, Z., Herben, T. & Ehrlén, J. (2006) Metapopulation dynamics of a perennial plant, *Succisa pratensis*, in an agricultural landscape. *Ecological Modelling,* **199,** 464-475.

Miller, T. E. X., Louda, S. M., Rose, K. A. & Eckberg, J. O. (2009) Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs,* **79,** 155-172.

Oostermeijer, J. G. B., Brugman, M. L., DeBoer, E. R. & DenNijs, H. C. M. (1996) Temporal and spatial variation in the demography of Gentiana pneumonanthe, a rare perennial herb. *Journal of Ecology,* **84,** 153-166.

Palmisano, S. & Fox, L. R. (1997) Effects of mammal and insect herbivory on population dynamics of a native Californian thistle, *Cirsium occidentale*. *Oecologia,* **111,** 413-421.

Rautio, P., Huhta, A. P., Piippo, S., Tuomi, J., Juenger, T., Saari, M. & Aspi, J. (2005) Overcompensation and adaptive plasticity of apical dominance in Erysimum strictum (Brassicaceae) in response to simulated browsing and resource availability. *Oikos,* **111,** 179-191.

Rose, K. E., Russell, F. L. & Louda, S. M. (2011) Integral projection model of insect herbivore effects on *Cirsium altissimum* populations along productivity gradients. *Ecosphere,* **2**.

Silvertown, J., Franco, M. & McConway, K. (1992) A demographic interpretation of Grime's triangle. *Functional Ecology,* **6,** 130-136.

Stubben, C. & Milligan, B. (2007) Estimating and analyzing demographic models using the popbio package in R. *Journal of Statistical Software,* **22,** 1-23.

Vanhoenacker, D., Ågren, J. & Ehrlén, J. (2009) Spatial variability in seed predation in *Primula farinosa*: local population legacy versus patch selection. *Oecologia,* **160,** 77-86.

von Euler, T., Ågren, J. & Ehrlén, J. (2014) Environmental context influences both the intensity of seed predation and plant demographic sensitivity to attack. *Ecology,* **95,** 495-504.

Williams, J. L., Miller, T. E. X. & Ellner, S. P. (2012) Avoiding unintentional eviction from integral projection models. *Ecology,* **93,** 2008-2014.

5 Paper IV

Opposing selective pressures of pollinators and seed predators cause plants to distribute their reproductive effort over time

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5.1 Abstract

Iteroparous plants face the problem of how to distribute their reproductive investment over time, i.e. how frequently to flower and with what size inflorescence. Plasticity in reproductive investment distribution may represent plant adaption enabling response to multiple selection pressures, such as from various biotic interactions. In the present article, we focus on the roles of pollinators and pre-dispersal seed predators in shaping long-term flowering strategies in the wet meadow perennial *Succisa pratensis*.

We performed an individual-based demographic study at two sites, monitoring plant individuals for five years. We recorded their seed production together with pollinator visitation and seed predation, while correcting for plant phenology and reproductive vestment. We classified observed flowering patterns into two strategies – investing in only one flowering event vs. flowering more than once during four years.

After accounting for reproductive investment, the influence of seed predators was approximately 2.5 times that of pollinators. Seed predators, unlike pollinators, occurred disproportionately more on larger plants. Reproductive success was affected by plant phenology only in interaction with pollinators. After accounting for total reproductive investment, plants that flowered more frequently had 0-25% higher four-year seed production. Their advantage increased with higher absolute values of reproductive investment. Plants flowering more frequently were favoured mainly by disproportionately lower levels of seed predation.

Pollinators and seed predators exerted opposing selection pressures on plants, resulting in non-linear selection for inflorescence size. This translated into differential long-term success of the two flowering strategies, which could not be detected by a one- or two-year-long study. Our results thus emphasize the necessity of including the temporal dimension into studies of impacts of biotic interactions on iteroparous plants.

Keywords

reproductive ecology, flowering strategy, lifetime fitness, Succisa pratensis*, Syrphidae, iteroparous perennial, predispersal seed predation.*

5.2 Introduction

Individuals of most perennial plant species delay their reproduction until they attain a certain threshold size (e.g. Mbeau-Ache and Franco, 2013, Jacquemyn et al., 2010, Mildén et al., 2006). Such behaviour is thought to maximise plant fitness in conditions of varying mortality and reproductive success (Koons et al., 2008), provided both mortality and reproductive success vary predictably with a plant's environment and age/size (e.g. Sletvold, 2002, Petanidou et al., 1995, Mildén et al., 2006, Wesselingh et al., 1997). Similar factors presumably influence plants' temporal allocation of reproductive effort, creating a continuum of flowering strategies ranging from flowering virtually every year after reaching threshold size to flowering only infrequently, with large inflorescences.

An extreme case of delayed and concentrated reproductive effort, i.e. semelparity, has been shown to be advantageous if there is a non-linear relationship between plant size and fitness or there are high costs of reproduction, e.g. high mortality from specialist herbivores (Sletvold, 2002, Williams, 2009). When examining iteroparous species per se, we can find similar evidence from *Orchis purpurea*, where variation in costs of reproduction results in an intraspecific continuum of reproductive effort concentration (Miller et al., 2012). Besides this example, the role of environmental variation in shaping iteroparous plant flowering strategies has not been systematically explored.

Plant reproductive success (and mortality) can be influenced by many environmental factors, such as climate (Ågren et al., 2008) and local abiotic conditions (Shefferson and Roach, 2010), as well as biotic interactions (Kolb and Ehrlén, 2010). While the possibilities for a plant to influence the first two groups of factors are rather limited, the structure of a plant's biotic interactions often depends on size parameters, and thus its growth and flowering strategy (Cariveau et al., 2004, Sletvold and Grindeland, 2008, Kolb and Ehrlén, 2010, Thomson, 1988, but see also Brody and Mitchell, 1997). Since changes in intensity of biotic interactions are often non-linear with respect to plant size, biotic interactions often exert selection pressures on plant size (e.g. Kolb and Ehrlén, 2010, Sletvold et al., 2010). These selection pressures can be antagonistic, as has frequently been shown in studies of the effects of pollinators and seed predators (Ågren et al., 2013, Asikainen and Mutikainen, 2005, Herrera et al., 2002, Herrera, 2000), although this scenario is also common in other settings (Parachnowitsch and Caruso, 2008, Brody and Mitchell, 1997). Nevertheless, there still remains the question of how such selection pressures translate into iteroparous perennial lifetime fitness, since most studies on biotic interactions observe the given plant individuals for only a year (but see Ehrlén, 2000).

Most variation in seed predation or pollination occurs among individuals within a population (Ågren *et al.*, 2008, KolbEhrlén *et al.*, 2007, Leimu *et al.*, 2002), which to a large extent precludes the use population-scale fitness metrics such as the population intrinsic growth rate, λ, a measure typically employed when studying lifetime fitness in iteroparous perennials (e.g. Kolb and Ehrlén, 2010, Ehrlén, 2002). Thus, a study of fitness of the same individuals for multiple years (in an ideal case over their whole lifespans) is necessary to assess the results of selection pressures from both pollinators and seed predators. Moreover, comparing the fitness of individuals within the same population is biologically more meaningful, since they compete with each other, and differences in selection pressures can translate directly into future population composition (Ågren et al., 2013).

The ultimate aim of our study is to determine which flowering strategy is the most successful under given conditions and with equal reproductive investment. More specifically, we investigate whether it is better for plants to flower less frequently but with larger inflorescences or, alternatively, to flower more frequently with smaller inflorescences. We consider this in light of the size-dependent selection exerted on the plants by their pollinators and seed predators. To address these issues, we conducted, at two sites, a five-year observational demographic study of *Succisa pratensis*, a long-lived iteroparous meadow perennial.

5.3 Materials and methods

5.3.1 Study species

Succisa pratensis Moench (*Dipsacaceae*) is a typical wet meadow iteroparous perennial, with its leaves arranged in a rosette at ground-level. It occurs from northern Spain to central Siberia in nutrient-poor habitats varying in pH and humidity (Adams, 1955, Meusel and Jäger, 1992). In the study area, this species flowers from mid-August until the second hay cut at the beginning of September. At our sites, the leaf rosettes form 1 to 4 stalks bearing 1 to 18 flower heads (median 3; inter-quartile range 2-6) each consisting of 14 to 105 florets (53.5 ± 16.1; mean ± SD). Each floret can produce a maximum of one achene, containing one seed.

S. pratensis is mainly (ca. 90%) pollinated by large eristaline hoverflies. Other pollinator groups are rather marginal except for flies (Muscidae), which form ca. 6% of all visitors (Janovský et al., 2013). Flower heads with developing seeds are attacked by several predispersal seed predators, with the caterpillars of tortricid moths being the most common (Říhová, D. unpublished data).

5.3.2 Study sites

Our study was conducted in meadows K Handrkovu (N 49.84662, E 15.14987, WGS 1984; hereafter site K) and Na nové kopanině (N 49.84231, E 15.15446; hereafter site N) near the village Vernýřov, Central Bohemia, Czech Republic. The local climate is moderately sub-oceanic (annual mean temperature around 8°C and annual precipitation around 650 mm; Tolasz, 2007). The sites are approximately 600 metres apart and are both situated in nutritionally poor grassland vegetation. At both sites, *S. pratensis* forms large populations. Productivity at site N is a bit higher as indicated by higher vegetation (mean±SE [n]; site K: 12.7±0.7 cm [21], site N: 14.9±0.7 cm [15]) and higher reproductive investment of *S. pratensis* individuals (see Results).

5.3.3 Study design

We established 1×1 metre permanent plots at our study sites (7 plots at site K in 2008 and 5 plots at site N in 2009). The plots were selected in a stratified random fashion within the *S. pratensis* populations so that the whole range of flowering plant densities was covered. In each plot, we located and measured all *S. pratensis* individuals in five field seasons during the period 2008– 2012. The plants were found again each year in mid-August based upon coordinates relative to permanent marking, and all newly established plants were also followed. All inflorescences produced were harvested each year in the first week of September, approximately two days before the hay cut, in order to determine both seed production and seed predation.

5.3.4 Measured plant traits and derived variables

The measured plant traits used in this study consisted only of reproductive traits (stalk height, number of flower heads). The harvested inflorescences were dissected, and all achenes (or florets) were assigned to one of three categories: (1) florets, aborted or undeveloped seeds (soft when touched by tweezers); (2) well-developed seeds; and (3) seeds consumed by seed predators. We calculated plant investment in reproduction as the total of all florets produced (hereafter, reproductive investment). This measure also determined maximum fitness achievable by a given individual in a given year, since each floret may give rise to only one seed. The number of well-developed seeds served as the measure of plant fitness (for the analysis excluding the effect of seed predators, the number of predated seeds was added, see data analysis section). The number of seeds consumed corresponded to the intensity of seed predation (for testing seed predator preferences, this was standardised to 100 florets in order to control for trivial effect of inflorescence size). We also calculated mean flower head size (*# florets/# flower heads*), which we used as a predictor in analyses.

We also collected phenological data on flowering individuals for the purpose of calculating relative plant phenology as well as for standardisation of the pollinator visitation data. We estimated percentages, rounded to the nearest 5%, of budding, flowering and senescent florets on a daily basis during the period of pollinator observations. Since plants with more inflorescences tend to flower for a longer period, we defined as the key event for phenological comparison the point at which more than 50% of the total number of florets has opened. Plants were divided into three groups: (1) meeting this criterion of flowering + senescent florets > 50% before the observation period; (2) meeting it during the observation period; and (3) meeting it after the observation period. The plants were further ranked within the groups according to the sum of flowering and senescent florets on the first day (group 1) or the last day of the observation period (group 3) or by the date of reaching the criterion (group 2). For each field season, a total ranking comprising the plants from all three groups was then constructed. The resulting rankings for individual seasons were then standardised by z-transformation to make relative rankings comparable across the seasons.

5.3.5 Pollinator censuses

In each field season, we recorded pollinator visitation of flowering individuals within the plot on at least three days with weather favourable to insect activity between 15th and 26th August. Observations consisted of 10-minute blocks randomly assigned to individual permanent plots, with at least 10 blocks of observations assigned to each plot during the observation period. Frequencies and durations of pollinator visits to plant individuals were recorded. For the purpose of this study, we used only standardised total visit durations (detailed data on pollinators will be published elsewhere – Pavlíková et al., unpublished data). We standardised the duration of visits to 100 open florets (# *open florets = total number of florets* × *that day's percentage of open florets*) for each observation block and plant individual. For each plant individual, we then calculated the mean across all observations, and used this in the analyses.

5.3.6 Data analysis

We constructed two datasets from collected data. The first dataset consisted of plants that lived throughout the period 2009–2012 and flowered at least once during this period. This dataset ("longitudinal dataset") was aimed at answering the main question, that of identifying which flowering strategy most advantageous (in terms of seeds developed per florets produced). The second dataset ("one-year dataset") listed all individual flowering events separately by year (and included plants that died during the 2009–2012), provided that both pollinator visitation and seed predation were recorded within the given year. This dataset was used for examining the preferences of pollinators and seed predators for plant traits as well as pollinator and seed predator relative influences on one-year fitness

Seed predator and pollinator preferences were analysed in the one-year dataset using the following as predictors: plant reproductive investment (number of florets produced), stalk height, mean flower-head size, relative phenology, site (i.e. productivity) and all corresponding second-order interactions. For analyses of one-year fitness, we also included as predictors standardised pollinator visitation, seed predation intensity and all their second-order interactions. The analyses of long-term fitness, i.e. from the longitudinal dataset, contained as explanatory variables reproductive investment, flowering strategy (flowered only once/at least twice), site and their corresponding second-order interactions. In these analyses, we used as responses both long-term fitness (number of well-developed seeds produced in four years) and long-term fitness with the effect of seed predators filtered out (number of well-developed seeds + number of predated seeds produced in four years). All analyses undertaken also contained plot identity or plot × year as a factor to account for the correlation structure in the data. We applied linear regressions and checked assumptions of the models by inspecting diagnostic plots. Square-root transformation of the response variable (number of well-developed seeds) in plant fitness models was necessary in order to meet the assumption of homoscedasticity. Because of this transformation, we also applied square-root transformation to plant reproductive investment since there is no reason to expect a quadratic relationship between the number of produced florets and number of seeds produced (the linear relationship is the best null hypothesis, since one floret can produce one developed seed at most). Some of the response variables were transformed (pollinator visitation by square-root and seed predation by natural logarithm) in order to meet the assumption of homogeneity of variance.

5.4 Results

Over the entire 2008 to 2012 study period, we recorded a total of 321 flowering events by 197 individual plants. We included all 228 flowering events from the 146 individual plants that lived throughout 2009-2012 (out of which 62 flowered more than once) in the flowering-strategy dataset. In the one-year dataset, we included the 264 flowering events (from 197 individual plants) for which we had data on both pollinator visitation and seed predation. The total of 8013 individual pollinator visits was recorded during 764 observation blocks.

The four-year plant fitness (seed production) was best explained by plant reproductive investment (Table 1), which corresponds to reproductive investment spanning two orders of magnitude. The differences among individuals with the same reproductive investment but different flowering strategies increased with reproductive investment (Fig. 1), rendering an advantage to plants flowering at least twice over those flowering only once (Fig. 2), with the former reaching almost 25% higher fitness than the latter. Interestingly, most of the plants that flowered at least twice produced in total more than 200 florets (Fig. 3), i.e. above the approximate total-reproductive-investment threshold, above which it is advantageous to flower more frequently. The same analysis done including the total of both well-developed and predated seeds as the measure of long-term fitness (i.e. excluding the effect of seed predation) showed an effect of flowering strategy in the same direction, but it was not significant (Table 1, p = 0.11), suggesting that a substantial part of the difference among the two flowering strategies was due to seed predation.

Table 1: Results of linear regression of four-year seed production of *S. pratensis* with and without seed predation exclusion. Expl. var. – percentage of explained variation. Response variable and reproductive investment are both square-root transformed (for details see Materials and Methods).

Predictor	**Df**	**Well-developed seeds**			**Well-developed + predated seeds**		
		Effect	**Expl. var.**	**p-value**	**Effect**	**Expl. var.**	**p-value**
plot identity	11		22.8%	<0.001		17.3%	<0.001
reproductive investment	1	+	58.9%	<0.001	+	65.4%	<0.001
flowered more than once	1		n.s.			n.s.	
rep. inv. x flowered > 1	1	+	0.8%	0.016		n.s.	
rep. inv. x site	1	+ (site N)	0.8%	0.012		n.s.	
flowered > 1 x site	1		n.s.			n.s.	
rep. inv. x flowered > 1 x site	1		n.s.			n.s.	
residuals	127		16.1%			16.2%	

Other than reproductive investment, the factors most influencing yearly seed production were seed predation, pollinator visitation and the interactions of each of these with flower head size (Table 2). The effects of seed predators and pollinators were antagonistic, with each of them stronger in plants with lower numbers of flower heads (controlled for total reproductive investment, i.e. total number of florets produced). The effect of seed predators was more than two times that of pollinators. The effect of pollinators was stronger in later flowering plants. At the more productive site, N, the plants produced more seeds per unit reproductive investment (number of florets produced). A weak interaction of site and plant height appeared, indicating that at site K taller plants had slightly higher one-year fitness, with this effect absent at site N.

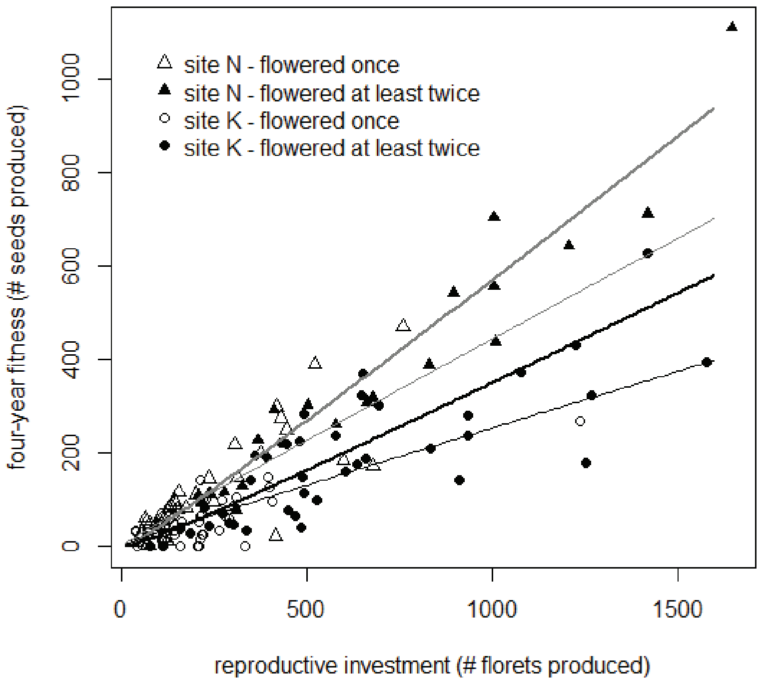

Fig. 1: Relationships of four-year fitness and reproductive investment for plants that flowered only once (empty symbols and thinner regression lines) and plants that flowered at least twice (full symbols and thicker regression lines). Grey regression lines and triangles correspond to site N, black lines and circles to site K).

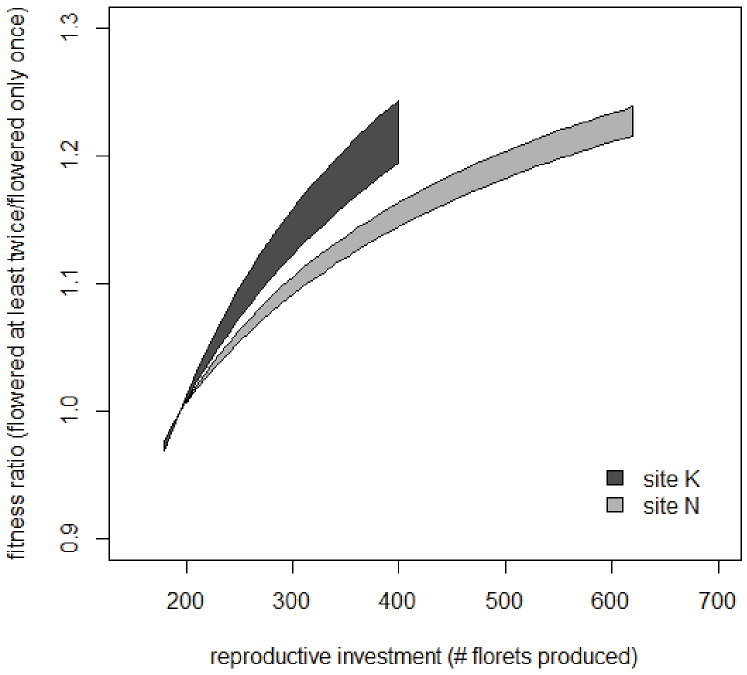

Fig. 2: Relative reproductive advantage of plants flowering twice compared to those flowering only once with the same reproductive investment (ratio of model predictions, see Table 1). The shaded areas depict the variability among individual permanent plots at a given site (constructed from the plots with minimum and maximum intercepts at the given site). The span of each shaded area along the x-axis corresponds to the overlap of the 5th to 95th percentiles of reproductive investment for the two strategies (i.e. area of co-occurrence of the two strategies).

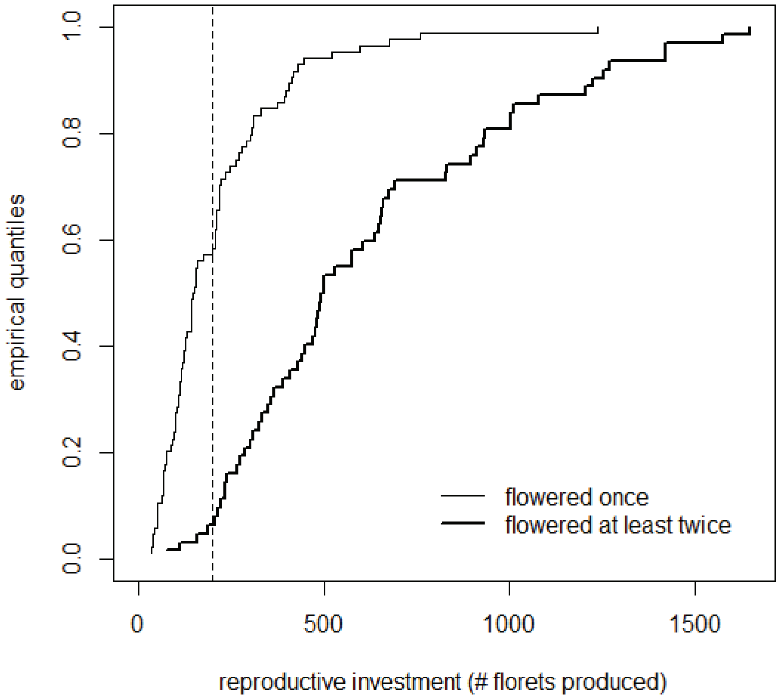

Fig. 3: Empirical distribution functions of reproductive investment of plants that flowered only once in four years and those that flowered at least twice. Broken line denotes reproductive investment of producing 200 florets, i.e. the boundary from which plants flowering at least twice have higher fitness than those flowering only once.

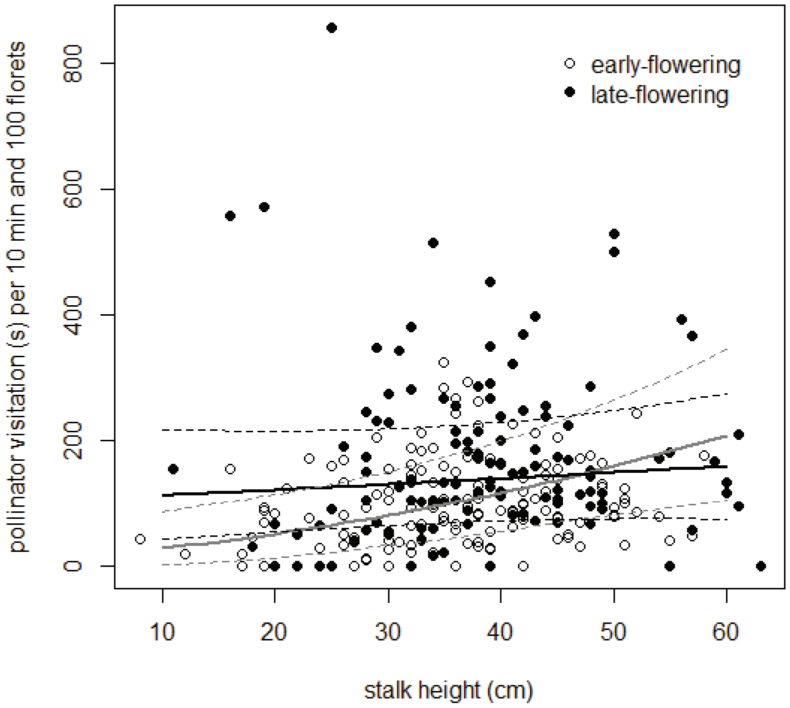

Fig. 4: Dependence of pollinator visitation (total duration of pollinator visits to 100 florets per 10 min of observation) on plant stalk height and relative plant phenology (classified for illustrative purposes only into above- or below-average, i.e. earlier or later than average plants). Regression lines correspond to the 25th percentile (grey line) and 75th percentile (black line) of relative plant phenology; dashed lines denote 95% confidence intervals.

Table 2: Results of linear regression of one-year seed production of *S. pratensis*; only main effects and significant interaction terms are shown. Expl. var. – percentage of explained variation. Response variable and reproductive investment are both square-root transformed (for details see Materials and Methods).

Predictor	**Df**	**Effect**	**Expl. var.**	**p-value**
plot identity x year	48		48.5%	<0.001
reproductive investment	1	+	30.1%	<0.001
flower head size	1		n.s.	
stalk height	1		n.s.	
relative phenology	1		n.s.	
pollinator vistation	1	+	0.6%	0.008
seed predation intensity	1	-	1.9%	<0.001
rep. inv. x site	1	+ (site N)	1.1%	<0.001
fl. head size x pollinators	1	+	0.5%	0.015
fl. head size x predation	1	-	0.7%	0.004
stalk height x site	1	- (site N)	0.3%	0.049
phenology x pollinators	1	+	0.4%	0.030
residuals	185		14.3%	

Unlike pollinator visitation, the intensity of seed predation depended on inflorescence size (Table 3). This, combined with the greater overall effect of seed predators, constitutes a selection pressure for smaller inflorescence size and more frequent flowering (Figs. 1 and 3). The interactions of both seed predators and pollinators with plant relative phenology were not trivial. The importance of plant traits for pollinators decreased in later flowering plants (Fig. 4), whereas seed predation generally decreased with time and its effect was stronger in taller plants.

Table 3: Results of linear regressions of pollinator visitation (total duration of pollinator visits to 100 florets per 10 min of observation) and intensity of seed predation (# seeds consumed per 100 florets); only main effects and significant interaction terms are shown. Pollinator visitation is square-root transformed and seed predation is log-transformed. Expl. var. – percentage of explained variation.

Predictor	**Df**	**Pollinator visitation**			**Seed predation**		
		Effect	**Expl. var.**	**p-value**	**Effect**	**Expl. var.**	**p-value**
plot identity × year	48		27.7%	0.001		39.6%	<0.001
reproductive investment	1		n.s.		+	2.5%	0.002
stalk height	1	+	4.0%	<0.001		n.s.	
flower head size	1		n.s.			n.s.	
relative phenology	1	+	2.3%	0.006	-	5.1%	<0.001
rep. inv. × stalk height	1		n.s.		-	1.0%	0.042
stalk height × phenology	1	-	2.0%	0.010	-	1.0%	0.049
fl. head size × phenology	1	+	1.3%	0.035		n.s.	
residuals	198		59.3%			48.8%	

5.5 Discussion

We demonstrated that plant individuals that distributed their reproductive investment into more than one flowering season had higher fitness than individuals which made the same investment in only one season. Seed predators disproportionately decreased the fitness of plants with larger inflorescences, while pollinators only weakly discriminated among *S.*

pratensis individuals based on other traits that were not connected to flowering strategy. No other measured plant traits besides reproductive investment were correlated with plant one-year fitness, but both pollinators and seed predators influenced one-year fitness, with the effects of seed predators being more than twice as great. Excluding the effect of seed predators (i.e. performing the analysis including consumed along with well-developed seeds) indicated that the substantial part of the advantage of flowering more frequently was probably caused by the selection pressure of seed predators favouring small inflorescences.

5.5.1 Pollinator and seed predator preferences

We found seed predators to relatively strongly discriminate among individuals with respect to inflorescence size, which is a relatively common pattern in other systems (Kolb and Ehrlén, 2010, Sletvold and Grindeland, 2008, Brody and Mitchell, 1997, Asikainen and Mutikainen, 2005, Cariveau et al., 2004). In contrast, pollinators slightly favoured taller *S. pratensis* individuals but not those with larger inflorescences. Such a result is in line with similar studies measuring pollinator visitation not per plant, but per unit inflorescence, which report only a proportionate (Brody and Mitchell, 1997, Parachnowitsch and Caruso, 2008, Thomson, 1988) or less-than-proportionate increase in visitation with inflorescence size (Cariveau et al., 2004, Klinkhamer and de Jong, 1990). A lesser role of pollinators in shaping adaptive landscapes of iteroparous perennials may thus be a much more common phenomenon, although examples of pollinators substantially shaping the adaptive landscape also exist (Vanhoenacker et al., 2013).

5.5.2 Is reproductive investment the only plant trait influencing fitness?

The number of florets produced (reproductive investment) was the main plant-influenced driver of the system, but the distribution of florets, i.e. mean flower head size, interacted with intensities of pollinator visitation and seed predation. This could be due to the fact that the individual flower head is the actual unit that seed predators and pollinators interact with and the same explanation could apply regarding total inflorescence size, i.e. the bigger the more attractive. Relative flowering phenology and stalk height influenced the occurrence of seed predators and pollinators, but their effects were relatively weak and did not translate into fitness effects (also due to correlation of both plant traits with reproductive investment). However, *S. pratensis* individuals experienced favourable conditions at both sites – low surrounding vegetation, high densities of pollinators, etc. For example, with lower pollinator densities, the relative intensities of both biotic interactions could change and thus also the strength and direction of selection pressures (Vanhoenacker et al., 2013). Also, variation in plant height is now relatively small (especially when compared to the two orders of magnitude in reproductive effort), and taller surrounding vegetation (e.g. due to cessation of management) could increase the selective advantage of taller plants (Ågren et al., 2006).

5.5.3 Limits of coexistence of the two flowering strategies

There were obvious differences in the ranges of four-year reproductive investment as well as in the slopes of the effect of reproductive investment at the two sites (Figs. 1 and 2). Interestingly, these differences did not translate into between-site differences in the ranges of advantage of the more-frequent-flowering strategy (in both cases 0-25%) or the lower threshold of reproductive investment by the plants flowering at least twice (ca. 200 florets produced, Fig. 3). On the other hand, the upper limit of reproductive investment, where both strategies still occur, differed between the sites, possibly due to the more frequent occurrence of larger individuals that flowered only once at the more productive site, N.

The lower boundary of reproductive investment, 200 florets, for more-frequently-flowering plants seems to be constrained by inflorescence architecture. The smallest but still well-developed *S. pratensis* inflorescence (i.e. bearing one stalk with a dichasium of three flowerheads; Philipson, 1947) contains ca. 100 florets (10% quantile = 93.8 for plants with 3 inflorescences; Z. Janovský, unpublished data), thus rendering a 200-floret boundary for plants flowering at least twice.

5.5.4 A continuum of iteroparous plant flowering strategies

Perennial plants exhibit a continuum of temporal concentration in reproductive effort (Mbeau-Ache and Franco, 2013 and citations therein), and plasticity in this trait constitutes an important means of plant response to the environment (Williams, 2009, Miller et al., 2012). In present study, we expanded this to biotic interactions, especially seed predation, which we show to be the principal (but likely not sole) driver in fitness differentiation between the two flowering strategies distinguished in our system (see Table 1).

The question remains regarding what mechanisms maintain the existence of the whole spectrum of flowering strategies within populations. A possible explanation can be year-to-year variation in environmental conditions (Shefferson and Roach, 2010). Spatial and temporal differences accounted for a large portion of variation even in our system, and thus the strategy of flowering more frequently can be viewed as a kind of bet-hedging strategy. In contrast, concentrating reproductive effort constitutes a riskier strategy but with the possibility of higher gains in years of low seed predation (such as 2009, data not shown).We believe that within-population variation in flowering strategies may serve as a much more common plastic response to environmental conditions or biotic interactions than has previously been recognized, since its detection requires long-term, individual-based demographic studies (Miller et al., 2012 and this study).[†]

5.6 References

Adams, A. W. (1955) *Succisa pratensis* Moench (*Scabiosa succisa* L.). *Journal of Ecology,* **43,** 709-718.

Ågren, J., Ehrlén, J. & Solbreck, C. (2008) Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology,* **96,** 334-345.

Ågren, J., Fortunel, C. & Ehrlén, J. (2006) Selection on floral display in insect-pollinated *Primula farinosa*: effects of vegetation height and litter accumulation. *Oecologia,* **150,** 225-232.

Ågren, J., Hellström, F., Toräng, P. & Ehrlén, J. (2013) Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. *Proceedings of the National Academy of Sciences,* **110,** 18202-18207.

Asikainen, E. & Mutikainen, P. (2005) Preferences of pollinators and herbivores in gynodioecious *Geranium sylvaticum*. *Annals of Botany,* **95,** 879-886.

Brody, A. K. & Mitchell, R. J. (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia,* **110,** 86-93.

Cariveau, D., Irwin, R. E., Brody, A. K., Garcia-Mayeya, L. S. & von der Ohe, A. (2004) Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos,* **104,** 15-26.

Ehrlén, J. (2000) The dynamics of plant populations: Does the history of individuals matter? *Ecology,* **81,** 1675-1684.

Ehrlén, J. (2002) Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). *Perspectives in Plant Ecology, Evolution and Systematics,* **5,** 145-163.

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Herrera, C. M. (2000) Measuring the effects of pollinators and herbivores: Evidence for non-additivity in a perennial herb. *Ecology,* **81,** 2170-2176.

Herrera, C. M., Medrano, M., Rey, P. J., Sánchez-Lafuente, A. M., García, M. B., Guitián, J. & Manzaneda, A. J. (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 16823-16828.

Jacquemyn, H., Brys, R. & Jongejans, E. (2010) Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. *Journal of Ecology,* **98,** 1204-1215.

Janovský, Z., Mikát, M., Hadrava, J., Horčičková, E., Kmecová, K., Požárová, D., Smyčka, J. & Herben, T. (2013) Conspecific and Heterospecific Plant Densities at Small-Scale Can Drive Plant-Pollinator Interactions. *Plos One,* **8,** e77361.

Klinkhamer, P. G. L. & de Jong, T. J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos,* **57,** 399-405.

Kolb, A. & Ehrlén, J. (2010) Environmental context drives seed predator-mediated selection on a floral display trait. *Evolutionary Ecology,* **24,** 433-445.

Kolb, A., Ehrlén, J. & Eriksson, O. (2007) Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution and Systematics,* **9,** 79-100.

Koons, D. N., Metcalf, C. J. E. & Tuljapurkar, S. (2008) Evolution of delayed reproduction in uncertain environments: A life-history perspective. *American Naturalist,* **172,** 797-805.

Leimu, R., Syrjänen, K., Ehrlén, J. & Lehtilä, K. (2002) Pre-dispersal seed predation in *Primula veris*: among-population variation in damage intensity and selection on flower number. *Oecologia,* **133,** 510-516.

Mbeau-Ache, C. & Franco, M. (2013) The time distribution of reproductive value measures the pace of life. *Journal of Ecology,* **101,** 1273-1280.

Meusel, H. & Jäger, E. J. (1992) *Vergleichende Chorologie der zentraleuropäischen Flora, Band III.* Gustav Fischer Verlag, Jena, Germany.

Mildén, M., Münzbergová, Z., Herben, T. & Ehrlén, J. (2006) Metapopulation dynamics of a perennial plant, *Succisa pratensis*, in an agricultural landscape. *Ecological Modelling,* **199,** 464-475.

Miller, T. E. X., Williams, J. L., Jongejans, E., Brys, R. & Jacquemyn, H. (2012) Evolutionary demography of iteroparous plants: incorporating non-lethal costs of reproduction into integral projection models. *Proceedings of the Royal Society B-Biological Sciences,* **279,** 2831-2840.

Parachnowitsch, A. L. & Caruso, C. M. (2008) Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. *Ecology,* **89,** 1802-1810.

Petanidou, T., den Nijs, J. C. M., Oostermeijer, J. G. B. & Ellis-Adam, A. C. (1995) Pollination ecology and patch-dependent reproductive success of the rare perennial *Gentiana pneumonanthe* L. *New Phytologist,* **129,** 155-163.

Philipson, W. R. (1947) Studies in the development of the inflorescence II. The capitula of *Succisa pratensis* Moench and *Dipsacus fullonum* L. *Annals of Botany,* **11,** 285-297.

Shefferson, R. P. & Roach, D. A. (2010) Longitudinal analysis of *Plantago*: adaptive benefits of iteroparity in a short-lived, herbaceous perennial. *Ecology,* **91,** 441-447.

Sletvold, N. (2002) Effects of plant size on reproductive output and offspring performance in the facultative biennial *Digitalis purpurea*. *Journal of Ecology,* **90,** 958-966.

Sletvold, N. & Grindeland, J. M. (2008) Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. *Acta Oecologica-International Journal of Ecology,* **34,** 21-25.

Sletvold, N., Grindeland, J. M. & Ågren, J. (2010) Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist,* **188,** 385-392.

Thomson, J. D. (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evolutionary Ecology,* **2,** 65-76.

Tolasz, R. (2007) Atlas podnebí Česka [Climatic atlas of the Czech Republic]. pp. 255. Český hydrometeorologický ústav, Praha.

Vanhoenacker, D., Ågren, J. & Ehrlén, J. (2013) Non-linear relationship between intensity of plant-animal interactions and selection strength. *Ecology Letters,* **16,** 198-205.

Wesselingh, R. A., Klinkhamer, P. G. L., de Jong, T. J. & Boorman, L. A. (1997) Threshold size for flowering in different habitats: Effects of size-dependent growth and survival. *Ecology,* **78,** 2118-2132.

Williams, J. L. (2009) Flowering life-history strategies differ between the native and introduced ranges of a monocarpic perennial. *American Naturalist,* **174,** 660-672.

6 Paper V

Reproduction timing and pollinator abundance, but not site quality and isolation, drive reproductive success of two common plant species in an agricultural landscape

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6.1 Abstract

Habitat loss and fragmentation, together with changes in management practices, affect many aspects of wild plants, including their sexual reproduction. Reproductive success is directly affected by plant reproductive allocation and pollinator availability, whereas the effects of (i) site quality (semi-natural meadows/verge sites); (ii) population size, i.e. mate availability; (iii) plot attractiveness to pollinators; and (iv) timing of flowering may be both direct and indirect. We aimed to disentangle the relative effects of these factors on the reproductive success of two meadow plant species common to agricultural landscapes of Central Europe.

We measured plant traits and reproductive success, while simultaneously conducting pollinator censuses and measuring plot attractiveness for pollinators. We sampled (sub)populations within landscape in three intervals corresponding to beginning, peak and end of flowering season of focal species.

Pollinator densities dropped sharply as the flowering season progressed. The influnce of plot attractiveness and focal plant density was detectable but and order of magnitude weaker. The drop in pollinator densities and indirectly timing of flowering were the main driver of plant reproductive success. Contrary to expectations, the differences between semi-natural and verge sites were negligible.

In our conditions, the likeliest driver of flowering time was the timing of hay cuts, a management feature which can be altered with zero to low costs and mitigate the current temporal mismatch between the plants and their pollinators. Small, overlooked verge sites can serve as efficient sources of propagules for colonisation of newly created sites, at least in our system.

Keywords
Achillea millefolium, Achillea ptarmica, *hoverflies, pollination crisis, semi-natural meadows, Syrphidae, road verges, Central European grasslands, habitat fragmentation, Allee effect*

6.2 Introduction

The intensification of agriculture in the last 150 years has led to both a considerable decrease in the area of semi-natural grasslands and their consequent fragmentation (e.g. Lennartsson and Oostermeijer, 2001). Both processes have negative impacts on many aspects of plant populations, not least on plant sexual reproduction (Hadley and Betts, 2012). In most plant species, sexual reproduction is crucial for both colonisation of new sites and maintenance of genetic diversity of extant populations, thus directly influencing long-term species survival at the landscape scale.

There are multiple potential mechanisms behind disruption of plant sexual reproduction in agricultural landscapes: (i) decrease in plant densities; (ii) decrease in pollinator densities; and (iii) temporal or spatial mismatch between plant flowering and pollinator occurrence (Hadley and Betts, 2012). Habitat loss and fragmentation result in sharply decreased plant diversity and population densities in remaining habitat fragments (e.g. Helm *et al.*, 2006) and are often prone to Allee effects (e.g. Lamont *et al.*, 1993, Luijten *et al.*, 2000). However, habitat fragmentation also creates various verge sites (such as road verges, ditch banks or midfield islets) at boundaries of new landscape features. Some of these verge sites may constitute surrogate sites for at least some plant species of semi-natural grasslands (Cousins, 2006, Norderhaug *et al.*, 2000).These species do not necessarily have to form fully viable populations there, as they may merely be represented by remnant populations (sensu Eriksson, 1996). Long-term persistence of such fragmented populations can be inhibited by disruption of one or more life-cycle stages due to different management practices in the new habitat fragment (e.g. Auestad *et al.*, 2010, Yates and Ladd, 2005).

Importantly, the failure of plant sexual reproduction (i.e., the setting and maturation of seed) does not have to be caused solely by processes within the fragmented plant population itself, as it can be also caused by lack of pollinators (major pollen vector of grassland plants), which are severely affected by habitat loss and fragmentation as well (Montero-Castaño and Vilá, 2012, Pauw, 2007). Additionally, changes in landscape structure and management lead to substantial changes in pollinator species composition and abundances (Biesmeijer *et al.*, 2006, Bommarco *et al.*, 2012, Goulson *et al.*, 2005). Furthermore, pollinators that remain under changed conditions tend to concentrate in well-connected attractive patches determined mainly by amount and diversity of accumulated floral resources (Hegland and Boeke, 2006, Öckinger and Smith, 2007, Sowig, 1989, Bhattacharya *et al.*, 2003, Cranmer *et al.*, 2012).

Even if a landscape supports enough available mates and pollinators, plant sexual reproduction may still fail due to a temporal mismatch between plant flowering period and pollinator emergence. Causes of such mismatches may be either climatic (although recently debated, see Bartomeus *et al.*, 2011) or more local phenomena such as the timing of hay cutting (e.g. Noordijk *et al.*, 2009, Reisch and Poschlod, 2009). Shifts in flowering phenology due to hay cut timing are not likely to influence pollinator emergence (unlike climate, Forrest and Thomson, 2011, Bartomeus *et al.*, 2011) and variation in hay cut timing is relatively high (often up to three weeks due to various agricultural considerations). The mowing regimes of verge sites (if any) are typically unconnected to meadows and even less regular, since they are mown for different reasons (e.g. road safety Kiviniemi and Eriksson, 1999, Sýkora *et al.*, 2002). Finally, mismatch between plants flowering after the hay cut and pollinator emergence can threaten pollination of early-flowering plants too, since late-flowering species constitute an important resource for maintaining pollinator populations into the following year (Persson and Smith, 2013, Memmott *et al.*, 2010, Noordijk *et al.*, 2009).

Changes in agricultural landscapes often actually comprise several simultaneous alterations, e.g. habitat fragmentation is frequently coupled with changes in management practices of the remaining semi-natural habitat fragments as well as of the new verge sites. These changes translate into changes in plant reproductive success via both direct and indirect effects. The impacts of these complex changes have so far been documented mainly in rare species (e.g. Luijten *et al.*, 2000, Oostermeijer *et al.*, 1998), which usually show only the one or few of these effects to which they are most vulnerable, although others might have been operative as well. Most common plant species with still-numerous, better-connected populations are usually thought to be safe for now, yet they too may be in peril, as several of these processes may be operating simultaneously, with their separate effects weaker and thus harder to detect.

In the present study, we attempt to assess both direct and indirect effects of: (i) flowering timing (mainly induced by hay cut timing); (ii) site type (verge/meadow); (iii) plot attractiveness to pollinators; and (iv) population density (number of available mates) on reproductive success (quantity and quality of progeny) of two common plant species of European agricultural landscapes. We also aim to decompose the indirect effects of these factors into components attributable to effects acting through plant characteristics and effects acting through pollinator availability.

6.3 Materials and methods

6.3.1 Study species

Achillea millefolium agg. (hereafter *A. millefolium*) and *A. ptarmica* are both polycarpic perennial herbs forming corymbs of flower heads after the first hay cut. Whereas *A.*

millefolium forms numerous small flower heads (in the study area: mean = 75.5, range 8-360), *A. ptarmica* relies on fewer, larger flower heads (mean = 24.8, range 1-136). All species of *Achillea* genus are autoincompatible (de Nettancourt, 1977). Both study species can also reproduce vegetatively at short distances using underground rhizomes. The essential pollinators of both species in our study area were hoverflies, which made up 74.0% and 76.3% of all pollinators visiting flowers of *A. millefolium* and *A. ptarmica*, respectively (other Diptera were the only other group exceeding 10%; Pavlíková, A. et al. unpublished data; see Appendix 1).

Achillea millefolium consists of numerous microspecies, which relatively freely hybridise with each other (Guo *et al.*, 2004). In our study area, only two microspecies occur, *A. pratensis* and *A. millefolium* s.s., with these microspecies differing in ploidy level (Danihelka and Rotreklová, 2001) among other characteristics. We tested the seedlings by means of flow cytometry (for details of the protocol and analysis please refer to Appendix 2) and excluded all hybrids from further analyses. We then ran preliminary analyses (as described below) with *A. pratensis* and *A. millefolium* s.s. samples, focusing on effects of species identity and their interactions with other predictors. Since we found no such effects, we merged both species' data for further analyses.

6.3.2 Study area

We conducted our study in an area of approximately 3 × 1.5 km (area 3,24 km^2) centred around Pivnisko village (N 49.85155, E 15.13602; WGS 1984) in Central Bohemia, Czech Republic. The local agricultural landscape is intensively managed, but a relatively high proportion (17.9%) of meadows has been retained. Management of virtually all the meadows is subsidised by Czech agro-environmental programs and carried out synchronously by a single farming company. The hay cut takes place twice a year: at the end of May or beginning of June and the end of August or beginning of September. Verge sites vary in size and position. Approximately two thirds of them (those larger in size, along public roads or larger private roads or field margins) are mown once a year at various times, while the rest are mown at most haphazardly once in several years.

The focal plant species are quite common within the study area. *A. millefolium* occurs in almost all of the meadows, while *A. ptarmica* is restricted to the wetter half of the meadow habitat in the area, and is thus absent from more sites. Of the two species, *A. ptarmica* is more common in at verge sites. Both species often co-occur within several metres in verges, unlike in meadows, and their flowering overlaps almost completely there. In contrast, in meadows, the hay cut timing causes an approximately two to three week delay in flowering of *A. ptarmica*, whereas the phenology of *A. millefolium* is not shifted.

6.3.3 Study design

We assessed reproductive success of *Achillea millefolium* and *A. ptarmica* in terms of seed production per flower head and seed germinability. To reach this goal, we sampled plots within local populations of *Achillea* in two types of biotopes: (i) semi-natural meadows; and (ii) various small verge sites, such as road verges, field margins, and ditch banks. The plots were established and observed during 2012 within three distinct periods (July 13th to 18th, July 26th to 31st and August 13th to 18th) roughly corresponding to the beginning, peak and end of flowering of both *Achillea* species (hereinafter referred as timing). The criteria for choice of study plots were the following: (i) at least five actively flowering individuals (with pollen presenting florets) of one of the focal species present within 5 metres from the plot centre; (ii) the plots chosen for observation within the same period could not be less than 30 metres apart, and plots chosen for observation in the different periods could not be closer than 10 metres; and (iii) the plots of all four types (two species × two site types) had to be spread across the whole study area approximately uniformly during each time interval in order to avoid spatial autocorrelation of results (see Appendix 3). In each plot, availability of floral resources, focal *Achillea* population density, and hoverfly pollinator density were assessed within subplots (described below) during the period in which the plot was observed.

The five randomly chosen actively flowering *Achillea* individuals within a 5-metre radius from the plot centre were tagged and approximately two to three weeks later harvested to determine seed production and to provide seeds for germination assessment. During the harvest, stalk height (as proxy for plant vegetative size) and number of flower heads (proxy for reproductive allocation) were counted. From each tagged plant, 15 (*A. millefolium*) or 5 (*A. ptarmica*) flower heads were collected for estimating seed production and germination. We estimated the mean number of developed seeds per flower head based on seeds counted from at least 5 (*A. millefolium*) or 2 (*A. ptarmica*) flower heads per individual and obtained 50 seeds per individual for the germination experiment. If less than 50 seeds were present in all collected flower heads from a given individual, we would use all the seeds it had, never less than 20.

The germination experiment was conducted in a growth chamber during the autumn and winter of 2012. The seeds were germinated at 20° C in a 12-hour light regime. All the seeds from each individual were arranged in a separate Petri dish, placed in the chamber and watered regularly. Seedlings were counted and removed once a week and the Petri dish monitored for a total of five consecutive weeks (with most seeds germinating within three weeks from the start).

For further analysis, we averaged the data obtained from all individuals originating from one plot in order to avoid pseudoreplication, as data on pollinator density and plot attractiveness were collected at the plot level.

6.3.4 Assessment of plot attractiveness and Achillea local population density

We assessed the amount of available floral resources in each plot by a semi-quantitative survey of flowering plants. At the centre of each study plot, we established a subplot of 4 × 4 m in meadows and 2 × 4 m in verge sites (because in verge sites it is usually impossible to delimit a continuous plot larger than this). The vegetation subplot would be divided into a regular grid of 64 or 32 (respectively) 0.5 × 0.5 m squares. Presence or absence of flowering stalks of all plant species in each square was recorded, thus yielding a semi-quantitative estimate of floral abundance for each species. In the reported analyses, we used only two variables constructed from the data: (i) plot attractiveness, corresponding to the sum of abundances of all species standardized by the number of subplots; and (ii) focal *Achillea* species local population density, defined as the proportion of occupied subplots in a given vegetation plot.

6.3.5 Pollinator censuses

Pollinator censuses were conducted in vegetation subplots during the periods for which they were chosen. Each subplot was censused at least five times within the given timing period (stratified with respect to time of day), with each census consisting of insect trapping by net for five minutes. Here we report only data on the total count of hoverflies caught divided by the number of censuses. The counts obtained from meadows had to be rescaled to those from verge sites due to different vegetation subplot sizes. The rescaling was done by multiplying the numbers of hoverflies trapped in meadows by 0.6250.

This coefficient was obtained from a calibration dataset collected in the same study area at a subset of sites during the *Achillea* peak flowering period in 2013. We established 20 meadow vegetation subplots (4 × 4 m), which were each divided into two 2 × 4 m halves. The subplots were chosen according to the same criteria as in the main study and partly overlapped with its vegetation subplots. Using the same trapping method as in the main study, pairs of 5-minute insect censuses were conducted, with ca. 30 minutes between each of the two censuses, and one of them covering a randomly chosen half of the subplot, and the other covering the whole subplot (and the order switched after each census pair). There were 120 such pairs of censuses conducted and more than 1800 hoverflies trapped. The ratio half/whole predicted by a binomial GLM had an overall average of 0.6250 (Janovský, Z. et al. unpublished data), thus providing the coefficient used in the main study.

6.3.6 Data analysis

The resulting datasets for each species were analysed by a series of linear regressions, since we were aware that unbalanced design of our study precludes use of path-analysis due to its sensitivity to colinearity of predictors (Petraitis *et al.*, 1996). Therefore we used linear regressions with Type I sums of squares, which are more appropriate for unbalanced designs, since the effect overlaps due to colinearity of predictors can be resolved by the order of predictors entered into the model with the later entered terms being corrected for the effect of already entered terms.

For purpose of entering the terms into the model, we recognized two types of predictors – primary predictors (timing, site type, patch attractiveness, population density), corresponding to exogenous variables in path analysis; and secondary predictors (plant characteristics, pollinator density, seed production per flower head), which acted in some analyses as response variables and in others as predictors (corresponding to endogenous variables). We followed two rules in establishing the order of predictors for each analysis. First, the secondary predictors preceded the primary ones (to distinguish direct and indirect effects of primary predictors). The order of secondary predictors followed the order of analyses. For example, we assumed that plant vegetative size (stalk height) could be affected only by primary predictors, whereas reproductive investment (number of flower heads) could be affected by both primary predictors and plant vegetative size, therefore reproductive investment preceded vegetative size in further analyses (to separate net effect of vegetative size from the indirect effects of reproductive investment). Second, concerning the order of primary predictors, timing preceded the site-related predictors, since the sample sizes of meadow and verge sites and the ratios between them varied greatly among the three sampling periods (see above for explanation). Plot type preceded plot attractiveness and population density in analyses with plant characteristics as response variables, since in those cases it was a more direct proxy of site characteristics than were the amount of flowering plants or *Achillea* population density.

The satisfaction of the assumptions necessary for linear regression was assessed from diagnostic graphs, and a suitable transformation was applied where necessary. The same transformation was also applied to these variables when used as predictors. The amount of variability explained by each significant factor (the direct effects) was computed. Besides the direct effects of primary predictors, we also computed their indirect effects by multiplication of the explained variability values along the effect path (e.g. the indirect effect of timing on seed production via stalk height was computed as the product of variability in stalk height explained by timing and the variability in seed production explained by stalk height).

All computations were done in the R statistical environment, version 2.15.3 (accessible at www.r-project.org).

6.4 Results

The linear regressions (Table 1) indicate that for both focal plant species, site type primarily affects plant vegetative size and consequently reproductive allocation (no. of inflorescences). Generally, the plants in verge sites were taller (also bearing more leaves) with greater effort allocated to reproduction (flower head production). Direct and indirect effects of site type (Table 1) on reproductive success were marginal (*A. ptarmica*) or absent (*A. millefolium*).

On the other hand, reproduction timing influenced the seed production of *A. millefolium* and *A. ptarmica* rather indirectly through a very strong influence (55.7% and 69.7% respectively) on hoverfly densities in plots (Fig. 2). The strong negative effect of timing on hoverfly densities translates into negative effects on seed production per flower head (5.6% and 14.2% respectively; Fig. 1, Table 2). Hoverfly densities constituted the only detected influence on seed production per flower head in the case of *A. millefolium*. For *A. ptarmica*, the effects of plant size and population density were each less than half the size of the hoverfly density effect.

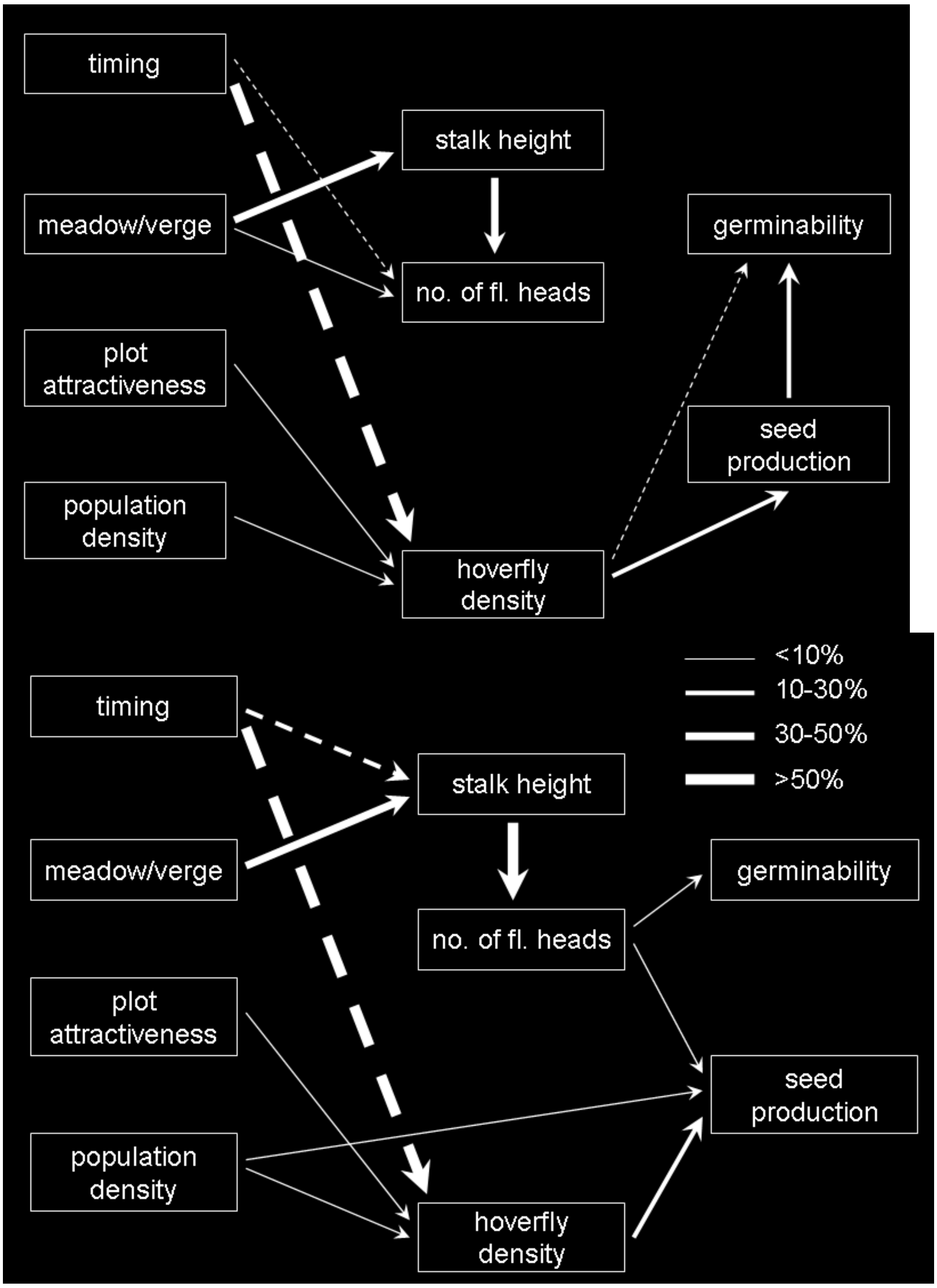

Fig. 1: Diagrams depicting the structures of detected direct effects among tested variables in the (A) *A. millefolium* dataset and (B) *A. ptarmica* dataset. Solid lines denote positive effects, broken lines negative ones. Line width corresponds to effect size.

Table 1: Results of linear regressions with effect directions, percentages of explained variability and p-values reported. Predictors are listed in the same order that they were entered into the model (see Materials and Methods for details). "---" denotes the predictor was not considered in the model. Timing was treated as a categorical variable in order to account for possible non-linearities; however, all detected effects were monotonic (reported in direction early to late). Site type (meadow/verge) values represent difference of verges compared to meadows.

A. millefolium	**Df**	**Stalk height**	**No. of flower heads (log)**	**Hoverfly density (log)**	**Seed production**	**Germinability**
Seed production	1	---	---	---	---	+ 23.7% <0.001
Hoverflies (log)	1	---	---	---	+ 10.1% 0.0217	- 6.2% 0.037
No. of fl. heads (log)	1	---	---	---	n.s.	n.s.
Stalk height	1	---	+ 39.3% <0.001	---	n.s.	n.s.
Timing	2	n.s.	- 9.4% 0.010	- 55.7% <0.001	n.s.	n.s.
Meadow/verge	1	+ 33.8% <0.001	+ 6.7% 0.010	n.s.	n.s.	n.s.
Plot attractiveness	1	n.s.	n.s.	+ 10.1% <0.001	n.s.	n.s.
Pop. density	1	n.s.	n.s.	+ 4.1% 0.013	n.s.	n.s.
Residuals	44-48	60.0%	43.4%	29.7%	80.0%	59.2%

A. ptarmica	**Df**	**Stalk height**	**No. of flower heads (log)**	**Hoverfly density (log)**	**Seed production**	**Germinability**
Seed production	1	---	---	---	---	n.s.
Hoverflies (log)	1	---	---	---	+ 19.6% <0.001	n.s.
No. of fl. heads (log)	1	---	---	---	+ 5.9% 0.037	+ 8.4% 0.036
Stalk height	1	---	+ 56.4% <0.001	---	n.s.	n.s.
Timing	2	- 17.3% <0.001	n.s.	- 69.7% <0.001	n.s.	n.s.
Meadow/verge	1	+ 35.6% <0.001	n.s.	n.s.	n.s.	n.s.
Plot attractiveness	1	n.s.	n.s.	+ 4.9% 0.001	n.s.	n.s.
Pop. density	1	n.s.	n.s.	+ 7.9% <0.001	+ 9.3% 0.010	n.s.
Residuals	40-44	45.4%	42.2%	17.5%	52.4%	71.7%

The primary predictors considered do not appear to have congruent effects on germinability in the two species. Plot attractiveness and focal *Achillea* species population density influenced focal species' reproductive success negligibly and mostly indirectly, through effects on hoverfly densities, with the only exception being the effect of *A. ptarmica* population density on its seed production per flower head.

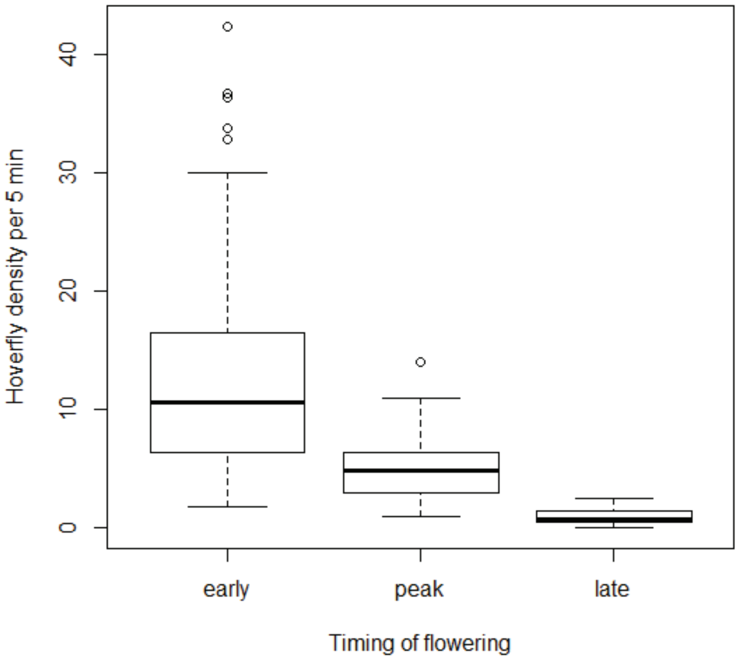

Fig. 2: Boxplot of hoverfly densities per 5 minutes (mean of all censuses per plot). Boxes denote inter-quartile range; thick lines denote medians; observations lying further from the median than 1.5 times the inter-quartile range are depicted as outliers.

Table 2: Summary of positive and negative indirect effects of primary predictors on measures of focal plant species' reproductive success (expressed as percentage of explained variability of a given response variable).

Primary predictor	***A. millefolium***				***A. ptarmica***			
	Seed production		**Germinability**		**Seed production**		**Germinability**	
	+	-	+	-	+	-	+	-
Timing	0.0%	5.6%	3.5%	1.3%	0.0%	14.2%	0.0%	0.8%
Meadow/verge	0.0%	0.0%	0.0%	0.0%	1.2%	0.0%	1.7%	0.0%
Plot attractiveness	1.0%	0.0%	0.2%	0.1%	1.0%	0.0%	0.0%	0.0%
Population density	0.4%	0.0%	0.1%	0.3%	1.6%	0.0%	0.0%	0.0%

6.5 Discussion

The most important driver of seed production per flower head was neither site type nor site attractiveness but the timing of reproduction, whose influence was mainly indirect, as it was mediated by pollinator availability. For both species, seed germination rates were not well predicted by the evaluated predictors. Notably, almost none of our primary predictors affected measures of reproductive success directly, with the exception of local population density having a small effect on seed production of *A. ptarmica*. Site type directly influenced only plant characteristics, whereas site attractiveness and *Achillea* population size directly influenced only availability of pollinators. Our results thus indicate that reproduction timing is the main driver of seed production in our system. This provides land managers a substantial opportunity (and responsibility) to influence reproductive success of common plants by manipulating hay cut timing, which is often the strongest driver of flowering timing.

6.5.1 Timing of reproduction and drivers of pollinator densities

Timing affected *Achillea* reproductive success mainly indirectly, through mismatch with its key pollinators, i.e. hoverflies. The local populations flowering at the end of the flowering period experienced approximately 8-10 times lower hoverfly densities. Larvae of the main hoverfly pollinators (ca 85% of all individuals caught) *Sphaerophoria scripta* agg., *Episyrphus baltheatus*, *Melanostoma mellinum*, *Syrphus* spp. (nomenclature follows van Veen, 2004) feed on aphids. Therefore, the numbers of adults probably diminished in response to the decline in abundance of aphids after frequent June and July storms hit the study area (Janovský, Z. personal observation). The same declines in abundance of adult aphid-feeding hoverflies have also been observed in a similar landscape in Germany (Ssymank, 2001). Larvae of many of the pollinating hoverflies feed on tree aphids rather than on grass aphids (Ssymank, 2001); thus, the potential for landscape managers to shift hoverfly phenology by manipulating hay cut timing is rather limited.

On the other hand, opportunities of landscape managers to influence timing of flowering of *Achillea* species are relatively large. Even in our relatively small and homogeneous study area the span of flowering time from the first to the last populations to flower is more than one month. The likeliest driver of this variation is the hay cut timing, which has been known to induce shifts in reproduction timing in other plant species (Reisch and Poschlod, 2009, Warwick and Briggs, 1979). Another likely cause, the differences in microclimate can almost be ruled out. Flowering time of *A. millefolium* did respond to differences in microclimatic conditions in the study of Johnston and Pickering (2006), yet the magnitude of changes in microclimate was in their study far greater than in our study area with shifts of only ca 10 days in detected flowering peaks. Hay cuts would mostly shift flowering time of meadow populations, since verges are mown mainly in early autumn. The hay-cut-induced delay in flowering seems to have been more pronounced in *A. ptarmica* which was shifted by approximately three weeks, compared to the approximately 10 day shift in *A. millefolium*. Such hay-cut-induced shifts in flowering timing are more pronounced than the effects of several decades of climate warming on flowering of most grassland species (see Fitter and Fitter, 2002).

Late-flowering meadow populations of both species also suffered much higher losses of total seed production due to an untimely second mowing (Appendix 3, Table 2). Thus, the total unfavourability of flowering late is greater than our model based on data only from harvested plots would suggest.

The effects of availability of floral resources at the plot were rather expected and are in line with previous research (e.g. Hegland and Boeke, 2006). A more interesting feature is the interdependence of site type and timing with plot attractiveness (see Appendix 5). The observed pattern of plot attractiveness is partly congruent with that of pollinator densities and can thus be one of the factors contributing to the influence of timing, besides the direct effects of timing on the study species. Unfortunately, this hypothesis cannot be verified without a manipulative experiment.

The interpretation of effects of flowering timing on plant characteristics is less straightforward. The effect of timing of reproduction on plant height in *A. ptarmica* may simply be an artefact of our study design, since *A. ptarmica* flowered in the beginning of the flowering period only at verge sites, where the plants were generally taller than in meadows (see Appendix 6). On the other hand, the lower number of flower heads of late flowering *A. millefolium* individuals formed an overall pattern and thus was not explainable by an unbalanced design (even in terms of unbalanced proportions of the two micro species – data not shown). The indirect effects of timing on reproductive output through plant characteristics were an order of magnitude weaker than the indirect effects through pollinator availability.

6.5.2 The role of verge sites

Our results indicate only weak or even no differences among the meadow and verge sites regarding the reproductive success of the studied species. On the other hand, the verge sites differed very much from meadows in plant characteristics. The plants in verges were bigger, and allocated more energy to reproduction, with their total seed production per plant thus much higher (data not shown). This was probably due to higher nutrient availability and different hay cut timing (later or irregular hay cut not limiting plant size). Verge populations under such management can thus serve as an effective source of propagules for site colonisation and recolonisation. However, the long-term persistence of such populations is questionable, due to low seedling recruitment and survival in verge sites with high vegetation and litter cover (Auestad *et al.*, 2011). A shifting mosaic of mowing and unmown treatments has been recommended to promote long-term stability of verge plant populations (Auestad *et al.*, 2010, Noordijk *et al.*, 2009) and to stabilise the flower supply for insects throughout the season (Noordijk *et al.*, 2009). Such season-long stability in flower availability used to be ensured in traditional Central European agricultural landscapes by meadows in different ecological conditions occurring close to each other.

6.5.3 Limitation by available mates – the effect of population size

Both studied species are self-incompatible, which would imply higher dependence on availability of pollinators and populations of available mates, as predicted by evolutionary models (Young *et al.*, 2012). However, it is not clear from the model whether observed pollinator and available mate densities are low enough to be limiting sexual reproduction of the study species. Possible limitation by mate availability was suggested by the positive effect of population size on seed production in *A. ptarmica*. *A. ptarmica* was less common than *A. millefolium*, but still it had several tens of populations within the study area, typically with tens of flowering conspecifics within the 15 metre radius (for more information see Appendix 4). However, the number of co-flowering populations was effectively lower due to temporal mismatch, which in *A. ptarmica* is more pronounced due to greater delay in flowering of meadow populations following the hay cut. This result is rather alarming, if we consider that *A. ptarmica* is still one of the commonest plant species in our study landscape, which moreover contains quite a high proportion of semi-natural grasslands (17.9%).

6.5.4 The effects on seed germinability

We detected generally only weak (and inconsistent between the two species) effects of our studied predictors on seed germinability. Whereas germinability was positively associated with seed production and negatively with pollinator densities in *A. millefolium*, it was dependent only on the plant's allocation to reproduction in *A. ptarmica*. We tend to agree with the interpretation of this phenomenon proposed by Andersson (1993), who experimented with *A. ptarmica* and its potential for selective seed abortion. He interpreted his findings of high variation in seed abortion rate and germination rate as connected to mother plant genotypes, possibly their genetic load. Similar variation in reproductive success unrelated (or only weakly related) to plants' environments has also been found in other species (e.g. Kärkkäinen *et al.*, 1999)

6.5.5 Implications and recommendations for landscape management

Our study produced three main findings of interest for landscape management: (i) even common species may be pollinator- or mate-limited irrespective of whether at semi-natural or verge sites; (ii) shifts in flowering phenology probably caused by shifts in hay cut timing seem to be the key driver of reproductive output of our study species; (iii) in terms of reproductive success, verge sites are equally valuable as semi-natural sites (and in total numbers per plant far better) and may efficiently serve as important seed sources for recolonisation of newly restored sites. The extent of pollinator and mate limitation in our study species is quite surprising, yet our results add to the line of evidence that the common and frequently visited (from a community perspective) species may be the most strongly pollen-limited (Hegland and Totland, 2008), due, e.g. to lower effectiveness of pollen carry-over. The effects of this limitation may be mitigated by (i) enhancing pollinator densities and/or (ii) directly enhancing flowering plant reproductive success.

Enhancing hoverfly densities cannot be easily achieved, since adults live only several days (e.g. Ottenheim *et al.*, 1999). Thus, measures promoting diversity and duration of nectar and pollen sources recommended for other pollinators, such as bumblebees (Memmott *et al.*, 2010), would have little or no effect. However, management could affect larvae of aphidophagous hoverflies, which feed on grass aphids. Synchronous mowing of meadows abruptly deprives these larvae of food. Whether desynchronisation of mowing would substantially improve hoverfly densities cannot be determined without further investigation.

On the other hand, flowering plant reproductive success can be relatively easily manipulated at the landscape scale in several ways. The easiest and least costly is by means of manipulating hay cut timing in order to minimize the temporal mismatch between pollinator densities and plant flowering. In our system, this would mean shifting the first mowing of the meadows earlier. This could be achieved in the case of *A. millefolium*, but the necessary shift for *A. ptarmica* would not be feasible for hay production reasons (too little biomass in the meadows). However, *A. ptarmica* could still be helped by postponing the time of the second hay cut, so that the seeds of late-flowering meadow populations could mature. Another option would be in some years to omit the first hay cut (which is currently against the rules of the Czech agro-environmental subsidies). Such a management practice would also promote other late-flowering wet meadow species, such as *Sanguisorba officinalis* and *Serratula tinctoria*. If different meadows omit the first hay cut in different years, this would yield a mosaic of managements, similar to schemes suggested and successfully tested for verge populations of plants and pollinators (Auestad *et al.*, 2010, Noordijk *et al.*, 2009, Jantunen *et al.*, 2007). If such schemes were permissible, their costs would be relatively negligible for several reasons: (i) the nutritional value of hay from wet meadows is relatively low; (ii) wet meadow areas usually constitute only small islands of approximately 20% of the whole meadows; (iii) hay- cutting is already timed to obtain optimal hay quality from moderately dry meadow areas, as it produces more valuable biomass than the wet parts (J. Dittrich, main agro-technician of the local farming company, pers. comm.); and (iv) income from superfluous low quality hay is minimal compared to the agro-environmental subsidies. Therefore, it should be possible to reduce plant-pollinator temporal mismatch and thereby improve reproductive success of wild plants in European agricultural landscapes without sacrificing agricultural income, and we believe that this possibility should be explored.[*]

6.6 References

Andersson S. 1993. The potential for selective seed maturation in *Achillea ptarmica* (*Asteraceae*). Oikos 66: 36-42.

Auestad I., Rydgren K. and Austad I. 2011. Road verges: potential refuges for declining grassland species despite remnant vegetation dynamics. Annales Botanici Fennici 48: 289-303.

Auestad I., Rydgren K., Jongejans E. and de Kroon H. 2010. *Pimpinella saxifraga* is maintained in road verges by mosaic management. Biological Conservation 143: 899-907.

Bartomeus I., Ascher J.S., Wagner D., Danforth B.N., Colla S., Kornbluth S. and Winfree R. 2011. Climate-associated phenological advances in bee pollinators and bee-pollinated plants. Proceedings of the National Academy of Sciences of the United States of America 108: 20645-20649.

Bhattacharya M., Primack R.B. and Gerwein J. 2003. Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? Biological Conservation 109: 37-45.

Biesmeijer J.C., Roberts S.P.M., Reemer M., Ohlemüller R., Edwards M., Peeters T., Schaffers A.P., Potts S.G., Kleukers R., Thomas C.D., Settele J. and Kunin W.E. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313: 351-354.

Bommarco R., Lundin O., Smith H.G. and Rundlöf M. 2012. Drastic historic shifts in bumble-bee community composition in Sweden. Proceedings of the Royal Society B-Biological Sciences 279: 309-315.

Cousins S.A.O. 2006. Plant species richness in midfield islets and road verges - The effect of landscape fragmentation. Biological Conservation 127: 500-509.

Cranmer L., McCollin D. and Ollerton J. 2012. Landscape structure influences pollinator movements and directly affects plant reproductive success. Oikos 121: 562-568.

Danihelka J. and Rotreklová O. 2001. Chromosome numbers within the *Achillea millefolium* and the *A. distans* groups in the Czech Republic and Slovakia. Folia Geobotanica 36: 163-191.

de Nettancourt D. 1977. Springer Verlag, Berlin, Heidelberg et New York.

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

Fitter A.H. and Fitter R.S.R. 2002. Rapid changes in flowering time in British plants. Science 296: 1689-1691.

Forrest J.R.K. and Thomson J.D. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecological Monographs 81: 469-491.

Goulson D., Hanley M.E., Darvill B., Ellis J.S. and Knight M.E. 2005. Causes of rarity in bumblebees. Biological Conservation 122: 1-8.

Guo Y.P., Ehrendorfer F. and Samuel R. 2004. Phylogeny and systematics of *Achillea* (Asteraceae-Anthemideae) inferred from nrITS and plastid trnL-F DNA sequences. Taxon 53: 657-672.

Hadley A.S. and Betts M.G. 2012. The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. Biological Reviews 87: 526-544.

Hegland S.J. and Boeke L. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. Ecological Entomology 31: 532-538.

Hegland S.J. and Totland Ø. 2008. Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? Oikos 117: 883-891.

Helm A., Hanski I. and Pärtel M. 2006. Slow response of plant species richness to habitat loss and fragmentation. Ecology Letters 9: 72-77.

Jantunen J., Saarinen K., Valtonen A. and Saarnio S. 2007. Flowering and seed production success along roads with different mowing regimes. Applied Vegetation Science 10: 285-292.

Johnston F.M. and Pickering C.M. 2006. Phenology of the environmental weed *Achillea millefolium* (Asteraceae) along altitudinal and disturbance gradients in the Snowy Mountains, Australia. Nordic Journal of Botany 24: 149-160.

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Kärkkäinen K., Savolainen O. and Koski V. 1999. Why do plants abort so many developing seeds: bad offspring or bad maternal genotypes? Evolutionary Ecology 13: 305-317.

Kiviniemi K. and Eriksson O. 1999. Dispersal, recruitment and site occupancy of grassland plants in fragmented habitats. Oikos 86: 241-253.

Lamont B.B., Klinkhamer P.G.L. and Witkowski E.T.F. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. Oecologia 94: 446-450.

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

Luijten S.H., Dierick A., Oostermeijer J.G.B., Raijmann L.E.L. and den Nijs H.C.M. 2000. Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. Conservation Biology 14: 1776-1787.

Memmott J., Carvell C., Pywell R.F. and Craze P.G. 2010. The potential impact of global warming on the efficacy of field margins sown for the conservation of bumble-bees. Philosophical Transactions of the Royal Society B-Biological Sciences 365: 2071-2079.

Montero-Castaño A. and Vilá M. 2012. Impact of landscape alteration and invasions on pollinators: a meta-analysis. Journal of Ecology 100: 884-893.

Noordijk J., Delille K., Schaffers A.P. and Sýkora K.V. 2009. Optimizing grassland management for flower-visiting insects in roadside verges. Biological Conservation 142: 2097-2103.

Norderhaug A., Ihse M. and Pedersen O. 2000. Biotope patterns and abundance of meadow plant species in a Norwegian rural landscape. Landscape Ecology 15: 201-218.

Öckinger E. and Smith H.G. 2007. Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. Journal of Applied Ecology 44: 50-59.

Oostermeijer J.G.B., Luijten S.H., Krenova Z.V. and Den Nijs H.C.M. 1998. Relationships between population and habitat characteristics and reproduction of the rare Gentiana pneumonanthe L. Conservation Biology 12: 1042-1053.

Ottenheim M.M., Wertheim B., Holloway G.J. and Brakefield P.M. 1999. Survival of colour-polymorphic *Eristalis arbustorum* hoverflies in semi-field conditions. Functional Ecology 13: 72-77.

Pauw A. 2007. Collapse of a pollination web in small conservation areas. Ecology 88: 1759-1769.

Persson A.S. and Smith H.G. 2013. Seasonal persistence of bumblebee populations is affected by landscape context. Agriculture Ecosystems & Environment 165: 201-209.

Petraitis P.S., Dunham A.E. and Niewiarowski P.H. 1996. Inferring multiple causality: The limitations of path analysis. Functional Ecology 10: 421-431.

Reisch C. and Poschlod P. 2009. Land use affects flowering time: seasonal and genetic differentiation in the grassland plant Scabiosa columbaria. Evolutionary Ecology 23: 753-764.

Sowig P. 1989. Effects of flowering plants patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera, Apidae). Oecologia 78: 550-558.

Ssymank A. 2001. Vegetation und blütenbesuchende Insekten in der Kulturlandschaft - Pflanzengesellschaften, Blühphänologie, Biotopbindung und Raumnutzung von Schwebfliegen (Diptera, Syrphidae) im Drachenfelser Ländchen sowie Methodenoptimierung und Landschaftsbewertung, Bonn - Bad Godesberg.

Sýkora K.V., Kalwij J.M. and Keizer P.-J. 2002. Phytosociological and floristic evaluation of a 15-year ecological management of roadside verges in the Netherlands. Preslia 74: 421-436.

van Veen M.P. 2004. Hoverflies of Northwest Europe. KNNV Publishing, Utrecht.

Warwick S.I. and Briggs D. 1979. Genecology of lawn weeds. 3. Cultivation experiments with *Achillea millefolium* L., *Bellis perennis* L., *Plantago lanceolata* L., *Plantago major* L. and *Prunella vulgaris* L. collected from lawns and contrasting grassland habitats. New Phytologist 83: 509-&.

Yates C.J. and Ladd P.G. 2005. Relative importance of reproductive biology and establishment ecology for persistence of a rare shrub in a fragmented landscape. Conservation Biology 19: 239-249.

Young A.G., Broadhurst L.M. and Thrall P.H. 2012. Non-additive effects of pollen limitation and self-incompatibility reduce plant reproductive success and population viability. Annals of Botany 109: 643-653.

7 Appendices

7.1 Appendices of Paper I

This supplementary material contains figures of damage types by different herbivore groups under study (Appendix 1), table of population characteristics (Appendix 2), details on classification of vegetation into types (Appendix 3) and summarisation of species composition in individual vegetation types for the final Twinspan analysis (Appendix 4).

7.1.1 Paper I – Appendix 1 – Damage caused by herbivore functional groups

Fig. 1: Examples of damage caused by invertebrate folivores

Fig. 2: Foliar damage caused by generalist vertebrate folivores.

Fig. 3: Gregarious pre-hibernation larvae of specialist folivore, butterfly *Euphydryas aurinia*.

Fig. 4: Stalks grazed by roe deer, biting off the stalk with all inflorescences is by far the commonest way of damaging stalks, although sometimes a minority of lower-placed inflorescences remains.

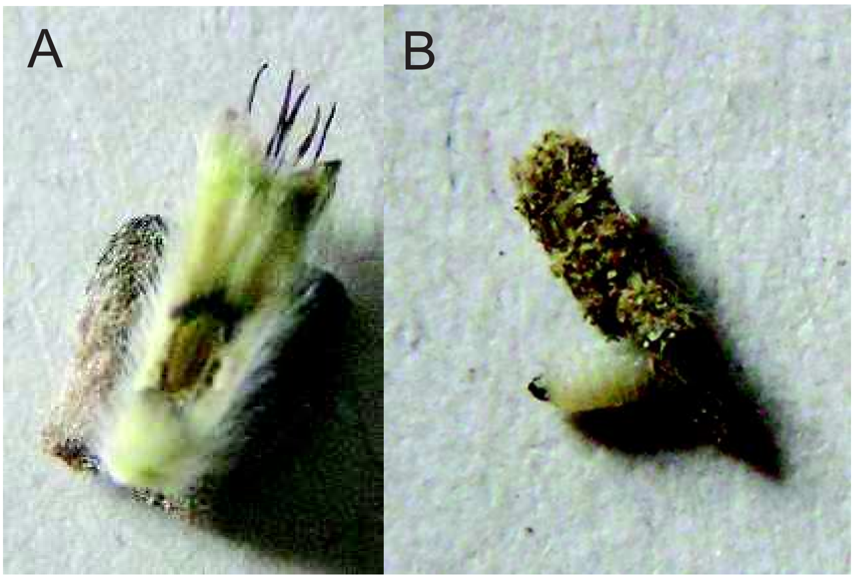


Fig. 5: Damage caused by seed predation; A) predated seed, B) seed predator larva (presumably of Tortricidae family) with rests of seeds

7.1.2 Paper I – Appendix 2 – Study population characteristics

Table 1: Summary of study population characteristics and sampling effort.

Population	# measured subpopulations	Represented veg. types	# measured plants		Plant density per m^2	Land use	Coord. (WGS 1984)	
			2010	2011			E	N
EN1*	4	6	142	143	3.38	pasture	12.8278	49.9803
EN2	6	4,5,7,8	80	38	0.78	abandoned meadow	12.8168	49.9840
EN3	4	7	81	76	2.99	recently abandoned pasture	12.8187	49.9813
HK2*	4	8	89	95	0.71	mown fen	12.8036	49.9586
HK3	4	8	84	88	0.58	meadow	12.7970	49.9564
HK4	4	1	84	87	1.42	late successional stage – shrubs	12.8152	49.9608
L10	4	1	92	97	5.90	meadow	12.7947	49.9863
LU1	4	7	64	84	0.26	meadow	12.8077	49.9909
LU2*	4	7,8	126	113	1.39	meadow	12.7869	49.9883
LU4*	4	2,4,7	164	166	3.97	abandoned meadow	12.8102	49.9898
LU5	3	2	86	81	3.40	abandoned meadow	12.7754	49.9898
LU6	2	3	103	119	19.01	meadow	12.7780	49.9893
LU7	3	4	80	60	5.88	abandoned fen	12.7759	49.9926
LU8	3	4,6	88	83	6.05	abandoned fen	12.8134	49.9887
LU9	2	4,7	82	90	6.78	abandoned meadow	12.7838	49.9833
OG1*	4	8	164	167	3.43	abandoned meadow	12.7966	49.9775
OG2*	4	4,7	150	147	2.01	abandoned fen	12.8104	49.9742
OG3	3	7,8	88	88	7.66	meadow	12.7953	49.9761
OG4	4	7,8	86	98	4.81	meadow	12.8016	49.9735
OG5	2	3	80	90	17.67	abandoned meadow	12.8104	49.9739
RO1*	4	7	125	154	2.60	abandoned meadow	12.7632	49.9870
RO2	6	6	89	100	1.62	recently abandoned pasture	12.7586	49.9872
RO3	4	2,3,7	107	117	3.61	abandoned meadow	12.7642	49.9877
WE1	4	5	83	72	1.61	mown fen	12.8089	49.9477
WE2	4	5,7	82	42	1.59	abandoned fen	12.8077	49.9533
WE3	4	5	84	89	2.46	abandoned fen	12.8148	49.9431
WE4	4	3,6	83	79	1.10	abandoned meadow	12.8184	49.9556

* Seven of the populations were involved also in another study of the species (Z. Janovský, unpublished data) and the sampling protocol slightly differed, namely not all plants within the transect were tagged. Therefore the estimate of population density was constructed only on thoroughly searched 3×3 metres around the vegetation sample.

7.1.3 Paper I – Appendix 3 – Details of BIC-informed selection of the most suitable vegetation classification

We considered Multivariate regression trees (De'Ath, 2002), classical Twinspan analysis (Hill, 1979, as modified by Roleček et al., 2009) and sample scores from Detrended correspondence analysis (DCA) for classification of vegetation samples defining the subpopulations. We used for classification both the qualitative information on species composition of vegetation samples and semi-quantitative estimates of species abundance on the Braun-Blanquet scale.

Based upon the 1 SE of cross-validated relative error criterion (De'Ath, 2002), we resulted with a model defining 6 clusters from Multivariate regression tree analysis. The Twinspan analysis does not possess any objective criterion for the final number of clusters; therefore we considered solutions producing 2 to 15 clusters. We did not use a solution with 13 resulting clusters in subsequent computations, since Twinspan analysis delimited there a group containing one vegetation sample, to which no *S. pratensis* individuals were assigned, i.e. the resulting predictor was the same as the solution featuring 12 clusters. In case of sample scores from DCA, we considered 4 models using as predictors sample scores from the first axis, first two axes, first three axes and first four axes and in all possible second order interactions. DCA was computed by means of CANOCO 4.56 programme (ter Braak and Šmilauer, 2002), Twinspan was applied in the recommended environment of JUICE 7.0 programme (Roleček et al., 2009)

We fitted linear models to response *S. pratensis* patch-level plant characteristics and herbivore occurrence (Appendix 3, Table 2) and calculated for each predictor obtained from different classifications model BIC (Schwarz, 1978). Afterwards, we summed the obtained BICs for a given vegetation classification and picked the classification having the minimal sum.

death hill roleček schwarz ter Braak

De'Ath, G. (2002) Multivariate regression trees: a new technique for modeling species-environment relationships. *Ecology,* **83,** 1105-1117.

Hill, M.O. (1979) TWINSPAN - A FORTRAN Program for Arranging Multivariate Data in an Ordered Two-Way Table by Classification of the Individuals and Attributes. Cornell University, Ithaca, NY.

Roleček, J., Tichý, L., Zelený, D. & Chytrý, M. (2009) Modified TWINSPAN classification in which the hierarchy respects cluster heterogeneity. *Journal of Vegetation Science,* **20,** 596-602.

Schwarz, G. (1978) Estimating the Dimension of a Model. *The Annals of Statistics,* **6,** 461-464.

ter Braak, C.J.F. & Šmilauer, P. (2002) CANOCO reference manual and CanoDraw for Windows user’s guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca.

Table 2: Model BICs for each classification and response variable used in analyses; for regression trees only the solution with six clusters is taken into account (see text for explanation); T2-T15 denote Twinspan classifications with corresponding number of resulting clusters; DCA ax1,2 denotes model taking into account first two DCA axes and all their second order interactions (all other models based on DCA follow the same pattern); the classfication with minimal BIC sum is reported in bold.

Classification	**Df**	**Mortality**	**Prop. of flowering individuals**			**Vegetative size**			**Invertebrate folivores**			**Vertebrate folivores**			**Eupyhdryas aurinia**			**BIC sum**
			2010	**2011**	**pooled**	**2010**	**2011**	**pooled**	**2010**	**2011**	**pooled**	**2010**	**2011**	**pooled**	**2010**	**2011**	**pooled**	
reg. tree	7	-20.4	-4.6	21.2	-17.9	196.3	212.5	193.5	-43.1	-51.0	-59.8	-302.8	-466.3	-419.2	-226.6	-173.1	-229.8	-1391.1
T2	3	-35.1	-21.7	-0.5	-37.6	175.1	188.2	169.9	-60.3	-54.6	-72.1	-317.4	-479.5	-435.8	-240.7	-186.4	-245.5	-1654.1
T3	4	-30.7	-17.3	3.1	-33.6	172.1	189.0	168.3	-55.9	-50.5	-67.5	-314.9	-476.8	-432.0	-237.0	-182.2	-241.6	-1607.1
T4	5	-26.1	-12.9	7.6	-29.0	176.1	192.9	172.0	-51.3	-53.6	-64.9	-310.4	-472.3	-427.5	-236.6	-178.6	-239.6	-1554.3
T5	6	-22.3	-8.4	12.0	-24.5	178.9	193.9	173.7	-46.7	-49.7	-60.5	-305.9	-470.5	-424.0	-233.8	-181.0	-241.4	-1510.1
T6	7	-25.4	-7.5	13.8	-23.6	174.3	198.0	175.0	-65.4	-76.4	-85.4	-305.4	-472.4	-426.4	-229.5	-176.4	-236.8	-1569.7
T7	8	-20.8	-13.4	8.9	-33.0	174.9	201.2	176.9	-61.0	-72.8	-81.4	-302.9	-471.2	-425.7	-226.1	-172.0	-232.3	-1550.7
T8	**9**	**-16.4**	**-17.2**	**8.0**	**-37.3**	**144.7**	**189.8**	**155.6**	**-65.3**	**-68.3**	**-80.0**	**-300.7**	**-470.9**	**-421.5**	**-233.9**	**-206.3**	**-267.9**	**-1687.5**
T9	10	-11.8	-12.7	12.2	-33.0	147.2	192.7	158.1	-60.8	-64.5	-75.9	-297.3	-466.4	-418.3	-229.3	-201.7	-263.3	-1624.9
T10	11	-8.2	-16.6	16.6	-33.3	151.7	196.4	162.1	-60.0	-62.0	-74.3	-301.2	-469.2	-426.8	-230.1	-197.7	-261.7	-1614.3
T11	12	-3.6	-12.1	20.1	-29.6	156.0	201.0	166.7	-55.4	-58.1	-69.9	-296.7	-464.6	-422.3	-226.0	-193.1	-257.2	-1544.8
T12	13	-4.5	-9.2	23.3	-27.0	159.9	202.9	169.6	-51.3	-53.6	-65.4	-297.2	-462.1	-425.4	-221.4	-188.9	-252.6	-1502.8
T14	14	-20.5	-14.8	19.8	-37.4	163.7	204.8	171.8	-69.5	-62.4	-83.9	-292.7	-458.6	-420.8	-216.8	-184.3	-248.0	-1549.5
T15	15	-16.1	-10.5	23.8	-33.5	167.4	209.0	175.7	-65.3	-59.3	-80.4	-295.0	-454.0	-422.7	-212.4	-179.6	-243.5	-1496.2
DCA ax1	3	-34.2	-23.3	1.0	-38.1	176.3	198.8	175.9	-62.9	-69.0	-80.9	-320.3	-478.2	-435.8	-240.7	-184.5	-244.2	-1660.1
DCA ax1,2	5	-26.8	-16.1	6.3	-31.9	179.8	205.1	180.7	-58.0	-61.6	-74.7	-312.0	-472.1	-429.1	-235.1	-179.4	-237.3	-1562.2
DCA ax1,2,3	8	-18.0	-9.5	13.8	-26.8	188.2	215.9	189.8	-51.0	-52.6	-66.2	-299.4	-460.7	-417.5	-228.0	-166.6	-226.4	-1415.2
DCA ax1,2,3,4	12	-7.5	5.0	23.3	-15.5	196.5	223.0	196.8	-41.2	-41.6	-55.9	-282.4	-449.8	-401.8	-214.6	-151.9	-213.2	-1230.7

7.1.4 Paper I – Appendix 4 – Synoptic tables of vegetation composition

Table 3: Synoptic tables of vegetation composition of individual vegetation types identified by means of Twinspan classification method; the first column corresponding to each vegetation type contains information on percentage of samples of a given vegetation type, which contain a given plant species; second column contains median non-zero cover of a given plant species

Plant species	veg. t. 1		veg. t. 2		veg. t. 3		veg. t. 4		veg. t. 5		veg. t. 6		veg. t. 7		veg. t. 8	
	n = 8		n = 6		n = 12		n = 13		n = 12		n = 15		n = 24		n = 24	
Agrostis canina	0	0	0	0	0	0	8	2	0	0	0	0	0	0	0	0
Agrostis capillaris	50	3	17	2	8	2	0	0	0	0	7	8	4	2	0	0
Agrostis stolonifera	0	0	0	0	8	2	0	0	33	5	0	0	8	5	0	0
Achillea millefolium agg.	50	2	17	2	25	2	15	1	0	0	20	2	8	2	4	2
Ajuga reptans	0	0	0	0	17	1	8	3	0	0	0	0	4	3	17	1
Alchemilla vulgaris s.lat.	25	1	17	2	0	0	0	0	0	0	27	1	0	0	0	0
Alopecurus pratensis	0	0	17	2	0	0	0	0	0	0	40	3	0	0	0	0
Anemone nemorosa	0	0	0	0	0	0	0	0	8	2	0	0	0	0	0	0
Angelica sylvestris	25	2	83	2	33	1	62	2	50	3	80	3	92	2	88	2
Anthoxanthum odoratum s.lat.	100	8	100	3	75	3	8	2	17	3	47	3	63	3	75	3
Anthriscus sylvestris	0	0	33	1	0	0	0	0	8	1	13	2	4	1	0	0
Arnica montana	0	0	0	0	17	2	0	0	0	0	0	0	0	0	0	0
Avenula pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	1
Avenula pubescens	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0
Bistorta major	38	2	100	3	8	1	69	3	8	2	60	2	79	8	58	3
Briza media	13	1	50	2	50	2	8	2	17	2	7	2	38	2	33	2
Caltha palustris	0	0	0	0	0	0	0	0	25	8	0	0	25	1	33	3
Cardamine pratensis	13	2	0	0	17	2	54	2	75	2	0	0	33	2	71	2
Carex canescens	0	0	0	0	0	0	15	3	8	3	0	0	0	0	29	2
Carex davalliana	0	0	0	0	0	0	0	0	33	3	0	0	4	2	0	0
Carex echinata	0	0	0	0	0	0	0	0	17	2	0	0	13	8	0	0
Carex hirta	25	2	17	2	0	0	0	0	0	0	20	2	0	0	0	0
Carex leporina	0	0	0	0	0	0	0	0	0	0	33	2	8	5	0	0
Carex nigra	13	2	33	2	75	2	46	8	92	8	87	8	50	8	88	18
Carex pallescens	13	3	0	0	25	3	0	0	0	0	40	2	8	2	4	2
Carex panicea	0	0	100	2	50	5	23	2	58	8	73	2	79	8	71	3
Carex pilulifera	0	0	17	4	58	3	0	0	0	0	13	3	0	0	4	2
Carex pulicaris	0	0	0	0	17	2	0	0	0	0	0	0	4	4	0	0
Carex rostrata	0	0	17	3	0	0	85	38	0	0	0	0	0	0	4	2
Carex vulpina	13	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea jacea	0	0	0	0	0	0	0	0	0	0	0	0	13	2	0	0
Cerastium arvense	13	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerastium holosteoides	25	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cirsium arvense	0	0	0	0	8	2	0	0	0	0	0	0	0	0	0	0
Cirsium heterophyllum	0	0	17	8	67	13	31	5	0	0	13	2	33	3	13	2
Cirsium oleraceum	0	0	0	0	0	0	0	0	0	0	0	0	4	2	8	3
Cirsium palustre	13	3	83	3	58	1	77	3	92	3	53	2	75	3	83	3
Clinopodium vulgare	0	0	0	0	0	0	0	0	0	0	0	0	13	2	0	0
Crepis mollis	38	2	100	2	50	2	46	1	0	0	7	2	88	2	71	2
Crepis paludosa	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0
Dactylis glomerata	0	0	0	0	0	0	0	0	0	0	0	0	4	8	4	1
Dactylorhiza majalis	0	0	0	0	0	0	0	0	0	0	0	0	21	1	17	1
Deschampsia cespitosa	75	8	83	2	58	3	8	18	8	3	93	5	96	8	17	2
Epilobium angustifolium	0	0	0	0	8	1	0	0	0	0	13	2	8	1	8	3

Plant species	**veg. t. 1**		**veg. t. 2**		**veg. t. 3**		**veg. t. 4**		**veg. t. 5**		**veg. t. 6**		**veg. t. 7**		**veg. t. 8**	
Epilobium palustre	13	1	0	0	0	0	46	2	50	2	7	1	58	2	50	2
Equisetum fluviatile	0	0	0	0	42	2	31	2	25	2	7	1	38	2	46	1
Equisetum palustre	0	0	50	2	33	1	85	2	33	2	0	0	88	2	42	2
Equisetum sylvaticum	0	0	33	1	25	2	0	0	8	2	0	0	29	2	4	1
Eriophorum angustifolium	0	0	0	0	8	2	38	2	50	3	0	0	4	3	46	3
Festuca ovina	25	2	100	38	100	28	38	3	17	28	53	3	88	38	88	18
Festuca pratensis s.str.	0	0	0	0	0	0	0	0	8	38	13	1	4	1	0	0
Festuca rubra agg.	38	18	33	3	8	3	31	2	75	8	100	38	21	3	42	3
Filipendula ulmaria	0	0	0	0	0	0	31	10	25	18	7	2	8	1	13	2
Fragaria vesca	13	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo agg.	13	4	0	0	0	0	0	0	17	5	13	2	0	0	0	0
Galium palustre agg.	0	0	0	0	8	2	38	2	58	2	40	2	13	1	33	2
Galium uliginosum	63	2	67	2	58	2	92	2	100	2	93	2	83	2	92	2
Galium verum agg.	13	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Geranium pratense	0	0	0	0	0	0	0	0	0	0	0	0	8	1	0	0
Geum rivale	0	0	17	2	0	0	8	2	0	0	0	0	17	8	4	18
Glyceria species	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1
Hieracium species	25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Holcus lanatus	50	2	83	2	50	1	15	2	17	1	40	2	75	2	58	2
Holcus mollis	13	2	0	0	0	0	0	0	0	0	0	0	0	0	4	3
Hypericum maculatum	63	4	50	1	8	2	0	0	0	0	13	2	4	1	4	1
Juncus articulatus	0	0	0	0	0	0	0	0	0	0	7	2	0	0	0	0
Juncus conglomeratus	0	0	0	0	8	2	23	2	8	2	20	3	71	3	42	3
Juncus effusus	0	0	0	0	17	2	23	2	50	3	60	3	13	3	13	3
Juncus filiformis	0	0	0	0	0	0	0	0	17	2	47	3	8	2	8	20
Knautia arvensis agg.	13	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lathyrus pratensis	38	2	67	2	17	1	23	2	17	2	60	3	50	2	88	2
Leucanthemum vulgare agg.	0	0	0	0	17	2	0	0	0	0	7	1	0	0	0	0
Lotus pedunculatus	0	0	0	0	0	0	0	0	0	0	0	0	4	8	8	2
Luzula campestris agg.	50	2	100	2	92	2	23	1	0	0	27	2	38	2	75	2
Lycopus europaeus	0	0	0	0	0	0	0	0	17	2	0	0	0	0	0	0
Lychnis flos-cuculi	25	1	33	1	8	1	8	2	33	2	33	2	29	2	46	2
Lychnis viscaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2
Lysimachia vulgaris	0	0	0	0	0	0	8	18	67	2	7	2	0	0	0	0
Melampyrum sylvaticum	0	0	0	0	0	0	0	0	17	1	0	0	4	2	0	0
Mentha arvensis	0	0	17	2	0	0	23	2	25	3	40	2	42	2	25	1
Menyanthes trifoliata	0	0	0	0	0	0	0	0	8	2	0	0	0	0	0	0
Myosotis palustris agg.	0	0	0	0	0	0	23	2	33	5	47	2	46	2	0	0
Nardus stricta	50	50	100	8	100	28	8	3	0	0	33	3	58	6	63	18
Persicaria amphibia	0	0	0	0	0	0	0	0	8	1	0	0	0	0	0	0
Peucedanum palustre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3
Phleum pratense agg.	0	0	0	0	0	0	0	0	0	0	20	4	0	0	0	0
Pimpinella saxifraga s.str.	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0
Plantago lanceolata	38	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa palustris	0	0	17	2	0	0	23	2	100	3	60	2	38	2	29	2
Poa pratensis s.lat.	75	23	33	1	0	0	0	0	0	0	20	2	4	2	4	1
Poa trivialis	0	0	0	0	0	0	0	0	8	8	0	0	4	8	0	0
Polygala vulgaris	0	0	0	0	67	2	0	0	0	0	0	0	0	0	0	0
Potentilla erecta	88	2	100	3	92	3	77	3	75	3	67	2	92	3	100	3
Potentilla palustris	0	0	0	0	0	0	38	18	58	18	7	3	13	8	46	3
Ranunculus acris	100	2	83	2	50	2	23	2	75	2	80	2	75	2	96	2
Ranunculus auricomus agg.	0	0	67	2	8	2	23	2	58	2	53	2	58	2	79	2
Ranunculus flammula	0	0	0	0	0	0	0	0	0	0	7	1	0	0	4	2

Plant species	veg. t. 1		veg. t. 2		veg. t. 3		veg. t. 4		veg. t. 5		veg. t. 6		veg. t. 7		veg. t. 8	
Ranunculus repens	0	0	17	3	0	0	0	0	0	0	13	5	4	2	0	0
Rumex acetosa	50	2	33	1	8	3	69	2	75	2	67	2	79	2	83	2
Rumex crispus	0	0	0	0	0	0	0	0	8	8	0	0	0	0	0	0
Rumex species	0	0	0	0	0	0	8	1	8	2	0	0	0	0	4	1
Sanguisorba officinalis	0	0	0	0	0	0	0	0	0	0	7	3	0	0	0	0
Scirpus sylvaticus	0	0	0	0	0	0	8	38	0	0	0	0	0	0	0	0
Scorzonera humilis	0	0	50	18	0	0	0	0	0	0	0	0	0	0	8	2
Scutellaria galericulata	0	0	0	0	0	0	8	2	8	2	0	0	13	2	13	2
Succisa pratensis	75	3	83	3	83	5	92	3	58	3	80	3	75	3	71	3
Taraxacum sect. Ruderalia	0	0	17	1	0	0	0	0	0	0	7	1	0	0	0	0
Tephroseris crispa	0	0	0	0	0	0	31	2	17	2	0	0	29	2	58	2
Trifolium medium	0	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0
Trifolium pratense	50	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium repens	0	0	0	0	0	0	0	0	0	0	33	2	0	0	0	0
Trifolium spadiceum	0	0	0	0	0	0	0	0	0	0	7	2	0	0	4	2
Vaccinium gaultherioides	0	0	0	0	8	3	0	0	0	0	0	0	0	0	0	0
Vaccinium vitis-idaea	0	0	0	0	8	2	0	0	0	0	0	0	0	0	0	0
Valeriana dioica	0	0	0	0	67	2	38	3	67	3	20	1	33	3	42	2
Valeriana officinalis	0	0	0	0	0	0	23	3	0	0	7	3	0	0	0	0
Veronica chamaedrys agg.	100	3	33	1	0	0	0	0	0	0	13	1	21	1	0	0
Veronica officinalis	0	0	0	0	33	2	0	0	0	0	0	0	4	2	0	0
Vicia cracca	25	2	0	0	8	1	8	2	0	0	13	2	8	1	0	0
Vicia species	0	0	67	1	0	0	23	2	0	0	13	1	0	0	13	1
Vicia tetrasperma	50	1	33	1	0	0	0	0	0	0	0	0	0	0	4	2
Viola canina	13	1	0	0	50	1	0	0	0	0	0	0	0	0	0	0
Viola palustris	0	0	0	0	8	1	15	1	33	2	0	0	8	1	0	0

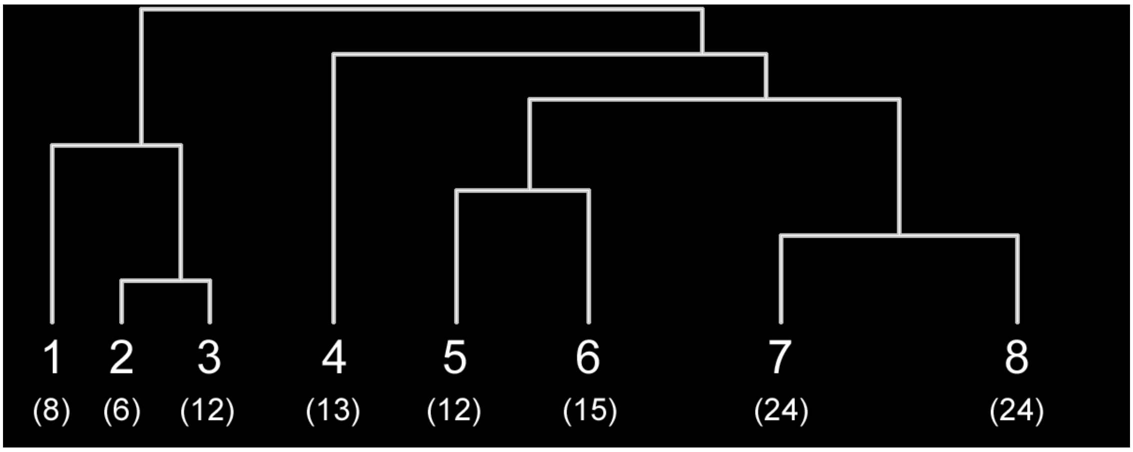


Fig. 6: Twinspan dendrogram describing the division of samples into clusters referred as vegetation types; numbers in brackets denote number of samples in a given cluster

7.2 Appendices of Paper II

This supplementary material contains six appendices:

Appendix 1 – Abundances and numbers of recorded pollinators for all plant species recorded in the study (Table A1, A2).
Appendix 2 – Results of DCA analysis of plot vegetation composition (Fig. A1, A2).
Appendix 3 – Maps of delimited sectors for individual plant species under study (see Fig. 1 in the article) and their pollinator assemblages (Fig. A3).
Appendix 4 – Maps of pollinator assemblages at individual plots with more than five recorded pollinators according to focal species (Fig. A4).
Appendix 5 – Ordination diagrams of the results of RDA analyses of pollinator spectra from individual plots (Fig. A5, A6, A7).
Appendix 6 – Maps of occurrence and abundance of the eight focal plant species in the studied plots (Fig. A8).

7.2.1 Paper II – Appendix 1 – Abundances and numbers of recorded pollinators for all plant species recorded in the study

Table 1: Abundances and numbers of recorded pollinators for plants species recorded by direct counting of the flowering stalks; species given in bold were included in analyses

Plant name	Plant abbr.	No. of occupied plots	No. of flowering stalks	No. of recorded pollinators
Ranunculus acris	**Ran_acr**	**68**	**2776**	**514**
Centaurea jacea	**Cen_jac**	**62**	**1707**	**926**
Achillea ptarmica	Ach_pta	56	847	111
Sanguisorba officinalis	**San_off**	**52**	**888**	**526**
Hypericum spp.*	**Hyp_spp**	**41**	**1732**	**291**
Pimpinella saxifraga	Pim_sax	35	379	156
Crepis biennis	Cre_bie	24	318	76
Selinum carvifolia	**Sel_car**	**24**	**285**	**355**
Succisa pratensis	**Suc_pra**	**17**	**203**	**414**
Serratula tinctoria	Ser_tin	16	96	25
Achillea millefolium agg.	Ach_mil	13	113	69
Leucanthemum vulgare agg.	Leu_vul	12	28	25
Cirsium palustre	Cir_pal	10	19	13
Angelica sylvestris	**Ang_syl**	**9**	**100**	**1281**
Daucus carota	Dau_car	9	29	134
Heracleum sphondylium	Her_sph	6	14	166
Cirsium arvense	Cir_arv	5	13	9
Lythrum salicaria	Lyt_sal	4	57	34

* *Hypericum maculatum* and *H. perforatum* are reported here together, since they were not distinguished during the pollinator census. *H. maculatum* is much more common with 37 occupied plots and 1710 flowering stalks compared to *H. perforatum* with 9 occupied plots and 22 flowering stalks.

Table 2: Abundances and numbers of recorded pollinators for plants species recorded semi-quantitatively by direct counting of the flowering stalks; species given in bold were included in analyses

Plant name	Plant abbr.	No. of occupied plots	No. of occupied subplots	No. of recorded pollinators
Trifolium repens	Tri_rep	72	1003	132
Trifolium hybridum	**Tri_hyb**	**66**	**902**	**327**
Plantago lanceolata	Pla_lan	63	1380	148
Lathyrus pratensis	Lat_pra	51	489	39
Prunella vulgaris	Pru_vul	51	368	21
Lotus corniculatus	Lot_cor	45	819	21
Galium album	Gal_alb	43	695	13
Potentilla erecta	Pot_ere	41	582	16
Alchemilla sp.	Alc_spe	40	159	0
Trifolium pratense	Tri_pra	34	256	75
Mentha arvensis	Men_arv	27	152	7
Ranunculus flammula	Ran_fla	26	250	36
Cerstium holosteoides	Cer_hol	20	57	0
Myosotis sp.	Myo_arv	18	61	3
Vicia tetrasperma	Vic_tet	16	48	0
Galium uliginosum	Gal_uli	15	36	0
Potentilla anserina	Pot_ans	10	64	5
Polygonum sp.	Pol_spe	7	21	0
Epilobium sp.	Epi_spe	7	9	0
Campanula patula	Cam_pat	7	7	0
Lysimachia vulgaris	Lys_vul	4	45	0
Anagallis arvensis	Ana_arv	4	20	0
Lysimachia nummularia	Lys_num	4	9	0
Stellaria graminea	Ste_gra	4	4	0
Leontodon hispidus	Leo_his	3	44	34
Gnaphalium sylvaticum	Gna_syl	3	5	0
Scutellaria galericulata	Scu_gal	2	10	0
Trifolium dubium	Tri_dub	2	4	0
Leontodon autumnalis	Leo_aut	2	2	0
Vicia hirta	Vic_hir	1	6	0
Lycopus europaeus	Lyc_eur	1	5	0
Odontites vernus	Odo_ver	1	2	0
Vicia cracca	Vic_cra	1	2	0
Vicia sepium	Vic_sep	1	2	0
Cirisium canescens	Cir_can	1	1	18
Lychnis flos-cuculi	Lyc_flo	1	1	0
Medicago lupulina	Med_lup	1	1	0
Polygonum aviculare	Pol_avi	1	1	0

Table 3: Factors included in forward selection of variables in RDA analyses of pollinator composition on focal species. Abbreviations: A. syl. – *Angelica sylvestris*, C. jac. – *Centaurea jacea*, H. spp. – *Hypericum* spp., R. acr. – *Ranunculus acris*, S. off. – *Sanguisorba officinalis*, S. car. – *Selinum carvifolia*, S. pra. – *Succisa pratensis*, T. hyb. – *Trifolium hybridum*

factors included in forw. selection	**A. syl.**	**C. jac.**	**H. spp.**	**R. acr.**	**S. off.**	**S. car.**	**S. pra.**	**T. hyb.**
DCA axis 1	+	+	+	+	+	+	+	+
DCA axis 2	+	+	+	+	+	+	+	+
meadow/verge	+						+	
log abund. A. syl.	+							
log abund. C. jac.		+	+	+	+	+	+	+
log abund. H. spp.		+	+		+	+		+
log abund. R. acr.	+	+	+	+	+	+	+	+
log abund. S. off.	+	+	+	+	+	+	+	+
log abund. S. car.		+	+		+	+		+
log abund. S. pra.				+			+	
log abund. T. hyb.	+	+	+	+	+	+		+

Table 4: Synoptic table of flowering plant species composition of the sectors at the study meadow. Species occurrences expressed as percentage of plots within the group a given species occurs in; plant species sorted according to decreasing overall frequency.

Sector	1	2	3	4	5	6	verges
No. of plots	22	15	12	25	8	11	10

Species				**Relative frequency (%)**			
Trifolium repens	59	100	58	80	100	82	0
Trifolium hybridum	32	100	83	88	75	55	0
Plantago lanceolata	18	100	100	96	75	18	0
Ranunculus acris	55	60	58	56	100	82	0
Centaurea jacea	50	73	75	80	38	9	10
Achillea ptarmica	82	33	50	36	88	36	70
Sanguisorba officinalis	64	53	100	32	25	45	30
Lathyrus pratensis	32	80	75	64	88	0	0
Prunella vulgaris	32	40	50	76	75	64	0
Lotus corniculatus	9	67	50	92	38	0	10
Galium album s.lat.	0	67	67	88	13	18	0
Potentilla erecta	91	40	25	12	13	27	50
Alchemilla species	0	67	92	76	0	0	0
Pimpinella saxifraga s.str.	0	47	58	80	13	0	0
Trifolium pratense	18	47	17	56	38	36	0
Hypericum maculatum	0	53	42	72	0	0	10
Mentha arvensis	32	7	8	24	38	64	20
Ranunculus flammula	41	7	0	0	38	100	20
Crepis biennis	0	53	25	52	0	0	0
Selinum carvifolia	0	27	75	36	0	9	10
Cerastium holosteoides	0	13	50	32	0	36	0
Myosotis arvensis	9	7	0	28	38	45	0
Succisa pratensis	50	13	0	0	0	0	40
Serratula tinctoria	14	20	75	4	0	0	0
Vicia tetrasperma	0	27	8	44	0	0	0
Galium uliginosum	27	13	8	4	13	36	0

Achillea millefolium agg.	0	0	17	44	0	0	0
Leucanthemum vulgare	0	27	17	24	0	0	0
Cirsium palustre	23	13	0	0	13	9	10
Potentilla anserina	0	20	8	16	25	0	0
Daucus carota	0	7	8	28	0	0	0
Hypericum perforatum	0	0	17	16	0	27	0
Angelica sylvestris	0	0	8	0	0	18	60
Campanula patula	0	7	8	20	0	0	0
Epilobium species	0	0	8	8	13	18	10
Polygonum species	0	0	0	4	13	45	0
Heracleum sphondylium	0	27	0	8	0	0	0
Cirsium arvense	0	0	8	12	0	9	0
Lythrum salicaria	5	0	0	0	0	9	20
Lysimachia nummularia	0	0	0	0	38	9	0
Anagallis arvensis	0	0	0	16	0	0	0
Stellaria graminea	0	0	0	16	0	0	0
Lysimachia vulgaris	0	0	0	0	0	0	40
Leontodon hispidus	0	0	0	12	0	0	0
Gnaphalium sylvaticum	0	0	0	8	0	9	0
Trifolium dubium	0	0	8	4	0	0	0
Leontodon autumnalis	0	0	0	4	13	0	0
Scutellaria galericulata	0	0	0	0	0	0	20
Vicia hirsuta	0	7	0	0	0	0	0
Vicia sepium	0	7	0	0	0	0	0
Lychnis flos-cuculi	5	0	0	0	0	0	0
Odontites vernus	0	0	8	0	0	0	0
Cirsium canum	0	0	8	0	0	0	0
Polygonum aviculare	0	0	0	4	0	0	0
Medicago lupulina	0	0	0	4	0	0	0
Vicia cracca agg.	0	0	0	4	0	0	0
Lycopus europaeus	0	0	0	0	0	0	10

7.2.2 Paper II – Appendix 2 – Results of DCA analysis of plot vegetation composition

Altogether 57 flowering plant species were recorded within 103 plots. The first axis explained 14.9% of variability in lowering plant species composition; the second axis explained 7.7% of variation. Downweighting of rare species was applied. The length of the gradient of the first axis was 5.441 suggesting the selected unimodal technique was an appropriate choice. The depicted axes could be interpreted as wetness and nutrient or meadow/verge gradient respectively (Figs. 1 and 2).

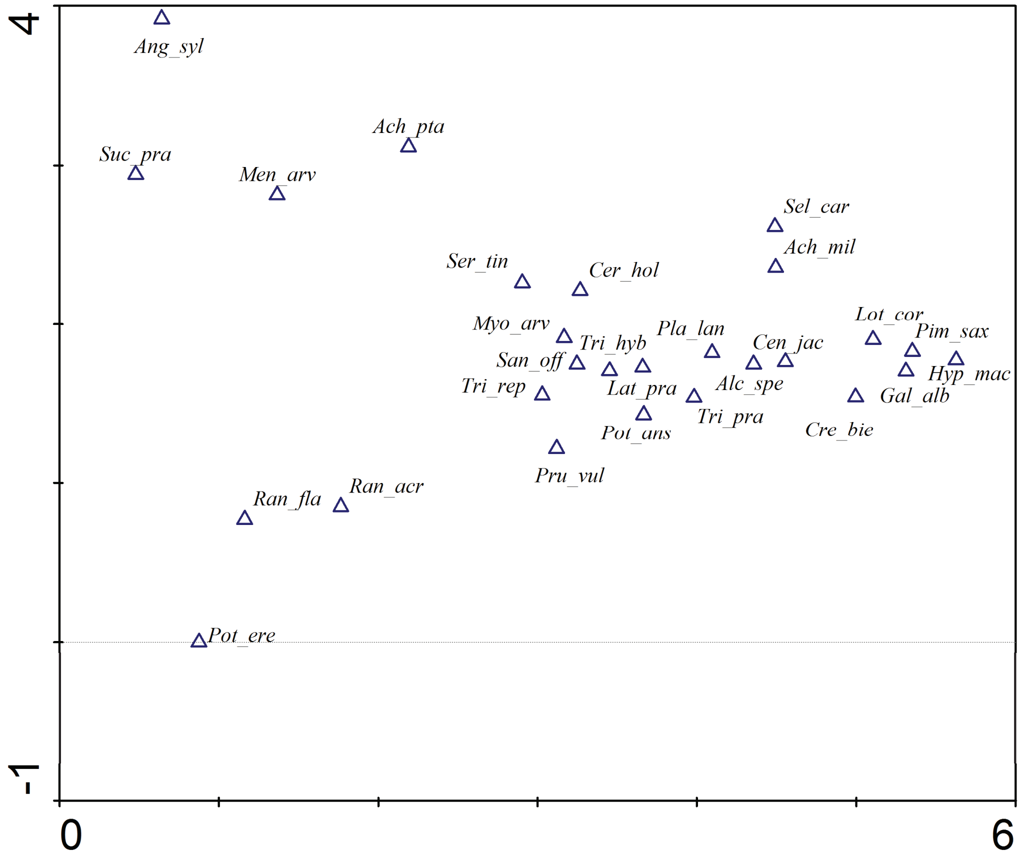


Fig. 1: Ordination diagram of species centroids for DCA of flowering species composition; first and second axis depicted with 14.9% and 7.7% of variability explained respectively; only species with weight greater than 2% shown; for explanation of abbreviations, see Appendix 1, Tables 1 and 2.

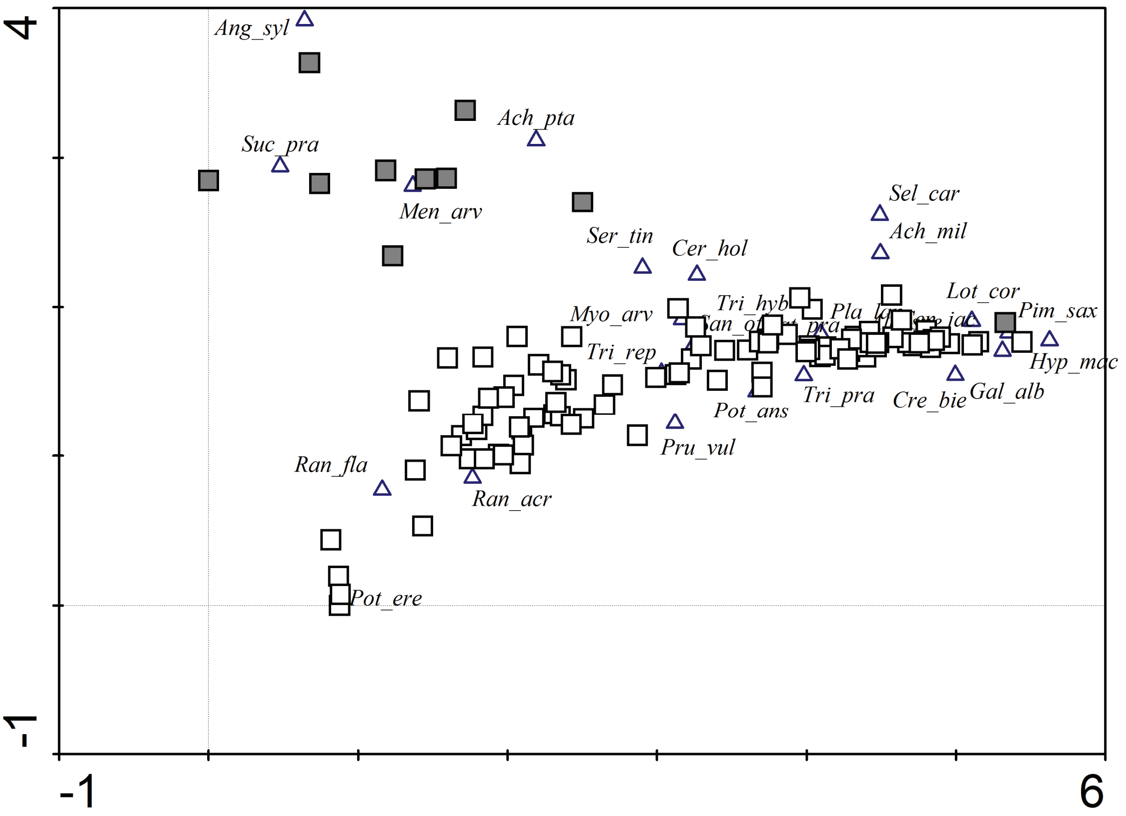


Fig. 2: Ordination diagram for species centroids (triangle) and sample scores for DCA of flowering species composition; first and second axis depicted with 14.9% and 7.7% of variability explained respectively; white squares denote meadow plots and grey ones verge plots; only species with weight greater than 2% shown; for explanation of abbreviations, see Appendix 1, Tables 1 and 2.

7.2.3 Paper II – Appendix 3 – Maps of delimited sectors for individual plant species under study and their pollinator assemblages.

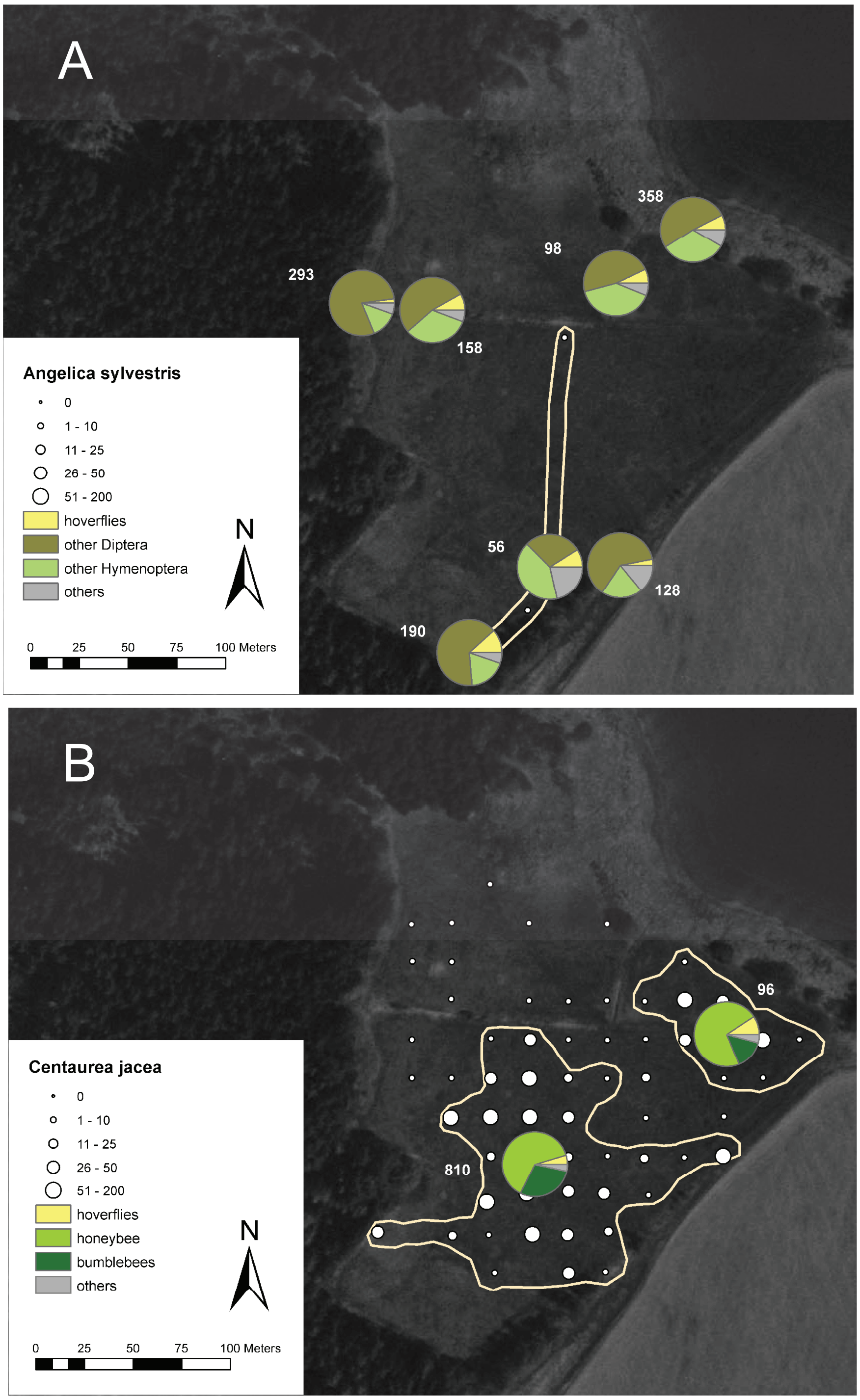


Fig. 3: Maps of delimited sectors for individual plant species under study and their pollinator assemblages; numbers next to pies indicate number of pollinators the pie is based on. Others denotes always all remaining distinguished pollinator functional groups, which do not have a separate field; abundance of focal plant species depicted on background, (for complete legend please see Appendix 6, Fig. 8); A) *A. sylvestris*; B) *C. jacea*.

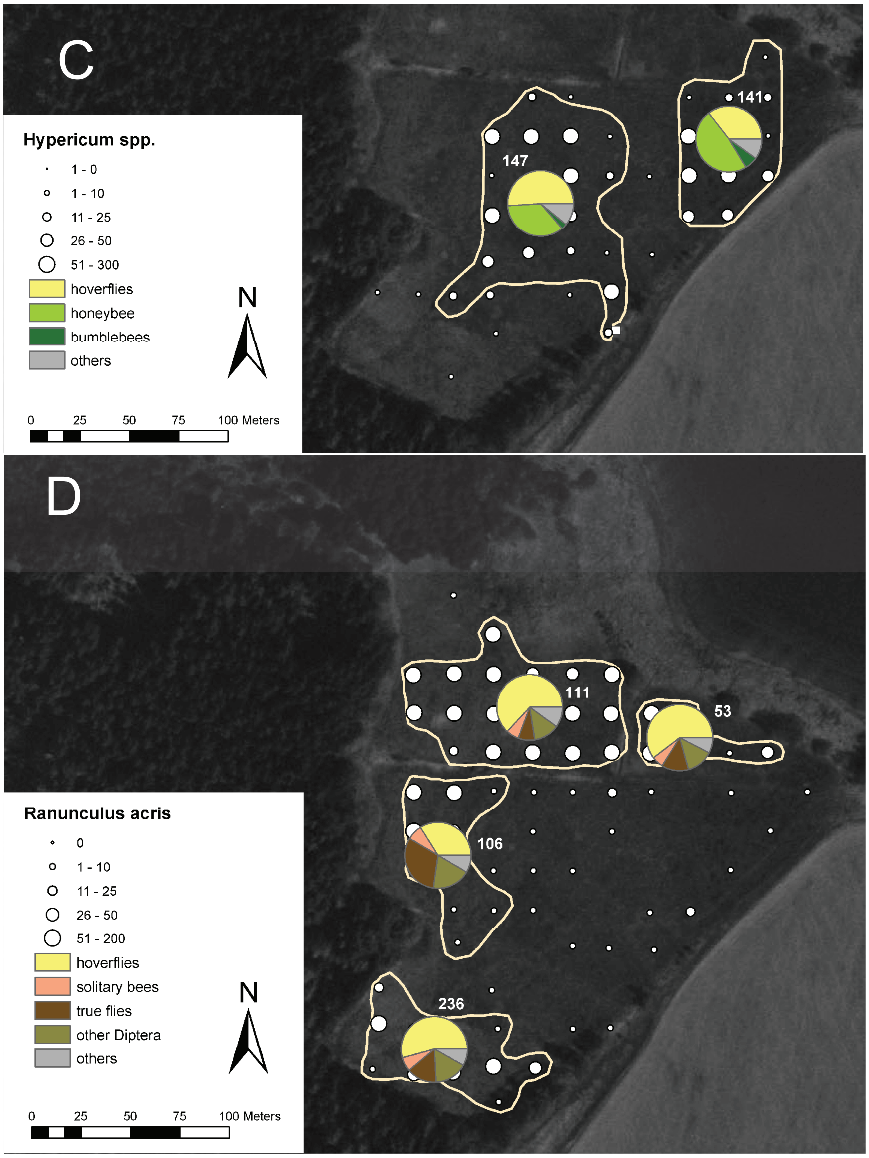


Fig. 3 (continued)**:** Maps of delimited sectors for individual plant species under study and their pollinator assemblages; numbers next to pies indicate number of pollinators the pie is based on. Others denotes always all remaining distinguished pollinator functional groups, which do not have a separate field; abundance of focal plant species depicted on background, (for complete legend please see Appendix 6, Fig. 8); C) *Hypericum* spp.; D) *R. acris*. Please note that only one sector was delimited in *S. carvifolia* and therefore it was not included into analysis of pollinator assemblages according to sectors.

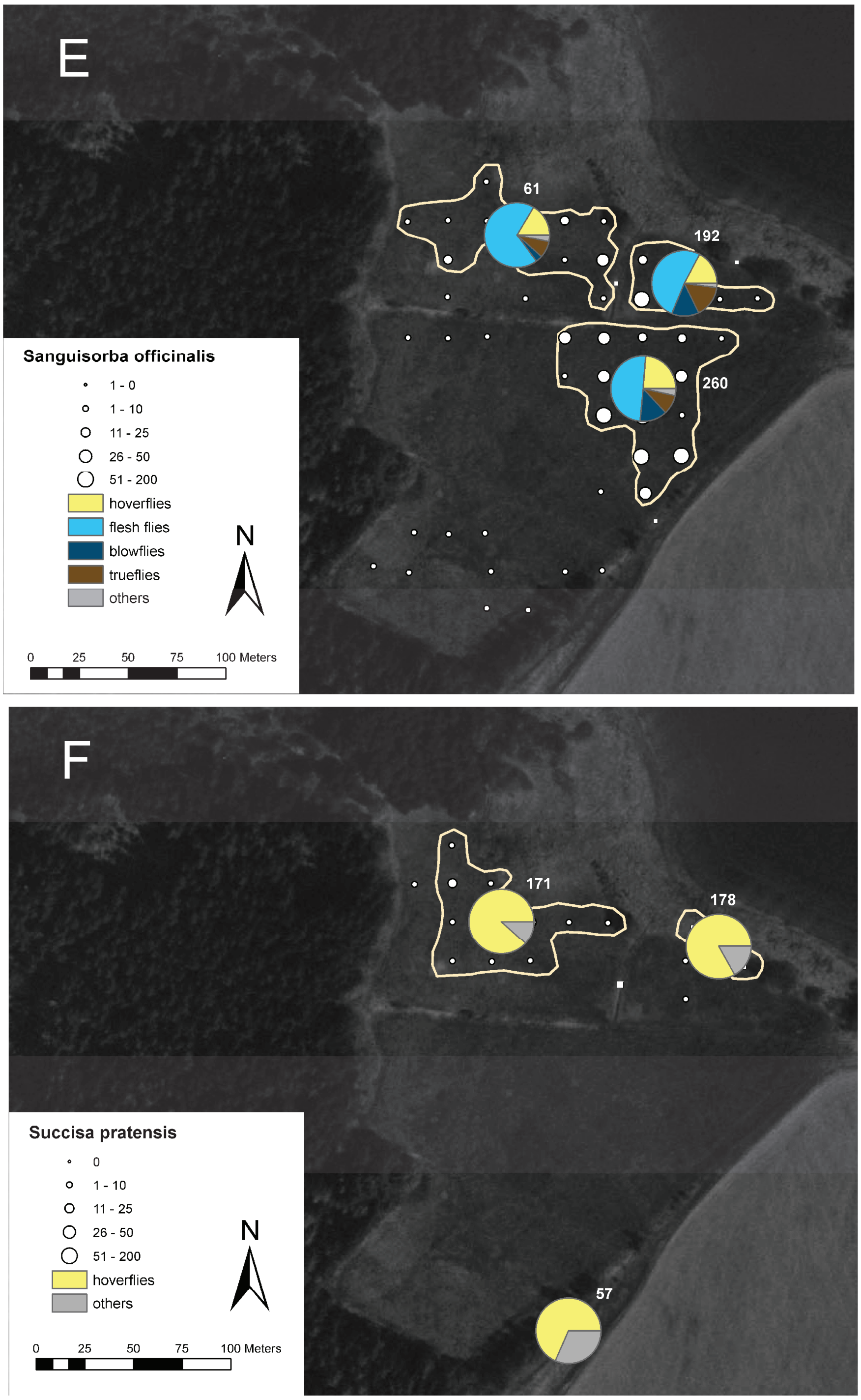


Fig. 3 (continued)**:** Maps of delimited sectors for individual plant species under study and their pollinator assemblages; numbers next to pies indicate number of pollinators the pie is based on. Others denotes always all remaining distinguished pollinator functional groups, which do not have a separate field; abundance of focal plant species depicted on background, (for complete legend please see Appendix 6, Fig. 8); E) *S. officinalis*; F) *S. pratensis*.

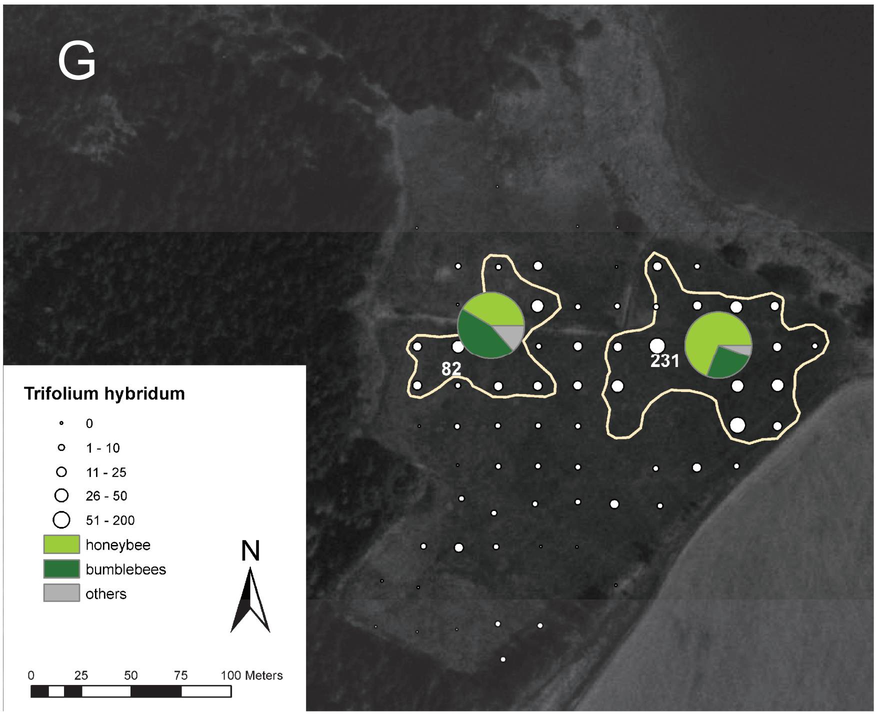


Fig. 3 (continued)**:** Maps of delimited sectors for individual plant species under study and their pollinator assemblages; numbers next to pies indicate number of pollinators the pie is based on. Others denotes always all remaining distinguished pollinator functional groups, which do not have a separate field; abundance of focal plant species depicted on background, (for complete legend please see Appendix 6, Fig. 8); G) *T. hybridum*.

7.2.4 Paper II – Appendix 4 – Maps of pollinator assemblages at individual plots with more than five recorded pollinators according to focal species.

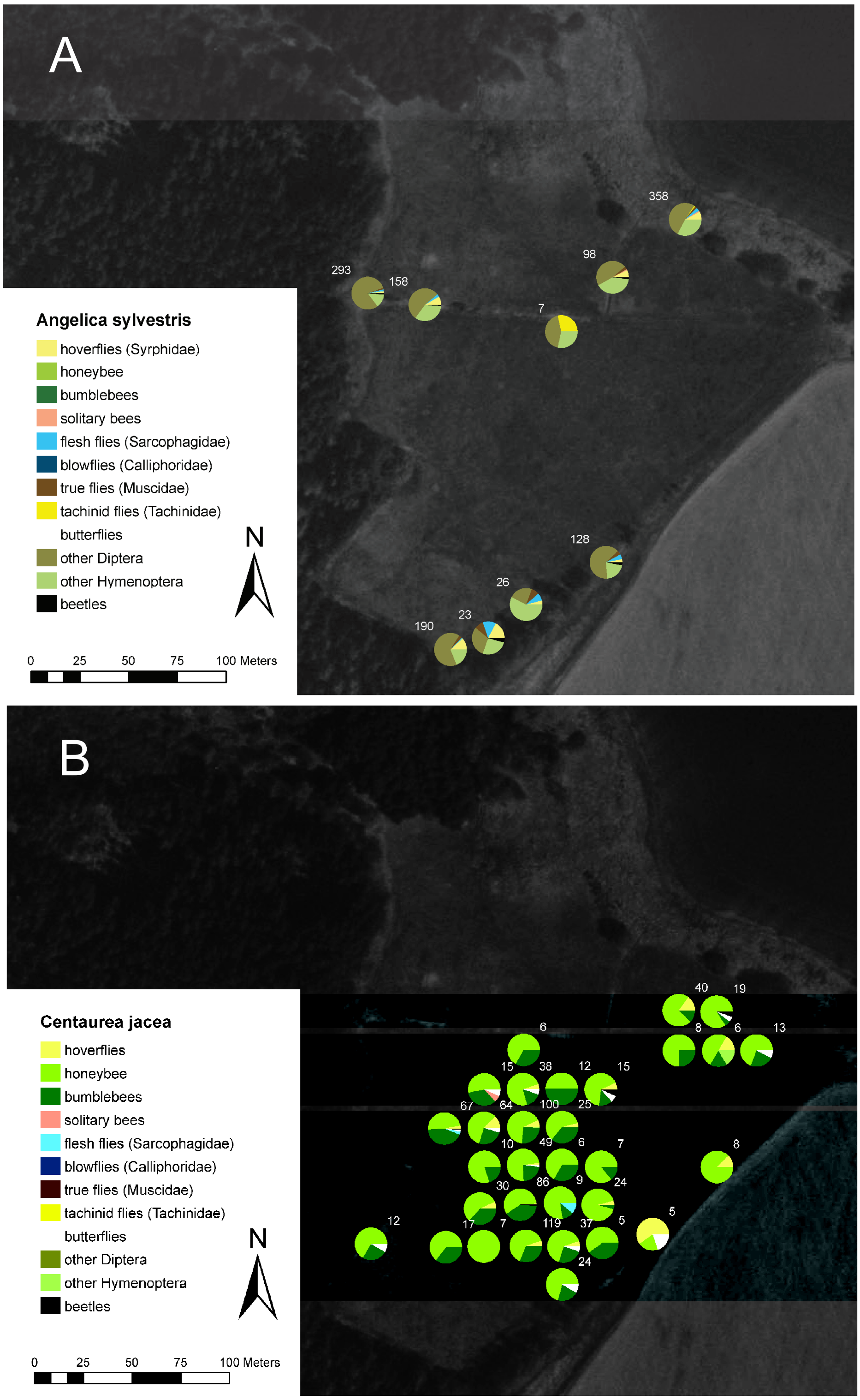


Fig. 4: Pollinator assemblages at individual plots with more than five recorded pollinators on focal species; numbers next to pies indicate number of pollinators the pie is based on. A) *A. sylvestris*; B) *C. jacea*.

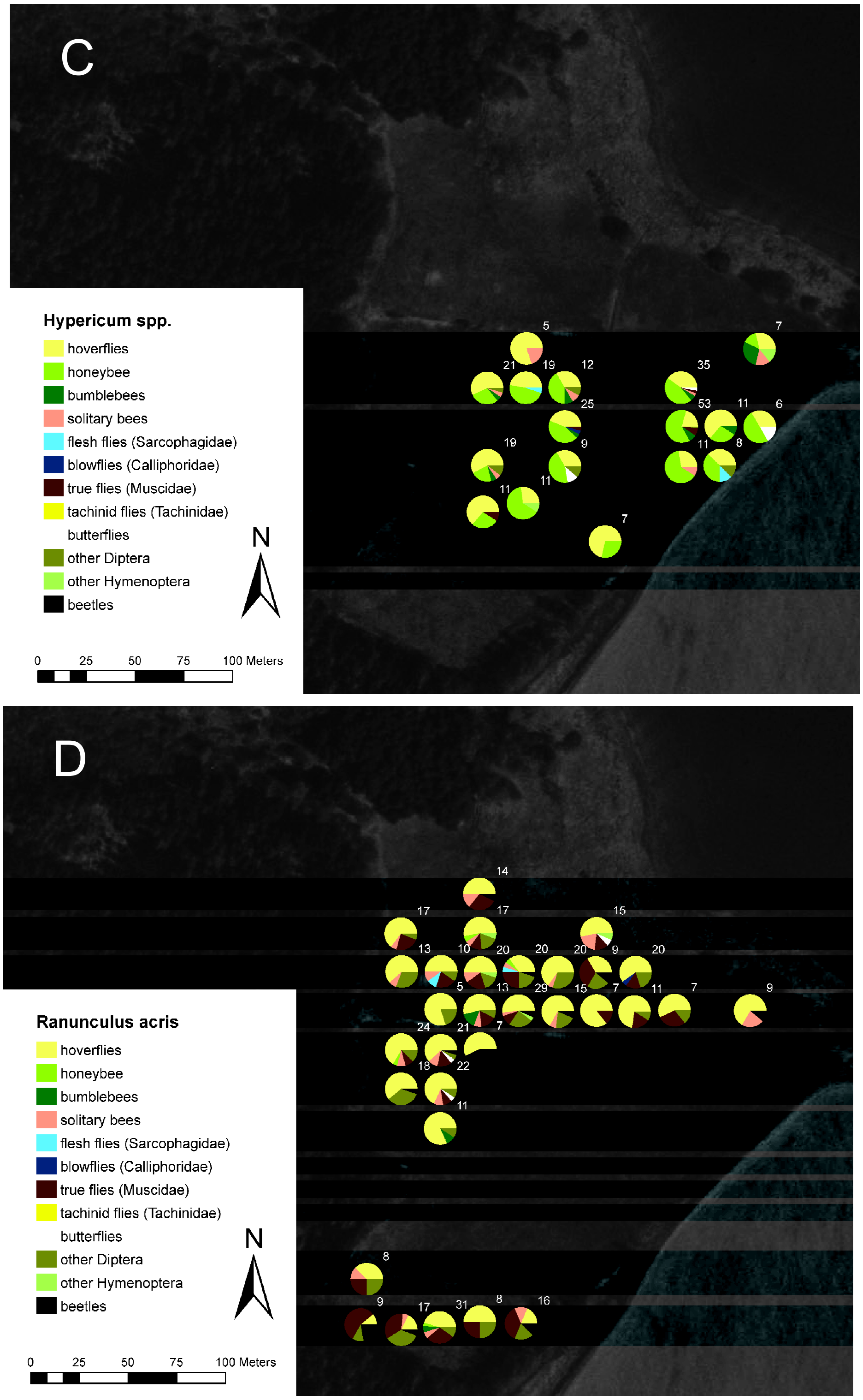


Fig. 4 (continued)**:** Pollinator assemblages at individual plots with more than five recorded pollinators on focal species; numbers next to pies indicate number of pollinators the pie is based on. C) *Hypericum* spp.; D) *R. acris*.

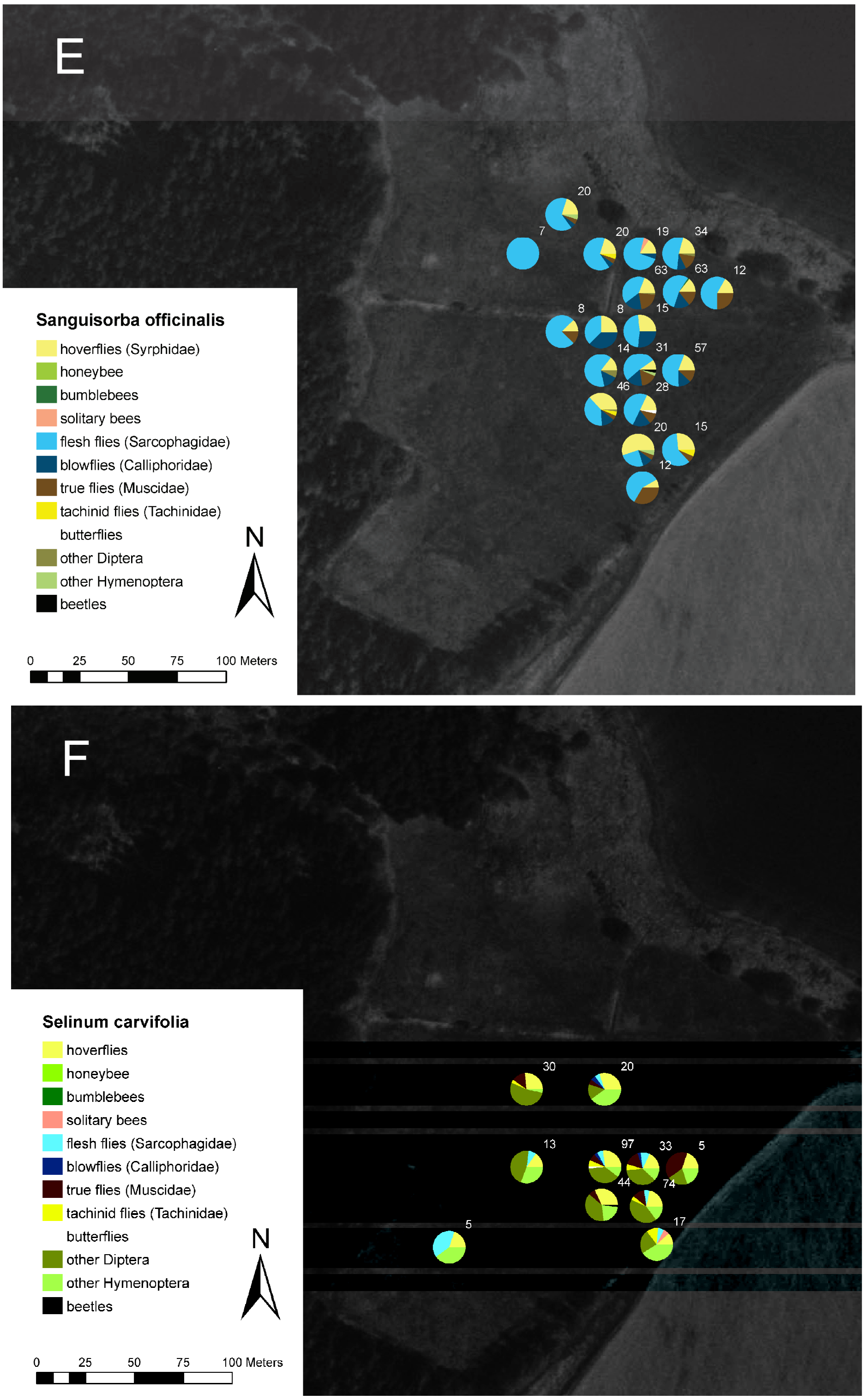


Fig. 4 (continued)**:** Pollinator assemblages at individual plots with more than five recorded pollinators on focal species; numbers next to pies indicate number of pollinators the pie is based on. E) *S. officinalis*; F) *S. carvifolia*.

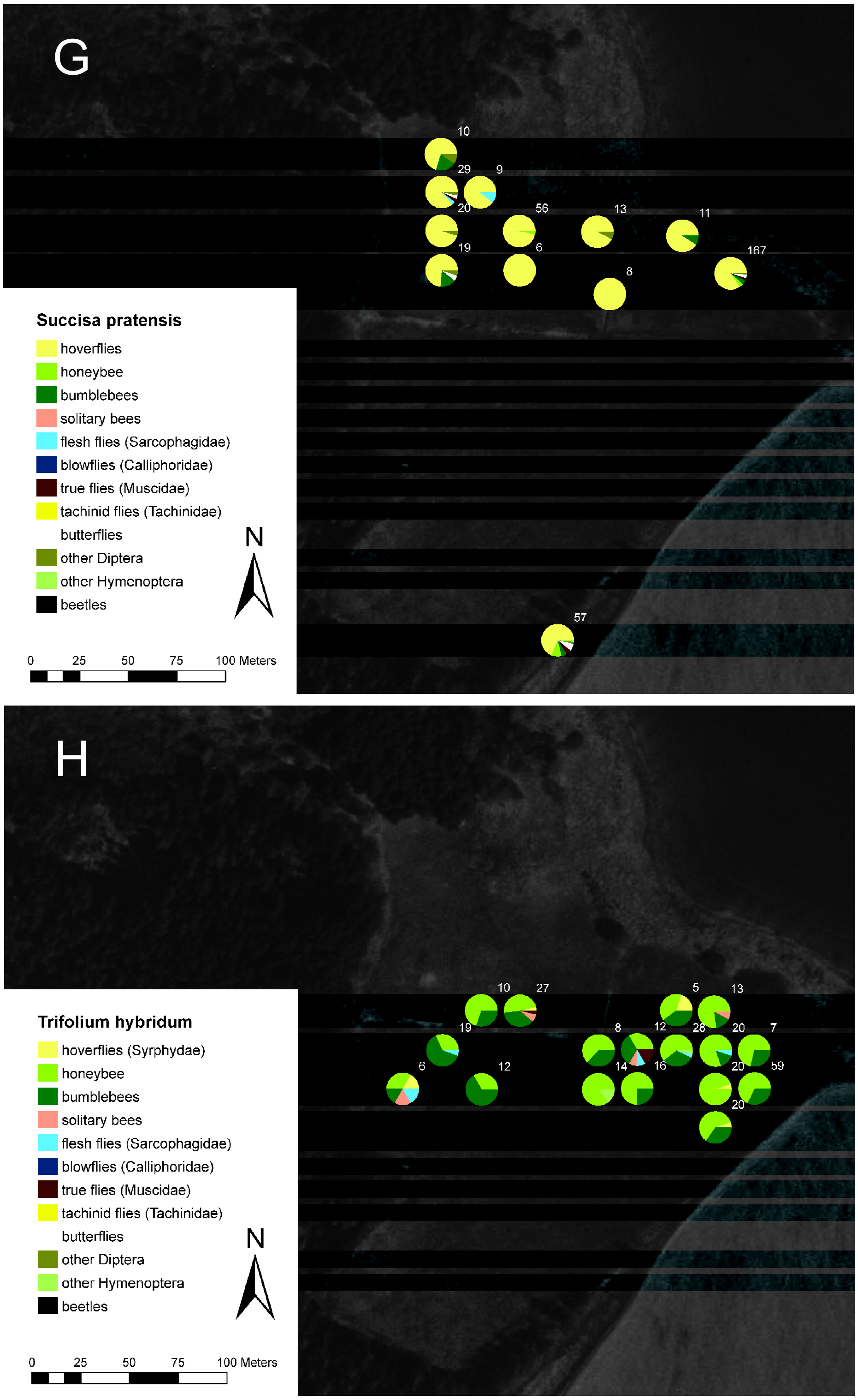


Fig. 4 (continued)**:** Pollinator assemblages at individual plots with more than five recorded pollinators on focal species; numbers next to pies indicate number of pollinators the pie is based on. G) *S. pratensis*; H) *T. hybridum*.

7.2.5 Paper II – Appendix 5 – Ordination diagrams of the results of RDA analyses of pollinator spectra from individual plots

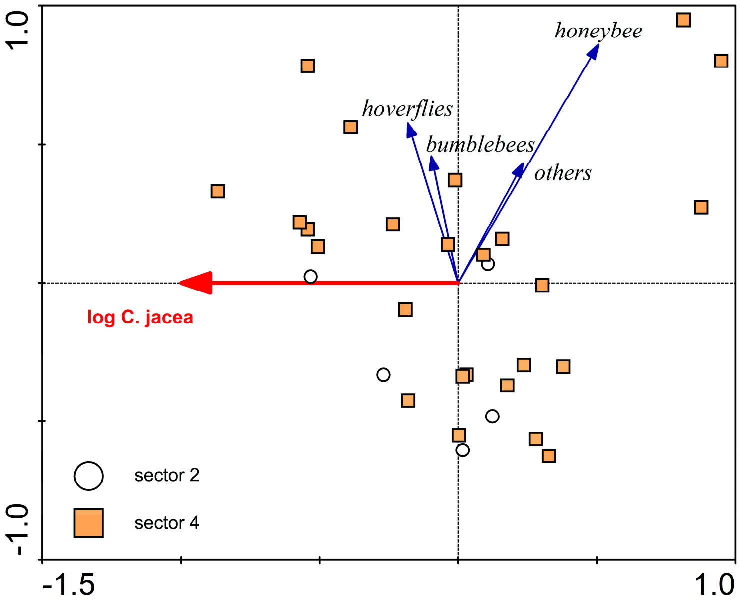


Fig. 5: Ordination diagram of RDA analysis of pollinator densities on *Centaurea jacea*, forward selection has identified as environmental variables included into the final model only logarithm of flowering stalk abundance of *C. jacea* (log C. jacea); plots are categorized according to the sector of origin (see Fig. 1 for definition of sectors); 1st ordination axis explains 21.9% of total variability in pollinator density, 2nd axis explains 66.0%.

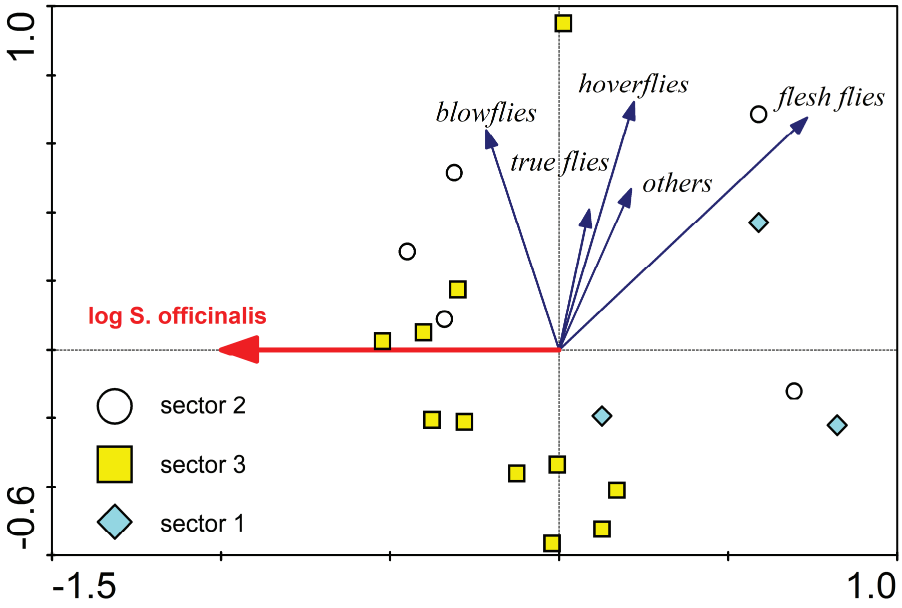


Fig. 6: Ordination diagram of RDA analysis of pollinator densities on *Sanguisorba officinalis*, forward selection has identified as environmental variables included into the final model only logarithm of flowering stalk abundance of *S. officinalis* (log S. officinalis); plots are categorized according to the sector of origin (see Fig. 1 for definition

of sectors); 1st ordination axis explains 46.0% of total variability in pollinator density, 2nd axis explains 44.4%.

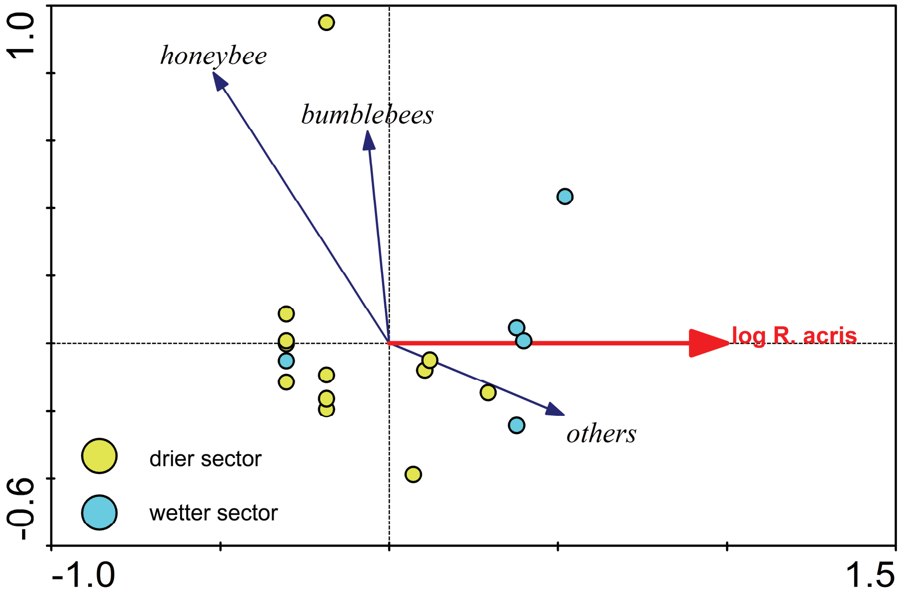
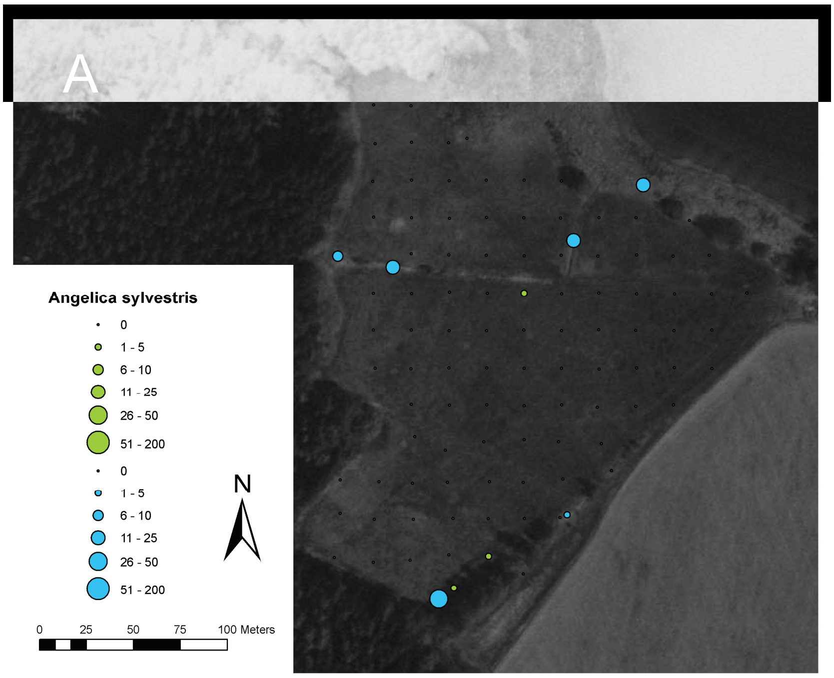


Fig. 7: Ordination diagram of RDA analysis of pollinator densities on *Trifolium hybridum*, forward selection has identified as environmental variables included into the final model only logarithm of flowering stalk abundance of *Ranunculus acris* (log R. acris); plots are categorized according to the sector of origin (see Fig. 1 for definition of sectors); 1st ordination axis explains 18.7% of total variability in pollinator density, 2nd axis explains 54.4%.

7.2.6 Paper II – Appendix 6 – Maps of occurrence and abundance of the eight focal plant species in the studied plots



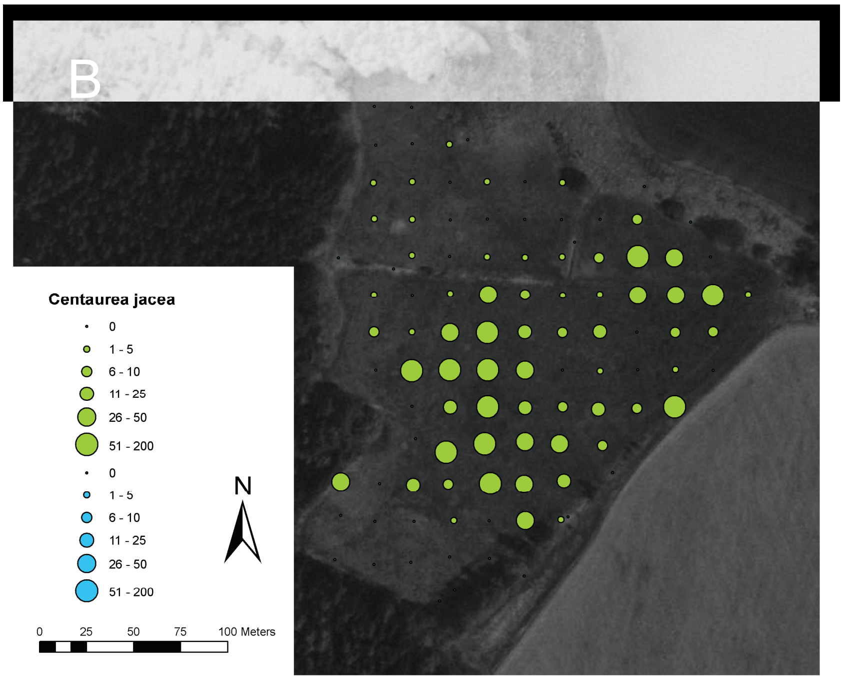


Fig. 8: Maps of occurrence and abundance of the eight focal plant species pollinator assemblages at individual plots; the symbol sizes indicate abundance categories as noted in legend; light green symbols stand for meadow plots and light blue for verge plots; please note the different scale in *T. hybridum* referring to the number of subplots occupied instead of number of flowering stalks; A) *A. sylvestris*; B) *C. jacea*.

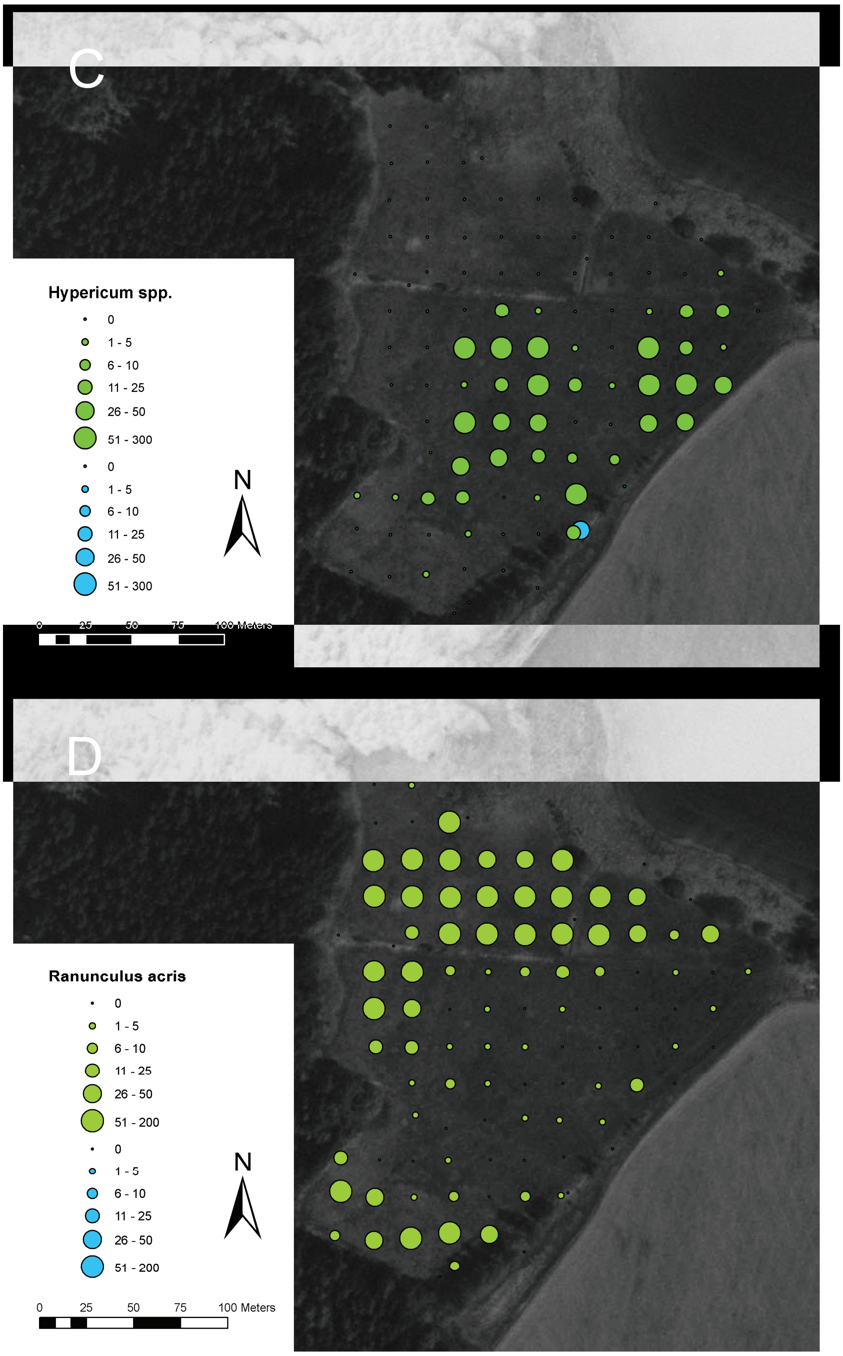


Fig. 8 (continued)**:** Maps of occurrence and abundance of the eight focal plant species pollinator assemblages at individual plots; the symbol sizes indicate abundance categories as noted in legend; light green symbols stand for meadow plots and light blue for verge plots; please note the different scale in *T. hybridum* referring to the number of subplots occupied instead of number of flowering stalks; C) *Hypericum* spp.; D) *R. acris*.

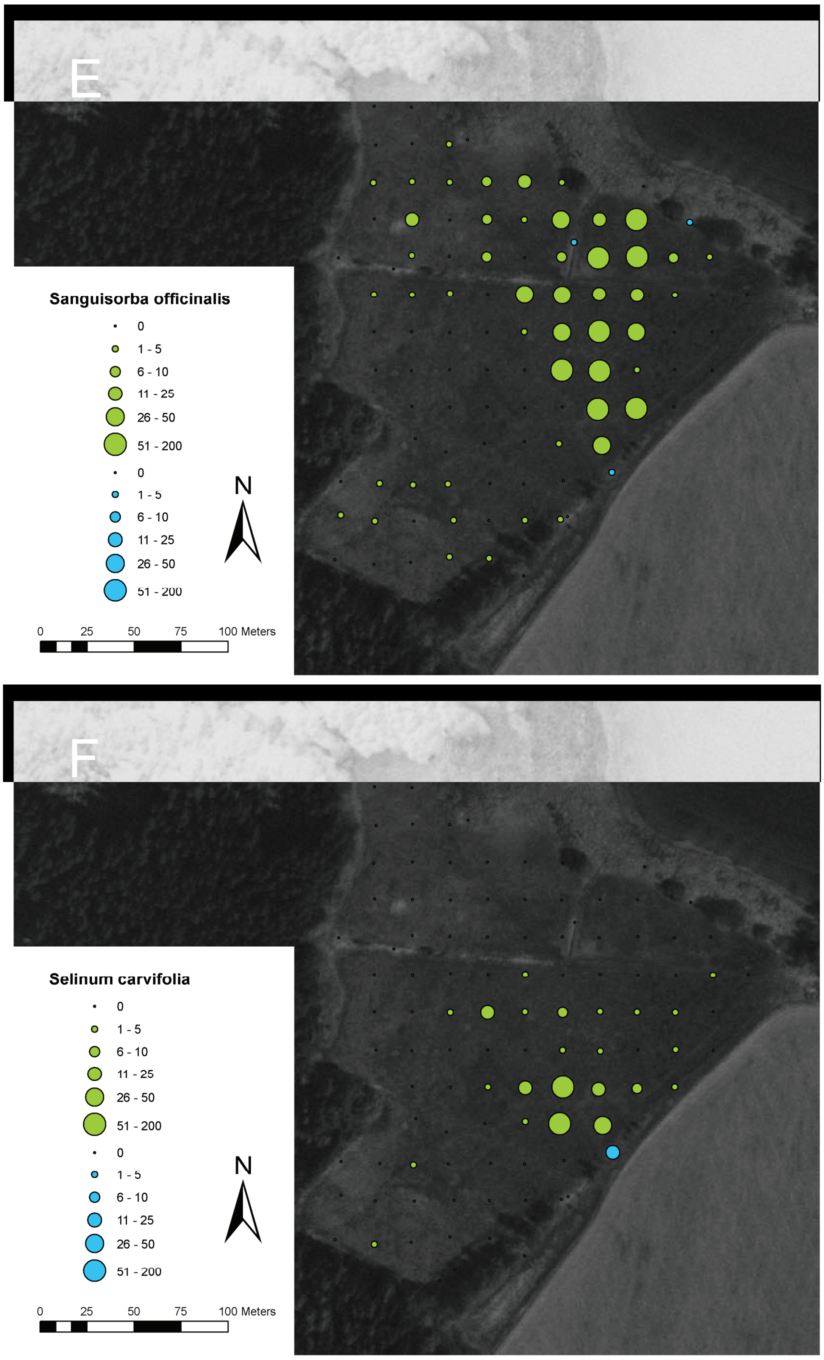


Fig. 8 (continued)**:** Maps of occurrence and abundance of the eight focal plant species pollinator assemblages at individual plots; the symbol sizes indicate abundance categories as noted in legend; light green symbols stand for meadow plots and light blue for verge plots; please note the different scale in *T. hybridum* referring to the number of subplots occupied instead of number of flowering stalks; E) *S. officinalis*; F) *S. carvifolia*.

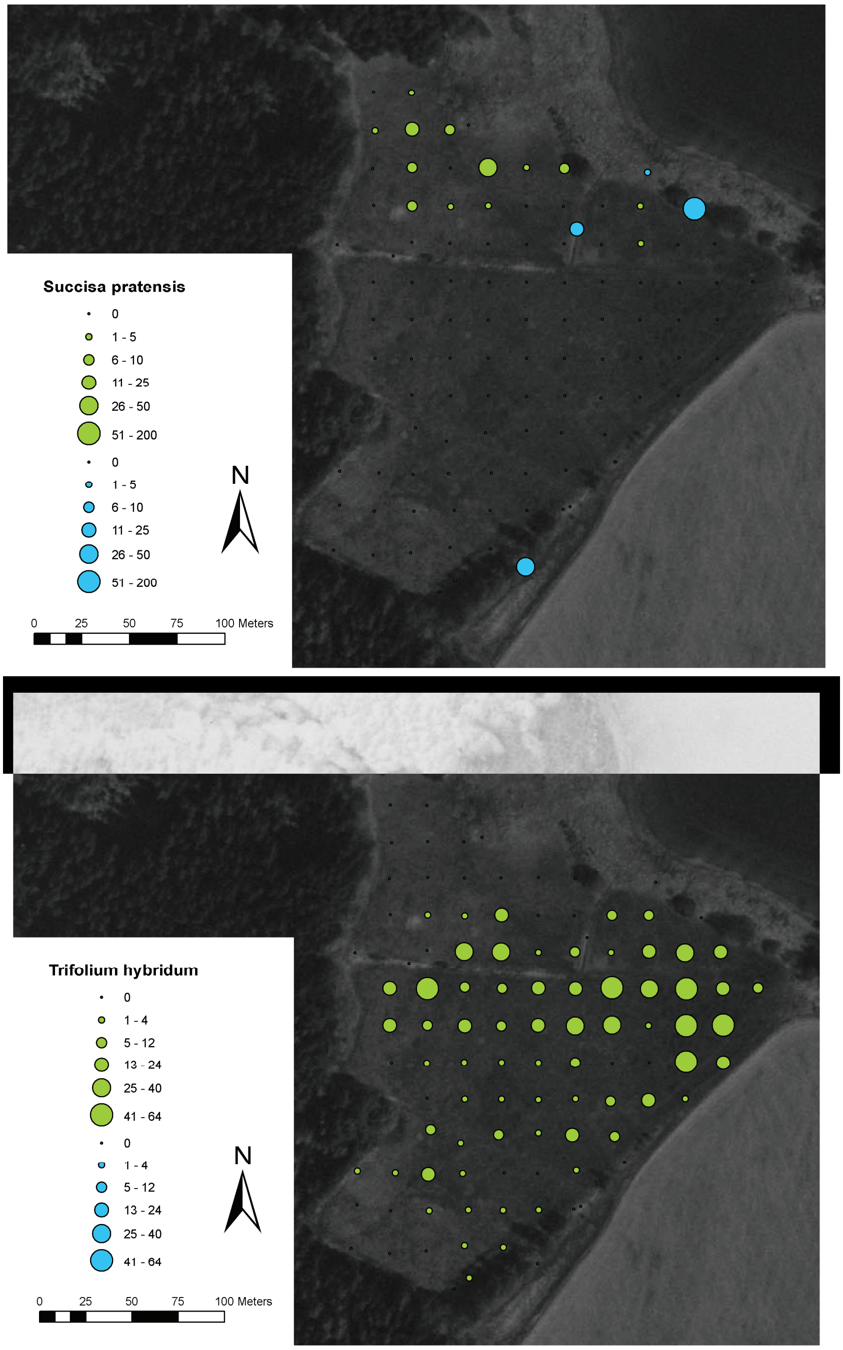


Fig. 8 (continued)**:** Maps of occurrence and abundance of the eight focal plant species pollinator assemblages at individual plots; the symbol sizes indicate abundance categories as noted in legend; light green symbols stand for meadow plots and light blue for verge plots; please note the different scale in *T. hybridum* referring to the number of subplots occupied instead of number of flowering stalks; G) *S. pratensis*; H) *T. hybridum*.

7.3 Appendices of Paper III

This supplementary material contains following Appendices:

Appendix 1 – Damage caused by herbivore functional groups
Appendix 2 – Information on study sites
Appendix 3 – Detailed results of all vital rate regressions
Appendix 4 – Details on construction of herbivory - plant size relationship
Appendix 5 – Coefficients of all constructed IPMs
Appendix 6 – Details on modelling floret correction coefficient
Appendix 7 – Visualisations of constructed IPM kernels
Appendix 8 – Observed plant size distributions in different vegetation types and effects of vegetation type on elasticities
Appendix 9 – Elasticity figures of vegetation-no no-herbivory and vegetation-no general-herbivory IPMs

7.3.1 Paper III – Appendix 1 – Damage caused by herbivore functional groups

This appendix is identical Appendix 1 of Paper I, see page 106.

7.3.2 Paper III – Appendix 2 – Information on study sites

Table 1: Summary of study population characteristics and sampling effort.

Population	# measured subpopulations	Represented veg. types	# measured plants 2010	# blocks in seedling est. experiment	Land use	Coord. (WGS 1984)	
						E	N
EN1	4	6	142	9	pasture	12.8278	49.9803
EN2	6	4,5,7,8	80	4	abandoned meadow	12.8168	49.9840
EN3	4	7	81	4	recently abandoned pasture	12.8187	49.9813
EN4	-	6	-	4	pasture	12.8239	49.9803
HK1	-	8	-	4	mown fen	12.7959	49.9568
HK2	4	8	89	10	mown fen	12.8036	49.9586
HK3	4	8	84	4	meadow	12.7970	49.9564
HK4	4	1	84	-	late successional stage - shrubs	12.8152	49.9608
HK5	-	6	-	4	pasture	12.7962	49.9478
L10	4	1	92	4	meadow	12.7947	49.9863
L11	-	1	-	4	meadow	12.8109	49.9891
L12	-	1	-	4	meadow	12.8125	49.9825
LU1	4	7	64	4	meadow	12.8077	49.9909
LU2	4	7,8	126	10	meadow	12.7869	49.9883
LU3	-	8	-	4	meadow	12.7921	49.9869
LU4	4	2,4,7	164	10*	abandoned meadow	12.8102	49.9898
LU5	3	2	86	-	abandoned meadow	12.7754	49.9898
LU6	2	3	103	4	meadow	12.7780	49.9893
LU7	3	4	80	4	abandoned fen	12.7759	49.9926
LU8	3	4,6	88	-	abandoned fen	12.8134	49.9887
LU9	2	4,7	82	4	abandoned meadow	12.7838	49.9833
OG1	4	8	164	10*	abandoned meadow	12.7966	49.9775
OG2	4	4,7	150	10*	abandoned fen	12.8104	49.9742
OG3	3	7,8	88	4	meadow	12.7953	49.9761
OG4	4	7,8	86	-	meadow	12.8016	49.9735
OG5	2	3	80	-	abandoned meadow	12.8104	49.9739
RO1	4	7	125	10*	abandoned meadow	12.7632	49.9870
RO2	6	6	89	-	recently abandoned pasture	12.7586	49.9872
RO3	4	2,3,7	107	-	abandoned meadow	12.7642	49.9877
RO4	-	6	-	4	abandoned meadow	12.7623	49.9882
WE1	4	5	83	-	mown fen	12.8089	49.9477
WE2	4	5,7	82	-	abandoned fen	12.8077	49.9533
WE3	4	5	84	-	abandoned fen	12.8148	49.9431
WE4	4	3,6	83	4	abandoned meadow	12.8184	49.9556

7.3.3 Paper III – Appendix 3 – Detailed results of all vital rate regressions

Vital rate regressions of vegetation-yes IPMs

Table 2: Results of logistic regression of survival probability from *t* to *t*+1

	Df	**McFadden's pseudo-R^2**	**Deviance**	**Resid. Df**	**Resid. Dev**	**Pr(>Chi)**
NULL				2664	2046.3	
size(t)	1	0.8%	15.96	2663	2030.3	6.47E-05
size(t)^2	1	1.4%	28.65	2662	2001.7	8.67E-08
flowering(t)	1	0.0%	0.145	2661	2001.5	0.703281
invertebrates(t)	1	0.5%	9.726	2660	1991.8	0.001817
vertebrate folivory(t)	1	0.0%	0.259	2659	1991.5	0.610904
E. aurinia(t)	1	0.0%	0.803	2658	1990.7	0.370189
veg. type	7	4.4%	89.237	2651	1901.5	< 2.2e-16
size(t):flowering(t)	1	0.0%	0.387	2650	1901.1	0.534083
size(t):invertebrates(t)	1	0.3%	6.412	2649	1894.7	0.011332
size(t):vert. fol.(t)	1	0.0%	0.613	2648	1894.1	0.433693
size(t):*E. aurinia*	1	0.1%	1.306	2647	1892.8	0.253095
size(t):veg. type	7	1.4%	29.303	2640	1863.5	0.000127
size(t)^2:flowering(t)	1	0.1%	1.492	2639	1862	0.221942
size(t)^2:invertebrates(t)	1	0.1%	1.511	2638	1860.5	0.218926
size(t)^2:vert. fol.(t)	1	0.0%	0.649	2637	1859.8	0.420397
size(t)^2:*E. aurinia*(t)	1	0.0%	0.086	2636	1859.8	0.76925
size(t)^2:veg. type	7	0.6%	12.937	2629	1846.8	0.073652
flowering(t):invertebrates(t)	1	0.0%	0.085	2628	1846.7	0.77121
flowering(t):vert. fol.(t)	1	0.1%	2.878	2627	1843.8	0.089813
flowering(t):*E. aurinia*(t)	1	0.4%	9.077	2626	1834.8	0.002588
flowering(t):veg. type	7	0.7%	13.734	2619	1821	0.056121
invertebrates(t):vert. fol.(t)	1	0.0%	0.513	2618	1820.5	0.47401
invertebrates(t):veg. type	7	3.3%	67.555	2611	1753	4.60E-12
vert. fol.(t):veg. type	7	0.4%	8.761	2604	1744.2	0.270298
E. aurinia(t):veg. type	5	0.4%	7.251	2599	1737	0.202606

Table 3: Results of GLS regression of sqrt(size(*t*+1)); resid. df.: 2241; variance modelled with power function

	numDF	**F-value**	**p-value**
(Intercept)	1	94802.48	<.0001
size(t)	1	2381.42	<.0001
size(t)ˆ2	1	156.51	<.0001
invertebrates(t)	1	7.51	0.0062
vertebrate folivory(t)	1	0.67	0.4129
E aurinia(t)	1	0.14	0.7099
vegetation type	7	18.84	<.0001
size(t):invertebrates(t)	1	1.37	0.2415
size(t):ver. fol.(t)	1	13.59	0.0002
size(t):*E. aurinia*(t)	1	8.36	0.0039
size(t):veg. type	7	8.06	<.0001
size(t)ˆ2:invertebrates(t)	1	2.46	0.1173
size(t)ˆ2:ver. fol.(t)	1	2.54	0.1108
size(t)ˆ2:*E. aurinia*	1	0.14	0.708
size(t)ˆ2:veg. type	7	1.74	0.0946
invertebrates(t):ver. fol.(t)	1	0.05	0.8248
invertebrates(t):veg. type	7	4.32	0.0001
ver. fol.(t):veg. type(t)	7	0.64	0.7257

Table 4: Results of logistic regression of flowering probability in *t*+1

	Df	**McFadden's pseudo-R^2**	**Deviance**	**Resid. Df**	**Resid. Dev**	**Pr(>Chi)**
NULL				2288	3172.8	
size(t)	1	18.8%	597.73	2287	2575	< 2.2e-16
size(t)ˆ2	1	0.6%	17.47	2286	2557.6	2.93E-05
invertebrates(t)	1	0.6%	19.14	2285	2538.4	1.21E-05
vertebrate folivory(t)	1	0.0%	0.2	2284	2538.2	0.65249
E aurinia(t)	1	0.1%	3.85	2283	2534.4	0.04985
vegetation type	7	1.5%	48.52	2276	2485.8	2.81E-08
size(t):invertebrates(t)	1	0.0%	0.05	2275	2485.8	0.81467
size(t):ver. fol.(t)	1	0.0%	0.86	2274	2484.9	0.35492
size(t):*E. aurinia*(t)	1	0.1%	3.64	2273	2481.3	0.05628
size(t):veg. type	7	0.4%	12.77	2266	2468.5	0.078
size(t)ˆ2:invertebrates(t)	1	0.1%	3.28	2265	2465.2	0.07021
size(t)ˆ2:ver. fol.(t)	1	0.0%	0.6	2264	2464.6	0.43874
size(t)ˆ2:*E. aurinia*	1	0.0%	0.79	2263	2463.9	0.37526
size(t)ˆ2:veg. type	7	0.3%	8.9	2256	2455	0.25984
invertebrates(t):ver. fol.(t)	1	0.0%	0.69	2255	2454.3	0.40495
invertebrates(t):veg. type	7	0.3%	10.38	2248	2443.9	0.16802
ver. fol.(t):veg. type(t)	7	0.2%	5.51	2241	2438.4	0.59847
E. aurinia(t):veg. type	5	0.1%	1.84	2236	2436.5	0.87078

Table 5: Results of OLS regression of log(flower heads(*t*+1)+1)

	Df	**R^2**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
stalk grazing(t+1)	1	54.2%	638.03	638.03	1804.472	< 2.2e-16
size(t+1)	1	8.7%	101.79	101.79	287.8899	< 2.2e-16
size(t+1)ˆ2	1	0.0%	0.08	0.08	0.233	0.629402
veg. type	7	0.8%	9.6	1.37	3.8785	0.000349
stalk gr.(t+1):size(t+1)	1	0.1%	0.98	0.98	2.7594	0.096965
stalk gr.(t+1):size(t+1)ˆ2	1	0.0%	0.02	0.02	0.0673	0.795322
stalk gr.(t+1):veg. type	7	1.2%	14.58	2.08	5.8913	9.70E-07
size(t+1):veg. type	7	0.6%	6.98	1	2.8206	0.006461
size(t+1)ˆ2	7	0.5%	5.76	0.82	2.3289	0.023167
Residuals	1127	33.9%	398.49	0.35		

Table 6: Results of OLS regression of no. of florets in flower head

	Df	**R^2**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
flower heads(t+1)	1	16.0%	77814	77814	186.6291	< 2.2e-16
size(t+1)	1	6.8%	33055	33055	79.279	< 2.2e-16
size(t+1)ˆ2	1	1.0%	4664	4664	11.185	0.000863
veg. type	7	4.1%	20136	2877	6.8991	5.51E-08
fl. heads(t+1):size(t+1)	1	1.8%	8751	8751	20.9886	5.36E-06
fl. heads(t+1):size(t+1)ˆ2	1	1.1%	5170	5170	12.4004	0.000454
fl. heads(t+1):veg. type	7	0.2%	1210	173	0.4147	0.89352
size(t+1):veg. type	7	0.7%	3338	477	1.1439	0.333307
size(t+1)ˆ2:veg. type	7	0.2%	743	106	0.2547	0.970694
Residuals	794	68.1%	331055	417		

Table 7: Results of quasibinomial GLM of ratio of well-developed seeds in terminal flower head to no. of florets produced; link function: logit; dispersion factor $\Phi = 25.55$

	Df	**McFadden's pseudo-R^2**	**Deviance**	**Resid. Df**	**Resid. Dev**	**F**	**Pr(>F)**
NULL				835	25984		
c(sezr/boule)	1	7.3%	1894.29	834	24090	74.1357	< 2.2e-16
boule	1	1.1%	284.03	833	23806	11.1157	0.000897
strb1	1	0.1%	19.58	832	23786	0.7662	0.381651
sqrt(velik1)	1	0.1%	24.15	831	23762	0.945	0.331309
velik1	1	0.0%	10.18	830	23752	0.3986	0.528007
shluk	7	1.1%	283.3	823	23468	1.5839	0.136695
c(sezr/boule):boule	1	0.0%	4.47	822	23464	0.1749	0.675903
c(sezr/boule):strb1	1	0.1%	16.28	821	23448	0.6371	0.424998
c(sezr/boule):sqrt(velik1)	1	0.0%	5.36	820	23442	0.2099	0.646983
c(sezr/boule):velik1	1	0.2%	43.35	819	23399	1.6966	0.193114
c(sezr/boule):shluk	7	0.3%	65.04	812	23334	0.3636	0.923331
boule:strb1	1	0.0%	0.07	811	23334	0.0028	0.958105
boule:sqrt(velik1)	1	0.3%	67.94	810	23266	2.6587	0.103387
boule:velik1	1	0.0%	12.64	809	23253	0.4949	0.481977
boule:shluk	7	1.2%	307.92	802	22945	1.7216	0.100661
strb1:sqrt(velik1)	1	0.0%	0.1	801	22945	0.0041	0.949172
strb1:velik1	1	0.1%	24.86	800	22920	0.9727	0.324303
strb1:shluk	7	0.5%	120.92	793	22799	0.676	0.692507
sqrt(velik1):velik1	1	0.0%	0.46	792	22799	0.0179	0.893605
sqrt(velik1):shluk	7	0.8%	206.05	785	22593	1.152	0.328366
velik1:shluk	7	1.0%	248.17	778	22345	1.3875	0.20725

Table 8: Results of GLS regression of log(seedling establishment+1); variance modelled by a power function of the fitted value; residual df: 129

	numDF	**F-value**	**p-value**
(Intercept)	1	53.89363	<.0001
shluk	7	5.52062	<.0001

Table 9: Results of GLS regression of seedling size; variance modelled by a power function of the fitted value; residual df: 211

	numDF	**F-value**	**p-value**
(Intercept)	1	1535.512	<.0001
shluk	7	6.5071	<.0001

Vital rate regressions of vegetation-no IPMs

Table 10: Results of logistic regression of survival rate

	Df	**Deviance**	**Resid. Df**	**Resid. Dev**	**Pr(>Chi)**
NULL			2664	2046.3	
size(t)	1	15.9602	2663	2030.3	6.47E-05
size(t)ˆ2	1	28.6504	2662	2001.7	8.67E-08
invertebrates	1	9.8429	2661	1991.8	0.001705

Table 11: Results of GLS regression of square-root transformed size(*t*+1); residual df: 2286

	numDF	F-value	p-value
(Intercept)	1	83531.28	<.0001
size(t)	1	2765.93	<.0001
size(t)ˆ2	1	184.03	<.0001
invertebrates	1	6.18	0.013

Table 12: Results of logistic regression of flowering probability in *t*+1

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			2288	3172.8	
size(t)	1	597.73	2287	2575	< 2.2e-16
size(t)ˆ2	1	17.47	2286	2557.6	2.93E-05
invertebrates	1	19.14	2285	2538.4	1.21E-05

Table 13: Results of OLS regression of log(flower heads+1)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
stalk grazing	1	638.03	638.03	1691.517	<2e-16
size(t+1)	1	101.79	101.79	269.8687	<2e-16
size(t+1)ˆ2	1	0.08	0.08	0.2184	0.6403
Residuals	1157	436.41	0.38		

Table 14: Results of OLS regression of no. of florets

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
flower heads	1	77814	77814	182.138	< 2.2e-16
size(t+1)	1	33055	33055	77.371	< 2.2e-16
size(t+1)ˆ2	1	4664	4664	10.916	0.000995
fl. heads:size(t+1)	1	12299	12299	28.789	1.05E-07
fl. heads:size(t+1)ˆ2	1	6924	6924	16.207	6.20E-05
Residuals	822	351181	427		

7.3.4 Paper III – Appendix 4 – Details on construction of herbivory - plant size relationship

Dataset and analyses

We used the same dataset as for the main part of vital rate regressions with one important difference. Whereas the datapoint consisted of transition of individual from t to t+1 (models of survival, growth and flowering probability) or from the state of the individual in t+1 (models of fecundity) in the vital rate regressions, here we used as datapoints each unique situation plant × year, i.e. amount of datapoints was approximately two times higher for ital rate regressions.

Since our aim was rather describing the observed relationship in the two years of observation, we used locally weighted quadratic regression (LOESS) with span parameter set to 0.75 for the purpose of IPM parameterisation. We did not extrapolate the LOESS regressions outside the range of data (i.e. very small and large plants, see Materials and methods for criteria for setting the minimum maximum plant sizes in the model). We assigned the predicted herbivory values of the observed boundary sizes to sizes outside the observed size range (see Figs. S6 and S7). For each of the three herbivore groups entering IPMs, we used the corresponding appropriate dataset, i.e. data on all plants for invertebrate folivores, data on all flowering plants for stalk grazing and data on all plants with collected terminal flower heads for seed predation. The predicted values of these regressions served as input for general-herbivory parameterisation. In case of invertebrate folivores, we also constructed LOESS regressions for each vegetation type separately, which served as input for vegetation-specific herbivory parameterisation (together with general-herbivory parameterisations for the other two groups, see Materials and Methods for explanation).

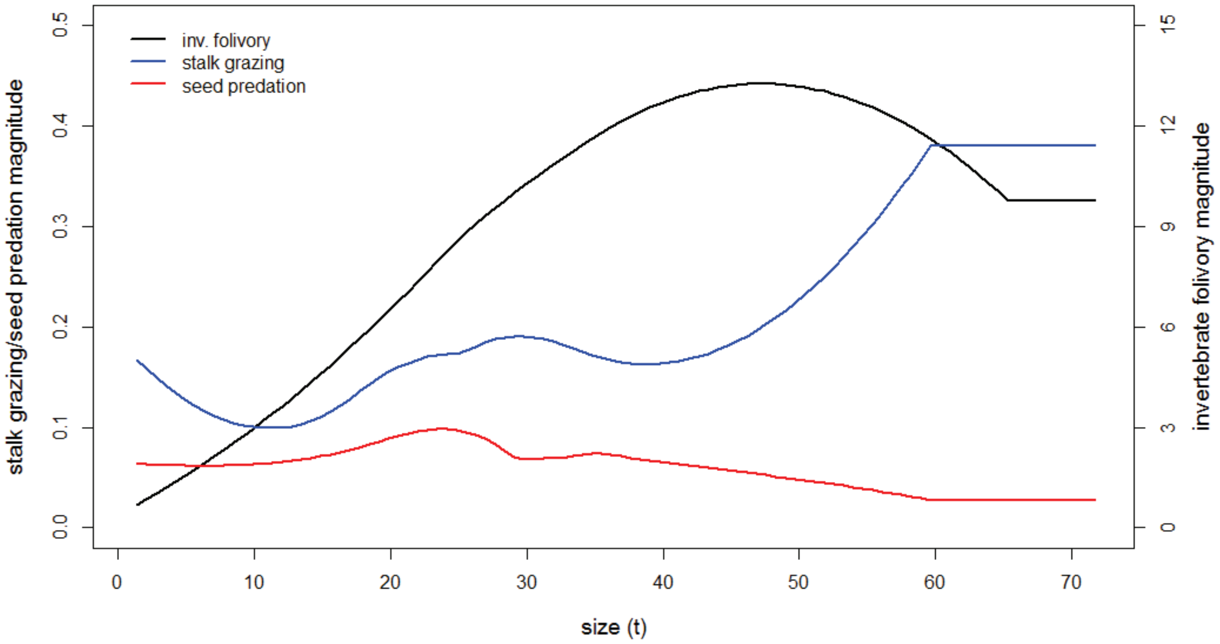


Fig. 6: Predicted values of LOESS of herbivory magnitude (general-herbivory parameterisation); please note different units and axes (see Paper III, Table 1 for details)

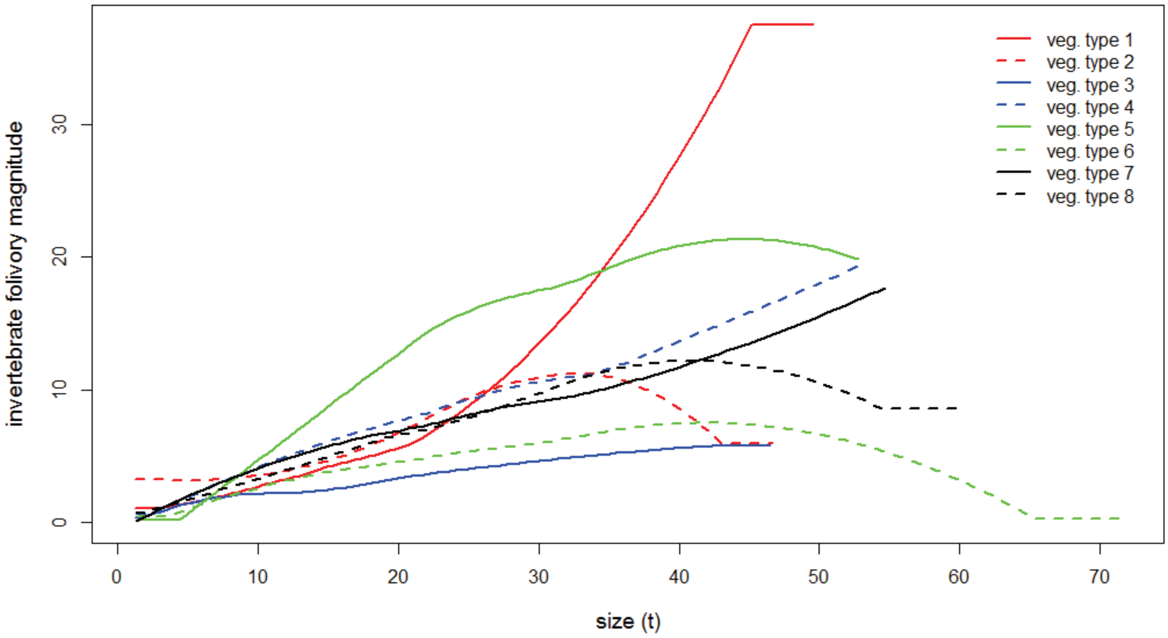


Fig. 7: Predicted mean values of LOESS for invertebrate folivory magnitude in individual vegetation types (input for vegetation-specific herbivory)

7.3.5 Paper III – Appendix 5 – Coefficients of all constructed IPMs

Table 15: Coefficients for equations underlying constructed IPMs (for the form of equations see Table 3 in the paper)

Coefficient	veg. type 1	veg. type 2	veg. type 3	veg. type 4	veg. type 5	veg. type 6	veg. type 7	veg. type 8	veg. type not included
Mortality intercept	0.769257	1.944742	1.988503	-0.26522	-2.29387	0.306758	-0.4877	1.720491	0.426551
Mortality size	0.252468	0.196581	0.051238	0.201117	0.16066	0.22327	0.201643	0.201602	0.140794
Mortality size^2	-0.00284	-0.00284	-0.00284	-0.00284	-0.00284	-0.00284	-0.00284	-0.00284	-0.00233
Mortality inv. folivory	-0.14598	-0.00557	0.167676	-0.07994	0.110951	-0.06271	-0.07033	-0.04389	-0.02836
Growth intercept	1.978734	2.331491	2.072573	2.625908	2.469139	1.888494	2.294916	1.817017	1.961128
Growth size	0.13068	0.147438	0.146283	0.142927	0.117597	0.17868	0.146297	0.162025	0.16163
Growth size^2	-0.00163	-0.00163	-0.00163	-0.00163	-0.00163	-0.00163	-0.00163	-0.00163	-0.00174
Growth inv. folivory	0.008666	-0.02719	-0.0187	-0.0154	0.023149	-0.03184	0.00031	0.005378	-0.00608
Growth RSE	0.330095	0.330095	0.330095	0.330095	0.330095	0.330095	0.330095	0.330095	0.317479
Growth power	0.507457	0.507457	0.507457	0.507457	0.507457	0.507457	0.507457	0.507457	0.560133
Flowering intercept	-4.08636	-5.30781	-4.38577	-5.2411	-4.83882	-4.71611	-4.63261	-5.17593	-4.2632438
Flowering size	0.289111	0.289111	0.289111	0.289111	0.289111	0.289111	0.289111	0.289111	2.59E-01
Flowering size^2	-0.00277	-0.00277	-0.00277	-0.00277	-0.00277	-0.00277	-0.00277	-0.00277	-2.44E-03
Flowering inv. folivory	-0.02551	-0.02551	-0.02551	-0.02551	-0.02551	-0.02551	-0.02551	-0.02551	-3.15E-02
Flower head intercept	2.356802	0.764537	0.767223	1.425379	1.390649	0.798813	1.726554	1.042108	1.111
Flower head stalk grazing	-1.24063	-1.00092	-1.56629	-0.77893	-1.01873	-1.51811	-1.78237	-1.34854	-1.489
Flower head size	-0.00284	0.080615	0.070707	0.014534	0.043289	0.051779	0.009794	0.047552	0.04133
Flower head size^2	0.005418	-0.00109	-0.00023	0.000381	-0.00078	-0.00019	0.000374	-0.00026	-8.6E-05
Floret intercept	22.93871	37.09999	35.68816	40.49509	41.27643	41.34501	35.15387	32.28151	24.91148
Floret flower head	6.23625	6.23625	6.23625	6.23625	6.23625	6.23625	6.23625	6.23625	6.979004
Floret size	2.873726	2.873726	2.873726	2.873726	2.873726	2.873726	2.873726	2.873726	3.457567
Floret size^2	-0.029	-0.029	-0.029	-0.029	-0.029	-0.029	-0.029	-0.029	-0.03494
Floret fl. head x size	-0.28436	-0.28436	-0.28436	-0.28436	-0.28436	-0.28436	-0.28436	-0.28436	-0.32538
Floret fl. head x size^2	0.003547	0.003547	0.003547	0.003547	0.003547	0.003547	0.003547	0.003547	0.004054
Seed intercept	-0.19577	-0.19577	-0.19577	-0.19577	-0.19577	-0.19577	-0.19577	-0.19577	-0.19577
Seed predation	-2.43059	-2.43059	-2.43059	-2.43059	-2.43059	-2.43059	-2.43059	-2.43059	-2.43059
Seed floret	0.005176	0.005176	0.005176	0.005176	0.005176	0.005176	0.005176	0.005176	0.005176
Seedling establishment	0.000393	0.006126	0.006613	0.005926	0.008024	0.003696	0.007106	0.006047	0.005258
Seedling size mean	1.265785	3.223689	3.376887	2.863557	3.480163	4.052917	3.169613	2.599776	3.063717
Seedling size sd	0.367986	1.223385	1.298597	1.050629	1.349855	1.641792	1.197076	0.92793	1.236867

7.3.6 Paper III – Appendix 6 – Details on modelling floret correction coefficient

Dataset

Data used for modelling floret correction coefficient form a subset of one-year dataset of Paper IV. Namely, we took only data on such individuals which in a given year did not suffer any stalk grazing nor lost any stalks due to mowing machines. Thus we resulted with 244 observations of plants with all their flower heads censused for number of florets instead of 321 in the original dataset of Janovský et al.

Considered model

Based upon the observation that terminal flower heads are always the largest on a given stalk, we can build a reasonable assumption that the relationship between (# florets in terminal flower head)/(# florets in average flower head) (hereafter only the ratio) and # flower heads will be hyperbolic, i.e. if a plant has one flower hand, the ratio is necessarily 1 and than by adding more flower heads of non-zero size but always smaller than the terminal must result in some sort of monotonous decrease of approximately hyperbolic nature. Therefore our predictors were power-transforms of 1/(no. of flower heads). The intercept under such model than describes the assymptote, to which the ratio converges in plants with many flower heads. We tested relationship of predictors to the response variable by means of OLS regression.

Table 16: Results of OLS regression of the ratio; $R^2 = 0.5317$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
1/sqrt(flower heads)	1	2.4766	2.47655	272.3068	<2e-16
1/flower heads	1	0.0013	0.0013	0.143	0.7056
1/sqrt((flower heads)^3)	1	0.0004	0.0004	0.0442	0.8337
Residuals	240	2.1827	0.00909		

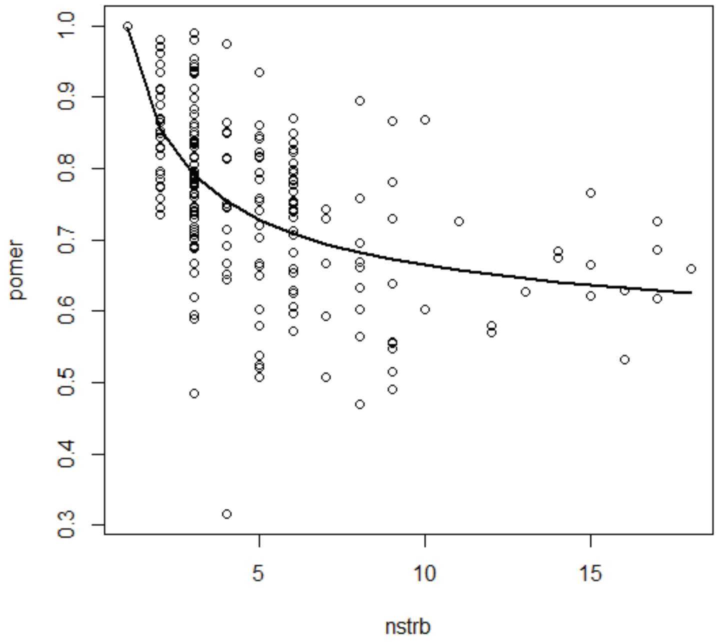


Fig. 8: Relationship of the ratio (noted as pomer) to no. of flower heads (noted as nstrb); thick line depicts regression line of the model back-transformed to the original scale

7.3.7 Paper III – Appendix 7 – Visualisations of constructed IPM kernels

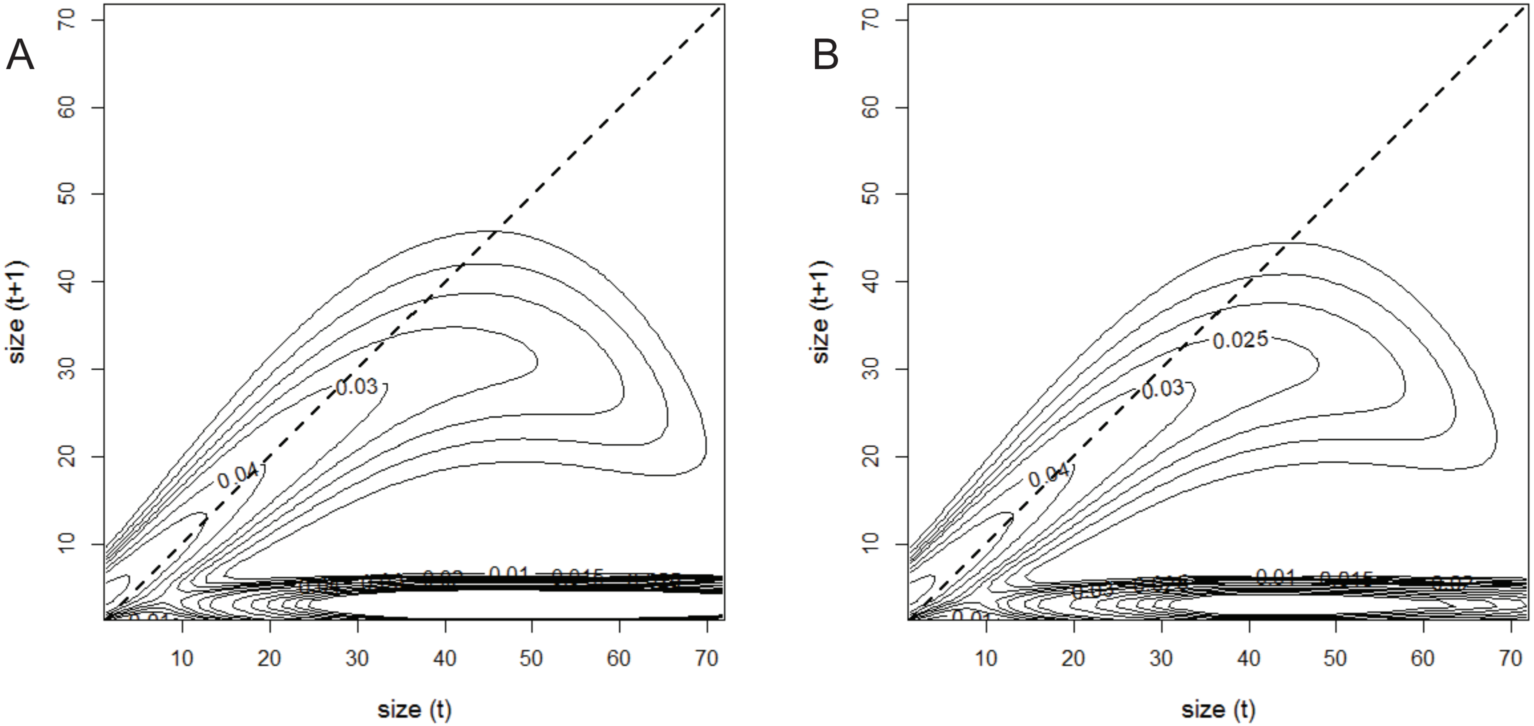


Fig. 9: Contour plots of vegetation-no IPM kernels; A) no-herbivory parameterisation; B) general-herbivory parameterisation; dashed line denotes size(*t*) = size(*t*+1)

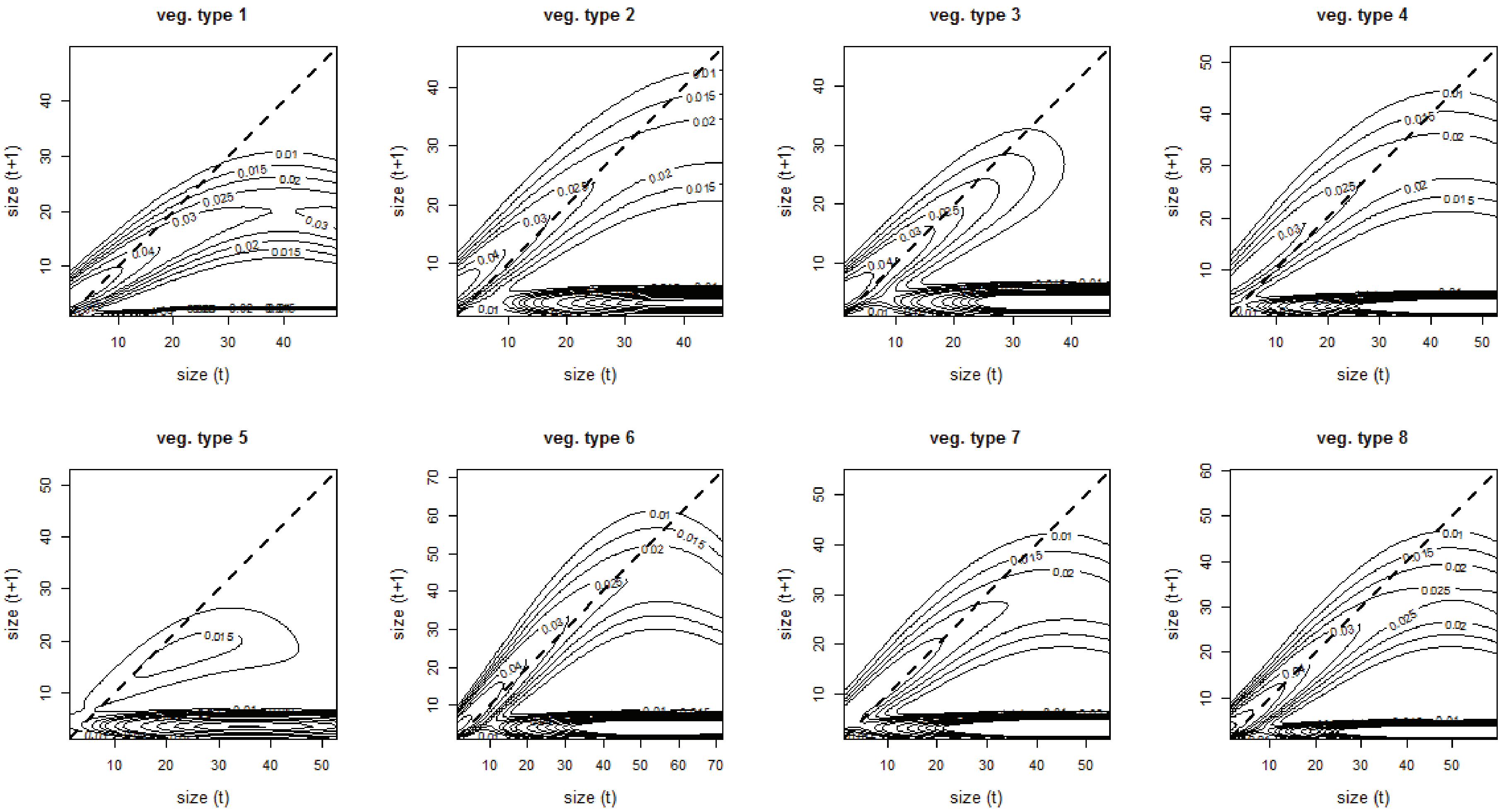


Fig. 10: Contour plots of vegetation-yes IPM kernels - no-herbivory parameterisation; dashed line denotes size(t) = size(t+1)

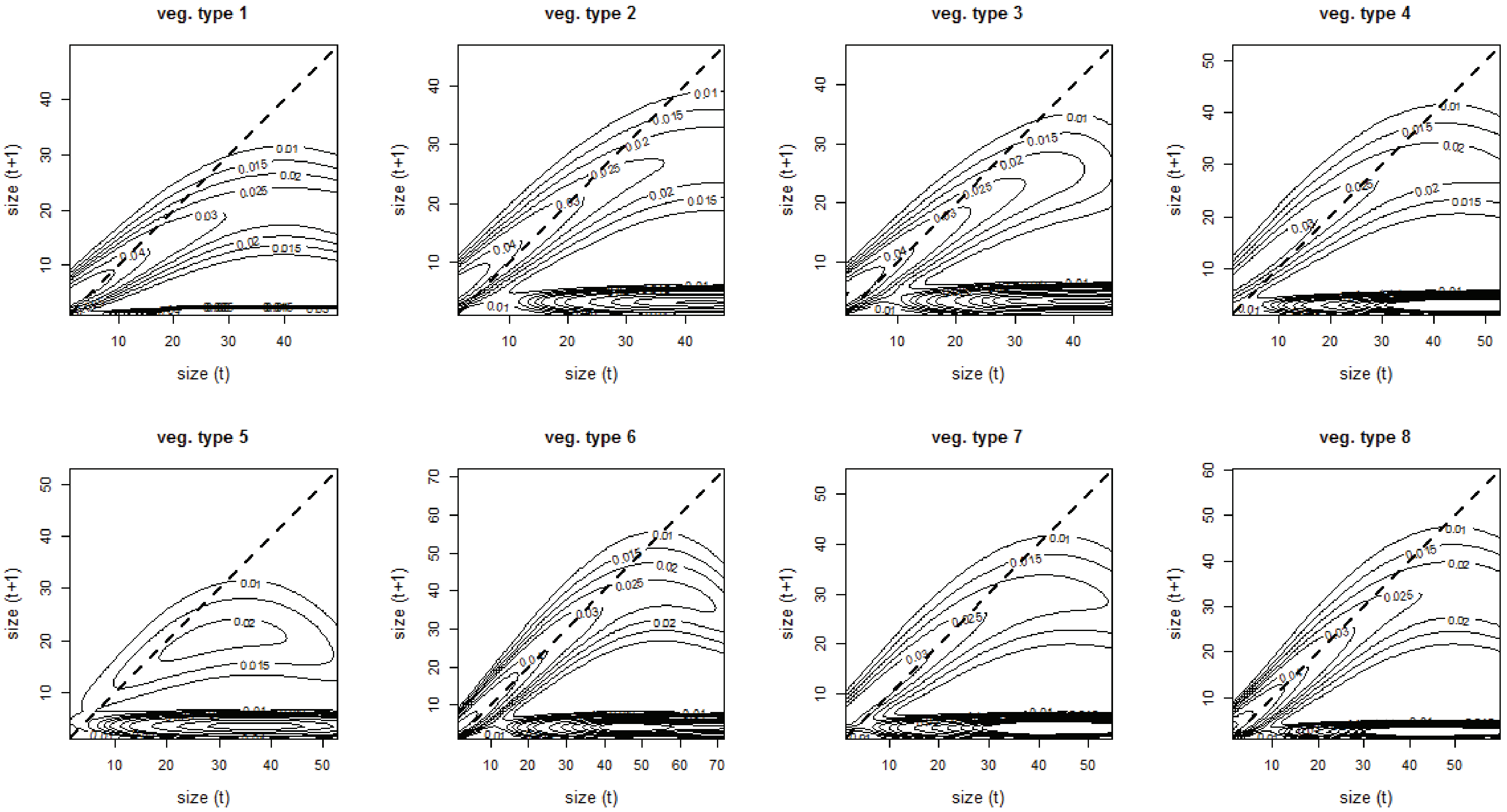


Fig. 11: Contour plots of vegetation-yes IPM kernels - general-herbivory parameterisation; dashed line denotes size(t) = size(t+1)

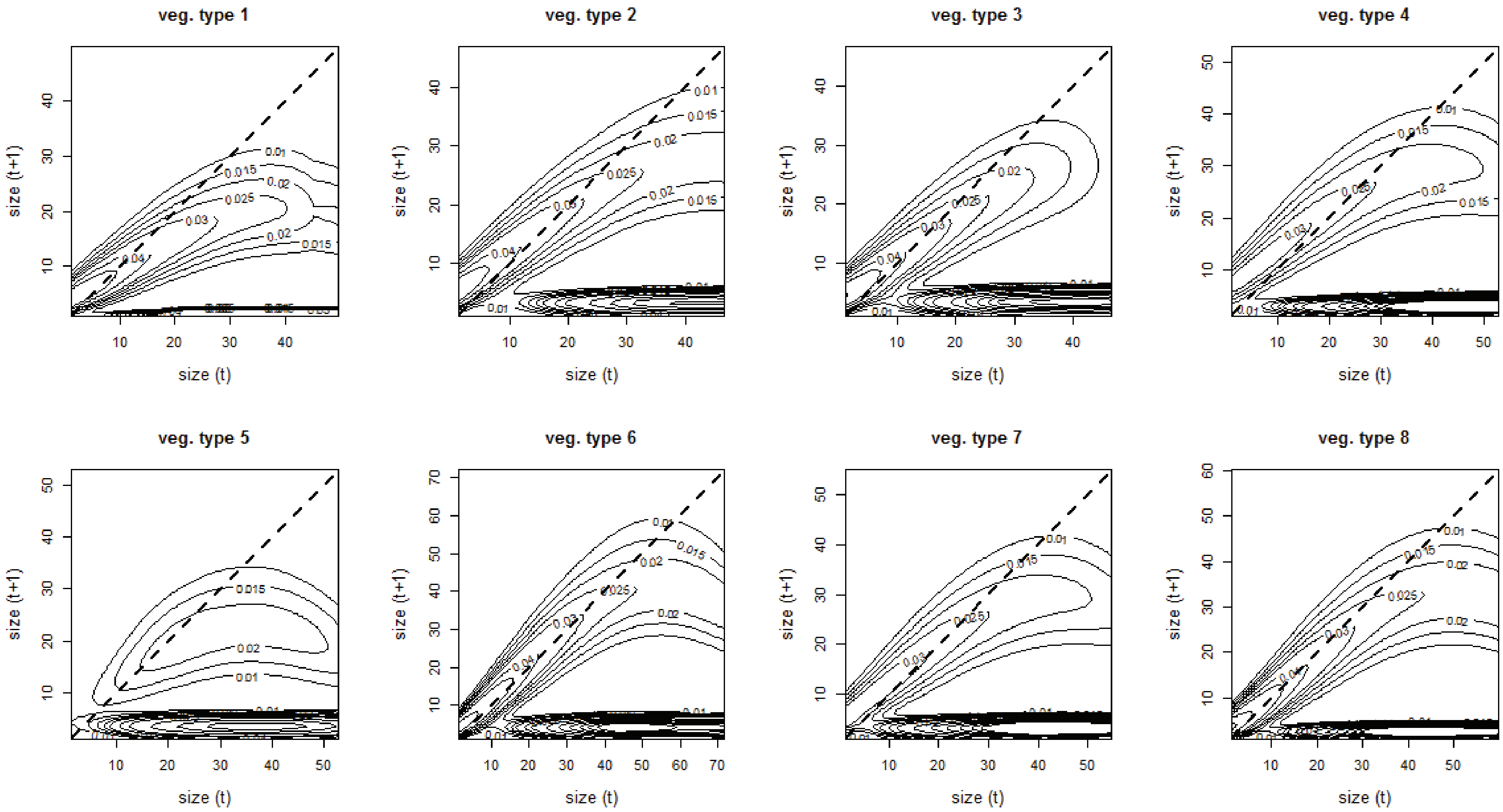


Fig. 12: Contour plots of vegetation-yes IPM kernels – vegetation-specific-herbivory parameterisation; dashed line denotes size(*t*) = size(*t*+1)

7.3.8 *Paper III – Appendix 8 – Observed plant size distributions in different vegetation types and effects of vegetation type on elasticities*

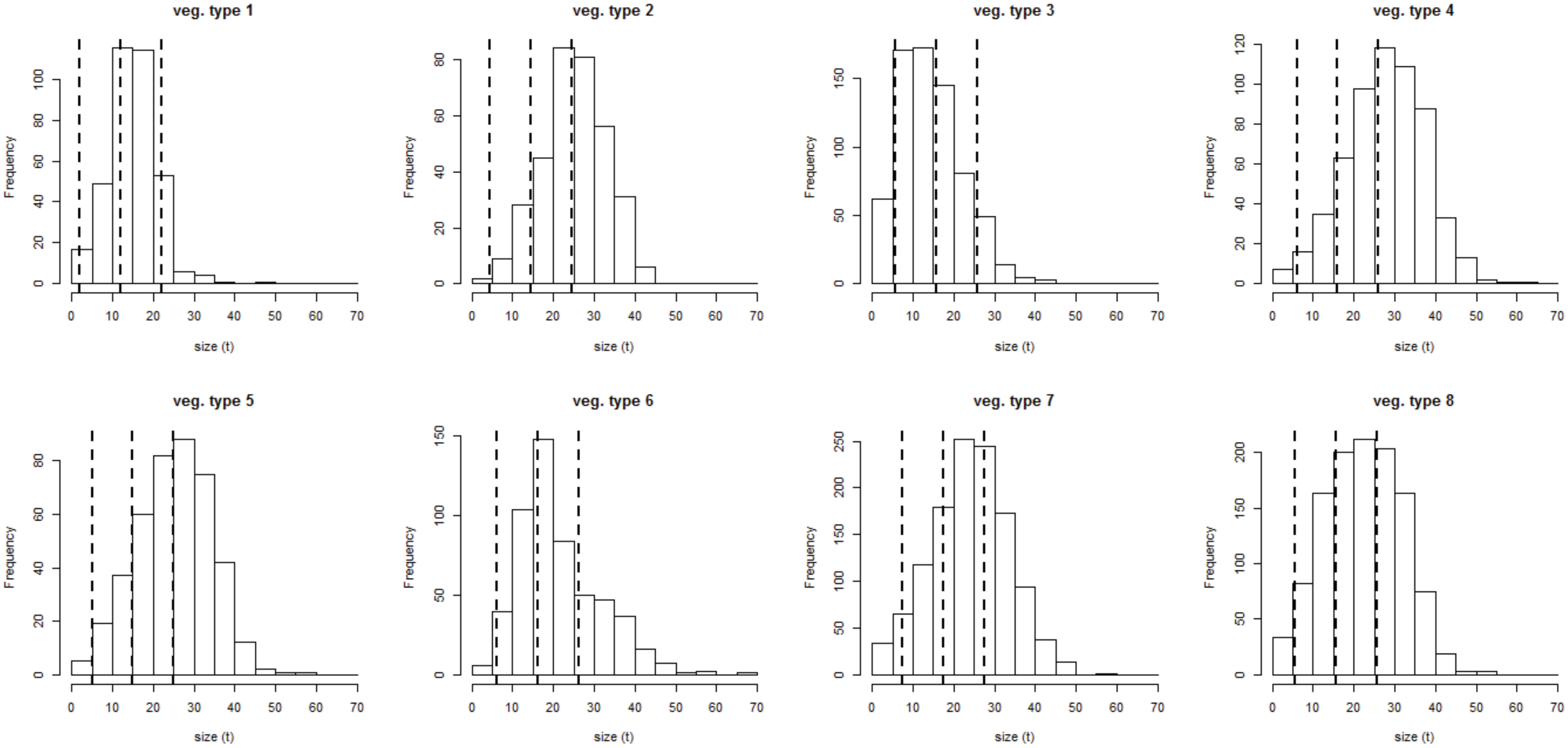


Fig. 13: Histograms of observed plant-size distributions in individual vegetation types; dashed lines denote size classes in the order seedlings, small, medium and large plants as defined for the purpose of summing elasticities (see Materials and Methods for details)

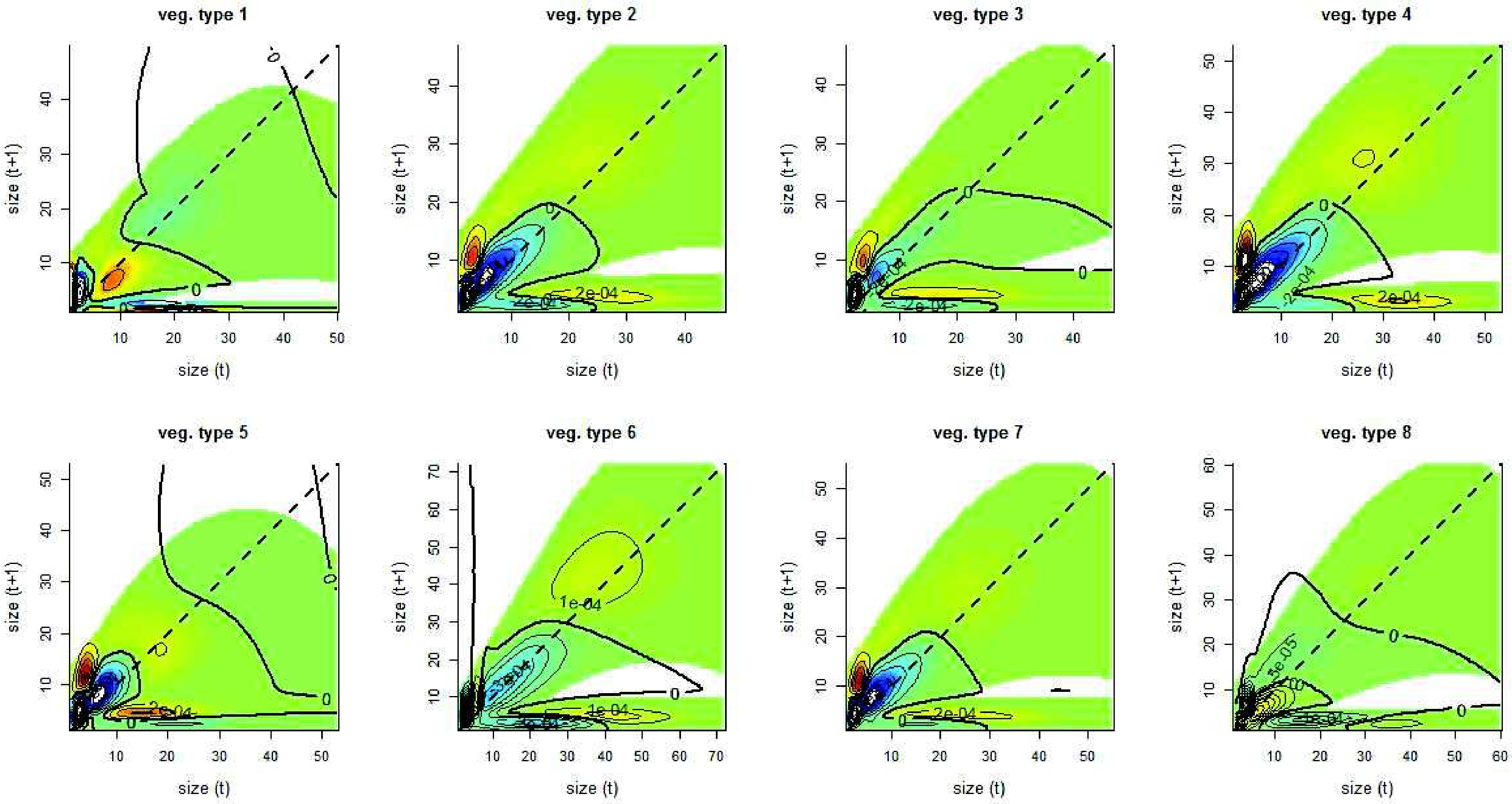


Fig. 14: Differences in elasticities between vegetation-yes no-herbivory IPM and vegetation-no no-herbivory IPM; positive differences indicate increase in importance of a given transition under the vegetation-yes model; coloured area denotes area with transition values greater than 0.001 (i.e. more than 0.1% plants from a given size enter this new size) or belonging to reproduction section of IPM

7.3.9 Paper III – Appendix 9 – Elasticity figures of vegetation-no no-herbivory and vegetation-no general-herbivory IPMs

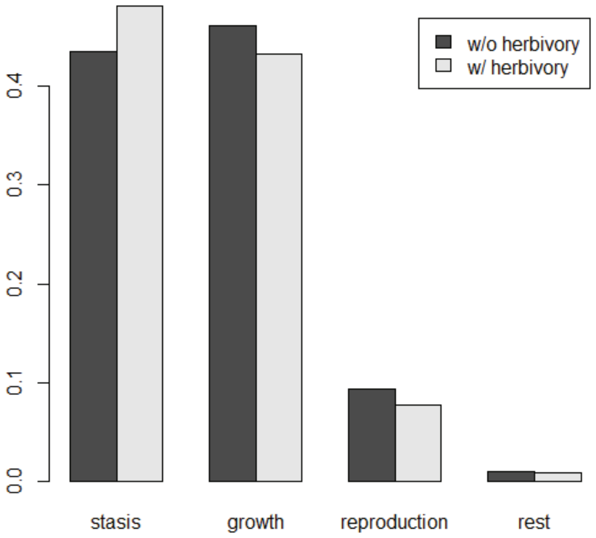


Fig. 15: Elasticities of vital rates for vegetation-no IPMs; dark-grey – no-herbivory parameterisation, light grey – general-herbivory parameterisation; growth was defined as increase in size greater than 20%; reproduction as all transitions to sizes smaller than 98% quantile of Normal distribution of seedling sizes; rest denotes elasticities of transitions with value lower than 0.001 (i.e. less than 0.1% plants entering from a given size the given new size)

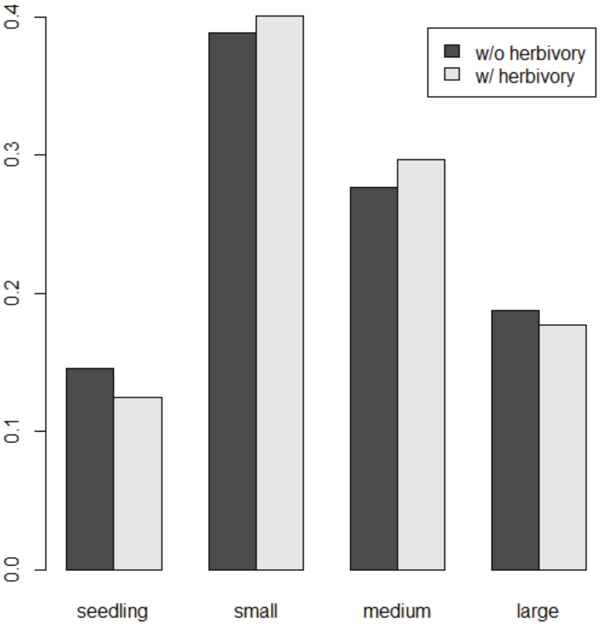


Fig. 16: Elasticities of size categories (see Materials and Methods section for delimitation) for vegetation-no IPMs; dark-grey – no-herbivory parameterisation, light grey – general-herbivory parameterisation;

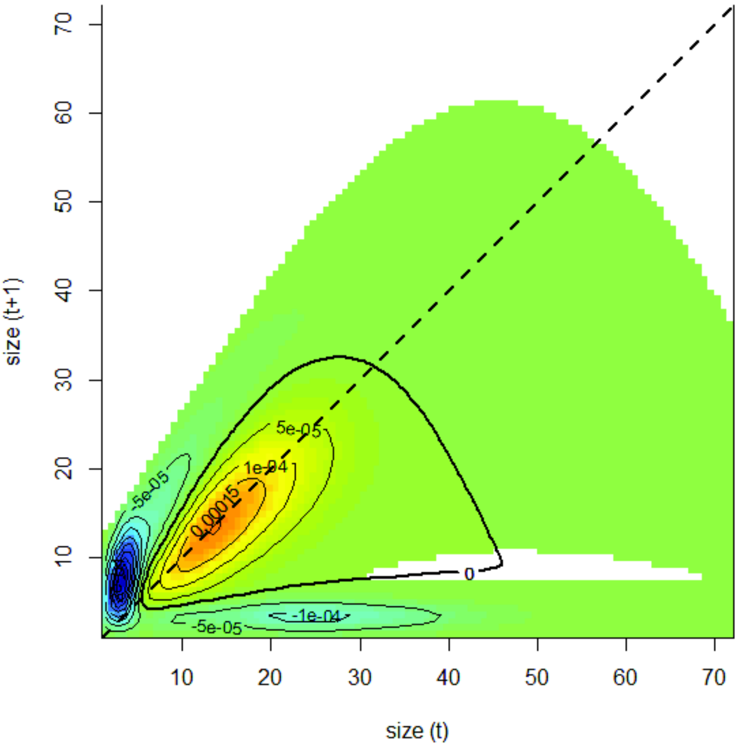


Fig. 17: Differences in elasticities between vegetation-no general-herbivory IPM and vegetation-no no-herbivory IPM; positive differences indicate increase in importance of a given transition under the general-herbivory; coloured area denotes area with transition values greater than 0.001 (i.e. more than 0.1% plants from a given size enter this new size) or belonging to reproduction section of IPM

7.4 Appendices of Paper V

7.4.1 Paper V – Appendix 1 – Pollinator spectrum of species under study

Table 1: Pollinator spectra of *A. millefolium* and *A. ptarmica* based upon camera observations (Pavlíková A., et al., unpublished data). The camera was always aimed at a single plant in different populations within the study area in 2011. The data is based on 80 individuals of *A. millefolium* and 75 individuals of *A. ptarmica* for 48, respectively and 53 hours. Only the flower visitors touching repeatedly the plant's reproductive structures, were considered as pollinators.

Pollinator group	***A. millefolium*** **n = 342**	***A. ptarmica*** **n = 409**
Hoverflies	74.0%	76.3%
Other diptera	13.7%	10.5%
Beetles	3.5%	5.4%
Solitary bees	3.5%	4.2%
Butterflies	2.3%	0.0%
Other hymenoptera	2.9%	3.7%

7.4.2 Paper V – Appendix 2 – Flow cytometry analysis of A. millefolium *progeny*

The material for the flow cytometry analysis was obtained from the seedlings of individual *A. millefolium* plants germinated in germination experiment. We took three seedlings from each analysed *A. millefolium* individual, in case of presence of more than one peak, the sample was reanalysed with two more seedlings. The samples were analysed according to a standardized protocol (Doležel et al. 2007). The leaves of *Bellis perennis* were used as internal standard against which the *Achillea* DNA content was compared. The samples were analysed on Partec PA 2 flow cytometer (Partec, Münster, Germany) equipped with HBO 100 (Osram, München, Germany). The positions of sample peaks were then compared with peaks of internal standard and their ratio computed. The samples identified as tetraploid *A. pratensis* had ratio sample to internal standard between 3.02 and 3.22, the hexaploid *A. millefolium* s.s. between 4.32 and 4.54 and pentaploid hybrids between 3.68 and 3.86.

From offspring of 203 tested *A. millefolium* agg. individuals, 65 belonged unambiguously to tetraploid *A. pratensis*, 119 to hexaploid *A. millefolium* s.s. and 19 samples of seedlings were identified as containing pentaploid hybrids. Such samples were excluded from all analyses.

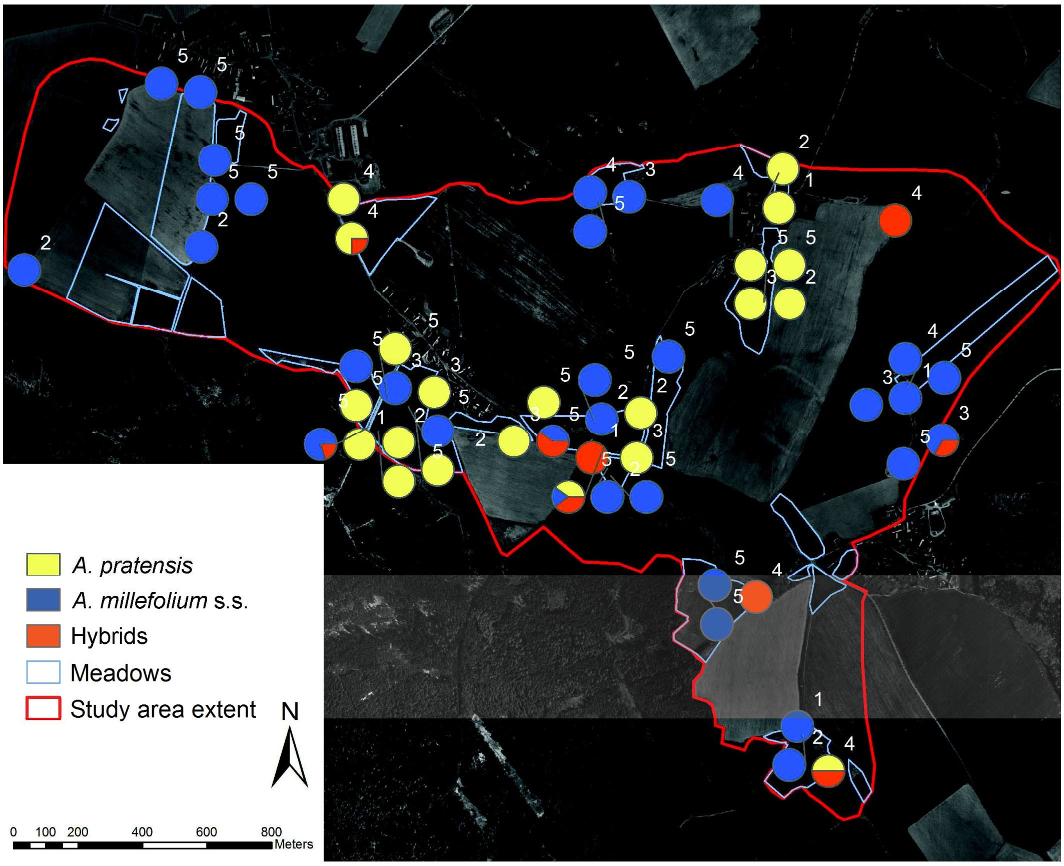


Fig. 1: Distribution of *Achillea millefolium* agg. microspecies and their hybrids within the study area. Numbers beside the pies indicate the number of plants collected at the sampling point.

Doležel, J. & Göhde, W. (1995) Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high-resolution flow cytometry. *Cytometry* 19: 103-106.

7.4.3 Paper V – Appendix 3 – Spatial distribution of sampled populations

Table 2: Summary of sampling effort in terms of sampled plots (*Achillea* populations); const. = the number of plots constructed; harv. = the number of plots in which the seeds ripened and could be harvested (not subjected to untimely hay cut).

Timing of flowering	***A. millefolium***				***A. ptarmica***			
	Verge		**Meadow**		**Verge**		**Meadow**	
	const.	**harv.**	**const.**	**harv.**	**const.**	**harv.**	**const.**	**harv.**
Early flowering (13.7.-18.7.)	17	13	20	20	23	21	-	-
Peak flowering (26.7.-31.7.)	11	10	13	10	15	13	6	4
Late flowering (13.8.-18.8.)	8	5	13	6	10	9	11	4
Total	36	28	46	36	48	43	17	8

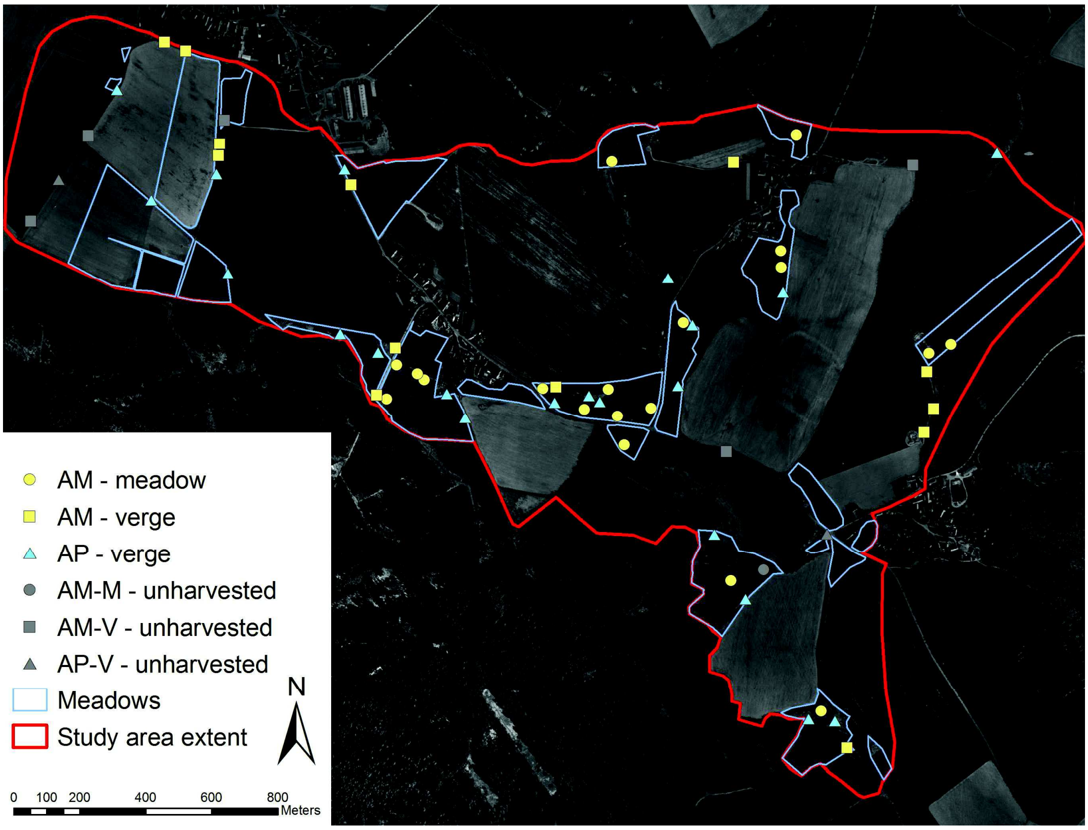


Fig. 1: Spatial distribution of populations sampled during the beginning of flowering (1st sampling interval; 13th to 18th July); AM – *A. millefolium*, AP – *A. ptarmica*

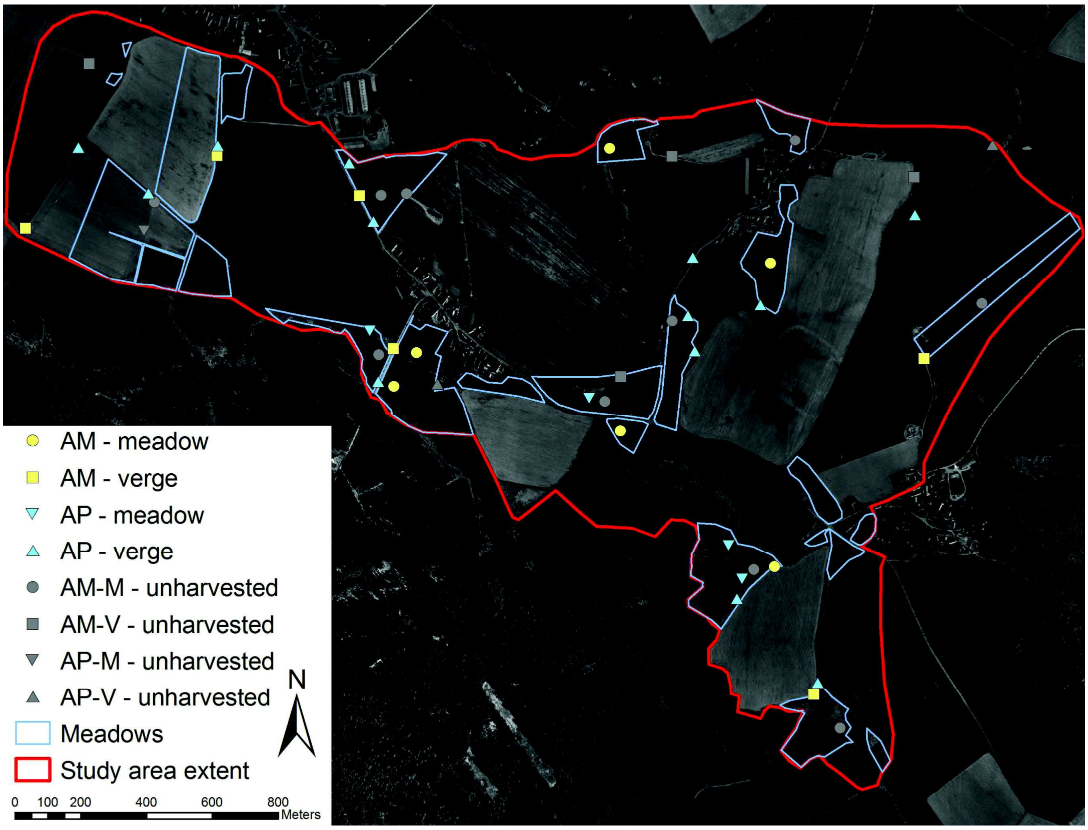


Fig. 2: Spatial distribution of populations sampled during the peak flowering (2nd sampling interval; 26th to 31st July); AM – *A. millefolium*, AP – *A. ptarmica*

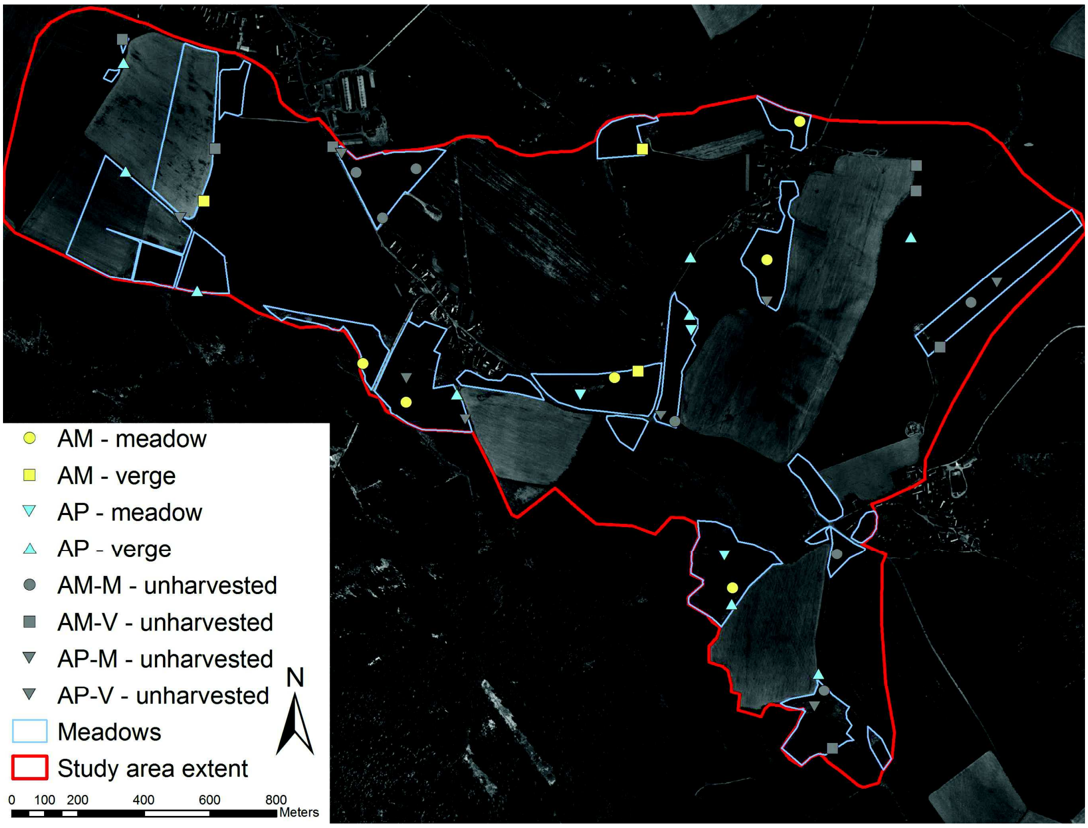


Fig. 3: Spatial distribution of populations sampled during the end of flowering (3rd sampling interval; 13th to 18th August); AM – *A. millefolium*, AP – *A. ptarmica*

7.4.4 Paper V – Appendix 4 – Estimates of pool of available mates in the 15 m radius of the sampled plots

Besides estimating conspecific population density within vegetation plots, we also estimated the number of flowering conspecifics in the 15 metre radius at semiquantitative scale. The scale was defined as follows: 0 – no flowering conspefics outside the vegetation plot; 1 – 1-10 conspecifics; 2 – 11-100 conspecifics; 3 – 101-1 000 conspecifics; 4 – 1 001-10 000 conspecifics; 5 – more than 10 000 conspecifics. The obtained variable was not used in the data analysis, since it was correlated with population density within the vegetation plot.

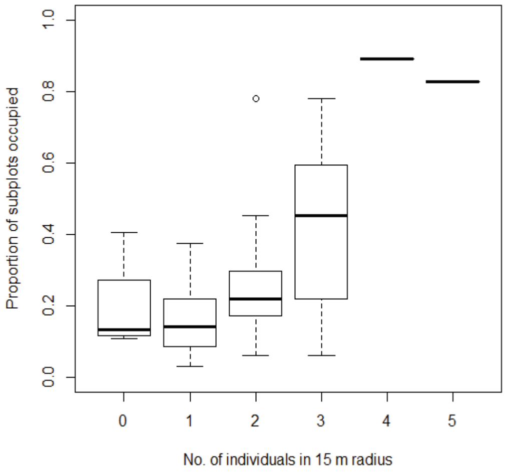


Fig. 4: Relationship between number of flowering *A. millefolium* individuals in 15 metre radius and proportion of subplots occupied by flowering *A. millefolium* plants within the vegetation plot

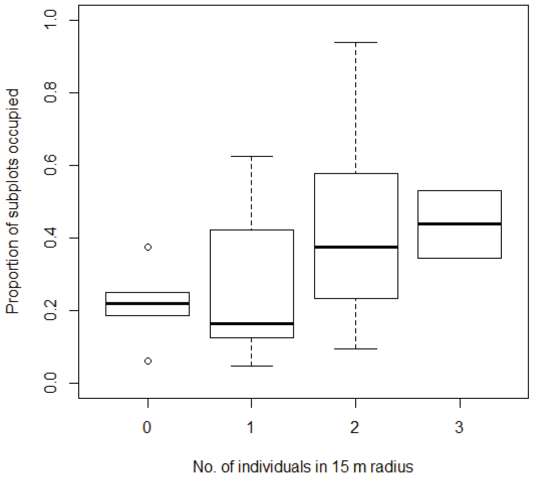


Fig. 5: Relationship between number of flowering *A. ptarmica* individuals in 15 metre radius and proportion of subplots occupied by flowering *A. ptarmica* plants within the vegetation plot

7.4.5 Paper V – Appendix 5 – Relationship of plot attractiveness to timing and site type

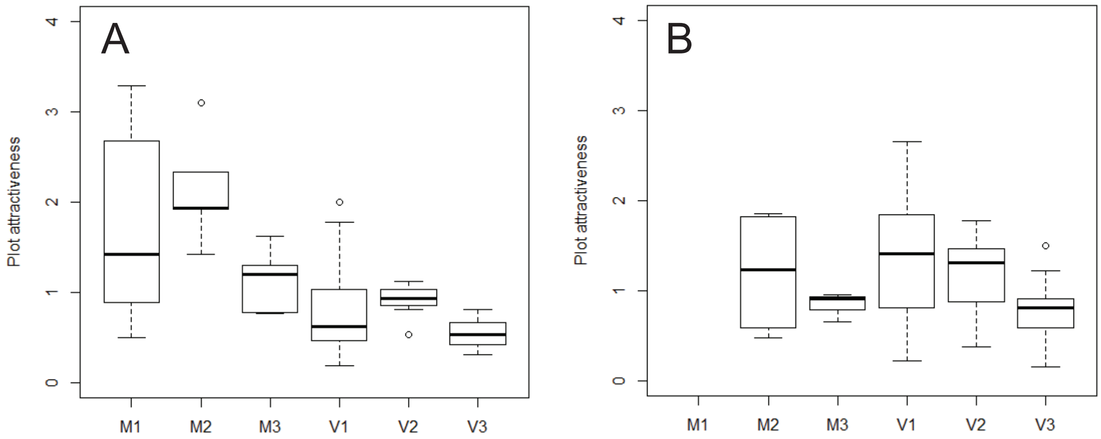


Fig. 6: Dependence of plot floral attractiveness for pollinators (for definition see Materials and Methods section) on timing and site type for A) *A. millefolium* dataset and B) *A. ptarmica* dataset. M – meadow sites, V – verge sites, 1 – early flowering, 2 – peak flowering, 3 – late flowering.

7.4.6 Paper V – Appendix 6 – Descriptive statistics of Achillea *plant characteristics according to site type and timing*

Table 3: Descriptive statistics (mean ± SE) of average per plot plant characteristics of *A. millefolium*; M – meadow sites, V – verge sites, 1 – early flowering, 2 – peak flowering, 3 – late flowering.

Site/timing	N	Stalk height	No. of flower heads	Seeds per flower head	Germinability
M1	20	32.3 ± 1.9	52.3 ± 4.9	9.4 ± 1.0	0.497 ± 0.028
M2	10	33.0 ± 3.4	39.4 ± 13.2	5.8 ± 1.5	0.533 ± 0.024
M3	6	43.4 ± 2.3	33.1 ± 7.1	5.9 ± 0.6	0.613 ± 0.031
V1	13	58.3 ± 7.0	115.9 ± 15.4	7.7 ± 1.2	0.513 ± 0.048
V2	10	51.9 ± 5.5	116 ± 23.6	7.0 ± 1.4	0.525 ± 0.028
V3	5	51.7 ± 8.6	72.7 ± 23.6	7.3 ± 2.4	0.539 ± 0.020

Table 4: Descriptive statistics (mean ± SE) of average per plot plant characteristics of *A. ptarmica*; M – meadow sites, V – verge sites, 1 – early flowering, 2 – peak flowering, 3 – late flowering.

Site/timing	N	Stalk height	No. of flower heads	Seeds per flower head	Germinability
M1	0	-	-	-	-
M2	4	35.3 ± 4.2	10.0 ± 4.2	10.6 ± 1.6	0.492 ± 0.079
M3	4	34.2 ± 4.2	6.0 ± 1.1	17.5 ± 4.0	0.437 ± 0.126
V1	21	75.4 ± 3.1	27.8 ± 4.8	25.6 ± 2.7	0.412 ± 0.029
V2	13	74.8 ± 3.5	28.5 ± 4.2	20.1 ± 3.0	0.496 ± 0.030
V3	9	62.6 ± 5.4	18.6 ± 3.1	16.7 ± 4.7	0.425 ± 0.082