The role of indirect plant-plant interactions via shared pollinators: a combined experimental and theoretical study in species-rich temperate grasslands.

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

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aus Chimay, Belgien

Tübingen

2014

Tag der mündlichen Qualifikation: 11 July 2014

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Citation

Hanoteaux, S. 2014. The role of indirect plant-plant interactions via shared pollinators: a combined experimental and theoretical study in species-rich temperate grasslands. *Ph.D. Thesis.* University of Tübingen, Germany.

Publication

During this thesis, the following article has been published :

Declaration of my own contribution to the present manuscript

The programming of the model used in the first chapter was entirely my work. The field experiment was jointly designed with Eva-Maria Hoch. I designed the common garden experiment and gathered all the data concerning pollinator visits for the two last chapters. The statistical data analysis of the three chapters was entirely my work.

During the course of the thesis I was advised by Dr. Merav Seifan, with whom I discussed the results. Dr. Merav Seifan and Prof. Dr. Katja Tielbörger contributed to advanced drafts of the first chapter, the published manuscript, as co-authors. The other two chapters were entirely written by me, but proofread by Dr. Merav Seifan and Prof. Dr. Katja Tielbörger.

Contents

Acknowledgements

Long was the way, I would not have made it alone...

First of all, I'm very grateful to **Dr. Merav Seifan** for having introduced me to the field of pollination ecology, for her guidance and support, constructive criticisms on my work, for always finding the time to discuss my results or improving my manuscripts, for her encouragements and, yes also for Sandman. Thank you so much Merav!

I also thank **Prof. Dr. Katja Tielbörger** for giving me the chance to write my PhD in her working group, for the prime times she spent correcting my manuscripts, for her support during the time in Tübingen and especially to have triggered that reaction when motivation was gone. I would not have made it without it. Thank you Katja!

I owe a deep debt to **Sara Bangerter**, who translated my application form into German and as such allowed me to get funded for two years. Thank you Sara!

Evidently, I also would like to thank the Land Baden-Württenberg for funding through the Landesgraduiertenförderung Baden-Württemberg fellowship.

During the time in Tübingen, I met many people who were the source of inspiring thoughts. I would like to thank **Pierre** for teaching to me what ecology is about and that it can (should?) be done drinking coffee and smoking; **Peter** who thaught me much about modelling with just one question; **Tal** for always finding the time to answer my "little questions" and for spending hours answering them, greatly improving my understanding of programming; **Mark** for his help in many aspects of my PhD, **Wolfgang** for

having made my life much easier in terms of computer use and for helping me spreading R through the world; **Mira**, not only for the nicest summer ever in the field but also for Johnossi; **Betty** and **Astrid** for teaching to me the nicest word in German: "*Notstro-maggregat*"; **Marcos** for teaching to me how to make my work attractive; **Ortrun** for her invaluable help in setting up experiments and raising plants; **Andrea** and **Christiane** for making German paperwork look easy and **Michael** for teaching me the names of plants.

For the help received in different ways, I thank **Sabine**, **Srijana**, **Christian**, **Johannes**, **Tom**, **Philipp**, **Nicole**, **Jan**, **Mirka**, **Hongchun**, **Michal**, **Udi**, **Yolanda**, **Anne**, **Benedikt**, **Raúl**, **Nina**, **Sara**, **Sara and Sara**.

Not necessarily involved in the scientific aspects of my PhD, but certainly involved in the social aspects of it, I would like to thank **Thierry**, **Kim**, **Paco**, **Ines**, **Ingo**, **Mark**, **Kris**, **Niklas**, **Jonas**, **Bertrand**, **Antoine**, **Fix** & **Emina** & **PJ**, **Sébastien**, **Nicolas**, **Jean-Pascal**, **Bert, Johannes, Germain, Laurent, Denis** and **Fabrice.**

I also would like to thank all persons involved in the development of free operating systems and softwares for making such a great job!

Thank you so much **Maman**, **Armand**, **Malika** and **Danaë**, I guess you know why.

A special thanks to **Ingo** - it all started because of you!

Abstract

Anthropogenic activities are rapidly changing the world. The ongoing climate change (and its associated shifts in flowering phenologies), biological invasions and increased fragmentation of ecosystems are all inducing rapid changes in structural characteristics of plant communities. In communities, where many species depend on the service of pollinators for their reproduction, changes in species composition, floral densities and spatial distribution will undoubtedly further affect plant-pollinator interactions.

Due to the interdependence of plant and pollinator species for their reproduction, plant-pollinator interactions are central in the maintenance of both plant and pollinator species and hence of biodiversity in many ecosystems. Therefore, understanding how structural characteristics of plant communities are affecting plant-pollinator interactions, would enable a better anticipation of the ecological consequences of destructive human activities.

The present thesis investigated the impact of changing structural characteristics of European grassland plant communities on plant-pollinator interactions and on the outcome of indirect plant-plant interactions mediated through shared pollinators. Firstly, a spatially explicit model was used to theoretically examine the interplay between the densities and the spatial distribution of two differently attractive species on the plant species survival. Secondly, the results of the model were put to the test in a field experiment in which the changes in plant-pollinator structure induced by changes in densities and spatial patterns were tracked using a network approach. Finally, a common garden experiment allowed us to test the importance of species identities on the role they play on the outcome of indirect plant-plant interactions.

The overall findings clearly indicate that the small-scale spatial patterning in plant communities is an important factor shaping the outcome of indirect plant-plant interactions

11

by manipulating the behaviour of pollinators. Spatial aggregation in plant species can result in pollinators being trapped in mono-specific patches, substantially increasing the quality and the quantity of pollinator services received by the aggregated plant species. This spatial mechanism is especially strong when the aggregated species grows at high density. This mechanism was shown to affect not only the survival of a less attractive species in a theoretical model but also the structure of plant-pollinator interactions under natural situation, by altering patterns of resource use by pollinators. Hence, spatial distribution of plant species at small-scale and its impact on the pollinator behaviour should be considered as an important process in shaping the general characteristics of plant-pollinator networks.

Further, the identities of plant species and their associated set of floral traits are undoubtedly influencing choices made by foraging pollinators and hence, shape the outcome of indirect plant-plant interactions via shared pollinators. Our results showed that the outcome of these indirect interactions is likely to be dictated by the difference in attractiveness among species rather than by the attractive character of only one species. The occurrence and intensity of both intra- and interspecific density dependent responses in the pollinator behaviour were shown to be conditioned not only by the identity of a focal species but also by the identity of its neighbours.

Future research should try to incorporate the small-scale spatial distribution of species and a characterisation of the attractiveness differential among flowering plant species in the set of ecological factors important in shaping the outcome of indirect plant-plant interactions. This would enable a better anticipation of the impact of anthropogenic changes on plant-pollinator interactions and by extension on plant reproductive success and as such on patterns of species coexistence shaping the structure of plant communities.

Introduction

A vast majority of plant species rely on the services of pollinators for their reproduction (Ollerton *et al.* 2012). Further, pollinator species are as well dependent on the floral reward offered by visited plant species for their survival and reproduction (Westrich 1989). As such, plant-pollinator interactions are crucial to the maintenance of both plant and pollinator species, and hence of biodiversity in many ecosystems (Geber & Moeller 2006, Waser & Ollerton 2006, Mitchell *et al.* 2009), a central question in ecology (see e.g. Loreau *et al.* 2001 for a recent review). Moreover, the insight that most pollinator species are generalists in their use of floral resources (Waser *et al.* 1996, Waser & Ollerton 2006) induces a strong potential for indirect plant-plant interactions mediated through shared pollinators (Rathcke 1983, Waser & Ollerton 2006, Sargent & Ackerly 2008, Hegland *et al.* 2009). In diverse plant communities dependent on the services of pollinators for their reproduction, these indirect interactions may play an important role in shaping patterns of species coexistence (Sargent & Ackerly 2008, Mitchell *et al.* 2009).

The outcome of such interactions can either be positive (i.e. facilitation, Rathcke 1983, Laverty 1992, Johnson *et al.* 2003, Feldman *et al.* 2004, Moeller 2004, Moragues & Traveset 2005, Ghazoul 2006, Bartomeus *et al.* 2008, Muñoz & Cavieres 2008, Hegland *et al.* 2009), negative (i.e. competition, Rathcke 1983, Grabas & Laverty 1999, Moragues & Traveset 2005, Bartomeus *et al.* 2008, Hegland *et al.* 2009) or neutral (no interactions, Grabas & Laverty 1999, Moragues & Traveset 2005, Muñoz & Cavieres 2008, Hegland *et al.* 2009). The major determinant of the nature of these indirect interactions is to be sought in

the way pollinators will respond to what they perceive while foraging and the subsequent choices made (Kunin & Iwasa 1996, Chittka & Thomson 2001, Feldman *et al.* 2004, Lãzaro & Totland 2010). Hence, plant communities structural characteristics such as the identity, density and spatial distribution of species in community are all likely to determine the nature of the effects co-flowering species exert on each other (Rathcke 1983, Grabas & Laverty 1999, Moragues & Traveset 2005, Muñoz & Cavieres 2008, Lãzaro & Totland 2010) as these factors are shaping the structure of the visual and olfactory landscape of foraging pollinators.

One of the first attempts to articulate a model predicting the outcome of these indirect plant-plant interactions mediated through shared pollinators, is the visitation – density relationship developed by Rathcke (1983). This graphical model states that a low floral density, visitation rate is very low and any small increase in density, created by either the same species' individuals or by another species which shares pollinators, will have a positive effect on the visitation rate and thus also on the reproductive success. However, as the number of pollinators available at a given place and time is finite, the outcome of such indirect interactions would shift from facilitation to competition with further increase in floral density (Ratchke 1983). Another well-known example of (positive) indirect plant-plant interactions, is the so called "magnet species effect" (Laverty 1992, Johnson *et al.* 2003, Moeller 2004). By locally increasing pollinator abundance, an attractive, highly rewarding species can increase the pollination success of rewardless or less attractive neighbouring species (e.g. Laverty 1992, Johnson *et al.* 2003, Molina-Montenegro *et al.* 2008). However, the close proximity with a highly attractive species could also result in strong competition if pollinators focus their foraging efforts more on the attractive species (Chittka & Schürkens 2001, Muñoz & Cavieres 2008) or if the close vicinity of the attractive species increases improper pollen transfer (e.g. Brown *et al.* 2002, Cariveau. & Norton 2009).

However, choices made by pollinators while foraging in patches are not only defined by the floral density and attractiveness of species (Chittka & Thomson 2001). Even if some pollinators species are known to travel substantial distances to find resources patch (Osborne *et al.* 1999, Pasquet *et al.* 2008, Hagen *et al.* 2011), they tend to fly short distances between consecutive visits (Waser 1982) and have restricted maximum detection ranges of visual and olfactory cues (Ne'eman & Kevan 2001). This implies that foraging bouts in patches are conducted within a restricted spatial extent. Hence, the spatial patterning of species can also affect the behaviour of pollinators (Goulson 1994, Morales & Vazquez 2008), by manipulating their foraging landscape. For example, a spatially clumped species might offer to a visiting pollinator a locally increased availability of that species in each clump (Goulson 1994, Feldman *et al.* 2004). This will tend to increase the visit quan-

tity (Rathcke 1983) in these clumps and increase the visit quality (by reducing improper pollen transfer rates, Rathcke 1983). The inverse is expected when species are spatially well mixed in communities, as pollinators will perceive a more heterogeneous foraging landscape, potentially inducing a switching behaviour and as such a decrease in visitation quality (Rathcke 1983, Brown *et al.* 2002). The potential effects of the spatial patterning of species within community on the reproductive success of plant species becomes even more complex when considering that they also depend on the ability of pollinators to discern among species (Chittka & Thomson 2001), on their innate preferences (Giurfa *et al.* 1995, Chittka *et al.* 1999, Chittka & Thomson 2001, Raine & Chittka 2007, Ings *et al.* 2009) and on change in preferences of pollinators with recent foraging experience (Dukas & Real 1993, Keasar *et al.* 1996) or floral resource availability (Goulson 1994, Kunin & Iwasa 1996, Chittka & Thomson 2001).

Even if often acknowledged, the occurrence and intensity of such spatial processes were never thoroughly investigated in pollination ecology (Goulson 1994, Feldman *et al.* 2004). In order to fill this gap, we used a spatially explicit individual based model to investigate the role played by relative densities and spatial patterning on the survival of two species differing only in their attractiveness to pollinators. Unlike many previous models (Bobisud & Neuhaus 1975, Waser 1978, Goulson 1994, Feldman *et al.* 2004), the model incorporated an elaborate pollinator behaviour which included innate preferences, the ability to discriminate among species and changes in preferences according to the foraging experience, reflecting hence a more realistic pollinator behaviour.

In species rich natural communities such as temperate grasslands, the impact on pollination patterns of the two above mentioned factors will be far more complex to predict than when considering only two species (Dunne *et al.* 2002, Bascompte *et al.* 2003, Olesen *et al.* 2007, Hegland *et al.* 2009). In such communities, plant-pollinator interactions are forming highly complex networks of interactions, the so-called pollination networks (Jordano 1987, Dunne *et al.* 2002, Bascompte *et al.* 2003, Blüthgen *et al.* 2008, Olesen *et al.* 2011). Due to the high interconnection between the two trophic levels in these networks, changes in the plant community composition and structure and the subsequent adaptation in the pollinator behaviour will propagate along the many network connections and can substantially affect the plant reproductive success of species in these communities (Bascompte *et al.* 2003, Waser & Ollerton 2006, Blüthgen *et al.* 2008, Olesen *et al.* 2011).

The use of networks in pollination ecology is relatively new (Jordano 1987) but the study of general characteristics of pollination networks has known a rapid development (Jordano 1987, Dunne *et al.* 2002, Bascompte *et al.* 2003, Blüthgen *et al.* 2006, Olesen *et al.* 2007, Blüthgen *et al.* 2008, Dormann *et al.* 2009, Olesen *et al.* 2011). The development of this branch of pollination ecology is fortunate as it allows to tackle highly complex problems

with a relatively simple methodological approach. As such, we now have a large set of network describers available (the so-called network metrics, Dormann *et al.* 2008, 2009) allowing not only the characterisation of general network properties (Bascompte *et al.* 2003, Vazquez & Aizen 2004, Olesen *et al.* 2007) but also allowing to track changes in pollination patterns induced by changes in structural characteristics of plant communities (Lopezaraiza-Mikel *et al.* 2007, Tylianakis *et al.* 2007, Bartomeus *et al.* 2008, Morales & Vazquez 2008) and to relate them to the occurrence of biological processes (Santamaría & Rodríguez-Gironéz 2007, Blüthgen *et al.* 2008, Vazquez *et al.* 2009).

In the second part of the present thesis, a network approach was used in order to evaluate the ecological consequences of introducing a highly attractive plant species in a species rich grassland community. By manipulating the density and the spatial patterns of the introduced species, we aimed at investigating changes in the network structure induced by our experimental manipulations and hence put the results of the model developed in the first chapter, to the test. We focussed on the analysis of ecological relevant indices related to the general organisation of interactions within these networks, to diversity and evenness of the interactions, to patterns of resource use by pollinators and finally to specialisation in pollinator behaviour.

Further, in the results of studies investigating the outcome of indirect plant-plant interactions, all types of interactions were found: positive, negative or neutral (see above for relevant references). This discrepancy in the results of such studies reflects that even though we have acquired a good understanding of the possible mechanisms shaping these indirect interactions (Rathcke 1983, Laverty 1992, Feldman *et al.* 2004, Seifan *et al.* 2014), we are still unable to predict their outcome. The effects of that a species exerts on its neighbours are undoubtedly species specific as it will depend on both its floral traits and the cognitive ecology of the visiting pollinators. As such, it seems logical to think that an attractive species will be more likely to affect its neighbours than a "less" attractive species (Laverty 1992). However, the concept "attractiveness" itself is relative and is only defined in a given ecological context. A species' attractiveness will not only be determined by its floral traits but also by the identity (and hence floral traits) of its neighbours, i.e. the "attractiveness differential" and not the absolute attractiveness will be important. This may explain why the above mentioned results are not conclusive.

Hence in the last part of this thesis, we aimed at investigating how changes in the species identities would affect the behavioural responses of pollinators to changing relative densities of two plant species. We conducted a common garden experiment in which we created artificial communities composed of two species, along a gradient of their relative densities. By systematically exchanging the identity of the species in our communities, we aimed at investigating the role played by the floral traits of both a focal species and the floral

traits of its different neighbours.

In summary, the present study aimed at disentangling the effects the floral density, the spatial patterns and the identity of plant species on the outcome of indirect plant-plant interactions mediated through shared pollinators in species rich grasslands. This thesis presents a unique combination of theoretical, observational and experimental approaches whose aim was to investigate largely understudied aspects of pollination ecology. The importance of this work goes beyond the sole investigation of the impact of unexplored structural community characteristics on the outcome of indirect plant-plant interactions. Indeed, the recent decline in pollinator populations and diversity (Kearns *et al.* 1998, Biesmeijer *et al.* 2006, Potts *et al.* 2010) could have severe impacts on these interactions and cascades of extinctions are to be expected if the trend in species loss continues (Waser & Ollerton 2006, Olesen *et al.* 2007). Additionally to species loss, anthropogenic changes such as biological invasions (Traveset & Richardson 2006, Morales & Traveset 2009), landscape fragmentation (see Aguilar *et al.* 2006 for a recent review) and shifts in flowering phenologies due to global warming (Visser & Both 2005, Elzinga *et al.* 2007, Miller-Rushing *et al.* 2010, González-Varo *et al.* 2013), have brought substantial changes in structural characteristics of plant communities. Furthermore changes in species identities, densities and spatial distribution of species in communities undoubtedly affect the foraging experience of pollinators and hence their behaviour (Schemske 1981, Rathcke 1983, Dukas & Real 1993, Keasar *et al.* 1996, Chittka & Thomson 2001, Lãzaro & Totland 2010). Alterations in the pollinator behaviour will have repercussions on the plant-pollinator interactions and by extension on both plant and pollinator species reproduction and survival. Hence, it is capital to understand how pollinators adapt their behaviour to the different structural characteristics of plant communities in order to be able to better anticipate further anthropogenic changes.

Thesis objectives and organisation

The present thesis is organised in three distinct chapters. The overall aim of this thesis was to investigate the effects of structural plant community characteristics on the outcome of indirect plant-plant interactions through shared pollinators. Hence, each chapter reflects a separate investigation of the combination of the floral density with such a structural aspect. Explicitly, the objectives of the individual chapters were as follows.

Chapter 1: This chapter aimed at theoretically investigating the impact of spatial patterning and its interplay with relative floral density on the reproductive success of

two species differing only in their attractiveness for pollinators. This was done using a spatially explicit individual based model coupled with an agent based model allowing the modelling of a complex pollinator behaviour.

Chapter 2: This chapter aimed at assessing changes in the structure of plant-pollinator interactions induced by the introduction of an attractive species in semi-natural grasslands, via the analysis of pollination networks. The impacts of the spatial distribution and the density of the attractive species were tested by analysing a large set of network indices related to ecologically relevant aspects of the structure of plant-pollinator interactions.

Chapter 3: This chapter aimed at testing whether the density responses in the behaviour of pollinators foraging in artificial two species plant communities is altered by changes in plant species identities. Systematically changing the identities of the two species allowed us to investigate the effects of both species on both intra- an interspecific density dependence in the pollinator behavioural responses.

Key Results

Chapter 1:
Effects of spatial patterns on the pollination success of a less attractive species

By using a spatially explicit individual based model coupled with an agent based model allowing the modelling of a complex pollinator behaviour, we evaluated the effects of the relative densities and spatial distribution of two species differently attractive to pollinators on the survival of the less attractive species.

We found that, at low relative density, the less attractive species had a higher survival when spatially uniformly distributed than when spatially aggregated. On the other hand, when the less attractive species was more abundant (i.e. at high relative density), its survival was higher when spatially aggregated in mono-specific patches than when uniformly distributed. These results indicate that spatially aggregated species can trap pollinators in mono-specific patches. These results were consistent as long as the scale of the plant spatial aggregation was similar to or larger than the pollinators' detection range. Finally, a certain degree of generalisation in the pollinator behaviour was the necessary condition for the effect of spatial patterns to emerge.

Chapter 2:
Density and spatial distribution of an attractive species alter plant-pollinator interaction structure in grasslands.

In order to put the predictions of the theoretical model developed in the first chapter of this thesis, to the test, we introduced an attractive species into semi-natural grasslands and manipulated its density and spatial distribution in a full factorial way. A large set of network indices reflecting important ecological processes in plant-pollinator interactions were analysed in order to track the changes in network structure following the introduction of the attractive species and the manipulation of its density and spatial distribution.

Our results suggest that the neutrality hypothesis can explain the changes in diversity and evenness of plant-pollinator interactions following the introduction of the attractive species and its density manipulation. However, it fails to explain the observed impact of spatial patterns. Indeed, we found that a regular spacing of the attractive species induced a higher exclusivity and lower similarity in resource use by pollinators than when the attractive species was spatially clumped. We have proven that small-scale spatial mechanisms are at work in pollination patterns and confirmed the predictions of the previous chapter. Our results further suggest that the introduced species act as a strong competitor for the services of pollinators, especially at high density.

Chapter3:
Identity of neighbouring species alters the response of pollinators to floral density in artificial plant communities

We created artificial communities composed of two differently attractive species and built a gradient of their relative floral densities. By systematically exchanging the two species, we aimed at investigating the effects of their identities (i.e. of both the focal species and of its neighbour) on both intra- and interspecific density dependent responses in the behaviour of several important pollinator groups.

We found that the identity of neighbouring species can induce and/or alter both intra- and interspecific density dependent response in quantitative aspects of the pollinator behaviour. Both positive and negative interspecific interactions among plant species were found and always enabled by the main shared pollinators and conditioned by the identity of the neighbouring species. Changing the identity of the neighbouring species can hence

alter the outcome of indirect plant-plant interactions. These results were explained in the light of the difference in attractiveness among plant species for pollinator, leading to the brief introduction of the concept of "attractiveness differential".

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CHAPTER 1

Effects of spatial patterns on the pollination success of a less attractive species

Abstract

Plant individuals rely on pollinator services for their reproduction and often have to share these services with co-occurring neighbours, creating complex indirect plant-plant interactions. Many current theoretical models focus on the effect of floral resources' density on the net outcome of these indirect plant-plant interactions, often neglecting the identity of plant species in the communities and especially the species' spatial pattern. To fill this gap, we created a spatially explicit model whose goal was to study the interplay between relative densities and spatial distribution patterns of two plant species differing in their attractiveness for pollinators. Since theory predicts that pollinator behaviour strongly governs the outcome of pollination, we allowed the pollinators to systematically change their plant preferences based on their foraging experience. Thus the interplay between density and spatial pattern of plants was tested over a continuum of behaviours from specialists to generalists. Our most striking finding was that reproductive success of the less attractive was affected in an opposite way by spatial patterns depending on whether the species had relatively low or high densities. Namely, when the less attractive species was highly abundant, its survival was higher when aggregated in large monospecific patches than when uniformly distributed. On the other hand, when the attractive species was more abundant, the less attractive species survived better when uniformly distributed.

These results were consistent as long as the scale of the plant spatial aggregation was similar to or larger than the pollinators' detection range. Our results suggest that aggregated plant spatial patterns manipulate pollinator behaviour by trapping them within monospecific patches. This effect was sufficiently strong to enhance the survival of a competitively inferior species and hence to act in a way similar to the more familiar niche or temporal separation among plant species.

Introduction

Reproduction of many plant species is subject to pollination success and often pollinator services are shared among co-flowering species (Campbell & Motten 1985, Feinsinger 1987, Geber & Moeller 2006, Mitchell *et al.* 2009). Therefore, community composition and the identity of neighbouring plants are likely to affect individual reproductive success. For example, the preferences and foraging patterns of pollinators (and hence plant reproductive success) are not merely the outcome of species-specific floral traits, but are greatly affected by the floral composition of the entire plant community (Kunin 1997, Chittka & Thomson 2001, Ghazoul 2006, Lãzaro & Totland 2010). In particular, pollinator foraging patterns may be strongly affected by the identity of the neighbouring plants because of differential attractiveness of floral display (Clegg & Durbin 2000), variation in reward content and quality (Dukas & Real 1993a, Klinkhamer & van der Lugt 2004), or because of inherent preferences and foraging behaviour of different pollinator groups (Sih & Baltus 1987, Lãzaro & Totland 2010). The intensity by which plants affect reproductive success of their neighbours is obviously affected by their relative attractiveness, i.e. the impact of attractive species on 'unattractive' ones is most likely much larger than vice-versa. Therefore, a useful approach to study the impact of neighbourhood community structure on the outcome of shared pollinator services is to focus on less attractive species, and determine their reproductive success as a function of modified features of their neighbouring plant species.

The factors enhancing the success of less attractive species in a community with attractive plants may be theoretically classified into two groups: the first is composed of factors which act against the negative effect of attractive neighbours. Since attractive plants are, by definition, preferred by pollinators, any factor that reduces the pollinator's ability to choose among species and forces it to visit the less attractive species will increase the less attractive species's reproductive success. One such factor is the relative density of the species in the community (Sih & Baltus 1987). If a species's density is relatively high, visitation rate may be increased simply due to the functional and/or numerical

response of pollinators, irrespective of its attractiveness (see e.g. Klinkhamer & van der Lugt 2004). The second group of factors is connected to the potential ability of the more attractive species to facilitate reproductive success of its neighbours. In these cases, the attractive species serves as a magnet species, increasing local pollinator activity and thus actively increasing not only its own reproductive success but also that of its less attractive neighbours (Laverty 1992, Johnson *et al.* 2003, Moeller 2004, Juillet *et al.* 2007).

Previous studies attempted to generate a robust theoretical background for predicting the outcome of both processes and hence to unravel the prevailing factors acting when plants share pollinators. One of the earliest models predicted that pollinator visitation rate per flower will increase with increasing plant density until pollinator visits are saturated and competition for pollinator visits starts dominating (Rathcke 1983). At low densities, visitation rate is very low and any small increase in density, created by either the same species' individuals or by another species which shares pollinators, will have a positive effect on the visitation rate and thus will increase reproductive success. However, as density increases further, competition for pollinators will become increasingly important. Kunin & Iwasa (1996) found similar results, but showed that the relative disadvantage of the low density species can be reduced by a manipulation of the pollinator's foraging choices, namely a specialisation of some pollinators on the low density species. A positive effect of a heterogeneous species composition at low plant densities was supported by an analytical model by Feldman *et al.* (2004) which showed that if the pollinator visitation rate is an initially accelerating function of total flower density, plant species showed higher reproductive success and longer survival time in the presence of another species relative to monocultures.

Next to the identity and density of neighbours, an important but largely understudied factor that may determine reproductive success of unattractive species is spatial arrangement of plant individuals. For example, within a plant community, a spatially clumped distribution of a certain species may offer a locally increased resource availability within each clump of that particular species. This will tend to increase pollinator visits in these clumps (increased visit quantity, Rathcke 1983), and in addition reduces the negative effects of improper pollen transfer (increased visit quality, Rathcke 1983). When the species are spatially well-mixed within a community, this tendency is expected to be reversed because the pollinator may perceive a more heterogeneous foraging landscape which may reduce visitation quality. Therefore, spatial segregation of floral resources could be beneficial for species due to lower interspecific competition (Goulson 1994, Jakobsson *et al.* 2009), similar to the positive effects of intraspecific clumping related to other resources (Stoll & Prati 2001).

The effects of spatial patterns on reproductive success become even more complex when

considering that they depend also on pollinator traits, i.e. their ability to differentiate between plant species and to respond to the perceived vegetation patterns. Unfortunately, many previous models used relatively simple rules for pollinator foraging behaviour (Bobisud & Neuhaus 1975, Waser 1978, Goulson 1994, Feldman *et al.* 2004), and there is a lack of studies combining the recipient and provisioning part of pollination in determining plant community structure. This discrepancy between the two aspects of pollination ecology is unfortunate, because there are clear indications that pollinator decision-making plays a significant role in the outcome of such systems (Kunin & Iwasa 1996, Chittka & Thomson 2001). For example, pollinators are known to fly shorter distances between two consecutive successful visits (Waser 1982) which reduces the spatial extent of the foraging bout. This observation, coupled with restricted maximum detection ranges of visual and olfactory cues (Ne'eman & Kevan 2001), indicates the importance of plant community composition and structure. Furthermore, many pollinator groups have certain innate preferences for flowering traits such as colours (Giurfa *et al.* 1995, Chittka *et al.* 1999, Chittka & Thomson 2001, Raine & Chittka 2007, Ings *et al.* 2009). However, these preferences can change during foraging activity depending on the availability of floral resources (Goulson 1994, Chittka & Thomson 2001) and recent foraging experiences (Dukas & Real 1993b, Keasar *et al.* 1996). This implies that constancy may change according to the relative density and the identity of species found during a foraging bout (Grindeland *et al.* 2005, Hegland & Totland 2005, Cariveau. & Norton 2009). Plant community spatial distribution can therefore alter pollinator behaviour (and thus constancy) by changing the available (i.e. detectable) resources, inducing different foraging experiences and hence affecting pollination success. Taking these considerations into account, the reproductive success of species should depend on their attractiveness, abundance and spatial distribution relative to the other species in the community.

In this study, we used an individual based simulation model to study the effect of plant community spatial pattern and pollinator characteristics on the probability of a less attractive species to survive. We employed a model where pollinators were foraging in a plant community composed of two species with different attractiveness to the pollinators. We varied the size of monospecific patches within the community and the relative densities of the two plant species in order to conduct an analysis of the interplay between density and spatial patterns. Since we assume that pollinator decision-making plays an important role, we tested the effect of three components: pollinator constancy (i.e. generalist *vs.* specialist behaviour), reward variation among plants, and pollinator detection range (Field Of View). Finally, we conducted a sensitivity analysis to test for the effects of different pollinator population sizes and plant dispersal distance on the model outcomes.

We used this combined approach (i.e. considering pollinators and plant individuals explic-

itly) in order to test the following hypotheses: (i) in a community dominated by attractive species, less attractive individuals will be avoided if they grow in clumps, due to the high availability of preferred floral resources. However, (ii) if the less attractive species is randomly dispersed among attractive ones, pollinators will be more homogeneously distributed in space, increasing the number of chance visits to the less attractive species. On the other hand, (iii) if the community is mainly composed of less attractive individuals, a spatial aggregation of the less attractive species may manipulate the pollinators' foraging landscape by reducing their choices over large areas of the plant community. This should increase the reproductive success of the less attractive species relative to a random distribution, where the attractive species can be detected and visited by pollinators from any position in the plant community.

Model

In order to investigate the effects of spatial patterns on the survival probability of a less attractive species, we developed a spatially explicit and time discrete model with a two-species plant community and a pollinator population. We used a grid based Individual Based Model (IBM) to model two self-incompatible flowering plant species. In this model, plant species reproductive success, and thus their ability to survive in the community, was governed by pollinator behaviour. To achieve that, pollinators were modelled as agents which interacted with plant individuals during their foraging bouts. The spatial aspects of the vegetation model combined with an interactive model, explicitly considering plants and pollinators separately but interacting with each other, allowed us to test the influence of spatial characteristics of both plants and their pollinators (initial spatial distribution of plant species and spatial movement of pollinators) in addition to the effects of non-spatial characteristics (e.g. densities of plant species, number of pollinators, floral constancy between visits).

The model contained a grid of 100×100 cells. Each cell represented a site in which a single plant can establish, flower, reproduce and die. We let the plants and pollinator interact with each other for 50 years and used the number of cells occupied by each plant species as a measure for its success. To avoid edge effects, the grid was designed as a torus. In the following, we briefly describe our model assumptions concerning plant species traits, pollinator traits, and the interactions among the two trophic levels.

Plant community

Plant species

The plant community was composed of two annual plant species, which differ only in their attractiveness to pollinators (A; $A \in \{0.1 , 0.9\}$; where higher A values indicate higher attractiveness; see Appendix 3 for the motivation for choosing attractiveness values). At the beginning of each model generation (i.e. one plant year), plant individuals of the two species were introduced to the grid as adult flowering individuals. Each individual flower, regardless of its identity, could contain reward with a probability P_R. As a default, both plant species had a reward probability of 0.5 (i.e. pollinator landing on a flower had 50% chances to be rewarded). However, as part of the model sensitivity test, we also studied situations in which both species had different reward probabilities (see section Simulation experiments). For simplicity, plants did not refill their reward during a generation. If a plant individual was pollinated, it produced N_{Seed} seeds that were then dispersed among the surrounding grid cells at the end of the generation. Dispersal distance was based on a normal distribution with mean 0 and standard deviation d_{disp}. The direction of dispersal was defined as an angle drawn from a uniform distribution ([0-360°]) (details see Appendix 4). At the end of each generation, all plant individuals died and the grid occupancy for the next generation was determined, taking into account the relative seed number of each species (weighted lottery; Warner & Chesson 1985; Appendix 4). For simplicity, no further competitive interactions between species were incorporated in the model (Straw 1972, Bobisud & Neuhaus 1975). Each plant individual could also die before reproducing with a probability $P_{Death} = 0.05$ and each cell had a probability $P_{Empty} = 0.05$ to stay empty during a generation.

Community structure: spatial patterns

To systematically study the effects of spatial patterns on the outcome of plant-pollinator interactions, we manipulated the level of intraspecific spatial aggregation of plant communities. An example may be seen in Figure 1. The first pattern generated was a random pattern (S1), where each cell was randomly assigned to one of the two species with 0.5 probability. This random pattern can be seen as the random distribution of square monospecific patches with a one cell edge. To create more aggregated spatial patterns, we increased intra-specific aggregation by randomly assigning a square monospecific patch with an edge length of five (S5) or ten (S10) cells (i.e. patches of 25 and 100 conspecific individuals, respectively) to one of the two species. As a control we also generated a regular arrangement of the two species among the grid (Reg; see details below).
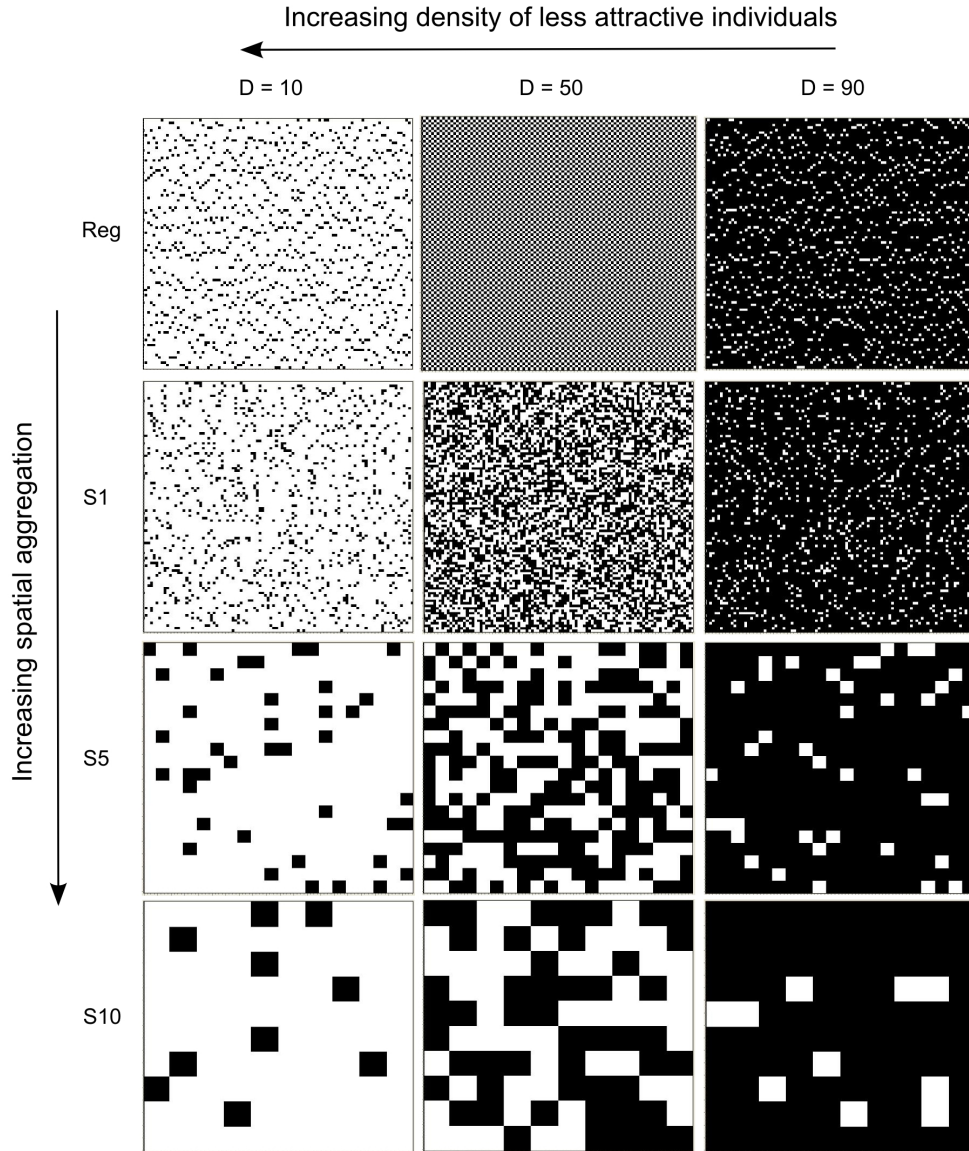
Figure 1: Example of initial spatial patterns and relative species densities combinations used in this study. Black cells represent areas occupied by the attractive species and white cells represent areas occupied by the less attractive species. For simplicity, empty cells are not represented although they were present with a probability of $P_{Empty} < 0.05$.

Community structure: plant density

Because previous studied indicated that species density may have a strong effect on reproductive success, we tested the spatial patterns with three different population densities, defined by the relative abundance of the attractive species ($D = 10$, 50 or 90 percent of the non-empty cells were occupied by the attractive species). To create intraspecific aggregated patterns (S1, S5, and S10) with different species' densities, we divided the grid into the appropriate number of square patches which were then assigned randomly to a species according to its density: 10%, 50% or 90% of the patches were assigned to the attractive species, respectively. The remaining patches were then filled by the less attractive species. During this process, we took into account that a cell had a P_{Empty} probability to be unsuitable for plant growth. To generate a regular pattern (Reg) for each density level, we used a more elaborate algorithm: when the two species densities were equally abundant ($D = 50$), the pattern was easily generated using a "checkerboard" pattern with alternate occupation of cells by the two species. During this process, each cell had a probability of P_{Empty} to remain empty. This resulted in an alternating pattern in which the direct neighbours (four nearest cells) of each cell were either empty or occupied by individuals of the other species. When the attractive and less attractive species' densities were not equal ($D = 90$ or $D = 10$), we divided each grid row into arrays of 10 cells. In each array, one cell was randomly assigned to either the less attractive species ($D = 90$) or to the attractive one ($D = 10$). The remaining cells were filled with the other species or stayed empty with a probability P_{Empty} . We repeated this process ten times in each row before filling the remaining rows in the same fashion. An example for the generated spatial patterns for the three density levels is shown in Figure 1.

Pollinators

The pollinator population represented one pollinator species with N individuals. The pollinators could distinguish between the two flower species (unlike, for example, Straw 1972, Bobisud & Neuhaus 1975, Feldman *et al.* 2004) and were inherently more attracted to one of the plant species, i.e. at the beginning of each model generation, pollinators had a higher preference for the attractive species. During each generation, pollinators could change their preference according to their foraging experience (see section Pollinator foraging rules). While foraging, pollinators had a restricted detection range, called here Field Of View (FOV). The FOV was defined by a Moore neighbourhood with a radius of R_{FOV} cells (Wolfram 1983) which did not change across simulations, i.e. the number of cells in the neighbourhood was equal to $(2 \times R_{FOV} + 1)^2$. We assumed that pollinators were only attracted by flowering individuals within their FOV. They had no information

about total reward distribution and there was no information exchange among pollinators (Pasquale & Jacobi 1998). We chose a pollinator population size that was smaller than the number of flowers in the field to ensure that there was a potential for competition among flowers for pollinator visits (Straw 1972, Bobisud & Neuhaus 1975).

Pollinator foraging rules

To prevent artificial aggregation of pollinators, each pollinator was randomly positioned in the plant community grid at the beginning of each model generation. From this random point, pollinators started their foraging bout using a specific decision rule. First, pollinators needed to decide about the first flower sampled in the field. This decision is usually assumed to be based on the inherent preferences of the pollinators. However, the initial choice may also be affected by a more general perception of the floral resources, because pollinators have a restricted ability to discern among floral resources at long distances (Ne'eman & Kevan 2001). Therefore, pollinators created a "rescaled attractiveness" landscape of the flowers in their field of view (FOV), i.e. a landscape of attractiveness values which depended not only on the specific flower species in the cell, but also on the species growing in neighbour cells of increasing distances, as described in Equation 1:

$$A'_{ij} = \frac{\left(\sum_{\substack{k=i-R_{FOV} \\ k \neq i}}^{i+R_{FOV}} \sum_{\substack{l=j-R_{FOV} \\ l \neq j}}^{j+R_{FOV}} \frac{A_{kl}}{d_{(ij),(kl)}} \right) + A_{ij}}{A'_{ij,max}} \tag{1}$$

where A'_{ij} is the rescaled attractiveness of cell (i,j), A_{ij} is the attractiveness of the flower located in (i,j), $d_{(ij),(kl)}$ is the distance between a cell situated in (k,l) and the focal cell in (i,j), and $A'_{ij,max}(R_{FOV})$ is the maximum value of rescaled attractiveness for the plant at position (i,j) (i.e. if all cells in the neighbourhood are occupied by flowers of maximum attractiveness $(A = 1)$). It should be noted that $A'_{ij,max}(R_{FOV})$ is a function of the size of the pollinators' FOV. Empty cells had a (rescaled) attractiveness of zero.

Once the rescaled attractiveness (A'_{ij}) was obtained for all plant individuals, we let the pollinators move from their randomly assigned positions in the direction of the steepest positive gradient of rescaled attractiveness within their specific FOV (i.e. in the direction of the more attractive part of the community). Pollinators followed that gradient until three conditions were met: (i) the chosen cell was not empty; (ii) no other pollinators were present in the chosen cell; and (iii) the numeric value of the steepest gradient was lower than a threshold value $(T = 0.05)$. A threshold was chosen because otherwise, all pollinators were finally aggregated in local (or global) maxima of the rescaled attractiveness

landscape (see Appendix 5). Therefore, the threshold parameter may be interpreted as a measure of the "pickiness" of pollinators concerning the starting point of their foraging bout. When two pollinators landed on the same grid cell, the later to arrive changed its starting random position by flying away for a distance of 25 cells in a randomly drawn direction (angle in [0,360°[) and started its search again until all three conditions were met.

After all pollinators chose their starting position, a second set of rules was applied. This second phase in the pollinator movements reflected the common foraging mode of most pollinators, where short distances between consecutive visits are preferred (Waser 1982). This implies that pollinators were able to distinguish between different floral resources by detecting the individual attractiveness (Ne'eman & Kevan 2001). To create a short distance foraging rule, pollinators searched for the most attractive flower within their FOV. The decision was made by each pollinator by calculating a score for all the cells within its FOV. This score was based on the distance between the location of the pollinator, the location of the plant individual and the instantaneous pollinator preference G (which was affected by its experience while foraging) as calculated in Equation 2:

$$S = G + \frac{1}{(d+1)} \tag{2}$$

where S is the instantaneous score for a certain plant individual in a specific cell, G is the instantaneous pollinator preference and d is the distance of the cell from the pollinator (adapted from Ohashi & Thomson 2005).

The cell with the highest score value (S) in the pollinator's field of view was chosen as the pollinator's next destination, as long as it was not occupied by another pollinator, and it was not the pollinator's last visited flower in this generation. If a pollinator was already present in the chosen cell, the last arrived flew 25 cells away in a randomly chosen direction (angle in [0,360°[). If more than one flower within the FOV met the conditions and had an equal score, the next position was randomly chosen among these flowers. To make sure that no bias was created during this step, pollinator order in this second phase was random.

Reward collection and pollination

Once the choice of the next flower was made (based on the second step of the pollinator foraging rules described above), the pollinator landed in that cell. As described in section Plant community, each plant individual had a P_R probability to contain a reward. If the flower provided a reward, it was emptied by the visiting pollinator. Pollinators always took

Table 1: Shift in preferences of pollinators depending on the last species visited (rows) and the rewarding character of this last visit (columns). Numbers in brackets give the probability of the events to happen. P_{Shift} is the constancy parameter of pollinators.

		Rewarding character of last visit	
		Rewarding visit	Non rewarding visit
Last species	attractive	preference set to attractive: Stay (1)	preference set to less attractive: Shift (P_{Shift})
visited	less attractive	preference set to attractive: Stay (1-P_{Shift})	preference set to attractive: Shift (1)

pollen from the last visited flower and carried it only until the next visit. If the species of pollen carried matched the species of the currently visited flower, pollination occurred. If the species did not match, no pollination took place, but no additional negative effect was applied, because the limitation put on the pollen carryover time is akin to strong negative effects of heterospecific pollen transfer (Feldman *et al.* 2004). This foraging behaviour was repeated 50 times for each pollinator. Hence, each pollinator had the possibility of creating a maximum of 50 pollination events within one model generation (no pollen was carried by the pollinators at the beginning of a new vegetation generation).

Changes in pollination preferences during a bout

Pollinators were modelled as having an innate preference, dictating an inclination towards the attractive species at the beginning of each generation. However, within a generation, the pollinator preferences could shift according to a set of decision rules (Table 1). Depending on the pollinator's constancy parameter (P_{Shift}) used in our "probabilistic Win-Stay-Loose-Shift Behaviour" algorithm (Ohashi & Thomson 2005), we could model a "constant" (i.e., specialist; $P_{Shift} = 0$) as well as a "shifting" behaviour (i.e. generalist; $P_{Shift} = 1$). Using this approach, the pollinator's past experience affected the attractiveness of species. Numerically, this was achieved by setting the pollinator preference values equal to the adequate plant attractiveness values for each individual pollinator. In this way, a pollinator that was not rewarded by an attractive species in a visit had a P_{Shift} probability of changing its preference towards the less attractive species in its next visit. For this specific pollinator, the instantaneous pollinator preference G for the less attractive species changed its value to equal the value of the attractive species and *vice-versa*.

Simulation experiments

To test the effect of pollinator characteristics on plant-pollinator interactions, we ran all the model simulations, i.e. all combinations of spatial patterns and density, using several pollinator trait combinations (see Table 2 for all the combinations of the parameter values used in the simulations). First, we tested the effects of pollinator constancy (P_{Shift}) on the dynamics of the model, tuning the pollinators from specialist behaviour (to the attractive species; $P_{Shift} = 0$) toward a more realistic scenario in which pollinators presented a shifting behaviour with innate preference towards the attractive species ($P_{Shift} = 1$). To do this, we considered 50 pollinators ($N = 50$) and used a priori defined values for the other parameters ($P_R = 0.50$, $R_{FOV} = 5$ and $d_{disp} = 5$). Because the changes in preferences of pollinators were driven by the rewarding character of their visit (Keasar *et al.* 1996), we investigated the effect of promoting the pollinators' staying behaviour by increasing rewarding probability ($P_R = 1$; i.e. each plant individual is rewarding at the start of each generation and thus no reward variation occurred within species). In a further step, we aimed at exploring the interplay between the size of the monospecific vegetation patches and the maximum detection distance of pollinators. Hence, we conducted simulations in which the values for the pollinator's FOV were altered ($R_{FOV} = 2$, 5 and 10). Finally, to estimate the generality of our findings, we conducted a sensitivity analysis for seed dispersal distances ($d_{disp} = 2.5$, 5 and 10) and pollinator population sizes ($N = 25$, 50 and 100). For each set of parameter values, 50 replications were made for each of the four starting patterns (Reg, S1, S5 and S10) and for the three relative density levels ($D = 10$, 50, 90).

Statistical analyses

The main goal of the statistical analysis was to estimate the probability of the less attractive species to survive in the community under different combinations of plant and pollinator characteristics (see Table 2 for all the combinations of the parameter values used in the simulations). Therefore, we mainly used survival analysis techniques, testing for differences between Kaplan-Meyer estimates of the survival curves for the different spatial patterns within the same set of parameter values (Kleinbaum & Klein 2005). If data was censored, we used Log-Rank tests, whereas if no censoring was present, we used Mann-Whitney U tests (Kleinbaum & Klein 2005). As a first step, we tested for differences in survival between the four spatial patterns. If the appropriate test was significant, we used the false discovery rate correction method to detect pairwise differences (Benjamini & Hochberg 1995). All statistical analyses were conducted in R version 2.13.1 (R

Table 2: Parameter value combinations tested in the simulation experiments, where P_{Shift} is the constancy parameter, R_{FOV} is the Field Of View (pollinator detection distance, in cells), d_{disp} the standard deviation of the seed dispersal distance, N is the size of the pollinator population and P_R is the rewarding probability (i.e. reward variation) of each plant individual. All parameter sets were run for all the combinations of four starting spatial patterns (S1, S5, S10 and Reg) and three densities ($D = 10$, 50 and 90).

Simulation N°	Effect	P_{Shift}	P_R	R_{FOV}	d_{disp}	N
1	constancy high	0	0.5	5	5	50
2	constancy interm.	0.5	0.5	5	5	50
3	constancy low	1	0.5	5	5	50
4	no reward variation	0.5	1	5	5	50
5	no reward variation	1	1	5	5	50
6	FOV small	1	0.5	2	5	50
7	FOV large	1	0.5	10	5	50
8	dispersal short	1	0.5	5	2.5	50
9	dispersal long	1	0.5	5	10	50
10	polli. pop. small	1	0.5	5	5	25
11	polli. pop. large	1	0.5	5	5	100

Development Core Team 2005). The graphical results of the survival analysis of all the parameter combinations tested are presented in Appendix 2.

Results

Effect of density and spatial patterns of the plant communities

The spatial patterns and relative densities of the species composing the community significantly affected the survival rate of the less attractive species, even if eventually it went extinct in many simulations. Generally, the effect of spatial patterns on the survival rate of the less attractive species varied among relative densities: when the less attractive species was dominant at the beginning of a simulation ($D = 10$) it survived longer when spatially aggregated (e.g. pattern S10). Vice-versa, at high density of the attractive species ($D = 90$), the less attractive species profited from being spatially dispersed (e.g. pattern S1; Table 3: simulations 3, 6, 8, 10, Figure 2: simulations 3, 6, Figure 3: simulations 8, 10 and Appendix 2 Figure A.1: simulations 3, 6, 8, 10). At intermediate densities, the results were similar to the effect found at high density of the attractive species, though weaker. In addition, the relative increase in density of the attractive species negatively affected

Table 3: Results of the survival analysis for all parameter combinations. Each simulation experiment was defined according to N = number of pollinators in the model, R_{FOV} = size of pollinator Field Of View, P_{Shift} = shifting parameter of the pollinator behaviour (0: specialist behaviour; 1:generalist behaviour), D = relative density of the attractive species, d_{disp} = standard deviation of the normal distribution used for modelling seed dispersal, P_R = probability of plant individuals to be rewarding). For each simulation experiment, the median survival time (i.e. the generation at which 50% of the simulations led to extinction of the less attractive species) is given for the four spatial patterns: Reg – regular pattern; S1, S5 and S10 – clumped spatial patterns with monospecific square patches with edge length of one, five or ten cells, respectively; the p-value of the adequate test (LR: Log Rank test; U: Mann Whitney U test) and the p-values of the pairwise comparisons (false discovery rate). Values for p<0.05 are in italics, p<0.01 are underlined, bold values indicate p<0.001. If less than 50% of the simulations led to the extinction of the less attractive species, the median could not be computed, implying that the species survived until the end of the simulation (indicated by >50).

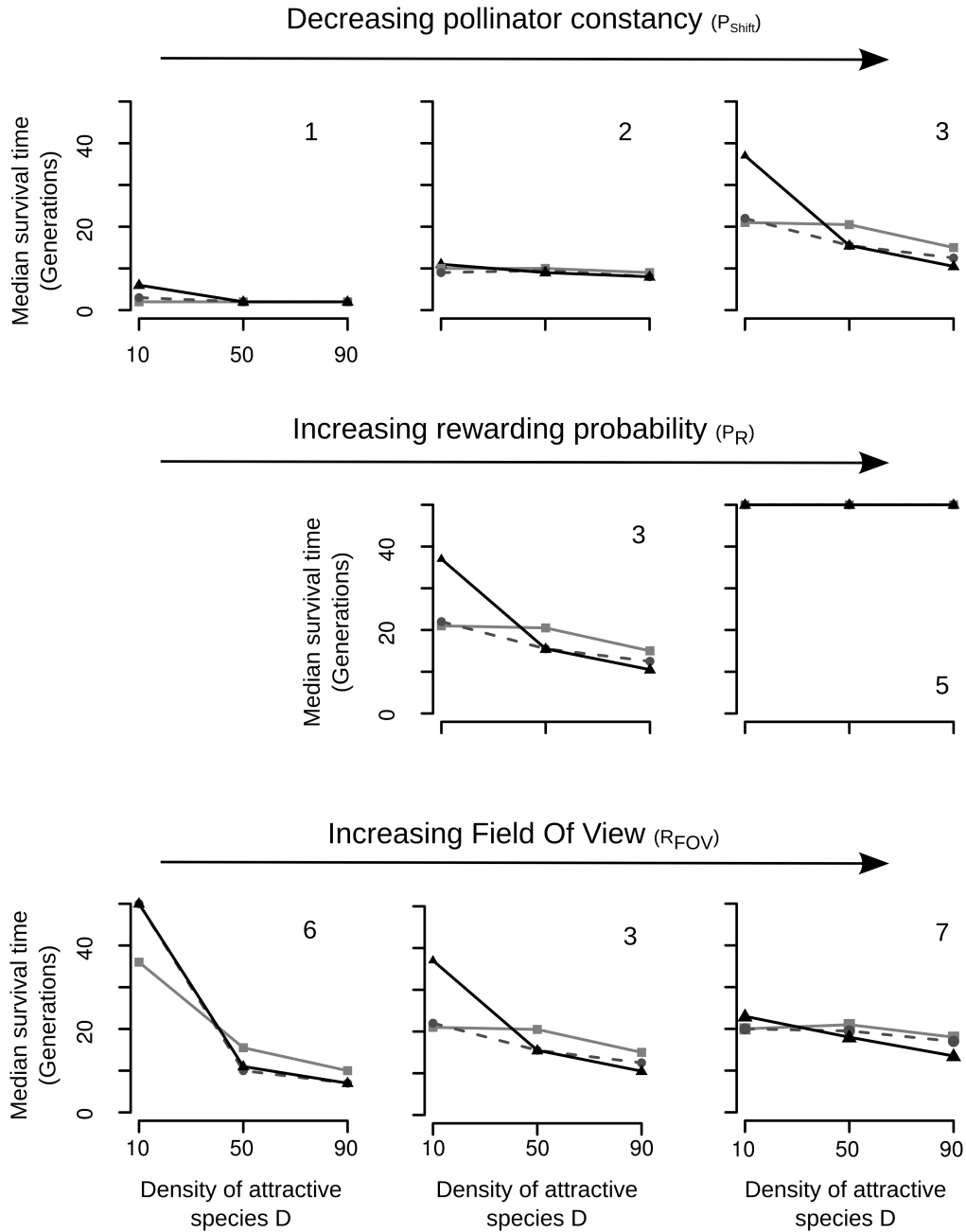| N	R_{FOV}	P_{Shift}	D	d_{disp}	P_R	Median Reg	Median S1	Median S5	Median S10	test	p-value survival	S1-S5	S1-S10	S1-Reg	S5-S10	S5-Reg	S10-Reg
1	5	0	10	5	0.5	2	2	3	6	U	**p<0.001**	**p<0.001**	**p<0.001**	1	**p<0.001**	**p<0.001**	**p<0.001**
1	5	0	50	5	0.5	2	2	2	2	U	0.110	0.719	0.719	0.114	0.719	0.639	0.017
1	5	0	90	5	0.5	2	2	2	2	U	1						
2	5	0.5	10	5	0.5	9	10	9	11	U	0.329	0.607	0.626	0.425	0.051	0.539	0.038
2	5	0.5	50	5	0.5	10	9.5	9.5	9	U	*0.021*						
2	5	0.5	90	5	0.5	8	8	8	8	U	*0.044*						
2	5	0.5	10	5	0.5	10	10	11	37	U	*0.044*	0.607	0.084	0.425	0.051	0.539	0.038
3	5	1	10	5	0.5	16	15	15	15	LR	**p<0.001**	0.192	0.716	0.716	0.763	**p<0.001**	**p<0.001**
3	5	1	50	5	0.5	20	20	20	15	LR	**p<0.001**	0.002	0.006	0.440	0.440	**p<0.001**	**p<0.001**
3	5	1	90	5	0.5	20	20	15.5	10	U	**p<0.001**	*0.017*	*0.016*	0.726	0.726	*0.015*	**p<0.001**
3	5	1	10	5	0.5	21	22	22	37	LR	*0.045*	0.607	0.084	0.425	0.051	*0.045*	*0.038*
4	5	0.5	90	5	1	23	21	37	15	LR	**p<0.001**	0.607	0.084	0.425	0.051	0.539	0.038
4	5	0.5	50	5	1	8	9	8	8	LR	**p<0.001**	**p<0.001**	**p<0.001**	0.626	**p<0.001**	**p<0.001**	**p<0.001**
4	5	0.5	10	5	1	10	10	9	10	LR	**p<0.001**	0.002	0.006	0.440	0.440	**p<0.001**	**p<0.001**
4	5	0.5	90	5	1	27	31	26	24	LR	**p<0.001**	**p<0.001**	**p<0.001**	0.770	0.516	**p<0.001**	**p<0.001**
5	5	1	50	5	1	>50	>50	>50	>50	LR	*0.020*	0.409	0.968	0.141	0.409	0.957	0.409
5	5	1	10	5	1	>50	>50	>50	>50	LR	1						
5	5	1	90	5	1	>50	>50	>50	>50	LR	1						
5	5	1	10	5	1	>50	>50	>50	>50	LR	1						
6	2	1	90	5	0.5	39	36	36	>50	LR	*0.045*	0.624	0.357	0.983	0.347	0.983	0.285
6	2	1	50	5	0.5	15	15	7	7	LR	0.099	0.969	0.008	0.059	0.008	0.003	
6	2	1	10	5	0.5	39	36	15	11	LR	**p<0.001**	0.986	0.006	0.976	*0.034*	0.029	0.976
6	2	1	90	5	0.5	>50	>50	>50	>50	U	**p<0.001**						
7	10	1	10	5	0.5	10	10	7	7	U	**p<0.001**	**p<0.001**	**p<0.001**	0.626	**p<0.001**	**p<0.001**	**p<0.001**
7	10	1	50	5	0.5	22	21	19.5	18	LR	*0.045*	0.624	0.357	0.983	0.347	0.983	0.285
7	10	1	90	5	0.5	20	20	20	23	LR	0.099	0.969	0.008	0.059	0.008	0.003	
7	10	1	10	5	0.5	21.5	20	19	38	LR	0.624	0.969	0.357	0.983	0.347	0.983	0.285
8	5	1	50	2.5	0.5	49	49	31.5	>50	LR	**p<0.001**	**p<0.001**	0.006	0.149	0.814	0.464	0.984
8	5	1	90	2.5	0.5	28	27	19	9	LR	0.005	0.986	0.006	0.976	*0.034*	0.029	*0.013*
8	5	1	10	2.5	0.5	2.5	2.5	2.5	>50	LR	**p<0.001**	0.054	0.416	0.149	0.276	0.976	
8	5	1	90	2.5	0.5	27	30	28	38	LR	**p<0.001**	0.824	0.189	0.770	0.427	0.427	0.237
9	5	1	50	10	0.5	12	17	9.5	12	LR	**p<0.001**	0.002	0.006	0.440	0.440	**p<0.001**	**p<0.001**
9	5	1	10	10	0.5	10	11	10	10	LR	**p<0.001**	**p<0.001**	0.006	0.149	**p<0.001**	**p<0.001**	**p<0.001**
9	5	1	90	10	0.5	23.5	33	19	10	LR	0.587	0.824	0.008	0.189	0.008	0.427	0.237
9	5	1	10	10	0.5	10	10	10	10	LR	0.519	0.024	0.038	0.222	**p<0.001**	0.539	*0.038*
10	25	1	10	5	0.5	16	17	9.5	12	LR	**p<0.001**	*0.024*	0.038	0.222	**p<0.001**	0.539	*0.038*
10	25	1	50	5	0.5	12	10	9	6	LR	**p<0.001**	*0.41*	0.146	0.429	0.263	*0.036*	0.651
10	25	1	90	5	0.5	12	10	9	6	LR	**p<0.001**	0.424	**p<0.001**	0.612	0.248	0.248	**p<0.001**
11	5	1	10	5	0.5	41.5	40.5	45.5	>50	LR	*0.013*	0.572	*0.014*	0.612	0.276	0.572	0.572
11	100	5	50	5	0.5	46	46	39.5	39	LR	0.189		0.212				
11	100	5	90	5	1	46	47	41	43.5	LR	0.716	0.572					0.572

40

Figure 2: Median survival time of the less attractive species in relation to factors affecting pollinator behaviour (pollinator constancy, reward probability and detection range) for the different spatial patterns and relative densities combinations. The number of each sub-figure refers to the simulation number in Table 2. Grey continuous lines with ■ represent the median survival time for the S1 pattern; dashed dark grey lines with ● represent the S5 pattern and black continuous lines with ▲ represent the median survival time for the S10 pattern. For simplicity, we did not visualize the curves for the regular pattern, which did not differ from the S1 pattern. If more than 50% of the repetitions led to coexistence of the two species, the value for the median survival time could not be computed and we visualized this on the figure by setting the median survival time to 50 generations. Note that simulation 3 is presented three times, to enable better visual comparison with other simulations.
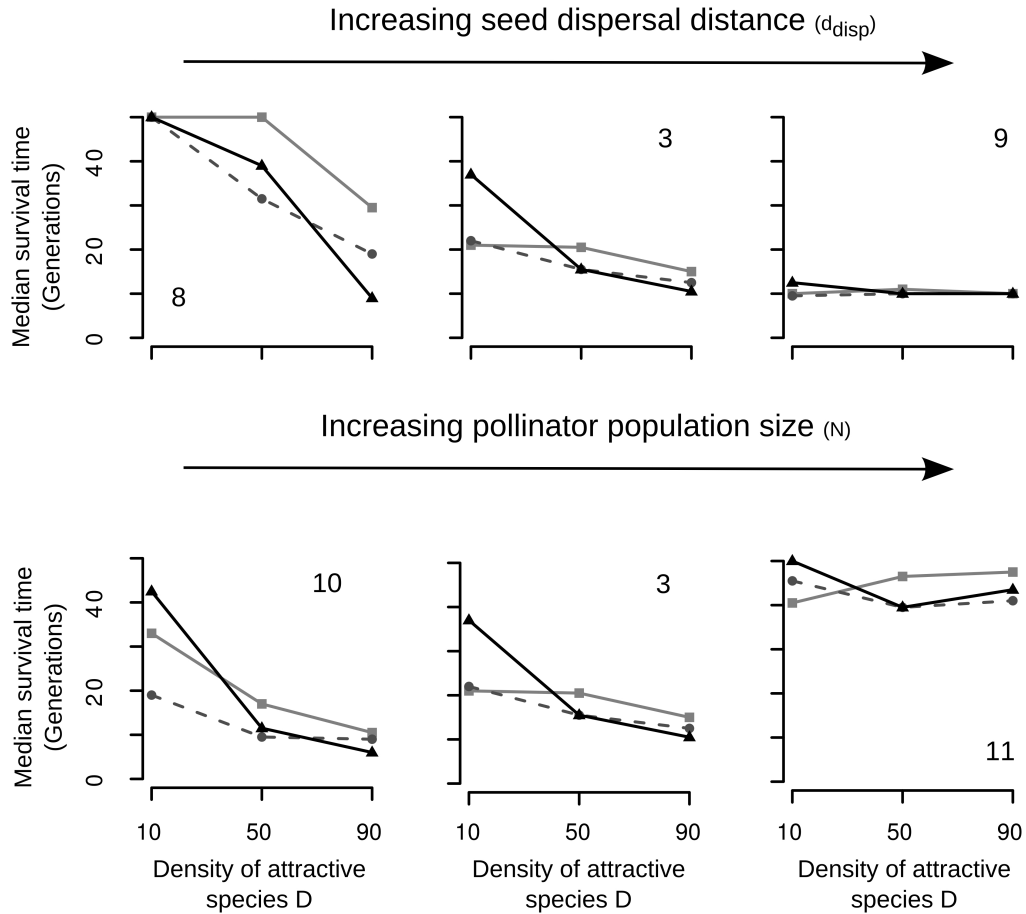
Figure 3: Median survival time for the less attractive species in relation to the factors tested in the sensitivity analysis (plant dispersal distance and pollinator population size) for the different spatial patterns and relative densities combinations. The number of each sub-figure refers to the simulation number in Table 2. Grey continuous lines with ■ represent the median survival time for the S1 pattern; dashed dark grey lines with ● represent the S5 pattern and black continuous lines with ▲ represent the median survival time for the S10 pattern. As in Fig. 2, we did not visualize the curves for the regular pattern and the value for the median survival time was set at 50 generations when it could not be computed. Note that the simulation 3 is presented twice, to enable better visual comparison with other simulations.

median survival time of the less attractive species regardless of spatial pattern (Table 3: simulations 3, 6, 8, 10, Figure 2: simulations 3, 6, Figure 3: simulations 8, 10).

Effect of factors shaping pollinators' behaviour

Constancy

The less attractive species's median survival time was longer for all spatial patterns when pollinators tended to be generalists (i.e. increasing the P_{Shift} value, Table 3 and Figure 2: simulation 1, 2, 3). However, the general outcome was also dictated by the interaction between spatial patterns and density as described above. The largest differences in survival curves were found for highly generalist pollinators ($P_{Shift} = 1$), where survival rate of the less attractive species increased with decreasing spatial aggregation at low and intermediate density of less attractive individuals (i.e. $D = 50, 90$), whereas at high density (i.e. $D = 10$), the survival rate of the less attractive species increased with aggregation. Both species' population dynamics supported the results of the surviving time analysis. When pollinators showed a more generalist behaviour, a strong decrease in the community size was created at early generations (see example in Figure 4). Although the population of the attractive species generally reached a stable size of approximately 5000 individuals (Appendix 6, Table A2), the time needed to reach these equilibrium densities was subjected to the interplay between spatial patterns and densities. In particular, when the density of the attractive species was low (i.e. D=10), the time needed to reach equilibrium was longer when plant community was highly aggregated (S10). When the density of the attractive species was higher, stronger spatial aggregation caused the population to reach its stable state faster (Figure 4).

Reward probability

Not surprisingly, pollinator decisions were affected by the reward probability of the plant individuals. Without reward variation, survival rate of the less attractive species was unaffected by density and initial spatial pattern, regardless of the constancy values tested (Table 3: simulation 4 and 5, Figure 2: simulation 5, compare with simulations with reward variation: simulation 3). In addition, the general model dynamics in terms of mean plant population sizes was neither affected by density nor spatial pattern (results not shown). Moreover, when studying the effect of pollinator constancy, we found that with generalist pollinators, the less attractive species always survived in the system (Figure 2, simulation 5). The mean total plant community size in this case was lower (approximately 3000 plant individuals) than in a situation of lower reward probability (approximately 5000
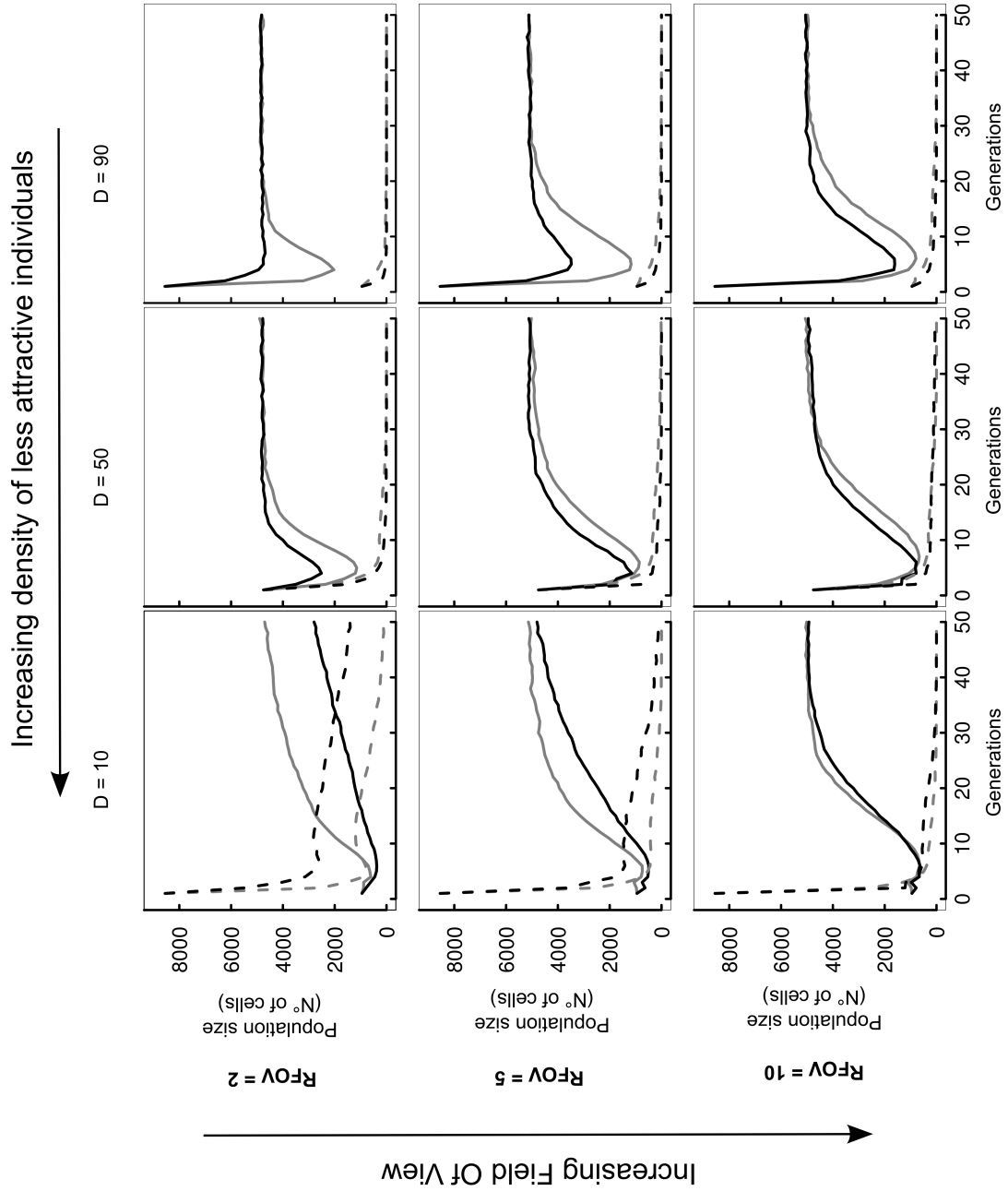
Figure 4: Influence of pollinator detecting range (field of view – R_{FOV}) on mean population size of the two plant species within a simulation. Continuous lines denote the mean population size of the attractive species; dashed lines represent the mean population size for the less attractive species. For clarity, we visualized the dynamics with only two initial spatial patterns: black lines represent the S10 patterns and grey lines represent the S1 pattern. Overall, the regular spatial pattern (Reg) did not differ from the S1 pattern. The dynamic of the intermediate aggregated pattern (S5) was always between the one observed for S1 and S10. Values of the remaining parameters in the presented simulation experiments were $N = 50$, $P_{Shift} = 1.00$, $P_R = 0.50$, $d_{disp} = 5$.

plant individuals, when all other model parameters were similar; details see Appendix 6 Table A2). Interestingly, although no significant pairwise differences were found, when pollinators were defined as intermediate generalists ($P_{Shift} = 0.50$), the survival curves suggested that the less attractive species had higher survival when highly dispersed among very dense attractive species (Appendix 2 Figure A.1: simulation 4).

Pollinators' detection range

When considering generalist pollinator behaviour ($P_{Shift} = 1$), a decrease in the pollinator FOV ($R_{FOV} = 2$) combined with aggregated population structure strengthened the general trends described for density and spatial pattern (Table 3 and Figure 2: simulation 6, 3 and 7). When the less attractive species was dominant ($D = 10$), a reduction in FOV led to a higher survival rate for that species. The median survival time was longest for the spatially aggregated community (S10). Moreover, at higher levels of aggregation, the less attractive species always survived in the system (Figure 2 simulation 6 and Figure 4 for $R_{FOV} = 2$). When density of the attractive species was higher ($D = 50, 90$), a reduction in FOV led to a lower survival rate for the less attractive species regardless of spatial pattern, and the reduction of median survival time was strongest for spatially well mixed patterns (S1, and Reg).

Sensitivity analysis

Seed dispersal distance

When the less attractive species was dominant at the beginning of simulations, decreasing the dispersal distance ($d_{disp} = 2.5$) of both plant species resulted in higher survival rate under aggregated patterns (Table 3 and Appendix 2, Figure A.1 simulation 8). When density of the less attractive species decreased, high aggregation became disadvantageous for the less attractive species, lowering its survival curve relative to more dispersed patterns. Increasing dispersal distance ($d_{disp} = 10$) weakened the differences between the spatial patterns in relation to the less attractive species's survival (Figure 3: simulation 9). When the less attractive species was dominant, the highly aggregated pattern (S10) survival curve was significantly higher than all other spatial patterns (although note that for the regular pattern, differences were not significant: Table 3, simulation 9). With decreasing less attractive species densities, no significant effects were detected.

Pollinator population size

The general trends of the survival analysis results were not affected by pollinator population size, though the effect of the spatial patterns was somewhat dampened (Table 3 and Figure 3: simulation 10, 3 and 11). The main differences found were in the population size of the attractive species (Appendix 6 Table A.3). Nevertheless, it should be noted that a decrease in pollinator population size led to some inconsistencies in the above trends: the less attractive species's survival rate was reduced in the intermediately aggregated pattern (S5) when the less attractive one was dominant or when both species were equally represented (Table 3 and Figure 3: simulation 10). However, generally, increasing the size of the pollinator population resulted in a higher survival rate for all densities and spatial configurations.

Initial locations of pollinators

In our model, pollinators appeared at random positions in the field. Although, this was not subjected to a sensitivity analysis, the results of the model should not be affected by other ways of initializing pollinator positions. This simply because even if pollinators were randomly initially positioned, they did not start their foraging bouts at random positions: during the first step of the foraging algorithm, they directed themselves towards parts of the plant community which they perceived as more attractive. Hence, the spatial distribution of the pollination activity was dictated by the plant community composition (dictated by the relative densities and spatial patterns). Because all pollinators were following similar movement rules while plant community composition did not differ according to the pollinator position within the grid, the pollinators' initial locations (before choosing a first flower) could only affect the spatial distribution of the pollination activity, but not the model outcome.

Discussion

Our model shows that the success of a less attractive species competing for the services of pollinators with an attractive species is strongly influenced by the relative density and spatial distribution of the two species. The spatial distribution of the more abundant species affected the success of its co-occurring species by manipulating the foraging landscape of the pollinators and hence the spatial availability of the other plant species. By growing spatially aggregated, a species is apparently able to locally "trap" pollinators and thus to reduce the local availability of pollinators for coexisting species. Trapped

pollinators no longer act as shared pollinators and therefore behave as local specialists, foraging only on one plant species. In such spatially aggregated situations, the more abundant species receives only its own pollen (higher visit quality sensu Rathcke 1983) which results in locally increased reproductive success, and a longer survival time in the community. This demonstrates that spatial arrangement of communities cannot be ignored when investigating the outcome of plant-plant indirect interactions mediated by shared pollinators.

The notion that separation of pollination services is profitable for plant species is well founded in pollination theory. Clearly, specialisation and speciation are driven by similar seclusion on an evolutionary time scale (Rathcke 1983, Harder & Barrett 2006). On an ecological time scale, species avoid interspecific competition by temporally separating their flowering times and thus separating their pollination services (Rathcke & Lacey 1985, Ishii & Higashi 2001). However, our model is highly novel in that it indicates how separation of pollination services can be modified solely by the spatial pattern of the component species, even if they flower simultaneously. (Waser 1978) demonstrated previously that coexistence of two species sharing pollinators is possible if spatial separation is included (i.e. when refugia were modelled), which he interpreted as a difference in edaphic requirements of the two competing species. Our findings demonstrate that the spatial arrangement of a community is a sufficient condition for enhancing survival of a less attractive species without any niche differentiation. Namely, spatial separation through aggregated distributions can benefit species by locally manipulating the foraging landscape of their pollinators even when species that share pollination services coexist and exhibit no other means of niche separation (e.g. temporal or evolutionary separation). Given the ubiquity of spatial aggregation in natural communities and the restricted spatial extent of foraging animals, our results strongly suggest that reproductive success may be largely affected by spatial structure.

The intriguing consequence of these findings is that one condition for less attractive species survival is their ability to indirectly manipulate pollinator behaviour by their vegetative spatial growth structure. Manipulation of pollinator behaviour is fundamental for plant-pollinator interactions (Kunin 1993, Waser & Ollerton 2006, Chittka & Thomson 2001). However, to the best of our knowledge, the mechanism we detected here has not yet been described before. It is usually assumed that the chance of correct conspecific pollen transfer increases with pollinator constancy within a bout (Rathcke 1983, Waser 1986). Therefore, flower traits that increase pollinators' tendency to continue visiting the same species are evolutionarily and ecologically favoured (see Harder & Barrett 2006). In accordance with this assumption, a species which is less preferred by the pollinator community needs to manipulate the inherent pollinators' preference and constancy towards its own

flowers. This may be achieved by several strategies. For example, the visually less attractive species may offer much higher reward (e.g. Goulson 1994). However, such a situation seems to be relatively unrealistic, because reward is considered costly for the plant (Pyke 1991) and especially because inherent pollinators' preferences (i.e. attractiveness) are assumed to be related to reward level (e.g. Cohen & Shmida 1993). On the other hand, if reward amount and availability are similar among species, a random sampling of the less attractive species may result in a constancy shift. As Goulson (1994) noted (but did not test), a simple strategy to increase the chance of such a shift is to aggregate the less attractive individuals. This spatial pattern strongly decreases the pollinator's foraging choices within the bout and increases the likelihood of further visits and with them the plant species' reproductive success (e.g. Jakobsson *et al.* 2009).

Moreover, our model indicates that the effect of spatial pattern is also a function of the mobility of pollinators. Namely, the spatial availability of plant species to a pollinator can substantially change when the scale of aggregation (size of monospecific patches) is at least as large as the pollinator detection range (FOV; Ne'eman & Kevan 2001). When the detection range was larger than the average monospecific patch size, the effect of spatial pattern on foraging decisions was weakened. A similar decrease in the ability to differentiate between clustered and random foraging landscapes was found in birds when their movement decision scale (equivalent to our FOV) was manipulated (Morales & Vazquez 2008). The consequences of these findings may be relevant for other situations where the size of an organism's detection range interacts with the spatial distribution of its food resources, e.g. for behaviour of animals in a fragmented landscape.

The importance of maintaining spatial separation among the species for higher reproductive success is expected to favour plant traits that contribute to spatial segregation. This was demonstrated in our model by the effect of seed dispersal distances on the less attractive species's survival time in the community. Namely, under limited dispersal, the chances of creating and maintaining monospecific patches are higher (Levine & Murrell 2003), further enhancing the trapping effect of pollinators and increasing reproductive success. Interestingly, this may lead to a positive feedback when more seeds are produced and thus more offspring germinate in the same patch in the following years. Therefore, a chance arrival of pollinators in a less attractive species's patch may trigger local spatial separation that will suffice to secure the survival of that species. A similar mechanism was found by Ishii & Higashi (2001) for temporal segregation. In their model, winning the competition for pollination during a sub-period of the total flowering period was sufficient for persistence in the community. Interestingly, in our spatially explicit case, locally winning the competition by decreased seed dispersal in early generations had a similar effect.

The effect of community spatial patterns on less attractive species reproductive success cannot be decoupled from the effect of population densities on pollinator behaviour. When overall flower density is high and pollination saturation is unlikely, pollinators usually profit from specialising on a single species (e.g. Kunin & Iwasa 1996), often the dominant one (e.g. Goulson 1994). Our results support this view because the less attractive species usually showed higher survival under increased density. However, we also found a clear interaction between density and spatial patterns. As previously mentioned, when the less attractive species was relatively abundant, its reproductive success was considerably increased by additional spatial structure that increased the tendency of pollinators to switch their preferences. However, when the less attractive species's densities were low, its survival time increased (though generally not leading to stable coexistence) when highly dispersed among the more attractive species. This result suggests an improved visitation rate of a rare species in the presence of additional neighbours, even of a different species, as was first suggested by Rathcke (1983; but see Feldman 2008). One possible explanation for such an observation may be that in relatively dense communities, pollinators tend to distinguish less among individuals, so that less attractive flowers are also more frequently visited (Klinkhamer & van der Lugt 2004). Another possibility may be that at low densities, dynamics similar to the magnet species effect (e.g. Laverty 1992, Johnson *et al.* 2003, Moeller 2004) are created. Here, the less attractive species profits from chance visits from pollinators which were attracted to the area because of the more attractive species.

Our results suggests that competitively inferior species may be able to persist longer in a community solely due to its spatial aggregation, creating a situation similar to "weak facilitation" *sensu* Feldman *et al.* (2004). Moreover, our model showed that coexistence of the two species is possible ("strong facilitation" *sensu* Feldman *et al.* 2004) when reward variation is low. An explanation may be that without reward variation, the inherent pollination bias toward the attractive species disappears. Each visit to a less attractive individual triggered a change in preference toward the species of the visited individual, thus dividing the pollinator community into two separate groups of "specialists", each foraging on another species. Because the scenario tested in our model was of relatively high reward chances (with low variation), almost all the flowers were rewarding and a random change of preference early in a bout resulted in an increased visitation rate to the less attractive species. This was enough to secure the less attractive species' survival in all simulations.

Our theoretical study relied on a model that was a simplification of the real world. Among others, in our model plant species characteristics were similar apart from their attractiveness to the pollinators. However, the fact that even with such restricted differences among species we observed an elongation of the less attractive species survival or even stable co-

existence among species when spatially aggregated, emphasizes the importance of space for our understanding of foraging dynamics. Hence, we argue that in addition to the well-known role of plant density for pollinator behaviour, community spatial structure should be taken into account (e.g. Rathcke 1983, Feldman *et al.* 2004). For example, changes in spatial distribution of different plant species in a community may alter the spatial distribution of the foraging pollinators and thus may separate pollination services among competing plant species. This may further affect the conditions in which facilitative processes may occur. Namely, an accelerating response in number of pollinators at low plant densities (Feldman *et al.* 2004) might not lead to facilitation if a strong spatial separation of the pollinator services is created by the plant community spatial structure. Such results may have a significant impact on pollination ecology beyond the borders of pure theory. Especially, we suggest that the spatial arrangement of the plant species of interest relative to their neighbours may play an important role in explaining variation in reproductive success in the field.

Conclusions

Our study aimed at disentangling the effect of several aspects of competition for pollinators which have not been studied in detail. First, we have shown that the spatial structure of communities cannot be ignored when plant species are competing for pollinator services (Straw 1972, Bobisud & Neuhaus 1975, Waser 1978, Goulson 1994, Feldman *et al.* 2004). Moreover, our model indicates that the ability of aggregated spatial patterns to manipulate pollinator behaviour by trapping is sufficient to enable persistence of a competitively inferior species. Another advantage of our modelling approach is that a continuum of pollinator behaviours was taken into account and their interactions with the explicitly modelled plant individuals were tested. With this new approach, we unravelled dynamics within plant communities emerging from spatial effects and affecting the outcome of indirect plant-plant interactions through pollinator sharing.

We conclude that at a restricted temporal and spatial scale, the spatial distribution of plant species in a community affects their reproductive success. This effect is mainly dictated by the manipulation of the foraging landscape of pollinators by the spatial structure of the plant community. This manipulation favours spatial separation of pollinator activity and acts at two temporal scales: whereas the size of monospecific patches is responsible for trapping pollinators within a foraging bout, plant traits such as dispersal distance can reinforce this effect across the next generations. Moreover, our results may serve as additional motivation to study spatial effects on plant-pollinator interactions in

the field. Because plants' spatial patterns are predicted to affect pollinators' foraging behaviour, it is crucial to consider (and manipulate) them when studying plant reproductive success in natural conditions.

Acknowledgements

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Appendices

Appendix 1: Model Parameters:

Values used for the model parameters in the simulations. If more than one value is given, combinations of these values were tested, as described in the Simulation experiments section.

- A_i: attractiveness of species i, $A_i \in \{0.1, 0.9\}$ (see Appendix 3)

- P_R: probability for a plant individual to contain reward (0.5, 1)

- μ_{Seed}: mean of the normal distribution for the number of produced seeds (40)

- δ_{Seed}: standard deviation of the normal distribution for the number of produced seeds (2.5)

- d_{disp}: standard deviation of the normal distribution used for the dispersal of seeds (2.5, 5, 10)

- P_{Empty}: probability of an establishment site to be unsuitable (0.05)

- P_{Death}: probability of death before seed set (0.05)

- D: percentage of attractive individuals at the beginning of the simulation (10, 50, 90)

- R_{FOV}: radius of the Field Of View of the pollinators (also used in the calculation of the rescaled attractiveness) (2, 5, 10)

- N: number of pollinators in the population (25, 50, 100)

- P_{Shift}: constancy parameter (0, 0.50, 1)

- T: threshold value in the first pollinator movement rule (0.05)
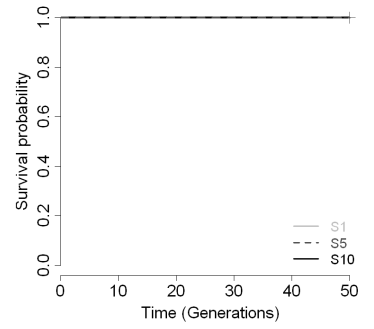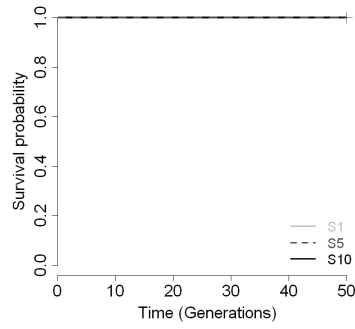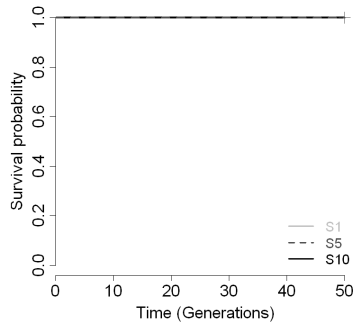
Appendix 2: Graphical results of the survival analysis
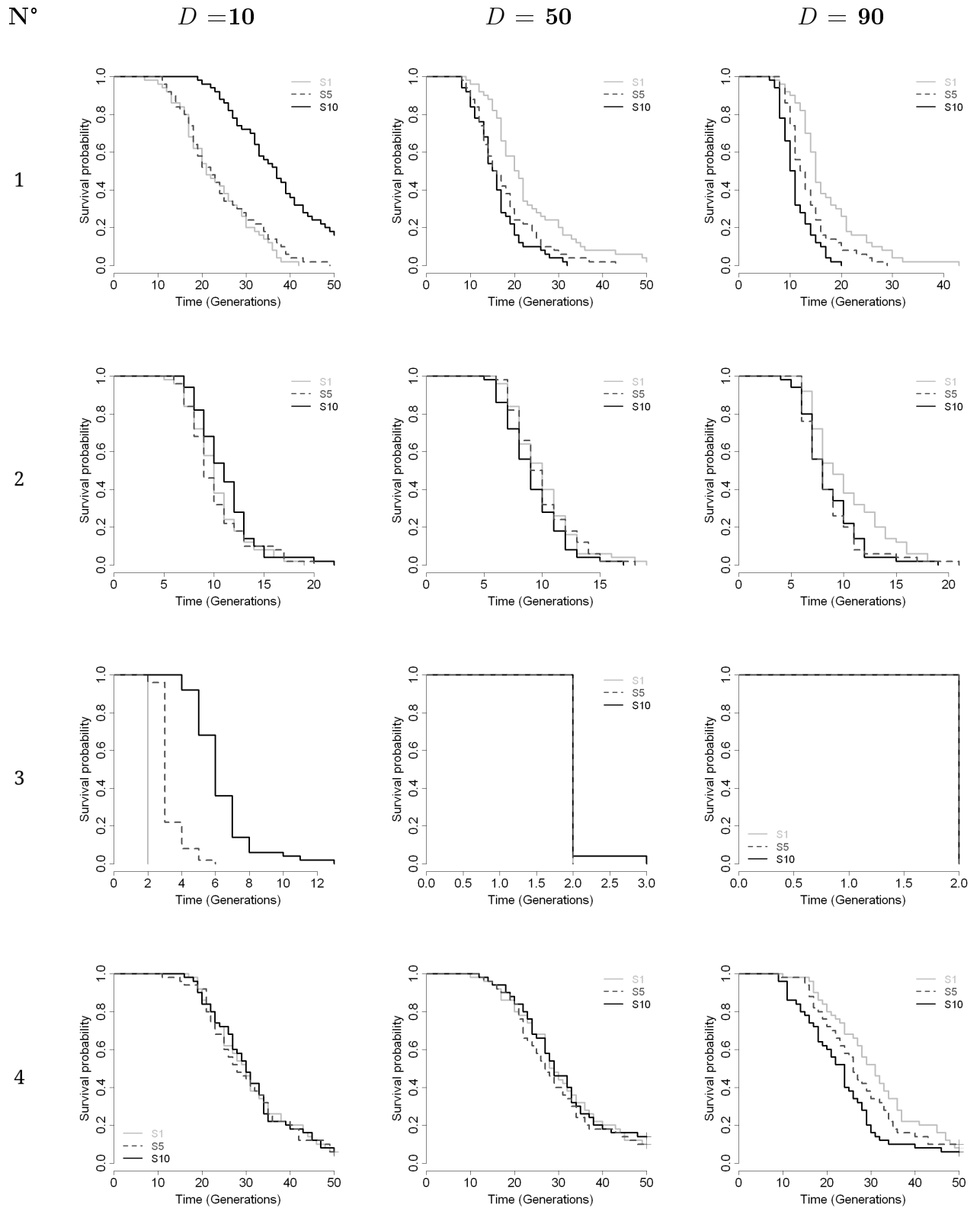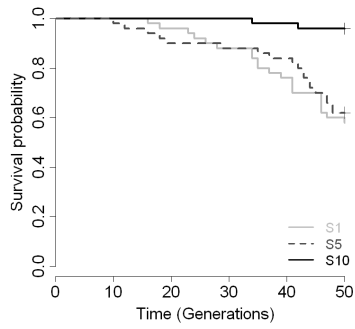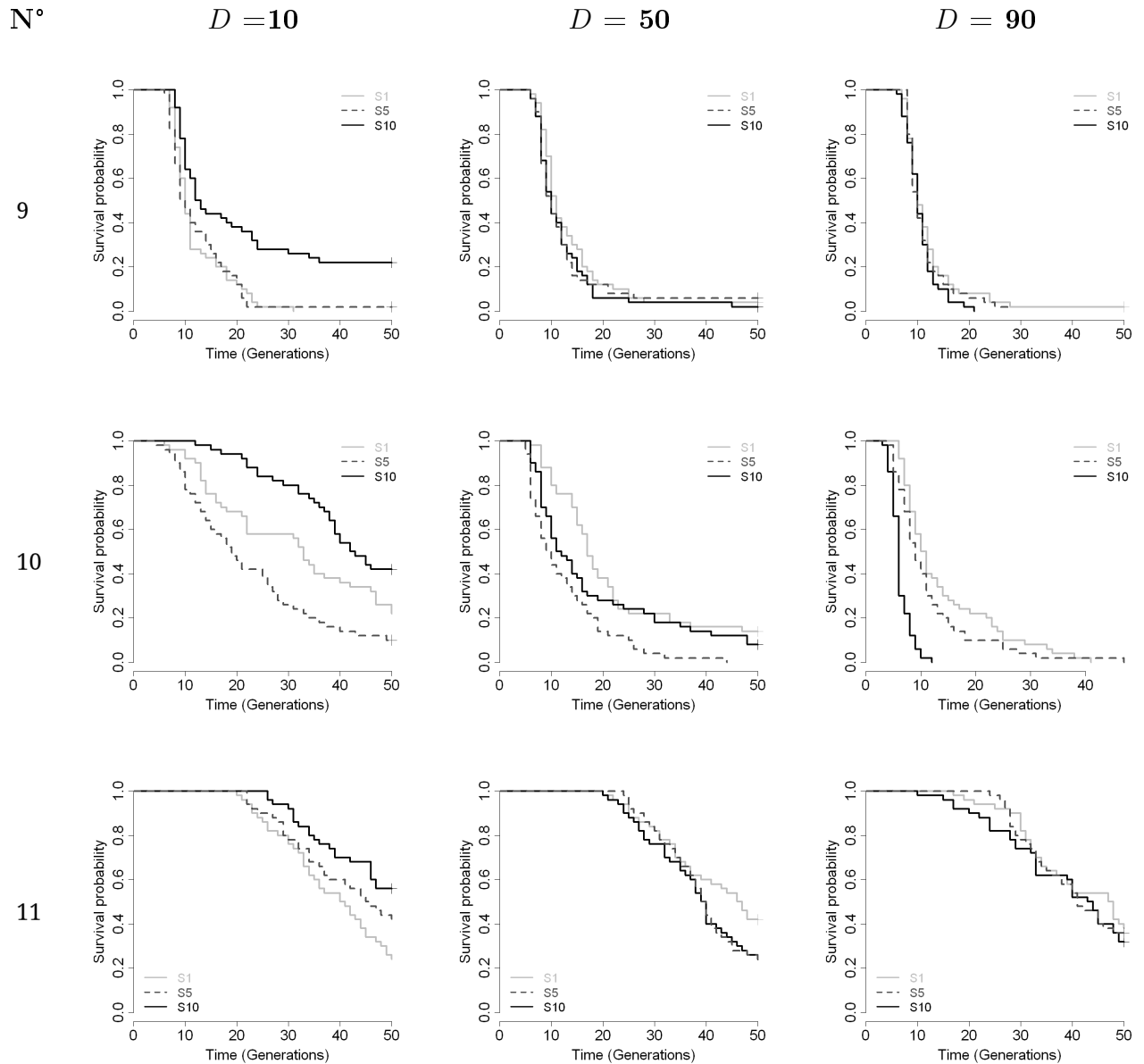
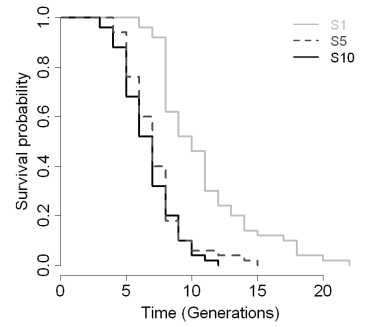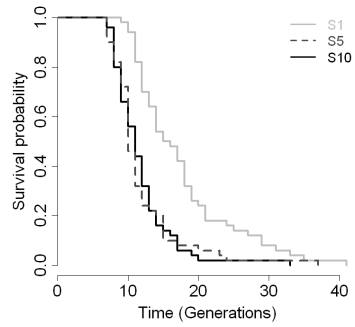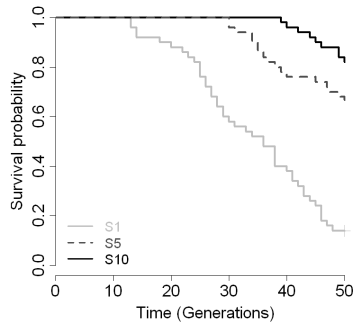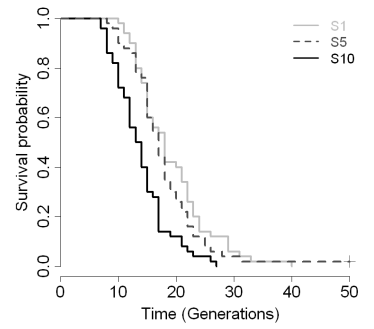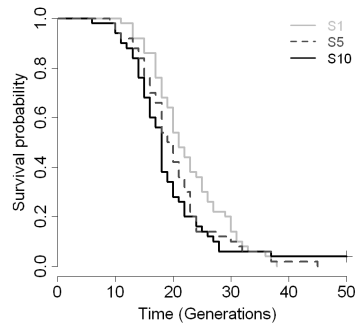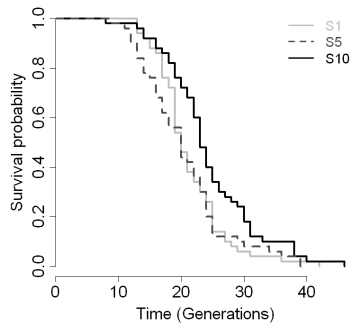**Figure A.1:** Kaplan-Meyer estimates of the survival curves (probability of survival in time) for the less attractive species for all the tested sets of parameters. The numbers of simulations correspond to the number given in Table 2 for each set of parameters. Continuous black lines depict the survival curves for the S10 (strong monospecific aggregation) patterns, dashed grey lines for the S5 (intermediate monospecific aggregation) pattern and light grey lines for the S1 (weak monospecific aggregation) pattern. Results of the survival analysis are given in Table 3.
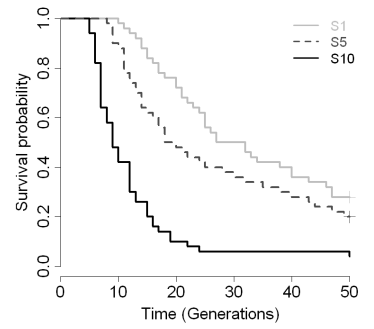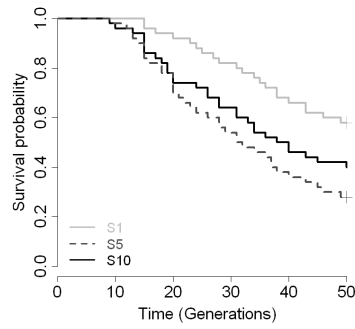
Appendix 3: Choice of the attractiveness values

The instantaneous pollinator preference G, as described in Equation 2 (main text) motivated the choice of the parameter values for the attractiveness (A) of the plant species. Our aim was to enable pollinators to search and finally select a plant individual using

their detection range (FOV), while taking into account two factors: a) individuals belonging to the preferred species (i.e. the species which is more attractive according to the pollinator's experience) should always be chosen over the less preferred individuals; b) closer plants are more likely to be selected than distant ones (due to energetic considerations), for plants having the same instantaneous pollinator preference. In the most extreme case of having only one preferred flower located at the corner cell of a pollinator's FOV (longest possible distance within its FOV) and the rest of the FOV occupied with less preferred individuals, this preferred flower should be chosen. In order to satisfy this rule, the instantaneous score S of the preferred flower in the corner must be higher than the score of an adjacent less preferred flower. To calculate this, we compared the instantaneous score S of a less attractive plant individual close by (i.e. adjacent cell) for a pollinator with that of the more preferred plant species at the corner of the pollinator's field of view, using the rule described in Equation A.1:

$$S_{NP}^{d=1} < S_P^{d=\left(R_{FOV}.\sqrt{2}\right)} \tag{A.1}$$

where $S_{NP}^{d=1}$ is the score for a less preferred flower at distance $= 1$ cell , and $S_P^{d=\left(R_{FOV}.\sqrt{2}\right)}$ is the score of a preferred flower at the corner cell of the pollinator's FOV (i.e. greatest distance possible in the FOV). Using Equation 2, inequality A.1 can be rewritten as:

$$G_{NP} < G_P + \frac{1}{R_{FOV}.\sqrt{2}} - \frac{1}{2} \tag{A.2}$$

where G_{NP} and G_P are the instantaneous preference value for a less preferred and a preferred flower, respectively. Assigning the value of 0.9 for G_P, critical values for G_{NP} can be computed. These values are given in Table A.1. In order to make sure that a pollinator would move to the preferred flower even if this is located in the corner cell of its FOV, the instantaneous preference value of a less preferred flower should be smaller than 0.47. To ensure these differences, we chose the value of 0.1 for the instantaneous preference of a less preferred flower. Hence, the attractiveness value of the less attractive species was also equal to 0.1.

Appendix 4: Reproduction and dispersal of plant individuals

If a plant individual was pollinated, it produced N_{Seed} seeds, N_{Seed} being drawn from a normal distribution with mean μ_{Seed} and standard deviation δ_{Seed} . Seeds produced by plant individuals were then dispersed locally among the grid. The cell to which a certain seed was dispersed was determined by a distance from the mother plant and an angle. The angle was randomly generated out of a uniform distribution while the distance was drawn

Table A.1: Critical values for instantaneous preference values for the less preferred species ($G_{NP} <$) as well as the values for the score of a less preferred flower at distance 1 ($S_{NP}^{d=1}$) and the score values for preferred flowers at the corner at the FOV of pollinators ($S_P^{d=\left(R_{FOV}\cdot\sqrt{2}\right)}$)

R_{FOV}	$G_{NP} <$	$S_{NP}^{d=1}$	$S_P^{d=\left(R_{FOV}\cdot\sqrt{2}\right)}$
2	0.66	0.6	1.23
5	0.52	0.6	1.07
10	0.47	0.6	0.99

out of a normal distribution with mean zero and standard deviation d_{disp}. Once seeds were dispersed, the grid occupancy for the next generation was determined. Each cell had a probability $P_{Empty} = 0.05$ to be unsuitable for establishment of plant individuals. If a cell was suitable for plant growth, species k had a probability of $P_k = \frac{N_k}{\Sigma N}$ ($k = 1, 2$) to occupy that cell for the next generation, with N_k being the amount of seeds of species k in the cell and N the total amount of seeds in the cell. We also considered mortality: each individual had a probability P_{Death} of 0.05 to die before seed set.

Appendix 5: Threshold value used in the pollinators' first movement rule:

In the first step of the pollinator behaviour, a threshold value T was used. This parameter reflects the "pickiness" of pollinators when choosing the position from which they will start their foraging bouts. This parameter value was chosen to ensure that the innate pollinators' preference will be taken into consideration while distributing pollinators among grid cells before the actual start of their foraging bouts. When a pollinator is at the edge of a patch composed only by attractive individuals and the patch is of a size equal to the pollinator's FOV, the threshold value determines how far the pollinator will penetrate this patch and on which cell it will land at the beginning of its foraging bout (Figure A.2). We can then calculate the rescaled attractiveness values (see section Pollinator foraging rules, main text) of all the cells in this pollinator's FOV and the differences in rescaled attractiveness values associated with the movement of the pollinator in the indicated direction (Figure A.3), for each of the three FOV sizes tested. Depending on the size of the patch, pollinator can move 3, 6 or 11 cells (for $R_{FOV} = 2, 5, 10$ respectively) before reaching the centre of the patch.

As can be seen in Figure A.3, pollinators with a $R_{FOV} = 2$ will land in the middle of the patch (i.e. after moving three times). For pollinators having a larger FOV, the threshold will be reached after moving 5 or 4 cells into the patch, for $R_{FOV} = 5$ and 10, respectively. Hence, we can assume that this threshold value is appropriate as it will guide pollinators

to land on flowers deep within patches of attractive individuals. This implies that our model involved a conservative behavioural rule which reflects the innate preferences of pollinators.
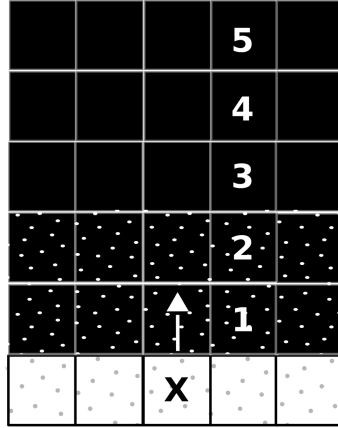
Figure A.2: Pollinator at position X (on less attractive flower, white cells) facing a patch of attractive individuals (black cells). Dotted areas represent the portion of the field within the pollinator's FOV (for a $R_{FOV} = 2$; only part of the FOV is depicted). The arrow indicates the direction of the pollinator's movement.
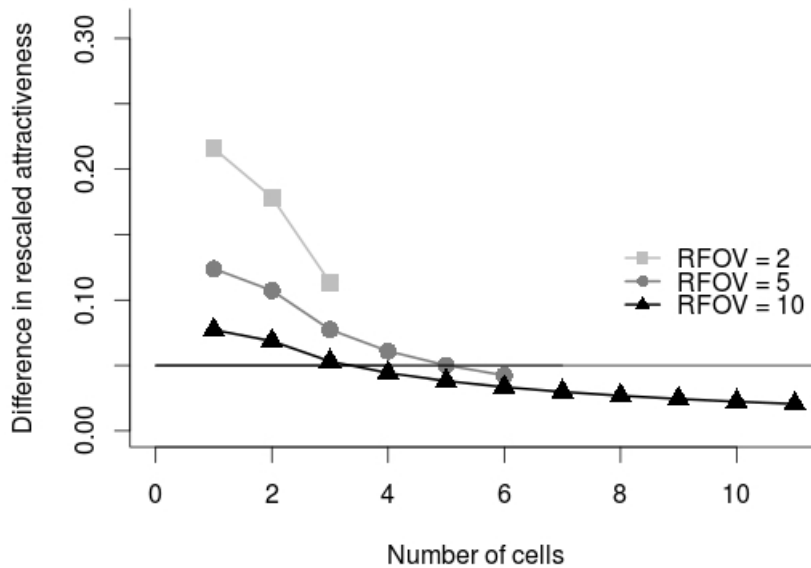
Figure A.3: Differences of rescaled attractiveness for a pollinator facing a patch of attractive species (as depicted in Figure A.2) as a function of the pollinator's movement distance (in number of cells), from the edge of the attractive flower patch (position X in Figure A.2). The line at 0.05 indicates the threshold value (T) used in the model. Pollinators will land on the first cell for which this value falls below the 0.05 line.

Appendix 6: Species final abundance as a function of reward variation and pollinator population sizes

Table A.2: Mean population sizes at generation 50 for the attractive (A) and the less attractive (NA) species in relation to starting densities of attractive plant individuals ($D = 10$: low density of the attractive species and high density of the less attractive one; $D = 50$: similar densities of the two plant species; $D = 90$: high density of the attractive species and low density of the less attractive one) and to reward variation ($P_R = 0.5$ or $P_R = 1$; with or without variation). Mean population sizes are rounded and given in numbers of cells. Remaining parameter values were $P_{Shift} = 1$, $R_{FOV} = 5$, $d_{disp} = 5$ and $N = 50$.

P_R	Species	$D = 10$				$D = 50$				$D = 90$			
		Reg	S1	S5	S10	Reg	S1	S5	S10	Reg	S1	S5	S10
0.5	A	5081	5085	5071	4647	5016	5039	4991	5095	5111	5083	5123	5131
0.5	NA	2	3	2	199	83	41	4	0	4	3	0	0
1	A	2068	2048	1960	2019	2009	2011	2004	1999	2006	1976	2022	1994
1	NA	1157	1199	1197	1191	1191	1204	1199	1187	1190	1204	1189	1191

Table A.3: Mean population sizes at generation 50 for the attractive (A) and the less attractive (NA) species along a gradient of three starting densities ($D = 10$: low density of the attractive species and high density of the less attractive one; $D = 50$: similar densities of the two plant species; $D = 90$: high density of the attractive species and low density of the less attractive one) for the three pollinator populations sizes (N). All the population sizes are rounded and given in numbers of cells. Remaining parameter values were $P_{Shift} = 1$, $R_{FOV} = 5$, $d_{disp} = 5$ and $P_R = 0.5$.

N	Species	$D = 10$				$D = 50$				$D = 90$			
		Reg	S1	S5	S10	Reg	S1	S5	S10	Reg	S1	S5	S10
25	A	2117	2012	2213	1480	2075	2065	2271	2136	2185	2218	2218	2212
25	NA	84	189	66	461	90	109	5	64	23	6	3	0
50	A	5081	5085	5071	4647	5016	5039	4991	5095	5111	5083	5123	5131
50	NA	2	3	2	199	83	41	4	0	4	3	0	0
100	A	7599	7903	7778	7391	7710	7724	7970	7976	7697	7828	7919	7820
100	NA	70	40	58	91	66	68	35	33	73	64	47	61

Density and spatial distribution of an attractive species alter plant-pollinator interaction structure in grasslands - A network approach.

Abstract

Plant-pollinator interactions in natural communities form highly complex networks. The structure of such networks affects the reproductive output of plant species and hence the plant community structure. The recent increased interest in pollination networks has resulted into a better appreciation of the ecological consequences of some of their proprieties such as nestedness, modularity, specialisation asymmetry, etc... . However as biological processes are responsible for the general characteristics of pollination networks , their structure is likely to be strongly affected by changes in plant communities structural characteristics. And until now, not much is known about how their structure would evolved with changing structural plant community characteristics, such as species composition, densities and spatial patterns. Whereas the neutrality hypothesis could help in predicting changes of the two first factors, the potential impact of spatial patterning in plant communities is totally unexplored. In order to test for the impact of these three community characteristics, we conducted an experimental study in semi-natural grasslands. We introduced an attractive species into two semi-natural communities and experimentally manipulated its density and spatial distribution in a full factorial fashion. For each treatments, pollination networks were recorded and compared. We found that the neu-

trality hypothesis could explain the recorded density and diversity of the plant-pollinators interactions in the different treatments but it fails to explain observed changes in patterns of resource use and similarity induced by the treatments. These changes were caused by the difference in spatial distribution of the introduced species, indicating the existence of a spatial mechanism acting at small scale in plant-pollinator interactions. These results corroborate previous theoretical work and add a new spatial perspective in the processes responsible for the general characteristics of plant-animal interaction networks.

Introduction

Plant communities are complex assemblies of spatially co-occurring species. In the vast majority of these communities, the species depend on the services of pollinators for their reproduction (Geber & Moeller 2006, Waser & Ollerton 2006, Mitchell *et al.* 2009), and when flowering phenologies overlap, pollinators have to be shared (Waser & Ollerton 2006). Such sharing may give rise to complex indirect plant-plant interactions, which can be either competitive or facilitative (Rathcke 1983, Feldman *et al.* 2004, Ghazoul 2006). Whether or not and to which extent these interactions are positive or negative may be a function of plant community structure on one hand, and of pollinator behaviour on the other hand (Kunin 1993, Klinkhamer *et al.* 2001, Lãzaro & Totland 2010). Since plant-plant interactions ultimately determine coexistence and thus the structure of plant communities, the study of pollinator services and the conditions under which these affect plant-plant interactions is crucial for understanding the assembly of natural communities (Mitchell *et al.* 2009).

One of the aspects of plant communities affecting the net outcome of these indirect plant-plant interactions is the density of floral resources (Rathcke 1983, Kunin & Iwasa 1996, Fontaine *et al.* 2008, Jakobsson *et al.* 2009, Lãzaro & Totland 2010). When plant species achieve a higher attraction of pollinators (i.e. higher aggregative and or numerical response of pollinators) because they grow in higher density (Rathcke 1983, Sih & Baltus 1987), the presence of flowering neighbours is beneficial for an individual and facilitation occurs (Rathcke 1983). It has been proposed that this increase and its positive consequences for the reproduction of neighbours is only occurring at relatively low densities of plant species (Rathcke 1983) because at a higher density of floral resources, the attraction for pollinators will saturate, due to the finite number of pollinators available. Hence, the number of visits per individual flower would decrease, and thus competitive effects will dominate (Rathcke 1983). It has been shown that for this scenario to occur, an initial accelerating aggregative response of pollinators is a necessary condition (Feldman *et al.* 2004).

However, within this theoretical framework, the identity of plant species has been mostly ignored and the pollinators were assumed to not distinguish between the different floral resources they are attracted to (Rathcke 1983, Feldman *et al.* 2004). Whereas this might be the case for an assembly of a few similar species, it is certainly not the case in species rich natural communities where much more species are sharing the services of pollinators (see e.g. Lãzaro & Totland 2010). Differences in flower morphologies (Stang *et al.* 2006), inborn preferences (Chittka & Thomson 2001), learning and handling capacity (Chittka & Thomson 2001) of the different pollinator species and outcomes of co-evolutionary processes (Harder & Barrett 2006) determine the importance of species identity in pollination ecology. For example, recent reports about highly attractive plant species, so-called "magnet species", indicate that these may enhance their neighbours reproductive success by their sole presence (Laverty 1992, Johnson *et al.* 2003, Moeller 2004, Juillet *et al.* 2007). However, the magnet species concept was mostly described for only a few interacting plant species (Laverty 1992, Johnson *et al.* 2003, Moeller 2004, Juillet *et al.* 2007), and we do not know whether the same mechanism can easily be extrapolated to more natural species-rich communities. In such communities, complex effects may occur that differ between the individual level and the community level. For example, if some species are more attractive for pollinators, their presence can facilitate their direct neighbours by locally increasing pollinator abundance. However, this effect may then reverse to competitive interactions via the density-dependence explained above (Rathcke 1983). At a community level, though, the presence of the attractive species may be beneficial, as long as the magnet species' density is not too large.

Next to species composition and floral density, the spatial structure of the communities, e.g. the degree of clumping of attractive flowers, may also play a key role in affecting pollinator behaviour. For example, when species are dominant and spatially aggregated, the clumps may act as 'traps' for pollinators and thus enhance intraspecific success by increasing the visit quality to their own kind (Hanoteaux *et al.* 2013). In another simulation study on plant-animal interactions, Morales & Vazquez (2008) showed that the structure of plant-frugivore interactions was not only influenced by the spatial distribution of plant individuals but also by the mobility of frugivorous birds. Due to the reduced mobility of birds (comparable to the tendency of pollinators to fly short distance bewteen consecutive visits, Waser 1982), spatial aggregation of resources led to less realised interactions between partners and lower interaction evenness.

It is clear that these three characteristics of plant communities' structure all affect the potential outcome of indirect plant-plant interactions by manipulating pollinator behaviour (Lãzaro & Totland 2010). These changes in behaviour induced by the plant community structure find their repercussions in the reproductive success of the species composing

these communities and hence affect their dynamics in time and space. Even if a certain body of evidence has been gathered about the separate effects of density (Kunin 1993, Bosch & Waser 1999, Grindeland *et al.* 2005, Hegland & Boeke 2006, Fontaine *et al.* 2008, Dauber *et al.* 2010) and diversity (Hegland & Boeke 2006, Fründ *et al.* 2010, Lãzaro & Totland 2010) of floral resources, much less is known about the effects of the spatial patterning (but see Morales & Vazquez 2008, Hanoteaux *et al.* 2013). Furthermore, we are still lacking an understanding of the combined effects of these three structural characteristics on the behaviour of pollinators and hence on the structure of plant-pollinator interactions.

Plant-pollinator interactions are complex and their analysis requires appropriate tools. One way of tackling this complexity is to consider plant-pollinator interactions as bipartite networks (see e.g. Blüthgen *et al.* 2008, Dormann *et al.* 2009), typically composed of two trophic levels interacting with each other (see Newman 2003 for definition). The topology of these networks can be analysed using network metrics or indices (e.g. Dormann *et al.* 2009). Recently, the analysis of ecological networks has received a lot of attention and indices and methods relevant for using them in tackling ecological questions are rapidly developed. For example, the nestedness of networks (Bascompte *et al.* 2003), the ecological importance of the specialisation asymmetry (Vazquez & Aizen 2004), modularity (Olesen *et al.* 2007), the degree distribution (Dunne *et al.* 2002, Jordano *et al.* 2003), and specialisation (Waser & Ollerton 2006) of mutualistic and antagonistic networks have been investigated, in parallel with the development of new indices or new methods (see for example for the modularity: Guimerá & Amaral 2005, for the H_2' index : Blüthgen *et al.* 2006 and for a new nestedness index WINE: Galeano *et al.* 2009). Here, we used this network approach in order to track the putative changes in plant-pollinator structure resulting from the introduction of new plant individuals in existing plant communities. We focused on the analysis of network indices representing relevant ecological information. Specifically, we used metrics related to the size and organisation of links within networks, to similarity of resource use by species and competition, to specialisation and to the diversity and evenness of interactions in the networks.

Material and methods

In order to test for the potential effects of identity, density and spatial arrangement of plant species on the structure of plant-pollinator interactions, we used natural plant communities in which we introduced plant individuals belonging to an "attractive species". The "attractive species" was defined as having a floral morphology that is not too specialised such that its floral resources are easily available for the majority pollinator groups.

This flowering species should, hence, get visited by a large variety of pollinator species. The visual advertisement of the species should be conspicuous and the species should be highly rewarding. Our choice was to work with *Centaurea cyanus* L., as we know from previous studies that this species has the three desirable proprieties to act as an attractive species (Hoch 2012, Seifan *et al.* 2014).

Study sites and experimental Set-Up

We selected two semi-natural grasslands in the Swabian Alb, South-West Germany: Dettingen (440 m a.s.l., 48.44°N 8.92°E) and Pfullingen (790 m a.s.l., 48.45°N 9.27°E). In each site, we delimited a working area of approximately 50 by 50m in which we conducted the experiment. Within the working area in both sites, we randomly chose 15 plots (2 by 2 meters) with similar community composition. Each of these 15 plots was randomly assigned to one of the following treatments: three plots were left untouched (controls), in six plots we introduced our attractive species at high density (28 individuals) and in the six others, the attractive species was introduced at low density (10 individuals). These two sets of six plots represent the two density levels applied in this study. Each set of six plots was divided into two groups: three plots were assigned a clumped spatial distribution of the introduced individuals and the three others were assigned a regular spatial distribution (see Figure 1). Hence, each treatment had 3 replications, for a total of 15 plots in each site. The clumped spatial pattern was achieved by randomly introducing three clumps of attractive individuals, taking care of spacing them by at least 50 cm (three clumps of 10, 9 and 9 individuals, respectively, were installed in the high density and clumped treatment and three clumps of 3, 3 and 4 individuals, respectively, for the low density). The regular spatial spacing was achieved by placing introduced individuals along lines at equal distances (see Figure 1). These two sets of six plots (each within a density level treatment) represent the two levels of spatial distribution applied in this study (Figure 1). We will refer throughout the text to R and C for the regular and clumped spatial treatments, respectively, and to H and L for the high and low density, respectively. For example, R-H refers to the treatment with regular spatial distribution at high density.

We conducted this experiment in two different grasslands in order to assess the validity of the general patterns of changes in plant-pollinator interaction structure under different scenarios of alteration in plant communities.
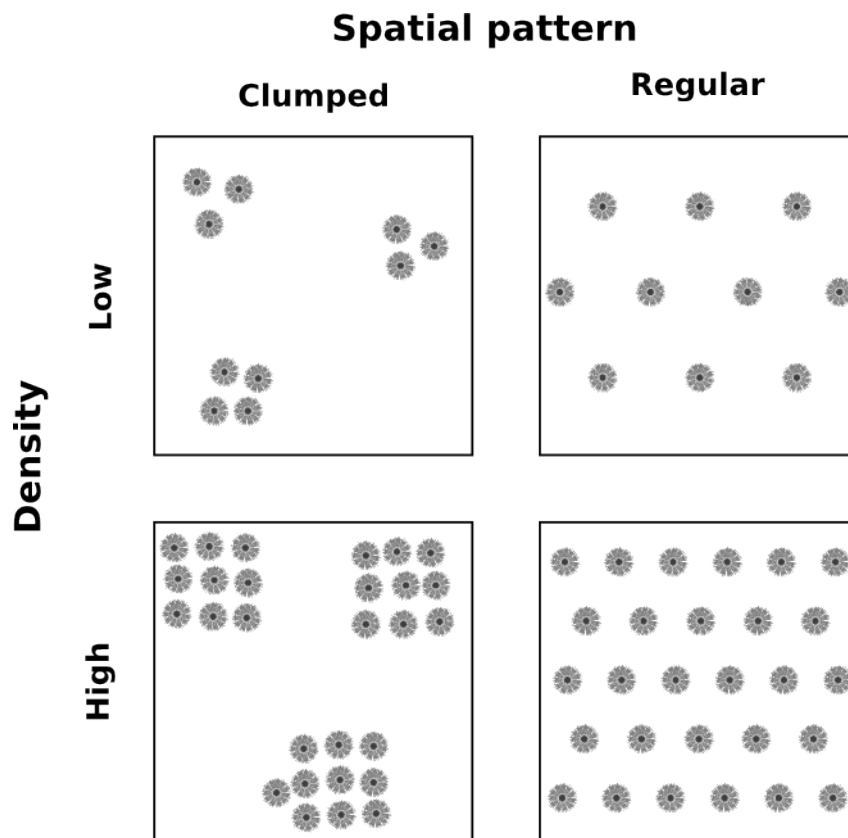
Figure 1: Study design as applied in each site. Each plot had a size of 2 by 2 meters. The combinations of the two levels of both experimental factors (density and spatial patterns) are represented. Control treatments are not shown (n=3).

Vegetation survey

Before each observation day, the number of inflorescences or flowers (hereafter flowers) was counted in the plots. We used the number of inflorescences for certain species (e.g. *Centaurea* species) rather than the number of flowers for the obvious reason that the inflorescences of these species will act as only one unit possibly attracting pollinators (Cohen & Shmida 1993, Hegland & Boeke 2006, see Appendix 1 Table A.1 for the list of species and details on the attraction units used in each site). Further, when calculating the floral density, evenness and diversity, the number of flowers in each plot for each species was weighted by a species specific average floral area (Hegland & Boeke 2006), in order to take species-specific differences in flower sizes into account (Appendix 1 Table A.1). If data were missing, or no counts were done on some observation days, we used a simple regression between the two closest dates in order to compute the missing values. In both sites, very similar species were occurring. These species were pooled together in the flower counts because, when conducting pollinator observations (see below), the distinction between the respective species was not always possible (see Appendix 1 Table A.1 for the name of these species).

Pollinator observation

Pollinators were observed during 5 min observation periods, under appropriate weather conditions, always between 10 A.M. and 6 P.M.. Each site was observed during its specific peak flowering season (Dettingen: from the 5th of June till the 3rd of July; Pfullingen: between the 6th of July and the 16th of July 2010). Each day observations were conducted (10 days in Dettingen, 9 days in Pfullingen), we observed pollinators in only one location. During observation days, we observed at least the 15 plots once. However, on some days, 2 or 3 observation rounds were conducted, i.e. each plot in a field was observed 2 or 3 times. Sufficiently long breaks were taken between two consecutive observation rounds in order to make sure that although the plots were permanent, we could consider the observations being independent. During the 5 min observations, we recorded the identity of both plant species and insect visitors, and used these data to build the networks (see below). As the majority of the pollinators in the area are protected by law (Bundesartenschutzverordnung Deutschland 2005), we could not catch them for identification. Therefore, we decided to determine them *in-situ* and lumped difficult groups into broader taxonomic classes. We are aware of the high probability of a discrepancy in the resolution of the determination among different taxonomic groups, but as this discrepancy is the same in all plots, it should not affect our ability to detect qualitative differences among treatments.

Networks

Networks were built with the data gathered during the pollinator observations. For each plot, a separate network was constructed based on all the visits (here a visit was defined as the contact between a pollinator body and reproductive organs of a plant species) recorded during our observations. As some observation data were lost due to unpredictable weather conditions and technical issues with our recording devices, there was a slight unbalance in the observation time of different plots. We corrected the networks for the potential differences in observation time. This was achieved by multiplying the entries of an under-observed network by the ratio of the maximum number of observations for plots in a field divided by the number of observations for that plot. Here, the maximum number of observations of plots within a field corresponded to the realised number of observations for the majority of plots. This because the correction applied to plots for which we lost observation data. However, this correction resulted in non-integer entries in the webs. In order to format all the network entries equally and because some indices should be computed with integers (see bipartite package manual, `http://cran.rproject.org/web/packages/bipartite/index.html` and Dormann *et al.* 2008), we rounded the non integer entries to the smallest integer. Table A.2 in Appendix 2 gives the overall relative interaction frequencies of all the observed pollinator species in both sites.

Network indices

Once the corrected networks for all the plots were obtained, we calculated indices relevant for our investigation, using the "bipartite" package of R (Version 2.13, R Development Core Team 2005 ; see Dormann *et al.* 2008, 2009). Indices were only calculated at the network level. We selected from the list of available network indices relevant indices to test our predictions (see Dormann *et al.* 2009 and references therein for a detailed explanation of the different indices). We classified these indices into four classes, representing the ecological information they can provide. Table 1 gives the calculated indices, their assigned group, their value range and their type (weighted (W) and unweighted (U)). The four classes of indices were related to (1) the size and general organisation of the networks, (2) similarity of resource use by species and competition, (3) specialisation of species and (4) diversity and evenness of the interactions. It should be noted that some of these indices are highly correlated (Dormann *et al.* 2009) and that this classification was only used in order to describe more efficiently different mechanisms possibly occurring within the communities and that these classes don't have fixed boundaries.

Remark. HTL en LTL refer to "Higher Trophic Level" and "Lower Trophic Level" respectively and the two abreviations will be used henceforth.

Table 1: List of the different indices used in this study and their assigned "class". HTL en LTL refer to "higher trophic level" and "lower trophic level" respectively. The type of index was either qualitative (U: unweighted metrics) or quantitative (W: weighted metrics). S represents the number of species involved in the calculation.

Indices	Index class	Value Range	Type
Number of higher trophic species	Size	$1-\infty$	U
Number of lower trophic species	Size	$1-\infty$	U
Connectance	Organisation	0-1	U
Togetherness	Competition	$0-\infty$	U
C-Score	Competition	0-1	U
Mean number of shared hosts	Similarity	$0-\infty$	W
Mean number of shared predators	Similarity	$0-\infty$	W
Niche overlap HTL	Resource use	0-1	W
Niche overlap LTL	Resource use	0-1	W
Links per species	Specialisation	$1-\infty$	U
Specialisation asymmetry	Specialisation	-1 - 1	W
H_2'	Specialisation	0-1	W
Generality	Specialisation	$(0)1 - \infty$	W
Vulnerability	Specialisation	$(0)1 - \infty$	W
Linkage density	Specialisation	$(0)1 - \infty$	W
HTL mean interaction diversity	Diversity	$0 - \ln(S)$	W
LTL mean interaction diversity	Diversity	$0 - \ln(S)$	W
Shannon diversity	Diversity	$0 - \ln(S)$	W
Interaction evenness	Evenness	0-1	W

Statistical analysis

As most of the indices values were normally distributed (tested with Shapiro test, see Appendix 3 Table A.3 for the p-values for the different indices), we used linear models for assessing the importance of density and spatial arrangement of the introduced species. However, some indices were log transformed (number of lower trophic species and the higher trophic level niche overlap) or square root transformed (the mean interaction diversity for both trophic levels) in order to achieve normality. Indices were equally transformed for both sites.

Additionally to the impact of our treatments, characteristics of the remaining plant community (density, diversity and evenness of floral resources) can affect the recorded network indices values and we wanted to account for their potential impact in our analysis. Therefore, for each index in each site, we fitted 10 different models (see Table 2) and selected the most parsimonious model based on their AIC values. The different fitted models always contained a term representing the cumulative floral density of *Centaurea cyanus* (either not transformed or log transformed density), the spatial patterns (grouping factor, 3 levels: clumped, regular or control) and their interaction as main effects. The fitted models differed in the presence (or absence) of one of the of the following 4 covariates (see Table 2): the overall cumulative density (untransformed or log transformed), the cumulative diversity or the cumulative evenness of floral resources in plots.

The cumulative values were obtained simply by adding the respective values over all the observation days for each site separately and were all computed using the floral surfaces rather than the number of flowers of the different plant species (see section Vegetation survey). Furthermore, working with these cumulative values allowed us to account for temporal changes in plant community characteristics (Vazquez *et al.* 2009). For each index, a model was also fitted without any covariate.

Subsequently, we extracted the p-values associated with the main effects in the most parsimonious models (spatial pattern, density and their interaction). For each of the best models, we ran a Breusch-Pagan Test in order to test for the invariance of the variance along the values of the response variable (i.e. index values). The covariates selected in the final models for all the analyses are given in Appendix 3 Table A.4. The structures of the best models were kept (i.e. we refit a new model with the same covariate combinations as in the most parsimonious models) and used in new models where we replaced the main effects by the different treatments (5 levels). This allowed us to pairwise compare among treatments using Tukey Honest Significant Differences (Tukey HSD).

Further, the three plant community characteristics (floral abundance, diversity and evenness of floral resources) in our experimental plots were analysed in order to assess the

Table 2: Structure of the 10 models fitted for in each index in each site. Combination of covariate and their respective transformation (log) in addition with the *Centaurea cyanus* (CC) density term used in the linear models. The cumulative values (Cumul.) represent the sum of the respective values over all the observation days.

Covariate + [transformation]	Variable 1
None	Cumul. density of CC
Cumul. floral density	Cumul. density of CC
log[Cumul. floral density]	Cumul. density of CC
Cumul. floral diversity	Cumul. density of CC
Cumul. floral evenness	Cumul. density of CC
None	log[Cumul. density of CC]
Cumul. floral density	log[Cumul. density of CC]
log[Cumul. floral density]	log[Cumul. density of CC]
Cumul. floral diversity	log[Cumul. density of CC]
Cumul. floral evenness	log[Cumul. density of CC]

homogeneity of the vegetation communities before introducing our attractive species. The details and results of the analysis of the plant communities for both sites are given in Appendix 4 (Table A.5 and Figure A.1). From this analysis, we could conclude that our assumption of similar communities before the introduction of the attractive species was met.

Results

Networks

Observations were conducted for 10 days in Dettingen resulting in 15 observations per plot (maximum value used for the observation time correction) and for 9 days in Pfullingen giving 16 observations per plot (maximum value used for the observation time correction). In total 5259 and 5444 visits were recorded in Dettingen and Pfullingen, respectively, and used for building the different networks (Table 3).

In total, 67 (morpho-) species of pollinators were identified in Dettingen and 59 in Pfullingen. The pollinator community composition was highly different among the two sites (Figure 2). In Dettingen, the pollinator community was highly dominated by *Apis mellifera* (accounting for 79 % of the recorded interactions). In Pfullingen, the distribution of pollinator species was much more even with *Bombus lapidarius*, *Apis mellifera* and

Table 3: Mean number of interactions, mean number of plant and animal species and mean sampling densities for the different treatments for the two sites. These values are averaged among the plots constituting the respective treatments (n = 3). Values between brackets are standard deviation of the respective means.

Field	Density	Spatial patterns	Mean # of interactions	Mean # of plant species	Mean # of animal species	Sampling density
Dettingen	Control	Control	339 (69)	9 (2)	20 (6)	2.03 (0.62)
	High	Regular	531 (104)	8.33 (1.53)	12 (2.65)	5.42 (0.60)
	High	Clumped	629 (120)	8.67 (0.58)	20 (4.36)	3.67 (0.45)
	Low	Regular	508 (54)	10 (2)	17.33 (3.21)	3.06 (0.74)
	Low	Clumped	529 (36)	8 (0.58)	18.33 (1.53)	3.49 (0.46)
Pfullingen	Control	Control	146 (64)	8 (1)	18 (2)	1.4 (0.47)
	High	Regular	629 (157)	8 (1)	19.67 (1.53)	4.11 (1.46)
	High	Clumped	408 (59)	9.67 (2.52)	19.67 (2.31)	2.37 (1.14)
	Low	Regular	287 (62)	7.33 (2.08)	17.33 (0.58)	2.30 (0.33)
	Low	Clumped	344 (131)	8.33 (1.53)	16 (1.73)	2.78 (1.41)

Bombus terrestris accounting for 35, 25 and 10 % of all the recorded interactions, respectively. A complete list of the pollinator (morpho-) species in the network is presented in Appendix 2. The evenness of the distribution of the higher trophic level based on the networks are given in Appendix 5 Figure A.2.

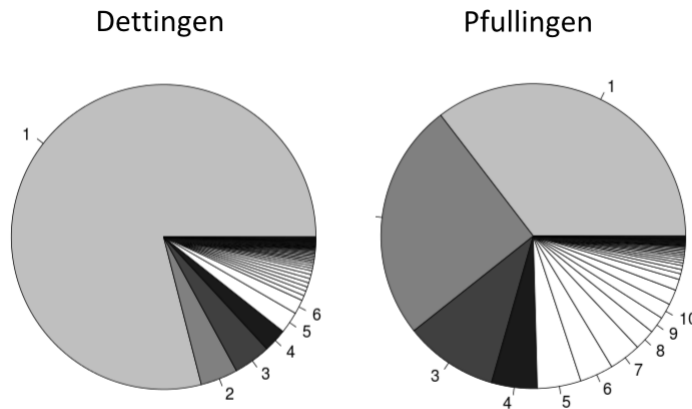**Figure 2:** Interaction frequency distribution of the different pollinator species in the two sites. Numbers represent pollinator species given in Table A.2 (see Appendix 2). Not all the numbers are given for better readability. In Dettingen, number 1 represent *Apis mellifera*. In Pfullingen, *Bombus lapidarius*, *Apis mellifera* and *Bombus terrestris* and represented by 1, 2 and 3, respectively

Network indices

Size and connectance

In both sites, neither the number of plant species, the number of pollinators species composing the networks, and the proportion of realised links (connectance) differed between treatments (Table 4 and 5).

Competition and resource use

The factors affecting competition indices were not consistent among the two sites. In Dettingen, a regular spacing of the introduced species lowered the togetherness but the values for that index in the spatially clumped treatments did not differ from the control (Figure 3 A and Table 4). The C-score was higher in the regularly spaced treatments, though, here again, the values both spatial configurations did not differ from the control values (Figure 3 B and Table 4). The presence of the attractive species decreased the HTL niche overlap and this effect was stronger when *Centaurea* was regularly spaced (Figure 3 C and Table 4) whereas a decrease in LTL niche overlap was substantially lower without the introduced species (Figure 3 D and Table 4).

In Pfullingen, the density of introduced individuals was the determining factor in rather small and undirected changes in the HTL niche overlap (Figure 4 A; Table 5).

Specialisation

The impact of our treatments on the different specialisation indices was much more pronounced in Dettingen than in Pfullingen. In Dettingen, the density of introduced individuals increased the specialisation asymmetry (Figure 3 E and Table 4) and the presence of the attractive species strongly decreased the linkage density (Figure 3 G and Table 4). Further, the all the model terms for the H_2' index (i.e. the density of the attractive species, the spatial patterns and their interactions) were statistically significant (Table 4). The regular treatments had higher H_2' values which increased with the density of attractive individuals. This effect was more pronounced for the clumped treatments than for the regular treatments (Figure 3 F and Table 4)

In Pfullingen, the presence of the introduced species lowered values for the H_2' index (Table 5, Figure 4 B). However, only the R-H treatments was significantly lower than most of the other treatments (except the C-L).
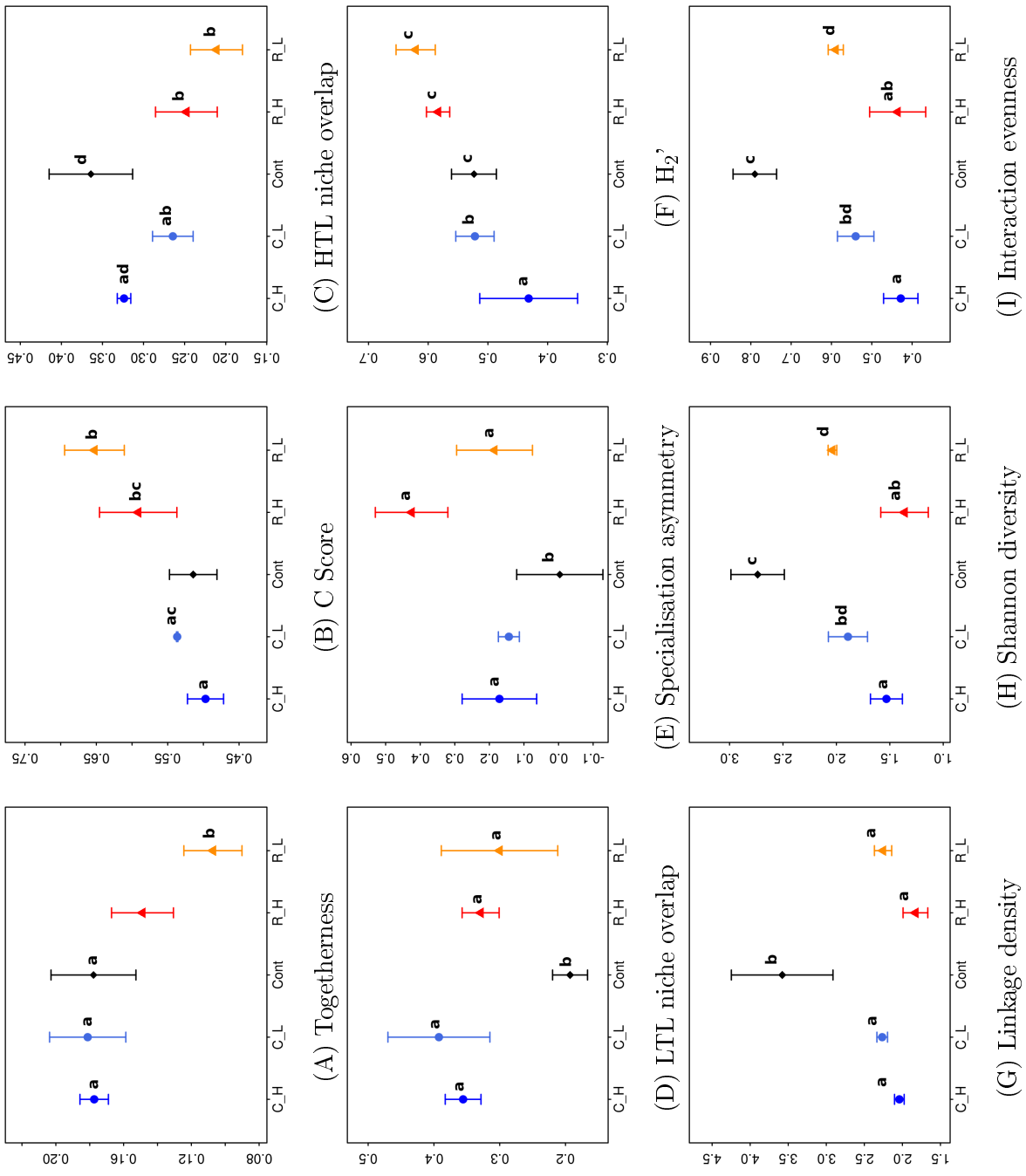
Table 4: Results of the statistical analysis for **Dettingen**. Only significant p-values associated with the best selected model terms are given. The same applies for the p-values for pairwise comparisons. Bold value are smaller than 0.05, italic values smaller than 0.01 and values smaller than 0.001 are indicated as such. Only the pairwise comparisons for models showing an effect in one of the three terms (spatial pattern, density of *Centaurea cyanus* and the interaction) in the first fitted models are shown. The transformation (T) applied to indices is indicated. Interaction diversity has been as inter. div. abreviated

Indices	Best model terms			Pairwise comparison										T
	pattern	density	inter.	CH CL	CH Cont	CH RH	CH RL	CL Cont	CL RH	CL RL	Cont RH	Cont RL	RH RL	
# of pollinator species														log
# of plant species														
connectance														
links per species														
mean # of shared hosts														
mean # of predators														
togetherness	**0.035**						**0.032**			**0.019**		**0.014**		
C score	**0.016**					**0.031**	*0.005*			**0.034**				
specialisation asymmetry		**0.039**			**0.041**						*0.0033*	**0.048**		
HTL niche overlap	*0.008*					**0.030**	**0.021**				*0.006*	*0.002*		log
LTL niche overlap	**0.024**				*0.007*			**0.026**	*0.004*		**0.020**	**0.031**		
generality														
vulnerability									*0.002*	*0.005*				
linkage density			**0.046**		*0.002*						p<0.001	*0.005*		
HTL mean inter. div.														sqrt
LTL mean inter. div.														sqrt
interaction evenness	p<0.001	p<0.001		**0.0456**	p<0.001	**0.013**		*0.002*	*0.005*		p<0.001	*0.004*	**0.049**	sqrt
Shannon diversity	p<0.001	p<0.001		**0.037**	p<0.001	**0.012**		p<0.001	*0.002*		p<0.001	*0.002*	**0.049**	
H2'	p<0.001	**0.015**	**0.024**					*0.002*	p<0.001		**0.049**	**0.015**	**0.016**	

Figure 3: Mean values of different indices (+/- standard errors) affected by the spatial patterns and / or the density of *Centaurea cyanus* for the different treatments in **Dettingen**. Treatments are coded as follows: R stands for the regular pattern, C for the clumped, H for the high density treatment and L for the low density treatments. Different letters in the graphs indicate pairwise differences between the treatments (Tukey HSD test).

Table 5: Results of the statistical analysis for **Pfullingen**. Only significant p-values associated with the best selected model terms are given. The same applies for the p-values for pairwise comparisons. Bold value are smaller than 0.05, italic values smaller than 0.01 and values smaller than 0.001 are indicated as such. Only the pairwise comparisons for models showing an effect in one of the three terms (spatial pattern, density of *Centaurea cyanus* and the interaction) in the first fitted models are shown. The transformation (T) applied to indices is indicated. Interaction diversity has been as inter. div. abreviated

Indices	Best model terms			Pairwise comparison										T
	pattern	density	inter.	CH CL	CH Cont	CH RH	CH RL	CL Cont	CL RH	CL RL	Cont RH	Cont RL	RH RL	
# of pollinator species														log
# of plant species														
connectance														
links per species														
mean # of shared hosts														
mean # of predators														
togetherness														
C score														
specialisation asymmetry														
HTL niche overlap														log
LTL niche overlap		**0.030**												
generality														
vulnerability														
linkage density														
HTL mean inter. div.														sqrt
LTL mean inter. div.														sqrt
interaction evenness		p<0.001			**0.027**			**0.024**			p<0.001		**0.024**	
Shannon diversity		**0.020**					**0.014**				**0.020**	*0.002*		
H₂'		*0.002*									**0.024**		*0.005*	

80

(A) HTL niche overlap

(B) H$_2$'

(C) Shannon diversity
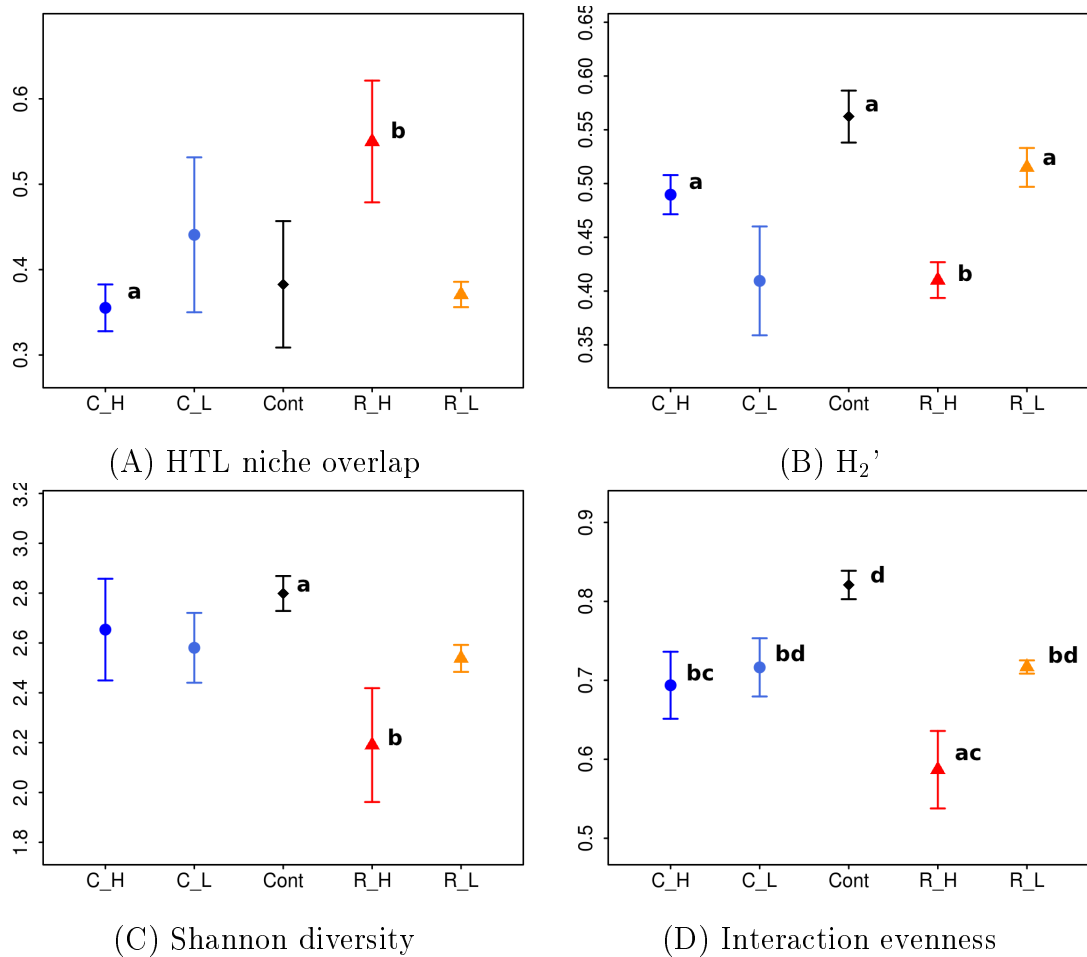
(D) Interaction evenness

Figure 4: Mean values of different indices (+/- standard errors) affected by the spatial patterns and / or the density of *Centaurea cyanus* for the different treatments in **Pfullingen**. Treatments are coded as follows: R stands for the regular pattern, C for the clumped, H for the high density treatment and L for the low density treatments. Different letters in the graphs indicate pairwise differences between the treatments (Tukey HSD test).

Diversity and evenness

The mean interaction diversities of the trophic levels when considered separately were not affected by our treatments in both sites (Table 4 and 5). However, both the Shannon diversity and evenness of interactions were reacting to the presence and the density of the attractive species in both sites (Table 4 and 5, Figure 3 H & I; Figure 4 C & D).

In Dettingen, the diversity and evenness of interactions between the two trophic levels were higher when the attractive species was absent and the values of both indices decreased with increasing density of the attractive flowers (Table 4 and Figure 3 H & I).

In Pfullingen, the trend for the two indices was approximately the same. Only the relative position of the clumped treatments changed and became more similar to the value of the control treatment, this for both indices (Table 5 and Figure 4 C & D).

Theoretical and methodological considerations

The study of the ecological consequences of the organisation of multi-trophic interactions is relatively recent (Jordano 1987) and some of its aspects are still in debate. As such, there is an ongoing discussion about the possibility that several factors can cause sampling artefacts (Vazquez *et al.* 2009). Such factors include the relative abundance of the species (more specifically the evenness of the marginal sums, Blüthgen *et al.* 2008), the relative sampling density (defined as the mean number of interactions per possible links in networks, Dormann *et al.* 2009), spatio-temporal effects and phenotypic effects (Vazquez *et al.* 2009). Due to the experimental design and the correction conducted for observation time, our results could only be impacted by the sampling density and the evenness of the marginal sums. However, we claim that the resulting indices are representative for the situation in nature as higher pollinator activity (higher sampling density) and different behaviour (higher preference toward the attractive species results in less even marginal sums distributions) are an entire part of the effects we wanted to test. Furthermore, the influence of these factors were not always in the expected direction and some indices have been shown to be resistant against changes in these factors (Blüthgen *et al.* 2006). A detailed description of the effects of networks proprieties on networks indices is given in Appendix 5.

Discussion

By introducing an attractive species at different densities in a semi-natural community, we impacted the floral diversity and evenness within these communities. These changes were

reflected in the structure of plant-pollinator networks, more specifically in the Shannon diversity and evenness of the network interactions. This indicates that the so-called "neutrality hypothesis" (Bascompte *et al.* 2003, Ollerton *et al.* 2003, Vazquez & Aizen 2004, Santamaría & Rodríguez-Gironés 2007) could explain a part of the structure recorded in our network. However, patterns of resource use by pollinators where strongly impacted by the attractiveness (and hence pollinator preferences) and the spatial distribution of our introduced species. This highly novel result suggest the existence of a spatial mechanism shaping the structure of plant-pollinator networks and leaves no doubt about the importance of spatial distribution of plant species within communities on pollinator foraging decisions (Morales & Vazquez 2008, Hanoteaux *et al.* 2013, Seifan *et al.* 2014) and hence on plant reproductive success (Hanoteaux *et al.* 2013, Seifan *et al.* 2014).

One of the possible explanation for the structure of plant-pollinator networks, is the so-called "neutrality hypothesis". This hypothesis states that the interactions among individuals are random and hence proportional to their abundances (Bascompte *et al.* 2003, Ollerton *et al.* 2003, Vazquez & Aizen 2004, Santamaría & Rodríguez-Gironés 2007). Under the assumption that species of two trophic levels interact randomly with each other, changes in evenness and diversity of plant communities should be reflected in the evenness and diversity of interactions. The clear match between these characteristics of the two communities and the network indices when the introduced species was present strongly indicates that neutrality can partly explain these results in both sites. However, the evenness and diversity of interactions was much more affected by the presence of the attractive species than would be expected under the assumption of random encounters. This exacerbated effect indicated that the introduced species had specific properties that were not interchangeable with other species (i.e. being indeed more attractive to pollinators). When conceiving a resource being more attractive than others, pollinators will tend to visit that resource more than would be expected by its abundance alone (Laverty 1992, Muñoz & Cavieres 2008, Hegland & Totland 2012, Seifan *et al.* 2014). This increased visitation rate towards the attractive species explains the much lower linkage density as well as the higher specialisation asymmetry found in Dettingen when the attractive species was present. Hence, the attractive species is exerting increasing competition with increasing abundance on its neighbours and pushes the outcome of indirect plant-plant interactions towards competition (Seifan *et al.* 2014).

However, the entirety of our results can not be explained by the abundance of species and the relative attractive character of the introduced species. The more exclusive and less similar use of resources by pollinators in the regular spatial pattern treatments in Dettingen suggest that pollinators exhibit different behavioural responses depending on the spatial distribution of the introduced plant species. This implies that spatial patterns

affected pollinator decisions beyond the sole effect of density. When the spatial pattern was regular, pollinators had to fly longer distances between individuals of the attractive species increasing the likelihood of encountering an individual of another species possibly triggering a change in visited plant species (Hanoteaux *et al.* 2013). This would increase resource use exclusiveness (H_2') and decrease similarity in resource use (increase C Score and decrease togetherness) in the regular pattern treatments compared to the clumped treatments.

On the other hand the spatial clumping of the attractive species reduced considerably the distance between flowers and induced a higher utilisation of that resource by pollinators due to its locally increased availability (Laverty 1992, Morales & Vazquez 2008, Hanoteaux *et al.* 2013). The spatial availability of an attractive floral resource resulted in a high proportion of pollinators foraging in clumps never encountering individuals of other species during their bouts, hence diminishing the chance for a change in species visited. This resulted in more pollinators visiting the same species, inducing a higher similarity in resource use and a lower exclusivity. This spatial mechanism is likely to substantially increase the reproductive output of the attractive species as both the quantity and the quality of the visits was increased (Rathcke 1983, Hanoteaux *et al.* 2013).

Even if the aim of this study was not to assess visitation rates and seed set as this was done elsewhere (Seifan *et al.* 2014), the results presented here are in perfect accordance with the results found in that sister study and confirm previous theoretical work (Hanoteaux *et al.* 2013). First, the observation that *Centaurea cyanus* is a strong competitor for the services of pollinators for its neighbour is a result common to the two studies. Secondly, the more visits to less conspicuous neighbours when the attractive species was spatially aggregated confirms that small scale spatial segregation can "trap" pollinators (Goulson 1994, Hanoteaux *et al.* 2013, Seifan *et al.* 2014). The more pollinators are trapped, the more individuals are using the same resources resulting in a lower exclusivity and higher similarity of resource use. At the same time, Seifan et al. (2014) found that the more attractive neighbours (congeneric and more conspicuous individuals) received more visits when the attractive species was regularly spaced. These individuals are more likely to trigger a switch in the pollinator choices than less conspicuous individuals and induced as such the higher resource use exclusivity and decrease similarity in resource use (Seifan *et al.* 2014).

In the light of recent biological invasions, our results suggest that the effects of species invading plant communities might not only be conditioned by the density of the invasives (Bartomeus *et al.* 2008) but also by their spatial distribution. Negative effects of invasive plant species on their neighbours have been documented (see Morales & Traveset 2009 for a review) and these negative effects could be enhanced with the tendency of invasive species

to grow in dense and spatially aggregated clumps (Gurevitch *et al.* 2011). Furthermore, the evenness of the composition of the pollinator community in invaded areas could affect the outcome of the invasion. Networks dominated by few abundant pollinator species are likely to be more affected by the introduction of new species because the attraction of one species dominates (Chittka & Schürkens 2001), the pollination system will leave the rest of the plant community with much less pollination opportunities, as suggested here by the stronger impact of spatial patterning in Dettingen where *Apis mellifera* was from far the dominant pollinator. The higher the degree of generalisation of the abundant pollinator species the higher the number of plant species potentially suffering competition from the newly arriving species. With the recent decline in diversity of pollinators (Biesmeijer *et al.* 2006, Potts *et al.* 2010) and the range expansion of some invasive pollinators (e.g. *Apis mellifera*, Schneider *et al.* 2004), the likelihood of having a pollinator community highly dominated by one or two species will increase and hence the potentially negative consequences of plant species invasion amplified.

Acknowledgements

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Appendices

Appendix 1: List of plant species, attraction units and average floral surfaces.

In order to take the differences in floral morphology and size among the different species used in this study, an "attraction unit" was defined for each species (Hegland & Boeke 2006). The definition of this species specific attraction unit specified what has to be counted and measured in the field in order to calculate floral surfaces (as a proxy for floral density). Depending on the species specific floral morphology, we considered different shapes of attraction units for the different species and adapted the measurements and formulas used to calculate their surfaces. Once the attraction unit for each species was defined, we measured characteristic dimensions (Table A.1) of 20 randomly chosen attraction units in randomly chosen plots and computed a mean floral surface for each species. Measurements were taken separately in each site.

In this study, we considered that the attraction units could have three different shapes which are listed below, together with their respective characteristics dimension(s) and the formulas used in the calculation of their surface.

Shape	Surface formula	Measured dimension(s)
Circle	$\pi \cdot r^2$	radius (r)
Ellipse	$\pi \cdot \frac{r_1 \cdot r_2}{2}$	smallest and largest radii (r_1 and r_2)
Rectangle	$L \cdot l$	length (L) and width (l)

In Pfullingen, the mean floral surface of attraction units of three species were not measured (*Euphorbia cyparissias*, *Plantago lanceolata* and *P. media*) and we set their surfaces to 1 cm². However, this should not affect our results because only 3 individuals of *E. cyparissias* were present across all plots and only 5 for the two *Plantago* species.

Table A.1: List of the plant species contained in the networks when pooling all observations together for both sites . The chosen attraction unit (A.U.), the shape (F; C: cirle, E: ellipse and R: rectangle) and the calculated mean surface (S) are given for each species (I: inflorescence, F: flower). If two species names appear on the same line, this species were pooled together in the network analysis.

Dettingen				Pfullingen			
Species	A.U.	F	S (cm²)	Species	A.U.	F	S (cm²)
Centaurea cyanus	I	C	10.68	*Centaurea cyanus*	I	C	10.68
Centaurea jacea	I	C	11.17	*Centaurea jacea*	I	C	11.73
Centaurea scabiosa	I	C	11.17	*Scabiosa columbaria*	I	C	6.49
Knautia arvensis	I	C	8.85	*Asperula cynanchica*	F	C	0.47
Achillea millefolium	I	E	6.20	*Campanula patula & C. rotundifolia*	F	C	1.70
Campanula patula & C. rotundifolia	F	C	1.70	*Galium mollugo & G. pumilum & G. verum*	I	R	2.3
Crepis biennis	I	C	9.72	*Helianthemum nummularium*	F	C	2.82
Galium mollugo & G. pumilum & G. verum	I	R	2.30	*Lotus corniculatus*	F	R	1.25
Lathyrus pratensis	F	R	1.12	*Onobrychis viciifolia*	F	R	3.50
Leontodon hispidus	I	C	9.72	*Rhinanthus glacialis*	F	R	2.64
Lotus corniculatus	F	R	1.25	*Euphorbia cyparissias*	I	R	1
Onobrychis viciifolia	F	R	5.90	*Stachys officinalis*	F	R	1.9
Ranunculus acris & R. bulbosus	F	C	2.13	*Thymus pulegioides*	I	C	0.86
Salvia pratensis	F	R	40.90	*Trifolium pratense*	I	C	2.00
Trifolium pratense & T. campestre	I	C	5.28	*Trifolium montanum*	I	C	2.00
Trifolium. repens	I	C	2.24	*Plantago lanceolata and P. media*	I	C	2.00
Vicia sativa	F	R	3.9	*Thesium pyrenaicum*	I	R	0.86

Appendix 2: List of pollinator species within the networks

Table A.2: List of all the pollinator species recorded in each site when pooling all the observations together. Names given to the species refer to the codes used in the field and are different from nomenclature. The overall relative frequency of interaction (RVF) is given for each species.

	Dettingen			Pfullingen	
#	Species	RVF	#	Species	RVF
1	*Apis mellifera*	0.7899	1	*Bombus lapidarius*	0.3542
2	*Bombus lapidarius*	0.0396	2	*Apis mellifera*	0.2526
3	*Empis tesselata*	0.0392	3	*Bombus terrestris*	0.099
4	*Cephus pygmaeus*	0.0249	4	*Andrenoida*	0.0485
5	*Holia argentea*	0.023	5	*Episyrphus balteatus*	0.0463
6	*Andrenoida sp.*	0.0152	6	*Melanargia galathea*	0.0356
7	*D too small*	0.0053	7	*Spraerophoria scripta*	0.0338
8	*Hyponephele lycaon*	0.0051	8	*Bombus bohemicus*	0.022
9	*Bombus terrestris*	0.0046	9	*Bombus not terrestris*	0.0176
10	*Muscoid sp.*	0.0046	10	*Bombus pascuorum*	0.0165
11	*Nemophora metallica*	0.0046	11	*Maniola jurtina*	0.0162
12	*Volucella bombylans*	0.0044	12	*Eristalis tenax*	0.0118
13	*Andrenoide Small*	0.004	13	*Zygaena sp.*	0.0061
14	*Sarcophaga carnaria*	0.0029	14	*Andrenoida Small*	0.0046
15	*Melanargia galathea*	0.0027	15	*Lysandra sp.*	0.0042
16	*Muscidae 1*	0.0025	16	*Argynnis aglaja*	0.0029
17	*Ants*	0.0019	17	*Chrysotoxum bicinctum*	0.0028
18	*Bombus pascuorum*	0.0017	18	*Tachina fera*	0.0028
19	*Eristalis tenax*	0.0017	19	*Muscoidea*	0.0022
20	*Tenthredo arcuata*	0.0017	20	*Sarcophaga carnaria*	0.002
21	*Small not Curculionidae*	0.0015	21	*Melanostoma sp.*	0.0018
22	*Symphita red abdomen*	0.0015	22	*Dipteran Too small*	0.0017
23	*Syrphida long*	0.0013	23	*Anthophora sp.*	0.0017
24	*Syrphide long mask*	0.0013	24	*Bombus pratorum*	0.0015
25	*Bombus not terrestris*	0.0011	25	*Sarcophagidae2*	0.0011
26	*Curculionidae*	0.001	26	*Lycaena tityrus*	0.0009
27	*Muscidae? 2*	0.001	27	*Volucella pelluscens/Merodon*	0.0007
28	*Muscidae? 1small*	0.0008	28	*Cercopidae*	0.0006
29	*Panorpa sp.*	0.0008	29	*Melicta sp.*	0.0006
30	*Symphita?*	0.0008	30	*Midge beak*	0.0006
31	*Dipteran 1?*	0.0006	31	*Scaeva pyrastri*	0.0006
32	*Midge*	0.0006	32	*Symphita1*	0.0006

	Dettingen			Pfullingen	
#	Species	RVF	#	Species	RVF
33	*Bombylius major*	0.0004	33	*Adscita geryon*	0.0004
34	*Coccinellidae*	0.0004	34	*Chrysotoxum taucum*	0.0004
35	*Elateridae*	0.0004	35	*Panorpa*	0.0004
36	*Lasiommata megera*	0.0004	36	*Pipiza bimaculata*	0.0004
37	*Maniola jurtina*	0.0004	37	*Small not Curculionidae*	0.0004
38	*Miridae 2*	0.0004	38	*Symphita2*	0.0004
39	*Miridae 4*	0.0004	39	*Syrphida1*	0.0004
40	*Moth*	0.0004	40	*Agrypnus murinus*	0.0002
41	*Small Ogive*	0.0004	41	*Asilidae1*	0.0002
42	*Symphita sand coloured*	0.0004	42	*Asilidae2*	0.0002
43	*Agrypnus murinus*	0.0002	43	*Conopidae*	0.0002
44	*Lycaena sp.*	0.0002	44	*Conopidae1:Sicus sp.?*	0.0002
45	*Cantharis vulgaris*	0.0002	45	*Cteniopus flavus*	0.0002
46	*Cercopidae 1*	0.0002	46	*Eupeodes sp.*	0.0002
47	*Conopidae ?*	0.0002	47	*Leptura melanura*	0.0002
48	*Green Carabidae*	0.0002	48	*Melanostoma scalare*	0.0002
49	*Hadrodemus m-flavum*	0.0002	49	*Moth*	0.0002
50	*Leptoterna dolobrata*	0.0002	50	*Small Maniola*	0.0002
51	*Long solitary bee*	0.0002	51	*Physocephala rufipes*	0.0002
52	*Miridae 1*	0.0002	52	*Pieris brassicae*	0.0002
53	*Miridae 3*	0.0002	53	*Platycheirus sp?*	0.0002
54	*Miridae 5*	0.0002	54	*Small not Curculionidae sand*	0.0002
55	*Muscidae2 small*	0.0002	55	*Small Ogive*	0.0002
56	*M Green*	0.0002	56	*Symphita red abdomen*	0.0002
57	*Oedemeridae*	0.0002	57	*Syrphus sp.*	0.0002
58	*Small Lycaena*	0.0002	58	*Tephritidae*	0.0002
59	*Maniola small*	0.0002	59	*Volucella bombylans*	0.0002
60	*Small black Butterfly*	0.0002			
61	*Heteropteran transparent*	0.0002			
62	*Stenotus binotatus*	0.0002			
63	*Symphita 1*	0.0002			
64	*Syrphida circle*	0.0002			
65	*Syrphida Big*	0.0002			
66	*Syrphida orange line*	0.0002			
67	*Tephritidae*	0.0002			

Appendix 3: Normality and residuals tests, covariates for the different models

Table A.3: P values for the normality test (P Norm, Shapiro test) and for the residual test of the selected best model (BP test, Breush-Pagan test) for all the indices for both sites. Underlined values are smaller than 0.001 as indicated.

Indices	Dettingen		Pfullingen	
	P Norm	BP test	P Norm	BP test
Number of higher trophic species	0.955	0.184	0.063	0.954
Number of lower trophic species	0.057	0.197	0.428	0.149
Connectance	0.079	0.643	0.282	0.329
Links per species	0.621	0.781	0.374	0.566
Mean number of shared hosts	0.083	0.101	0.495	0.605
Mean number of predators	0.679	0.432	0.705	0.311
Togetherness	0.956	0.615	0.595	0.495
C score	0.350	0.701	0.357	0.483
Specialisation asymmetry	0.454	0.204	0.822	0.226
HTL niche overlap	0.776	0.328	0.614	0.706
LTL niche overlap	0.854	0.381	0.103	0.544
Generality	0.829	0.181	0.416	0.311
Vulnerability	$\underline{p < 0.001}$	0.072	0.424	0.335
linkage density	$\underline{p < 0.001}$	0.081	0.853	0.695
HTL mean interaction diversity	0.110	0.169	0.199	0.579
LTL mean interaction diversity	0.087	0.368	0.138	0.425
Interaction evenness	0.887	0.629	0.734	0.064
Shannon diversity	0.832	0.491	0.334	0.393
H_2'	0.224	0.308	0.912	0.580

Table A.4: Covariates selected in the best fitting models for the analysis for both sites. NoCov refers to no covariates included in the model (other than the density of *C. cyanus*), Dens refers to the cumulative total flower abundance in the plots, LogDens is the log transformed cumulative total flower abundance, Eve is the cumulative evenness, and Div is the cumulative diversity. If the log transformed density of *Centaurea cyanus* was used "_LOG" is added after the name of the selected covariate.

Indices	Dettingen Covariate	Pfullingen Covariate
Number of higher trophic species	LogDens_LOG	Eve_LOG
Number of lower trophic species	Div_LOG	LogDens
Connectance	Eve_LOG	Eve_LOG
Links per species	Eve	NoCov_LOG
Mean number of shared hosts	Eve	Eve_LOG
Mean number of predators	Dens_LOG	Eve_LOG
Togetherness	Div_LOG	Eve_LOG
C score	Dens	Eve_LOG
Specialisation asymmetry	LogDens	Dens
HTL niche overlap	Div	Eve
LTL niche overlap	LogDens	Eve
Generality	Eve_LOG	LogDens
Vulnerability	Div	Dens
linkage density	Div	Dens_LOG
HTL mean interaction diversity	LogDens_LOG	LogDens
LTL mean interaction diversity	Eve_LOG	Dens
Interaction evenness	LogDens	NoCov
Shannon diversity	LogDens	NoCov
H_2'	Dens	Div

Appendix 4: Plant community analyses

In order to control for the homogeneous composition of the community in our plots, we conducted a short analysis of the flower abundance, diversity and evenness. In order to take temporal aspects into consideration and because we were interested in an overall measure of abundance, diversity and evenness, we summed all these values across all the observation dates (Vazquez *et al.* 2009). As no deviations from normality were found in the untransformed cumulative values (tested with Shapiro tests), we used linear models assessing the effect of our treatments on the three response variables (Table A.5 , Figure A.1). We found that our plots represented indeed similar communities as they never

differed in cumulative floral abundances, diversities and evenness, this consistently in both sites (results not shown). Surprisingly, when incorporating the attractive species into the analysis, only the diversity of floral resources in Pfullingen seemed to be affected (Table A.5). Pairwise comparisons revealed that only C-L was higher than the Control (p = 0.0038) and than R-H treatments (p = 0.0060).

Table A.5: Results of the community composition statistical analyses in both sites. Values for the test of normality (P Norm) and for the treatment effect in the linear model (P model). Bold values are smaller than 0.05. Tests were conducted with the introduced species.

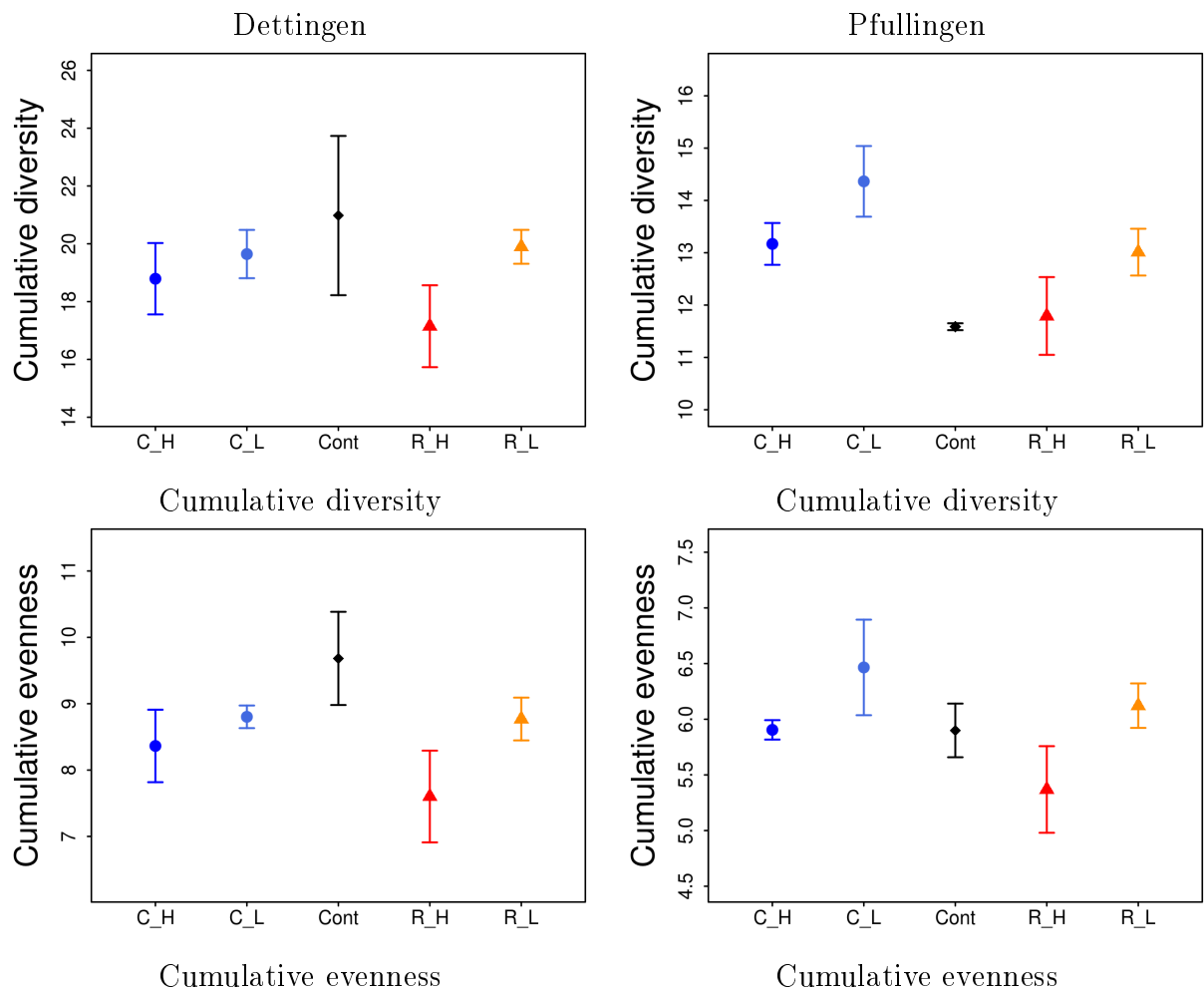| | Dettingen | | Pfullingen | |
	P Norm	PModel	PNorm	P Model
Density	0.9024	0.2421	0.6215	0.313
Diversity	0.5013	0.5304	0.9958	**0.0221**
Evenness	0.6416	0.1647	0.512	0.2044

Figure A.1: Mean values (+/- standard errors) for the cumulative density and diversity of the plant communities according the treatments for both sites.

97

Appendix 5: Theoretical and technical considerations

It has been shown that the values of different network metrics are highly dependent on some network properties such as network size (Dormann *et al.* 2009), the evenness of the distribution of the marginal sums (Blüthgen *et al.* 2008) and the sampling density (Dormann *et al.* 2009). In our analysis we did not find differences among treatments for the number of species composing the networks. It is hence unlikely that network size will affect our results (for example, no differences were found in connectance; see Table 4 & 5). However, differences in sampling densities and/or in the evenness of the marginal sums of both trophic levels could affect our results.

As such, we tested for potential differences among these network characteristics. First, we used Shapiro tests to test for deviation from normality on the untransformed values of the sampling densities and the evenness of the marginal sums. Only the values for the evenness of the lower trophic level marginal sums for Dettingen needed to be arcsine square root transformed in order to achieve normality. After applying the necessary transformations, we analysed the differences among treatments with linear models using Tukey Honest Significant Differences (Tukey HSD). The results of this analysis are given in Table A.6 and in Figure A.2. From this we can conclude that the tested networks characteristics were substantially different among treatments. Only the sampling density for Pfullingen was not affected by our treatments. The sampling density in Dettingen and the evenness of the marginal sums of both trophic levels in both sites differed considerably among treatments. This can have far reaching consequences for our results and we can tackle this in two ways. First, we can consider that these differences are the result of our experiment and hence are an entire part of the results. Secondly, we could consider that these network properties are affecting our results and that we should account for these differences.

Here I will shortly review the putative effects of these network properties on the indices discussed in this study. I base my short review mainly on two papers: for the effects of the evenness of the marginal distributions, see Blüthgen *et al.* (2008), and for the effect of network sizes and sampling densities, see Dormann *et al.* (2009).

Table A.6: Results of the analysis of the sampling density and evenness of the marginal sum distribution for both sites (D: Dettingen and P: Pfullingen). The p-values of the Shapiro test (p norm) and of the linear models (p model) are given together with the p-values associated with the different pairwise comparisons (Tukey HSD). Bold values are < 0.05, values in italic are < 0.01 and values < 0.001 are indicated as such.

|	Field	p norm	p model	CH-CL	CH-Cont	CH-RH	CH-RL	CL-Cont	CL-RH	CL-RL	Cont-RH	Cont-RL	RH_RL
Sampling density	D	0.88	*0.001*	0.716	*0.006*	*0.004*	0.230	**0.012**	*0.002*	0.388	P<0.001	0.055	P<0.001
Evenness HTL	D	0.26	**0.012**	0.485	*0.003*	0.687	0.212	**0.011**	0.281	0.556	*0.002*	**0.030**	0.111
Evenness LTL	D	0.06	**0.035**	0.066	*0.006*	0.687	**0.038**	0.179	0.181	0.756	**0.016**	0.287	0.109
Sampling density	P	0.39	0.064	0.651	0.159	0.076	0.938	0.075	0.161	0.597	*0.006*	0.180	0.066
Evenness HTL	P	0.92	**0.047**	0.807	0.127	0.065	0.847	0.084	0.098	0.663	*0.004*	0.173	**0.046**
Evenness LTL	P	0.92	**0.014**	0.671	0.058	**0.038**	0.441	0.120	**0.018**	0.723	*0.001*	0.211	**0.010**
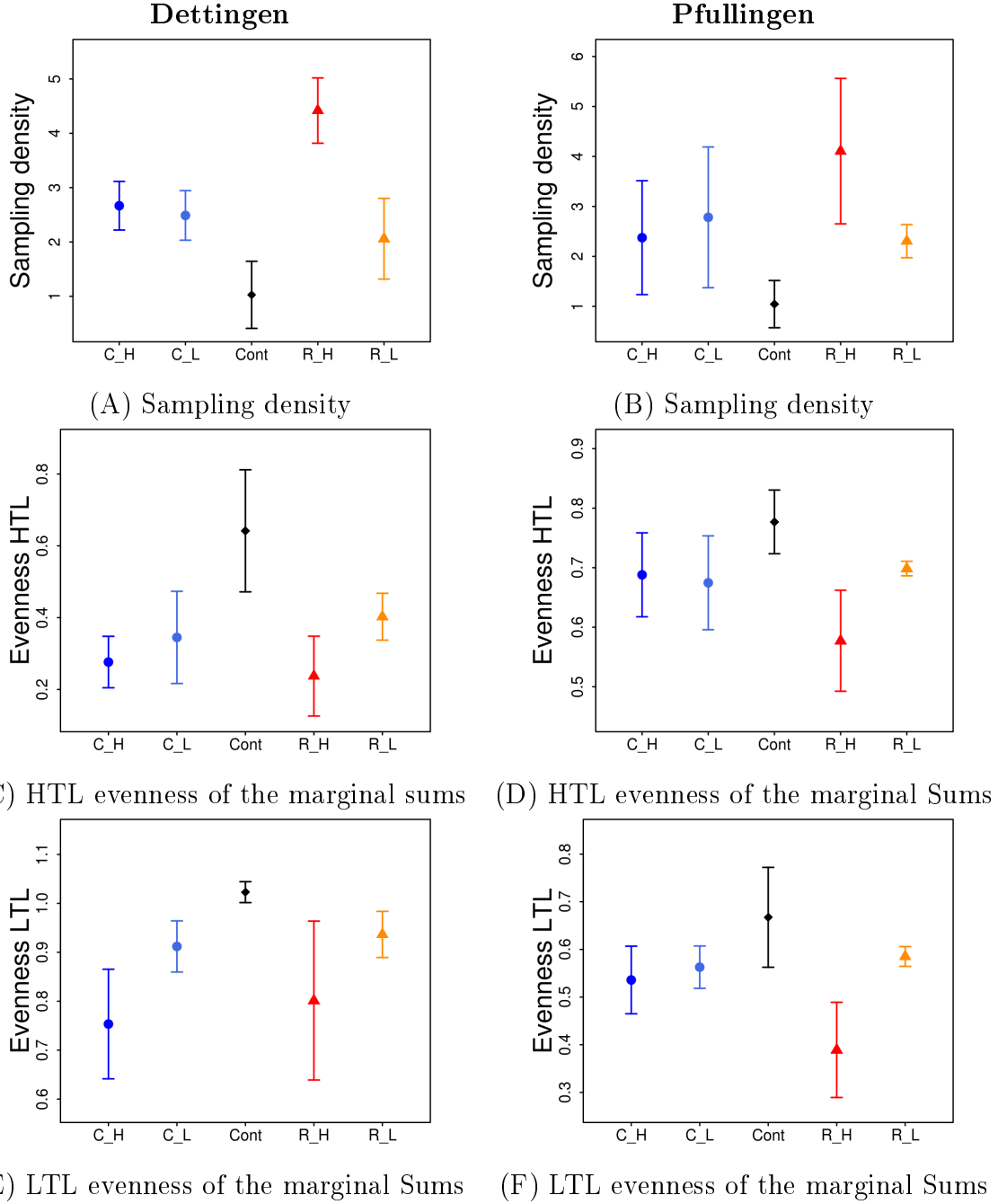
Figure A.2: Means of sampling density, of the evenness of the higher trophic level (HTL) and lower trophic level (LTL) (+/- standard errors) for the different treatments in both sites. C refers to the clumped treatment; R refers to the regular treatments; L and H refer to the low and high density treatments, respectively; Cont was the control .

The connectance, interaction evenness and the generality can be affected by the evenness of the marginal sums. These three indices are supposed to increase with increasing evenness. We found no difference for the connectance between our treatments although the marginal distributions differed. At the contrary, the differences in interaction evenness in both sites were reflecting the differences in evenness of the marginal distribution

(compare Figure 3 I with Figure A.2 C & E and Figure 4 D with Figure A.2 D & F). When graphically comparing the interaction evenness of the treatments and these of the marginal sums, it seems clear that similarities in the differences among the treatments are present. These similarities are clearly found back within the pairwise comparisons (compare Table 4 and 5 with Table A.6 for Dettingen and Pfullingen).

For the H_2' index , differences were found among treatments but the evenness of the marginal sums can not possibly explain them (compare Figure 3 F with Figure A.2 C & E for Dettingen and Figure 4 B with Figure A.2 D & F for Pfullingen).

The sampling density in both fields was the highest for the R-H treatment and the lowest for the control. Comparing the trend described in our results with the trend in the results of Dormann *et al.* (2009) (see there Figure 3), we find contradicting trends between e.g. the C-score, the H_2' and the HTL niche overlap for Dettingen and the expected effects of the sampling density on the values of these indices (compare Figure 3 B, C, F with Figure A.2 A). Further, the differences in interaction evenness could possibly be explained by differences in sampling density. In Pfullingen, I could not reject the idea that the differences between the values of the HTL niche overlap, the H_2' and the evenness indices in the different treatments could be (partly) explained by the differences in the sampling densities (compare Figure 4 A, B, D with Figure A.2 B).

Hence, it became clear that some of the trends found in this study could be emerging from differences in sampling density and or evenness of the marginal sums of the different networks. In order to elucidate the impact of these network proprieties on the values of the computed indices, I tested for a relation between these indices and the three networks proprieties (LTL and HTL evenness of the marginal sums and the sampling density, Table A.7 & A.8).

As expected, the majority of the tested values seemed to be, at least partly, explainable by the values of one of the three independent variables, with however mostly only a small amount of the variance explained by the regressions (except for the evenness and diversity indices which showed rather high R^2 values). Interestingly, all the indices that were found to be affected by the spatial distribution of the attractive species (i.e. the C-Score, the togetherness, the HTL niche overlap and the H_2'in Dettingen and none in Pfullingen, see Table 4 & 5) didn't exhibit a significant relation with one of the three network characteristics. These results strongly confirm that the neutrality principle (Bascompte *et al.* 2003, Ollerton *et al.* 2003, Vazquez & Aizen 2004, Santamaría & Rodríguez-Gironés 2007) certainly explains a part of our results but fails to describe every aspects of them. Manipulating the density and evenness of plant communities will affect the interaction structure of organisms randomly interacting, because abundant species interact more fre-

Table A.7: Results of the regression analysis of the influence of the sampling density and evenness of both trophic levels on the values of different indices for **Dettingen**. The p-values, slopes and R^2 are given for each regression. Bold value are smaller than 0.05, italic values are smaller than 0.01 and values smaller than 0.001 are indicated as such. Indices in bold are indices for which at least one of the three regressions had a slope significantly different from 0.

Indices	Sampling density			LTL marginal sums evenness			HTL marginal sums evenness		
	p	slope	R^2	p	slope	R^2	p	slope	R^2
# of pollinators	P < 0.001	-2.721	0.607	0.816	2.291	0.004	0.088	11.160	0.207
# of species	0.118	-0.459	0.177	0.669	-1.312	0.014	0.936	0.173	0.001
Connectance	*0.004*	0.022	0.490	0.836	0.019	0.003	0.492	-0.043	0.037
Links per species	0.736	0.009	0.009	0.925	0.024	0.001	0.951	-0.011	0.000
Mean number of shared hosts	0.266	0.058	0.094	0.514	-0.347	0.033	0.327	-0.362	0.074
Mean number of predators	0.280	0.033	0.089	0.804	-0.078	0.005	0.384	-0.034	0.002
Togetherness	0.921	0.001	0.001	0.920	0.009	0.001	0.878	0.055	0.059
C score	0.944	0.001	0.000	0.854	0.033	0.003	0.429	-0.098	0.049
Specialisation asymmetry	*0.004*	0.116	0.491	**0.039**	-0.889	0.289	P < 0.001	-0.940	0.654
HTL level niche overlap	0.240	-0.020	0.105	0.994	-0.001	0.000	0.141	0.169	0.159
LTL trophic level niche overlap	**0.022**	0.051	0.341	0.462	-0.180	0.042	**0.028**	-0.346	0.318
Generality	0.849	-0.018	0.003	**0.017**	1.989	0.366	0.813	0.154	0.004
Vulnerability	*0.010*	-0.835	0.412	0.080	6.055	0.218	P < 0.001	7.876	0.745
Linkage density	*0.006*	-0.426	0.455	0.090	4.022	0.406	P < 0.001	4.015	0.820
HTL mean interaction diversity	**0.033**	-0.071	0.305	0.685	0.080	0.013	0.850	-0.026	0.003
LTL mean interaction diversity	0.189	0.025	0.129	P < 0.001	1.113	0.807	P < 0.001	0.831	0.910
Interaction evenness	P < 0.001	-0.085	0.466	P < 0.001	3.851	0.722	P < 0.001	3.086	0.939
Shannon diversity	P < 0.001	-0.355	0.612	P < 0.001	3.851	0.722	P < 0.001	3.086	0.939
H_2'	0.784	-0.006	0.006	0.166	0.287	0.142	0.307	0.152	0.080

Table A.8: Results of the regression analysis of the influence of the sampling density and evenness of both trophic levels on the values of different indices for **Pfullingen**. The p-values, slopes and R^2 are given for each regression. Bold value are smaller than 0.05, italic values are smaller than 0.01 and values smaller than 0.001 are indicated as such. Indices in bold are indices for which at least one of the three regressions had a slope significantly different from 0.

Indices	Sampling density			LTL marginal sums evenness			HTL marginal sums evenness		
	p	slope	R^2	p	slope	R^2	p	slope	R^2
# of pollinators	0.901	0.054	0.001	0.289	-5.383	0.086	0.397	-5.715	0.056
# of species	0.102	-0.538	0.193	0.867	0.693	0.002	0.376	4.774	0.061
Connectance	**0.026**	0.023	0.328	0.959	-0.007	0.000	0.202	-0.218	0.122
Links per species	0.096	0.062	0.199	0.717	-0.171	0.010	0.170	-0.825	0.140
Mean number of shared hosts	**0.013**	0.205	0.387	0.474	-0.792	0.040	0.081	-2.423	0.217
Mean number of predators	*0.007*	0.121	0.444	0.396	-0.517	0.056	0.060	-1.431	0.247
Togetherness	*0.004*	0.038	0.482	0.415	-0.151	0.052	0.062	-0.431	0.243
C score	P < 0.001	-0.075	0.624	0.098	0.502	0.196	**0.012**	0.944	0.399
Specialisation asymmetry	0.857	0.004	0.003	0.156	0.326	0.149	0.483	-0.219	0.039
HTL level niche overlap	*0.001*	0.064	0.554	*0.006*	-0.694	0.446	**0.010**	-0.874	0.407
LTL trophic level niche overlap	0.108	0.016	0.187	0.437	-0.094	0.047	0.189	-0.205	0.129
Generality	0.186	-0.065	0.130	**0.034**	1.188	0.301	0.247	0.908	0.101
Vulnerability	**0.042**	-0.273	0.282	0.804	0.432	0.005	*0.005*	5.507	0.460
Linkage density	**0.012**	-0.169	0.395	0.366	0.810	0.063	*0.001*	3.208	0.571
HTL mean interaction diversity	0.586	-0.019	0.023	0.233	0.479	0.107	0.296	0.557	0.084
LTL mean interaction diversity	0.895	0.003	0.001	0.170	0.371	0.140	0.999	0.000	0.000
Interaction evenness	P < 0.001	-0.063	0.872	P < 0.001	0.645	0.631	P < 0.001	1.042	0.946
Shannon diversity	P < 0.001	-0.204	0.804	P < 0.001	2.105	0.598	P < 0.001	3.171	0.781
H$_2$'	**0.025**	-0.031	0.329	**0.038**	0.356	0.292	**0.032**	0.481	0.307

quently and with more species than rare species (Vazquez *et al.* 2009). Hence, under the assumption of neutrality, changes in plant communities will equally affect the marginal sums of networks. Clues for the neutrality hypothesis were especially strong in Dettingen were *Apis mellifera* was super abundant. However, the underlying supposition of random interactions between organisms failed to explain the effects of the spatial distribution of the introduced plant species in the very same field. This reinforces our conclusion that decisions of pollinators were certainly at least partially driven by the spatial distribution of attractive floral resources. Hence, the general patterns of visits of pollinators at small spatial scale did not obey the tyranny of the neutrality hypothesis.

CHAPTER 3

Identity of neighbouring species alters the response of pollinators to floral density in artificial plant communities

Abstract

Indirect plant-plant interactions mediated through shared pollinators may play an important role in shaping patterns of coexistence through their impact on the reproductive success of flowering plant species. Recently, much efforts have been developed in order to investigate the factors affecting the outcome of such interactions. The floral density is one of the major determinant of the nature of these indirect interactions. Further, it has been recently demonstrated that the spatial patterning within plant communities can alter the effect of the floral density by manipulating the pollinator behaviour. As the pollinator behaviour determines the net outcome of indirect interactions, the identity of species involved in such interactions will undoubtedly play an important role. In this study, we aimed at investigating the changes in pollinator behaviour induced by changes in species identities and relative densities. In order to achieve this goal, we created artificial communities composed of two species and gradients of changing relative densities of the two species. Further, in order to assess the role species of identity, the component species were systematically exchanged. We found that the identity of neighbouring species can induce and/or alter both intra- and interspecific density dependent responses in quantitative aspects of the pollinator behaviour. As such, the occurrence and intensity of both negative and positive density dependence were conditioned by the identity of

the neighbouring species. Finally, we briefly discuss the importance of the attractiveness differential in further studies as it might help resolving the discrepancy reigning in the results of studies investigating the role of species in indirect plan-plant interactions.

Introduction

When sharing pollinators, the pollination success of plant species in communities may depend not only on the species's own floral traits but also on the floral traits and densities of sympatric co-flowering species (Campbell & Motten 1985, Feinsinger 1987, Geber & Moeller 2006, Mitchell *et al.* 2009). Structural plant community characteristics such as the densities, identities and spatial distribution of species, are indeed likely to affect pollination patterns by inducing changes in the behaviour of pollinators attracted to and foraging in plant communities (Jakobsson *et al.* 2009, Hegland & Totland 2012, Hanoteaux *et al.* 2013). In turn, such changes can modify indirect interspecific interactions among plants which are mediated by shared pollinators (Rathcke 1983, Feldman *et al.* 2004).

A key factor affecting the net outcome of such interspecific interactions is the density of neighbouring flowering individuals (Rathcke 1983, Feldman *et al.* 2004, Muñoz & Cavieres 2008, Hanoteaux *et al.* 2013). The presence at low density of other flowering individual can increase the overall attractiveness of the vegetation patch to shared pollinators, ultimately ensuring more visits and a higher reproductive output (facilitation, Rathcke 1983, Feldman *et al.* 2004). However, as the number of available pollinators is finite, the nature of such indirect plant-plant interactions should shift from positive (i.e. facilitation) to competition as the overall density of floral resources increases (Rathcke 1983).

This model is based on a saturating density-visitation relationship which shape will certainly vary among different plant assemblies (as originally stated in Rathcke 1983, see also Sih & Baltus 1987, Lãzaro & Totland 2010). Indeed, due to the diversity of floral morphologies and pollinator species, the impacts of changing densities on the pollinator attraction and behaviour will also depend on the plant species identities (and hence floral morphologies) and on the identities (and hence cognitive ecology) of the available pollinators (Sih & Baltus 1987, Feldman 2006, Seifan *et al.* 2014).

The effect of the identities of co-flowering individuals is related to their floral traits and hence to their similarity but also to the ability of pollinators to discriminate among them. The more similar the floral traits among species are, the less pollinator will discriminate among them (Schemske 1981, Dukas & Real 1993, Clegg & Durbin 2000, Chittka & Schürkens 2001, Goulson 2010). In the extreme case, when plant species are so similar that pollinators do not distinguish between species, the outcome of indirect plant-plant

interactions will be defined by the overall floral density (see above, Rathcke 1983, Feldman *et al.* 2004). Further, if co-flowering plant species are morphologically very dissimilar, the indirect interactions among plant individuals could disappear if pollinators cannot access the floral resources of a species (morphological constraints, forbidden links, Olesen *et al.* 2011). However, in most cases, pollinators will behave as generalists and visit even morphologically dissimilar species (Waser *et al.* 1996, Waser & Ollerton 2006). In such situations, the outcome of indirect plant-plant interactions will be much more difficult to predict as it will depend on both the floral traits of the plant species involved and the impacts of these traits on the pollinator behaviour (Chittka & Thomson 2001, Goulson 2010, Lãzaro & Totland 2010).

As such, the relative attractiveness of the plant species involved in the indirect interactions could determine the net effect of a species on another (Laverty 1992, Chittka & Schürkens 2001, Johnson *et al.* 2003, Hanoteaux *et al.* 2013). Considering species that differ in relative attractiveness for pollinators, the intensity of mechanisms shaping the density-visitation relationship could increase. Indeed, the number of pollinators attracted by a certain density of the more attractive species will be higher than compared to the number of pollinators attracted by the same density of a "less" attractive species (Laverty 1992, Johnson *et al.* 2003, Molina-Montenegro *et al.* 2008, Seifan *et al.* 2014). Hence, the density of the neighbouring species at which facilitation would occur and the density at which the shift towards competition occurs, would be lower compared to a situation where the neighbouring species is "less" attractive. This means that considering two species mixtures having one species in common and where the densities are the same, the species in common could be facilitating or competing with the other species, depending on the relative attractiveness of the second species. Hence, the effect of that common species on its neighbours depends on the identity of its neighbour.

Furthermore, we argue that, additionally to the absolute floral density, the relative floral densities of plant species (i.e. the species specific proportion of floral density in plant assemblies), will also help defining the role a species plays in indirect plant-plant interactions. Where the absolute floral density will determine the overall attractiveness of a patch and hence the number of pollinators attracted to this patch (Rathcke 1983, Feldman *et al.* 2004, Ghazoul 2005, Hegland & Boeke 2006, Lãzaro & Totland 2010), the relative floral densities will manipulate the pollinator behaviour after pollinators chose to forage in that patch (Hanoteaux *et al.* 2013). If a species is well represented in a foraging patch (i.e. relatively high relative density), it will offer a locally increased food availability. This could induce a preference for the pollinators to forage on that species, inducing a disproportionate increase in the visitation rate on the species (Kunin & Iwasa 1996, Hanoteaux *et al.* 2013). This ultimately implies that the species with the highest relative density

107

would act as a strong competitor for the other species by manipulating the pollinator behaviour towards more specialisation (Kunin & Iwasa 1996, Muñoz & Cavieres 2008, Hanoteaux *et al.* 2013). However, the net effect of increasing the relative density for a species will again depend on the identity of the co-flowering individuals and on their relative attractiveness. Indeed, one might expect that an attractive species might need to increase less its relative density in order to be disproportionately more visited than a less attractive species (but see Muñoz & Cavieres 2008).

In summary, we argue that the role a species plays on the outcome of indirect plant-plant interactions mediated by shared pollinators is not only determined by the absolute floral density (Rathcke 1983), but by a complex interplay among the absolute density, the identities of all the plant species involved (including the neighbour identity) and the relative densities of the co-flowering species. In this study, we aimed specifically at testing for the importance of the identities and relative densities of plant species on the pollinator visitation patterns foraging in species. Concretely, the aim of this study was to investigate the role of a focal species density and the role of the floral density and identity of its neighbour on the behaviour of pollinators foraging on the focal species, this along a gradient of changing relative floral densities. To this end, we created artificial plant communities where we varied the relative densities of two morphologically different species. A multi-species approach enabled us to test whether the putative role of a given species will be the same if the identity of the neighbours change.

Material and methods

Study species and artificial communities

In order to investigate the effect of species identities on the pollinator visitation patterns on co-occurring species, we paired two species along a gradient of changing plant community relative densities. In order to insure that the chosen species would share pollinators we chose to work with *Centaurea jacea* L. (hereafter *Centaurea*) and *Scabiosa columbaria* L. (hereafter *Scabiosa*). These two "generalist" species have relative large floral displays, tubular open floral structure and are visited by a broad spectrum of pollinators (choice motivated by previous studies: Seyfang 2010, Hoch 2012, Hanoteaux 2014). These species were paired with 3 species having different level of attractiveness, according to their colour and floral shape: *Achillea millefolium* L. (hereafter *Achillea*), *Salvia farinacea* Benth. (hereafter *Salvia*) and *Matricaria recutita* L. (hereafter *Matricaria*). With these species, artificial communities composed of 5 plant individuals (we refer hereafter to a community as to a"plot") were created with the following compositions (Figure 1):
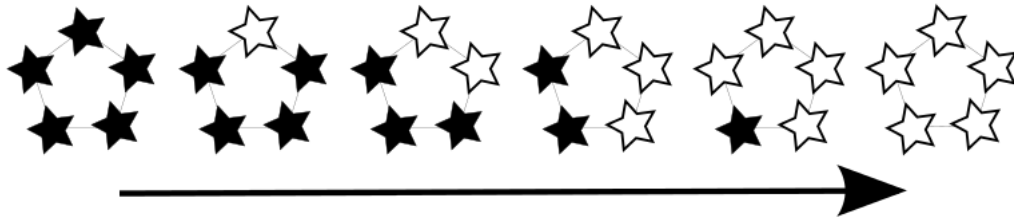
Figure 1: Schematic representation of the artificial density gradient created for one species pair. The stars represent plant individuals and the different colours (black and white) represent different species. The black arrow represents the increasing relative density of the white species. The radius of the pentagon was 15 cm long.

- monoculture of each species (5 species, hence 5 monocultures)

- for all possible combinations of one of the "generalist" species (2 species, i.e. *Centaurea* and *Scabiosa*) with each one of the other species (3 species, i.e. *Achillea*, *Matricaria* and *Salvia*), a gradient ranging from 1 "generalist" individual and 4 other individuals to 4 "generalist" individuals and 1 other individual was created (a total of 6 possible species pairs in 4 combinations).

With 5 replicates for plot type, this resulted in a total of of 145 plots (5 x (5 monocultures + 24 mixtures)).

It should be noted that whereas the number of plant individuals in each community was fixed (5 individuals), the overall absolute floral density in the communities was not, as we did not control for the number of flowers on plant individuals.

Experimental set up

Before setting up the experiment, all plants were removed from a common garden (Tübingen, 48.55°N 9.04 °E) and the experimental area was covered with a black plastic sheet to prevent any other plants to grow within plots. The plant individuals used in the experiment were raised in a common garden nearby and placed in the experimental garden on the 18th of May 2011 (prior to flowering). The different treatments were randomly assigned a position on a virtual grid with 2m spacing between the centre of the plots (the plots were distributed in 12 rows and 13 columns with the last column only partially filled). The 5 plant individuals within each plot were arranged in a pentagonal fashion around the centre of the plot at a distance of approximately 15 cm (see Figure 1), with a random orientation. During the experiment, the surroundings of the experimental area were frequently mown to remove all flowers and prevent any edge effects.

Pollinator observations and pollinator behavioural responses

From the 14th of June (date at which enough plots were having five flowering plant individuals) until the 26th of July 2011 (for a total of 18 days of observations), we conducted plot-based pollinator observations. As often as possible and under appropriate weather conditions, plots were observed during 5 min (between 10 A.M. and 5 P.M.) in a randomly chosen order. Not all the plots could be observed within the same observation day but we tried to have an approximately equal number of observations for each treatment.

During the observations, we recorded the identity of floral visitors and followed their movements within the observed plots. Pollinators were originally identified and classified into the following groups: honey bees, bumblebees, hoverflies, solitary bees, muscid flies and butterflies. Following all the movements of pollinators in the plots enabled us to calculate different relevant responses:

1. Arrival rate: measured as the number of new pollinator individuals arriving to a specific species in a plot. This value reflects the long distance attraction of the species within a plot;

2. Visitation rate: measured as the number of visits conducted by the pollinator individuals on a specific species within a plot. This value reflects the visit quantity *sensu* Rathcke (1983).

3. Bout length: measured as the number of consecutive visits conducted by each pollinator on a specific species within a plot. This value reflects the way pollinator individuals use floral resources and is a good indicator of the visit quality *sensu* Rathcke (1983). Only bouts larger than or equal to 2 were included in the analysis. For bouts including more than one switch, a correction was applied which is described in Appendix 1.

Each of the above mentioned response was calculated separately for each flower species per plot and for each pollinator groups separately (including a calculation for the pollinator community, i.e. all the pollinators pooled together). It should be noted that we did not incorporate the same responses for the whole plot, as it is out of the scope of this study.

Floral density

In order to be able to investigate the role of changing species densities on the pollinator activity in our plots, the number of inflorescences or flowers (hereafter flowers) was recorded for all the plant individuals in all the plots after every observation days. In order to take

species-specific differences in flower sizes into account, the number of flowers in each plot for each species was weighted by a species-specific average floral area (Hegland & Boeke 2006, see Appendix 2 Table A.1 for details). As it was not always possible to count all the flowers each day, when missing, the numbers of flowers in plots were estimated using a linear regression between the two closest recording events.

Statistical analysis

The aim of this study was to investigate the role of a focal species density and the role of the floral density and identity of its neighbour on the behaviour of pollinators foraging on the focal species, this along a gradient of changing relative floral densities. Hence, for each species in each possible plant species combination (i.e. for each gradient in which the focal species was included) and for each pollinator response measured (i.e. arrival rate, visitation rate and bout length), a GLM was fitted with the focal species own floral density and the floral density of its neighbours as explanatory variables and the considered pollinator behavioural response as dependent variable. In order to account for differences in flower size, the densities were introduced in the model as floral surfaces (see section Floral density, Hegland & Boeke 2006). This model structure in which the two floral densities (i.e. the floral density of the focal species and the density of the neighbouring species) were included separately, allowed us to separate between intra- and interspecific density effects on each aspect of the pollinator behaviour for each species in each species pair. Further, comparing the results of the GLM's for the same species but with different neighbours allowed us to test for the influence of the identity of the neighbouring species on both the intra- and interspecific density effects. All the GLMs were fitted with a quasi-Poisson distribution and a log link function as recommended for count data. Further, these models were fitted for the whole pollinator community (i.e. all pollinator individuals pooled together) and each major pollinator group (bumblebees (BB), solitary bees (SB), hoverflies (SY) and muscid flies (FLY)) for the six species pairs separately. Due to the rare occurrence of the remaining pollinator groups (honey bees and butterflies), even when pooled together, they were not considered in the analysis. All the analyses were conducted in R (2.13, R Development Core Team 2005).

Results

Pollinator community composition

During the 147 observation periods, a total of 1261 pollinators were recorded for a total of 4789 visits (the number of observations conducted for each particular treatment is given in Appendix 3, Table A.2). Overall, bumblebees were the main pollinators (52.58 % of all the recorded pollinator individuals), followed by solitary bees (25.12 %), hoverflies (12.84 %) and muscid flies (5.15%). The remaining 4.31 % of the pollinator community were honey bees (1.81%), butterflies (1.60%) and unidentified floral visitors (0.9 %).

Shared pollinators

The first condition enabling indirect plant-plant interactions is realised when pollinators are shared among plant species. To assess in which extent pollinators were shared between the species pairs, we compared the spectrum of attracted pollinators in monoculture of the different species (Figure 2). We found that the proportion of shared pollinators between plant species in the different species pairs was highly dependent on the specific plant species combinations (Figure 2). The rather similar pollinator community composition for *Centaurea* and *Salvia* observed in the monocultures led to a very high proportion of pollinators shared between these species when associated together (Figure 2). Due to the absence of bumblebees foraging on *Achillea* and *Matricaria* (both species visited by a very similar pollinator community), the proportion of shared pollinators between these species and *Centaurea* was much lower and reduced to mainly solitary bees and hoverflies (Figure 2). Due to the high proportion of honey bees in the pollinator spectra of *Scabiosa*, this species shared a smaller proportion of pollinators with its associated species than *Centaurea* did. This proportion was especially low when *Scabiosa* was associated with *Achillea* where only hoverflies were shared or when associated with *Matricaria* with which honey bees and hoverflies were shared. The full pollinator spectrum of *Salvia* was shared with *Scabiosa*.

Effects of floral densities on the pollinator behaviour

Effects of intraspecific density

Overall, the effects of a species' density on the number of arrivals and / or the number of visits on that plant species was that both responses always increased with increasing
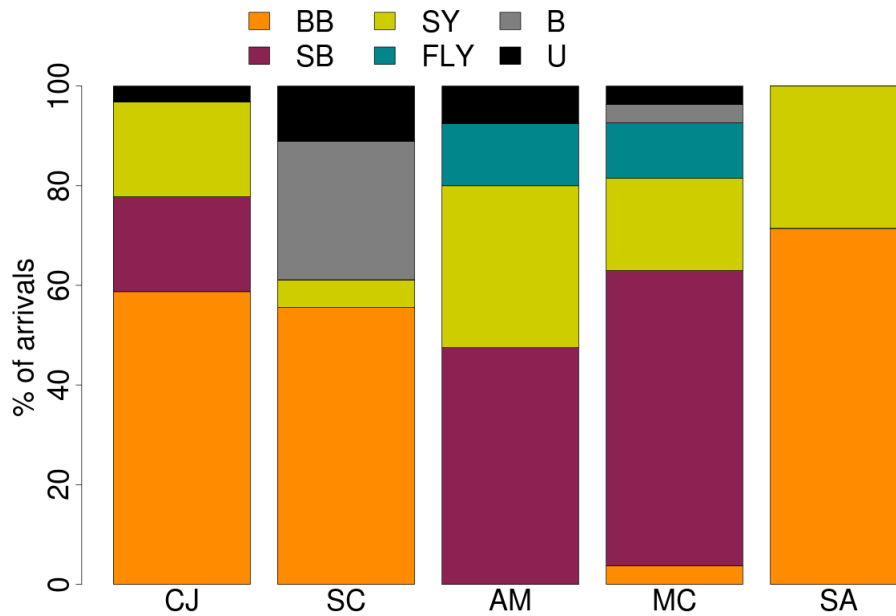
Figure 2: Pollinator community composition for the different species monitored in monocultures (CJ: *Centaurea*, SC: *Scabiosa*, AM: *Achillea*, MC: *Matricaria* and SA: *Salvia*). Calculations are based on the number of pollinator individuals of BB: bumblebees, SB: solitary bees, SY: hoverflies, FLY: muscid flies, B: honey bees, U: others

intraspecific density (see Table 1 A, column Dens = O for *Achillea*, *Matricaria* and *Salvia* and Table 1 B, column Dens = O for *Centaurea* and *Scabiosa*, details of the analysis are given in Appendix 4, Table A.3 and A.4), irrespective of the plant species identity.

These increases were always recorded for the whole pollinator community and mostly caused by the main pollinator groups of the species under consideration. Hence, more arrivals and visits of solitary bees were recorded on *Achillea* and *Matricaria* when their respective densities increased (Table 1 A) and the same was true for bumblebees on *Centaurea* and *Scabiosa* (Table 1 B).

Interestingly, the identity of the neighbouring species affected the occurrence of intraspecific density responses on the number of arrivals and / or the number of visits in some cases. For example, hoverflies did not respond to change in density of *Achillea* and *Matricaria* when the two species where associated with *Centaurea,* while a density response was found when associated with *Scabiosa* (Table 1 A). Similar differences were found for bumblebees on *Salvia* (absence of density responses when associated with *Centaurea* whereas they were present when *Scabiosa* was the second species, Table 1 A) and for flies on *Achillea* (the presence of *Scabiosa* as neighbouring species seemed to induce density responses, Table 1 A).

The bout length was only rarely affected by intraspecific density. Only in the species pair *Matricaria-Scabiosa*, solitary bees made shorter bouts on *Scabiosa* when its density

increased (see Table 1 B).

Effect of interspecific species density

The density of the neighbouring species affected the arrival and visitation rate on a focal species in two different and opposite ways:

1. Negative interspecific density effects: the number of arrivals/visits to species decreased with increasing density of the neighbouring species. In these cases, we compared the value of the responses for the species and pollinator groups under consideration along the density of the neighbouring species, to the value of the monoculture of that species (e.g. we compared the number of solitary bees foraging on *Achillea* along increasing density of *Centaurea* to the number of solitary bees foraging in monoculture of *Achillea*). This allowed us to distinguish between two different cases: in some cases, the negative effect of the density of the neighbouring species was present all along the gradient of relative density of two species, i.e. even at very low density of the neighbouring species, the arrival and/or visitation rate to a focal species was lowered in comparison with the monoculture of the focal species (see Figure 3 A, B & C; these cases are indicated with red cells in Table 1 A & B, see also Appendix 4 Table A.3 & A.4 for details). This was the case in the species pair *Achillea* - *Centaurea* for the whole pollinator community foraging on *Achillea* (Table 1 A); for solitary bees foraging on *Achillea* (Figure 3 A, Table 1 A) and on *Centaurea* (Figure 3 B, Table 1 A); and for solitary bees foraging on *Centaurea* in the species pair *Matricaria – Centaurea* (Figure 3 C, Table 1 B).

   In other cases, a shift in the nature of the effect of the density of the neighbouring species occurred: at low density the values of the responses were higher than the values in monocultures and with increasing density these values dropped below that of the monoculture (see Figure 3 D & E, these cases are indicated with green cells in Table 1 A & B, see also Appendix 4 Table A.3 & A.4 for details) This was the case for the number of visits by solitary bees to *Matricaria* in the species pair *Matricaria* - *Centaurea* (Figure 3 D, Table 1 A; the same was observed at the whole pollinator community level, Table 1 A) and for the number of bumblebees arriving on *Salvia* when associated with *Centaurea* (Figure 3 E, Table 1 A).

2. Positive interspecific density effects: the number of bumblebees arriving on *Scabiosa* increased with increasing density of *Salvia* (reflected by the same effect at the level of the whole pollinator community, indicated by yellow cells in Table 1 B, see also Figure 3 F and Appendix 4 Table A.4).

Table 1: Schematic representation of the results of the GLM analysis for the different responses (Arrivals: number of pollinators arriving on the respective species, Visits: Number of visits on the respective species, Consec.: number of consecutive visits) **(A) on AM: *Achillea*, MC: *Matricaria*, SA: *Salvia*** and on **(B) CJ: *Centaurea* and SC: *Scabiosa*** for the different pollinator groups (All: the whole pollinator community, BB: bumblebees, SB: solitary bees, SY: hoverflies, FLY: muscid flies) when associated with different neighbouring species (the identity of the neighbouring species is given in the column headings). Significant slopes (O: for the own density, H: for the density of the other species) are represented by their respective signs (i.e. a "+" represent a significant positive slope in the fitted GLM whereas a "-" represent a significant negative slope, empty cells indicate a non-significant effect of the respective densities). Dark shaded cells are missing values (no occurrence). The meaning of the coloured cells is given in the text.

(A)

Response	Polli. Dens.	CJ ALL O	H	BB O	H	SB O	H	SY O	H	FLY O	H	SC ALL O	H	BB O	H	SB O	H	SY O	H	FLY O	H
Arrivals	AM	+		+	+	+	+					+		+	+	+	+	+	+	+	+
Visits		+	+	+	+	−	−					+				+	+	+		+	
Consec.		−	−	▓	▓													▓			
Arrivals	MC	+				+	+			+		+		+		+	+	+		+	
Visits		+	+	+	+	+	−					+	+	+	+			+			
Consec.		−	−			−				▓	▓										
Arrivals	SA	+	+	−				+				+	+	+	+	+	+	+	+	+	+
Visits																					
Consec.																▓					

(B)

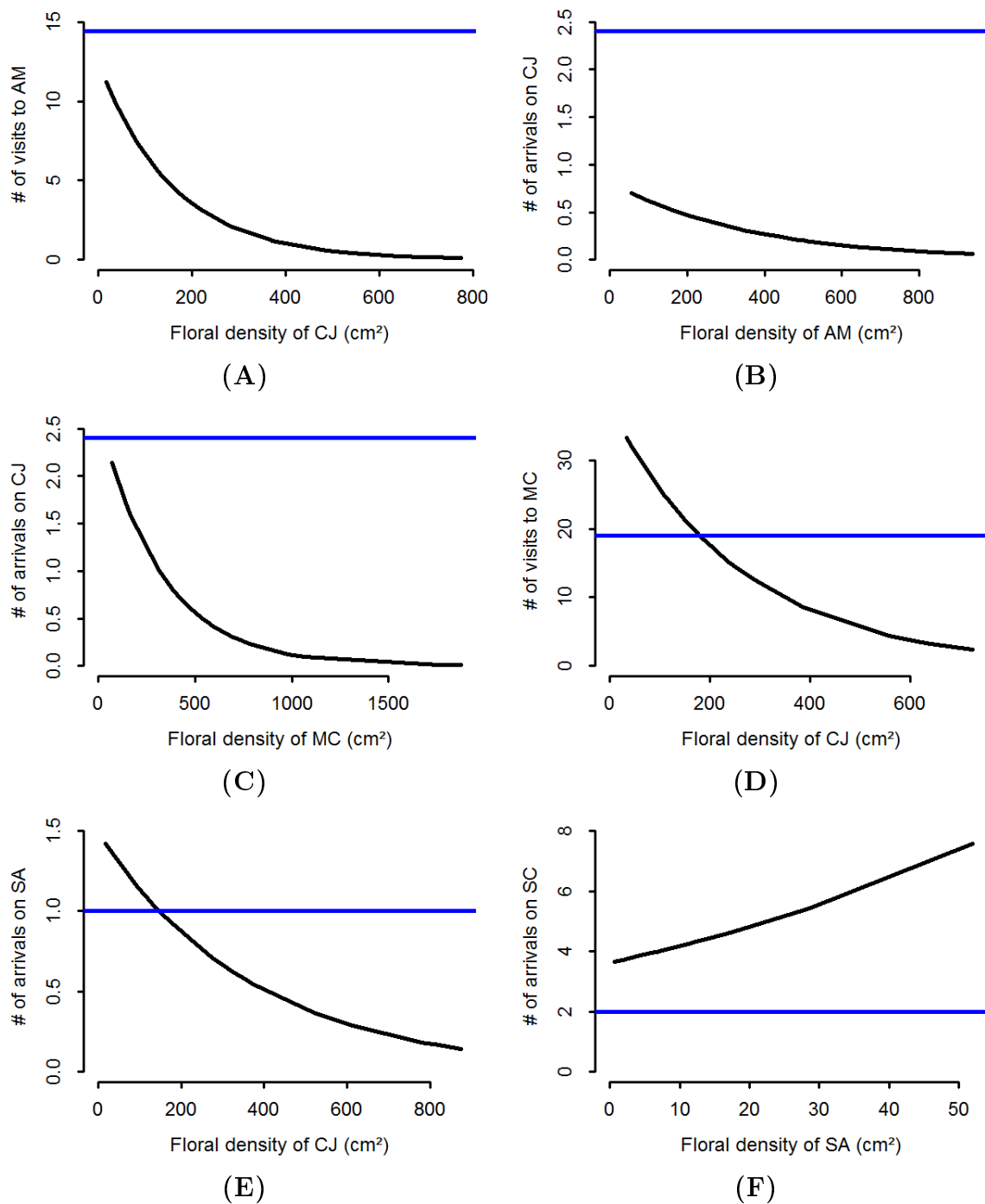| Response	Polli. Dens	MC ALL O	H	BB O	H	SB O	H	SY O	H	FLY O	H	SA ALL O	H	BB O	H	SB O	H	SY O	H	FLY O	H
Arrivals	CJ	+	+	+	+	−	−			▓	▓	+	+	+	+	+	+	+	+		
Visits		+	+	+	+	−	−	+	+	▓	▓	+	+	+	+	+🟨	+🟨	+	+		
Consec.																					
Arrivals	SC	+	+	+			+	+		▓	▓	+	+	+	+	+	+	+	+		
Visits						−		+		▓											
Consec.																					

115

Figure 3: Effects of the density of the neighbouring species on different pollinator responses (black continuous line) for (A) the number of visits of solitary bees on *Achillea* (AM) along the density of *Centaurea* (CJ); (B) the number solitary bees arrivals on *Centaurea* (CJ) along the density of *Achillea* (AM); (C) the number solitary bees arrivals on *Centaurea* (CJ) along the density of *Matricaria* (MC); (D) the number of visits by solitary bees on *Matricaria* (MC) along the density of *Centaurea* (CJ); (E) number of bumblebees arrivals on *Salvia* (SA) along the density of *Centaurea* (CJ) and (F) the number of bumblebees arrivals on *Scabiosa* (SC) along the density of Salvia (SA). Blue continuous lines represent the average of the respective responses in the monoculture of the species for which the response is presented (i.e. *Achillea* for A; *Centaurea* for B and C; *Matricaria* for D; *Salvia* for E and *Scabiosa* for F). Note the difference in the values of axes.

116

Interestingly, all negative density dependences found for the number of arrivals and the number of visits was observed for species pairs containing *Centaurea* but not in communities with *Scabiosa* (see Table 1 A & B).

Effects of the density of the neighbouring species on the number of consecutive visits on a focal species were only found in three cases (see Table 1 A & B and Appendix 3 Table A.3 & A.4). Solitary bees shortened their bout lengths on *Matricaria* and on *Scabiosa* when the density of *Centaurea* and *Matricaria*, respectively, increased (see Table 1 A & A.3 for the species pair *Matricaria - Centaurea* and Table 1 B & A.4 for *Matricaria - Scabiosa*). Bumblebees made longer bout lengths on *Centaurea* when the density of Salvia increased (see Table 1 B and A.4).

Discussion

The results of our study clearly demonstrate that both intra- and interspecific densities of floral resources are important factors in determining arrival and visitation rates of pollinators on these floral resources (Kunin 1997, Hegland & Boeke 2006, Jakobsson *et al.* 2009, Dauber *et al.* 2010, Seifan *et al.* 2014).

In most of the cases, an increase in intraspecific floral density resulted in more pollinators attracted and more visits conducted on species (Kunin 1993, Hegland & Boeke 2006, Feldman 2008, Seifan *et al.* 2014). More interesting though, is the fact that the intensity of these intraspecific density dependence on the arrival and visitation rates depended on the identity of the neighbouring species.

For example, hoverflies foraging on *Achillea* and *Matricaria* increased their arrivals and visitation rate when the density of these species increased, but this was only detected when *Scabiosa* was the neighbouring species and not with *Centaurea* present, and a similar pattern was found for bumblebees foraging on *Salvia*. At the same time, an increase in the density of *Centaurea* resulted in increased visitation and arrival rates on *Centaurea* for hoverflies, whereas this pollinator group did not respond to changes in the density of *Scabiosa*. These examples clearly indicates that *Centaurea* is more attractive than *Scabiosa*. It also suggest that *Centaurea* can act as a strong competitor for the services of pollinators (Hegland *et al.* 2009) and that the occurrence of positive intraspecific density dependence in quantitative aspects of the pollinator behaviour on a species can depend on the relative attractiveness (and hence identity) of neighbouring species.

Further, muscid flies also seemed to exhibit enhanced arrival and visitation rates with increasing density of *Achillea* when *Scabiosa* was the neighbouring species but not in

communities including *Centaurea*. The explanation here can not be linked with an attractiveness differential as both *Scabiosa* and *Centaurea* were only sporadically visited by flies. A possible explanation might lies in the difference of habitus between the two neighbouring species. The fact that *Centaurea* is taller and produces a generous amount of leaves and stems in comparison with *Scabiosa*, might have made *Achillea* easier to detect when growing in the vicinity of *Scabiosa*. This confirms again that vegetative characteristics of neighbouring species can influence quantitative aspects of pollinator behaviour (and hence the reproductive success of plant species) by altering the detectability of floral resources (Toräng *et al.* 2006). It should be noted that the same explanation can not be advanced for the hoverflies as the recorded density responses to changes in the attractive (i.e. *Centaurea*) species density strongly hints towards an active process of decision making by the hoverflies (Goulson 1999, Chittka & Thomson 2001).

The floral density of heterospecific neighbours was found to influence behavioural responses of pollinators in two opposite ways strongly hinting towards competitive or facilitative mechanisms. Here again, the occurrence of such effects was conditioned by the identity of the neighbouring species.

Firstly, competitive mechanisms between two plant species for the service of pollinators are typically characterised by a decrease in arrival or visitation rate with increasing density of a co-occurring species (Rathcke 1983, Brown *et al.* 2002, Moragues & Traveset 2005). In each species pair where *Centaurea* was present, a decrease in visitation rate relative to its neighbours was observed when its density increased. This confirms the attractive character of that particular species and adds another example on the growing body of evidence that attractive species can deter pollination service to their neighbours (Brown *et al.* 2002, Muñoz & Cavieres 2008, Seifan *et al.* 2014) and hence have negative repercussions on the reproduction success of co flowering neighbours (Grabas & Laverty 1999, Chittka & Schürkens 2001). However, depending on the identity of its neighbour, competition was either found all along the gradient of relative density (i.e. only the presence of one individual was enough to lower visitation rate to the neighbouring species, e.g. with *Achillea* as a neighbour), or only at higher densities (Rathcke 1983, Muñoz & Cavieres 2008, Seifan *et al.* 2014). In the latter case, at low density, *Centaurea* exerted a positive effect on the visitation rates of some of its neighbours (i.e. *Matricaria* and *Salvia*, Rathcke 1983, Seifan *et al.* 2014). With increasing density, this effect soon became negative. This implies that the very same species at low density can have contrasting effects on their neighbours depending on their identity (Bartomeus *et al.* 2008) and we argue that this can be explained by the difference in attractiveness between plant species. If the attractiveness differential between two species is large, one individual of the more attractive species will be enough to lower visitation rate to its neighbours, as the additionally attracted

pollinators will forage on the attractive species and some of the pollinators foraging on the less attractive species will switch to the attractive species. However, decreasing the attractiveness differential between the species, might allow the less attractive species to keep its pollinators and to receive some of the newly attracted pollinators. The importance of the attractiveness differential will further determine at which density of the attractive species, competition will prevail.

Interestingly, in two cases, we found that solitary bees, were inducing reciprocal negative effects between the two species, i.e. increasing density of each species caused a decrease in visitation rates to the other species. This may seem contradictory at first glance, but it can be explained by a segregation in the behaviour within the rather diverse group of solitary bees related to their body size (Westrich 1989). Very small solitary bees may specialise on species with smaller inflorescences (such as *Achillea* and *Matricaria*) whereas larger solitary bees are not able to access these resources, and hence specialise on accessible resources (here *Centaurea*).

Secondly, positive interspecific density dependence (i.e. facilitation), was also found between two species, here again enabled by the main shared pollinator group and conditioned by the identity of the neighbouring species. This facilitative indirect interaction was revealed in our analysis by an increased in the number of pollinators attracted to *Scabiosa* when associated with *Salvia*, which hence acted as a "magnet species" (Laverty 1992, Molina-Montenegro *et al.* 2008, Yang *et al.* 2013). In this particular case, the presence of the neighbour was enough to enhance arrival rate all along the gradient of relative density. Interestingly, this facilitative mechanism was found between two species having very different floral morphologies. This difference might greatly enhance the putative positive effect on the reproductive success of *Scabiosa*. Indeed, due to the cost of switching between two species, or the limited ability of pollinators to retain and/or retrieve handling skills (Darwin's interference hypothesis, Goulson 1999), constancy will be favoured, greatly enhancing the visit quality on *Scabiosa* in addition to the increased visit quantity. This is yet another example that facilitative interactions among dissimilar floral resource occur more often than previously thought (Hegland *et al.* 2009).

Quite logically, the occurrence of all the indirect interactions found in this study were enabled by the main shared pollinator groups among species, i.e. solitary bees or bumblebees. This shows the importance of these two groups for the pollination of grassland species and should serve as an additional incentive to protect them. Additionally, although present in the area, the honey bee (*Apis mellifera*) did not play an important role as pollinator. This adds to the body of evidence that in many ecosystems, the importance of the honey bee as "essential pollinator" is probably overrated (Ollerton *et al.* 2012).

The number of consecutive visits made by pollinators was only rarely affected by the changes in community composition. This is highly surprising as optimal foraging theory predicts that bout length on a species should increase with increasing density of that species (Kunin & Iwasa 1996). One potential explanation for this is that the distance between our plots was not perceived as long enough by pollinators. This could induce a behaviour based on the overall density of the preferred species, resulting in a number of visits per plant guided by this overall density and not the local (i.e. in a plot) density of that species.

To conclude, we have shown that the impact of intra- and interspecific density changes on quantitative aspects of the pollinator behaviour foraging on a focal species are also affected by the identity of neighbouring species. This implies that the outcome of indirect plant-plant interactions is shaped by the identities of all the species involved in these interactions. We have shown that the role a species plays in the outcome of indirect plant-plant interactions at a certain density could change drastically according to the identity of its neighbours. This points to an overriding importance of the attractiveness differential, rather than the absolute attractiveness among plant species. We believe that a stronger consideration of this differential in future studies could help in resolving some discrepancies observed in studies of indirect plant-plant interactions mediated through shared pollinators (Bartomeus *et al.* 2008, Hegland *et al.* 2009).

Acknowledgements

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Appendices

Appendix 1: Calculation of the number of consecutive visits with pollinators switching multiple times

In the calculation of the number of consecutive visits by individual pollinators, two distinct cases were considered. If the recorded visits of a pollinator individual were all to the same plant species or if the pollinator switched only once between species, the number of consecutive visits to a certain species was directly observed and no calculations were required. However, if a pollinator individual switched more than once (e.g. a pollinator visiting A switched to B to switch back again to A), we set the number of consecutive visits to a certain species (e.g. species A) equal to the total number of visits to that species divided by the number of foraging sequences on the species (the example above includes 2 foraging sequence on A). As the whole pollinator bouts were recorded, the number of sequences on a particular species could easily be calculated. However, as the analysis of count data with (quasi) Poisson distribution requires integers, we rounded the calculated mean number of consecutive visit to the first larger integer. We argue that, as the observation period was arbitrary defined and limited in time, we possibly missed some visits prior and after the observation period in each plot. Hence, the small correction applied on the resulting means corrects for these missing observations.

Appendix 2: Attraction units and floral surface calculations

In order to take the differences in floral morphology and size among the different species used in this study, an "attraction unit" was defined for each species (Hegland & Boeke 2006). The definition of this species specific attraction unit specified what has to be counted and measured in the field in order to calculate floral surfaces (as a proxy for floral density). Depending on the species specific floral morphology, we considered different shapes of attraction units for the different species and adapted the measurements and formulas used to calculate their surfaces. Once the attraction unit for each species was defined (see Table A.1), we measured characteristic dimensions (see Table A.1) of 20 randomly chosen attraction units in randomly chosen plots and computed a mean floral surface for each species.

125

Table A.1: Attraction units, geometrical form (Shape), measurements and formulas used in the calculation of the floral surface (surface in cm²) of the attraction units for each species.

Species	Attraction unit	Shape	Measurement	Formula	Surface (cm²)
Centaurea jacea	inflorescence	circle	radius	π . radius²	16.83
Scabiosa columbaria	inflorescence	circle	radius	π . radius²	8.92
Achillea millefolium	capitulum	ellipse	smallest & largest radii	$\pi . \frac{radius_1 . radius_2}{2}$	12.01
Matricaria recutita	inflorescence	circle	radius	π . radius²	5.39
Salvia farinacea	flower	circle	radius of the lip	π . radius²	0.65

Appendix 3: Number of observation periods of 5 min conducted for each treatment

Table A.2: Number of observations conducted for each treatment. Mono: Monoculture of species, numbers under "Community composition" indicate the plant community composition (first number gives the number of individuals from CJ or SC), CJ: *Centaurea*, SC: *Scabiosa*, AM: *Achillea*, MC: *Matricaria*, SA: *Salvia*

		Community composition				Monocultures	
		1-4	2-3	3-2	4-1	CJ	5
AM	CJ	7	5	3	4	SC	5
	SC	6	7	4	4	AM	5
MC	CJ	6	5	5	4	MC	5
	SC	5	4	5	6	SC	5
SA	CJ	6	5	5	5		
	SC	6	4	5	6		

Appendix 4: Details of the analysis of density effects on pollinator visitation patterns

Table A.3: Details of the analysis of the effect of the plant species densities on pollinator visitation patterns for the species **Achillea**, **Matricaria** and **Salvia**. For each type of response (Arrivals: Number of pollinator individuals arriving on the species, Visits: total number of visits, Consec. : number of consecutive visits), each species pairs (rows, AM: *Achillea*, MC: *Matricaria*, SA: *Salvia*, CJ: *Centaurea*, SC: *Scabiosa*) and each pollinator group (columns: all the pollinator community, BB: Bumblebees, SB: Solitary bees, SY: Hoverflies), the intercept (Inter.), the significant coefficients (slope) and their p-values (p; p-values <0.05 are in bold, p < 0.01 are underlined, p-values < 0.001 are indicated as such in the table) associated with both plant species densities (densother: density of the neighbouring species, densown: density of the focal species) are indicated. #obs refers to the number of 5 minutes observations round incorporated in each model. The table contains indications for the cases where some pollinator groups were not recorded, or if their occurrence was too low.

| | | | | ALL | | | | | |
| | | | | densother | | | owndens | | #obs |
Response			inter.	slope	p	slope	p		
Arrivals	AM	CJ	0.786	-0.001		0.002	*0.003*		24
Visits	AM	CJ	1.962	-0.002	**0.012**	0.002	P<0.001		24
Consec.	AM	CJ	1.881	-0.001	**0.042**	0.000			72
Arrivals	AM	SC	0.679	-0.001		0.003	P<0.001		26
Visits	AM	SC	1.407	0.003		0.004	P<0.001		26
Consec.	AM	SC	1.662	0.000		0.000			76
Arrivals	MC	CJ	1.503	0.000		0.001	*0.003*		25
Visits	MC	CJ	2.994	-0.002	**0.044**	0.001	P<0.001		25
Consec.	MC	CJ	1.877	-0.002	*0.004*	0.000			107
Arrivals	MC	SC	0.478	-0.001		0.002	P<0.001		25
Visits	MC	SC	1.623	0.000		0.002	P<0.001		25
Consec.	MC	SC	1.671	0.000		0.000			50
Arrivals	SA	CJ	-0.167	-0.001		0.039	**0.026**		26
Visits	SA	CJ	1.491	-0.001		0.049	**0.032**		26
Consec.	SA	CJ	1.665	0.001		0.004			26
Arrivals	SA	SC	-0.303	-0.003		0.037	*0.002*		26
Visits	SA	SC	1.682	-0.002		0.038	*0.009*		26
Consec.	SA	SC	2.013	-0.002		0.003			31

| | | | | BB | | | | | |
| | | | | densother | | | owndens | | #obs |
Response			inter.	slope	p	slope	p		
Arrivals	AM	CJ	-4.513	-0.002		0.003			24
Visits	AM	CJ	-4.513	-0.002		0.003			24
Consec.	AM	CJ		No Occurence					0
Arrivals	AM	SC	-2.386	-0.007		-0.002			26
Visits	AM	SC	-4.104	-0.001		0.004			26
Consec.	AM	SC		No Occurence					0
Arrivals	MC	CJ	-1.518	-0.002		0.001			25
Visits	MC	CJ	-1.548	-0.002		0.001			25
Consec.	MC	CJ		Not Enough Occurence					1
Arrivals	MC	SC		No convergence					25
Visits	MC	SC		No convergence					25
Consec.	MC	SC		No Occurence					0
Arrivals	SA	CJ	-0.328	-0.002	**0.029**	0.030			26

Response			inter.	slope	p	slope	p	#obs
Visits	SA	CJ	1.410	-0.001		0.043		26
Consec.	SA	CJ	1.720	0.001		0.010		19
Arrivals	SA	SC	-0.573	-0.004		0.043	P<0.001	26
Visits	SA	SC	1.631	-0.004		0.040	**0.011**	26
Consec.	SA	SC	2.140	-0.003		0.002		27

SB

			densother			owndens		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	-0.751	-0.005	*0.007*	0.004	*0.003*	24
Visits	AM	CJ	0.752	-0.006	*0.005*	0.003	*0.006*	24
Consec.	AM	CJ	1.907	-0.002		0.000		29
Arrivals	AM	SC	-0.367	-0.001		0.003	P<0.001	26
Visits	AM	SC	0.805	0.000		0.004	P<0.001	26
Consec.	AM	SC	1.721	0.000		0.000		40
Arrivals	MC	CJ	0.858	-0.001		0.001	**0.018**	25
Visits	MC	CJ	2.557	-0.003	**0.025**	0.001	P<0.001	25
Consec.	MC	CJ	2.066	-0.002	**0.034**	0.000		61
Arrivals	MC	SC	-0.107	-0.002		0.002	*0.006*	25
Visits	MC	SC	0.791	0.002		0.003	P<0.001	25
Consec.	MC	SC	1.619	0.001		0.000		31
Arrivals	SA	CJ	-2.439	-0.001		0.032		26
Visits	SA	CJ	-0.924	-0.002		0.057		26
Consec.	SA	CJ			Not Enough Occurence			3
Arrivals	SA	SC	-3.421	0.009		0.029		26
Visits	SA	SC	-1.962	0.010		0.031		26
Consec.	SA	SC	0.221	0.005		0.043		4

SY

			densother			owndens		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	0.541	-0.001		0.001		24
Visits	AM	CJ	1.682	-0.002		0.001		24
Consec.	AM	CJ	1.843	-0.001		0.000		30
Arrivals	AM	SC	-0.468	-0.001		0.002	*0.002*	26
Visits	AM	SC	0.064	0.006		0.003	*0.007*	26
Consec.	AM	SC	2.161	-0.002		-0.001		17
Arrivals	MC	CJ	0.066	0.001		0.001		25
Visits	MC	CJ	1.106	0.001		0.000		25
Consec.	MC	CJ	1.150	0.000		0.000		28
Arrivals	MC	SC	-0.829	0.001		0.001	**0.021**	25
Visits	MC	SC	0.475	0.000		0.001		25
Consec.	MC	SC	2.143	0.001		-0.002		9
Arrivals	SA	CJ	-2.249	-0.001		0.064		26
Visits	SA	CJ	-2.179	-0.001		0.097	*0.003*	26
Consec.	SA	CJ	0.521	-0.001		0.024		4
Arrivals	SA	SC	0.468	-1.926		-0.073		26
Visits	SA	SC	0.468	-1.926		-0.073		26
Consec.	SA	SC			No Occurence			0

FLY

			densother			owndens		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	-1.427	0.001		0.002		24
Visits	AM	CJ	-0.588	0.001		0.003		24
Consec	AM	CJ	1.859	-0.001		-0.001		13

Response			inter.	slope	p	slope	p	#obs
Arrivals	AM	SC	-1.048	0.000		0.003	**0.022**	26
Visits	AM	SC	-0.490	0.003		0.004	**0.019**	26
Consec	AM	SC	0.698	0.005		0.001		18
Arrivals	MC	CJ	-0.568	0.000		0.001	**0.012**	25
Visits	MC	CJ	1.410	-0.002		0.000		25
Consec	MC	CJ	2.000	-0.002		0.000		15
Arrivals	MC	SC	-2.171	0.000		0.003	**0.017**	25
Visits	MC	SC	1.053	-0.016		0.001		25
Consec	MC	SC	2.477	-0.008		-0.001		6
Arrivals	SA	CJ			No Occurence			26
Visits	SA	CJ			No Occurence			0
Consec	SA	CJ			No Occurence			0
Arrivals	SA	SC			No Occurence			26
Visits	SA	SC			No Occurence			0
Consec	SA	SC			No Occurence			0

Table A.4: Details of the analysis of the effect of the plant species densities on pollinator visitation patterns for the species ***Centaurea***, and ***Scabiosa***. For each type of response (Arrivals: Number of pollinator individuals arriving on the species, Visits: total number of visits, Consec. : number of consecutive visits), each species pairs (rows, AM: *Achillea*, MC: *Matricaria*, SA: *Salvia*, CJ: *Centaurea*, SC: *Scabiosa*) and each pollinator group (columns: all the pollinator community, BB: Bumblebees, SB: Solitary bees, SY: Hoverflies), the intercept (Inter.), the significant coefficients (slope) and their p-values (p; p-values <0.05 are in bold, p < 0.01 are underlined, p-values < 0.001 are indicated as such in the table) associated with both plant species densities (densother: density of the neighbouring species, densown: density of the focal species) are indicated. #obs refers to the number of 5 minutes observations round incorporated in each model. The table contains indications for the cases where some pollinator groups were not recorded, or if their occurrence was too low.

				ALL				
				owndens		densother		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	1.409	0.0012	*0.001*	-0.0002		24
Visits	AM	CJ	2.607	0.0015	p<0.001	-0.0003		24
Consec.	AM	CJ	1.280	0.0002		0.0000		110
Arrivals	AM	SC	1.168	0.0029		-0.0001		26
Visits	AM	SC	2.011	0.0056	*0.004*	-0.0001		26
Consec.	AM	SC	1.208	0.0014		-0.0001		75
Arrivals	MC	CJ	1.612	0.0010	*0.010*	-0.0003		25
Visits	MC	CJ	2.691	0.0014	*0.002*	-0.0004		25
Consec.	MC	CJ	1.459	0.0000		-0.0001		104
Arrivals	MC	SC	0.617	0.0065	**0.010**	0.0000		25
Visits	MC	SC	1.850	0.0082	*0.005*	-0.0002		25
Consec.	MC	SC	1.430	0.0019		-0.0004		77
Arrivals	SA	CJ	1.580	0.0011	p<0.001	-0.0101		26
Visits	SA	CJ	2.654	0.0015	p<0.001	-0.0072		26
Consec.	SA	CJ	1.272	0.0001		0.0148	**0.035**	125
Arrivals	SA	SC	0.893	0.0059	p<0.001	0.0126	**0.043**	26
Visits	SA	SC	1.910	0.0082	p<0.001	0.0081		26
Consec.	SA	SC	1.618	-0.0002		-0.0017		106

	BB	
owndens	densother	#obs

Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	1.050	0.0011	*0.002*	0.0003		24
Visits	AM	CJ	2.283	0.0016	p<0.001	0.0001		24
Consec.	AM	CJ	1.265	0.0004		-0.0001		88
Arrivals	AM	SC	0.884	0.0014		0.0002		26
Visits	AM	SC	1.650	0.0050	**0.016**	0.0004		26
Consec.	AM	SC	1.142	0.0018		0.0000		57
Arrivals	MC	CJ	1.203	0.0009	**0.024**	0.0000		25
Visits	MC	CJ	2.343	0.0015	*0.002*	-0.0002		25
Consec.	MC	CJ	1.465	0.0001		-0.0002		80
Arrivals	MC	SC	0.049	0.0068	**0.013**	0.0004		25
Visits	MC	SC	1.234	0.0092	*0.003*	0.0000		25
Consec.	MC	SC	1.418	0.0027		-0.0007		51
Arrivals	SA	CJ	1.247	0.0009	*0.004*	-0.0127		26
Visits	SA	CJ	2.418	0.0015	p<0.001	-0.0070		26
Consec.	SA	CJ	1.212	0.0002		0.0230	*0.004*	90
Arrivals	SA	SC	0.253	0.0073	p<0.001	0.0181	**0.021**	26
Visits	SA	SC	1.300	0.0097	p<0.001	0.0170		26
Consec.	SA	SC	1.677	-0.0004		0.0008		77

			SB					
				owndens		densother		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	0.723	-0.0001		-0.0042	**0.023**	24
Visits	AM	CJ	1.877	-0.0005		-0.0036	**0.024**	24
Consec.	AM	CJ	1.537	-0.0006		-0.0001		13
Arrivals	AM	SC	-2.507	0.0125	**0.014**	-0.0002		26
Visits	AM	SC	-2.068	0.0153	P<0.001	0.0009		26
Consec.	AM	SC	-0.767	-0.0030		0.0176		6
Arrivals	MC	CJ	1.029	-0.0002		-0.0031	*0.004*	25
Visits	MC	CJ	1.952	-0.0004		-0.0029		25
Consec.	MC	CJ	1.706	-0.0008		-0.0003		13
Arrivals	MC	SC	-2.600	0.0099		0.0008		25
Visits	MC	SC	-1.505	0.0096		0.0013		25
Consec.	MC	SC	0.694	-0.0041	**0.027**	0.0041	**0.019**	6
Arrivals	SA	CJ	0.392	0.0001		-0.0429		26
Visits	SA	CJ	1.157	0.0001		-0.0332		26
Consec.	SA	CJ	1.509	-0.0006		-0.0051		16
Arrivals	SA	SC	-1.369	0.0060		-0.0341		26
Visits	SA	SC	-1.039	0.0096		-0.0374		26
Consec.	SA	SC	2.518	0.0013		-0.1895		4

			SY					
				owndens		densother		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	-2.432	0.0033	p<0.001	-0.0009		24
Visits	AM	CJ	-1.009	0.0028	p<0.001	-0.0018		24
Consec.	AM	CJ	0.976	0.0003		-0.0007		8
Arrivals	AM	SC	-2.514	0.0134	**0.018**	-0.0045		26
Visits	AM	SC	-1.158	0.0085		-0.0004		26
Consec.	AM	SC	2.457	-0.0079		-0.0080		4
Arrivals	MC	CJ	-1.169	0.0021	*0.004*	-0.0014		25
Visits	MC	CJ	-0.277	0.0022	*0.002*	-0.0018		25
Consec.	MC	CJ	0.707	0.0006		-0.0002		10
Arrivals	MC	SC	-1.124	0.0052		-0.0056		25
Visits	MC	SC	-0.423	0.0098		-0.0071		25

Response			inter.	slope	p	slope	p	#obs
Consec.	MC	SC	Not enough Occurence					3
Arrivals	SA	CJ	-0.831	0.0019	p<0.001	0.0067		26
Visits	SA	CJ	-0.188	0.0021	p<0.001	-0.0059		26
Consec.	SA	CJ	0.930	0.0003		-0.0151		16
Arrivals	SA	SC	-0.259	-0.0009		-0.0125		26
Visits	SA	SC	0.706	0.0015		-0.0257		26
Consec.	SA	SC	0.608	0.0094		-0.0248		8

			FLY					
			owndens			densother		#obs
Response			inter.	slope	p	slope	p	
Arrivals	AM	CJ	No Occurence					0
Visits	AM	CJ	No Occurence					0
Consec.	AM	CJ	No Occurence					0
Arrivals	AM	SC	-4.098	0.0082		-0.0001		26
Visits	AM	SC	-4.098	0.0082		-0.0001		26
Consec.	AM	SC	No Occurence					0
Arrivals	MC	CJ	No Occurence					0
Visits	MC	CJ	No Occurence					0
Consec.	MC	CJ	No Occurence					0
Arrivals	MC	SC	No Occurence					0
Visits	MC	SC	No Occurence					0
Consec.	MC	SC	No Occurence					0
Arrivals	SA	CJ	No Occurence					0
Visits	SA	CJ	No Occurence					0
Consec.	SA	CJ	No Occurence					0
Arrivals	SA	SC	-1.427	-0.0095		-0.1233		26
Visits	SA	SC	-0.734	-0.0095		-0.1233		26
Consec.	SA	SC	Not enough Occurence					1

Discussion

The major objective of this thesis was to investigate the role played by structural characteristics of plant communities such as the identities, densities and spatial distribution of the component species, on the outcome of indirect plant-plant interactions mediated through shared pollinators.

Using a spatially explicit modelling approach in the first chapter of this thesis, we were able to demonstrate that the spatial patterning of species can strongly interfere with the relative density of plant species and determine the survival chance of a less attractive species. Moreover, we found that a less attractive species at low density at higher chance of surviving when spatially regularly distributed whereas at high density, its survival chances where substantially higher when spatially aggregated. These results suggested that species are able to "trap" pollinators within their own occurrence range (Goulson 1994). By growing spatially aggregated, a species can enhance both the quantity and the quality of the visits it receives (Rathcke 1983). The crucial aspect shaping the outcome of indirect plant-plant interaction can hence be phrased in terms of "spatial availability" of flower resources for pollinators. Even if suggested by Goulson (1994), this spatial mechanism favouring constancy has never been thoroughly investigated before. Further, additionally to the inclusion of spatial patterns into pollination ecology, a complex pollinator behaviour was used in our original approach. As such, pollinators were able to distinguish among different floral resources (Chittka & Thomson 2001) and were also able to change their preferences according to recent foraging experiences (Dukas & Real 1993, Keasar *et al.* 1996), reflecting a behaviour closer to reality. This complexity in the pollinator behaviour has mostly been ignored by previous models (Bobisud & Neuhaus 1975, Waser 1978, Goulson 1994, Feldman *et al.* 2004). From this, it resulted that the effect of spatial

patterning is, logically, only emerging when a certain degree of generalisation exists in the pollinator behaviour (Waser *et al.* 1996, Waser & Ollerton 2006).

Due to the diversity of both plant and pollinator species in natural communities, the effect of spatial patterning will undoubtedly be much more complex than when considering only two plant and one pollinator species. In the second chapter of this thesis, we were able to tackle this complexity in plant-pollinator interactions, using a network approach. By introducing an attractive species into semi-natural communities, we showed that only the diversity and evenness of plant-pollinator interactions were following the trend that the neutrality hypothesis would impose on these interactions (Bascompte *et al.* 2003, Vazquez *et al.* 2009). The recorded effects on these two network metrics were further enhanced by the attractive character of the introduced species showing that the set of floral traits of plant species can exacerbate its effects on the pollinator behaviour. This corroborates the idea that highly attractive species can act as strong competitor for the services of pollinators on co-flowering sympatric plant individuals (Moragues & Traveset 2005, Muñoz & Cavieres 2008). However, our most prominent result is that the organization in pollination networks cannot be explained alone by the neutrality hypothesis (Olesen *et al.* 2007, Santamaría & Rodríguez-Gironéz 2007, Vazquez *et al.* 2009) as some aspects of the organisation of the interactions in such networks was highly influenced by the spatial distribution of plant species at small scale. Especially, patterns of resource use by pollinators were more similar and less exclusive when the introduced species was spatially clumped than when it was spatially aggregated. These findings are proving that the spatial mechanisms described in the theoretical model are indeed occurring under natural conditions. Further, these results are in line with recent studies (Olesen *et al.* 2007, Santamaría & Rodríguez-Gironéz 2007, Vazquez *et al.* 2009) showing that linkage rules are best explained by a combination of different hypotheses such as complementary and barrier models, and not only by the neutrality hypothesis alone. Our findings clearly indicate that the spatial distribution of plant species can affect the general organisation of such networks. As such, further studies should investigate the role of spatial distribution of plant species in plant communities in shaping network structure and especially as a factor potentially explaining linkage rules of networks.

The last prominent result emerging from this work is that the occurrence and intensity of both intra- and interspecific density responses of quantitative aspects of the pollinator behaviour were conditioned not only by a focal species identity but also by the identity of its neighbouring species. This demonstrates that pollinators can and do distinguish among florally dissimilar species (Chittka & Thomson 2001) and that their choices are guided by the sets of floral traits of all the species present in the patches their forage in (Hegland *et al.* 2009). As such, the difference in attractiveness among species available for pollinators

could be a major determinant of the outcome of indirect plant-plant interactions. These observations should lead to studies aiming at identifying all the factors affecting the attractiveness of plant species. In this work, we have confirmed that the floral density is one such factor and discovered that spatial patterning can act as another one. Finally, the role of attractiveness differentials among co-occuring plant species on the outcome of indirect plant-plant interactions should be investigated.

Conclusion

This thesis created a unique bridge between theoretical and experimental evidences about the crucial role of the pollinator behaviour on the outcome of indirect plant-plant interactions and by extension about patterns of species coexistence in plant communities. By combining a spatially explicit modelling approach, an experimental network approach under natural conditions and a common garden experiment under more controlled conditions, we were able tackle the complexity of plant-pollinator interactions and to unmask the effects of structural characteristics of plant communities and their respective interplay on pollination patterns in species-rich grasslands.

Not unsurprisingly, the majority of the results presented here were linked with the difference in attractiveness of plant species. In most of studies on the same topic, it is often assumed that plant differ in their attractiveness and theories are built on this paradigm (Laverty 1992, Moeller 2004, Molina-Montenegro *et al.* 2008, Muñoz & Cavieres 2008, Peter & Johnson 2008, Hanoteaux *et al.* 2013, Seifan *et al.* 2014). However, mostly the attractive character of a species can only be proven *a posteriori*. As such, further studies should try to achieve a characterisation of the "attractiveness" in its broader ecological context. Even if this will be difficult as the "attractiveness" may include many ecological factors, this could allow pollination ecologists to understand the occurrence of contrasting effects of flowering species on each other (Bartomeus *et al.* 2008) and hence resolve the discrepancy reigning in the results of studies investigating the outcome of indirect plant-plant interactions.

Finally, the most novel aspect of this thesis is the inclusion of small-scale spatial mechanisms into pollination ecology. This work is to be considered as a first step and should serve as an incentive to further research the potential of spatial processes in shaping the outcome of plant-pollinator interactions for both trophic levels. As such, including spatially conditioned linkage rules as potential determinant of the general structure of mutualistic networks may help in unravelling the biological processes responsible for these general characteristics.

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