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PLANT-POLLINATOR NETWORKS: INCORPORATING INDIVIDUAL VARIATION AND FUNCTIONAL INFORMATION

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DECLARO:

Que la tesi doctoral que porta per títol

Plant-pollinator networks: incorporating individual variation and functional information

presentada per Cristina Tur Espinosa per a l'obtenció del títol de doctora, ha estat dirigida sota la meva supervisió i que compleix amb els requisits necessaris per optar a la Menció Europea en el títol de doctor.

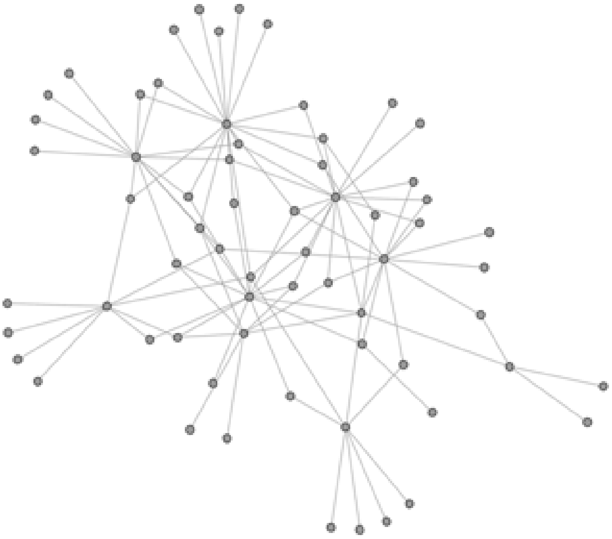
I perquè quedi constància d'això signo aquest document.

Palma de Mallorca, 26 de Juny 2015

Vistiplau del ponent:

Maurici Mus Amézquita

A la meva família.
A en Joan Pau.



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Outline and publications

This thesis is a compendium of publications, organized in four parts: Prologue, Main chapters, Epilogue, and Supplementary Materials and References. The prologue presents an overview of plant-pollinator interaction networks, introduces relevant background information of the specific topics studied in this thesis, and highlights the general objectives. The second part consists of four independent chapters, each one corresponding to a different research article. Three of them are already published, and one is in preparation for submission. The epilogue contains a general discussion of the main findings of the thesis, the final conclusions, and ideas for future investigation in this research line. Finally, supplementary material and a comprehensive list of references are included.

List of accepted publications included in this thesis:

Tur, C., Castro-Urgal, R., and Traveset, A. (2013). Linking plant specialization to dependence in interactions for seed set in pollination networks. *Plos One*, 8(10).
`<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0078294>`
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`<http://onlinelibrary.wiley.com/enhanced/doi/10.1111/1365-2656.12130>`
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Tur, C., Olesen, J. M., and Traveset, A. (2015). Increasing modularity when downscaling networks from species to individuals. *Oikos*, 124(5):581–592.
`<http://onlinelibrary.wiley.com/enhanced/doi/10.1111/oik.01668>`
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Resum

Les xarxes complexes serveixen als ecòlegs per a estudiar les interaccions de pol·linització a nivell comunitari. Actualment, és necessari construir xarxes que siguin representacions més realistes del procés de pol·linització per tal d'incrementar la seva utilitat pràctica. Per això, aquesta tesi es centra en incorporar a les xarxes la variació interindividual i informació sobre la magnitud i el signe (positiu, neutre o negatiu) dels efectes de les interaccions sobre la reproducció de les plantes.

Les espècies consten de poblacions d'individus amb fenotips, genotips i comportaments diferents, que per tant poden diferir en les interaccions. Aquesta variació intraespecífica és rellevant per molts processos ecològics i evolutius, però no s'ha tengut en compte en la majoria d'estudis de xarxes planta-pol·linitzador. En els capítols 1 i 2 s'estudien les càrregues pol·líniques d'exemplars d'insectes pol·linitzadors en dues comunitats de muntanya a Mallorca i es construeixen xarxes on els nodes dels pol·linitzadors representen individus en lloc d'espècies. Aquesta aproximació permet considerar la variació interindividual i aporta una nova perspectiva sobre l'estructura de les xarxes i els mecanismes que determinen les interaccions. Les xarxes de transport de pol·len a nivell d'individu tenen una densitat d'interaccions, connectància, grau d'anidament i diversitat d'interaccions més baixa que les mateixes xarxes a nivell d'espècies, i per contra una modularitat major. Es troben aquestes diferències perquè les espècies de pol·linitzadors generalistes estan formades per individus especialistes i heterogenis en l'ús de recursos. El grau d'especialització individual està associat a la intensitat de competència inter- i intraespecífica.

Per a que les xarxes planta-pol·linitzador representin millor les implicacions funcionals, han d'incloure mesures dels efectes de les interaccions sobre les espècies o de la magnitud real en què les espècies depenen d'aquestes interaccions. La freqüència d'interacció es considera una estimació vàlida de la magnitud de l'efecte del pol·linitzador sobre l'èxit reproductiu de la planta, però no aporta cap informació del seu signe. Per exemple, en plantes amb mecanismes de producció de llavors que no depenen d'insectes (e.g. autogàmia, anemogàmia), les interaccions poden tenir efectes neutres sobre la reproducció. En el capítol 3, per diferents

plantes de dues comunitats d'estudi (costa i muntanya) es quantifica el seu grau de dependència dels pol·linitzadors, comparant experimentalment la producció de llavors amb i sense insectes. L'objectiu és determinar si les espècies que són més depenents dels pol·linitzadors són també les que estan més connectades en les xarxes de visites florals, és a dir si tenen major centralitat, nombre i diversitat d'interaccions. Només en una de les dues comunitats estudiades es troba aquesta relació, fet que suggereix que pot dependre del contexte comunitari.

D'altra banda, hi ha interaccions que poden causar més efectes negatius que positius sobre l'èxit reproductiu de les plantes. Això pot passar quan els pol·linitzadors depositen pol·len conespecífic en els estigmes, però alhora també pol·len heterospecífic. La transferència interespecífica de pol·len és relativament comuna, perquè sovint les espècies de floració simultània comparteixen pol·linitzadors, i pot tenir efectes perjudicials per les plantes (e.g. pèrdua de pol·len, obstrucció dels estigmes). En el capítol 4, s'estudien les transferències de pol·len interespecífiques en tres comunitats andines al llarg d'un gradient altitudinal. Es construeixen xarxes dirigides representant la transferència de pol·len des de les espècies donants a les receptores i a cada interacció de la xarxa s'hi associa un signe per representar l'efecte. Aquest signe s'obté de la relació entre el pol·len conespecífic i heteroespecífic depositat sobre els estigmes. En totes les comunitats estudiades, les interaccions positives i neutres són predominants, particularment en la comunitat de major altitud. Aquesta troballa suggereix que la facilitació entre plantes d'una comunitat pot augmentar quan les condicions pel servei de pol·linització es tornen menys favorables.

Resumen

Las redes complejas sirven a los ecólogos para estudiar las interacciones de polinización a nivel comunitario. Actualmente, para incrementar su utilidad práctica, es necesario construir redes que sean representaciones lo más realistas posibles del proceso de polinización. Por este motivo, esta tesis se centra en incorporar a las redes la variación interindividual y información sobre la magnitud y el signo (positivo, neutro o negativo) de los efectos de las interacciones sobre la reproducción de las plantas.

Las especies constan de poblaciones de individuos con fenotipos, genotipos y comportamientos distintos, que por tanto pueden diferir en sus interacciones. Esta variación intraespecífica es relevante para muchos procesos ecológicos y evolutivos, pero no se ha considerado en la mayoría de estudios de redes planta-polinizador. En los capítulos 1 y 2 se estudian las cargas polínicas de ejemplares de insectos polinizadores en dos comunidades de montaña en Mallorca y se construyen redes donde los nodos de los polinizadores representan individuos en lugar de especies. Esto permite incorporar la variación interindividual y aportar una nueva perspectiva sobre la estructura de las redes y los mecanismos que determinan las interacciones. Las redes de transporte de polen a nivel de individuo tienen una densidad de interacciones, conectancia, anidamiento y diversidad de interacciones más baja que las mismas redes a nivel de especies, pero una modularidad mayor. Estas diferencias ocurren porque las especies de polinizadores generalistas están formadas por individuos especialistas y heterogéneos en la utilización de recursos. El grado de especialización individual está asociado a la intensidad de competencia inter- e intraespecífica.

Para que las redes planta-polinizador sean más representativas de las implicaciones funcionales deben incluir medidas de los efectos de las interacciones sobre las especies o de la magnitud real en que las especies dependen de estas interacciones. La frecuencia de interacción se considera una estimación válida del efecto del polinizador sobre el éxito reproductivo de la planta, aunque no aporta información sobre su signo. Por ejemplo, en plantas con mecanismos de producción de semillas independientes de los insectos (e.g. autogamia, anemogamia), las in-

teracciones pueden tener efectos neutros sobre la reproducción. En el capítulo 3, para diferentes plantas de dos comunidades de estudio (costa y montaña) se cuantifica el grado de dependencia de polinizadores comparando experimentalmente la producción de semillas con y sin insectos. El objetivo es determinar si las especies más dependientes de polinizadores son también las más conectadas en las redes, con mayor centralidad, número y diversidad de interacciones. Sólo en una de las comunidades de estudio se encuentra esta relación, sugiriendo que es variable en función del contexto comunitario.

Por otro lado, hay interacciones que pueden causar efectos más negativos que positivos sobre el éxito reproductivo de las plantas. Esto puede pasar cuando los polinizadores depositan polen conespecífico y heterospecífico en los estigmas. La transferencia interespecífica de polen es relativamente común, porque a menudo las especies de floración simultánea comparten polinizadores, y puede tener efectos perjudiciales para las plantas (e.g. pérdida de polen, obstrucción de estigmas). En el capítulo 4, se estudian las transferencias de polen interespecíficas en tres comunidades andinas a lo largo de un gradiente altitudinal. Se construyen redes dirigidas que representan la transferencia de polen desde las especies donantes a las receptoras y a cada interacción se le asocia un signo mostrando el efecto. Este signo se obtiene de la relación entre el polen conespecífico y heteroespecífico depositado sobre los estigmas. En todas las comunidades estudiadas, predominan las interacciones positivas y neutras, particularmente en la comunidad de mayor altitud. Esto sugiere que la facilitación en una comunidad puede aumentar cuando las condiciones para la polinización se vuelven menos favorables.

Abstract

Ecologists use network analysis to study pollination interactions at a community-wide level. The construction of plant-pollinator networks which are realistic representations of the pollination process is fundamental to increase their usefulness and ecological meaning. For that reason, this thesis focuses on incorporating to such networks individual variation and information about the magnitude and sign (positive, neutral or negative) of interaction effects on plant reproduction.

Species consist of populations of phenotypically, genetically and behaviourally diverse individuals which thus differ in their interactions and foraging decisions. Despite its relevance for many ecological and evolutionary processes, intraspecific variation has been overlooked in most plant-pollinator network studies. In chapters 1 and 2, pollen loads of insect pollinator individuals are studied in two mountain communities of Mallorca, and networks where pollinator nodes depict individuals instead of species are built. Such approach (network downscaling) permits to account for the variation within species and provides new insights on network interaction patterns and their causal mechanisms. Pollen-transport networks at the individual level had lower linkage density, connectance, nestedness and interaction diversity, than the same networks at the species level, but higher modularity. These differences occur because generalist pollinator species are composed of heterogeneous specialist individuals in the use of resources. The degree of individual specialization is associated with inter- and intraspecific competition.

Plant-pollinator networks which represent more accurately functional implications should include measures of interaction effects on species or estimates of the actual degree to which species depend on such interactions. In general, interaction frequency is considered a good surrogate of the magnitude of interaction effects, although it does not give any information about the effect sign. For instance, in plant species with mechanisms for producing seeds independently of pollinators (e.g. autogamy, anemogamy), interactions may have a neutral reproductive effect. In chapter 3, the degree of reproductive dependence on pollinators is quantified for several plant species in two study communities (coast and mountain), comparing seed set with and without insects. The objective is to determine whether plant species which depend more on pollinators are also more connected in flower-visitation networks, i.e. have high centrality, number and diversity of interactions. Such relationship is only found in one of the two study communities, which suggests that it is community-context dependent.

On the other hand, there are interactions which may cause more negative than positive effects on plant reproductive success. This may happen when flower-visitors deposit both conspecific and heterospecific pollen on stigmas. Such interspecific pollen transfer is common because co-flowering plants often share pollinators, and can have detrimental effects on plant fitness (e.g. pollen loss and stigma clogging). In chapter 4, interspecific pollen transfers are studied in three high-Andean communities along a mountain altitudinal gradient. Directed networks depicting pollen transfers from donor to receptor species are constructed, and a sign is associated to each network link to represent the effect. This sign is obtained from the study of the relationship between conspecific and heterospecific pollen deposited on stigmas. In all study communities, facilitative and neutral pollinator-mediated interactions among plants prevail over competition, particularly in the highest elevation community. This finding suggests that pollination facilitation in communities can increase under less favourable conditions for the pollination service, supporting previous predictions of higher incidence of facilitative interactions in stressful environments.

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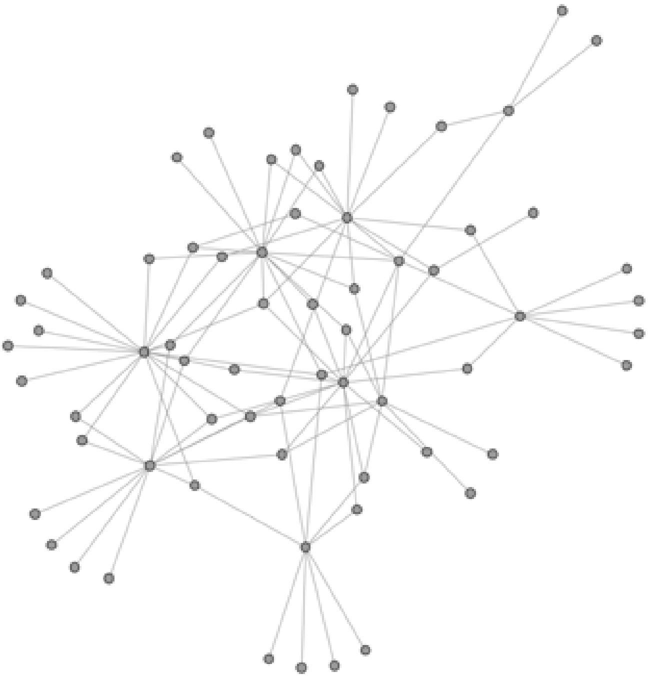
I | Prologue



General introduction

Plant-pollinator interactions: an important type of mutualism

Mutualistic interactions are widespread in nature, so much that virtually any species in Earth is involved in one or more of them (Bronstein et al. 2006). In particular, interactions between plants and animals for pollination are one of the most ecologically important mutualisms. During the pollination process, plants obtain dispersal services of pollen from anthers to target stigmas for fertilizing ovules and producing seeds, whereas pollinators obtain food or other rewards from plants, such as pollen and nectar. It is estimated that 87.5% of all flowering plants are pollinated by animals (Ollerton et al. 2011) and that approximately one third of the global crop production relies on pollinators (Klein et al. 2007). The major animal pollinators are insects (e.g. bees, butterflies, moths, flies and beetles) and nearly 290,000 flower-visiting insect species have been reported worldwide (Nabhan and Buchmann 1997). Plant-pollinator interactions play a key role for plant reproduction, but also for population dynamics, biodiversity maintenance, diversification, species coevolution and community structure (e.g. Pellmyr 2002, Waser and Olleton 2006, Bascompte and Jordano 2007). Unfortunately, many pollinators, and therefore also the pollination services they provide, are increasingly threatened by human disturbances, such as habitat fragmentation, changes in land use, species introductions, agricultural practices or pollution (Traveset and Richardson 2006, Hegland et al. 2009b, Potts et al. 2010, Spiesman and Inouye 2013, González-Varo et al. 2013, Burkle et al. 2013). Thus, there is a growing concern about how to preserve not only species but also interactions (Kearns et al. 1998, Kaiser-Bunbury et al. 2010, Tylianakis et al. 2010).

Plant and animals do not function as isolated entitites; most plant species are visited by a taxonomically diverse array of pollinators and most pollinator species visit a diverse array of plants (Waser et al. 1996, Johnson and Steiner 2000). Therefore, species in communities are entangled in complex webs of interactions.

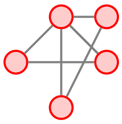
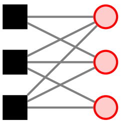
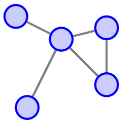
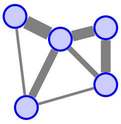
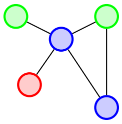
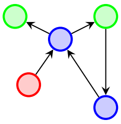
For that reason, plant-pollinator interaction studies, which traditionally focused on single species or pairwise interactions, expanded to the community-wide level (e.g. Jordano 1987, Memmott 1999). However, the complexity of multi-species communities dramatically increases with species richness and hence the myriad of possible interactions. Network analysis has proved to be an efficient tool for understanding such complex systems.

The network approach in ecology: a general overview

A network represents a set of interacting elements, i.e. a group of nodes (vertices) connected by links (edges). Depending on the properties and attributes of nodes and links several types of networks can be distinguished (Table 1). Multitude of systems can be described as networks, for instance, the Internet (computers linked by data connections), human societies (people linked by social relationships), molecular structures (atoms linked by chemical bonds) or food webs (species linked by predation interactions). Networks have been used in disparate disciplines – from neurobiology to economics – because they are a useful tool to study how the different components of a system are connected. The way connections are structured is not trivial, because the connectivity pattern affects in turn the functioning and behaviour of the whole system (e.g. Albert et al. 2000). As Aristotle said, 'the whole is greater than the sum of its parts'. Networks not only provide a holistic view of complex systems but also allow going beyond their structural parts in order to understand also the dynamical processes happening there. The graph theory developed in mathematics field (Erdös and Renyi 1959) provides the conceptual and analytical framework required to study any kind of networks.

'Network thinking' was imported to ecology around the 70's (Paine 1966, May 1973, Cohen 1977, Pimm 1979). However, in the last two decades, the use of a network approach in ecological studies has experienced an important outbreak (e.g Proulx et al. 2005, Pascual and Dunne 2006, Bascompte and Jordano 2007, Woodward 2010, Heleno et al. 2014) fuelled by emergent computation techniques and network theory advances in other research fields, such as social sciences, physics, computer science or mathemathics (Strogatz 2001, Albert and Barabási 2002, Newman 2003, Watts 2004). Ecologists borrowed concepts and tools from these diverse areas and re-applied them, for instance, to study predator-prey (e.g. Dunne et al. 2002), plant-pollinator (e.g. Memmott 1999), plant-disperser (e.g. Jordano 1987) or host-parasitoid interactions (e.g. Tylianakis et al. 2007) at a community-wide level. In particular, the analysis of plant-pollinator networks revealed several invariant structural patterns and unique properties (Bascompte

Table 1: Types of networks according to different attributes of nodes and links. In unipartite networks (Figure a) all nodes belong to the same category or set and any node can theoretically be connected to another node, whereas in bipartite networks (Figure b) nodes belong to two different subsets and can only be connected to nodes from the other subset. When links do not have an associated weight or strength, networks are unweighted or qualitative (Figure c), while weighted or quantitative networks (Figure d) have links with an associated weight. Moreover, if links do not have a particular direction the network is undirected (Figure e). On the contrary, when links have a direction and a focal node can have incoming and outgoing links, the network is directed (Figure f).

	Network classification	
Node categorisation	**(a)** Unipartite 	**(b)** Bipartite 
Link weight	**(c)** Unweighted 	**(d)** Weighted 
Link directionality	**(e)** Undirected 	**(f)** Directed 

et al. 2003, Jordano et al. 2003, Vázquez and Aizen 2004, Bascompte et al. 2006, Olesen et al. 2007, Bascompte and Jordano 2007, Vázquez et al. 2009a) determined in turn by common underlying principles or building mechanisms (e.g. Vázquez and Aizen 2006, Stang et al. 2007, Vázquez et al. 2009b, Eklöf et al. 2013). In the last years, the discipline has evolved from first descriptive studies to more functional and mechanistic perspectives of interaction networks.

Currently, the principal challenge in network ecology is to build more realistic and informative networks in terms of the true ecological processes described and the ecological meaning of what flows through network links (Ings et al. 2009, Olesen et al. 2011b, Heleno et al. 2014). To achieve this goal, two fruitful approaches have been proposed: scaling up networks to construct 'networks of networks', i.e. combining different types of interaction networks (such as food webs, parasitoid webs, seed dispersal networks or pollination networks) into a single super-network (Fontaine et al. 2011, Pocock et al. 2012, Evans et al. 2013); and scaling down from networks of species to networks of individuals, i.e. building networks where nodes are individuals instead of species (Dupont et al. 2011). In addition, because many ecosystem functions are a result of species interactions, it is needed to link network structural properties to functional consequences for the ecological systems (e.g. Gómez et al. 2011) and to provide good estimates of the magnitude and direction of the functional effects rather than simply the frequency of interactions.

In this thesis I aim to move forward the construction of increasingly representative plant-pollinator networks by incorporating individual variation within species and information about the effects of interactions for the reproductive success of plants.

From species-based to individual-based networks: the importance of intraspecific variation

Interaction networks, such as plant-pollinator networks, should be quantified based on what individuals do because the individual is the level of organization at which real interactions take place in nature (Ings et al. 2009, Olesen et al. 2010). Species consist of populations of phenotypically, genetically and behaviourally diverse conspecific individuals. Animal individuals can differ in size, sex, age or social status, whereas plant individuals can differ in traits such as floral display, plant height, flower morphology or amount of rewards. Thus, on a finer-grained level, each node in a species-based network may be viewed as formed by more nodes depicting individuals, but species-based networks absorb inter-individual variation. Individual-based networks can account for all this valuable information. The process of moving from species-based to individual-based networks can be defined as network downscaling (Figure 1).

Ecologists increasingly recognize the importance of individual variation within natural populations for many ecological processes (Bolnick et al. 2011, Dall et al. 2012, Wolf and Weissing 2012). Because conspecific individuals are not identical, their niches and pattern of interactions may differ (Figure 2). In general, a high niche variation exists within populations, sometimes even exceeding differences between species (van Valen 1965, Bolnick et al. 2003, Araújo et al. 2011). However, the role that within-population variation plays on the structure of ecological networks is largely unknown. Studies using simulation models, have shown that

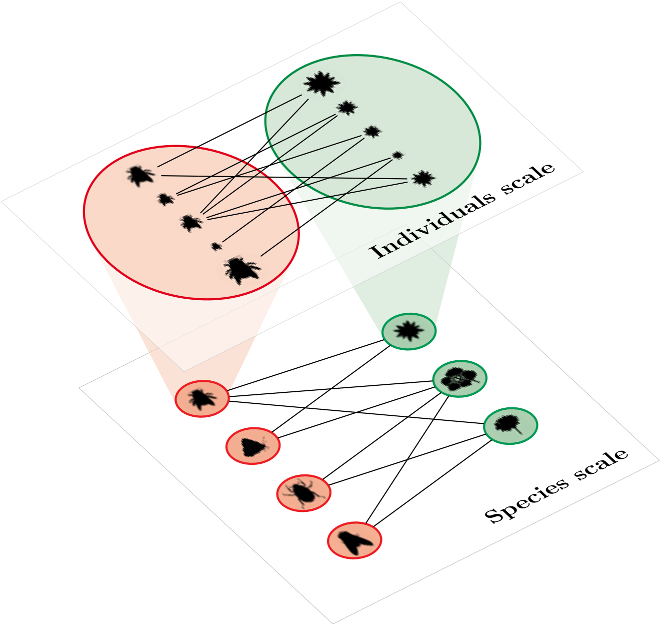


Figure 1: Illustration of the idea of network downscaling, the process of moving from the species scale to the individual scale. The plane below depicts a species-based bipartite network, i.e. a network where nodes represent pollinator and plant species whereas the plane above shows an individual-based network. All nodes in a species-based network can be disaggregated into more nodes representing their conspecic individuals, which differ in traits and thus interactions. Note that when downscaling, different networks may be obtained depending on whether both interacting subsets (plants and pollinators) are decomposed into their constituent individuals (individuals-individuals networks) or only a subset is decomposed (species-individuals networks). Moreover, individual-based networks can be unipartite if they represent interactions among individuals of the same subset or species.

intraspecific variance can have a strong effect on the properties of species-based networks, which might in turn potentially affect network dynamics and stability (Melián et al. 2011, Moya-Laraño 2011). In addition, individual-based networks have been used in empirical studies exploring intrapopulation patterns of resource partition in vertebrate species (Araújo et al. 2010, Pires et al. 2011, Tinker et al. 2012), changes in foraging preferences of fishes with intraspecific competition (Araújo et al. 2008), body size effects in prey-predator interactions (Woodward and Warren 2007, Yvon-Durocher et al. 2011), patterns in roosting sites of bats (Fortuna et al. 2009) and disease dynamics in rodent populations (Perkins et al. 2009).

In the case of pollination networks, the number of individual-based network studies is scarce, all focusing on one or two species (Fortuna et al. 2008, Dupont et al. 2011, Gómez et al. 2011, Gómez and Perfectti 2012, Dupont et al. 2014, Dáttilo et al. 2015). The reason for such limited number of studies is probably the labour-intensive fieldwork required to sample individuals from all species in a community, in addition to the problem of how to adequately sample them, especially in the case of animal species. For instance, mark-reobservation of individuals (Dupont et al. 2011) and micro-radio telemetry tracking (Hagen et al. 2011) have been successfully applied to study foraging patterns of bumblebees, but both methodologies seem unrealistic for a multi-species sampling of pollinators. Nevertheless, the few existing empirical pollination networks at the individual scale demonstrated that individual-specific plant traits (e.g. plant height, flower number, corolla shape) influence their connectivity within the network and even the overall network structure (Gómez and Perfectti 2012, Dupont et al. 2011, 2014), affecting in turn both plant individual and population fitness (Gómez et al. 2011, Gómez and Perfectti 2012).

In addition, moving beyond exploring networks of species to exploring networks of individuals sets us at the proper scale to test ecological questions and processes which occur at the individual level, such as foraging theory or natural selection. Optimal foraging theory (MacArthur and Pianka 1966, Schoener 1971, Stephens and Krebs 1986) provides a general context to understand how pollinator individuals discriminate between resources and why a particular individual uses a given set of resources from all available. According to optimal foraging theory, individuals choose resources to maximize the energy income per unit time. Thus, pollinator decisions on whether to spend time and energy probing a flower depend on tradeoffs between factors, such as the energetic content of alternative resources, handling times, search efficiencies, resource abundances or predation risk (Goulson 1999). As each individual differs from its conspecifics, the balance between tradeoffs and hence foraging decisions differ as well. Since individual foraging behaviour ultimately determines which interactions are realized and which are not, it has been recognized as a key driver of network structure (Kondoh

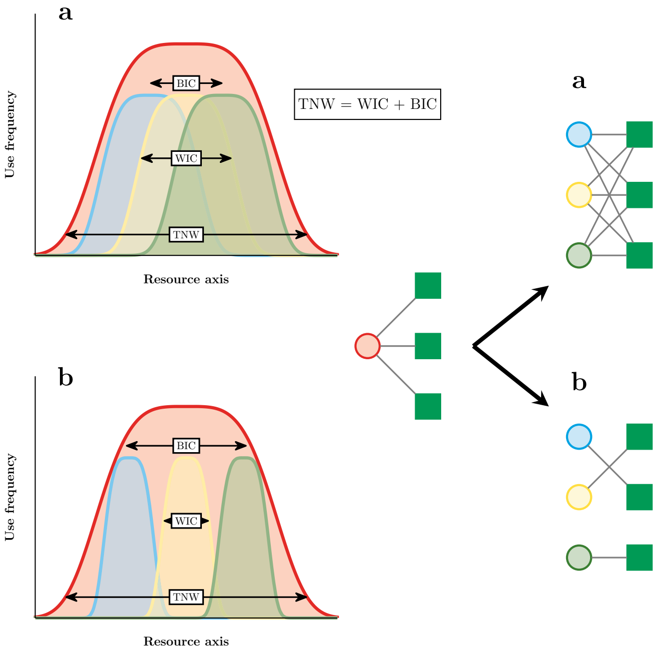


Figure 2: Schematic illustrations showing two alternative ways of how conspecific pollinator individuals (blue, yellow and green curves) may subdivide the population or species niche (red curve), and networks that would be expected under each alternative when downscaling from a species-based to an individual-based network (circles are pollinators and squares are plant resources). Following the intrapopulation niche width variation model (Roughgarden 1972, 1974) the total niche width of a species (TNW) can be partitioned in two components: a within-individual component (WIC), which is the average of the niche breadth of individuals; and a between-individual component (BIC), which is the niche breadth variance among individuals. The degree of individual specialization is measured as WIC/TNW and indicates how much narrower the niches of individuals are compared to the species niche (see Bolnick et al. 2002). (a) The species niche is subdivided among generalist individuals with broad niche breadths which overlap greatly among them and with the species niche. Under this scenario, WIC is a large part of TNW and WIC/TNW tends to 1, implying a weak individual specialization of the species. Therefore, the expected individual-based network is highly connected and individuals have the same linkage level as the species. (b) The species niche is subdivided among specialist individuals with narrow niche breadths which do not overlap with each other and overlap little with the species. Under this scenario, WIC is a small part of TNW and WIC/TNW tends to 0, implying a strong individual specialization of species. The expected individual-based network in this case is weakly connected and individuals have a smaller linkage level than the species. Figure modified from Bolnick et al. (2003).

2003, Beckerman et al. 2006, Petchey et al. 2008, Valdovinos et al. 2013). In individual-based pollination networks, foraging choices and division of resources among flower-visitors at small spatial scales have been shown to determine the network structure (Dupont et al. 2014) and could in turn affect patterns of pollen dispersal and mating within plant populations (Fortuna et al. 2008).

Finally, knowledge of the relationships among different hierarchical levels of organisation is essential for understanding complex systems and ecological processes across scales. Frequently, aspects of processes change with the scale at which they are studied or generate patterns at scales that may differ from those at which processes act (Levin 1992, Chave 2013). Thus, topology at one scale (individuals) might cascade up and influence topology at the other scale (species). Indeed, although we could view species interactions as merely the sum of individual decisions, interactions occurring between individuals might, alternatively, produce higher level attributes or collective dynamics, such as in self-organized social insect colonies (e.g. Fewell 2003).

In the two first chapters of the thesis I follow the downscaling approach to investigate which new insights do plant-pollinator networks at the individual level offer us compared with networks at the species level, exploring whether species-based and individual-based networks offer complementary or diverging information, and which network patterns and drivers at the individual-level differ from those at the species-level.

From maps of interactions to maps of functions: the effects of plant-pollinator interactions

The first ecological network studies provided detailed road maps of interactions (i.e. maps of who interacts with whom), but they lacked information on the relative contribution of each interaction to a particular ecological effect or function (e.g. seed dispersal, pollen transfer). The construction of quantitative or weighted networks was a first step to partially resolve this weakness, because in these networks interactions have a weight (i.e. interaction strength). However, to be ecologically meaningful the weight measure should be adequate to represent the functional effect or impact of the interactions. The use of a meaningful weight might in turn allow determining the sign (positive, neutral, negative) of the functional effect and formulating indirect relations between the elements of the network (e.g. plant-plant interactions) (Figure 3).

In the case of plant-pollinator interactions, the pollinator has an effect on plant reproductive success and the plant's rewards have a reciprocal impact on the pollinator's nutrition. Despite functional effects being bidirectional, most studies focus on the effects of interactions for the plant side due to experimen-

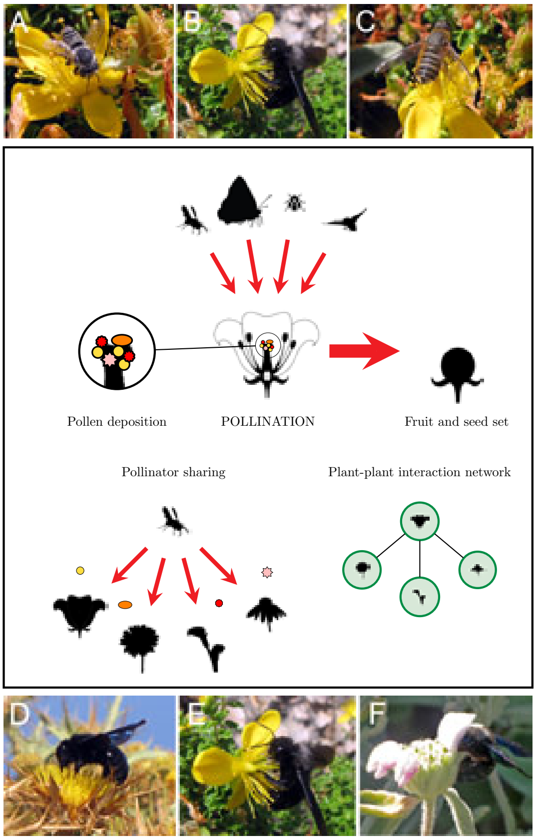


Figure 3: Ideally, quantitative pollination networks should include a link weight representing the magnitude and sign of the effect of interactions on plant reproduction. The magnitude of the effects of flower-visitors might be estimated measuring the contribution of visits to some component of plant reproductive fitness, such as the amount of conspecific pollen deposited on stigmas, pollen tubes, fruit set or seed set. The effects of flower-visitors can range from positive, when conspecific pollen is deposited effectively, to negative, for instance when flower-visitors rob nectar without depositing pollen, remove conspecific pollen deposited on stigmas or only deposit heterospecific pollen. Interspecific pollen transfer among plants is relatively common because plants are visited by many insects which carry pollen from other plant species visited before. This means that plants can interact among them through their shared pollinators, and these interactions can cause beneficial or detrimental effects on reproductive fitness. Pictures: *Megachile pilidens* (A), *Xylocopa violacea* (B) and *Villa* sp. (C) visiting flowers of *Hypericum balearicum*. *Xylocopa violacea* does not visit exclusively *Hypericum balearicum* (E), it also visits *Carlina corymbosa* (D) and *Phlomis italica* (F).

tal difficulties for estimating the effect for both interaction participants (but see Vázquez et al. 2012). The total effect of a pollinator species on plant reproduction depends on two components: the 'quality' component, and the 'quantity' component (interaction frequency) (e.g. Herrera 1987, 1989). However, a direct measure of the quality component (e.g. single visit pollen deposition, seed- or fruit-set) is more difficult to obtain in the field than a measure of the quantity component (e.g. visitation rate). The quality component is influenced by multiple factors, such as the amount of conspecific and foreign pollen carried by the pollinator, pollen compatibility, the genetic identity of pollen donors represented in the pollen load, the duration of the flower visit, or the pollinator foraging behaviour (see Ne'eman et al. 2010, and references therein). Interaction frequency has been considered a good proxy of the total effect and it is used as interaction strength in most quantitative networks, because the variability in the quantity component exceeds qualitative differences among pollinators (Vázquez et al. 2005, Sahli and Conner 2006). However, the most frequent pollinator is sometimes not the one with the highest impact on plant reproduction (Schemske and Horvitz 1984, Mayfield et al. 2001, Sánchez-Lafuente et al. 2012). Conversely, the most frequently visited or the most connected plant species in a network may not be the one receiving the highest pollination service. A key aspect to understand functional effects of interactions is the identification of which particular network attributes (e.g. connectance, interaction diversity, centrality) are linked to plant reproductive performance (Gómez et al. 2011, Gómez and Perfectti 2012).

Plant-pollinator networks built on visitation data may not always be good representations of pollination function because some flower-visitors do not carry or deposit conspecific pollen on stigmas (e.g. Alarcón 2010, Popic et al. 2013, King et al. 2013) and others can even act as nectar or pollen robbers (Genini et al. 2010). Alternatively, some studies constructed plant-pollinator networks based on data from pollen-loads carried by flower visitors (Gibson et al. 2006, Bosch et al. 2009, Alarcón 2010, Popic et al. 2013). Pollen-transport networks may be viewed as improved representations of effective pollination compared to visitation networks although they do not provide direct evidence of pollen deposition on stigmas. Pollen carried by insects, however, may be deposited in conspecific unreceptive stigmas, heterospecific stigmas or may be lost before deposition on any flower (e.g. Rademaker et al. 1997, Adler and Irwin 2006). A functional informative pollination network may be one which depicts directional pollen transfer (i.e. from anthers to stigmas) among plants within a population or among species in a community (Fang and Huang 2013).

In addition, indirect relationships among neighbouring plant species and the sign of the effects on plant pollination might be deduced from a network of interspecific pollen transfers. Interspecific pollen transfer is relatively common in natural communities (McLernon et al. 1996, Montgomery and Rathcke 2012, Ashman and Arceo-Gómez 2013) and can have detrimental effects on both male and female plant functions (Morales and Traveset 2008, Muchhala and Thomson 2012). It is a direct consequence of pollinator sharing among co-flowering plant species, which occurs frequently since many animal-pollinated plants are generalized (e.g. Waser et al. 1996). Indirect interactions established among co-flowering plants through shared pollinators have the potential to affect reproductive outcomes of species (Rathcke 1983, Mitchell et al. 2009). The effects of pollinator sharing on reproductive success range from negative (competition), neutral, to positive (facilitation) (Moragues and Traveset 2005, Bjerknes et al. 2007, Morales and Traveset 2008, Hegland et al. 2009a). Competitive interactions occur when the presence of a plant species reduces pollinator visits to other co-flowering species (Brown et al. 2002, Mitchell et al. 2009, Tscheulin and Petanidou 2013) and/or increases heterospecific deposition on their stigmas causing stigma clogging or allelopathic inhibition of conspecific pollen germination (Sukhada and Jayachandra 1980, Galen and Gregory 1989, Murphy and Aarssen 1995, Brown and Mitchell 2001, Matsumoto et al. 2010). On the other hand, facilitative interactions take place when the presence of a plant species increases visitation rates and reproductive success of co-flowering species (Moeller 2004, Ghazoul 2006, Molina-Montenegro et al. 2008, Liao et al. 2011). Sometimes, plant-plant interactions for pollination are neutral if the presence of a co-flowering species does not induce changes in the reproductive success of another species despite sharing pollinators (e.g. Armbruster and McGuire 1991). Pollinator-mediated interactions among plants have potential ecological and evolutionary implications for plant community structure (Feinsinger 1987, Sargent and Ackerly 2008, Mitchell et al. 2009, Muchhala et al. 2010). Therefore, these indirect interactions should be also taken into account in networks which attempt to represent functional impacts of pollinator interactions.

In the third chapter of this thesis, in order to link flower-visitation networks with pollination function, I study the relationship between plant connectivity within the network and the magnitude of insect-pollination contribution to seed set. Finally, the fourth chapter explores the sign of the effects of pollinator-mediated interactions among co-flowering plant species in three communities along an altitudinal gradient.

General objectives of this thesis

In this thesis I aim to construct and explore plant-pollinator networks which incorporate: individual variation within pollinator species (chapter one: **'Downscaling pollen transport networks to the level of individuals'**, and chapter two: **'Increasing modularity when downscaling networks from species to individuals'**); and information about the effects of interactions on plant pollination, specifically their contribution to plant reproductive success (seed set) (chapter 3: **'Linking plant specialization to dependence in interactions for seed set in pollination networks'**) and the sign of the effects (negative, neutral, positive) of shared pollinators (chapter 4: **'Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities'**).

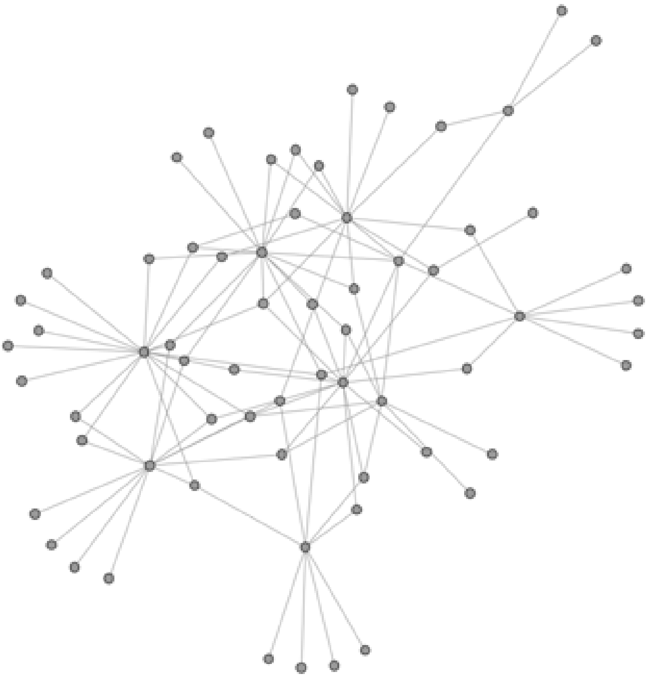
Below I outline the particular objectives addressed within each chapter:

1. (Chapter one) To identify which structural parameters of pollen-transport networks change when downscaling from species-species to individuals-species networks.
2. (Chapter one) To evaluate the incidence and magnitude of individual specialization in pollen resource use, i.e. to study intraspecific niche variation in different pollinator species.
3. (Chapter one) To investigate the potential influence of various ecological factors (particularly, inter- and intraspecific competition, insect species abundance, and insect species phenophase) on the degree of individual specialization in pollen use by pollinators.
4. (Chapter two) To determine whether the pattern of modularity (i.e. densely connected groups of species with sparse connections to species in other groups) is consistent or not when downscaling from species-species to individual-species networks using three different modularity metrics: unipartite modularity (Newman and Girvan 2004, Guimerà and Amaral 2005a,

Olesen et al. 2007), bipartite modularity (Barber 2007) and weighted bipartite modularity (Dormann and Strauss 2014).

5. (Chapter two) To analyze how conspecific individuals are organized into modules. Conspecific individuals might belong to the same or different modules depending on the pattern of within-species resource partition.
6. (Chapter two) To identify the drivers of modularity in individuals-species networks, and assess whether species or individual's traits – such as generalization, abundance or phenophase – influence their topological roles within the network.
7. (Chapter three) To estimate the degree of reproductive dependence on pollinators for several abundant plant species in two study communities. Dependence is defined here as the magnitude of seed set reduction when plant species are not pollinated by insects, i.e. the contribution of total pollinator interactions to plant seed production.
8. (Chapter three) To assess whether the degree of plant dependence on pollinators is associated with the number of interactions, the diversity of interactions and the closeness centrality of species within the network. Highly dependent species might tend to occupy central positions in the network and to have a high number and diversity of interactions.
9. (Chapter four) To describe plant-plant pollination interactions at a community-wide level by constructing a directed network representing all interspecific pollen transfers due to shared pollinators among plants.
10. (Chapter four) To estimate the sign (negative, neutral or positive) of the effect of pollinator-mediated interactions on pollen receptor species, both in terms of pollination quantity and quality. The balance between conspecific and heterospecific deposition per stigma might provide an estimate of the relative cost or benefit obtained from shared pollinators.
11. (Chapter four) To determine the relative frequency of facilitative, neutral or competitive pollination interactions among plant species within each study community.
12. (Chapter four) To assess whether the incidence of pollination facilitation or competition changes across the altitudinal gradient studied.

II | Main chapters



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Downscaling pollen-transport networks to the level of individuals

Content of this chapter is published as:
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`<http://onlinelibrary.wiley.com/enhanced/doi/10.1111/1365-2656.12130>`
DOI: 10.1111/1365-2656.12130

Data and R scripts for analysis are deposited in the Dryad Digital Repository:
`<http://datadryad.org/resource/doi:10.5061/dryad.63fp5>`

Abstract

1. Most plant-pollinator network studies are conducted at species level whereas little is known about network patterns at the individual level. In fact, nodes in traditional species-based interaction networks are aggregates of individuals establishing the actual links observed in nature. Thus, emergent properties of interaction networks might be the result of mechanisms acting at the individual level.
2. Pollen loads carried by insect flower-visitors from two mountain communities were studied to construct pollen-transport networks. For the first time, these community-wide pollen-transport networks were downscaled from species-species (sp-sp) to individuals-species (i-sp) in order to explore specialization, network patterns and niche variation at both interacting levels. We used a null model approach to account for network size differences inherent to the downscaling process. Specifically, our objectives were: (i) to investigate whether network structure changes with downscaling, (ii) to evaluate the incidence and magnitude of individual specialization in pollen use, and (iii) to identify potential ecological factors influencing the observed degree of individual specialization.
3. Network downscaling revealed a high specialization of pollinator individuals, which was masked and unexplored in sp-sp networks. The average number of interactions per node, connectance, interaction diversity and degree of nestedness decreased in i-sp networks, because generalized pollinator species were composed of specialized and idiosyncratic conspecific individuals. An analysis with 21 pollinator species representative of two communities showed that mean individual pollen resource niche was only c. 46% of the total species niche.
4. The degree of individual specialization was associated to inter- and intraspecific overlap in pollen use and it was higher for abundant than for rare species. Such niche heterogeneity depends on individual differences in foraging behaviour and likely has implications for community dynamics and species stability.
5. Our findings highlight the importance of taking inter-individual variation into account when studying higher–order structures such as interaction networks. We argue that exploring individual-based networks will improve our understanding of species-based networks and will enhance the link between network analysis, foraging theory and evolutionary biology.

Key-words: species-based networks, individual-based networks, individual specialization, ecology of individuals, linkage level, niche overlap, pollen-load analysis, foraging behaviour, resource partition, generalization.

1.1 Introduction

In the last couple of decades, community-studies of plant-animal interactions have been explored with the aid of network theory (e.g. Memmott 1999, Jordano et al. 2003)). In such interaction-networks, animal and plant species are nodes, and links represent the interactions between them. As each node is a different species, these networks are species-based. However, each node in a species-based network consists of a population of conspecific individuals, which are the true interacting actors in nature. Operating exclusively at species level may obscure individual behaviour, resulting in loss of valuable information (Ings et al. 2009). The relevance of scaling down species-based pollination networks to individual-based networks has previously been stressed (e.g. Olesen et al. 2010, Dupont et al. 2011), as patterns and forces acting at the individual level may be important drivers of structure and dynamics at species level. Moreover, relative invariance of network patterns and lack of differences found among different species-based networks (e.g. Jordano et al. 2006, Petanidou et al. 2008) could be a consequence of not resolving networks at the proper scale.

Despite the recognized importance of individual variation within natural populations for many ecological processes (Bolnick et al. 2011, Dall et al. 2012, Sih et al. 2012, Wolf and Weissing 2012), only a few empirical studies to date have applied network theory as a tool for exploring ecological interactions at the individual level. For example, individual-based networks have been used to study intrapopulation patterns of resource partition (Araújo et al. 2010, Pires et al. 2011, Tinker et al. 2012), changes in foraging preferences at increasing levels of intraspecific competition (Araújo et al. 2008), body size effects in prey-predator interactions (Woodward and Warren 2007, Yvon-Durocher et al. 2011), patterns in roosting sites of bats (Fortuna et al. 2009) and disease dynamics (Perkins et al. 2009). In the case of pollination networks, the number of individual-based network studies is very scarce, all focusing on one or two species (Fortuna et al. 2008, Dupont et al. 2011, Gómez et al. 2011, Gómez and Perfectti 2012). A likely explanation for the scarcity of such studies is the labour-intensive sampling required to resolve community-wide species networks into individual-based networks covering all species present.

Network data at individual level may be used to test important niche breadth questions, and this is a natural progressional step and promising avenue for future network research (Ings et al. 2009). It is well known that conspecific individuals vary in their resource use (van Valen 1965, Roughgarden 1972). Individual specialization occurs when individuals exploit only a small subset of the total resources used by the entire population, and it is a ubiquitous phenomenon in both vertebrate and invertebrate populations (Bolnick et al. 2003). As individuals within a population vary genetically and phenotypically, their resource choice may differ as well. For example, physiology, criteria of optimal diet shifting, behavioural skills or social status (see Araújo et al. 2011, for a review on the ecological causes of individual specialization), all influence individual resource use and preferences. Several indices have been developed to quantify the degree of individual specialization (Bolnick et al. 2002), allowing researchers to compare the magnitude of individual specialization across a variety of ecological situations in nature. Indeed, intrapopulation variation in resource use has been proposed to affect population dynamics and ecological interactions (Bolnick et al. 2011), whereas these in turn affect the magnitude and incidence of intrapopulation niche variation in a feedback loop way (Araújo et al. 2011).

Pollination networks are usually built based on field observations of plant and flower-visitor interactions. However, some studies have used data from pollen loads carried by flower visitors (e.g. Bosch et al. 2009, Alarcón 2010) finding that they give complementary information. The analysis of pollen loads, which provides a record of individual foraging patterns across time, addresses one component of pollinator effectiveness and actually gives a good indication of probable pollinators of a given plant species (Popic et al. 2013). Construction of pollen-transport networks has also been useful in applied studies (e.g. Forup and Memmott 2005, Gibson et al. 2006, Lopezaraiza-Mikel et al. 2007). Here we used pollen–loads from insect flower-visitors (pollinators hereafter) in two mountain scrublands to construct pollen-transport networks at both the species level (species-species network; hereafter sp-sp), and the individual level of the pollinators (individuals-species network; hereafter i-sp). Plants were only analysed at species level. To our knowledge, no previous study has downscaled a whole pollination network using this approach.

Our first goal was to investigate whether network structure changes when downscaling from sp-sp to i-sp and if so, which network topological parameters change in particular. The network-downscaling process inherently implies an increase in the number of network nodes, because species are composed of groups of conspecific individuals. Given that most network metrics are influenced by network size (Dormann et al. 2009), we built null models that account for size-related differences. Changes in network structure when downscaling might be expected due to differences in individual pollen use resulting from different foraging patterns, individual behaviours and trait variability among conspecifics. We defined niche of a pollinator species both qualitatively (number of interactions to plant pollen types, i.e. linkage level) and quantitatively (diversity of pollen types carried by insects). Given that linkage level of a species (L_{sp}) expresses the sum of all links established by its individuals (L_i), we hypothesize that pollinator species niche may be determined by means of two possible mechanisms: (i) individuals are as generalized as their species, i.e. all individuals have similar feeding niche (null hypothesis: $L_{sp} \approx L_i$), or (ii) individuals are more specialized than the species (alternative hypothesis: $L_i \ll L_{sp}$). If the alternative hypothesis is true, we would predict changes in topological parameters beyond those related to size differences when downscaling from sp-sp to i-sp networks.

Our second goal was to evaluate the incidence and magnitude of individual specialization in pollen use. First, we compared species and individual generalization levels for all pollinators (i.e. population *vs.* individual niche). Moreover, for a subset of abundant and representative pollinator species, we compared the mean empirical linkage levels with those obtained from the null models and tested whether the distribution of their species linkage level among conspecific individuals was nested, as found previously in other studies (Araújo et al. 2010, Pires et al. 2011). In addition, we measured quantitatively the relative degree of individual pollen specialization and determined its significance.

Finally, our third goal was to identify potential ecological factors influencing individual specialization in pollen use. Specifically, using structural equation modeling we tested the effect of factors known to influence individual specialization from other studies: (i) inter- and intraspecific overlap (competition, i.e. amount of resources shared by individuals of different or same species), (ii) insect species abundance and (iii) insect species phenophase, i.e. temporal extent of network membership. In the context of Optimal Foraging Theory (Stephens and Krebs 1986), we expected a high degree of individual specialization to be associated to: (i) low amounts of interspecific overlap, because release from resource competition favours species niche expansion through increased variation in resource use between individuals (Costa et al. 2008, Bolnick et al. 2010); (ii) high amounts of intraspecific overlap, because strong intraspecific competition promotes resource use diversity among conspecifics (e.g. Svanbäck and Bolnick 2005, 2007, Araújo et al. 2008); (iii) high abundance of species, because at high densities of foragers the availability of preferred resources decreases causing individuals to add different subsets of resources (e.g. Fontaine et al. 2008, Tinker et al. 2008, Svanbäck et al. 2011, Tinker et al. 2012); and (iv) long species phenophases, because species with short phenophases might be composed by short-lived individuals using similar and narrower subsets of resources due to temporal restriction in resource pool compared to species with long phenophases.

1.2 Materials and methods

1.2.1 Study sites and data collection

Plant-pollinator interactions were studied at two sites on the highest mountain in Mallorca (Puig Major, 1445 m): (i) Sa Coma de n'Arbona (CN) at 1100 m a.s.l. (39°48′5″N 2°47′9″E) and (ii) Passadís de Ses Clotades (PC) at 1400 m a.s.l. (39°48′34″N 2°47′50″E). Plant-pollinator interactions were surveyed during the entire flowering season, from May to August 2010, on clear and calm days. Pollinator censuses of 5 min, focusing upon randomly selected plant individuals of every species in bloom, were carried out between 10 am and 5 pm three times a week. During each census we recorded: (i) taxonomic identity of plant species under observation; (ii) taxonomic identity of insect pollinators (species name if possible or morphospecies otherwise; hereafter species), and (iii) number of individuals of each insect species observed. After each census, flower-visiting insects were captured, placed separately in clean vials and stored in a freezer for later pollen analysis and expert identification. We carried out a total of 458 censuses at CN (38 h 10 min) and 377 at PC (31 h 25 min), capturing 73 and 61 insect species, respectively. At each site, abundance and phenophase of each insect species were estimated as total number of individuals observed in all censuses and total number of days between first and last observation date of flower-visiting individuals, respectively.

In the laboratory, each captured insect individual was examined for pollen loads by washing, identifying and counting all pollen grains from its body surface. Pollen was identified at species, species cluster or morphospecies level (pollen types hereafter). See section A.1 in Supplementary Material Chapter 1 for a detailed description of the pollen load analysis procedure. At CN, a total of 190 individuals (71 Diptera, 83 Hymenoptera, 33 Coleoptera, 3 Hemiptera) were examined for pollen, and 55 pollen types were identified. At PC, a total of 137 individuals (43 Diptera, 64 Hymenoptera, 26 Coleoptera, 4 Hemiptera) carried 49 pollen types. Lepidopterans were excluded because their pollen load could not be analysed according to our quantitative methodology protocol (they could not be washed).

1.2.2 Construction and analysis of sp-sp and i-sp pollen-transport networks

Data from pollen load analyses were used to construct plant-pollinator interaction matrices at two levels of resolution: (i) species-species (sp-sp network), representing interactions between insect species and pollen types and (ii) individuals-species (i-sp network), representing interactions between insect individuals and pollen types. Interaction weight was the number of pollen grains of a given type carried by either the insect species or the individual.

We selected the following parameters to describe sp-sp and i-sp network structure: number of pollinator nodes (A), number of pollen type nodes (P), total number of nodes (T), total number of interactions (I), linkage level (L), network size (N), linkage density (LD), connectance (C), nestedness (NODF), interaction diversity (H_2) and interaction evenness (E_2) (see Supplementary Table 9 for definitions). The significance of the NODF metric was assessed against 1,000 randomizations using the fixed row and column totals constrained model, i.e. node linkage level was fixed. We calculated the probability of randomly obtaining higher NODF values than that of the empirical matrix with a one-tailed Z-test. All network metrics were obtained with the *bipartite* (version 1.17 Dormann et al. 2008) and *vegan* (version 2.0-6 Oksanen et al. 2012) packages implemented in the R statistical software version 2.15.0 (R Development Core Team 2012).

To test whether network structure changes when downscaling from sp-sp to i-sp, accounting for differences in network size, we built 1,000 null i-sp pollen-transport networks of the same size and species composition as the empirical i-sp networks. These null networks were build combining simulated i-sp submatrices for each species generated with the Patefield algorithm (i.e. observed marginal totals maintained for matrix rows and columns). Each null i-sp subnetwork simulated that conspecific individuals act as generalists as their species, sampling each pollen type at a rate proportional to the corresponding species pollen use distribution. Thus, in null i-sp submatrices with a rows and p columns (a was number of individuals of species S and p number of plant pollen types carried by species S), each individual was reassigned the same pollen load as observed but pollen grains were randomly distributed among pollen types with a probability equal to the observed pollen type proportion used by the species. We calculated the abovementioned parameters also for the 1,000 null i-sp networks. When parameter values of empirical i-sp networks did not fall into 95% confidence intervals of values for the null i-sp networks, differences were thus attributed to individual specialization and not to a network size artifact.

1.2.3 Evaluation of incidence and magnitude of individual specialization

We compared linkage level of each insect species (L_{sp}) with those of their individuals (L_i) to explore specialization at both levels. For species with ≥ 5 sampled individuals (14 spp. at CN, 7 spp. at PC; see species list in Supplementary Material Chapter 1 Table 10), we calculated average $\bar{L_i}$ of a given species, and compared it to the $\bar{L_i}$ obtained in null networks. We concluded that a species was composed of individuals being significantly more specialized than the species when empirical $\bar{L_i} <$ 95% of 1,000 null $\bar{L_i}$. Within a species, both generalist and specialist individuals were frequently found, so we evaluated the presence of a nested pattern in species linkage level partition among conspecifics. We followed the same procedure explained above for NODF calculation with a set of 21 i-sp submatrices (matrices for species with ≥ 5 sampled individuals) (see Figure 6 for an example).

Relative degree of individual specialization in pollen use was estimated for all those 21 species with ≥ 5 sampled individuals in each network. We followed the intrapopulation niche width variation model suggested by Roughgarden (1972) and indices developed by Bolnick et al. (2002, 2007). Total niche width (TNW) can be partitioned into two components: a within-individual component (WIC) and a between-individual component (BIC), thus TNW = WIC + BIC. WIC is the average variance in the range of pollen types each conspecific individual is using, i.e. the average of individuals' niche breadth. BIC is the variance in mean pollen use among individuals of the particular species, i.e. it represents the niche variation among individuals. Relative degree of individual specialization is measured as the proportion of total niche width (TNW) explained by the within-individual component, WIC/TNW, and is thus a species-level metric. This metric approaches 1 when individuals' niches include the full range of pollen types used by their species, and tends to 0 when individuals use smaller, non-overlapping subsets of their species' resources. We applied Monte Carlo re-sampling procedures (Bolnick et al. 2002, Araújo et al. 2008, 2010) to test whether the observed individual specialization was significant, i.e. observed WIC/TNW values were less than 95% confidence interval of WIC/TNW values obtained for the 1,000 null i-sp submatrices of each species (null hypothesis that all individuals act as generalized as the species).

At the individual level, we estimated specialization by calculating a proportional similarity index (PS_i), which measures similarity in the use of pollen (diet overlap) between an individual i and its corresponding species S. All indices were obtained using R (version 2.15.0, R Development Core Team 2012), following formulae described in detail in Supplementary Material Chapter 1 section A.2.

1.2.4 Analysis of ecological factors affecting the degree of individual specialization

1.2.4.1 Interspecific overlap

To measure interspecific overlap, we transformed our two-mode pollen-transport networks into one-mode networks depicting the pattern of shared pollen resources. By definition, two-mode networks represent the pattern of interactions among two different subsets of nodes (e.g. pollinators and pollen types), whereas one-mode networks represent interactions among nodes of the same set (e.g. pollinators). We constructed one-mode projections of the sp-sp two-mode network matrices at each study site following the co-occurrences projection method (Opsahl 2009a, Padrón et al. 2011), which entails counting the number of pollen types shared among each pair of different insect species. We thus obtained a square symmetric matrix with a zero diagonal and with s rows and s columns, where s is the number of insect species and the value in each cell w_{ij} is the number of pollen types shared between them. Thus, total interspecific overlap for a species s_i can be defined as the sum of all its weighted links $\sum_j w_{ij}$ (degree for weighted networks in Barrat et al. 2004). For example, a species will get a total interspecific overlap of six by sharing one pollen type with six different insect species but also by sharing six different plant pollen types with one insect species. In order to compare species among sites, we calculated a standardized measure of interspecific overlap (species-species overlap, SPO), by dividing total interspecific overlap by the maximum overlap that a species can achieve in its network (i.e. when an insect species shares all pollen resources with all other insect species). Thus,

$$SPO = \frac{\displaystyle\sum_j w_{ij}}{p \cdot (s - 1)} \tag{1.1}$$

where p is total number of pollen types in the community and s is total number of insect species. SPO ranges from 0 (no interspecific overlap) to 1 (maximum overlap). For simplicity, we ignored phenological and size constraints and assumed all species were equally likely to share resources. SPO was calculated for each of the 21 selected insect species (section A.3 in Supplementary Material Chapter 1 gives an example of the procedure). All one-mode network analyses were done with the *tnet* R package (version 3.0.5, Opsahl 2009b).

1.2.4.2 Intraspecific overlap

To estimate the degree of intraspecific overlap for each species, i.e. the amount of overlap in pollen use among its individuals, the 21 two-mode submatrices (consisting of conspecific individuals and their pollen types) were transformed into one-mode weighted networks following the same approach as above. Each one-mode matrix consisted of a rows and a columns, with a being number of conspecific individuals of species S and the cell value (w_{ij}) was number of pollen types shared by the individuals i and j. The standardized measure of intraspecific overlap (individual-individual overlap, IO) was calculated as

$$IO = \frac{\displaystyle\sum_i \sum_j w_{ij}}{a \cdot n \cdot (a - 1)} \tag{1.2}$$

where $\sum_i \sum_j w_{ij}$ is the total overlap among all conspecific individuals in the subnetwork of S (sum of the link weights for all individuals of S) and the denominator is the maximum overlap for the subnetwork of S, i.e. when all the conspecific individuals share all pollen types used by the species (n = total number of pollen types used by S and a = total number of conspecific individuals of species S). See section A.4 in Supplementary Material Chapter 1 for an illustrative example of the detailed calculation method of intraspecific overlap. It is important to note that our estimates of inter- and intraspecific overlap in pollen use are only a 'proxy' of competition, as either individuals or species, respectively, might share the same flower species but use a different resource (e.g. pollen or nectar). We thus prefer to use the neutral term 'overlap' instead of competition because the real sign of the interaction is unknown.

1.2.4.3 Statistical analysis

Structural equation modeling (SEM) was used to determine the effects of inter- and intraspecific overlap, insect species abundance and species phenophase on the degree of individual specialization (WIC/TNW). This technique explores causal relationships among ecological variables and it allows decomposing total effects into direct and indirect effects (Grace 2006). We proposed a model with intra- (IO) and interspecific overlap (SPO) directly influencing WIC/TNW and also abundance and phenophase connected directly to WIC/TNW as well as indirectly through effects on IO and SPO (see full path diagram in Figure 8). Standardized path coefficients were estimated with maximum likelihood method, significance of each one determined with a Wald test and error terms expressed as $\sqrt{1 \quad R^2}$. Indirect effects were calculated by the product of the path coefficients connecting two variables of interest, and total effects were defined as the sum of direct and indirect effects. To assess whether the model fits the observed data we performed a Chi-square test of goodnes-of-fit (χ^2) and calculated the standardized root mean residuals (SRMR). A non-significant χ^2 indicates that predicted covariance among variables in the model is not distinguishable from the observed covariances, while SRMR calculates deviations between observed and predicted covariances. Variables were log transformed when necessary to meet the normality assumption. All analyses were done in R version 2.15.0 (R Development Core Team 2012) with package *lavaan* (Rosseel 2012).

1.2.5 Sampling limitations and study caveats

Downscaling a community-wide pollination network from sp-sp to i-sp is a methodologically complex task, and consequently several sampling limitations must be noted. First, owing to the difficulty in identifying pollen grains to species level, we made 'pollen type clusters', referring to pollen from closely related species (see section A.1 in Supplementary Material Chapter 1 for details). This means specialization and overlap levels might be overestimated in some cases. Downscaling may improve our understanding of networks, but achieving sufficient sampling to resolve them is hard, even more than in sp-sp networks (Chacoff et al. 2012). For that reason, the estimation of individual specialization is restricted to a small proportion of the total number of species in the networks

(19% for CN and 11% for PC). The number of replicates per species is relatively low (5-10 sampled individuals), mainly because quantifying pollen loads is a highly time-consuming task. However, we evaluated completeness of interaction detection for each species (see section A.5 in Supplementary Material Chapter 1) and 69% of the expected interactions were detected on average. Studies on the degree of individual specialization have not yet determined the minimum number of individuals of a population needed to be sampled to get reliable estimations (Araújo et al. 2011). By applying Monte Carlo resampling procedures, we partly overcame the problem of overestimating the measures of individual specialization due to low sample sizes (Bolnick et al. 2002, Araújo et al. 2008).

Temporal consistency of the observed individual specialization is important. Here, each individual pollen load sample, rather than a snapshot of the individual foraging behaviour, is a picture of the extended flower visitation history of the individual (Bosch et al. 2009). Thus, pollen loads can be reasonable estimators of individual's long-term foraging patterns because pollen grains can remain attached to insect bodies for long periods (Courtney et al. 1981). Obviously, the attachment time depends upon species-specific grooming behaviour and body surface hairiness, as well as pollen grain surface structure. Although we lack information about specific pollen attachment times, we identified pollen grains on insects even one month after the last flowering plant of a given species was blooming in the area (e.g. *Rosmarinus officinalis*).

1.3 Results

1.3.1 Structural parameters of sp-sp and i-sp networks

Downscaling from sp-sp to i-sp modified most network topological parameters studied (Table 2). The direction and magnitude of the observed changes depended on the foraging behaviour of insect individuals, as shown by the contrasting results obtained between null models and empirical networks. Empirical i-sp networks were obviously larger in size than sp-sp, as most species were resolved into several individuals except singleton observations (i.e. insect species observed only once and therefore consisting of one individual). Consequently, at both study sites, downscaling increased total number of interactions (I) 1.5-fold (Figure 4), although significantly less than the expected with null i-sp networks. Linkage density (LD) and network connectance (C) in empirical i-sp networks was nearly half the expected compared to the null hypothesis. Therefore, such differences between sp-sp and i-sp can be attributed to a significant decrease in the number of links per pollinator node in empirical i-sp networks (Table 2), rather than to an effect of increasing network size. Single individuals had a narrower foraging niche than their corresponding species. Mean pollinator linkage level (L_A) in i-sp net-

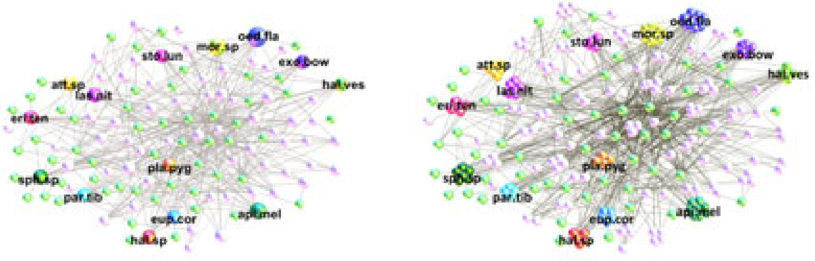
Table 2: Structural properties of the empirical sp-sp networks, empirical i-sp networks and null i-sp networks.

	sp-sp networks		**i-sp networks**			
	Empirical		Empirical		Null model	
	CN	PC	CN	PC	CN	PC
Qualitative network parameters						
Number of pollinator nodes (A)	73	61	190	137	190	137
Number of pollen type nodes (P)	55	49	55	49	55	49
Total number of nodes ($T = A + P$)	128	110	245	186	245	186
Network size ($N = A \times P$)	4,015	2,989	10,450	6,713	10,450	6,713
Total number of interactions (I)	434	360	681†	506†	1,342.48	881.78
Linkage density ($LD = I/(A + P)$)	3.39	3.27	2.78†	2.72†	5.48	4.74
Connectance ($C = I/A \times P$)	0.108	0.120	0.065†	0.075†	0.12	0.13
Nestedness (NODF)	34.45*	38.65*	26.99*†	29.67*†	44.29	43.77
Quantitative network parameters						
Interaction diversity (H_2)	3.38	2.89	3.63†	3.18†	3.95	3.36
Interaction evenness ($E_2 = H_2/H_{m\acute{a}x}$)	0.56	0.49	0.56†	0.51†	0.55	0.49
Node parameters						
Mean pollinator linkage level (L_A)	5.9	5.9†	3.5†	3.6†	7.07	6.44
Mean pollinator interaction diversity (H_A)	0.79	0.70	0.54†	0.54†	0.99	0.78
Mean pollen type linkage level (L_P)	7.89	7.35	12.38†	10.33†	24.41	18
Mean pollen type interaction diversity (H_P)	0.62	0.57	0.76†	0.69†	1.46	1.21

[*] Significance p-value < 0.001. That is the probability of getting by random a higher value of nestedness than the empirical one. See text for more details on the calculation.

[†] Observed values were outside of 95% confidence intervals of values obtained for 1,000 null i-sp networks.

works was c. 50% lower than that predicted by the null model. Mean interaction diversity for pollinators (H_A) was also significantly smaller when downscaling, because individuals transported fewer and a more variable load of pollen types than their corresponding species. Because of this idiosyncratic and heterogeneous individual behaviour, changes in network nestedness were also observed. Both sp-sp and i-sp networks were significantly nested; however, NODF values were consistently lower in empirical i-sp than in null i-sp networks. Furthermore, minor but statistically significant decreases of interaction diversity (H_2) were observed in empirical i-sp networks compared to null models, due to differences in the number of interactions, whereas interaction evenness (E_2) increased showing a reduction in the skewness in the distribution of link frequencies.



(A) sp-sp network *(B) i-sp network*

Figure 4: Pollen-transport networks at two scales of resolution: (A) species-species (sp-sp) and (B) individuals-species (i-sp). Pale green nodes are plant pollen types whereas the rest of nodes are pollinators. Coloured and labelled nodes are pollinators with ≥ 5 individuals captured (see Table 10 for full species names). In network (A) each pollinator node represents an insect species with a size proportional to the number of individuals captured in the field. The result of decomposing each insect species node into its conspecific individuals is seen in network (B), where each pollinator node represents an insect individual and individuals of the same species are clumped together in the graph. Networks were drawn with *Gephi 0.8beta* (Bastian et al. 2009).

1.3.2 Partition of species linkage level among conspecific individuals and relative degree of individual specialization

In general, species' linkage level - or species' niche width - was partitioned among specialized conspecific individuals. Most individuals had a much lower L_i than their species (L_{sp}) (Figure 5), i.e. individuals were always more specialized than their corresponding species (average ratio $L_i/L_{sp} = 0.55$). This was also observed when insect orders were treated separately (Supplementary Material Figure 22). For the 21 species with ≥ 5 individuals, $\bar{L}_i$ was significantly lower than expected under the null hypothesis (Supplementary Material Table 10). Therefore, a generalist species was composed of specialist individuals using different resources, and only in a few cases did individuals have a similar feeding niche as their corresponding species (Figure 6). When examining how resources are partitioned among individuals within a species, we found a nested pattern only in five out of 14 species at CN [NODF: *Halictus* spp. (*H. scabiosae* and *H. fulvipes*) = 60.39, *Halictus vestitus* = 52.71, *Oedemera flavipes* = 50.29, *Paragus tibialis* = 48.77, *Stomorhina lunata* = 49.46; $P < 0.001$] and in one out of seven species at PC (NODF: *Halictus* spp. = 50.90, $P < 0.001$). Thus, for most species, individuals were highly heterogeneous in their use of pollen, and interactions of the most specialized individuals were rarely proper subsets of those of more generalized individuals.

On average, an individual niche represented c. 46% of total species niche (mean WIC/TNW ± SD; CN = 0.45 ± 0.25, PC = 0.48 ± 0.27; empirical WIC/TNW < null WIC/TNW for all species) (Figure 7, Supplementary Table 10). At both sites, a large fraction of individuals (63.5% in CN and 54% in PC) had a niche which differed strongly from their species' niche, i.e. $PS_i < 0.5$ (Supplementary Material Figure 23). However, the intraspecific variability of PS_i was high (average coefficient of variance in mean PS_i of species was 59% at CN and 86% at PC). Thus, most species consisted of both generalist and specialist individuals (examples in Figure 6).

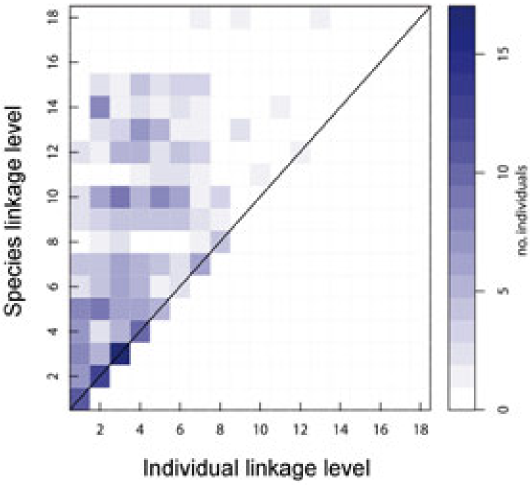


Figure 5: Comparison of species linkage level (L_{sp}) and individual linkage level (L_i). Cells represent combinations of L_i and L_{sp} (data from both sites, $n = 327$) with colour intensity correlating with number of individuals found at each position. Matrix diagonal is the species-individual isocline (i.e. perfect matching of species and individuals niche width, $L_i = L_{sp}$), while deviations to the left indicate individuals, being more specialized than their species ($L_i < L_{sp}$). The figure shows a high density of individuals with a linkage level lower than their species, although some individuals are positioned on the species-individual isocline (mainly species captured one or a few times). Notice that the upper right region representing highly generalized species with generalized individuals is completely empty.

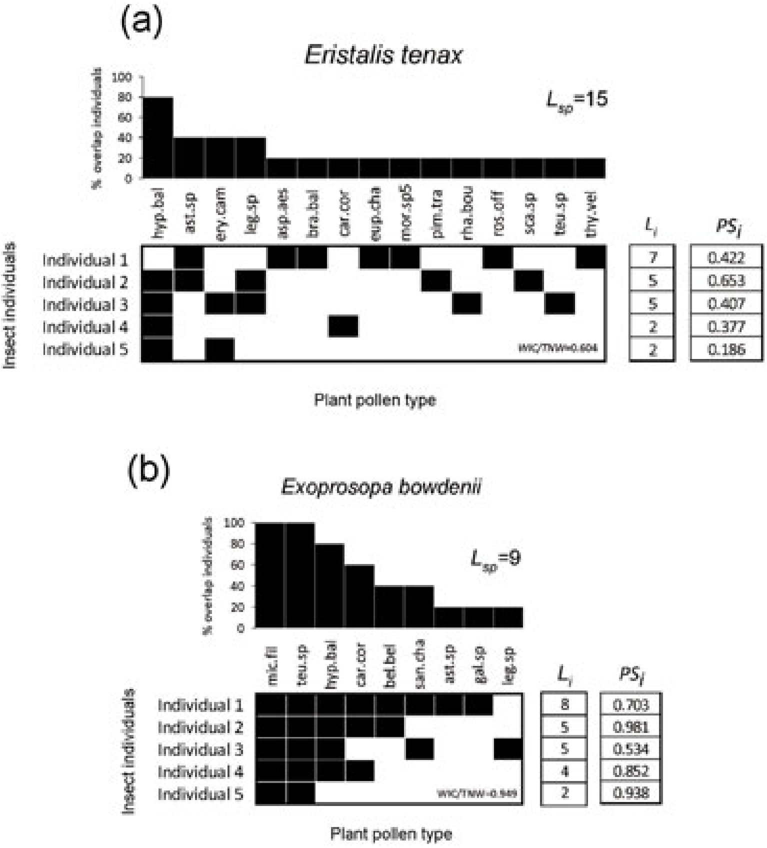


Figure 6: i-sp binary pollen-transport matrices of two dipteran species representing linkage level partitioning among conspecifics. There are two alternative mechanisms to evolve a wide species niche: (a) generalist species composed of relatively specialized individuals using different pollen types and (b) generalist species composed of relatively generalized individuals using broad and similar subsets of resources. Species with high heterogeneity in pollen use among conspecifics (example a) were more common. Bar plots on top of the matrices show percentage of conspecific individuals carrying each pollen type, so generalized species with specialized individuals have a long tail. (L_{sp}: species linkage level; L_i: individual linkage level; PS_i: proportional similarity index; WIC/TNW: degree of individual specialization).

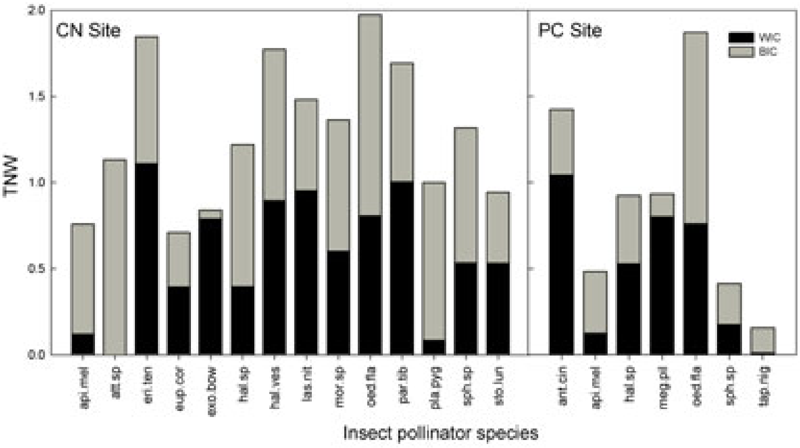


Figure 7: Total niche width (TNW) partition into within-individual (WIC) and between-individual (BIC) components for species with ≥ 5 individuals sampled ($n = 21$, 10 Hymenoptera spp., 7 Diptera spp. and 4 Coleoptera spp.) at both study sites (CN = Coma de n'Arbona, PC = Passadís de Ses Clotades).

1.3.3 Factors affecting the degree of individual specialization

Indices of inter- and intraspecific overlap in pollen use are summarized in Supplementary Materials Table 10. Results for the proposed SEM model are reported in Figure 8. Observed data fitted reasonably well the proposed model ($\chi^2 = 0.198$, $d.f. = 1$, $P = 0.66$). High levels of inter- and intraspecific overlap reduced individual specialization (increased WIC/TNW values). From all ecological factors included in the model, insect species abundance showed the strongest total effect on individual specialization (-0.561, $P = 0.002$), partly mediated through its significant negative association with intraspecific overlap (association with interspecific overlap was non-significant). This suggests that as species abundance increases, individuals use a smaller subset of the whole species niche, thereby reducing overlap between conspecifics. Likewise, species phenophase significantly affected intraspecific overlap, but not interspecific overlap, i.e. species with short phenophases had individuals with greater overlap among conspecifics than species with long phenophases. This relationship caused a negative indirect effect on WIC/TNW (-0.358, $P = 0.02$), although the total effect was non-significant (-0.168, $P = 0.35$).

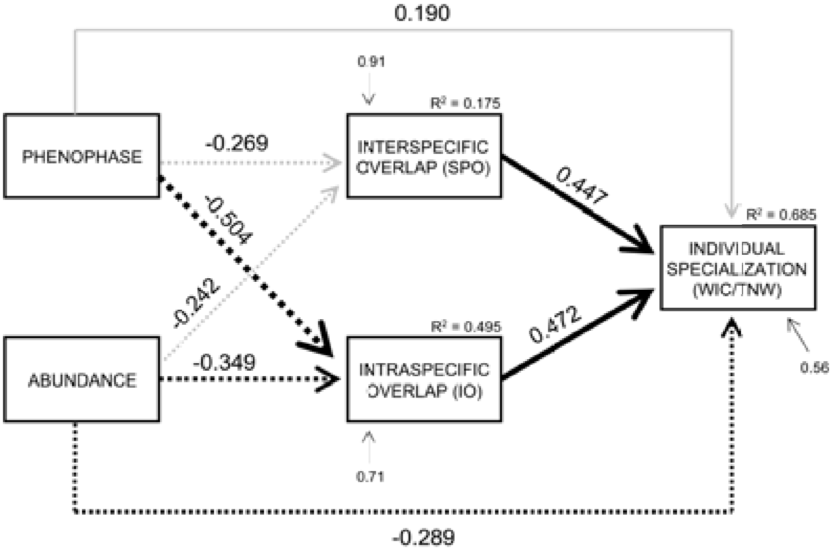


Figure 8: Path diagram showing the relative effect of several ecological factors (insect phenophase, insect abundance, inter- and intraspecific overlap (SPO and IO respectively) on the degree of individual specialization (WIC/TNW). Positive effects are indicated by solid lines and negative effects by dashed lines. Thickness of arrows is proportional to the standardized path coefficients indicated with numbers next to each path. Significant paths are coloured in black, whereas non-significant ones are in grey. R^2 and error terms are shown for each endogenous variable. Statistics of goodness of fit for this model are: $\chi^2 = 0.198$, $df = 1$, $P = 0.656$, SRMR = 0.02.

1.4 Discussion

Our analyses showed that when downscaling from sp-sp to i-sp pollen-transport networks different structural parameters changed significantly, specifically linkage density, connectance, nestedness and interaction diversity. The rationale for such changes appears to be the high degree of individual specialization for most pollinator species. This heterogeneity in pollen use and foraging behaviour among conspecific individuals has been overlooked in network studies, despite the potential misinterpretation of ecological dynamics and intra- and interspecific interactions occurring in the community. We discuss these results suggesting possible causes and implications of the main findings.

1.4.1 Network downscaling and individual foraging behavior

Downscaling to the individual level revealed a high degree of specialization and heterogeneity hidden within sp-sp networks. Generalist pollinator species were actually found to be composed of specialist individuals. Results showed empirical i-sp networks had lower linkage density, connectance, nestedness and interaction diversity than predicted by null models, because conspecific individuals were idiosyncratic in their food plant choice and foraging behaviour. Species linkage level was usually partitioned among specialist individuals (Figure 5), and this was true for all pollinators combined and also when separating species into orders (Hymenoptera, Diptera and Coleoptera, Supplementary Figure 22). Therefore, the most common mechanism for pollinator species to achieve a broad niche (here high L_{sp}) was to have individuals with different and narrow niches ($L_i \ll L_{sp}$) (Figure 6 a). Quantitative measures of individual specialization (WIC/TNW, PS_i) also confirmed this pattern and let us to reject the null hypothesis of individual generalist sampling from species pollen use distribution. Our average values of WIC/TNW were within the range reported by Araújo et al. (2011) for a broad array of taxa. Nevertheless, both generalist and specialist individuals were commonly found within a species. The frequency distribution of individual niche width was highly skewed, i.e. common species had only a few individuals with a wide niche. However, intraspecific partition of resources was quite overdispersed (i.e. non-nested) and the specialized individuals were not carrying a subset of the pollen types carried by more generalized conspecifics.

Network downscaling from species to individuals seems a promising way to connect pollination networks to pollinator foraging behaviour by further exploring mechanisms underlying the observed patterns. The foraging behaviour of insect pollinators is very flexible and a complex array of strategies for efficient collection of pollen and nectar have been described (Goulson 1999). For instance, generalist *Apis mellifera* individuals ($L_i/L_{sp} = 6/12$) may be scouts searching for new food resources independently of each other, whereas foraging workers, which are guided to food resources by specific waggle dances, only carry pollen from one or two species (Seeley 1983, Dupont et al. 2011). Furthermore, individual specialization in pollinator species may reflect the individual foraging behaviour described as flower constancy (Waser 1986). Flower constancy occurs in many pollinators when individuals restrict their visits to certain flowers, even ignoring more rewarding alternatives, although explanation of this behaviour is still in debate (Chittka et al. 1999). This individual specialization is likely to be beneficial to plants, since it might decrease heterospecific pollen deposition on conspecific stigmas thus preventing stigma clogging, as well as conspecific pollen loss on heterospecific flowers (Morales and Traveset 2008).

1.4.2 Factors influencing individual specialization

Variation in individual specialization depends both on intrinsic (e.g. sex, age, morphology, behavior and physiology) and extrinsic factors (e.g. ecological interactions, population density and diversity of resources) (Bolnick et al. 2003, Araújo et al. 2011). Among the extrinsic factors explored, we found evidence of a significant relationship between ecological interactions and inter-individual variation. Such relationship is likely to have ecological consequences for population and community dynamics (Bolnick et al. 2011, Wolf and Weissing 2012).

Low levels of interspecific overlap increased the degree of individual specialization, thus supporting the notion that in the absence of competing species individuals switch to different resources depending on their phenotypes (Costa et al. 2008, Bolnick et al. 2010). Populations can expand their diet breadth when individuals expand their niche and/or specialize on different niches (Bolnick et al. 2007, Tinker et al. 2008). Intraspecific competition has been documented to increase individual specialization (Svanbäck and Bolnick 2007, Araújo et al. 2008). However, this outcome depends on the type of rank preference variation among individuals, i.e. whether individuals have the same or different primary and secondary preferred resources (Svanbäck and Bolnick 2005, Araújo et al. 2011). Our results suggest a scenario where at low intraspecific overlap levels individuals are using different preferred resources, whereas at high levels they expand their niches adding the same resources, thereby reducing individual specialization.

In addition, population densities affect individual foraging decisions as each individual's choice depends on those made by other individuals depleting the floral resources. Our SEM model suggested that as species abundance increases, conspecific individuals become more specialized and heterogeneous in pollen choice, which is concordant with other studies (e.g. Svanbäck and Bolnick 2007, Tinker et al. 2012). Unexpectedly, species abundance was negatively associated to intraspecific overlap level. However, this might be explained when considering insect phenologies. Two species might be equally abundant by producing either a cohort with a high number of individuals during a short period or several cohorts with low numbers of individuals during a long period, but intraspecific competition would be stronger in the former case.

Finally, degree of individual specialization was not influenced by species phenophase. Species with intermediate-long phenophases (range 40-100 days) showed both high and low individual specialization, probably depending on whether they consisted of short-lived individuals, long-lived or both. Given that species phenophase was associated to intraspecific overlap (Figure 8), we might expect species with very short phenophases to consist of short-lived individuals with broad niches (relative to the corresponding species). On the other hand, species with long phenophases might consist of either short-lived individuals with narrow and non-overlapping niches or a combination of individuals with different phenophases and degree of specialization. Individual-based networks would certainly be a more informative tool to examine seasonal dynamics if, for instance, individuals present at the beginning of the season interact with early flowering plants whereas those present towards the end of the season do it with another set of plants.

1.4.3 Relevance of network downscaling

Downscaling networks from species to individuals is important in our efforts to explore mechanisms acting at the individual level, which further may upscale and shape species network structure (Olesen et al. 2010). Indeed, the individual-based networks reported here provide useful information to improve the understanding of species-based networks because most sp-sp networks contain a substantial proportion of singleton observations, which means they are based on observations of only one individual (e.g. 23% of an arctic network in Olesen et al. 2008, was based on singletons). Commonly, rare species in networks appear to be more specialized than they really are due to insufficient sampling of the rare interactions (Vázquez and Aizen 2003, Dorado et al. 2011). In concordance with this, our results suggest that a specialized behaviour of individuals compared to the species might be a possible explanation for the sampling bias in the estimation of linkage level in rare species. Similarly, most abundant species tend to be also the most generalized in pollination networks (e.g. Elberling and Olesen 1999, Olesen et al. 2008), but as we have shown here, this might actually cover a scenario where the conspecific individuals are specialized on different resources. Everything else being equal, individuals of abundant insect species are observed more frequently than those of rare species, and as new individuals are collected proportionally more new links are added to the species due to the specialized behaviour of the individuals. Thus, some broadly described specialization patterns in sp-sp pollination networks might have their origin in i-sp networks. Because ecological specialization is not a fixed species attribute and much variation exists within species, more studies are needed to explore ecological specialization across scales (Devictor et al. 2010).

Our findings highlight the importance of also taking inter-individual variation into account when studying higher–order structures such as networks, as part of our understanding of network structure and dynamics hidden on adjacent scale levels. For example, the high heterogeneity in pollen use among conspecifics enforces a high heterogeneity in interaction strength in species-based networks as well, which, so far, has been completely neglected. The strength of a sp-sp interaction depends on the number of individuals taking part in the interaction and the degree of their involvement. This has obviously important implications in the interpretation of community structure and dynamics (Bolnick et al. 2011, Sih et al. 2012). For instance, a pollen type is more likely to be an important resource to a species than other pollen types if a larger proportion of the population is using it. Consequently, having generalized species decomposed into dissimilar and specialized individuals might increase stability of species to the loss of a resource, because only a small proportion of the population would be affected (Wolf and Weissing 2012). By contrast, high individual specialization increases species vulnerability to the loss of individuals. A species composed of generalized individuals may lose a substantial proportion of its population before any effects are seen in the species-based network, whereas a species composed of specialized idiosyncratic individuals loses individuals and network links simultaneously. Therefore, our cross-scale level study suggests that individual foraging mode affects species persistence and, further, network stability. The demonstrated variation in individual resource use will affect the network outcome of disturbances, and information about the specific kind of disturbance will be important in our efforts to predict how network stability in detail is affected.

The intraspecific heterogeneity in pollen use might be related to trait variability among individuals, and most of these traits are subject to natural selection. Because there are trait-matching constraints in how links are distributed in networks, incorporating traits into models which predict species interactions have already helped to gain more insight in network structure and properties (e.g. Petchey et al. 2008, Stang et al. 2009, Ibanez 2012). Therefore, further research on individual-based networks would enable us to link network theory to evolutionary biology by working at the proper scale where natural selection takes place. Exploring all the potential bottom-up processes determining the emergent properties of interaction networks seems a promising avenue for future studies.

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2

Increasing modularity when downscaling networks from species to individuals

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Abstract

Downscaling networks from species to individuals is a useful approach to incorporate inter-individual variation and to investigate whether topology of species-based networks results from processes acting at the scale of individuals, such as foraging behaviour. Here, we analyzed pollen-transport networks at two scales, i.e. pollinator species-plant species (sp-sp) and pollinator individuals-plant species (i-sp), and assessed whether modularity – a prevalent pattern in most pollination networks – is consistent across both scales. To test this we use three different algorithms developed for the calculation of modularity (unipartite, bipartite and weighted bipartite modularity) and compare the results obtained. Downscaling networks revealed a higher modular structure in i-sp networks than in sp-sp networks, regardless of the modular metric used. Using a null model approach, we show that modularity at the individual scale is originated by the existence of a high heterogeneity and specialization in the partition of pollen resources among conspecific individuals, a pattern which obviously cannot be observed at the species level. Modules in i-sp networks consisted of individuals sometimes neither taxonomically nor functionally related, but sharing common pollen resources at different moments of the flowering season. Interestingly, conspecific individuals may belong to different modules. Both plant and insect phenologies were important drivers of the modularity detected in individual-based networks, even determining the topological roles of nodes in the networks. A temporal turnover of modules was identified, i.e. modules of individuals assembled and disassembled over time as species modify their foraging choices throughout the flowering season adjusting to ecological conditions. Downscaling from species to individual-based networks is a promising approach to study the interplay among structural patterns and processes at different, but interdependent organizational levels.

Key-words: species-based networks, individual-based networks, individual specialization, unipartite modularity, bipartite modularity, weighted bipartite modularity, topological roles, phenology.

2.1 Introduction

Interactions between plants and their pollinators can be represented at the community level using complex networks. Such real networks have topological features, which differ from randomly constructed networks (e.g. Bascompte and Jordano 2007). Detection of community structure within these networks is important in order to further identify the underlying ecological and evolutionary processes causing them (Lewinsohn et al. 2006) and to determine the potential consequences of such patterns for network stability and dynamics (Tylianakis et al. 2010). Nestedness and modularity are two of the patterns most frequently investigated in plant-pollinator mutualistic networks (Bascompte et al. 2003, Olesen et al. 2007). Both can coexist in the same network, being complementary rather than exclusive; even single modules are often nested (Lewinsohn et al. 2006, Olesen et al. 2007), although the correlation between modularity and nestedness depends on network connectance (Fortuna et al. 2010).

A species-species network has a nested link pattern if specialist species interact with proper subsets of the species interacting with the more generalist ones (Bascompte et al. 2003). On the other hand, a modular pattern consists of densely connected groups of species with sparse connections to species in other groups (Olesen et al. 2007). These strongly linked subgroups are called modules or compartments. Complex algorithms are needed to identify them (Guimerà and Amaral 2005b). Depending on the pattern of within and between-module connections, species can be classified into different topological roles (Guimerà and Amaral 2005a, Olesen et al. 2007). Especially, regarding their species composition, modules have been viewed as potentially co-evolutionary units of biological significance (Olesen et al. 2007, Dupont and Olesen 2009, Donatti et al. 2011).

A modular structure is derived from constraints in the interactions. Modularity in different kinds of network is indeed associated with a variety of ecological factors and explanatory processes (Bascompte and Olesen ress), such as: (i) convergence in pollination syndromes (Danieli-Silva et al. 2011); (ii) phylogeny and body mass in food webs (Rezende et al. 2009); (iii) trophic specialization and host range selection in plant-herbivore interactions (Prado and Lewinsohn 2004); (iv) species phenology in plant-pollinator networks (Bosch et al. 2009, Martín-González et al. 2012); (v) species niche organization and diet in food webs (Guimerà et al. 2010) and (vi) spatial or habitat segregation (e.g. Fortuna et al. 2009, Dupont et al. 2014). However, the degree of modularity and the number of modules in a network can be constant over time-cumulative periods (Dupont and Olesen 2012).

Modularity is a topological property important for network robustness. It may increase overall network stability because the spreading of perturbations across weakly connected modules occurs slowly so that effects stay embedded within modules (Fortuna et al. 2009, Stouffer and Bascompte 2011). The relationship between modularity and stability, however, may depend on whether the interaction is mutualistic or antagonistic (Thébault and Fontaine 2010) as well as on the specific type of perturbation.

Most networks studied to date are constructed at the species level, i.e. they represent interactions among species. However, a species is in fact a population of phenotypically diverse individuals, so species-based networks overlook the existing intraspecific variation. Individual variation within natural populations is a fundamental factor in ecological and evolutionary processes (Darwin 1859, Bolnick et al. 2011, Dall et al. 2012, Wolf and Weissing 2012). Ecologically, variation in traits (e.g. size, sex, age, social status) determines differences in foraging behaviours and resource use among individuals (Bolnick et al. 2003, Araújo et al. 2011), which in turn might affect structure, dynamics and stability of ecological interactions at a community scale (e.g. Beckerman et al. 2006, Bolnick et al. 2011). Therefore, downscaling networks from species-level to individual-level is a fundamental step for linking individuals to population dynamics and community structure (Ings et al. 2009, Beckerman et al. 2010), but also a way to link community biology to natural selection. For instance, further research is needed to investigate whether the structural properties described in species-based networks are maintained in individual-based networks or not (Tur et al. 2014), and which are the drivers behind patterns detected at the individual level.

Here, we investigate consistency of the modular pattern to network downscaling from species to individuals. We constructed pollen-transport networks from two mountain habitats at both species level (pollinator species-plant species network; hereafter sp-sp) and individual level (individual pollinator-plant species network; hereafter i-sp) by studying the pollen loads of insect flower-visitors. First, we explore modularity at both levels (i.e. species and individuals) using different modularity metrics – unipartite modularity (Newman and Girvan 2004, Guimerà and Amaral 2005a, Olesen et al. 2007), bipartite modularity (Barber 2007) and weighted bipartite modularity (Dormann and Strauss 2014) – and we address whether the pattern found is consistent across levels. We expect to detect modularity in i-sp networks if the pattern is already present in sp-sp networks, but we predict a stronger modularity in the former due to a high degree of individual specialization in the use of pollen resources (Tur et al. 2014) and the potential existence of individuals or groups with alternative foraging preferences within a species (Araújo et al. 2008, Tinker et al. 2012). To test this prediction, 100 null i-sp networks of same size and species composition as the empirical ones were constructed, but in which all conspecific individuals act as generalized as their species (i.e. there is no degree of individual specialization). The comparison of null and empirical i-sp networks allows identifying how much information is lost when intraspecific variation is not considered in interaction networks. Given that results may vary depending upon the modularity metric used, particularly in the identification of modules (Thébault 2013), we compare the different metrics. Second, we analyze how conspecific individuals are organized into modules. Conspecific individuals with similar interaction patterns are likely to aggregate in the same module. Alternatively, conspecific individuals specialized on different pollen resources might belong to different modules and thus the degree of individual specialization would in turn affect the degree of heterogeneity in module membership within species, i.e. species with a higher degree of individual specialization might have conspecific individuals spread into a higher number of modules than species with a low degree of individual specialization. Third, we discuss the drivers of modularity in i-sp networks and the underlying mechanisms influencing the distribution of individuals across modules. As only a few pollination networks have been studied at the individual level (see Dupont et al. 2011, 2014, Gómez and Perfectti 2012, for single species network approaches), the ecological factors causing modularity at this level are poorly known. Specifically, we focus upon pollen resource affinity among individuals and phenology as drivers of module composition. Finally, we identify the topological roles of insect individuals and plant species in i-sp modules, and explore the influence of abundance, phenophase and linkage level upon topological role assignment. As far as we know, our study is the first to investigate the interplay between two organizational levels in modularity pattern of pollination networks and to assess whether modularity is driven by the same or different factors at the two levels.

2.2 Materials and methods

2.2.1 Data sampling and network construction

We studied interactions between plants and insect flower-visitors at two locations from the highest mountain in Mallorca (Puig Major, 1445 m): (i) Sa Coma de n'Arbona (CN) at 1100 m a.s.l. (39°48′5″N, 2°47′9″E) and (ii) Passadís de Ses Clotades (PC) at 1400 m a.s.l. (39°48′34″N, 2°47′50″E). Fieldwork was conducted throughout the flowering season (May to August 2010). Observations of pollinators (i.e. insects visiting flowers and touching the reproductive parts of these) were carried out on randomly selected single plants or patches during 5–min surveys. We recorded identity and number of observed pollinator individuals. When possible, these pollinators were captured at the end of the survey and stored separately in clean vials for later taxonomical identification and pollen load analysis. All plant species in bloom in the area were surveyed three times a week, on calm and sunny days, between 10 am and 5 pm. Flower abundance (flowers/m^2) of each plant species was estimated every two weeks by counting the number of open flowers in fixed transects (nine in CN and three in PC). A total of 190 individuals (71 Diptera, 83 Hymenoptera, 33 Coleoptera, 3 Hemiptera) belonging to 73 distinct insect species were captured in CN and 137 individuals (43 Diptera, 64 Hymenoptera, 26 Coleoptera, 4 Hemiptera) from 61 species in PC. Number of individuals per species ranged from 1 to 10 (mean ± SD: 2.44 ± 1.81). In the laboratory, the pollen load of each pollinator specimen was examined. Using a pollen reference collection from the study sites, we identified the pollen types and counted all pollen grains from the body surface of each insect following the methodological procedure in Tur et al. (2014). In total, we recorded 55 pollen types on insects from CN and 49 pollen types on insects from PC. On average (mean ± SD), 17982 ± 80588.4 and 18654 ± 95421.8 pollen grains per insect individual were counted in CN and PC, respectively. Data from the pollen load analysis (available at *Dryad Digital Repository*: `http://dx.doi.org/10.5061/dryad.63fp5`) were used to construct pollen-transport networks depicting the interactions between plant–pollen types and insect pollinators (see Tur et al. 2014, for details about data). We built both binary and weighted interaction matrices for each study site at two scales of resolution: (i) sp-sp, i.e. insect species and plant–pollen types and (ii) i-sp, i.e. insect individuals and plant–pollen types. In binary matrices an interaction between an insect individual or species (in rows) and a flowering plant taxon (in columns) was present (i.e. corresponding cell filled with a 1), if pollen was detected on the body of the insect. In weighted matrices, interactions have an associated weight measured as the specific number of pollen grains from each pollen type identified on the body of insects.

2.2.2 Construction of null i-sp networks

Networks at different scales differ in size, because downscaling from sp-sp to i-sp networks increases the total number of nodes as many of the pollinator species were represented by several individuals. Given that most network descriptors are affected by network size (Dormann et al. 2009), a null model is needed to carry out comparisons across scales (species and individuals) accounting for network size-related differences. Therefore, we built 100 null i-sp weighted networks for each study site with same size and species composition as the empirical i-sp networks. In these null networks each conspecific individual was reassigned the same pollen load as observed, but pollen grains were redistributed among all pollen types used by the corresponding species with a probability equal to the observed pollen type proportion used by the species (see more details in Tur et al. 2014). Thus, in these null i-sp networks, individuals act as generalized as their species (i.e. there is no degree of individual specialization) and so the null i-sp weighted networks constructed for each site serve both as a control for network size and for individual specialization. A binary version of the null i-sp networks was obtained by transforming the null weighted matrices into presence-absence matrices.

2.2.3 Modularity analysis

For each pollen-transport network (i.e. empirical sp-sp networks and i-sp networks at both study sites), the level of modularity, number of modules and composition of modules were calculated using three different metrics: (a) unipartite modularity (Newman and Girvan 2004), (b) bipartite modularity (Barber 2007), and (c) weighted bipartite modularity (Dormann and Strauss 2014). The difference among these metrics is that each one was designed for a particular type of network (i.e. binary unipartite, binary bipartite and weighted bipartite networks). Thus, for the first two measures, the binary interaction matrices were used for the analysis, whereas in the last one we used the weighted matrices. All three metrics measure the extent to which interactions are organized into subgroups of tightly linked nodes, so that modularity is high when within- module connectance is high and between-module is low. Modules were identified using the simulated annealing method (Guimerà and Amaral 2005a,b), a strong and accurate modularity-detection algorithm (Danon et al. 2005) which randomly rearranges nodes and modules until a maximum modularity is achieved. The first modularity measure calculated was unipartite modularity M_U (Newman and Girvan 2004, Guimerà and Amaral 2005a, Olesen et al. 2007) defined as

$$M_U = \sum_{m=1}^{N_M} \left[\frac{l_m}{I} \quad \left(\frac{d_m}{2I} \right)^2 \right] \tag{2.1}$$

where N_M is the number of modules in the network, I is the total number of network links, l_m is the number of links between nodes in module m (within-module links) and d_m the sum of the number of links of all nodes belonging to module m. M_U ranges from 0 to $(1 \quad 1/N_M)$. M_U , number of modules and composition of each one were calculated with the program NetCarto (Guimerà and Amaral 2005a,b) with the following input parameters: iteration factor = 0.95, cooling factor = 0.99 and final temperature = 0.

The second modularity metric calculated was bipartite modularity M_B defined by Barber (2007) as follows

$$M_B = \sum_{m=1}^{N_M} \left[\frac{l_m}{I} \quad \left(\frac{d_m^A \cdot d_m^B}{I^2} \right) \right] \tag{2.2}$$

where N_M is the number of modules in the network, I is the total number of network links, l_m is the number of links between nodes in module m (within-module links) and d_m^A and d_m^B are the sum of the number of links of the nodes within module m which belong to A-set and B-set respectively. Therefore, M_B is an extension of Newman & Girvan's measure (M_U) but taking into account bipartiteness of the network, i.e. network nodes of set A can only interact with nodes of set B. M_B, number of modules and composition of each one were calculated with the program BIPMOD (Thébault 2013).

The last modularity metric calculated was bipartite weighted modularity M_{BW} as proposed by Dormann and Strauss (2014)

$$M_{BW} = \sum_{m=1}^{N_M} \left[\frac{W_m}{W} \quad \left(\frac{w_m^A \cdot w_m^B}{W^2} \right) \right] \tag{2.3}$$

where N_M is the number of modules in the network, W is the total sum of link weights in the network ($W = \sum_{ij} w_{ij}$, i.e. total column and row sums), W_m is the total sum of link weights between nodes within module m ($W_m = \sum_{ij \in m} w_{ij}$) and w_m^A and w_m^B are the sum of the link weights of the nodes within module m which belong to A-set (row sums within module m) and B-set respectively (column sums within module m). M_{BW}, number of modules and composition of each one were calculated using function *computeModules* within the *bipartite* R-package (Dormann et al. 2008). For the calculation of this measure we previously log-transformed all link weights with log10 (number of pollen grains +1) in order to avoid having very large numbers in the matrices.

For all the modularity metrics considered, significance was assessed by comparing observed values against modularity values of 100 random matrices of same size and linkage level rank distribution (null models with fixed column and row totals). We calculated Z-scores as the difference between the observed modularity and the mean modularity of randomizations divided by their standard deviation. Networks with a Z-score > 2 were considered as significantly modular.

We evaluated the differences among the three modularity metrics and the concordance of the modules identified by each metric (i.e. which individual belongs to each module) in i-sp networks at both study sites. As the simulated annealing algorithm is a stochastic optimization technique, even different runs of the algorithm can yield different classifications of nodes into modules (Guimerà and Amaral 2005a). Thus, 10 runs for each empirical i-sp network and modularity metric were performed. Concordance of modules identified, both within runs of the same metric and among runs of different metrics, was estimated with the mutual information index (Guimerà et al. 2007; Thébault 2013), which ranges from 1 (when partitions are identical) to 0 (when partitions are uncorrelated) (see section B.1 in Supplementary Material Chapter 2).

To determine whether differences in modularity among networks at both scales (species and individuals) were a result of individual specialization rather than an artifact of network size, we also calculated the above mentioned modularity metrics for the 100 null i-sp networks constructed (see previous section). Modularity in empirical i-sp networks was considered as significantly higher when it ranged above 95% of modularity values obtained for these null i-sp networks.

Finally, for each pollen-transport network and for all significant modules detected inside these networks we determined: number of insect pollinator nodes (A), number of pollen type nodes (P), total number of nodes ($A + P$), total number of interactions (I), linkage level of each node (L), connectance (C) and nestedness (NODF). Connectance is the proportion of realized links from all possible links. NODF is a measure of nestedness (Almeida-Neto et al. 2008), which ranges from 0 for non-nested matrices to 100 for perfectly nested matrices. To test whether NODF was significant, values were compared with those obtained from 1,000 random networks with fixed row and column totals. All these network metrics were calculated with the *bipartite* (Dormann et al. 2008) and *vegan* (Oksanen et al. 2012) packages implemented in R v2.15.0 (R Development Core Team 2012).

2.2.4 Distribution of individuals among modules

In order to explore how conspecific individuals were distributed across modules in null and empirical i-sp networks, we selected species for which we sampled $\geq$ 5 individuals (14 spp. in CN and seven spp. in PC). For these species we quantitatively measured the dispersion of conspecific individuals between different modules, i.e. species module membership heterogeneity, with an index of qualitative variation IQV (Wilcox 1973) calculated as follows

$$IQV = \frac{N_M}{N_M \quad 1} \cdot \left(1 \quad \sum_{m=1}^{N_M} p_m^2\right) \tag{2.4}$$

where N_M is the number of modules and p_m the proportion of individuals of species i in a module m. IQV was obtained for each of the selected species (species for which we sampled $\geq$ 5 individuals) and ranged from 0, when all conspecific individuals are distributed inside the same module, to 1 when conspecific individuals are evenly distributed among all modules. We calculated IQV using module membership assigned by each different metric in one of the runs of the algorithm to see whether results where consistent regardless of the modularity metric considered. In null i-sp networks species IQV is 0, as all conspecific individuals have exactly the same interactions and thus belong to the same module. However, in empirical i-sp networks we expected species with a high degree of individual specialization in the use of pollen resources (Tur et al. 2014) to be more heterogeneous in module membership than species with a low degree of individual specialization. Thus, using simple linear regression analysis, we tested if species module membership heterogeneity in empirical i-sp networks (IQV) was related to their degree of individual specialization in pollen resources measured as explained in Supplementary Material section B.2. To identify whether IQV values calculated were associated to potential sampling biases, such as differences in the number of individuals captured per species or differences in species phenophase length, we performed Spearman correlations among IQV and these two variables.

2.2.5 Relationships between biological factors and modularity

We explored if pollen resource niche partitioning among individuals was associated to the modularity pattern, i.e. whether individuals within the same module were more similar in their pollen niches than individuals in different modules. For this, we used a multi-response permutation procedure MRPP (Mielke and Berry 2001) to test whether within-module pollen niche dissimilarity was less than expected by random. Pollen niche dissimilarity was calculated with pairwise Bray-Curtis distance from presence/absence i-sp matrices. The overall weighted mean of within-module dissimilarity ($\delta = \sum_{m=1}^{N_M} \frac{n_i}{N} \cdot \bar{d}_m$; where N_M is the number of modules, n_i the number of individuals within module m, N the total number of individuals in the network and $\bar{d}_m$ the average dissimilarity among individuals within module m) was compared against δ-values obtained for 1,000 permutations, which shuffled randomly individuals across modules to assess the significance level. The statistic A, which is a measure of within-module homogeneity compared to random expectation ($A = 1 \quad$ observed δ / expected δ), provided an estimate of effect size. A ranges from 1 (when within-module homogeneity deviation from random is maximum) to 0 (when it is random). MRPP was performed with R package *vegan* (Oksanen et al. 2012, R Development Core Team 2012).

To evaluate the role of phenology as a driver of modularity, we first classified all network nodes into phenological categories: (1) May, (2) June, (3) July and (4) August. For plant pollen types, we used the date of the flowering peak, i.e. date of maximum flower abundance in the field, or date of maximum abundance of pollen grains on insects when field data were not available. For insect individuals, we used the date of field capture. We analyzed the phenological composition of each module in i-sp networks. To test whether modules were significantly associated to the phenology of nodes we performed randomization tests of independence. We generated 999 permutations of the empirical contingency tables using fixed column and row marginal sums (i.e. representing no association between variables) and then calculated χ^2 statistic for each one. We counted the number of times (x) the χ^2 statistic for null permutations was greater or equal to empirical χ^2 and a P value was calculated as x/number of permutations + 1.

2.2.6 Relationships between node features and topological roles

In empirical i-sp networks, based on the network partition into modules provided by the M_U metric, a topological role to each node was assigned depending on its connectivity. This topological role is described by two parameters: within-module degree (z, i.e. standardized number of links to other nodes in the module) and among-module connectivity (c, i.e. the level to which a node is linked to other modules) (Guimerà and Amaral 2005a,b). According to c and z, nodes (both plants and individuals) were classified into four roles (Olesen et al. 2007): (i) peripherals, which are specialists ($z \leq 2.5$ and $c \leq 0.62$); (ii) connectors, which are nodes with low z and high c acting as glue among different modules ($z \leq 2.5$ and $c > 0.62$); (iii) module hubs, which are highly connected nodes but mainly linked within their own module ($z > 2.5$ and $c \leq 0.62$); and (iv) network hubs, which are super–generalists ($z > 2.5$ and $c > 0.62$).

Moreover, all nodes in empirical i-sp networks were also characterized by a list of biological features (linkage level, pollen abundance, flowering period length, and flowering peak for plant pollen types; individual linkage level, species abundance, species phenophase length, individual phenophase, and sex for insects) (see details in Supplementary Material section B.3). To assess the effect of these biological features determining the role of a node in i-sp networks, we performed a multinomial logistic model for plants and a binary logistic model for insect individuals. In the multinomial logit model for the plants the response variable was 'role' coded as a factor with three levels (P = peripherals, MH = module hubs, and NH = network hubs). Connectors were excluded from this model as too few plant nodes had this role. The predictors included were: (1) flowering period length, and (2) pollen abundance (with logarithm base 10 transformation). Neither linkage level nor flowering peak were included as predictors to avoid strong collinearity in the former and because no differences were detected among roles in the latter. The model estimated the probability ratio of being assigned into a certain role based on the predictors included, so that separate odds ratios were determined for all predictors for each role except one, which is set as a reference level and omitted from the analysis (P was selected as the base role in our model). On the other hand, for insect individual nodes we performed a binary logistic model where the response variable was 'role' coded as a factor with two levels (P and C) and the predictors included were: (1) linkage level of individuals, (2) individual phenophase (coded here as a factor with two categories: 'early season', including individuals from May–June, and 'late season' including individuals from July–August), and (3) the interaction between those two variables. For a straightforward interpretation of the models, marginal effects for each predictor and effects displays were calculated by fixing the other predictors at mean values. Analyses included in this section were performed with R packages *mlogit* (Croissant 2012), *nnet* (Venables and Ripley 2002) and *effects* (Fox and Hong 2009). R script written to perform all analyses included in this paper is deposited on *figshare*: `http://dx.doi.org/10.6084/m9.figshare.1190856`.

2.2.7 The downscaling approach: sampling considerations

Downscaling a whole pollination network from the species to the individual level is a challenging methodological task. Mark-reobservation of individuals (Dupont et al. 2011) or micro-radio telemetry tracking (Hagen et al. 2011) are two techniques successfully used earlier in studies of individual spatio-temporal foraging patterns in bumblebees. Despite such methods might work for sampling interactions of individuals in some pollinator species, both seem rather unfeasible for a multi-species sampling with limited human and budget resources. Instead, here pollen load analysis was used to estimate visitation patterns of insect individuals. However, several methodological issues must be considered when using this kind of data, and below we point out some of them for our particular dataset (see Tur et al. 2014, for more details). First, pollen identification to species level is sometimes uncertain for closely related species, so in these cases (four in total) we had to lump pollen from several species into one type. Second, number of individuals captured per species was low, i.e. for only 15% of all pollinator species did we sample ≥ 5 individuals, because it was not possible to capture all species whenever they were seen in the field and besides some species were rarely observed. Despite this, we calculated with rarefaction curves (following Chacoff et al. 2012) that the number of individuals captured allowed an average detection of 69% of the expected interactions per species. Interaction rarefaction curves can be saturated with fewer samples because most specimens carry pollen from more than one species (Bosch et al. 2009). Moreover, individuals collected from the same species were sampled over their entire activity period (see Figure 10), although we do not have information about the exact lifespan of each individual. Finally, pollen loads are assumed to be a reasonable proxy for the individual's interaction pattern over time. Rather than a single snapshot, pollen load analysis provides a longitudinal record of the flower visitation history of individuals (Bosch et al. 2009), because pollen grains can remain attached to the body of pollinators for long periods. Pollen from flowers visited days or weeks before insect capture might be present in low numbers, in spite of the grooming behaviour of the insects. We detected pollen grains on insects even up to a month after the end of the flowering of a particular plant species. However it is very difficult to precise the foraging period which is represented in these insect pollen loads, as we lack information about the exact lifespan of each individual of the different species.

2.3 Results

2.3.1 Comparing network modularity at the species and individual levels with different metrics

Network parameters calculated for networks at species and individual level are summarized in Table 3. Downscaling from sp-sp to i-sp networks increased total number of nodes, and also number of interactions (Table 3). However, as expected, empirical i-sp networks had less interactions and hence lower connectance values than null i-sp networks of the same size, because conspecific individuals are more specialized than their corresponding species. Moreover, sp-sp networks were significantly nested (Table 3) and remained nested when downscaling to the individual level, although NODF values for empirical i-sp networks were lower than for null i-sp networks.

The degree to which networks at the species level were modular varied depending on the modularity metric considered (Table 4). The M_U metric did not detect modules in the sp-sp networks from both sites but the other two metrics did (Table 4). By contrast, downscaling from species to individuals turned the networks more modular consistently with the three metrics. Modularity values of different metrics were quite similar, although the number and identity of modules identified in i-sp networks varied depending on the metric used (Table 4 and Supplementary Material Table 11). The metric showing the highest variation (% coefficient variation) among different runs of the algorithm for same network was M_{WB}, being more variable both in the modularity value returned ($M_U = 0.17\%$, $M_B = 0.25\%$, $M_{WB} = 4.71\%$) and the number of modules identified ($M_U = 2.67\%$, $M_B = 6.29\%$, $M_{WB} = 15.82\%$). Congruence of the identities of modules identified within different runs of the metrics was high (mutual information > 0.8) in the case of M_U and M_B, but not for M_{WB} which showed a concordance between runs comparable to the concordance existent among M_U and M_B (Supplementary Material Table 11).

In both study sites, modularity values of empirical i-sp networks were above the range of values obtained for null i-sp networks of the same size due to the specialization of individuals. This result was consistent regardless of the modularity metric used (Figure 9).

2.3.2 Composition of modules in i-sp networks: distribution of individuals among modules

Hereafter, and unless otherwise is indicated, we only provide the results obtained with M_U because this modularity metric has been that mostly used in pollination network studies (e.g. Bosch et al. 2009, Dupont and Olesen 2009). Although the

Table 3: Parameters describing the structure of the empirical networks at the different scales studied (sp-sp = species – species and i-sp = individuals – species) and the null i-sp networks constructed for comparison. Mean ± SD values (n = 100) of the null networks are shown.

	sp-sp networks		**i-sp networks**			
	Empirical		Null		Empirical	
	CN	PC	CN	PC	CN	PC
Insect pollinator nodes (A)	73	61	190	137	190	137
Pollen type nodes (P)	55	49	55	49	55	49
Total nodes ($A + P$)	128	110	245	186	245	186
Total interactions (I)	434	360	1342.79 ± 6.0	882.74 ± 5.88	681	506
Connectance (C)	0.11	0.12	0.13 ± 0.001	0.13 ± 0.001	0.07	0.08
Nestedness (NODF)	34.45^*	38.65^*	$44.22 \pm 0.46^*$	$43.78 \pm 0.42^*$	26.99^*	29.67^*

* $P < 0.001$. Significance value of NODF tested using the fixed row and column totals null model.

Table 4: Modularity values obtained for empirical networks at different scales (sp-sp: species-species, i-sp: individuals-species) and number of modules identified using different metrics. Significance of modularity is shown with Z-scores (> 2 is significant), calculated as the difference between observed modularity and mean modularity of 100 randomizations (fixed-fixed column and row sums null model) divided by their standard deviation.

	sp-sp networks		**i-sp networks**	
	CN	PC	CN	PC
Unipartite modularity				
M_U	0.31	0.31	0.37	0.38
Number of modules	5	5	6	5
Z-score	0.73	0.89	3.51	5.25
Bipartite modularity				
M_B	0.32	0.32	0.38	0.39
Number of modules	6	6	9	7
Z-score	7.39	3.69	7.95	8.34
Weighted bipartite modularity				
M_{WB}	0.32	0.33	0.33	0.39
Number of modules	6	6	13	8
Z-score	92.88	86.35	76.63	104.38

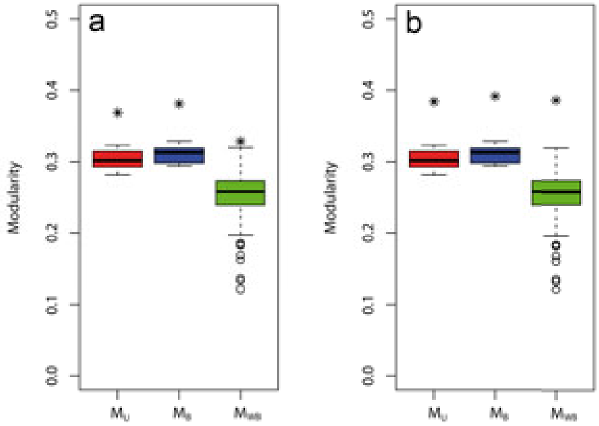

Figure 9: Modularity values of empirical i-sp networks obtained with three different metrics (M_U = unipartite modularity, M_B = bipartite modularity and M_{WB} = weighted bipartite modularity) compared to null i-sp networks without individual specialization (i.e. conspecific individuals act as generalized as their corresponding species). Box plots show the range of values obtained in 100 null i-sp networks whereas asterisks show the empirical modularity (a: CN site and b: PC site). Modularity in the empirical networks can be considered as significant when the asterisk falls outside the boxplot.

metric was originally designed for unipartite networks and makes no distinction between types of nodes, it performs well for bipartite networks and the modularity values obtained are similar to those obtained using the modularity metric designed for bipartite ones (Thébault 2013, this paper).

In the study site CN, the empirical i-sp network had six modules, with sizes ranging from 24 to 64 nodes, and two nodes disconnected from the main network (Supplementary Material Figure 25 a). However, all modules were strongly connected, as shown by the relatively high numbers of between-module links compared to within-module links (Table 5). At PC, the i-sp network had five modules: two large modules (49 – 65 nodes) with a high number of within-module links, two medium–sized modules (c. 30 nodes) with more between-module links than within-module links and finally, a small module of only two plants and four insect individuals (Supplementary Material Figure 25 b, Table 5). A nested pattern was found inside most modules (Table 5, Supplementary Figure 25). In more than half of the modules, insect individuals were structured around 1-3 plant pollen types, which acted as module hubs. Proportion of module and network hubs (always plant pollen types) was small in both networks (c. 2% of nodes). Most

Table 5: Composition and topological properties of modules detected in empirical i-sp networks at both study sites using the unipartite modularity metric.

	CN i-sp network						**PC i-sp network**				
	Mod1	Mod2	Mod3	Mod4	Mod5	Mod6	Mod1	Mod2	Mod3	Mod4	Mod5
Total nodes	64	38	29	24	33	55	32	49	6	34	65
Plant pollen types	18	10	7	4	5	10	10	22	2	9	6
Insect individuals	46	28	22	20	28	45	22	27	4	25	59
No. insect species	26	20	17	14	17	25	16	18	4	21	34
Within-module links	121	41	41	38	44	102	47	83	5	50	147
Between-module links	115	32	91	97	86	165	83	69	13	78	105
Nestedness (NODF)	46.98^{*}	18.72^{ns}	55.54^{*}	42.86^{ns}	56.13^{*}	51.65^{*}	39.28^{*}	38.35^{*}	50.00^{ns}	46.57^{*}	55.81^{*}
Connectance (C)	0.15	0.15	0.27	0.48	0.31	0.23	0.21	0.14	0.62	0.22	0.42
Topological roles of nodes											
Network hub	1	0	1	2	1	2	1	1	0	1	0
Module hub	2	1	1	0	1	0	0	1	0	0	3
Connector	10	3	13	16	16	9	8	1	3	7	1
Peripheral	51	34	14	6	15	44	23	46	3	26	61
Phenological composition											
(1) May	17	31	0	0	0	0	1	4	0	0	0
(2) June	28	3	3	0	0	3	3	44	0	26	6
(3) July	19	2	21	19	7	45	26	1	4	8	42
(4) August	0	2	5	5	26	7	2	0	2	0	17

* $P < 0.001$

^{ns}Non-significant

nodes (75%) acted as peripherals and the remaining as connectors (Supplementary Material Figure 26). The proportion of connectors in CN site was high (28%) compared to PC (11%), and they were mainly hoverflies and small bees.

Interestingly, when downscaling networks to the level of individuals, individuals of the same species did not belong to the same module. Instead, species module membership changed throughout the flowering season (Figure 10) and conspecific individuals were heterogeneously distributed among modules. In empirical i-sp networks, only two species, out of the 21 studied with ≥ 5 individuals captured, had all conspecifics grouped together within the same module (*Sphaerophoria* sp. in module 1 and *Exoprosopa bowdenii* in module 4 in the CN i-sp network). On average, a species belonged to 2.7 modules and the range was 1 – 5. Heterogeneity in module membership of conspecifics was 0.55 ± 0.27 at CN (mean $IQV \pm SD$; $n = 14$ species) and 0.63 ± 0.18 at PC ($n = 7$ species). A similar result was also obtained when M_B and M_{WB} metrics were used (M_B: $IQV_{CN\ site} = 0.59 \pm 0.14$; $IQV_{PC\ site} = 0.71 \pm 0.16$; M_{WB}: $IQV_{CN\ site} = 0.49 \pm 0.22$; $IQV_{PC\ site} = 0.61 \pm 0.19$). In null i-sp networks, as expected, individuals of the same species were always grouped inside the same modules, thus suggesting that individual specialization is a driver of module dispersion of conspecific individuals. However, the degree of heterogeneity in module membership among conspecifics was not proportionally related to the degree

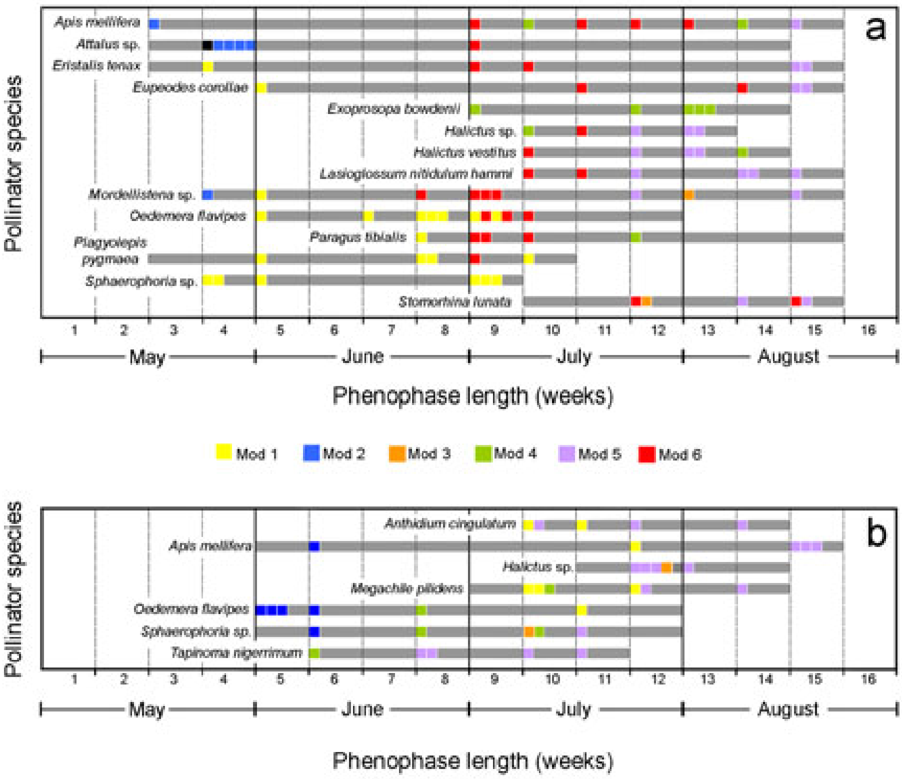

Figure 10: Grey bars represent active species phenophase duration estimated by field observations (a: CN site, b: PC site) and coloured squares indicate the time (sampling week) at which individuals were captured. Each square represents a single individual and the different colours show the module to which individuals belong. Squares representing captures of individuals within the same sampling week were coloured in the same order as individuals were captured. In most species conspecific individuals belong to different modules depending on the time of the season. Thus, species switch between modules, a behaviour that disappears at the sp-sp network level.

of individual specialization WIC/TNW ($R = \quad 0.05$, $P = 0.75$). Species with longer phenophases or those for which more individuals were sampled were not more dispersed among modules than species with shorter phenophases or with relatively few samples (Spearman's rho = 0.411, $P = 0.06$; Spearman's rho = -0.005, $P = 0.97$, respectively), rejecting possible influence from these variables.

2.3.3 Biological factors and modularity

Results from the MRPP analysis showed that within-module pollen niche dissimilarity was significantly less than expected by random in both i-sp networks, although the deviation was small (CN: $\delta = 0.59$, $A = 0.24$, $P < 0.001$; PC: $\delta = 0.64$, $A = 0.17$, $P < 0.001$). Thus, affinity in pollen resources was higher between individuals from the same module (CN: = 0.60; PC: = 0.62) than between individuals from different modules (CN: = 0.82; PC: = 0.84) (Supplementary Material Figure 27).

In addition, modularity in i-sp networks was also consistently associated to phenology (CN: Empirical $\chi^2 = 277.88$, mean permutations $\chi^2 = 15.18$, $df = 15$, $P = 0.001$; PC: Empirical $\chi^2 = 136.82$, mean permutations $\chi^2 = 12.23$, $df = 12$, $P = 0.001$). In most modules of the CN i-sp network we found a predominance of nodes from a certain month of the season (Figure 11a): (i) module 2 was mainly composed of plants flowering in May and insect individuals from the beginning of the season, (ii) module 1 contained 76% of all network plants and pollinator individuals from June, (iii) plants and pollinators from July were found in several modules, but 39% of insect individuals and 47% of flowering plants from this month belonged to module 6, and (iv) module 5 was made up of 57% of all plants and insect individuals from August. This seasonality in module composition was also detected in the PC i-sp network (Figure 11 b): (i) modules 2 and 4 included all plants with a flowering peak in June and 83% of pollinator individuals from this month, (ii) module 1 contained mainly plants and insect individuals from July, and (iii) module 5 was the largest module with 52% of total network nodes from July and 81% from August.

2.3.4 Association of node features with topological roles

Plant's and insect individual's topological roles in the network were determined by their biological features. Results from the multinomial and binary logistic models are reported in Supplementary Table 12. For plant pollen types, longer flowering periods and higher pollen abundances significantly increased the probabilities of being a network or module hub (Supplementary Figure 28a-b). The model estimated a very high probability for plants with low pollen abundances to be peripherals in the interaction network, whereas plants with high pollen abundance had a higher likelihood of becoming module hubs. Moreover, only plants with very long flowering periods (14 weeks) were likely to become network hubs. For insect individuals, as expected, increases in linkage level increased the likelihood of being a connector. For instance, for a node with $L_i = 2$ the model predicted a probability of being a connector to 0.08%, whereas for a node with $L_i = 10$ it was 90%. This positive effect of linkage level was higher for individuals present at the end of the season, as shown by the significant positive

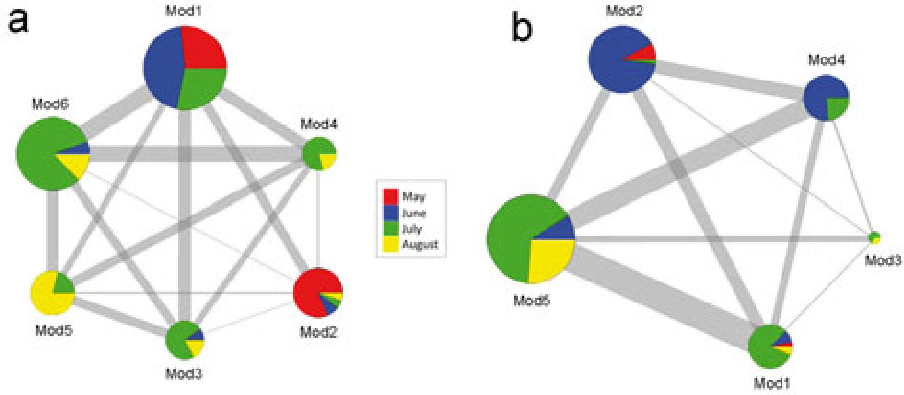

Figure 11: Representation of the two i-sp network graphs showing the change in phenological composition across modules in the two study communities: (a) CN, and (b) PC. Size of each pie chart is proportional to the number of nodes within each module and links connecting them are weighted by the number of between-module interactions. For insects, phenology (May, June, July, August) corresponds to the date the individual was captured whereas for plant pollen types it corresponds to the peak flowering date. A strong seasonality can be observed in each module.

interaction between individual linkage level and phenophase in the model (Supplementary Material Table 12). The average probability of being a connector in May–June (early season) was 26% whereas in July–August (late season) it was 65% (Supplementary Figure 28 c).

2.4 Discussion

Downscaling pollination networks to the individual level revealed a modularity pattern which can be hidden at the species level. Such modularity was associated to: (a) the heterogeneity and specialization in the partition of pollen resources among individuals, and (b) a dynamic switching of interactions within pollinator species during the season tracking plant flowering phenologies.

Results showed that when conspecific individuals are aggregated into species in the process of constructing species-based pollination networks, a misleading or incomplete picture of overall network patterns can be obtained because the existing inter-individual variation in flower foraging behaviours is not considered. For instance, in our study, empirical i-sp networks were less connected and nested than expected from the null models because generalized pollinator species were, in fact, composed of specialized and idiosyncratic conspecific individuals (Tur et al. 2014). Particularly, and in contrast to the results of Dupont and Olesen (2012) showing that a modular pattern was stable to changes in temporal scale, we found that modularity was not consistent across the two hierarchical scales of organization (i.e. species and individuals), regardless of the metric used to measure it. When downscaling, i-sp networks turned more modular than expected with our null model. The explanation for this is the strong specialization and heterogeneity in resource partitioning within species in empirical networks (Tur et al. 2014), as modularity tends to increase with higher specialization of interactions (Prado and Lewinsohn 2004, Lewinsohn et al. 2006). Therefore, our results suggest that individual specialization plays an important role in the magnitude of emergent modularity in i-sp networks. Further studies are needed to assess how consistent a modular pattern in pollination networks at the scale of individuals is. Exploring community structure at this level offers the opportunity to link network topology to the mechanisms underlying variation among conspecific individuals, such as differences in phenotypical traits, foraging preferences, sex, physiological condition or social status (Araújo et al. 2011, Dall et al. 2012), and thus ultimately differential natural selection regimes and evolution.

Resource partitioning and niche organization have been suggested as drivers of network modularity in previous studies at the species level (Prado and Lewinsohn 2004, Guimerà et al. 2010). Indeed, resource partitioning proved to be a driver of floral diversification in models (Rodríguez-Gironés and Santamaría 2010). Resource partitioning, however, operates at the individual scale, as foragers compare the available resources and make the choice providing the maximum energy intake (MacArthur and Pianka 1966). Differences in how conspecific individuals rank preferred resources can generate a modular network structure (Araújo et al. 2008, Tinker et al. 2012). Variation in how flower-visitor individuals forage through space can also determine the modular pattern of a network (Dupont et al. 2014). Here, modules in i-sp networks matched groups of individuals, which shared a common pool of pollen resources regardless of their species identities, i.e. individuals of the same species were not necessarily grouped into the same module. This means that, contrary to the traditional view in static species-based pollination networks, a species does not belong unambiguously to a single module, but, one may say, more or less to a module. For instance, a hoverfly individual of *Eristalis tenax* had a higher pollen resource affinity with a bee individual of *Osmia latrellei* than with another conspecific hoverfly individual. The identified modules were composed of functionally different pollinators (e.g. small bees, large bees, beeflies, hoverflies, flies) with overlapping pollen niches, so the view of modules as a set of species with convergent morphological traits (Olesen et al. 2007, Danieli-Silva et al. 2011) or taxonomical relatedness (Rezende et al. 2009) might not necessarily be the main rule at the individual level.

Conspecific individuals were distributed into different modules due to the heterogeneity in the use of pollen resources within species (Tur et al. 2014). By belonging to several modules a species might reduce intraspecific competition, as competition between modules can be lower than within modules (Rezende et al. 2009), although we did not test this hypothesis here. However, the degree of heterogeneity in module membership for each species was not proportionally related to a quantitative measure of the degree of individual specialization. This suggests that other factors might be important for the assignment of individuals to a particular module, such as species sociability traits, voltinism or other life history traits. Incorporating this kind of information in future studies as well as data on intraspecific trait variation will provide a better understanding on how interactions are distributed among individuals.

Phenology was one of the main drivers of modularity in the i-sp networks, implying that time means more than taxonomy. In most modules we detected predominance of plant pollen types and insect individuals present at a particular month of the season. Modularity in pollination networks has been associated to phenology in previous studies (Bosch et al. 2009, Martín-González et al. 2012). However, in our study, phenological compartmentalization was evident only when downscaling networks from species to individuals. At the individual level, a temporal dynamics hidden at species level appeared, revealing the existence of module turnover in the network. Modules changed through time during the flowering season, so as the season advanced new modules are formed and old ones dissolve. In fact, as the season progressed, pollinator species switched from one module to the next, a behaviour detectable only at the individual scale. Changes in species module membership through time might be a consequence of adjustment of foraging choices in response to changes in flower abundances, availability of resources and/or density of foragers throughout time (e.g. Goulson 1999). Therefore, a continuous interaction rewiring process is occurring at the species level which is driven by the dynamics of the adaptive foraging behaviour of individuals to resource fluctuations. These species switches in resource choice can enhance the stability of networks and community persistence (Kondoh 2003, Kaiser-Bunbury et al. 2010, Valdovinos et al. 2013).

Relatively few studies until now have attempted to correlate species traits with species roles (Donatti et al. 2011, Schleuning et al. 2014, Dupont et al. 2014). However, such knowledge might be relevant for the conservation of species interaction networks (Tylianakis et al. 2010). In our i-sp modular networks, phenology was an important determinant of network structure, and thus flowering period length of plant pollen types and phenophase of individual insect species turned out to be important attributes determining a node's topological role. Similar to other pollination networks (Dupont and Olesen 2009, 2012), the modules of individuals assembled around 1-3 plant pollen types, which were the module or network hubs. These plant hubs were species with long flowering periods (7- 10 weeks) and also with high abundances in the study area, such as *Hypericum balearicum*, *Santolina chamaecyparissus*, *Teucrium* spp., *Bellium bellidioides*, *Micromeria filiformis*, *Euphorbia characias* and several Asteraceae species. As modules detected in networks were related to a temporal dynamics, these network hubs become key species not only because of their importance to the cohesiveness of the entire network at a given point in time, but also because of their role acting as temporal couplers (Rasmussen et al. 2013). In contrast, no insect species acted as hubs, which seems to be an almost general trend in pollination networks (e.g. Dupont and Olesen 2009), also when sampling is not plant-centered like here. In particular, we note that even within a single pollinator species, individuals played different roles, thus not all individuals of a population are equivalent from a network structural point of view. This implies that the potential impacts of a disturbance might be different depending on whether affected individuals are connectors or peripherals. The loss of connector individuals, for example, might cause the isolation of modules (Olesen et al. 2007, Guimerà et al. 2010). Thus, our findings highlight the importance of considering intraspecific variation in foraging behaviours also for topological roles, although further studies are needed to determine which individual traits, in particular, define whether an individual acts as connector, peripheral or hub (Dupont et al. 2014). The downscaling approach improve our understanding of the structure and dynamics of species-based networks, as it assists in unravelling ecological processes which actually take place at the scale of individuals but act as potential network pattern drivers. This is, for instance, the case of individual foraging behaviour, which is known to be an important driver of network structure in food-webs (Beckerman et al. 2006, Petchey et al. 2008) as it ultimately determines which interactions are realized and which are not. In addition, the module turnover identified at the individual scale highlights the importance of studying networks from a temporal viewpoint, not only across years or seasons, but also in series of smaller temporal windows (Rasmussen et al. 2013) or at different organizational scales. Finally, network downscaling may facilitate bridging ecology and evolution through its focus upon determinants of individual fitness.

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3

Linking plant specialization to dependence on interactions for seed set in pollination networks

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Abstract

Studies on pollination networks have provided valuable information on the number, frequency, distribution and identity of interactions between plants and pollinators. However, little is still known on the functional effect of these interactions on plant reproductive success. Information on the extent to which plants depend on such interactions will help to make more realistic predictions of the potential impacts of disturbances on plant-pollinator networks. Plant functional dependence on pollinators (all interactions pooled) can be estimated by comparing seed set with and without pollinators (i.e. bagging flowers to exclude them). Our main goal in this study was thus to determine whether plant dependence on current insect interactions is related to plant specialization in a pollination network. We studied two networks from different communities, one in a coastal dune and one in a mountain. For ca. 30% of plant species in each community, we obtained the following specialization measures: (i) linkage level (number of interactions), (ii) diversity of interactions, and (iii) closeness centrality (a measure of how much a species is connected to other plants via shared pollinators). Phylogenetically controlled regression analyses revealed that, for the largest and most diverse coastal community, plants highly dependent on pollinators were the most generalists showing the highest number and diversity of interactions as well as occupying central positions in the network. The mountain community, by contrast, did not show such functional relationship, what might be attributable to their lower flower-resource heterogeneity and diversity of interactions. We conclude that plants with a wide array of pollinator interactions tend to be those that are more strongly dependent upon them for seed production and thus might be those more functionally vulnerable to the loss of network interaction, although these outcomes might be context-dependent.

Key-words: plant-pollinator networks, specialization, centrality, interaction effect, plant dependence on pollinators, seed production.

3.1 Introduction

Pollination is a very important ecosystem service (Costanza et al. 1997) because plants benefit from animal pollination for seed production. Nearly 85% of all flowering plants are pollinated by animals (Ollerton et al. 2011) and 35% of global crop production depends on pollinators (Klein et al. 2007). Thus, the study of plant-pollinator interactions and its functional consequences for plant reproduction have long interested ecologists. In the last decades, pollination ecology has expanded from studies focused in single species and involving pairs of interactions to wide community studies involving entire networks of interactions (Memmott 1999, Jordano et al. 2003, Dupont et al. 2003, Kaiser-Bunbury et al. 2009). Tools from network theory help to disentangle the structure and properties of these complex webs of interactions (Newman 2003, Proulx et al. 2005). This network approach revealed several interesting findings regarding pollination specialization/ generalization patterns at community level. The frequency distribution of species generalization (i.e. number of links per species or linkage level) follows a power-law distribution or truncated power-law (Jordano et al. 2003), i.e. there are many species with few interactions (specialists) and a few with many interactions (generalists or hubs). Specialist species tend to interact with proper subsets of the species that generalists interact with, thus leading to the broadly observed topological pattern of nestedness (Bascompte et al. 2003). Interestingly, interactions are asymmetric: (i) specialized plants tend to have generalized pollinators and vice versa (Vázquez and Aizen 2004) and (ii) the strength of each interaction is not reciprocal, so that if one plant is much dependent on a pollinator, that pollinator is not dependent upon that plant (Bascompte et al. 2006, Vázquez et al. 2007).

However, despite much information has been accumulated on the topology of pollination networks, studies linking network structure and functionality are still scarce (Gómez et al. 2011). The first studies have shown that network position of individual plants influences their fitness, individuals in central positions showing higher fitness than those in peripheral positions (Gómez and Perfectti 2012). Moreover, recent studies have made important advances providing field estimates of the magnitude of species impacts and interaction strengths (Vázquez et al. 2012). However, further research is needed to fill the existent gap of knowledge on the consequences of network links for plant reproductive success. This knowledge will help to determine the real plant functional dependence on such interactions and to make better predictions on how can they be affected by the loss of interactions.

Obviously, measuring the plant functional dependence in a per-interaction basis for all network links would require an enormous amount of fieldwork. Therefore, we propose a simplified approach consisting in measuring plant reproductive dependence on all pollinator interactions (i.e. pooling the effect of all pollinators). Dependence can be defined as the magnitude of seed set reduction when plant species are not pollinated by animals (Klein et al. 2007). Highly dependent plants are those for which a high reduction in seed set occurs when pollinators are excluded, i.e. plants for which animal pollination is essential. In the present study, we ask: are the plants with more links in the networks those that in turn are more dependent upon pollinators for seed production? Specifically, we want to assess whether the degree of plant dependence on pollinators to set seeds is associated with: (i) total number of interactions (i.e. linkage level), (ii) diversity of interactions or (iii) topological position of each plant species within the plant-pollinator network (closeness centrality). These indices have been proposed as measures of specialization in pollination networks (Dormann 2011). If the observed network links are contributing effectively to plant reproduction, we would expect number and diversity of interactions to positively influence seed set. Previous empirical studies have found a positive relationship between pollinator diversity and plant reproductive success (Kremen et al. 2002, Klein et al. 2003b,a, Fontaine et al. 2006, Hoehn et al. 2008, Albrecht et al. 2012). Therefore, plants with high diversity and number of links in the networks may be those depending more strongly on pollinators. However, some specific studies (Gómez et al. 2007) have found maximum reproductive success at intermediate levels of pollinator diversity, which suggests the existence of an optimal level of generalization. Moreover, plants in central positions in the network, i.e. highly connected to other plant species through shared pollinators, may experience a reduction in the amount of pollination received because of potential heterospecific deposition of pollen on stigmas by generalist pollinators (Morales and Traveset 2008). Alternatively, thus, plants with a high dependence on animal pollinators to produce seeds might rely just on a few but effective interactions.

3.2 Materials and methods

3.2.1 Sampling plant-pollinator networks

The study was conducted in two different communities from Mallorca (Balearic Islands, Spain): (i) a dune marshland community at sea level located in the northeast of the island (Son Bosc, 39°46′28.11″N; 3°7′45.34″E; SB hereafter) and (ii) a high mountain shrub community at ca. 1100 m above sea level (Sa Coma de n'Arbona in Puig Major, 39°47′59.51″N; 2°47′7.81″E; PM hereafter). Both communities differ in plant species composition (Bray-Curtis binary dissimilarity among sites is 0.9) and flower abundances, being much higher in the coastal (mean ± SD: 31.51 ± 145.58 flowers/m^2 per species) than in the mountain community (2.30 ± 5.893 flowers/m^2 per species). We sampled plant-pollinator interactions in both communities during two consecutive flowering seasons (years 2009 and 2010), from April to July at SB and from May to August at PM. Sampling method consisted of time-fixed (3 min in SB and 5 min in PM) pollinator censuses on randomly-selected plant individuals of every species in bloom. During each census, we recorded: (i) taxonomic identity of plant species observed, (ii) taxonomic identity of insect flower-visitors observed (pollinators, hereafter) and (iii) number of flower visits made by each pollinator species, i.e. number of pollinator contacts with flower reproductive parts. When pollinators were not identified in the field they were captured for further identification by taxonomist experts. All plant species in bloom in the communities were sampled weekly at each site, between 10:00 am - 5:00 pm on sunny and non-windy days. Weekly sampling effort was the same for all plant species in bloom regardless their abundance, although total census time accumulated throughout the sampling season differed across species, sites and years due to differences in plant species richness and flowering phenologies. In 2009, total census time was 42 h 18 min (SB) and 13 h 20 min (PM), while in 2010 it was 49 h 39 min (SB) and 38 h 15 min (PM).

For each study site, pollinator census data from the two years were pooled to construct a plant-pollinator weighted bipartite network. Plants and pollinators are nodes linked when an interaction between them was observed and each link has a specific weight depending on interaction frequency. These networks were represented by a quantitative interaction matrix $p \times a$, where p is the number of plant species in the community, a is the number of pollinator species and the value in each matrix cell n_{ij} is the interaction frequency measured as visits per flower per unit time made by pollinator j to plant species i. Interaction frequency is considered to be a good surrogate of total interaction effect of mutualist animals on plant reproduction (Vázquez et al. 2005, Sahli and Conner 2006, Vázquez et al. 2012). As simple descriptors of these networks we calculated: (i) network size (S), i.e. number of plant nodes × number of pollinator nodes; (ii) total number of interactions; (iii) average number of interactions per species (I); (iv) interaction diversity ($H2$), i.e. Shannon's diversity of interactions for the whole network; and (v) interaction evenness ($E2$), i.e. Shannon's evenness measuring the heterogeneity in the frequency of interactions across the network (0 = uneven, 1 = uniform).

3.2.2 Plant specialization level in networks

For a subset of selected plant species from our networks (27 species in SB and 11 species in PM, see next section for details), we calculated linkage level (L), diversity of interactions (H) and closeness centrality (CC). These indices result from different ways of measuring species specialization level in networks, matching different concepts and aspects of specialization (Dormann 2011). Linkage level

(L) is the total number of interactions for each plant species. A complete list of the observed insect pollinators and their interaction frequencies can be found in Supplementary Material Table 14 and Table 15. Diversity of interactions (H) is the Shannon-Wiener diversity calculated as

$$H = \sum_{j=1}^{S} p_j \cdot \ln p_j \tag{3.1}$$

where p_j is the interaction frequency among pollinator j and plant species i relative to total interaction frequency of i (row sum) and S is the total number of plant i's pollinators. Because diversity incorporates richness and evenness it can provide a much more accurate understanding of specialization, particularly when the number of flower visits is unevenly distributed across different pollinators. Closeness centrality (CC) was proposed as a measure of specialization in pollination networks (Martín-González et al. 2010) based on node position in the network. CC is the inverse of the average shortest distance between a focal plant species node and every other plant species nodes in a unipartite plant-plant network derived from the bipartite plant-pollinator network. In the unipartite network, two plant species are linked directly if they share at least one pollinator species. Therefore, CC measures the proximity of a plant species to other plant species. A plant is central when it has a high CC value which means is close to other plants in the network via shared pollinators. All indices were calculated using *bipartite* (Dormann et al. 2008, 2009) and *sna* packages (Butts 2010) in *R* statistical programme version 2.15 (R Development Core Team 2012). Software *Gephi 0.8* (Bastian et al. 2009) was used for network drawings.

3.2.3 Degree of plant dependence on insect pollination

For each study community, we evaluated the seed set -with and without pollinators- of several abundant and representative plant species. A total of 27 plant species were studied in SB, during 2009 and 2010, whereas 11 species were studied in PM in 2010. In both sites, the number of species selected (Table 6) represented ca. 30% of the entire plant assemblage, including 42% of all plant families present in SB and 35% in PM. These selected plant species covered the full range of specialization level in our networks (i.e. from specialist plants with one or two pollinators to generalist plants with more than 20 pollinators) and all were sampled a minimum observation time of 30 min in pollinator census. Two different treatments for each plant species studied were conducted: (i) Open pollination (OP), naturally pollinated flowers without manipulation and (ii) Pollinator exclusion (PE), in which flowers were covered with fine mesh bags that prevented insects visiting them but allowed wind- and self-pollination. Treatments started when plants had flowers at bud stage. Flowering branches or flower pedicels of each plant were marked, and flower units (flowers or inflorescences in the case of Asteraceae) were counted for each treatment. The number of flower units examined varied among individual plants and treatments depending on individual plant floral display and type of inflorescence (see table 13 in Supplementary Material). Plants were monitored until fruits were almost mature, and at this moment bags were removed and fruits were collected. In the laboratory, fruits were dissected and viable seeds counted under the stereomicroscope when necessary. Mean seed set for each treatment was calculated as the total number of seeds produced per marked flower unit.

Previous studies with crops (Klein et al. 2007) defined several levels of dependence on animal-mediated pollination by estimating the magnitude of seed set reduction comparing experiments with and without animal pollinators. Following the same approach, we calculated dependence on insect pollination (IPD) for each plant species as the percentage of open pollination seed set (SS_{OP}) attributable to insect pollinator interactions (i.e. open pollination seed set excluding self-pollination and wind-pollination seed set, $SS_{OP} \quad SS_{PE}$). Therefore, IPD ranges from 100 for plants which totally relied on pollinators for seed production (i.e. all seed set was a consequence of insect interactions), regardless of whether they produced many or few seeds, to 0 for plants that either selfed or were pollinated by wind. It is a useful index as it can be compared across different plant species and calculated using other measures of reproductive success different from seed set without losing meaning and interpretation. However, IPD cannot be considered as a measure of absolute plant species dependence on pollinators, as it may be contingent upon the current abiotic conditions, including resource availability, and we also need to consider the fact that plants may be pollen limited for several reasons (Ashman et al. 2004).

3.2.4 Data analysis

To test the relationship between IPD and plant specialization level we first performed simple linear and quadratic regressions. We retained the regressions providing the best fit (R^2) and lowest significance p-values (P). Variables were log-transformed when necessary to meet residuals' normality assumption. To ensure that results in the mountain community (PM) were not caused by a low statistical power due to the relative small number of species, we bootstrapped the data (1000 times resampling with replacement) to increase sample size from 11 to 27 species (same number of species as in the larger community, SB). Regressions were repeated with each bootstrap and the number of significant regressions was calculated.

The presence of phylogenetic related plant species in the community can produce biases in regression analyses, thus we performed the same regressions with Generalized Estimating Equations (*GEE*) (Paradis and Claude 2002). This method incorporates a correlation matrix of dependencies among observations in the modelling process. The correlation matrix is obtained from the phylogenetic tree of species in the community previously constructed with the free available software *Phylocom 4.2* (Webb et al. 2008). All phylogenetic analyses were done with function *compar.gee* from the *ape* package version 3.0-3 (Paradis et al. 2004) implemented in *R*. Tree polytomies were resolved randomly with function *multi2di*.

3.3 Results

Plant-pollinator networks studied had very different sizes and number of interactions. A total of 696 interactions between 80 plants and 162 insect species were recorded in SB and a total of 250 interactions between 34 plants and 92 insect in PM site (S_{SB} = 12960, S_{PM} = 8464). Both the average number (I_{SB} = 2.87, I_{PM} = 1.98) and the diversity of interactions per species ($H_{2\,SB} = 5.29, H_{2\,PM} = 4.53$) were higher in the coastal than in the mountain community, although the heterogeneity in interaction frequencies was similar in the two communities ($E_{2\,SB} = 0.80$; $E_{2\,PM} = 0.82$).

For the selected species ($N_{SB} = 27, N_{PM} = 11$), we report seed set obtained in each treatment in Appendix 13. Specialization indices and *IPD* are summarized in Table 6. Plants were less dependent on insects, on average, in SB (58.5 ± 38.1 %, mean ± SD) than in PM (70.9 ± 24.2 %). Results of the linear regressions between specialization indices and *IPD* are reported in Table 7. Results were consistent regardless phylogenetic relatedness among plants was controlled for or not. A significant relationship was found only in the larger and more heterogeneous coastal community (SB) (Figure 12). In this community, highly dependent plants tended to have more links and a higher diversity of interactions in the network than plants little dependent on pollinators (Figure 13 a,b). Furthermore, plants in central positions within the network (high *CC*), because they were visited by generalist pollinators which in turn visited many other plant species, showed also higher dependencies than plants occupying peripheral network positions (Figure 13 c). In the smaller mountain community (PM), the relationships between *IPD* and all three measures of plant specialization were non-significant (Table 7). Increasing the sample size with bootstrapping methods did not produce different results in the simple linear regressions (Appendix Figure 29), thus reducing the probability of an effect of statistical power and suggesting that there might be an ecological cause behind the lack of a relationship in this community.

Table 6: Specialization indices obtained for plant species studied in each site and degree of plant dependence on insect pollination (*IPD*). Obs. time: observation time accumulated in pollinator censuses (min), L: linkage level, H: diversity of interactions, CC: closeness centrality.

Site	**Plant family**	**Plant species**	**Obs. time (min)**	**L**	**H**	**CC**	**IPD(%)**
SB	Liliaceae	*Allium roseum*	50	11	1.50	0.87	79.72
SB	Liliaceae	*Asphodelus fistulosus*	135	12	1.94	0.92	0
SB	Scrophulariaceae	*Bellardia trixago*	71	4	0.93	0.78	0
SB	Gentianaceae	*Blackstonia perfoliata*	107	3	0.59	0.66	0
SB	Asteraceae	*Centaurea aspera*	120	17	1.66	0.88	46.42
SB	Gentianaceae	*Centaurium erythraea*	77	3	0.48	0.67	42.64
SB	Cistaceae	*Cistus salviifolius*	53	23	2.27	0.92	100
SB	Convulvulaceae	*Convolvulus althaeoides*	103	16	1.37	0.91	87.50
SB	Convulvulaceae	*Convolvulus arvensis*	113	25	1.63	0.95	87.03
SB	Asteraceae	*Crepis vesicaria*	67	15	2.14	0.92	97.74
SB	Apiaceae	*Daucus carota*	119	41	3.04	0.87	82.82
SB	Boraginaceae	*Echium sabulicola*	151	20	2.02	0.91	41.70
SB	Apiaceae	*Foeniculum vulgare*	42	10	1.78	0.78	61.31
SB	Asteraceae	*Helichrysum stoechas*	80	27	2.55	0.92	61.64
SB	Clusiaceae	*Hypochoeris achyrophorus*	68	11	2.02	0.86	96.43
SB	Asteraceae	*Hypericum perforatum*	80	9	1.66	0.80	19.74
SB	Fabaceae	*Lotus corniculatus*	147	18	2.38	0.89	100
SB	Fabaceae	*Lotus cytisoides*	89	9	1.43	0.88	100
SB	Fabaceae	*Medicago litoralis*	132	5	0.88	0.76	0
SB	Fabaceae	*Melilotus indica*	33	6	1.52	0.74	0
SB	Fabaceae	*Melilotus segettalis*	64	3	0.64	0.73	0
SB	Scrophulariaceae	*Parentucellia viscosa*	64	2	0.67	0.57	52.54
SB	Rosaceae	*Potentilla reptans*	86	28	2.62	0.96	98.96
SB	Asteraceae	*Scabiosa maritima*	120	24	1.87	0.95	78.45
SB	Caryophyllaceae	*Silene vulgaris*	70	3	0.36	0.77	96.77
SB	Lamiaceae	*Teucrium dunense*	92	28	2.08	0.94	63.84
SB	Scrophulariaceae	*Verbascum sinuatum*	101	11	1.49	0.78	85.02
PM	Caryophyllaceae	*Arenaria grandiflora*	75	8	1.75	0.73	77.02
PM	Asteraceae	*Bellium bellidioides*	135	13	2.20	0.86	75.81
PM	Asteraceae	*Carlina corymbosa*	80	18	1.86	0.82	96.72
PM	Asteraceae	*Crepis triasii*	85	14	2.12	0.89	94.68
PM	Rubiaceae	*Galium balearicum*	80	1	0	0.53	76.36
PM	Rubiaceae	*Galium cinereum*	85	2	0.28	0.70	25.61
PM	Cistaceae	*Helianthemum apenninum*	100	4	0.70	0.68	93.11
PM	Lamiaceae	*Rosmarinus officinalis*	45	9	1.39	0.79	42.40
PM	Asteraceae	*Santolina chamaecyparissus*	85	15	1.96	0.85	42.78
PM	Crassulaceae	*Sedum dasyphyllum*	90	8	1.84	0.82	89.90
PM	Lamiaceae	*Teucrium asiaticum*	135	13	1.93	0.74	65.25

Table 7: Results for simple linear regression analyses (LM) and phylogenetic linear regression analysis using GEE in the coastal community (SB) (*dfP*=11.33, phylogenetic degrees of freedom as defined in (Paradis and Claude 2002) and in the mountain community (PM) (*dfP*=5.7). Significant relationships (*p-values* in bold numbers) between plant specialization and degree of plant dependence on insect pollination (*IPD*) were only found in one of the communities.

Response	**Variable**	**Regression type**	**Site**	**Estimate**	**SE**	***t***	***P***
IPD	log (L)	LM	SB	23.506	7.626	3.082	**0.005**
			PM	5.768	8.556	0.674	0.517
		GEE	SB	17.798	4.964	3.585	**0.005**
			PM	0.626	7.744	0.081	0.939
IPD	H	LM	SB	24.429	9.681	2.523	**0.018**
			PM	8.795	10.0570	0.875	0.404
		GEE	SB	21.332	6.602	3.231	**0.009**
			PM	3.962	8.987	0.440	0.683
IPD	CC	LM	SB	187.58	64.300	2.917	**0.007**
			PM	24.960	76.350	0.327	0.751
		GEE	SB	99.974	43.069	2.321	**0.044**
			PM	-103.92	68.468	-1.518	0.209

3.4 Discussion

Our findings demonstrate that plants highly dependent on insects for pollination can be also those with high linkage levels, high diversity of interactions and occupying central positions in the network. We detected such relationship, however, only in one of the two communities studied (the largest, most diverse and most heterogeneous community), what suggests that the functional relationship is context-dependent and thus not consistent across all communities. If our results can be generalized to at least large pollination communities, it implies that plants dependent upon pollinators to seed set may ensure pollination by being generalists in the network, i.e. by attracting a wider array of pollinators. Generalization is considered to be a beneficial strategy, especially if pollinator abundances and interactions fluctuate across time, as found in most networks (Alarcón et al. 2008, Petanidou et al. 2008, Olesen et al. 2008, Dupont et al. 2009). Moreover, there is evidence of positive effects of pollinator species richness and diversity on pollination services (Kremen et al. 2002, Klein et al. 2003b,a, Hoehn et al. 2008, Albrecht et al. 2012), indicating thus that a greater generalization tends to translate into greater reproductive success. Several possible mechanisms may explain the increase in seed production with increasing pollinator diversity (Klein et al. 2008): (i) a sampling effect by which rich communities have more probabilities of including highly effective species or groups (Huston 1997); (ii) niche complementarity of pollinators, which occurs when species differ in their foraging patterns, for instance through space, time and/or environmental conditions (Blüthgen and Klein 2011, Fontaine et al. 2006, Hoehn et al. 2008, Albrecht et al. 2012); and (iii) functional facilitation, when the presence of a pollinator species enhances the performance of other species (Greenleaf and Kremen 2006).

However, the functional relationship between plant dependence on insect pollination and generalization level might be weaker or simply absent in some contexts

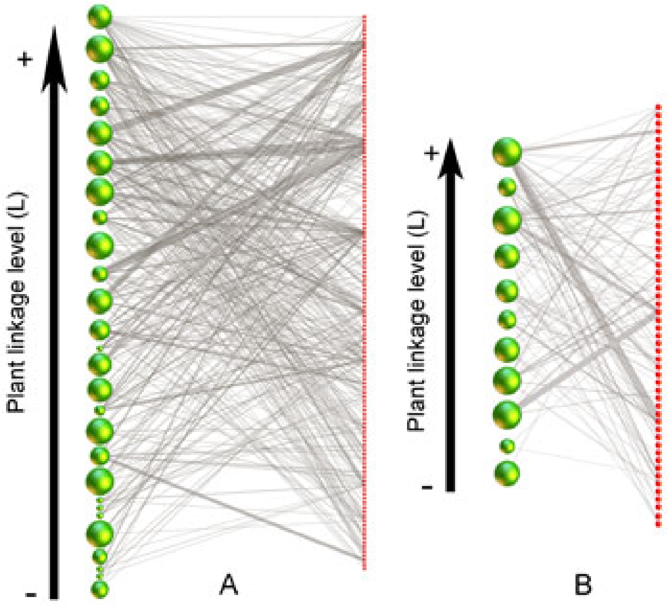

Figure 12: Bipartite representation of networks only including plant species whose seed set was studied: (a) SB site (27 plants × 126 insects) and (b) PM site (11 plants × 54 insects). Green nodes represent plant species, red nodes represent pollinator species and links are weighted by interaction frequency (visits per flower/min). Plant nodes are ordered by linkage level (L) from the most specialist (bottom) to the most generalist (top). Within each network plant node size is proportional to the insect pollination dependence (IPD) (be aware size of nodes cannot be compared among subnetworks because they have been rescaled to fit in the figure). In SB network, the smallest green nodes are mainly concentrated in the bottom of the figure, indicating plants with a small linkage level were those with the lowest dependences on insect interactions. This trend is not observed in PM network where plants with just a few interactions (low L) were relatively highly dependent. Phylogenetic relationships between plants are not considered here.

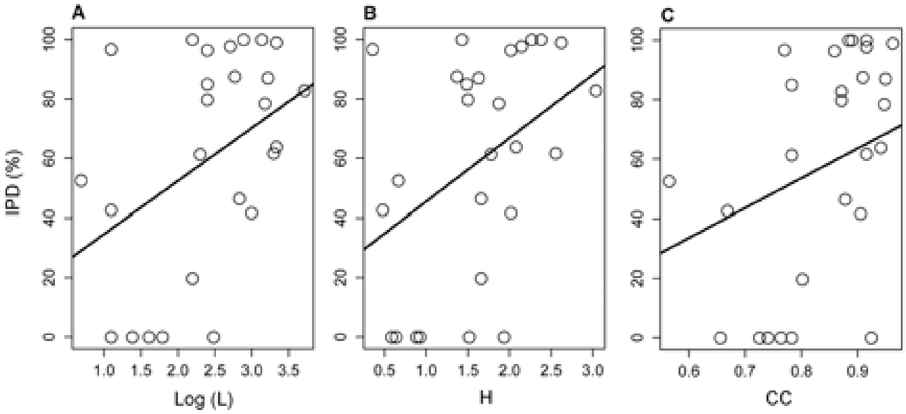

Figure 13: Relationships between plant dependence on insect pollination and plant specialization. Regressions obtained for the coastal community (SB). The degree of plant dependence on insect pollination (*IPD*) is the percentage of actual seed set attributed to pollinator interactions, i.e. excluding seed set caused by wind and self-pollination. Plant specialization is measured as: (A) linkage level (*L*), (B) diversity of interactions (*H*), and (C) closeness centrality (*CC*). Plotted lines are the fitted *GEE* models.

and communities. Previous studies have shown that, biodiversity has a higher impact on ecosystem functionality in naturally heterogeneous ecosystems - where niche complementarity can be most strongly expressed - and that resource heterogeneity may actually be required for a positive biodiversity-function relationship (Tylianakis et al. 2008). Our results are actually congruent with such findings, as the significant association between diversity of interactions and *IPD* was only found in the habitat with greater heterogeneity in flower-resource abundance and higher diversity of interactions. Interestingly, a theoretical approach (Perfectti et al. 2009) also suggested the diversity-function relationship can vary from negative to neutral to positive due to differences in effectiveness and abundance of pollinators. When the most abundant pollinators are also the most effective, it even may be beneficial for plants to be visited by a low diverse group of pollinators.

In addition, we found that topological position of a plant species within the network was also related to plant dependence on insect pollination. In individual-based one-mode networks, it has been recently found that plants occupying network central positions had higher fitness than those occupying peripheral positions, as chances of pollen outcrossing via shared pollinators with conspecific plants increase (Gómez and Perfectti 2012). Following the same rationale, but turned into our species-based networks, we hypothesized that a high closeness centrality (CC) may imply negative effects for plant reproductive success because insects which are already visiting flowers of other plant species (i.e. generalist pollinators) may carry heterospecific pollen which could potentially interfere with conspecific pollen when deposited to stigmas (Morales and Traveset 2008, Muchhala and Thomson 2012). For this reason, dependent plants might benefit from not being central in networks. Interestingly, the opposite was found: highly dependent plants had a high connection to other plants through shared pollinators, suggesting that sharing pollinators with other plant species does not necessarily have negative competitive effects on reproductive success. However, this could be interpreted more as a result of generalist species occupying also central network positions (Martín-González et al. 2010) rather than an absence of negative interspecific pollen transfer effects. Quantitative information such as the frequency of interaction among each pollinator shared, the amount of interespecific pollen carried, or the frequency at which pollinators are shifting among plant species should be considered in order to adequately evaluate the potential competition for pollinators among plants (Mitchell et al. 2009).

Our study is only a first step in the understanding of the functional impact of network interactions on plant reproductive success. Most plant-pollinator network studies describe the pattern of interactions which take place in a community, but without measuring the real functional consequences of each of these interactions for plant reproduction. This occurs because quantifying the contribution of pollinator species to the reproductive output of plant species for each single network interaction would require a prohibitive amount of fieldwork. As far as we know, there is only one study conducted to date (Vázquez et al. 2012) which quantified the reciprocal impact of plants on pollinators and vice versa for five selected species of a network. Here, instead of measuring each per-interaction effect, we propose an alternative and simplifying approach based on measuring total-interactions effect on plant seed set, i.e. the percentage of actual seed set which depended on insect interactions. Obviously, using this approach precludes knowing to what extent each specific plant-pollinator link contributes to total plant seed set. High variability on the functional effect of each link should be expected, as flower-visitors vary in their pollination ability and effectiveness (Schemske and Horvitz 1984, Herrera 1987). Indeed, sometimes such network links may even not have a functional effect on plant reproduction because observed interactions do not translate always into true pollination events. For instance, our approach allowed us to detect some plants (n = 6) which had several interactions in the network (between two to 12) but with no real functional impact on seed set, because plants were self-pollinating. These observations highlight that inferring pollinator function directly from network data must be done with caution. We further need to consider that the functional effect of such observed links for plant reproduction may change in time (Fishbein and Venable 1996, Ivey et al. 2003, Rader et al. 2012). Linking network structure to community function is one of the forthcoming challenges in network ecology (Thompson et al. 2012). This kind of knowledge might be important in the future as it will permit, for instance, to make more realistic predictions of disturbance effects on plant-pollinator networks, to assess potential functional impacts of species loss or to help in species management decisions.

3.5 Acknowledgements

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4

Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities

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Abstract

Information about the relative importance of competitive or facilitative pollinator-mediated interactions in a multi-species context is limited. We studied interspecific pollen transfer (IPT) networks to evaluate quantity and quality effects of pollinator sharing among plant species on three high-Andean communities at 1600, 1800 and 2000 m. To estimate the sign of the effects (positive, neutral or negative), the relationship between conspecific and heterospecific pollen deposited on stigmas was analyzed with GLMMs. Network analyses showed that communities were characterized by the presence of pollen hub-donors acting as 'magnet species'. In general, facilitative and neutral pollinator-mediated interactions among plants prevailed over competition. Thus, the benefits from pollinator sharing outweigh the costs (i.e. heterospecific deposition and conspecific pollen loss). The largest proportion of facilitated species was found at the highest elevation community, suggesting that under unfavourable conditions for the pollination service and at lower plant densities facilitation can be even more common.

Key-words: facilitation, competition, pollination interactions, multi-species, magnet-species, network, interspecific pollen transfer, heterospecific pollen deposition, conspecific pollen loss, pollinator sharing

4.1 Introduction

Nearly 87.5% of all flowering plants rely on animals (mainly insects) as vectors for effective pollen transport (Ollerton et al. 2011) and most of these animal-pollinated plants are generalized rather than specialized (Waser et al. 1996, Waser and Olleton 2006). Generalization in plant-pollinator interactions implies a high degree of pollinator sharing among co-flowering plant species in communities. A particular consequence of pollinator sharing is interspecific pollen transfer (IPT), since movements of shared pollinators can often result in pollen transfer from anthers of one species to the stigmas of another species. IPT seems to be common in natural plant communities as most plants receive heterospecific pollen, although its contribution to the total stigmatic load is variable (0-75%) (McLernon et al. 1996, Montgomery and Rathcke 2012, Fang and Huang 2013, Ashman and Arceo-Gómez 2013). However, almost nothing is known about community-wide patterns of IPT despite its potential ecological and evolutionary implications for plant community structure (Feinsinger 1987, Sargent and Ackerly 2008, Morales and Traveset 2008, Mitchell et al. 2009, Muchhala et al. 2010).

The outcomes of pollinator sharing on plant reproductive performance vary from positive (facilitation), neutral, to negative (competition) (Moragues and Traveset 2005, Hegland et al. 2009a, Morales and Traveset 2008, Bjerknes et al. 2007). However, the costs-benefits of pollinator sharing for plants have been mostly studied using isolated pairs of species, which do not allow any generalization of what pollination-mediated process, facilitation vs. competition, prevails in plant communities (but see Hegland et al. 2009a, Aizen and Rovere 2010). Here, we propose a conceptual framework to estimate the potential effects of pollinator-mediated interactions at a community-wide level through the study of the relationship between the heterospecific pollen (HP) and conspecific pollen (CP) deposited on stigmas. Deposition of HP on stigmas might be viewed as a 'service fee' that plant species have to pay for the pollination by shared mutualists. The balance between the overall amounts of CP and HP transferred to stigmas might indicate the relative cost or benefit obtained from pollinator sharing (Figure 14a-c), i.e. the sign of the effect of pollinator-mediated interactions. A positive linear relationship between HP and CP deposited on stigmas might be found when pollinator sharing implies an increase of HP deposition, but also an increase in CP deposition (Figure 14 a). For instance, a positive effect might be observed when the 'mass effect' of flowering together or the presence of a particular 'magnet species' attracts more pollinators to the area, increasing the quantity of visits to co-flowering plants on a per flower basis (Laverty 1992, Moeller 2004, Ghazoul 2006, Molina-Montenegro et al. 2008, Liao et al. 2011). On the other hand, a negative relationship between HP and CP deposited on stigmas might be found when shared pollinators deposit increasing amounts of

HP while decreasing CP deposition (Figure 14 b). This negative effect may occur when: (1) species compete for a limited number of pollinators and the presence of a certain plant species reduces pollinator visitation to other co-flowering plants (Brown et al. 2002, Tscheulin and Petanidou 2013, Mitchell et al. 2009), (2) there are increasing losses of CP during visitation to heterospecific flowers or more prolonged flights between conspecific flowers (Mitchell et al. 2009), or (3) deposition of HP blocks the stigma surface (stigma clogging) preventing adherence of CP grains (Galen and Gregory 1989, Brown and Mitchell 2001, Matsumoto et al. 2010, Runquist 2012). Finally, a neutral effect (i.e. no relationship between CP and HP receipt) might result when the facilitative effect of flowering via pollinator attraction balances the negative effects of pollinator sharing, e.g. via CP loss (Figure 14 c).

Although the above-mentioned cases refer to quantitative effects of pollinator-mediated interactions, the framework we propose can be expanded to qualitative effects. This can be achieved, for instance, through the study of the relationship between the amount of HP deposited on stigmas and the relative number of CP grains germinated per stigma, relative number of pollen tubes in the style, and/or ultimately number of seeds sired per pollen grain deposited on the stigma. In the case of pollen germination (Figure 14d-f), for instance, a positive relationship between HP and the proportion of germinated CP (germination ratio) might occur when the presence of co-flowering species promotes the arrival of more pollinators to individual plants that visit fewer flowers per plant. This will decrease the deposition of self pollen (mainly via reduced geitonogamy), while favouring the deposition of outcross pollen (Liao et al. 2011, Yang et al. 2013). On the other hand, a negative relationship between HP and CP germination ratio might be found in the opposite scenario (i.e. fewer visitors but more flowers visited per plant) and in cases where HP causes mechanical or allelopathic inhibition of CP germination (Sukhada and Jayachandra 1980, Murphy and Aarssen 1995).

In this study, we use for the first time this simple conceptual framework to estimate the sign of the effect (i.e. positive, neutral, or negative) of pollinator-mediated interactions at a community-wide level, both in terms of pollination quantity and quality. In order to achieve this goal, germinated and non-germinated CP and HP grains per stigma were counted and identified in co-flowering plant species from three alpine communities occurring at different altitudes (1600, 1800 and 2000 m) in the Patagonian Andes. Using this data we constructed directed plant-plant networks depicting all IPTs in each community (Fang and Huang 2013), i.e. a map of all pollinator-mediated interactions among co-occurring plants. Generalized linear mixed models (GLMMs) were used to estimate in each community the sign of (a) the overall quantitative and qualitative effect of all pollinator-mediated interactions on each plant receptor species, and (b) the quantitative and qualitative effect of each pairwise interaction. We also

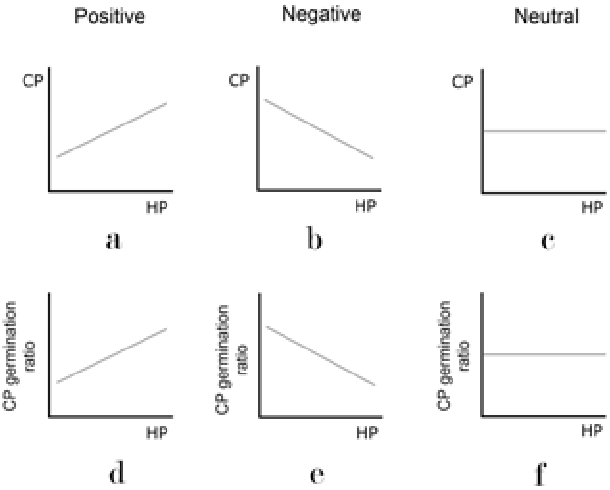

Figure 14: Simple conceptual model for the evaluation of quantitative (a-c) and qualitative (d-f) effects of pollinator-mediated interactions through the study of pollen deposition on stigmas (CP: conspecific pollen, HP: heterospecific pollen). HP transfer to stigmas is assumed to be a consequence of pollinator sharing among plants. Here graph intercept represents the average CP deposition when pollinators are not shared among plants. A positive effect of pollinator sharing (i.e. facilitation) is found when shared pollinators increase HP deposition on stigmas, but also increase CP deposition (a) and/or the proportion of CP pollen germinated (d). On the other hand, a negative effect of pollinator sharing (i.e. competition) is found when shared pollinators reduce the amount of CP load (b) and/or CP germination ratio (e). A neutral effect is found when HP deposition does not modify CP deposition (c) and/or CP germination ratio (f).

determined the relative frequency of each type of interaction (positive, neutral, negative) in each community, assessing whether the prevalence of facilitative, neutral and competitive pollinator – mediated interactions change across the altitudinal gradient studied. It has been proposed that facilitation is more common in habitats with adverse abiotic conditions, and that competition at low elevations can shift to facilitation at high elevations (Callaway et al. 2002). In the particular case of pollination, high elevations are harsh environments for insect-pollinated plants since the abiotic conditions there (e.g. low temperatures, strong winds, short snow-free growing seasons) limit abundance, diversity and activity of insect pollinators (Arroyo et al. 1982, Totland 1993). Changes in pollinator availability can then affect the direction and magnitude of pollinator-mediated interactions among plants (Lázaro et al. 2014, Ye et al. 2014). Moreover, pollinator-mediated effects vary also with plant density and flower abundance, so that for instance the effect of a focal species may change from facilitation to competition with increasing density (Munoz and Cavieres 2008, Seifan et al. 2014). We expect that plant-plant facilitation might be more frequent at higher than at lower altitudinal communities because, under limited pollinator services and at lower plant densities, the plant benefits obtained from sharing pollinators (e.g. increase in quantity and/or quality of CP deposition) outweigh their resulting costs (i.e. HP deposition and CP loss).

4.2 Materials and methods

4.2.1 Study sites and field sampling

The study was performed at the northern face of Cerro Challhuaco (2101 m) in Nahuel Huapi National Park (San Carlos de Bariloche, Río Negro, Argentina) over the 2010-2011 austral flowering season (from december, after snow melt, to march). We sampled plant species from the high Andean semidesert at three altitudinal levels above the *Nothofagus pumilio* timberline: (i) 1641 m above sea level (41°16.01′S, 71°18.08′W), (ii) 1807 m a.s.l (41°16.09′S, 71°18.45′ W), and (iii) 2101 m a.s.l. (41°16.003′S, 71°19.161′W). Hereafter, we refer to these three altitudes as 1600, 1800, and 2000 m, respectively. Plant cover in these high-elevation communities is low due to severe abiotic conditions. Mean annual temperature at about 1500 m is ca. 3°C and annual precipitation is ca. 1000 mm, mainly falling during winter in the form of snow. Westerly winds blow strongly very often, and the summer season is short, mild and dry, whereas winter is cold and wet. The vegetation is typically dominated by cushion plants and perennial herbs with patchy distribution. Asteraceae is the most common plant family, represented by a high diversity of native species from the genus *Senecio*. Pollinators are scarce and difficult to observe, but bumblebees, bee flies and butterflies are among the most active flower visitors (pers. obs.). Each altitudinal level was sampled once a day every two weeks during the whole study period (six times in total). On each sampling day, we identified all flowering plant species in bloom in a 100 m x 25 m transect per altitudinal level (a total of 48 species were identified, Table S1). We counted the number of individuals per species in transects to estimate species abundance. At each altitude we selected randomly five individuals for every plant species, whenever possible, and collected five senescent flowers (i.e. post-anthesis) per individual, which were stored separately in clean eppendorf tubes with ethanol 70%.

4.2.2 Analysis of pollen deposition on stigmas

In the laboratory, we counted and identified pollen from the stigmas of collected flowers. To facilitate pollen identification, we prepared a pollen reference collection of all species present in the study communities. Pollen from anthers of each plant species was collected, mounted on slides and stained with Alexander's solution (Alexander 1980). We took photographs from pollen grains at different planes and measured pollen size for all species (10 pollen grains per species) under the optical microscope at 400x magnification.

Senescent flowers were dissected under the stereomicroscope. Stigmas were removed, mounted on a microscope slide and stained with Alexander's solution. This staining solution contains malachite green, which stains cellulose in pollen walls, and acid fuchsine which stains the cell protoplasm, thus allowing clear distinction of germinated (i.e. without protoplasm) and non-germinated (i.e. with protoplasm) pollen grains (Galen and Gregory 1989). Slides were examined under the microscope (400x) and the number of CP (germinated and non-germinated) and HP grains were counted. The identity of HP grains was determined with assistance of the reference collection. When identification was uncertain (this occurred in only 8% of the cases, mainly involving pollen of con-generic or con-familiar species), pollen was assigned to the most abundant species in flower with that pollen morphology at the time of stigma collection. If pollen was not in the reference collection (i.e. from outside the sampled communities), it was designated as belonging to an unknown species (3 morphotypes in total, 1% of all identifications).

4.2.3 Construction of a plant-plant pollen transfer network

Data from pollen deposition on stigmas was used to construct unipartite directed networks depicting HP transfers among plant species for each altitude (i.e. 1600, 1800 and 2000 m). In these networks, nodes are plant species and links represent HP transfer from anthers of one species (donor) to stigmas of another species (receptor). For instance, a link is directed from species i to j ($i \rightarrow j$) when pollen of species i was detected on stigmas of species j (i.e. i is the donor species and j is the receptor). Thus, the presence of a link in one direction does not entail necessarily the presence of the reverse link. In directed networks, in-degree (k_i^{in}) and out-degree (k_i^{out}) are defined as the number of links incoming and outgoing a focal node i, respectively (Newman 2003). Hence, here k_i^{in} measures the number of different species from which plant species i receives pollen, whereas k_i^{out} measures to how many other species i donates pollen. The frequency distributions of the number of incoming and outgoing links per node (in-degree and out-degree distributions) were examined for each network. The Spearman rank correlation between in- and out- degree of species within each network was calculated ($r_{in \quad out}$). A significant positive correlation indicates that those species in the network which tend to donate to many other species also tend to receive pollen from many, whereas a negative relationship indicates that a 'successful' donor is a 'poor' receptor or viceversa. Species were classified as pollen donors ($k_i^{in} < k_i^{out}$), receptors ($k_i^{in} > k_i^{out}$) or balanced donor-receptors ($k_i^{in} = k_i^{out}$) considering incoming and outgoing pollen transfers. For instance, generalist plants in the community are likely to be pollen receptors (Fang and Huang 2013). For species sampled in more than one community (Table S1) the correlations between in-degree ($r_{in \quad in}$) and out-degree ($r_{out \quad out}$) at different communities were calculated to assess whether their role as donors and receptors was consistent across communities.

Because observed HP transfers are a consequence of pollinator sharing among plants, our networks depict all plant-plant pollinator-mediated interactions in the community. The sign of the effect of each particular interaction for the receptor species (positive, neutral or negative) was determined following the methods described in the next section. The open-source software *Gephi 0.8-beta* (`https://gephi.github.io/`) was used for network drawings and package *igraph* (Csardi and Nepusz 2006) in R software version 2.15 (R Development Core Team 2012) for the calculation of in- and out-degrees.

4.2.4 Models for the estimation of pollinator-mediated interactions effect: facilitation, neutrality or competition

We used generalized linear mixed-effects models (GLMMs) – also called multilevel generalized linear models (Gelman and Hill 2007) – to estimate: (a) whether the overall effect of all pollinator-mediated interactions for each plant species was positive, neutral or negative, and (b) the sign (i.e. positive, neutral or negative) for each plant-plant interaction occurring in the communities and represented in our networks. Data from pollen deposition on stigmas was used and models were fitted separately for each community using the *lmer* function in package *lme4* (Bates et al. 2014) in R software version 2.15 (R Development Core Team 2012).

For the first objective, two models were applied: model 1 to assess the quantitative effects of pollinator-mediated interactions, and model 2 to assess the qualitative effects (see conceptual framework Figure 14). Model 1 was a Poisson GLMM with number of CP grains per stigma as response variable, number of HP grains as predictor and sampling date as covariate (factor with 6 levels indicating the sampling round). Plant species (i.e. receptor species) and also plant individual were included as random effects (random slope and intercept, and random intercept, respectively). The resulting model was

$$y_n = e^{\alpha_{j[k[n]]} + \beta_{j[n]} \cdot x_n + \delta_{t[n]} + \varepsilon_n} \tag{4.1}$$

where y_n is the number of CP grains on stigma n, $\alpha_{j[k[n]]}$ is the specific intercept for plant individual k of receptor species j on which stigma n was sampled, $\beta_{j[n]}$ is the specific slope term for the receptor species j to which stigma n belongs, x_n is the number of HP grains on stigma n, $\delta_{t[n]}$ is the coefficient for the sampling date t of stigma n and ε_n the error term.

Model 2 was a binomial GLMM with the proportion of germinated CP grains per stigma or germination ratio (i.e. number of germinated CP grains/total CP grains deposited) as response variable, number of HP grains as predictor and sampling date as covariate. Plant species was included as a random effect, so that the resulting model was

$$p_n = logit\ \ ^{1}(\alpha_{j[n]} + \beta_{j[n]} \cdot x_n + \delta_{t[n]} + \varepsilon_n) \tag{4.2}$$

where p_n is the germination ratio of stigma n, $\alpha_{j[n]}$ is the specific intercept for species j to which stigma n belongs, $\beta_{j[n]}$ is the specific slope term for the receptor species j, x_n is the number of HP grains on stigma n, $\delta_{t[n]}$ is the coefficient for the sampling date t of stigma n and ϵ_n the error term. Plant individual was not included as random effect in model 2 since between-individual variance in one of the communities was not sufficiently large for model convergence.

Both models are varying-intercept and varying-slope multilevel models. Such multilevel modelling approach allows for reliable estimates, even for species with small sample sizes, while accounting for the inherent clustered structure of the data (Gelman and Hill 2007). Following our conceptual framework (Figure 14), the slope β_j estimated for each species (which in model 1 shows the relationship between HP and CP deposition, and in model 2 between HP and CP germination ratio) is an indicator of the effect of pollinator sharing from the perspective of the receptor plant species. We considered this slope to be evidence of an overall positive or facilitative effect when $\beta_j \pm 2\text{SE} > 0$, a neutral effect when $\beta_j \pm 2\text{SE}$ overlapped 0, and of a negative or competitive effect when $\beta_j \pm 2\text{SE} < 0$. The percentage of species experiencing each type of effect was determined for each community (i.e. 1600, 1800 and 2000 m).

For the second objective, two other models were constructed (model 3 for quantitative effects and model 4 for qualitative effects), but this time only considering stigmas with both CP and HP deposition (21% of observations) and incorporating the information from the identity of donor species. Model 3 was a Poisson GLMM with number of CP grains per stigma as response variable, number of HP grains transferred from a specific donor as predictor and sampling date as covariate. The model fitted was again a varying-intercept and varying-slope model, same as model 1 but this time including receptor and donor species as random effects (random slope and intercept), with donor nested within receptor species. Thus, for each plant-plant interaction (i.e. species pair combination donor:receptor) the model estimated a different slope and intercept. Lastly, model

4 was a binomial GLMM with CP germination ratio as response variable, number of HP grains transferred from a specific donor as predictor and sampling date as covariate. Again receptor species and donor nested within receptor, i.e. each donor:receptor combination, were included as random effects (random slope and intercept). In these models, we were interested in the sign of the slope estimation of each combination donor:receptor (β_{ij}). We considered this slope to be evidence of an overall positive or facilitative interaction when $\beta_{ij} \pm 2\text{SE} > 0$, a neutral interaction when $\beta_{ij} \pm 2\text{SE}$ overlapped 0, and of a negative or competitive interaction when $\beta_{ij} \pm 2\text{SE} < 0$. The percentage of each type of interaction was determined for each community (i.e. 1600, 1800 and 2000 m).

4.3 Results

We counted a total of 57,514 pollen grains (54,937 CP and 2,577 HP) on 2987 stigmas revealing an overall number of 264 IPT distinctive interactions among the different co-flowering plant species in the three communities (Table 8). The highest richness of plant species and IPTs was found in the intermediate community (1800 m). The average total pollen load per stigma was extremely variable among species (range: 0 – 180.84 grains). CP was identified in ca. 85% ($n = 2,513$) of all stigmas examined, whereas HP was detected in only ca. 21% ($n = 625$). In general, the amount of HP per stigma was small compared to CP deposition, which represented > 90% of the total pollen load per stigma (range: 36.7 – 100%) for most species. The maximum absolute number of HP grains on a stigma was 64 (*Hypochaeris tenuifolia*), and the maximum number of different pollen species identified on a single stigma was seven (*Valeriana carnosa*).

In all IPT networks, the frequency distributions of species in-degree and out-degree were heterogeneous and right-skewed (Figure 15). Most species received HP from one or two donors (ca. 60% species), but a few species acted as hub receptors and received pollen from many species. For instance, *Valeriana carnosa* (1600 m), *Armeria maritima* (1600 m), *Quinchamalium chilense* (1800 m) and *Leucheria millefolium* (1800 m) all received pollen from > 12 HP donors. A similar pattern was found for pollen donation (i.e. out-degree distributions), with most plant species donating pollen to a few receptors (ca. 65% of species donate to less than three species) and a few species donating to many (Figure 15). *Quinchamalium chilense* (1600 m), *Adesmia parviflora* (1800 m) and *Nassauvia pygmaea* (2000 m) showed the highest out-degree in their respective communities. The proportion of species acting as donors decreased from 48% in the lowest altitudinal network to 27% in the highest one, whereas the proportion of receptors increased with altitude (Table 8). Moreover, hub-receptors were not the same species acting as hub-donors, i.e. species which received pollen from many do

Table 8: Total number of species, stigmas and pollen grains (heterospecific and conspecific) sampled in each community. Interspecific pollen transfers are the total links identified in each plant-plant network. Species within each network were classified as pollen donors, receptors or balanced donor-receptors considering their incoming and outgoing pollen transfers, percentages are indicated in the table. The correlations between in- and out-degree for species within each network are also shown, in all cases values were non-significant (ns).

	1600 m	**1800 m**	**2000 m**
No. species	26	34	14
Total no. stigmas	925	1501	561
Total no. pollen grains	18960	29504	9050
Conspecific no. pollen grains	18174	27989	8774
Heterospecific no. pollen grains	786	1515	276
Interspecific pollen transfers	105	130	29
% donor species	48%	41.94%	27.27%
% receptor species	44%	51.61%	63.64%
% donor-receptor species	8%	6.45%	9.09%
In-out degree correlation ($r_{in\ \ out}$)	0.05^{ns}	0.33^{ns}	0.05^{ns}

not necessarily exported pollen to many species as shown by the non-significant correlations between in- and out-degree of species (Table 8). In-degrees of the same species present in different communities were correlated ($r_{in\ \ in} = 0.64$, $p < 0.001$), but not out-degrees ($r_{out\ \ out} = 0.25$, $p = 0.18$), suggesting that a species identity as a heterospecific pollen recipient is less variable than as a donor.

The overall effect of pollinator-mediated interactions on the receptor species – evaluated as the relationship between CP and HP deposition (model 1) – varied from positive, neutral to negative (Figure 16 a,c,e). In two of the communities (1600 and 2000 m) the effect for most receptor plants was positive (63% and 75% of species, respectively, Figure 16 a,e). However, in the intermediate altitudinal community (1800 m) neutral effects predominated (59 % of species), followed by positive effects of pollinator sharing (33% of species) (Figure 16 c). Interestingly, negative effects for receptor species in terms of pollen quantity (results from model 1) were rare in all communities (5%, 8% and 0% of species at 1600, 1800 and 2000 m respectively).

On the other hand, when qualitative effects of all pollinator-mediated interactions were evaluated through the relationship between total HP received per stigma and CP germination ratio (model 2), neutral effects prevailed (89 %, 67 % and 62 % of receptor species in 1600, 1800 and 2000 m respectively; Figure 16b,d,f). However, the percentage of species for which model 2 estimated positive

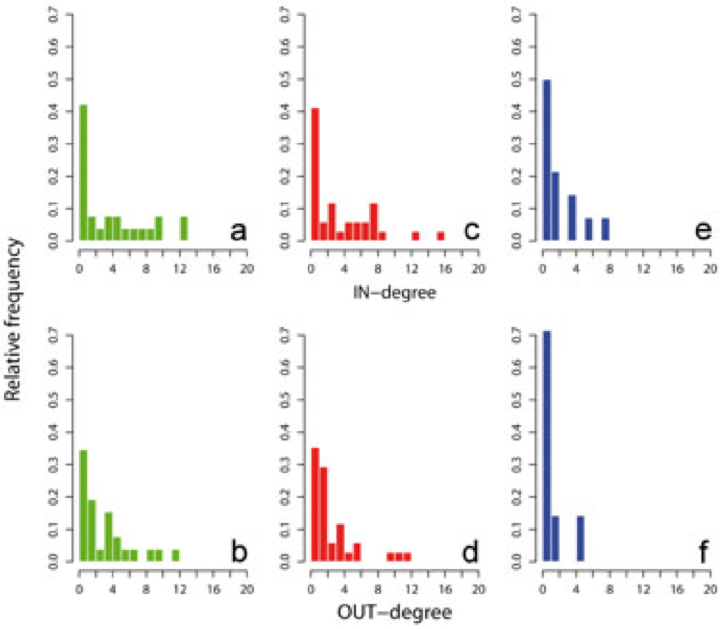

Figure 15: Frequency distributions of species in-degree and out-degree in the different IPT networks: (a-b) 1600 m network, (c-d) 1800 m network and (e-f) 2000 m network. In-degree frequency distributions represent the pattern of heterospecific pollen receipt on stigmas (pollen deposition), whereas out-degree frequency distributions show the pattern of pollen donation to heterospecific stigmas (pollen loss). In all cases there is a large number of species receiving from and donating to a small number of species (i.e. low in-degree and out-degree) and a small number of species receiving from and donating to a large number of species (i.e. high in-degree and out-degree).

effects increased with altitude, from 11% at 1600 m, to 17% at 1800 m and 25% of species at 2000 m. In total, the percentage of species showing positive effects was nearly twice as high as that of species exhibiting negative effects.

Quantitative effects estimated for each pairwise donor:receptor interaction in plant-plant pollen transfer networks at 1600 and 1800 m (model 3) were predominantly neutral (87% and 79% of all interactions, respectively), followed by positive effects (Figure 17 a,b). A few hub pollen-donor species within each of these two communities (i.e. species donating pollen to many other species) accounted for most positive effects exhibited by receptor species. For instance, *Quinchamalium chilense* and *Senecio argyreus* were responsible for more than

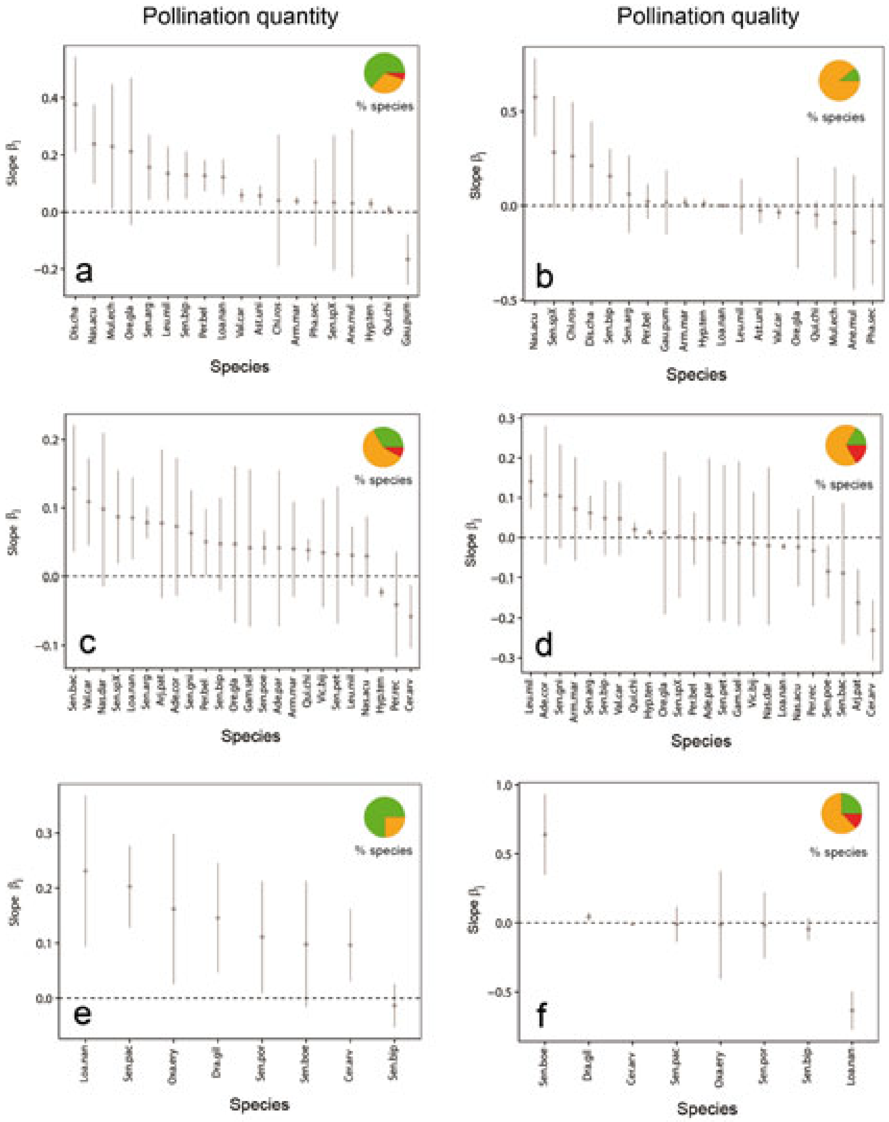

Figure 16: Estimated slopes ($\beta_j \pm 2\text{SE}$) for the effect of HP deposition on CP deposition (*pollination quantity*), and the effect of HP deposition on the proportion of CP grains germinated (*pollination quality*) for each receptor plant species at the three high-Andean semidesert communities studied: (a-b) 1600 m, (c-d) 1800 m, and (e-f) 2000 m. Coloured pie charts represent the percentage of species in each case which undergo an overall facilitative (green), neutral (orange) or competitive (red) effect from sharing pollinators with other plants in the community. Results from both models show that facilitation is more common than competition in these communities.

half of the positive effects on receptor species at 1600 m. Contrary to the results found in the two lower communities, the model showed a prevalence of positive interactions between plants in the highest altitudinal community (97% of all interactions, Figure 17 c). Thus, the proportion of positive interactions increased with altitude (9%, 12% and 97% at 1600, 1800 and 2000 m, respectively).

The sign of the effect of each interaction between pollen donor and receptor species changed depending on whether the quantitative or qualitative effects were considered (model 3 *vs.* model 4, Figure 17). Model 4 showed that neutral effects of IPTs on CP germination ratio predominated in the three communities, followed again by positives effects (Figure 17 d,e,f). The highest percentage of interactions with positive effects was found at the intermediate altitudinal community (41%).

4.4 Discussion

Facilitation prevailed over competition in pollinator-mediated interactions among plants in the study high-Andean communities. In general, receptor species showed more positive or neutral than negative effects of pollinator sharing. Total HP transfers due to interspecific movements of shared pollinators neither restricted CP deposition nor decreased CP germination on stigmas. Interestingly, at higher elevation the proportion of species experiencing a facilitative effect was larger than at lower altitudes. This suggests that pollinator-mediated facilitation is more common in plant communities under severe environments, similarly as reported for facilitative nurse effects (Callaway et al. 2002). Below we discuss the potential mechanisms explaining these results, their implications and the benefits and limitations of our novel multi-species approach.

4.4.1 Pollinator-mediated interactions among plants in a multi-species context: a wide range of mechanisms and outcomes

Our study confirms that IPT due to pollinator sharing among co-flowering plants is ubiquitous in natural communities, although HP contribution to total pollen load on stigmas is small on average (McLernon et al. 1996, Aizen and Rovere 2010, Montgomery and Rathcke 2012, Fang and Huang 2013, Ashman and Arceo-Gómez 2013). Despite pollinators move frequently between plants and carry pollen from more than one species, only a low proportion of stigmas receives HP grains (Murcia and Feinsinger 1996, Jakobsson et al. 2008, Bartomeus et al. 2008). Limited interspecific pollen exchange might occur whenever pollen is: (1) deposited on floral structures other than stigmas during visitation (Murcia and Feinsinger 1996), (2) actively removed from a pollinator's body during grooming,

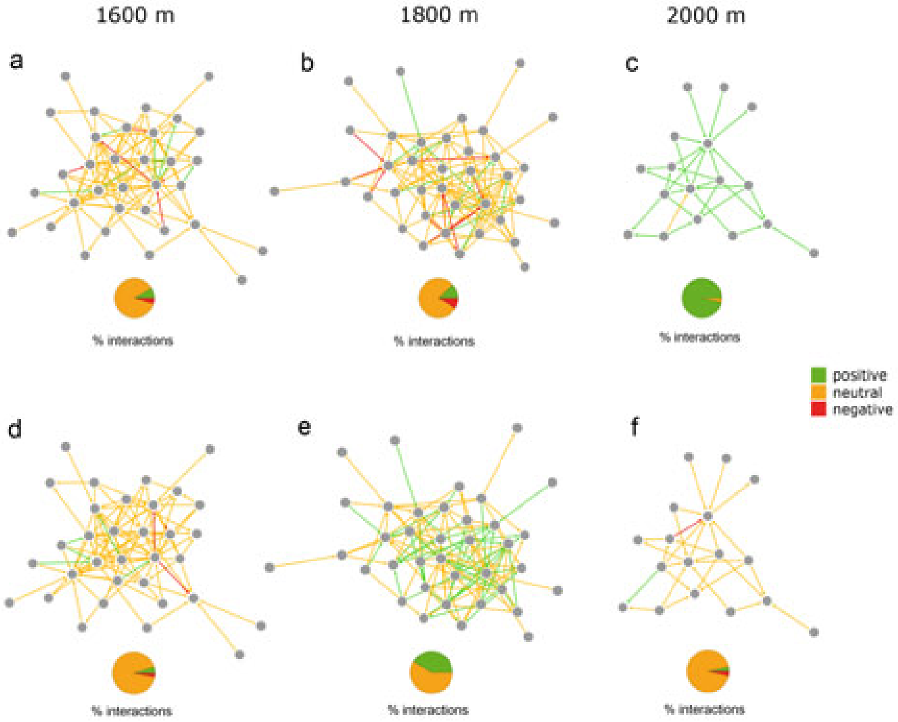

Figure 17: Plant-plant networks of the three communites studied (altitudinal levels: 1600 m, 1800 m and 2000 m) from the high-Andean semidesert. Nodes are plant species and links (i.e. arrows) among them represent HP transfers due to interspecific movements of shared pollinators. The arrows indicate the direction of pollen transfer from one species (donor) to another (receptor). Colours represent the sign of the quantitative effect estimated with model 3 (a, b, c) and the qualitative effect estimated with model 4 (d, e, f) of each particular plant-plant interaction. Pie charts show the percentage of each type of interactions (positive, neutral or negative) in each community.

or (c) passively fall from a pollinator's body during flight (Flanagan et al. 2009).

In the three plant communities we found species acting as hubs of interspecific pollen donation or receipt. Surprisingly, usually hub-receptors were not hub-donors, since patterns of interspecific pollen donation and receipt were asymmetric at the species level. Hub-donors (e.g. *Quinchamalium chilense*, *Senecio argyreus*, *Adesmia parviflora*, *Mulinum echinus*, *Nassauvia pygmaea*) may be viewed as the 'magnet species' of the communities (Laverty 1992). Although their effect on other plants varied depending on receptor identity, they accounted for ca. 50% of all interactions with positive quantitative effects in the low- and mid-elevation communities. Hence, hub-donors may play a relevant ecological role in attracting pollinators and enhancing visitation to co-occurring plants in the area. The magnitude of influence will probably depend on how important the donor species is as a resource (e.g. flower nectar and pollen production), accessibility of such resource and phylogenetic proximity to receptor species (Carvalheiro et al. 2014). On the other hand, hub-receptors can be considered the generalist plants of the community (Fang and Huang 2013). Interestingly, the role of a species as a receptor (expressed as the number of species from which it receives HP) was quite consistent across communities, whereas its role as a donor was not. This finding suggests that patterns of HP receipt are influenced by species-specific floral morphological traits (Caruso 2000, Montgomery and Rathcke 2012), whereas patterns of HP donation are more community-specific and largely influenced by relative abundance and traits of co-flowering species as well as by pollinators' preferences and behavior.

The fact that some species act more as donors and others more as receptors implies different selective pressures and potential evolutionary consequences (Feinsinger 1987). For donor plants, the fitness costs from pollinator sharing mainly occur through a reduction of male fitness via CP loss on heterospecific stigmas (pollen misplacement), while for receptor plants the effects on female fitness should be stronger, through either diminished CP or increased HP deposition (Johnson et al. 2005, Morales and Traveset 2008, Muchhala and Thomson 2012). Although we did not measure effects of IPT on male fitness, they are an unavoidable consequence of CP loss with probably a higher magnitude than effects on female fitness which are more contingent upon the occurrence of pollen limitation (Campbell and Motten 1985, Murcia and Feinsinger 1996, Flanagan et al. 2009, Aizen and Rovere 2010, Muchhala and Thomson 2012).

Pollination-mediated plant-plant interactions occur through two main interconnected mechanisms: (a) changes in flower visitation rates, and (b) changes in pollination quantity and quality (Mitchell et al. 2009). In the high-Andean study communities, we found that the net outcome of these mechanisms was neutral for many species, but that pollinator-mediated facilitative effects prevail over competitive effects. In a different multi-species approach, Hegland et al. (2009a) reported also more facilitation than competition for pollinator visitation among plant species in a temperate grassland. The combination of particularly rewarding 'magnet species' and aggregations of multiple species flowering together increases pollinator attraction (Laverty 1992, Moeller 2004, Ghazoul 2006, Molina-Montenegro et al. 2008), thus benefiting most plants with an increase in the number of visits which deposit CP. Increased CP deposition associated with increased visitation via magnet-species or multi-species attraction effects also involve increased HP deposition, a process which might counteract the abovementioned benefits. Nevertheless, we observed that HP deposition only occurred in a relatively small fraction of stigmas and the amounts of pollen transfered by shared pollinators were, for most species, not large enough to entail detrimental effects (Morales and Traveset 2008). However, the effect of HP receipt on species is likely to vary depending on pollen and stigma characteristics of both donor and receptor species (e.g. pollen size, stigma area), plant mating systems, phylogenetic relatedness, or arrival time of HP relative to CP (Caruso and Alfaro 2000, Ashman and Arceo-Gómez 2013, Fang and Huang 2013).

Interestingly, the sign of particular pollinator-mediated interactions and the frequency of each type of interactions in the communities changed depending on whether quantity or quality effects were considered. In general, facilitative effects were more common in terms of quantity than quality. This might be so because, although pollinator sharing might translate into an increase of CP deposition per flower, the quality of this CP load (Aizen and Harder 2007) might depend on the degree of kinship (self *vs.* outcross) between pollen and target stigma (Mitchell et al. 2009, Liao et al. 2011). Although 66.6% of the plant species in our communities have different mechanisms to reduce or even avoid autogamy (18 species are dichogamous, 3 species are dioecious, 3 species are herkogamous and at least 8 species are self-incompatible, Table S1), a fraction of CP deposited on their stigmas might come from either the same flower or from other flowers within the same individual (geitonogamy). Under both scenarios, a negative relationship might be detected between HP and CP germination ratio whenever self-pollen: (1) germinates less or slowler than outcross pollen (e.g. Montalvo 1992, Aizen et al. 1990), (2) does not germinate in sporophytic self-incompatible species (e.g. Galen et al. 1989, Waser and Price 1991, de Jong et al. 1993), or (3) its germination is affected by HP deposition (Arceo-Gómez and Ashman 2014). Perhaps more pollination quality effects might have been detected if, for instance, pollen-tube survival rather than germination rates had been measured, because pollen-tube growth is a better indicator of genetically- and environmentally-driven pollen-pistil interactions than pollen germination (e.g. Souto et al. 2002). Despite the larger paucity of pollination quality than quantity effects detected here, there was also a trend for the former to reflect facilitation rather than competition.

Community-wide studies of plant-plant pollination interactions are important to address whether the overall effect of simultaneous pollinator sharing among multiple species is a linear additive or alternatively a non-additive combination of the effect of pairwise interactions alone (Mitchell et al. 2009, Flanagan et al. 2011). Our findings support the latter, at least for the sign of the overall effect, and for instance, species with several neutral interactions had an overall negative or positive effect. This might occur if HP from diverse donors interacts synergistically or antagonistically on the stigma and their combined effect is more or less detrimental than the average of their independent effects (Arceo-Gómez and Ashman 2011). Even when the overall effect is similar to the addition of pairwise effects, it does not imply that the underlying competitive or facilitative mechanisms involved are the same (Flanagan et al. 2011).

At this time, it is worth asking whether other factors not considered in our models might generate positive or negative relationships between CP and HP, without implying any facilitative or competitive processes among co-flowering species. Specifically, intraspecific variation in rewards (pollen and/or nectar), number of flowers, or density of conspecifics (e.g. Real and Rathcke 1988, Petanidou and Vokou 1990, McDade and Weeks 2004) can generate small-scale spatial foraging patterns of pollinator individuals, potentially leading to heterogeneous (quantity and quality) CP transfer in space (Makino et al. 2007, Leiss and Klinkhamer 2005, Fortuna et al. 2008, Dupont et al. 2014). More rewarding or attractive plants within a population are likely to receive more visits per unit time, which should increase both CP and HP deposition (Ohashi and Yahara 2001, Cartar 2004, Thomson et al. 2012). However, HP transfer might depend on the distribution of co-flowering species in the area. For instance, more attractive patches might have more chances to receive also more HP only if heterospecifics are closer, but the likelihood will decrease if heterospecifics are farther away. Also, a concurrent increase of CP and HP in more rewarding or attractive plants might neither occur if, as expected, pollinators probe more flowers within plants in longer visit bouts. Therefore, overall we might expect that these sources of variation will actually tend to decouple the relationship between CP and HP rather than reinforce a positive or negative trend.

4.4.2 Why is plant-plant pollination facilitation more common at higher altitudes?

At 2000 m, the proportion of species receiving a facilitative effect from pollinator sharing was larger than in the two communities below. This finding is consistent with the idea that facilitation is more common in habitats with adverse environments (Callaway et al. 2002). Under low visitation rates, plants are expected to generalize to minimize pollen left undispersed in anthers (Muchhala et al. 2010), which increases the potential for pollinator sharing in plants growing at high elevations with reduced pollinator activity, abundance and diversity (Arroyo et al. 1982, Totland 1993). Moreover, plants in such stressful habitats for the pollinator service might be more prone to suffer from pollen limitation (but see García-Camacho and Totland 2009). Pollen-limited species are likely to be those benefitting most by the presence of neighbour plants. However, the effect of joint attraction of pollinators is a function of population density or relative abundance of floral resources (Feinsinger 1987). In general, pollinator-mediated facilitation is expected to occur at low to intermediate floral densities, but it turns into competition at relatively high densities (Feinsinger 1987, Moeller 2004, Munoz and Cavieres 2008, Seifan et al. 2014). In our study communities, floral density decreased with altitude. Thus, at the highest altitude, several species growing nearby in patches contributed to a 'mass effect' of concentration of pollinators. By contrast, at the lower altitudinal communities – in which patch sizes were larger – a dilution effect might occur because the pollinator pool becomes saturated and visits per flower decline (Feinsinger 1987). The high proportion of species-pairs interactions with positive quantitative effects at 2000 m did not translate into the same high proportion of positive qualitative effects. Due to floral resource limitation and a sparce plant distribution, at the highest altitude insects might visit more flowers per plant (Galloway et al. 2002) and deposit poor quality CP.

4.4.3 Concluding remarks

This work provides a feasible multi-species approach to assess the potential consequences of pollinator sharing in plant communities through the study of interspecific pollen transfers. This knowledge is key to understand which mechanisms drive broad scale ecological and evolutionary patterns, such as community structuring or floral diversification. Although the role of competition for pollination has been frequently emphasized, our results showed that facilitative interactions among plant species are predominant, particularly in habitats with challenging conditions for the pollinator service.

4.5 Acknowledgements

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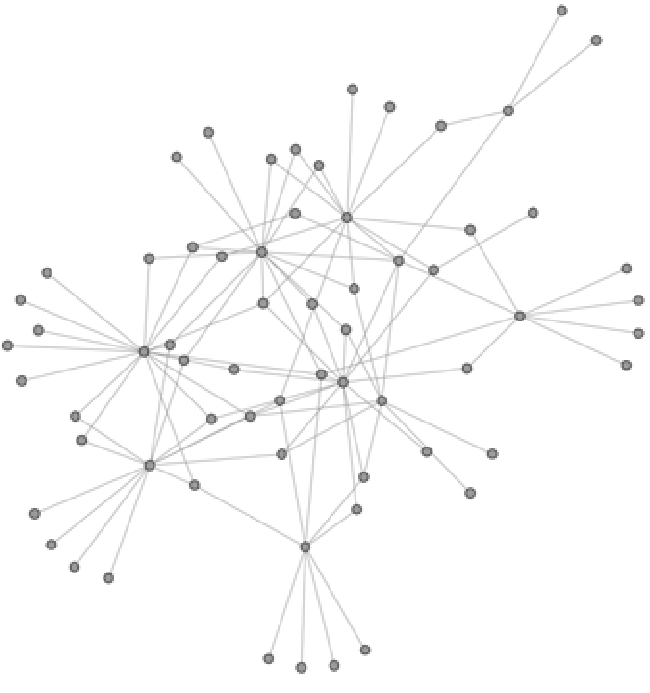
III | Epilogue



General discussion

Including intraspecific variation and information about interaction effects in pollination networks can largely affect the current view of: network structural properties, the specialization of pollinators, the implications of network structure for plant reproductive success, and the relative importance of facilitative or competitive processes among plants for pollination.

The structure of plant-pollinator networks across hierarchical levels of biological organization

There are several relevant aggregative hierarchical levels of biological organization within the realm of ecology, for example: individuals, species populations, communities and ecosystems. Ecological networks are mainly constructions describing the relationships between entities within each level (e.g. species within communities). However, because a hierarchical organization implies both horizontal and vertical relationships, a study of the relationships between networks across hierarchical levels is also needed in order to develop a hierarchical theory of ecological interaction networks (Olesen et al. 2010, Clauset et al. 2008, Jorgensen and Nielsen 2015). Such development is likely hampered by the many ways to analyze interactions within and across levels (e.g. unipartite individual-individual networks, bipartite individual-individual networks, bipartite individual-species networks), the limitations in human and budget resources for sampling these networks, and the lack of an appropriate methodology for multi-scale comparisons. A hierarchical theory of networks might help to identify potential between-scale coupling general principles, and top-down or bottom-up constraints in ecological interaction networks. It might also shed light on the extent to which this hierarchical organization of networks explains structural features and the functioning of ecological networks at higher or lower scales (Clauset et al. 2008).

Chapters 1 and 2 show that structural properties of plant-pollinator networks are not necessarily consistent across hierarchical levels of biological organization, namely species and individuals. Downscaling pollen-transport networks to the individual level may change most network descriptors studied, and reveal structural features which are hidden on species-based networks. The studied empirical networks have significantly lower linkage density, connectance, nestedness and interaction diversity at the individual scale than predicted by null models (chapter 1). Such differences are caused by individual specialization in pollen resources, i.e. conspecific individuals are heterogeneous in their interactions and specialized on a small subset of resources used by their corresponding species. This finding confirms the ubiquity of individual specialization (Bolnick et al. 2003, Araújo et al. 2011). The number and diversity of interactions per pollinator in individual-based networks reduces by half, compared to the species-based networks. In general, this supports the idea that the upper hierarchical level (species) limits connectance at the lower level (individuals) and that nodes at a lower hierarchical scale are likely to be less connected when there is a high heterogeneity (e.g. morphological, physiological, behavioural heterogeneity) within the larger scale. This might be expected for instance in a scenario of fine-scale functional division or complementarity (e.g. organelles within a cell, individuals within a colony). In such cases, fewer but diverse connections at the lower hierarchical scale might mean more connections at the higher scale. Thus, there might be a possible conflict of interest between both hierarchical scales in network stability against the loss of interactions and resources: individual specialization might increase vulnerability of individuals (mortality) but decrease species vulnerability (extinction risk) because only a fraction of the entire population would be affected.

The strong specialization found at the individual level increases network modularity of individual-based networks compared to species-based (chapter 2). Modules were expected to be ‘taxonomical units’ in the individual-based networks, i.e. to represent, to some extent, the hierarchical structure of individuals within species taxa. However, due to interaction heterogeneity of conspecifics, modules detected are not aggregations of individuals of the same species. In some sense, individuals seem to depart from what would be expected given their species identity. This finding highlights that taxonomical species are not always the most relevant study units, and some other aggregative criteria may define more appropriate operational units for particular ecological or evolutionary processes. Instead of taxonomical units, modules in the individual-based networks match phenological units, as previously found in species-based networks (Bosch et al. 2009, Martín-González et al. 2012). However, phenological compartmentalization appears only when downscaling, what suggests that phenology or time is a more important or traceable factor constraining interactions at the individual than at the species level. Because lower-level hierarchies are characterized by more rapid dynamics, small temporal windows of time such as days or hours might generate detectable imprints on network structure (Rasmussen et al. 2013). Moreover, phenological uncouplings often remain undetected at the species scale, but not at the individual scale. For instance, even when species pairs phenophases overlap, forbidden links (Olesen et al. 2011a) in individual-based networks might occur if individual lifespan does not match flowering interval or when individual foraging activity periods do not overlap with daily flower opening times of plants.

To determine the generality of the structural properties found in the pollen-transport networks, other multispecies plant-pollinator datasets resolved at the individual level would be needed. Unfortunately, currently available individual-based pollination networks focus exclusively on single species or pairs of species (Gómez et al. 2011, Gómez and Perfectti 2012, Dupont et al. 2011, 2014, Dáttilo et al. 2015).

In addition, changes on network patterns across hierarchical scales may differ depending on the specific network downscaling analytical approach followed. The across-scale network structural differences observed in chapters 1 and 2 are between individual – species and species – species networks. However, other differences may be found between individuals – individuals and species – species networks. For instance, Dupont et al. (2011) found concordances and divergences in several network properties when comparing a flower-visitation network of individual honeybees (*Apis mellifera*) and thistle plants (*Cirsium arvense*) to similar-sized species-based pollination networks from the literature. Their individual-based network had higher connectance than species-based and was non-modular. Dupont et al. (2011) proposed that individual-individual networks should be more densely linked than species-species networks because trait matching constraints are larger among different species in a community than among individuals within a population. Contrasting hierarchical-scale variance in connectance between both approaches might also be influenced by the time scale of each study (days vs. months), the studied pollinator species (honeybee vs. many insect species) or interaction sampling methodology applied (mark-reobservation of individuals vs. pollen load analysis).

In conclusion, the structure of pollination networks at different hierarchical levels of biological organization diverges, at least this is now known for the species and individual scales. The interaction heterogeneity among individuals within species is so high that ignoring intraspecific variation in plant-pollinator networks might give us an incomplete and even a misleading picture of network interaction patterns and specialization.

Individual specialization of pollinators and foraging behaviour

Intraspecific niche variation and individual specialization of pollinators governed most of the structural differences observed across hierarchical scales in chapters 1 and 2 and can have important implications for most ecological and evolutionary processes (Bolnick et al. 2011, Dall et al. 2012). There are a large number of mechanisms which may limit the range of resources used by an individual, thus causing individual specialization (Bolnick et al. 2003, Svanbäck and Bolnick 2008, Araújo et al. 2011). In the particular case of pollinators, individual specialization may be associated with the widespread behaviour of flower constancy (Waser 1986, Chittka et al. 1999, Grüter and Ratnieks 2011), which probably benefits plant reproduction, since it facilitates pollen transfer between conspecifics and minimizes pollen wastage and stigma clogging (Goulson 1999, Montgomery 2009).

In interaction networks, species linkage level might be viewed as a measure of niche breadth which results from integrating conspecific linkage levels. Niche is not a fixed attribute of species because conspecific individuals differ in niche breadth and, as shown in chapter 1, generalist species are, in fact, composed of heterogeneous specialist individuals (van Valen 1965, Roughgarden 1974, Bolnick et al. 2003, Araújo et al. 2011). Optimal foraging theory (MacArthur and Pianka 1966, Schoener 1971, Stephens and Krebs 1986) predicts that differences in niche breadth of individuals result from differences in resource use efficiencies which translate into distinct foraging choices. In general, individual foraging decisions result from the complex interaction between persistent phenotypical differences (variation in morphological, physiological or behavioural traits) and dynamic environmental differences (variation in resources, competitors or predators) (Figure 18).

Chapter 1 shows that the intensity of intra-, interspecific competition and species population abundance actually affect the species degree of individual specialization (Figure 19). High interspecific competition is related to weak individual specialization in several pollinator species studied. In the presence of many heterospecific competitors, species niche is reduced and all individuals are restricted to use the same resources. On the other hand, in the absence of competing species, species niche is expected to expand, but this occurs mainly via increased variation among individuals (between-individual component) rather than increased individual niche widths (within-individual component) because individuals switch to different resources depending on their phenotypes (Bolnick et al. 2007, 2010, Costa et al. 2008).

In general, intraspecific competition under high population density is expected to increase individual specialization and resource use variation among conspecifics (Svanbäck and Bolnick 2007, Araújo et al. 2008, Tinker et al. 2012) This may

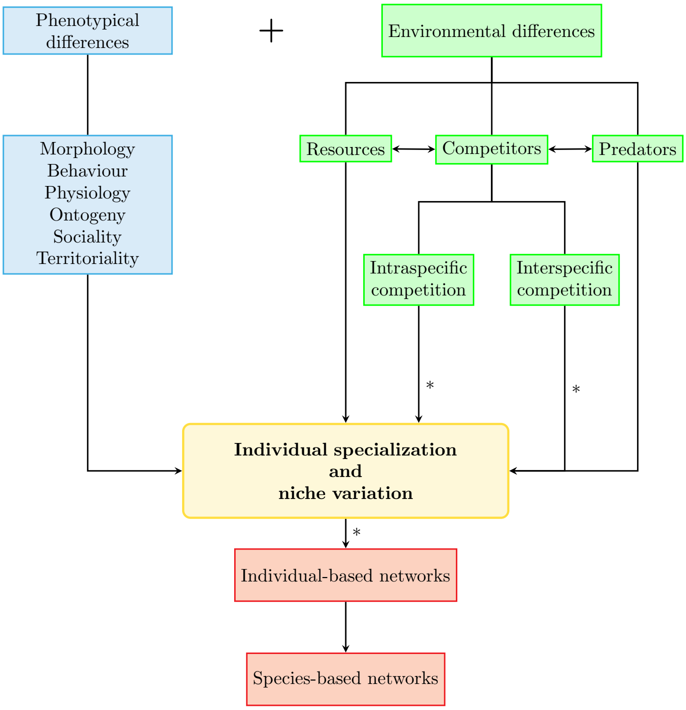


Figure 18: Diagram of the general relationships linking optimal foraging theory, intraspecific niche variation and interaction networks. Under the optimal foraging theory, niche variation among conspecific individuals results from phenotypical and environmental differences which lead to distinct trade-offs determining foraging decisions. Phenotypical differences, determined in turn by the genotype, include a broad range of traits of pollinators which may influence individuals' ability to find and handle resources. These traits are, for instance, sex of individuals (e.g. Ne'eman et al. 2006, de Jager and Ellis 2012), body size (e.g. Worden et al. 2005), foraging experience and learning capacities (e.g. Laverty 1980, Dukas 2008), individual ontogeny (e.g. chemical imprinting of pollen and nectar feeding during larval stage, Dobson et al. 2012), social status (e.g. division of labour in honeybees, Seeley 1983, Biesmeijer and de Vries 2001) or territoriality (e.g. Wainwright 1978, Batra 1978). On the other hand, optimal foraging strategies of pollinators change dynamically according to environmental conditions such as resource abundance (e.g. Pleasants 1981, Kunin and Iwasa 1996, Ohashi and Yahara 2001), predation risk (e.g. Reader et al. 2006, Wang et al. 2013) or density of conspecific and heterospecific competitors (Heinrich 1979a,b, Fontaine et al. 2008, Baude et al. 2011, Lázaro et al. 2011, Geslin et al. 2014). At high density of foragers, preferred resources may be depleted and individuals may modify foraging choices by switching to less preferred but non-depleted resources. Foraging decisions of individuals determine realized interactions in both individual-based networks and species-based, since species interactions are the result of what individuals do. Asterisks on arrows indicate relationships found in chapters 1 and 2.

depend, however, on how individuals with different phenotypes rank alternative resources and include them in order to expand the niche. Svanbäck and Bolnick (2005) proposed three models to explain how rank preference variation occurs within populations: (1) distinct preferences model, all phenotypes rank resources in a different order; (2) competitive refuge model, all phenotypes have the same first rank resource but differ in the order of lower-ranked resources; and (3) shared preferences model, all phenotypes rank resources in the same order but vary in the acceptance rate of lower-rank resources. In the competitive refuge and the shared preferences models, an increase of individual specialization is expected at high intraspecific competition because individuals add different resources or add resources at different moments thus increasing variation among individuals. Conversely, in the distinct preferences model, a decrease of individual specialization is expected because individuals end up including the same resources (Svanbäck and Bolnick 2005). Chapter 1 results are more congruent with this latter option, despite that the opposite outcome is found when species population abundance is considered instead of intraspecific competition (Figure 19). Outcomes depend on the particular form of competition which occurs: exploitative or interference competition. Changes in individual specialization with intraspecific competition may be more related to resource density than to forager density when exploitative competition is dominant (Svanbäck et al. 2011). Exploitative competition can take place because finite floral resources may be depleted at high densities of foragers (Hansen et al. 2002, Dupont et al. 2004). On the other hand, the effect of forager density on individual specialization is more important than resource density when interference competition dominates. Interference competition occurs, for instance, when pollinators such as honeybees or bumblebees deposit repellent scent-marks on visited flowers which temporarily deter subsequent flower-visitors (Stout and Goulson 2001, Yokoi et al. 2007).

In addition, each of the above-mentioned resource use models can translate into distinct observed patterns in empirical individual-resource networks (Pires et al. 2011). The distinct preferences and competitive refuge models generate modular networks (Tinker et al. 2012, Araújo et al. 2008), whereas the shared preferences model translates into nested networks (Araújo et al. 2010, Pires et al. 2011). In chapter 1, both pollen generalist and specialist individuals within a species population are frequently found, but pollen resources used by the most specialized individuals were rarely proper subsets of those used by the more generalized (only 28.5% of studied species), which suggests species-specific patterns, but low prevalence of the shared preferences model.

Foraging strategies of individual pollinator determine which interactions are realized and which are not, and thus drive individual-based network structure and ultimately even species-based network structure (Rodríguez-Gironés and Santamaría 2010). In fact, foraging behaviour has been already identified as a key pre-

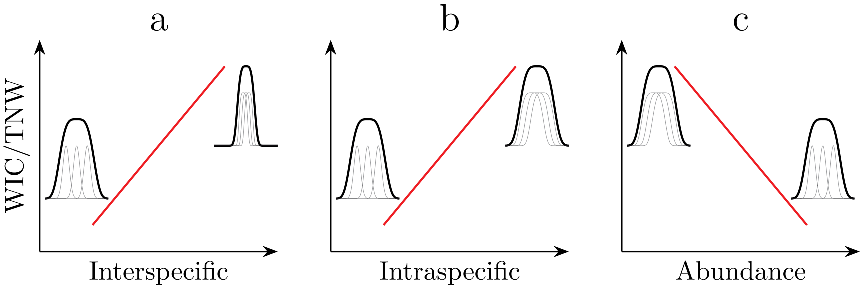


Figure 19: Relationships found in chapter 1 between estimates of interspecific, intraspecific competition and population abundance, and the degree of individual specialization (WIC/TNW) for several pollinator species (WIC= within-individual niche component and TNW= total niche width). Curves represent niche width of species (thick line) and individuals (thin lines). (a) Interspecific competition reduces the niche width of both the species and the individuals, thus decreasing the degree of individual specialization. (b) Intraspecific competition increases individual niches widths, however when all individuals end up adding the same resources individual specialization decreases. (c) Species with a higher population abundance tend to have a lower degree of individual specialization, which may be due to interference competition between individuals.

dictor of connectance and diversity in food-webs (Beckerman et al. 2006, Petchey et al. 2008, Melián et al. 2014). Moreover, niche partitioning of conspecific individuals can enhance the coexistence of plant species, especially when the number of pollinator species is small (Song and Feldman 2014). Since foraging behaviour is flexible, a continuous interaction re-wiring process takes place in networks in response to environmental fluctuations, a feature which may not be totally perceived in species-based networks. In individual-based networks, it is actually found that species switch from one module to another through time (chapter 2). Importantly, interaction switching and flexibility in foraging behaviour promotes biodiversity and increases network robustness to species loss (Kaiser-Bunbury et al. 2010, Thierry et al. 2011, Valdovinos et al. 2013).

Network structure and function: the effects of interactions on seed production

The construction of pollination networks would be partially incomplete without the estimation of the functional impacts of observed interactions on species fitness (Vázquez et al. 2012) and the identification of which particular network attributes are linked to community and species functions (Gómez et al. 2011, Gómez and

Perfectti 2012). Since networks represent the biodiversity of interactions, they may be used to test the effects of interaction diversity on ecosystem functioning (Loreau et al. 2001, Balvanera et al. 2006).

Quantifying the effects of each particular interaction in a network, however, is virtually impossible due to the myriad of possible interactions and hence reciprocal impacts among species. In chapter 3, a simplified alternative is proposed: to measure the effect of all interactions together as the relative actual contribution of insects to plant seed production. A greater connectivity within a plant-pollinator network (i.e. high linkage level, interaction diversity, closeness centrality) can translate into a higher contribution to seed production. Increases in the number and diversity of interactions are expected to enhance the pollination service, particularly when complementarity of pollinators occurs (Klein et al. 2003b, Hoehn et al. 2008, Albrecht et al. 2012). Pollinators can have complementary functional roles for plant reproductive success when they differ in the time of visitation, the position of flowers visited within a plant or the activity response to environmental conditions (Blüthgen and Klein 2011). Moreover, at a community-wide level, shifts in floral niches of pollinators due to interspecific competition are also expected to enhance the functional complementarity among species, thus affecting the biodiversity-function relationship (Fründ et al. 2013). Fontaine et al. (2006) were the first to provide experimental evidence that plant communities pollinated by functionally diverse assemblages of pollinators had higher reproductive success and plant recruitment richness.

Because all the above-mentioned mechanisms are highly context-dependent, results from chapter 3 also show that the 'connectivity-function' relationship is variable across communities. The outcome probably depends also on the total diversity of pollinators and differences in effectiveness and frequency among interactions in the community. For instance, in communities where the most abundant pollinators are the most effective, plant specialization may be more favourable than generalization (Stebbins 1970), because increasing the number of interactions may be accompanied by an increment of inefficient interactions. On the other hand, in communities with no relationship between interaction frequency and per-visit effectiveness generalization may increase seed production (Perfectti et al. 2009).

Despite networks have the potential to provide a good representation of pollen flow in plant communities or populations, few attempts have been made to link network structure and pollination efficiency or plant reproductive success. Specifically, Gómez et al. (2011) found that nestedness, connectivity and clustering in networks depicting shared pollinators among individual plants of *Erysimum mediohispanicum* were positively related to the number of per-capita juvenile plants produced per population. In another study Gómez and Perfectti (2012) reported that plants more connected to conspecifics (hubs) had higher plant fitness than plants with less connections in the network (peripherals). Therefore, these studies and findings from chapter 3 highlight that pollination network topology can have consequences for the reproductive success of plants.

However, direct inference of pollination function from interaction frequencies of flower-visitation networks must be done with caution, because visitation networks include in fact a mixture of interactions with neutral, positive and negative effects on plant reproductive success (i.e. there are neutral, mutualistic and antagonistic flower-visitors). For instance, not all visits observed in the network may result into effective conspecific pollen transfer to stigmas (King et al. 2013), because some flower-visitors may actually not carry pollen (Alarcón 2010, Popic et al. 2013), whereas others may be efficient at removing pollen from anthers but not at depositing pollen on stigmas (Wilson and Thomson 1991, Adler and Irwin 2006, Bartomeus et al. 2008). In addition, other flower-visitors may act as nectar or pollen robbers (Irwin et al. 2010). Therefore, it is very important to take into account that some visits observed may have more detrimental than beneficial effects for plants, and this might be an alternative explanation for the variability in the 'connectivity-function' relationship found across networks studied in chapter 3. Moreover, interactions may not have a real reproductive impact on plants if plant species are self-pollinated, something surprisingly not considered before in plant-pollinator network studies. Incorporating the reproductive dependence of plants on pollinators (breeding systems) is a key step to understand the real effects of observed interactions, and to predict plants' tolerance to pollinator loss (Vieira and Almeida-Neto 2015, Astegiano et al. 2015).

As shown in Vázquez et al. (2012), empirically estimating the number of encounters between species pairs could be enough to estimate the magnitude of species impacts (Vázquez et al. 2005, Sahli and Conner 2006, Vázquez et al. 2012) but not the particular sign of interactions, i.e. whether the interaction has a positive or a negative effect. The addition of a more realistic characterization of the effects of interactions in plant-pollinator networks would strengthen their informative value for community management and conservation (Forup et al. 2008, Memmott 2009, Tylianakis et al. 2010, Elle et al. 2012, Kaiser-Bunbury and Blüthgen 2015), for the prediction of network stability to disturbances (e.g. Memmott et al. 2004, Kaiser-Bunbury et al. 2010, Spiesman and Inouye 2013), and for a meaningful interpretation of the ecological and evolutionary implications of interactions.

The sign of interactions in networks: facilitative vs. competitive pollinator-mediated interactions

The estimation of the sign of interactions in networks is a key step to advance towards the construction of functionally informative pollination networks. Flower-visitors can deposit conspecific pollen but also pollen from other co-flowering species visited before, which may have detrimental effects on plant fitness (reviewed in Morales and Traveset 2008). Because most plants are visited by a wide array of pollinators (Waser et al. 1996, Johnson and Steiner 2000), pollinator sharing among plant species and hence interspecific pollen transfer (IPT) are common in natural communities (Morales and Traveset 2008, Ashman and Arceo-Gómez 2013). Therefore, a full network of indirect interactions among plant species in a community (i.e. pollinator-mediated interactions) may be deduced from the occurring IPTs. The relationship between conspecific and heterospecific pollen deposited on plant stigmas may provide a proxy of the relative cost or benefit obtained from shared pollinators (i.e. the sign of plant-plant interactions). This multi-species approach is followed in chapter 4 to study pollinator-mediated interactions in three high-Andean communities along an altitudinal gradient, and to determine the frequency of pollination facilitation and competition. Although interactions of all signs (positive, neutral, negative) are identified depending on the particular species involved, in general facilitative interactions prevail over competitive ones.

In chapter 4 the ubiquity of IPT in communities is confirmed, but also their small relative magnitude (McLernon et al. 1996, Aizen and Rovere 2010, Montgomery and Rathcke 2012). Interestingly, the IPT networks constructed reveal asymmetric patterns between pollen donation and receipt: many species receive from or donate to a few others, whereas a few species donate to or receive from many species (hubs); hub-receptor species are not the same species acting as hub-donors; and few pollen transfers among species are bidirectional. These structural patterns coincide with the only other existent study of IPT networks I am aware of (Fang and Huang 2013). However, the potential causes (floral morphology, flower abundances, pollinator behaviour) behind such common structural features still need further investigation (Montgomery and Rathcke 2012, Fang and Huang 2013, Ashman and Arceo-Gómez 2013), as well as their consequences on pollination efficiency and plant fitness. The donation-receipt asymmetry at the species level suggests, in turn, different selective pressures on reproductive fitness through costs to the male (pollen misplacement) or female functions (heterospecific deposition) (Johnson et al. 2005, Morales and Traveset 2008, Muchhala and Thomson 2012).

Previous field studies of pollinator sharing among co-flowering species documented both pollination competition and facilitation (e.g. Moeller 2004, Moragues and Traveset 2005, Mitchell et al. 2009). However, studies of isolated or pairwise species do not consider all interactions which occur simultaneously in a community, neither the potential non-additive effects which may take place reinforcing or weakening competition. Therefore, it is difficult to draw conclusions about the relative importance of positive and negative pollinator-mediated interactions in communities. So far, there is only one community-wide level study from Hegland et al. (2009a) which explored the relationship between conspecific and heterospecific floral densities and visitation rates in a temperate grassland. They found more positive intra- and interspecific interactions than negative ones, suggesting enhancement of pollinator attraction by co-flowering plants ('joint attraction effect'). Similarly, in the high-Andean communities studied, facilitation among plant species through shared pollinators prevails over competition. This finding reinforces the idea that co-flowering plant species benefit from magnet-species or multi-species attraction effects with increased visitation and increased conspecific pollen deposition, and that such positive outcomes frequently outweigh the associated cost of increased heterospecific pollen deposition due to shared pollinators. Nevertheless, positive effects in visitation can sometimes be accompanied by a reduction in the quality of pollen deposited. For this reason, facilitative effects are more common in terms of quantity than quality in chapter 4. Despite the role of pollination competition has received most attention, facilitation at the community level can be more important than competition, and plant species which share pollinators may be more likely to establish and persist in communities.

In addition, chapter 4 shows that in the community from the highest elevation, where the conditions for the pollinator service are less favourable – i.e. low temperatures, strong winds and snow limit pollinator abundance, activity and diversity (Arroyo et al. 1982, Totland 1993) – the proportion of species experiencing a facilitative effect is larger than in the communities from lower altitudes. This finding supports the stress-gradient hypothesis which predicts that occurrence of positive interactions among plants in natural communities increases with increasing abiotic/biotic stress (Bertness and Callaway 1994, Callaway et al. 2002, He et al. 2013). However, further empirical studies in other communities are needed to determine how generalized this hypothesis for plant-plant pollination interactions is. The reported increase in facilitation with altitude may be in part associated with the amelioration of pollen limitation in such stressful habitats when species are co-flowering (Moeller 2004) and with density-dependent effects (Feinsinger 1987, Munoz and Cavieres 2008). Moreover, shifts in the direction and magnitude of pollinator-mediated interactions among plants can occur when the availability of pollinators changes, as shown in a few manipulative experimental studies (Lázaro et al. 2014, Ye et al. 2014).

Therefore, the effects of pollination interactions on plant reproductive success are expected to be also strongly influenced by co-flowering neighbours. The study of the particular influence of plant-plant indirect interactions in the structure and functioning of pollination networks, community structure, population dynamics and plant evolution (competitive displacement or convergence) deserves further attention (e.g. Rathcke 1983, Feinsinger 1987, Aizen and Vázquez 2006, Sargent and Ackerly 2008). The estimation of an interaction sign in networks which depict pollination plant-plant interactions is a first step to improve our understanding of the importance of competitive and facilitative processes in communities.

General conclusions

1. The structure of networks from different organizational hierarchical levels (species and individuals) can diverge. The pollen-transport networks at the individual scale (pollinator individuals – plant species) had lower linkage density, connectance, nestedness and interaction diversity, but higher modularity than networks at the species scale (pollinator species – plant species). Differences were caused by individual specialization in pollen resources and heterogeneity in foraging behaviour among individuals. Given that species-based networks do not consider intraspecific variation, a misleading or incomplete picture of network patterns may be obtained.

2. Generalist pollinator species in networks are composed by specialist individuals. On average, individual pollen resource niche represented only c. 46% of the total species niche. The degree of individual specialization of pollinator species was associated with inter- and intraspecific competition, and was higher for abundant than for rare species. Such relationships highlight the importance of environmental factors and indirect interactions among species and individuals for determining how interactions are structured within communities.

3. The view of network modules as groups of species taxonomically or morphologically related may not hold at the individual level. Downscaling networks showed that a pollinator species does not belong unambiguously to a single module. Conspecific individuals were not aggregated into the same module because they did not use the same pollen resources. At the individual scale, phenology was a more important driver of modularity than taxonomy. Modules changed through time during the flowering season, so new modules were formed and old ones dissolved as the season progressed.

4. Not all individuals of a species are equivalent from a network structural viewpoint. Flowering period length, plant abundance, specialization and phenophase of insect individuals were important attributes determining the topological role of plant species and individual pollinators, respectively.

5. The connectivity pattern of a species within a plant-pollinator network can be associated to plant reproductive success. Plant species with a high centrality, number and diversity of interactions within a plant-pollinator network tend to more dependent on pollinators for seed production. However, this ‘connectivity-function’ relationship is context-dependent and, thus, variable across communities.

6. The study of interspecific pollen transfers at a community-wide level and the relationship between conspecific and heterospecific pollen deposition on stigmas can provide a feasible multi-species approach to estimate the sign of the effect (positive, neutral, negative) of shared pollinators on plant pollination quality and quantity.

7. Interspecific pollen transfer due to shared pollinators is widespread among plant species in communities, although its relative magnitude is small. Networks of pollen transfer among co-flowering species were monopolized by a few species acting as either pollen hub-receipts or hub-donors. Patterns of pollen donation and receipt were asymmetric, implying that species receiving pollen from many are not necessarily those exporting pollen to many.

8. Facilitative and neutral pollinator-mediated interactions among plants prevailed over competition in the three high-Andean communities studied. Facilitation was attributed to an enhancement of pollinator attraction by plants flowering together (‘joint attraction’) and by focal plants acting as ‘magnet-species’.

9. The incidence of facilitative, neutral, and competitive pollinator-mediated interactions in communities changes with altitude. A largest proportion of facilitated species was found in the highest elevation community, suggesting that facilitation can be even more common under less favourable conditions for pollination service and at low plant densities.

Ideas for future investigation

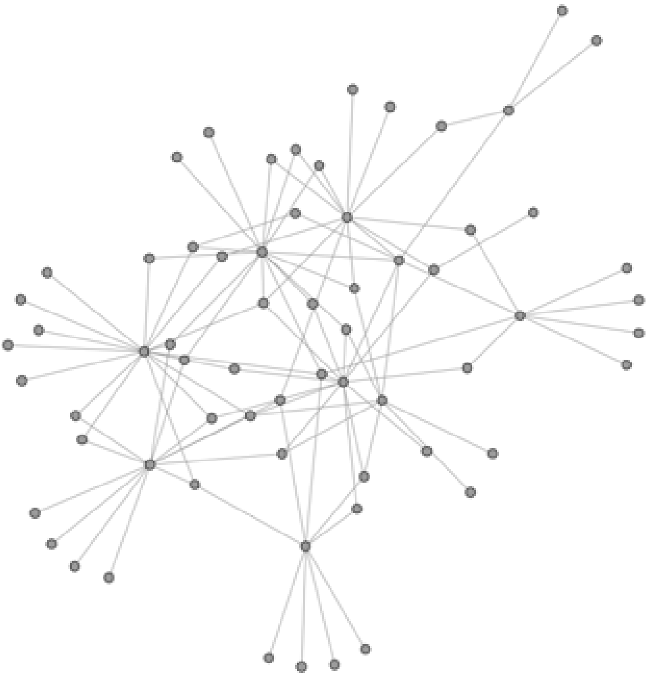
As usually occurs when doing science, the research conducted ends with more questions than answers. In this thesis, some of the results found open new questions offering novel opportunities for future investigation. Here, I would thus like to outline several ideas for further exploration:

First, we need more studies confirming widespread divergence of network structural patterns across hierarchical levels. Once we confirm a general pattern, it will then be interesting to link structure across levels. For this purpose, theoretical models may help testing whether particular network patterns at the individual level (e.g. nestedness, modularity) can upscale generating the same or alternatively new patterns at the species level. Moreover, empirical studies of multispecies interactions, such as pollination or seed-dispersal, resolved at the individual scale and including complete data from morphological traits of individuals will be essential to address, for instance: (1) how intraspecific trait variation influences species-based network structure, niche partitioning within species, and species connectivity within the network; and (2) which morphological traits in particular define highly connected individuals which give structural cohesiveness to the network. All this knowledge will help to incorporate an evolutionary perspective to interaction networks, since intraspecific niche variation means also variation in fitness and selective pressures. From an applied perspective, individual-based networks can be useful for management and conservation. For instance, when planning the conservation of a particular species an efficient strategy would be to focus in the protection of resources used by more individuals within the population. Moreover, strategies for preserving interactions in a population may concentrate more effort in highly connected (network hubs) than in weakly connected individuals (peripherals), because the loss of 'core' members can initiate secondary extinctions and network fragmentation. In other cases, understanding the structure of individual-based networks might be important to prevent and control the spreading of pathogens or diseases within-populations.

Pollination networks with a more realistic characterization of the functional effects of plant-pollinator interactions on plant reproduction will help modelling network robustness to the loss of species in a more realistic way, and will allow predicting also the function coupled to interactions that may be lost with extinctions. For instance, from a plant perspective, the loss of interactions with little impact on reproductive success may be less detrimental for plant persistence than the loss of pollinators that contribute much to its reproductive success. Moreover, plant species with a small animal pollination dependence (e.g. plants which are capable of self-pollinating) may survive even if a large fraction of the pollinators' community is lost.

Linking pollinator visitation patterns to plants and plants' patterns of pollen receipt will be an important step to determine to what extent visitation networks can be considered good representations of 'real' pollination networks ('networks of functions'). To achieve this goal, for instance, the concordance between visitation, pollen-transport and pollen deposition networks could be analyzed in the future. In addition, interspecific pollen transfer networks may be also useful tools to address the effect of conspecific pollen loss and heterospecific pollen deposition on pollination efficiency (i.e., the proportion of pollen produced on anthers which is deposited on conspecific stigmas), both at the species and the community level. Moreover, the study of potential mechanisms and factors driving the structure of interspecific pollen transfer networks (e.g. pollen production per flower, plant abundance, flower morphology, plant reproductive systems, pollinator groups, plant phylogeny) will help understanding the ecological and evolutionary implications of such transfers for plants and the processes of pollination competition and facilitation between plants in communities.

IV | Supplementary Materials and References



A

Supplementary Material Chapter 1

A.1 Insect pollen load analysis

In the laboratory, we studied the pollen loads of each insect individual captured. For frequently captured species, a maximum of 10 individuals per species was included in the analysis. An isotonic water solution (1.5 ml) was added to each vial containing a specimen and shaken for 5 sec in a vortex and then washed by agitation for 15 min in an ultrasonic bath to remove pollen grains from their body surface. For honeybees, pollen clumps in the corbicula of the hind legs were removed in advance. Afterwards, insects were pinned for later identification by taxonomist experts. Vials containing the pollen load solution were centrifuged at 13.000 rpm for 15 min and inspected for any presence of a pollen pellet at the bottom. In vials with a large pollen pellet, to facilitate pollen counting, we added a *Lycopodium* spore tablet containing 18,584 spores (batch no. 177745, Lund Univ., Sweden), whereas in the other vials the supernatant was carefully removed, and the droplet with the pollen was suspended on a microscopic slide. Pollen concentrated samples were homogenized with vortex agitation in order to dissolve the spore tablets and then three drops (replicates) were mounted on slides. We added a small pink fuchsine-stained jelly cube to the pollen smear, melted it and covered the final smear with a cover slip (Kearns and Inouye 1993). To avoid any pollen contamination, laboratory instruments were cleaned with ethanol between manipulation of samples. Slides were analyzed under microscope (100-400x) to estimate total number of pollen grains from each insect individual. All pollen grains were identified to the lowest possible taxonomic level and the number of pollen grains of each species was counted (on average 60% of total slide area was inspected for pollen). In slides from pollen-dense samples, we counted the number of pollen grains and also the number of spores. In these samples, total pollen number carried by the insect was estimated as the mean of the three replicates, calculated as the number of pollen grains counted in a droplet × (18,584/number of spores counted in droplet) (Jakobsson et al. 2008).

A pollen reference collection was made during the field season in order to facilitate pollen identification. Pollen grains of each species were measured and photographed under the microscope at 400x. Pollen grains of some species were indistinguishable from those of closely related species, and therefore classified into 'pollen type clusters' including more than one species: 'Galium' (including *Galium cinereum*, *G. crespianum* and *G. balearicum*), 'Teucrium' (including *Teucrium marum* and *T. asiaticum*), 'Geraniaceae' (including *Geranium colombinum*, *G. lucidum*, *G. molle*, *G. purpureum* and *Erodium cicutarium*), and 'Asteraceae' (including all Asteraceae at study sites except *Bellium bellidioides*, *Carlina corymbosa* and *Santolina chamaecyparissus*, which were identified to species). Unknown pollen grains were assigned to morphotype species.

A.2 Indices for quantifying individual specialization and niche components

We used formulae from Bolnick et al. (2002) to calculate each niche component (TNW, WIC and BIC; Roughgarden 1972, 1974) using categorical data on pollen types carried by insect specimens. Shannon diversity index is used as a proxy for variance in niche width, so the niche widens with addition of new resources and with increasing evenness in resource use. For each species with ≥ 5 individuals sampled (14 spp. at CN, 7 spp. at PC), we constructed a matrix with a rows and p columns, where a is the number of individuals of species S, p the plant pollen types found on the body of individuals of S, and the value in each cell (n_{ij}) is the number of j's pollen grains carried by the ith individual. Then, using those matrices for each species S, we calculated niche components as

$$WIC = \sum_i p_i \cdot \left(\quad \sum_j p_{ij} \cdot \ln(p_{ij}) \right) \tag{A.1}$$

$$BIC = \quad \left(\sum_i p_i \cdot \ln(p_{ij}) \right) \quad \left[\sum_j q_j \cdot \left(\quad \sum_i \gamma_{ij} \cdot \ln(\gamma_{ij} \right) \right] \tag{A.2}$$

$$TNW = \quad \sum_i q_j \cdot (q_j) \tag{A.3}$$

$$p_{ij} = \frac{n_{ij}}{\sum_j n_{ij}} \qquad p_i = \frac{\sum_j n_{ij}}{\sum_i \sum_j n_{ij}} \qquad q_j = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}} \qquad \gamma_{ij} = \frac{n_{ij}}{\sum_i n_{ij}} \tag{A.4}$$

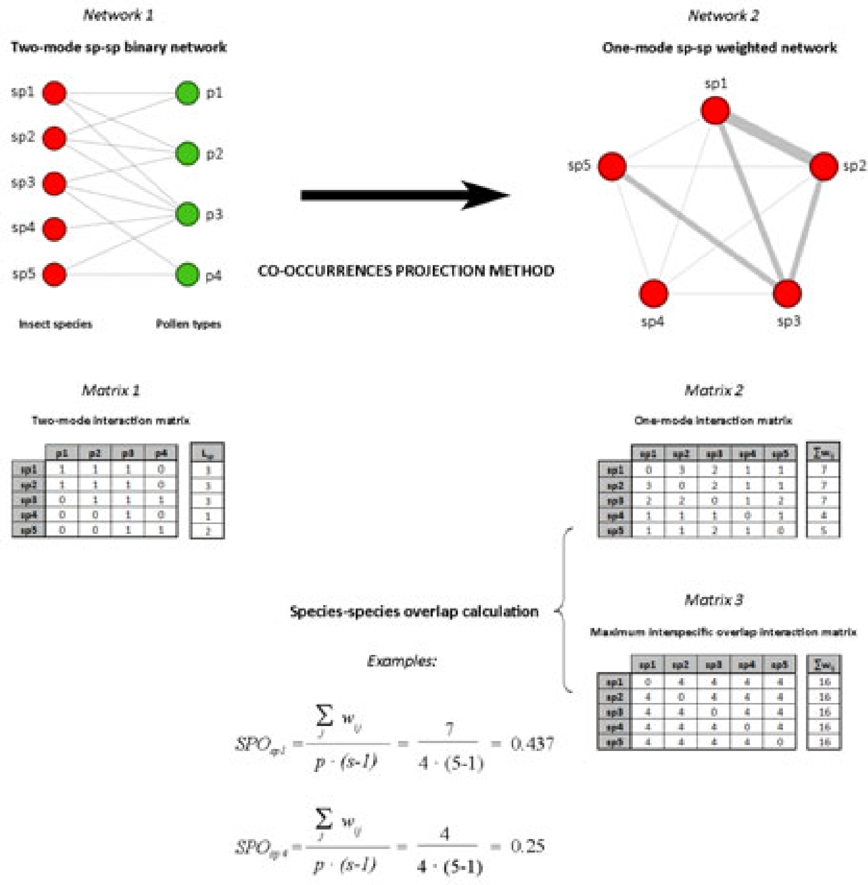
where, p_{ij} is the proportion of pollen type j in the total pollen load of individual i of species S, p_i is the proportion of all pollen types used by S that are used by individual i, q_j is the proportion of pollen type j in the total pollen load of S, and γ_{ij} is the proportion of total pollen type j used by S that was carried by individual i. Relative degree of individual specialization was obtained by dividing WIC by TNW, i.e. proportion of total niche width explained by the within-individual component.

Following Bolnick et al. (2007) we also calculated overlap in pollen resource use among each individual and the whole population as

$$PS_i = 1 \quad 0.5 \cdot \sum_j |p_{ij} \quad q_j| \tag{A.5}$$

where PS_i is the proportional similarity index, measuring the similarity in pollen resource use distribution of individual i and the corresponding species S (Feinsinger et al. 1981). Individuals using pollen resources in the same proportion as their species have a $PS_i = 1$, whereas $PS_i = q_j$ if they are specialized in just one pollen resource j.

A.3 Measuring interspecific overlap with one-mode weighted networks



	p1	p2	p3	p4	L_{sp}
sp1	1	1	1	0	3
sp2	1	1	1	0	3
sp3	0	1	1	1	3
sp4	0	0	1	0	1
sp5	0	0	1	1	2

	sp1	sp2	sp3	sp4	sp5	$\sum w_{ij}$
sp1	0	3	2	1	1	7
sp2	3	0	2	1	1	7
sp3	2	2	0	1	2	7
sp4	1	1	1	0	1	4
sp5	1	1	2	1	0	5

	sp1	sp2	sp3	sp4	sp5	$\sum w_{ij}$
sp1	0	4	4	4	4	16
sp2	4	0	4	4	4	16
sp3	4	4	0	4	4	16
sp4	4	4	4	0	4	16
sp5	4	4	4	4	0	16

$$SPO_{sp1} = \frac{\sum_j w_{ij}}{p \cdot (s-1)} = \frac{7}{4 \cdot (5-1)} = 0.437$$

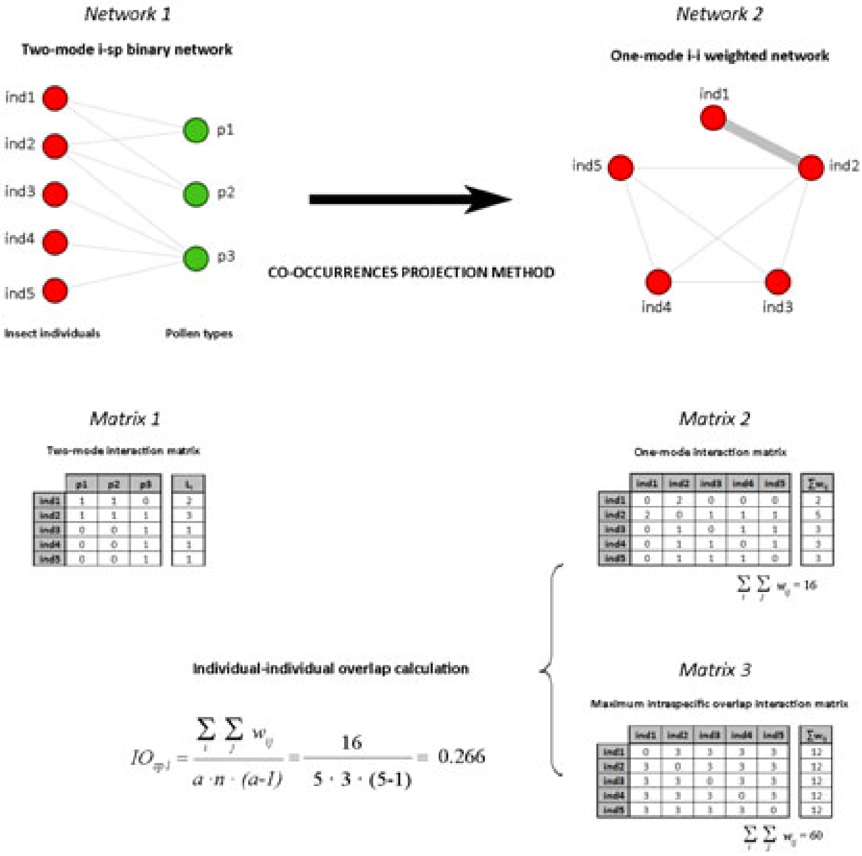
$$SPO_{sp4} = \frac{\sum_j w_{ij}}{p \cdot (s-1)} = \frac{4}{4 \cdot (5-1)} = 0.25$$

Figure 20: Example of interspecific overlap calculation in networks.

Here we present a simple hypothetical example to illustrate the procedure applied when estimating interspecific overlap (SPO) for insect species in our networks. *Network 1* is a two-mode binary pollen-transport network depicting interactions between five insect species (red nodes) and four plant pollen types (green nodes). Insect species are linked to plant pollen types if they carried pollen grains. *Net-*

work 1 can also be represented as an interaction matrix (*Matrix 1*) with s rows and p columns, where s is the number of insect species ($s = 5$), p is the number of plant pollen types ($p = 4$) and the value in each cell entry is 0 or 1 (i.e. absence or presence of pollen grains in insect's body, resp.). Linkage level of species (L_{sp}) is the total number of plant pollen types carried (i.e. *matrix 1* row sum). This two-mode binary network was transformed into a one-mode weighted network by counting the total number of plant pollen types shared among species (co-occurrences projection method as in Opsahl 2009a, Padrón et al. 2011). *Network 2* is the one-mode projection of *network 1*, where insect species are linked if pollen grains of the same plant pollen type were found on the body of both insects, and the weight of each link is the number of plant pollen types in common. For instance, *sp1* shares three pollen types with *sp2*, two pollen types with *sp3* and only one with *sp4* and *sp5*. Therefore, the maximum link weight possible in this kind of network projection is always the total number of plant pollen types present in the community (here $p = 4$). This one-mode weighted network depicting the pattern of shared pollen types among insect species can be also represented by an interaction matrix with s rows and s columns (*Matrix 2*) where the value in each cell entry (w_{ij}) is the number of plant pollen types shared. Therefore, sum of link weights ($\sum w_{ij}$, i.e. *matrix 2* row sum) is the overlap in plant pollen types among a certain species and all other species in the network. To get a standardized measure of interspecific overlap ranging from 0 to 1 (SPO), we divided $\sum w_{ij}$ by the maximum interspecific overlap possible for a species in the network, which was calculated as $p \cdot (s \quad 1)$, i.e. sum of node link weights $\sum w_{ij}$ in an hypothetical case where all insect species carried all plant pollen types from the community and therefore shared all (*Matrix 3*). We illustrate the complete calculation of species-species overlap (SPO) for two species in our example.

A.4 Measuring intraspecific overlap with one-mode weighted networks



Matrix 1

Two-mode interaction matrix

	p1	p2	p3	L_i
ind1	1	1	0	2
ind2	1	1	1	3
ind3	0	0	1	1
ind4	0	0	1	1
ind5	0	0	1	1

Matrix 2

One-mode interaction matrix

	ind1	ind2	ind3	ind4	ind5	$\sum w_{ij}$
ind1	0	2	0	0	0	2
ind2	2	0	1	1	1	5
ind3	0	1	0	1	1	3
ind4	0	1	1	0	1	3
ind5	0	1	1	1	0	3

$$\sum_i \sum_j w_{ij} = 16$$

Matrix 3

Maximum intraspecific overlap interaction matrix

	ind1	ind2	ind3	ind4	ind5	$\sum w_{ij}$
ind1	0	3	3	3	3	12
ind2	3	0	3	3	3	12
ind3	3	3	0	3	3	12
ind4	3	3	3	0	3	12
ind5	3	3	3	3	0	12

$$\sum_i \sum_j w_{ij} = 60$$

$$IO_{sp1} = \frac{\sum_i \sum_j w_{ij}}{a \cdot n \cdot (a-1)} = \frac{16}{5 \cdot 3 \cdot (5-1)} = 0.266$$

Figure 21: Example of intraspecific overlap calculation in networks.

Here we present an example illustrating the methodology used to estimate intraspecific overlap (IO) for insect species in our networks. *Network 1* is the two-mode binary pollen-transport network for *sp1* (see previous example in section A.3) depicting interactions between five insect individuals (red nodes) of *sp1* and three plant pollen types (green nodes). Therefore, *network 1* represents within-species partition of pollen resources among insect individuals. Insect individuals are linked to plant pollen types if they carried pollen grains. *Network 1* can also be represented as an interaction matrix (*Matrix 1*) with a rows and n columns, where a is the number of *sp1*'s insect individuals ($a = 5$), n is the number of plant pollen types visited by *sp1* ($n = 3$, i.e. L_{sp1}) and the value in each cell entry is 0 or 1 (i.e. absence or presence of pollen grains on insect's body, respectively). Linkage level of the corresponding species (L_{sp}) is the maximum linkage level possible for conspecific insect individuals (L_i, i.e. *matrix 1* row sum). This two-mode binary network (*Network 1*) was transformed into a one-mode weighted network (*Network 2*) by counting the total number of plant pollen types shared among individuals and using it as link weight w_{ij} (co-occurrences projection method as in Opsahl 2009a, Padrón et al. 2011). In the example, *ind1* and *ind2* have two pollen types in common and all other individuals share a pollen type among them. This one-mode weighted network can also be represented by an interaction matrix with a rows and a columns (*Matrix 2*) where the value in each cell entry (w_{ij}) is the number of plant pollen types shared among ind_i and ind_j, so $\sum w_{ij}$ (i.e. *matrix 2* row sum) represents the total number of pollen types shared among ind_i and all other conspecific individuals. Therefore, sum of all matrix link weights ($\sum_i \sum_j w_{ij}$, i.e. *matrix 2* row and column sum) is the overlap in plant pollen types among all conspecific individuals (i.e. intraspecific overlap). To get a measure of intraspecific overlap ranging from 0 to 1 (IO), we divided by the maximum intraspecific overlap possible for the corresponding species (see *Matrix 3*). As maximum number of pollen types in common (w_{ij}) among two individuals of *sp1* is n, maximum intraspecific overlap will occur when all conspecific individuals have the same linkage level as the species and therefore share all n pollen resources among them as represented in *Matrix 3* (i.e. $\sum w_{ij} = n \cdot (a \quad 1) = 12$ and $\sum_i \sum_j w_{ij} = a \cdot \sum w_{ij} = 12 \cdot 5 = 60$). Complete formulae for calculation of intraspecific overlap (i.e. individual-individual overlap, IO) is shown for *sp1* as example.

A.5 Evaluation of species sampling completeness

We aimed to determine the extent to which the number of individuals sampled per species allowed for a sufficient description of species interactions (L_{sp}). To evaluate completeness of our sample sizes we first computed sampled-based rarefaction curves for each species (Gotelli and Colwell 2001) using the package *vegan* (version 2.0-6, Oksanen et al. 2012) in the *R program* (version 2.15.0, R Development Core Team 2012). Following Chacoff et al. (2012), for each of the 21 species studied, we calculated the percentage of estimated asymptotic richness detected as,

$$\% S_{obs} = 100 \cdot \frac{S_{obs}}{S_e} \tag{A.6}$$

where S_{obs} is the observed pollen type richness in the samples (i.e. species linkage level, L_{sp}) and S_e is the asymptotic estimated pollen type richness (i.e. estimated species linkage level). To compute S_e we used the *Chao 2* non-parametric estimator in its bias-corrected form,

$$S_e = S_{obs} + \left[\frac{(a - 1)}{a}\right] \cdot \left[\frac{u \cdot (u - 1)}{2 \cdot (d + 1)}\right] \tag{A.7}$$

where a is the sample size (i.e. number of individuals of each species sampled for pollen load analysis), u is the number of uniques (i.e. plant pollen types that occur only in one sample) and d is the number of duplicates (i.e. plant pollen types that occur in two samples) (Chao 2005). *Chao 2* index relies on the principle that rare species in the samples carry most information on the number of un-observed species (Chao 1984) and is one of the least biased estimates for small sample sizes (Colwell and Coddington 1994).

A.6 Supplementary Figures Chapter 1

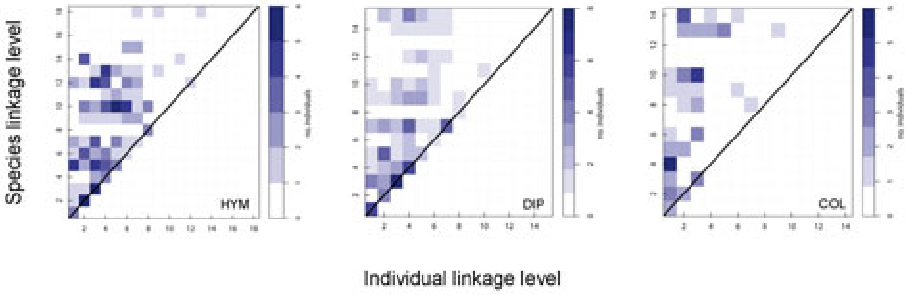


Figure 22: Relation between linkage level of species (L_{sp}) and individuals (L_i) for the main insect orders. Each matrix corresponds to a different insect pollinator order: Hymenoptera ($N_{Hym} = 147$ individuals), Diptera ($N_{Dip} = 114$) and Coleoptera ($N_{Col} = 59$). Colours represent number of individuals with a given L_i and L_{sp} configuration, so figures show where the highest density of individuals is in each matrix. Matrix diagonal (species-individual isocline) represents perfect matching of individual niche width and species niche width ($L_i = L_{sp}$), while deviations to the left indicate individuals being more specialized than their species ($L_i < L_{sp}$). Filled cells are located in the upper region of the diagonal because of the constraint $L_i \leq L_{sp}$. For all species in the different orders, specialist individuals predominate ($L_i/L_{sp} < 1$). The trend is more marked for beetles, probably because of their lower mobility.

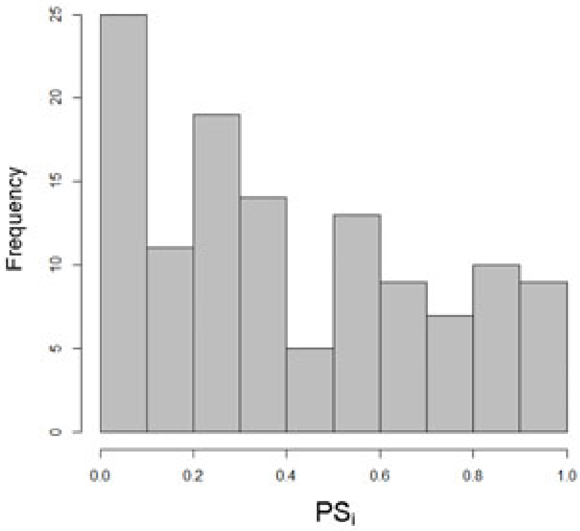


Figure 23: Frequency histogram ($N = 122$) of the proportional similarity indices of individuals (PS_i) from the 21 selected species in our networks. Individuals with narrower niches than their corresponding species are more frequent (60.7% individuals had a $PS_i < 0.5$), although some highly generalized individuals can be found as well.

A.7 Supplementary Tables Chapter 1

Table 9: Definitions of parameters used to describe sp-sp and i-sp networks. The first five metrics are qualitative, whereas the last two are quantitative.

Parameter	**Definition**
Linkage level (L)	Number of interactions of each network node, i.e. number of interactions per species (L_{sp}) in sp-sp networks and number of interactions per individual (L_i) in i-sp networks.
Network size (N)	Total number of possible interactions in the network, i.e. the number of cell entries in the interaction matrix (number of i rows multiplied by number of j columns).
Linkage density (LD)	Mean number of links per network node.
Connectance (C)	Realized proportion of all possible links (Dunne et al. 2002).
Nestedness (NODF)	Nestedness metric based on overlap and decreasing fills (Almeida-Neto et al. 2008). It measures to what extent the interaction pattern resembles a perfectly nested pattern where specialist species interact with a proper subsets of the species with which more generalized species interact. It ranges from 0 (non-nestedness) to 100 (perfect nestedness).
Interaction diversity (H)	Shannon diversity of links for a network node i $$H_i = \sum_j p_{ij} \cdot \ln p_{ij} \qquad \text{(A.8)}$$ where $p_{ij} = \frac{n_{ij}}{\sum_j n_{ij}}$ and n_{ij} is the interaction frequency between node i and j (here number of j's pollen grains carried by insect node i) Shannon diversity of links for the whole network $$H_2 = \sum_i \sum_j q_{ij} \cdot \ln q_{ij} \qquad \text{(A.9)}$$ where $q_{ij} = \frac{n_{ij}}{\sum_i \sum_j n_{ij}}$ (Bersier et al. 2002).
Interaction evenness (E_2)	Shannon's evenness of link frequency distribution in the whole network, calculated as $$E_2 = \frac{H_2}{\ln(I)} \qquad \text{(A.10)}$$ where I is the total number of links in the network and $\ln(I)$ the maximum diversity possible in the network i.e. $H_{máx}$). It measures the heterogeneity of interaction frequencies, ranging from 0 (uneven network) to 1 (uniform network). An uneven network is one with high skewness in the distribution of link frequencies (Tylianakis et al. 2007).

Table 10: List of individual specialization and overlap indices calculated for the 21 insect species of flower-visitors selected from our networks.

Insect species	Label	Order	Site	a	L_{sp}	$\bar{L_i}$	null $\bar{L_i}$	TNW	WIC	BIC	WIC/TNW*	SPO	IO
Apis mellifera	api.mel	HYM	CN	8	12	3.25	9.84	0.76	0.12	0.63	0.16	0.05	0.08
Attalus sp.	att.sp	COL	CN	6	4	1.00	3.59	1.13	0.00	1.13	0.00	0.02	0.05
Eristalis tenax	eri.ten	DIP	CN	5	15	4.20	13.59	1.84	1.11	0.73	0.60	0.06	0.06
Eupeodes corollae	eup.cor	DIP	CN	5	10	3.40	7.08	0.71	0.40	0.31	0.56	0.06	0.12
Exoprosopa bowdeni	exo.bow	DIP	CN	5	9	4.80	5.16	0.83	0.79	0.04	0.95	0.06	0.34
Halictus spp.	hal.sp	HYM	CN	5	10	4.40	9.80	1.19	0.37	0.82	0.31	0.06	0.20
Halictus vestitus	hal.ves	HYM	CN	5	11	5.40	11.00	1.76	0.89	0.87	0.51	0.06	0.25
Lasioglossum nitidulum hammi	las.nit	HYM	CN	6	12	4.00	9.09	1.41	0.89	0.52	0.63	0.06	0.16
Mordellistena sp.	mor.sp	COL	CN	9	10	2.44	9.10	1.35	0.60	0.75	0.44	0.06	0.09
Oedemera flavipes	oed.fla	COL	CN	10	13	4.20	12.95	1.97	0.81	1.16	0.41	0.06	0.14
Paragus tibialis	par.tib	DIP	CN	5	12	5.40	11.61	1.69	1.00	0.68	0.59	0.07	0.23
Plagyolepis pygmaea	pla.pyg	HYM	CN	5	5	1.80	3.72	1.00	0.09	0.91	0.09	0.04	0.10
Sphaerophoria sp.	sph.sp	DIP	CN	6	15	4.17	12.96	1.32	0.54	0.78	0.41	0.05	0.08
Stomorhina lunata	sto.lun	DIP	CN	5	7	3.00	6.90	0.92	0.52	0.40	0.56	0.05	0.21
Anthidium cingulatum	ant.cin	HYM	PC	5	13	5.00	9.84	1.42	1.04	0.37	0.74	0.07	0.19
Apis mellifera	api.mel	HYM	PC	5	14	3.80	11.47	0.48	0.13	0.35	0.27	0.07	0.06
Halictus spp.	hal.sp	HYM	PC	5	10	4.80	9.22	0.91	0.52	0.39	0.57	0.06	0.27
Megachile pilidens	meg.pil	HYM	PC	6	13	5.00	8.78	0.93	0.80	0.13	0.86	0.07	0.16
Oedemera flavipes	oed.fla	COL	PC	6	14	3.00	12.99	1.81	0.71	1.11	0.39	0.05	0.02
Sphaerophoria sp.	sph.sp	DIP	PC	5	14	5.00	9.84	0.40	0.17	0.23	0.42	0.08	0.12
Tapinoma nigerrimum	tap.nig	HYM	PC	6	7	2.00	2.80	0.14	0.01	0.14	0.05	0.05	0.06

HYM: Hymenoptera; COL: Coleoptera; DIP: Diptera; CN: Coma de n'Arbona; PC: Passadís de Ses Clotades; a: number of individuals captured for pollen load analysis; L_{sp}: linkage level of species; $\bar{L_i}$ mean linkage level of conspecific individuals; null $\bar{L_i}$: mean linkage level of conspecific individuals after 1,000 randomizations under the null hypothesis that individuals act as generalists sampling from species pollen resource distribution proportions; TNW: total niche width; WIC: within-individual niche component; BIC: between-individual niche component; WIC/TNW: degree of individual specialization; SPO: interspecific overlap; IO: intraspecific overlap.

* All WIC/TNW empirical values reported were below null WIC/TNW values obtained from 1,000 randomizations.

B

Supplementary Material Chapter 2

B.1 Comparison of module identification by different measures

We evaluated the concordance of modules identified by each modularity metric (M_U = unipartite modularity, M_B = bipartite modularity, M_{WB} = weighted bipartite modularity). Ten runs for each empirical network and modularity metric were performed. Partitions returned by these runs were compared by calculating and index of mutual information (I_{EF}) between pairwise runs E and F (Guimerà et al. 2007, Thébault 2013) with the following formula

$$I_{EF} = \frac{2\sum_{i=1}^{N_M^E}\sum_{j=1}^{N_M^F} n_{ij}^{EF} \cdot \log\left(\frac{n_{ij}^{EF} S}{n_i^E n_j^F}\right)}{\sum_{i=1}^{N_M^E} n_i^E \cdot \log\left(\frac{n_i^E}{S}\right) + \sum_{j=1}^{N_M^F} n_j^F \cdot \log\left(\frac{n_j^F}{S}\right)} \tag{B.1}$$

where N_M^E and N_M^F are the number of modules in partitions E and F respectively, n_i^E and n_j^F are the number of nodes in module i of partition E and in module j of partition F, and n_{ij}^{EF} is the number of nodes that are in module i of partition E and in module j of partition F. The mutual information between partition E and F is equal to 1 if both partitions are identical, and 0 if partitions are uncorrelated. We then calculated the average mutual information among runs of the same metric and among runs of different metrics for empirical i-sp networks at each study site.

B.2 Measure of the degree of individual specialization

According to the model developed by Roughgarden (1972), total niche width (TNW, i.e. the variety of resources exploited by a population) can be partitioned into two components: the variation in resource use within individuals (within-individual component, WIC), and the niche variation among individuals (between-individual component, BIC), so that TNW = WIC + BIC. The degree of individual specialization can be measured as the proportion of the total niche width (TNW) explained by the within-individual component, i.e. WIC/TNW. We applied formulae from Bolnick et al. (2002) to calculate each niche component using quantitative data of pollen load analysis. As described also in Tur et al. (2014), for each selected species (species with ≥ 5 individuals sampled, 14 spp. at CN and 7 spp. at PC), a matrix with a rows and p columns was built, where a is the number of individuals of species S, p the plant pollen types found on the body of individuals of S, and the value in each cell (n_{ij}) is the number of j's pollen grains carried by the ith individual. Then, using those matrices for each species we calculated niche components as

$$WIC = \sum_i p_i \cdot \left(\sum_j p_{ij} \cdot \ln(p_{ij}) \right) \tag{B.2}$$

$$BIC = \left(\sum_i p_i \cdot \ln(p_{ij}) \right) \left[\sum_j q_j \cdot \left(\sum_i \gamma_{ij} \cdot \ln(\gamma_{ij}) \right) \right] \tag{B.3}$$

$$p_{ij} = \frac{n_{ij}}{\sum\limits_j n_{ij}} \qquad p_i = \frac{\sum\limits_j n_{ij}}{\sum\limits_i \sum\limits_j n_{ij}} \qquad q_j = \frac{\sum\limits_i n_{ij}}{\sum\limits_i \sum\limits_j n_{ij}} \qquad \gamma_{ij} = \frac{n_{ij}}{\sum\limits_i n_{ij}} \tag{B.4}$$

where, p_{ij} is the proportion of pollen type j in the total pollen load of individual i of species S, p_i is the proportion of all pollen types used by S that are used by individual i, q_j is the proportion of pollen type j in the total pollen load of S, and γ_{ij} is the proportion of total pollen type j used by S that was carried by individual i. Finally, we obtained WIC/TNW, which measures the amount of variation in pollen resource use within a species due to heterogeneity and niche specialization of conspecific individuals. Therefore, WIC/TNW approaches 1 when the niches of individuals include the full range of pollen resources used by their species (i.e. homogeneous individuals being as generalized as their species), whereas it approaches 0 when individuals use smaller and non-overlapping subsets of the species resources (i.e. heterogeneous individuals being more specialized than their species). Significance of WIC/TNW values obtained was adressed by Monte Carlo re-sampling procedures (Araújo et al. 2010). For each species 1000 randomizations were conducted, in which each individual was reassigned the same pollen load as observed carrying, but pollen grains were distributed among pollen types with probabilities equal to pollen type proportions used by the corresponding species (i.e. null hypothesis that all individuals act as generalized as the species). The observed WIC/TNW values were significant when they were below 95% confidence interval of WIC/TNW randomization values.

B.3 Biological features studied to characterize network nodes and their topological roles

For each plant pollen type in empirical i-sp networks we determined: (a) linkage level, (b) pollen abundance, (c) flowering period length, and (d) flowering peak. Linkage level (L_{sp}) is the number of interactions between a plant pollen type and insect pollinator species in sp-sp networks. Pollen abundance was calculated as total number of pollen grains on insects for each plant pollen type and can be considered a proxy of flower abundance (Spearman's rho = 0.37, $P < 0.001$). The flowering period length was estimated by counting the number of weeks between date of first and last plant pollen type detection on insects or observation of plant species blooming in the field. Flowering peak was determined categorically (May, June, July, August) considering the date of maximum flower abundance or maximum pollen abundance. Similarly, for each insect individual we determined: (a) individual linkage level, (b) species abundance, (c) species phenophase length, (d) individual phenophase, and (e) sex of the specimen. Individual linkage level (L_i) is the number of interactions between each insect individual and plant pollen types in i-sp networks. Species abundance was calculated as the total number of individuals from each species observed visiting flowers during the whole sampling period. Species phenophase was estimated counting the number of weeks between date of first and last field observation of individuals. Individual phenophase was determined categorically (May, June, July, August) considering the capture date of each specimen.

We calculated the average values of these features for each topological role (plant pollen type and insect individuals separately) and performed Kruskal-Wallis tests and post-hoc multiple comparisons with Bonferroni correction to detect differences among roles. Differences in flowering peak, individual phenophase and sex were assessed using Fisher's exact test.

Plant pollen types with different roles in the modular networks varied in length of flowering period ($\chi^2 = 29.32$, *df* = 3, $P < 0.001$), species linkage level ($\chi^2 = 50.13$, *df* = 3, $P < 0.001$) and pollen abundance ($\chi^2 = 38.88$, *df* = 3, $P < 0.001$) (see figure below), but not in flowering peak (Fisher exact test $P = 0.58$). In turn, insect individuals acting as connectors and peripherals differed significantly in individual linkage level ($\chi^2 = 70.67$, *df* = 1, $P < 0.001$) and individual phenophase ($P < 0.001$), although they had similar species abundances ($\chi^2 = 0.62$, *df* = 1, $P = 0.43$) and species phenophase length ($\chi^2 = 0.29$, *df* = 1, $P = 0.59$) (see figure below). Both connectors and peripherals were also represented by the same sex ratio among their individuals.

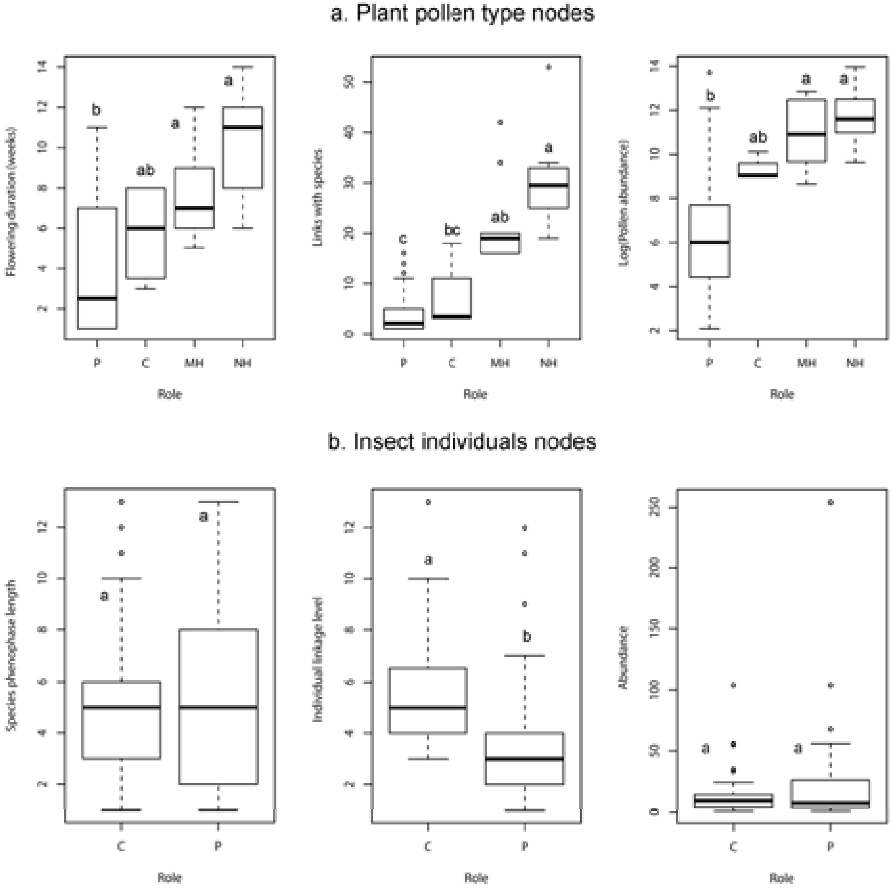


Figure 24: Boxplot of several features of plants (a) and insect individuals (b), which describe nodes classified into the following topological roles: peripherals (P), connectors (C), module hubs (MH) and networks hubs (NH). Different letters indicate significant differences according to a Kruskal-Wallis test post-hoc comparisons among topological roles.

B.4 Supplementary Figures Chapter 2

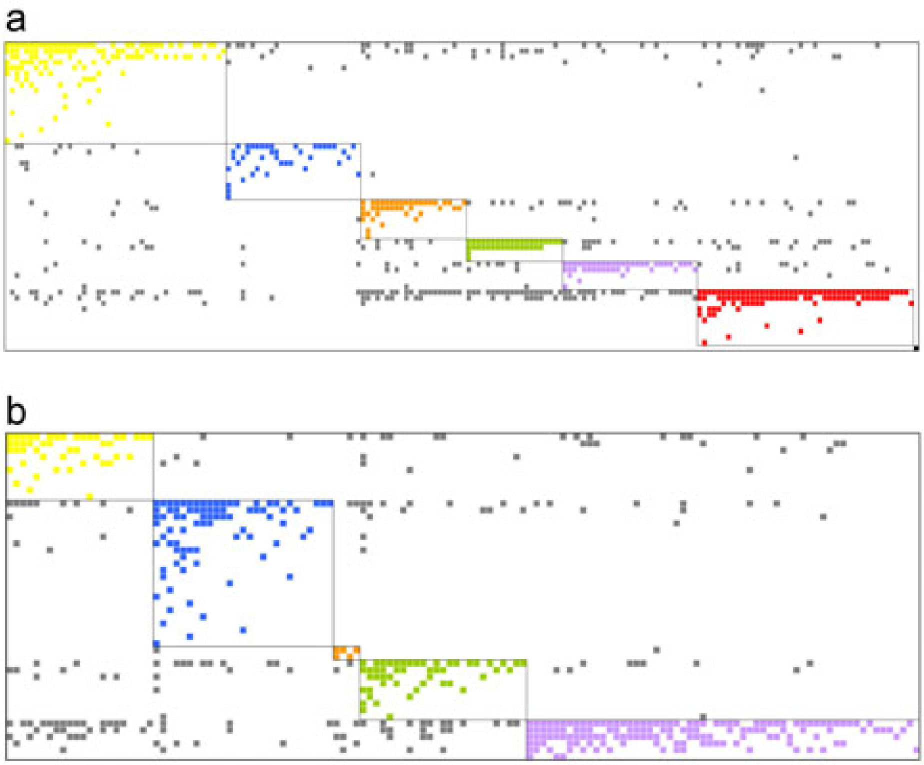


Figure 25: Modular pollen-transport interaction matrices for: (a) CN i-sp network, and (b) PC i-sp network. Matrix rows represent plant pollen types and columns represent insect individuals. Coloured cells indicate if pollen grains were detected on the body of insects. Frames delimit the modules identified in each network using Netcarto (M_U metric). Links connecting nodes between modules are all coloured in grey, whereas links connecting nodes within the same module have non-grey colours (yellow, blue, orange, green, purple and red, each one representing a different module). Inside each module, nodes are sorted in a nested way.

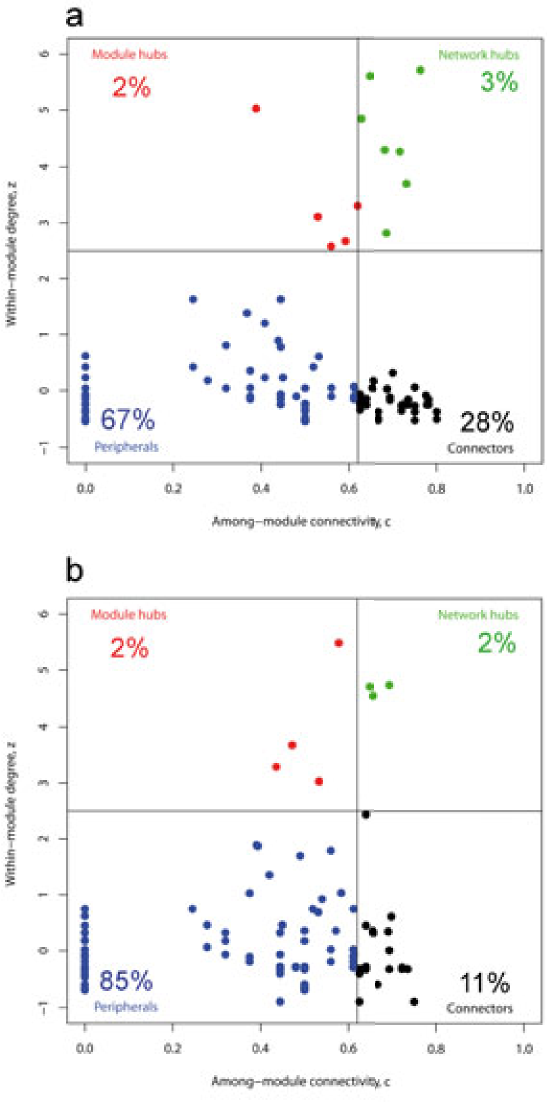


Figure 26: Distribution of network nodes according to their topological role based on within-module degree (z) and among-module connectivity (c): (a) CN i-sp network, and (b) PC i-sp network. Dots represent plant pollen types and insect pollinator individuals. Percentages of nodes assigned to each role are also shown in the plots.

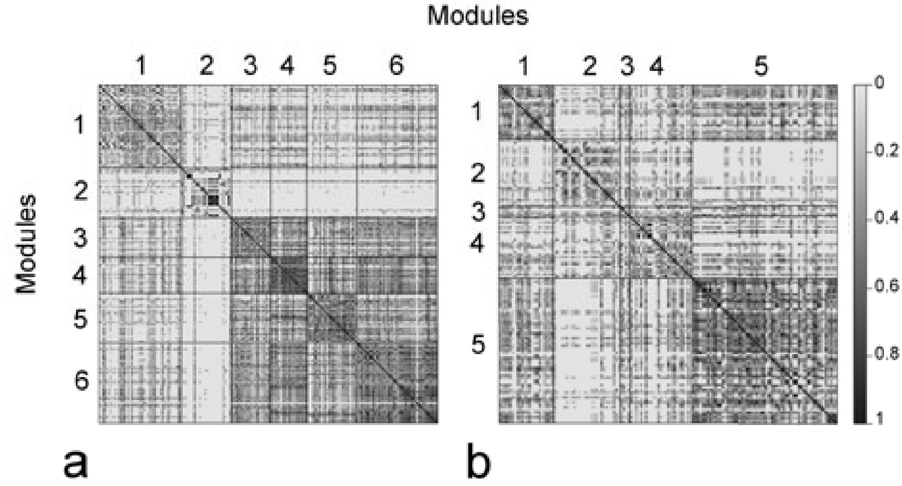


Figure 27: Matrices representing pairwise similarity in pollen resources among individuals within and between modules in both study sites: (a) CN i-sp network, and (b) PC i-sp network. Each row in the matrix is a pollinator individual and lines delimitate the modules, which appear numbered in both axes. Average affinity in pollen niche is higher within-modules than between-modules.

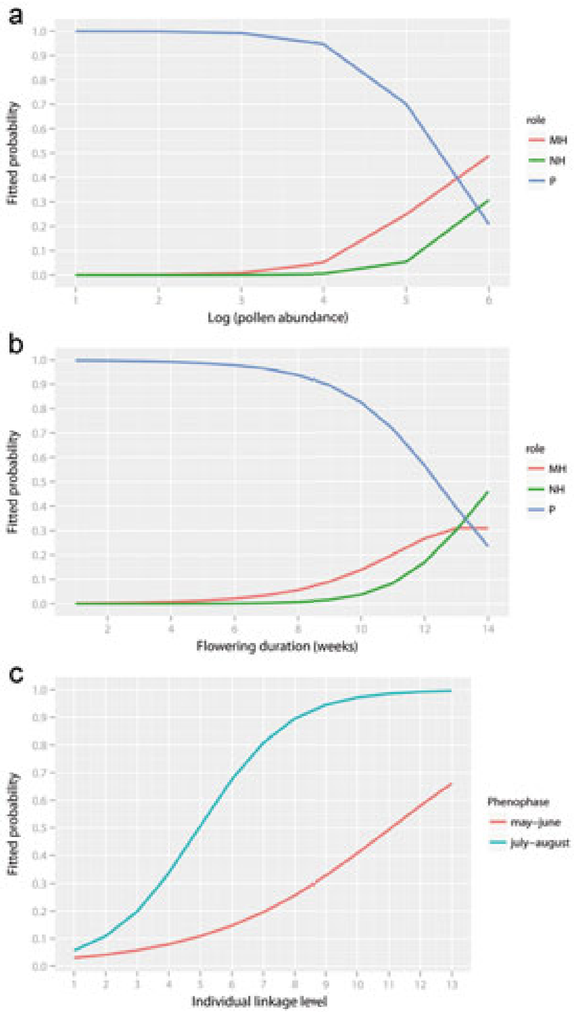


Figure 28: (a-b) Estimated effects of plant pollen abundance and flowering duration on the probability that a plant achieves a given topological role (P: peripheral, MH: module hub, NH: network hub). As flowering period length and abundance of plant pollen types increase, plants tend to act as module or network hubs. (c) Estimated effect of linkage level on the topological role developed by insect individuals (figure shows the probability of acting as connector). When the linkage level of an individual increases, the chance of being a connector is higher, especially at the end of the season (July–August).

B.5 Supplementary Tables Chapter 2

Table 11: Congruence between modules obtained for empirical i-sp networks using different metrics of modularity (M_U = unipartite modularity, M_B = bipartite modularity, M_{WB} = weighted bipartite modularity). Concordance of modules was estimated with the mutual information index, which ranges from 1 (when both partitions are identical) to 0 (when partitions are uncorrelated). The average values ± SD of this index among runs of the same metric and among runs of different metrics (10 runs for each network site) are shown.

	M_U	M_B	M_{WB}
M_U	0.825 ± 0.234	0.519 ± 0.056	0.319 ± 0.036
M_B		0.862 ± 0.091	0.396 ± 0.023
M_{WB}			0.597 ± 0.085

Table 12: Results from the multinomial logistic model fitted for plants and the binary logistic model for insect individuals. To estimate the model, peripherals (P) were set as the base role and the estimated coefficients (βs) of the other alternative roles (MH: module hub, NH: network hub) must be interpreted with reference to this base. Odds ratios were calculated as e^{β} and indicate the changes in the probabilities of being a MH or a NH per one unit change in the predictor.

(a) Multinomial logistic model for plants

Predictor variable	Alternative vs. base category	Estimated coeffs. (βs)	Std. error	t-value	P	Odds ratio
Flowering period length	MH vs. P	0.51	0.24	2.19	0.02	1.67
	NH vs. P	0.94	0.29	3.13	0.001	2.56
Log (pollen abundance)	MH vs. P	1.84	0.59	3.08	0.002	6.29
	NH vs. P	2.89	0.90	3.19	0.001	18.06

(b) Binary logistic model for insect individuals

Predictor variable	Estimated coeffs. (βs)	Std. error	Z-value	P	Odds ratio
Individual linkage level	0.35	0.11	3.11	0.002	1.42
Phenophase	0.33	0.85	0.38	0.702	1.38
Individual linkage level × Phenophase	0.36	0.16	2.27	0.023	1.43

C

Supplementary Material Chapter 3

C.1 Supplementary Figures Chapter 3

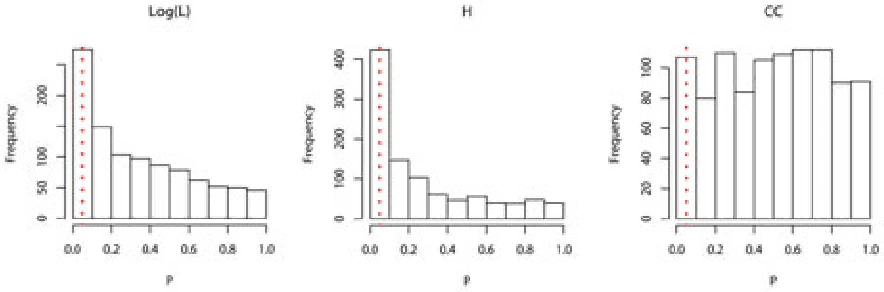


Figure 29: Histograms showing the frequency of significance levels (P) obtained for the linear regressions performed using 1000 bootstraps of PM data with sample size n= 27. Red dotted line indicates the boundary of $P = 0.05$. The percentage of cases resulting in a significant linear relationship among plant specialization indices (L: linkage level, H: diversity of interactions, CC: closeness centrality) and degree of plant dependence on insect pollination (IPD) is very low in this community even when increasing sample size: 17.9%, 30% and 5.7% of significant regressions, respectively.

C.2 Supplementary Tables Chapter 3

Table 13: List of plant species selected for estimating seed production. Here we indicate: study site, plant family, sample size as total number of plants and total number of flowers studied per treatment, mean seed set calculated as mean number of viable seeds per flower in each treatment.

			OPEN POLLINATION				**POLLINATORS EXCLUSION**			
Site	Family	Species	Plants	Flowers	Seeds	Seed set	Plants	Flowers	Seeds	Seed set
SB	Liliaceae	*Allium roseum*	3	33	7	0.212	3	23	1	0.043
SB	Liliaceae	*Asphodelus fistulosus*	3	35	81	2.314	3	15	36	2.400
SB	Scrophulariaceae	*Bellardia trixago*	3	19	4786	251.895	3	16	4952	309.500
SB	Gentianaceae	*Blackstonia perfoliata*	3	101	12598	124.733	3	83	14028	169.012
SB	Asteraceae	*Centaurea aspera**	3	3	7	2.333	3	4	5	1.250
SB	Gentianaceae	*Centaurium erythraea*	3	75	9623	128.307	3	78	5741	73.603
SB	Cistaceae	*Cistus salviifolius*	3	25	298	11.920	3	21	0	0.000
SB	Convulvulaceae	*Convolvulus althaeoides*	3	10	20	2.000	3	4	1	0.250
SB	Convulvulaceae	*Convolvulus arvensis*	3	24	20	0.833	3	37	4	0.108
SB	Asteraceae	*Crepis vesicaria**	3	38	761	20.026	3	31	14	0.452
SB	Apiaceae	*Daucus carota*	3	10845	7004	0.646	3	9400	1040	0.111
SB	Boraginaceae	*Echium sabulicola*	3	23	37	1.609	3	16	15	0.938
SB	Apiaceae	*Foeniculum vulgare*	4	700	718	1.026	3	917	364	0.397
SB	Asteraceae	*Helichrysum stoechas**	3	153	866	5.660	3	175	380	2.171
SB	Clusiaceae	*Hypericum perforatum*	3	253	118	0.466	3	203	76	0.374
SB	Asteraceae	*Hypochoeris achyrophorus**	3	7	56	8.000	3	7	2	0.286
SB	Fabaceae	*Lotus corniculatus*	3	18	214	11.889	3	24	0	0.000
SB	Fabaceae	*Lotus cytisoides*	3	15	20	1.333	3	15	0	0.000
SB	Fabaceae	*Medicago litoralis*	3	28	37	1.321	3	49	200	4.082
SB	Fabaceae	*Melilotus indica*	3	127	43	0.339	3	154	119	0.773
SB	Fabaceae	*Melilotus segettalis*	3	97	97	1.000	3	112	152	1.357
SB	Scrophulariaceae	*Parentucellia viscosa*	3	55	4867	88.491	3	77	3234	42.000
SB	Rosaceae	*Potentilla reptans*	3	5	481	96.200	3	3	3	1.000
SB	Asteraceae	*Scabiosa maritima**	3	3	181	60.333	3	3	39	13.000
SB	Caryophyllaceae	*Silene vulgaris*	3	46	374	8.130	3	38	10	0.263
SB	Lamiaceae	*Teucrium dunense**	3	24	426	17.750	3	31	199	6.419
SB	Scrophulariaceae	*Verbascum sinuatum*	3	97	1160	11.959	3	77	138	1.792
PM	Caryophyllaceae	*Arenaria grandiflora*	2	8	22	2.750	3	19	12	0.632
PM	Asteraceae	*Bellium bellidioides**	4	19	576	30.316	2	18	132	7.333

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			OPEN POLLINATION				**POLLINATORS EXCLUSION**			
Site	Family	Species	Plants	Flowers	Seeds	Seed set	Plants	Flowers	Seeds	Seed set
PM	Asteraceae	*Carlina corymbosa**	3	15	687	45.800	3	14	21	1.500
PM	Asteraceae	*Crepis triasii**	3	21	1397	66.524	2	13	46	3.538
PM	Rubiaceae	*Galium balearicum*	2	120	64	0.533	2	191	24	0.126
PM	Rubiaceae	*Galium cinereum*	2	381	110	0.289	4	390	84	0.215
PM	Cistaceae	*Helianthemum apenninum*	5	18	220	12.222	4	19	16	0.842
PM	Lamiaceae	*Rosmarinus officinalis*	3	55	76	1.382	3	54	43	0.796
PM	Asteraceae	*Santolina chamaecyparissus**	4	40	329	8.225	4	34	16	4.706
PM	Crassulaceae	*Sedum dasyphyllum*	3	59	483	8.186	4	127	105	0.827
PM	Lamiaceae	*Teucrium asiaticum*	4	81	238	2.938	3	48	49	1.021

* For these species number of flowers are inflorescences.

Table 14: List of plant-pollinator interactions observed in SB site for the 27 selected species. Interaction frequency is the number of flowers visited per unit time by each insect pollinator species.

PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq.
Liliaceae	*Allium roseum*	Apidae	*Apis mellifera*	0.52
		Anthomyiidae	*Delia platura*	0.04
		Bibionidae	*Dilophus antipedalis*	2.22
		Formicidae	*Linepithema humile*	0.42
		Nitidulidae	*Meligethes* sp.	0.72
		Apidae	*Osmia caerulescens*	0.06
		Apidae	*Osmia latreillei*	0.12
		Chrysomelidae	*Spermophagus* sp.	0.30
		Vespidae	*Stenodynerus f. fastidiosissimus*	0.04
		Formicidae	*Tapinoma madeirense*	0.04
		Curculionidae	*Tychius aureolus*	0.04
Liliaceae	*Asphodelus fistulosus*	Apidae	*Apis mellifera*	0.36
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.07
		Bibionidae	*Dilophus antipedalis*	0.12
		Eurytomidae	*Eurytomidae* sp.	0.05
		Apidae	*Lasioglossum griseolum*	0.01
		Apidae	*Lasioglossum villosulum*	0.01
		Formicidae	*Linepithema humile*	0.03
		Nitidulidae	*Meligethes* sp.	0.15
		Mordellidae	*Mordellistena* sp.	0.01
		Lygaeidae	*Nysius cymoides*	0.01
		Chrysomelidae	*Spermophagus* sp.	0.01
		Rhinophoridae	*Stevenia deceptoria*	0.01
Scrophulariaceae	*Bellardia trixago*	Apidae	*Lasioglossum malachurum*	0.01
		Nitidulidae	*Meligethes* sp.	0.18
		Apidae	*Osmia aurulenta*	0.08
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.06

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
Gentianaceae	*Blackstonia perfoliata*	Curculionidae	*Aulacobaris* sp.	0.06
		Vespidae	*Eumenes c. coarctatus*	0.02
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.03
Asteraceae	*Centaurea aspera*	-	*Acari* sp.	0.12
		Apidae	*Apis mellifera*	0.98
		Braconidae	*Braconidae* sp2	0.01
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	2.81
		Apidae	*Lasioglossum albocinctum*	0.01
		Formicidae	*Linepithema humile*	0.19
		Apidae	*Megachile apicalis*	0.08
		Apidae	*Megachile pilidens*	0.03
		Nitidulidae	*Meligethes* sp.	0.15
		Mordellidae	*Mordellistena* sp.	0.02
		Oedemeridae	*Oedemera caudata*	0.02
		Oedemeridae	*Oedemera flavipes*	0.01
		Oedemeridae	*Oedemera simplex*	0.03
		Apidae	*Osmia latreillei*	0.01
		Lycenidae	*Polyommatus icarus*	0.06
		Apidae	*Rhodanthidium septemdentatum*	0.05
		Nymphalidae	*Vanessa carduii*	0.01
Gentianaceae	*Centaurium erythraea*	Apidae	*Ceylalictus variegatus*	0.03
		Syrphidae	*Helophilus trivittatus*	0.09
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.03
Cistaceae	*Cistus salviifolius*	-	*Acari* sp.	0.30
		Apidae	*Andrena subgen. Micrandrena*	0.02
		Dermestidae	*Anthrenus miniopictus*	0.02
		Dermestidae	*Anthrenus pimpinellae*	0.02
		Apidae	*Apis mellifera*	0.36
		Chrysomelidae	*Bruchidius* sp1	0.09
		Chrysomelidae	*Bruchidae* sp3	0.02
		Milichiidae	*Desmometopa m-nigrum*	0.02

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Malachiidae	*Ebaeua apendiculatus*	0.04
		Apidae	*Eucera oraniensis*	0.28
		Sciomyzidae	*Euthycera alaris*	0.02
		Tenebrionidae	*Isomira* sp.	0.08
		Apidae	*Lasioglossum prasinum*	0.04
		Formicidae	*Linepithema humile*	0.17
		Nitidulidae	*Meligethes* sp.	0.91
		Mordellidae	*Mordellistena* sp.	0.02
		Stratiomyidae	*Nemotelus pantherinus*	0.04
		Oedemeridae	*Oedemera flavipes*	0.09
		Cetoniidae	*Oxythyrea funesta*	0.08
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.04
		Apidae	*Rhodanthidium septemdentatum*	0.04
		Chrysomelidae	*Spermophagus* sp.	0.26
		Rhinophoridae	*Stevenia deceptoria*	0.02
Convolvulaceae	*Convolvulus althaeoides*	Apidae	*Apis mellifera*	0.06
		Apidae	*Bombus terrestris*	0.23
		Pieridae	*Colias croceus*	0.03
		Malachiidae	*Ebaeua apendiculatus*	0.01
		Syrphidae	*Helophilus trivittatus*	0.01
		Nitidulidae	*Meligethes* sp.	0.13
		Stratiomyidae	*Nemotelus pantherinus*	0.01
		Oedemeridae	*Oedemera caudata*	0.01
		Oedemeridae	*Oedemera flavipes*	0.01
		Oedemeridae	*Oedemera simplex*	0.06
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.07
		Apidae	*Rhodanthidium septemdentatum*	0.05
		Chrysomelidae	*Spermophagus* sp.	1.83
		Calliphoridae	*Stomorhina lunata*	0.01
		Curculionidae	*Tychius aureolus*	0.01
		Nymphalidae	*Vanessa carduii*	0.01

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
Convolvulaceae	*Convolvulus arvensis*	-	*Acari* sp.	0.04
		Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.04
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.22
		Apidae	*Ceylalictus variegatus*	0.04
		Cerambicidae	*Chlorophorus trifasciatus*	0.01
		Pieridae	*Colias croceus*	0.04
		Malachiidae	*Colotes maculatus*	0.01
		Syrphidae	*Eristalinus aeneus*	0.03
		Apidae	*Lasioglossum gemmeus*	0.02
		Apidae	*Lasioglossum griseolum*	0.02
		Apidae	*Lasioglossum malachurum*	0.06
		Apidae	*Lasioglossum minutissimum*	0.01
		Apidae	*Lasioglossum villosulum*	0.02
		Nitidulidae	*Meligethes* sp.	0.16
		Mordellidae	*Mordellistena* sp.	0.04
		Stratiomyidae	*Nemotelus pantherinus*	0.09
		Oedemeridae	*Oedemera caudata*	0.02
		Oedemeridae	*Oedemera flavipes*	0.02
		Oedemeridae	*Oedemera simplex*	0.01
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.03
		Cantharidae	*Rhagonycha fulva*	0.02
		Chrysomelidae	*Spermophagus* sp.	1.66
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.20
		Calliphoridae	*Stomorhina lunata*	0.01
		Formicidae	*Tapinoma madeirense*	0.01
Asteraceae	*Crepis vesicaria*	Apidae	*Andrena subgen. Micrandrena*	0.03
		Byrrhidae	*Byrrhidae* sp.	0.03
		Apidae	*Eucera oraniensis*	0.75
		Syrphidae	*Helophilus trivittatus*	0.09
		Nitidulidae	*Meligethes* sp.	0.40
		Mordellidae	*Mordellistena* sp.	0.01

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Stratiomyidae	*Nemotelus pantherinus*	0.07
		Oedemeridae	*Oedemera caudata*	0.07
		Oedemeridae	*Oedemera flavipes*	0.06
		Apidae	*Osmia latreillei*	0.12
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.30
		Cantharidae	*Rhagonycha fulva*	0.01
		Chrysomelidae	*Spermophagus* sp.	0.18
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.03
		Nymphalidae	*Vanessa carduii*	0.07
Umbelliferae	*Daucus carota*	-	*Acari* sp.	0.42
		Dermestidae	*Anthrenus* sp.	0.13
		Braconidae	*Braconidae* sp1	0.03
		Ceratopogonidae	*Ceratopogonidae* sp.	0.01
		Apidae	*Ceylalictus variegatus*	0.63
		Chironomidae	*Chironomidae* sp.	0.15
		Cerambicidae	*Chlorophorus trifasciatus*	0.64
		Coccinelidae	*Coccinella septempunctata*	0.45
		Coccinellidae	*Coccinella undecimpunctata*	0.03
		Syrphidae	*Eristalinus aeneus*	0.45
		Syrphidae	*Eristalis arbustorum*	0.42
		Syrphidae	*Eristalinus sepulchralis*	0.13
		Gasteruptiidae	*Gasteruption undulatum*	0.26
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	0.42
		Chrysididae	*Holopyga fervida*	1.16
		Apidae	*Hylaeus* sp. *(clypearis + trinotatus + signatus + variegatus)*	1.09
		Apidae	*Lasioglossum gemmeus*	0.13
		Miridae	*Lepydargyrus ancorifer*	0.03
		Formicidae	*Linepithema humile*	1.89
		Tiphiidae	*Meria tripunctata*	1.54
		Mordellidae	*Mordellistena* sp.	1.05
		Stratiomyidae	*Nemotelus pantherinus*	0.29

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Muscidae	*Neomyia cornicina*	0.49
		Anthicidae	*Notoxus monoceros*	0.03
		Oedemeridae	*Oedemera caudata*	0.47
		Oedemeridae	*Oedemera simplex*	0.38
		Cetoniidae	*Oxythyrea funesta*	0.03
		Agromyzidae	*Phytomyza* sp.	0.01
		Vespidae	*Polistes* sp. *(dominulus + gallicus)*	1.46
		Sphecidae	*Prionyx kirbii*	0.03
		Muscidae	*Pyrellia vivida*	0.03
		Cantharidae	*Rhagonycha fulva*	2.45
		Sarcophagidae	*Sarcophaga villeneuveana*	0.25
		Sarcophagidae	*Senotainia tricuspis*	0.04
		Chrysomelidae	*Spermophagus* sp.	1.15
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.25
		Rhinophoridae	*Stevenia deceptoria*	0.95
		Syrphidae	*Syritta pipiens*	0.35
		Formicidae	*Tapinoma madeirense*	0.55
		Therevidae	*Thereva spiloptera*	0.13
		Curculionidae	*Tychius aureolus*	0.43
Boraginaceae	*Echium sabulicola*	Apidae	*Amegilla balearica*	0.50
		Apidae	*Amegilla quadrifasciata*	0.13
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	1.14
		Apidae	*Ceylalictus variegatus*	0.01
		Bibionidae	*Dilophus antipedalis*	0.02
		Curculionidae	*Gymnetron* sp.	0.01
		Apidae	*Hoplitis adunca*	0.03
		Apidae	*Hoplitis benoisti*	0.01
		Apidae	*Lasioglossum gemmeus*	0.03
		Apidae	*Lasioglossum griseolum*	0.02
		Miridae	*Lepydargyrus ancorifer*	0.01
		Nitidulidae	*Meligethes* sp.	0.48
		Anthocoridae	*Orius niger*	0.10

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Apidae	*Osmia adunca*	0.05
		Apidae	*Osmia aurulenta*	0.17
		Apidae	*Osmia caerulescens*	0.33
		Apidae	*Osmia versicolor*	0.07
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.01
		Apidae	*Rhodanthidium septemdentatum*	0.04
		Nymphalidae	*Vanessa carduii*	0.01
Umbelliferae	*Foeniculum vulgare*	Crabronidae	*Bembix occulata*	1.19
		Syrphidae	*Eristalinus sepulchralis*	0.24
		Apidae	*Lasioglossum gemmeus*	0.43
		Rhinophoridae	*Phyto melanocephala*	1.19
		Vespidae	*Polistes* sp. *(dominulus + gallicus)*	4.88
		Pteromalidae	*Pteromalidae* sp.	0.02
		Sarcophagidae	*Sarcophaga* sp.	1.19
		Sarcophagidae	*Sarcophaga unicurva*	0.19
		Sarcophagidae	*Sarcophaga villeneuveana*	0.36
		Chrysomelidae	*Spermophagus* sp.	2.24
Asteraceae	*Helichrysum stoechas*	-	*Acari* sp.	0.88
		Apidae	*Andrena subgen. Micrandrena*	0.25
		Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.19
		Apidae	*Andrena* sp3	0.31
		Apidae	*Apis mellifera*	0.19
		Blattodea	*Blattodea* sp.	0.04
		Byrrhidae	*Byrrhidae* sp.	0.01
		Cerambicidae	*Chlorophorus trifasciatus*	0.85
		Muscidae	*Coenosia tigrina*	0.04
		Apidae	*Colletes abeillei*	1.54
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	0.19
		Apidae	*Heriades rubicolus*	0.19
		Apidae	*Lasioglossum gemmeus*	0.06
		Apidae	*Lasioglossum minutissimum*	0.19

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Miridae	*Lepydargyrus ancorifer*	0.04
		Gryllidae	*Melanogrillus desertus*	0.01
		Nitidulidae	*Meligethes* sp.	0.04
		Stratiomyidae	*Nemotelus pantherinus*	2.63
		Muscidae	*Neomyia cornicina*	0.19
		Oedemeridae	*Oedemera caudata*	0.19
		Oedemeridae	*Oedemera flavipes*	0.05
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.04
		Cantharidae	*Rhagonycha fulva*	0.83
		Sarcophagidae	*Sarcophaga villeneuveana*	0.24
		Chrysomelidae	*Spermophagus* sp.	0.09
		Rhinophoridae	*Stevenia deceptoria*	0.28
		Syrphidae	*Syritta pipiens*	0.06
Guttiferae	*Hypericum perforatum*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.06
		Apidae	*Apis mellifera*	0.01
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.29
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	0.01
		Formicidae	*Linepithema humile*	0.01
		Apidae	*Rhodanthidium septemdentatum*	0.13
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.05
		Syrphidae	*Syritta pipiens*	0.04
		Apidae	*Xylocopa violacea*	0.01
Asteraceae	*Hypochoeris achyrophorus*	Buprestidae	*Anthaxia funerula*	0.04
		Braconidae	*Braconidae* sp1	0.01
		Bibionidae	*Dilophus antipedalis*	0.04
		Miridae	*Lepydargyrus ancorifer*	0.01
		Nitidulidae	*Meligethes* sp.	0.26
		Mordellidae	*Mordellistena* sp.	0.04
		Oedemeridae	*Oedemera caudata*	0.04
		Oedemeridae	*Oedemera flavipes*	0.03
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.37

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Chrysomelidae	*Spermophagus* sp.	0.22
		Curculionidae	*Tychius aureolus*	0.06
Leguminosae	*Lotus corniculatus*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.90
		Apidae	*Apis mellifera*	1.06
		Tachinidae	*Clairvillia pninae*	0.03
		Apidae	*Colletes abeillei*	0.34
		Bibionidae	*Dilophus antipedalis*	0.11
		Apidae	*Hoplitis leucomelans*	0.20
		Apidae	*Lasioglossum malachurum*	0.01
		Apidae	*Megachile apicalis*	0.05
		Apidae	*Megachile pilidens*	0.10
		Nitidulidae	*Meligethes* sp.	0.03
		Apidae	*Osmia andrenoides*	0.05
		Apidae	*Osmia aurulenta*	0.05
		Apidae	*Osmia caerulescens*	0.27
		Apidae	*Osmia versicolor*	0.70
		Lycenidae	*Polyommatus icarus*	0.34
		Apidae	*Rhodanthidium septemdentatum*	0.03
		Sarcophagidae	*Sarcophaga villeneuveana*	0.01
		Chrysomelidae	*Spermophagus* sp.	0.01
Leguminosae	*Lotus cytisoides*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.18
		Apidae	*Apis mellifera*	1.35
		Apidae	*Bombus terrestris*	0.01
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.06
		Bibionidae	*Dilophus antipedalis*	0.06
		Formicidae	*Linepithema humile*	0.01
		Nitidulidae	*Meligethes* sp.	0.10
		Apidae	*Osmia caerulescens*	0.02
		Apidae	*Rhodanthidium septemdentatum*	0.13
Leguminosae	*Medicago littoralis*	Curculionidae	*Curculionidae* sp1	0.01
		Nitidulidae	*Meligethes* sp.	0.26

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Syrphidae	*Paragus tibialis*	0.01
		Lycenidae	*Polyommatus icarus*	0.01
		Tachinidae	*Siphona* sp.	0.01
Leguminosae	*Melilotus indica*	Apidae	*Andrena subgen. Micrandrena*	0.61
		Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	1.97
		Apidae	*Andrena* sp4	0.45
		Apidae	*Lasioglossum griseolum*	0.45
		Cantharidae	*Rhagonycha fulva*	0.45
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.15
Leguminosae	*Melilotus segetalis*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	1.75
		Apidae	*Apis mellifera*	7.11
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.02
Scrophulariaceae	*Parentucellia viscosa*	Apidae	*Anthophora plumipes*	0.08
		Apidae	*Lasioglossum griseolum*	0.03
Rosaceae	*Potentilla reptans*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	0.03
		Apidae	*Apis mellifera*	0.43
		Blattodea	*Blattodea* sp.	0.01
		Chrysomelidae	*Bruchidius* sp1	0.01
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.01
		Cerambicidae	*Chlorophorus trifasciatus*	0.02
		Gasteruptiidae	*Gasteruption undulatum*	0.02
		Apidae	*Hoplitis leucomelans*	0.07
		Apidae	*Hylaeus pictus*	0.51
		Apidae	*Lasioglossum griseolum*	0.03
		Apidae	*Lasioglossum malachurum*	0.01
		Formicidae	*Linepithema humile*	0.05
		Nitidulidae	*Meligethes* sp.	0.01
		Mordellidae	*Mordellistena* sp.	0.02
		Stratiomyidae	*Nemotelus pantherinus*	0.03
		Oedemeridae	*Oedemera caudata*	0.20

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Oedemeridae	*Oedemera flavipes*	0.05
		Apidae	*Osmia caerulescens*	0.03
		Apidae	*Osmia versicolor*	0.17
		Syrphidae	*Paragus tibialis*	0.05
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.02
		Apidae	*Rhodanthidium septemdentatum*	0.01
		Chrysomelidae	*Spermophagus* sp.	0.60
		Syrphidae	*Sphaerophoria* sp. *(scripta+rueppellii)*	0.65
		Rhinophoridae	*Stevenia deceptoria*	0.01
		Syrphidae	*Syritta pipiens*	0.01
		Formicidae	*Tapinoma madeirense*	0.08
Rosaceae	*Potentilla reptans*	Curculionidae	*Tychius aureolus*	0.05
Dipsacaceae	*Scabiosa maritima*	-	*Acari* sp.	0.17
		Buprestidae	*Acmaoederella discoida*	0.01
		Apidae	*Apis mellifera*	5.03
		Crabronidae	*Bembix occulata*	0.04
		Bombyliidae	*Bombylius posticus*	0.08
		Apidae	*Bombus terrestris*	0.02
		Lycaenidae	*Celastrina argiolus*	0.01
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.46
		Pieridae	*Colias croceus*	0.02
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	2.99
		Syrphidae	*Helophilus trivittatus*	0.12
		Scoliidae	*Megascolia* sp.	0.02
		Nitidulidae	*Meligethes* sp.	0.43
		Mordellidae	*Mordellistena* sp.	0.03
		Stratiomyidae	*Nemotelus pantherinus*	0.01
		Oedemeridae	*Oedemera caudata*	0.04
		Oedemeridae	*Oedemera flavipes*	0.01
		Oedemeridae	*Oedemera simplex*	0.01
		Cetoniidae	*Oxythyrea funesta*	0.01

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Syrphidae	*Paragus tibialis*	0.01
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.03
		Apidae	*Rhodanthidium septemdentatum*	0.02
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.02
		Nymphalidae	*Vanessa carduii*	1.04
Caryophyllaceae	*Silene vulgaris*	Nitidulidae	*Meligethes* sp.	0.14
		Dasytidae	*Psilotrix* sp. *(illustris + cyaneus + aureolus)*	0.01
		Nymphalidae	*Vanessa carduii*	0.03
Labiatae	*Teucrium dunense*	Apidae	*Andrena* sp1 *(ovatula + fulvipes)*	1.14
		Apidae	*Andrena* sp2	0.87
		Apidae	*Andrena* sp3	0.54
		Apidae	*Apis mellifera*	40.82
		Crabronidae	*Bembix occulata*	3.15
		Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.16
		Cerambicidae	*Chlorophorus trifasciatus*	0.03
		Apidae	*Colletes dusmeti*	0.16
		Formicidae	*Crematogaster laestrygon*	0.01
		Syrphidae	*Eristalinus aeneus*	0.33
		Syrphidae	*Eristalinus megacephalus*	0.03
		Apidae	*Halictus* sp. *(scabiosae + fulvipes)*	0.57
		Apidae	*Heriades rubicolus*	0.09
		Apidae	*Lasioglossum gemmeus*	0.55
		Apidae	*Lasioglossum malachurum*	0.16
		Formicidae	*Linepithema humile*	0.71
		Scoliidae	*Megascolia hortorum*	0.58
		Scoliidae	*Megascolia* sp.	3.21
		Nitidulidae	*Meligethes* sp.	0.10
		Crabronidae	*Philanthus triangulum*	0.16
		Lycenidae	*Polyommatus icarus*	0.20
		Vespidae	*Polistes* sp. *(dominulus + gallicus)*	0.30
		Sphecidae	*Prionyx kirbii*	1.25

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq
		Apidae	*Rhodanthidium septemdentatum*	0.18
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.17
		Calliphoridae	*Stomorhina lunata*	0.11
		Syrphidae	*Syritta pipiens*	0.16
		Nymphalidae	*Vanessa carduii*	1.25
Scrophulariaceae	*Verbascum sinuatum*	Apidae	*Ceratina* sp. *(cucurbitina + dellatorreana)*	0.03
		Apidae	*Ceylalictus variegatus*	0.05
		Syrphidae	*Eristalinus aeneus*	0.02
		Apidae	*Hylaeus pictus*	0.02
		Formicidae	*Linepithema humile*	0.50
		Curculionidae	*Mogulones* sp.	0.01
		Cetoniidae	*Oxythyrea funesta*	0.01
		Syrphidae	*Paragus tibialis*	0.01
		Syrphidae	*Sphaerophoria* sp. *(scripta + rueppellii)*	0.05
		Calliphoridae	*Stomorhina lunata*	0.03
		Syrphidae	*Syritta pipiens*	0.01

Table 15: List of plant-pollinator interactions observed in PM site for the 11 selected species. Interaction frequency is the number of flowers visited per unit time by each insect pollinator species.

PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq.
Caryophyllaceae	*Arenaria grandiflora*	Anthomyiidae	*Adia cinerella*	0.01
		Bruchidae	*Bruchidius* sp.	0.03
		Syrphidae	*Eristalis tenax*	0.05
		Chrysididae	*Holopyga fervida*	0.03
		Oedemeridae	*Oedemera flavipes*	0.01
		Apidae	*Osmia latreillei*	0.04
		Syrphidae	*Paragus pecciolii*	0.04
		Sarcophagidae	*Sarcophaga nigriventis*	0.03
Asteraceae	*Bellium bellidioides*	Anthomyiidae	*Anthomyia pluvialis*	0.01
		Malachiidae	*Attalus* sp.	0.01
		Braconidae	*Chelonus* sp.	0.07
		Tachinidae	*Cylindromyia brassicaria*	0.04
		Tachinidae	*Gymnosoma* sp.	0.01
		Apidae	*Lasioglossum transitorium planulum*	0.01
		Sphingidae	*Macroglossum stellatarum*	0.01
		Oedemeridae	*Oedemera flavipes*	0.05
		Syrphidae	*Paragus pecciolii*	0.02
		Syrphidae	*Paragus tibialis*	0.07
		Lycaenidae	*Polyommatus icarus*	0.01
		Chloropidae	*Polyodaspis sulcicollis*	0.01
		Syrphidae	*Sphaerophoria* sp.	0.04
Asteraceae	*Carlina corymbosa*	Apidae	*Andrena* sp.	0.04
		Apidae	*Apis mellifera*	1.25
		Malachiidae	*Attalus* sp.	0.01
		Curculionidae	*Baris* sp.	0.01
		Syrphidae	*Chrysotoxum intermedium*	0.03
		Syrphidae	*Eristalinus taeniops*	0.03
		Syrphidae	*Eristalis tenax*	0.01

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq.
		Syrphidae	*Eupeodes corollae*	0.05
		Apidae	*Halictus scabiosae*	0.26
		Apidae	*Lasioglossum nitidulum hammi*	0.01
		Apidae	*Megachile pilidens*	0.01
		Mordellidae	*Mordellistena* sp.	0.03
		Lycaenidae	*Polyommatus icarus*	0.03
		Apidae	*Rhodanthidium septemdentatum*	0.03
		Calliphoridae	*Stomorhina lunata*	0.20
		Bombyliidae	*Villa hottentotta*	0.03
		Bombyliidae	*Villa* sp.	0.01
		Apidae	*Xylocopa violacea*	0.01
Asteraceae	*Crepis triasii*	Malachiidae	*Attalus* sp.	0.04
		Apidae	*Ceratina cucurbitina*	0.01
		Braconidae	*Chelonus* sp.	0.01
		Syrphidae	*Eristalis tenax*	0.07
		Apidae	*Halictus fulvipes*	0.01
		Apidae	*Halictus vestitus*	0.07
		Tenebrionidae	*Isomira* sp.	0.01
		Apidae	*Lasioglossum nitidulum hammi*	0.01
		Oedemeridae	*Oedemera flavipes*	0.14
		Apidae	*Osmia latreillei*	0.01
		Bombyliidae	*Phthiria pulicaria*	0.01
		Bombyliidae	*Phthiria* sp.	0.02
		Formicidae	*Plagiolepis pygmaea*	0.04
		Apidae	*Rhodanthidium septemdentatum*	0.04
Rubiaceae	*Galium balearicum*	Braconidae	*Chelonus* sp.	0.09
Rubiaceae	*Galium cinereum*	Mordellidae	*Mordellistena* sp.	0.02
		Oedemeridae	*Oedemera flavipes*	0.14
Cistaceae	*Helianthemum apenninum*	Pieridae	*Colias croceus*	0.01
		Oedemeridae	*Oedemera flavipes*	0.09

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq.
		Syrphidae	*Sphaerophoria* sp.	0.02
		Formicidae	*Temnothorax specularis*	0.02
Labiatae	*Rosmarinus officinalis*	Apidae	*Apis mellifera*	1.60
		Apidae	*Ceratina cucurbitina*	0.11
		Syrphidae	*Eristalis tenax*	0.02
		Apidae	*Eucera oraniensis*	0.02
		Syrphidae	*Helophilus trivittatus*	0.02
		Sphingidae	*Macroglossum stellatarum*	0.13
		Formicidae	*Plagiolepis pygmaea*	0.02
		Melyridae	*Psilotrix illustris*	0.02
		Nymphalidae	*Vanessa cardui*	0.07
Asteraceae	*Santolina chamaecyparissus*	Dermestidae	*Anthrenus pimpinellae*	0.01
		Malachiidae	*Attalus* sp.	0.01
		Syrphidae	*Chrysotoxum intermedium*	0.02
		Tachinidae	*Cylindromyia brassicaria*	0.15
		Tachinidae	*Dionaea aurifrons*	0.01
		Syrphidae	*Eristalis tenax*	0.06
		Apidae	*Lasioglossum transitorium planulum*	0.07
		Apidae	*Megachile pilidens*	0.01
		Mordellidae	*Mordellistena* sp.	0.19
		Oedemeridae	*Oedemera flavipes*	0.52
		Syrphidae	*Paragus tibialis*	0.12
		Bombyliidae	*Phthiria pulicaria*	0.09
		Bombyliidae	*Phthiria* sp.	0.02
		Chloropidae	*Polyodaspis sulcicollis*	0.08
		Calliphoridae	*Stomorhina lunata*	0.09
Crassulaceae	*Sedum dasyphyllum*	Apidae	*Halictus vestitus*	0.01
		Apidae	*Hoplitis leucomelans*	0.03
		Apidae	*Lasioglossum nitidulum hammi*	0.07
		Apidae	*Lasioglossum transitorium planulum*	0.21
		Oedemeridae	*Oedemera flavipes*	0.03

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PLANT		INSECT POLLINATOR		
Family	Species name	Family	Species or morphospecies name	Int. freq.
		Chloropidae	*Oscinella frit*	0.03
		Bombyliidae	*Phthiria pulicaria*	0.03
		Formicidae	*Plagiolepis pygmaea*	0.13
Labiatae	*Teucrium asiaticum*	Apidae	*Amegilla quadrifasciata*	0.40
		Apidae	*Anthidium cingulatum*	0.01
		Apidae	*Anthidium manicatum*	0.09
		Apidae	*Apis mellifera*	0.41
		Syrphidae	*Chrysotoxum intermedium*	0.01
		Apidae	*Halictus vestitus*	0.02
		Apidae	*Lasioglossum nitidulum hammi*	0.09
		Sphingidae	*Macroglossum stellatarum*	0.39
		Apidae	*Megachile pilidens*	0.52
		Apidae	*Protosmia minutula*	0.01
		Apidae	*Rhodanthidium septemdentatum*	0.04
		Bombyliidae	*Villa* sp.	0.01
		Apidae	*Xylocopa violacea*	0.01

D

Supplementary Material Chapter 4

D.1 Supplementary Table Chapter 4

Table 16: Plant species sampled at each altitudinal community. Plant's sexual system is based on field observations except when a reference is indicated. H = hermaphroditic, Di = dioecious, D = dichogamous (protandry), Hk = herkogamous, SI = self-incompatibility.

Plant species	**1600 m**	**1800 m**	**2000 m**	**Sexual system**
Adesmia corymbosa	X	X		H
Adesmia parviflora		X		H, D
Anemone multifida	X	X		H
Arjona patagonica		X		H, SI[1]
Armeria maritima	X	X		H, SI[5]
Asteraceae unidentified	X			-
Azorella monantha	X			H, SI[1]
Cerastium arvense		X	X	H, SI[3]
Chiliotrichium rosmarinifolium	X	X		H, D
Discaria chacaye	X			H
Discaria nana		X		H, D, SI[1]
Draba gilliesii			X	H
Erigeron leptopetalus		X	X	H, D
Gamocarpha selliana	X	X		H
Gaultheria pumila	X	X	X	H
Geranium sessiliflorum		X		H
Huanaca andina			X	Di
Hypochaeris tenuifolia	X	X		H, D
Leucheria millefolium	X	X		H, D
Loasa nana	X	X	X	H
Moschopsis caleofuensis	X	X		H
Mulinum echinus	X		X	Di
Mulinum leptacanthum	X	X		Di
Nassauvia aculeata	X	X		H, D
Nassauvia darwinii		X		H, D
Nassauvia pygmaea		X		H, D
Nassauvia revoluta			X	H
Nastanthus spathulatus	X			H, SI[4]
Oreopolus glacialis	X	X		H, Hk
Oxalys erythrorhiza			X	H, Hk, SI[1]
Perezia bellidifolia	X	X		H
Perezia recurvata		X		H, D
Phacelia secunda	X			H
Polygala salasiana	X	X		H

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Plant species	**1600 m**	**1800 m**	**2000 m**	**Sexual system**
Quinchamalium chilense	X	X		H
Senecio argyreus	X	X		H, D
Senecio baccharidifolius		X		H, D
Senecio bipontinii	X	X	X	H, D, SI[2]
Senecio boelckei			X	H
Senecio gnidioides		X		H, D
Senecio pachyphyllos			X	H, D
Senecio peterianus		X		H, D
Senecio poeppigii		X		H
Senecio portalesianus			X	H, D
Senecio sp.	X	X		H, D
Sisyrinchium arenarium		X		H
Valeriana carnosa	X	X		H, Hk
Vicia bijuga		X		H

1. Medan et al. (2002), 2. Rivero Gutiérrez (1991), 3. Lundqvist (1990), 4. Ladd and Arroyo (2009), 5. Eisikowitch and Woodell (1975).

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