Spatial variability of bee communities: from local assemblages to interaction networks

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"Rather than love, than money, than fame...

give me truth."

Henry David Thoreau

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Abstract

Organisms are heterogeneously distributed following spatial patterns, which are the consequence of many biotic and abiotic factors. One of the main goals in ecology is to understand how species interactions with other organisms and with the environment determine these observed species distribution patterns. In this thesis, we analyze the factors explaining spatial variability of bee communities and associated interaction networks at local scale. The study was conducted in the Garraf Natural Park, in a 32km^2 area homogeneously occupied by Mediterranean scrubland, and lacking strong gradients or ecological barriers. Because bees rely on floral and nesting resources to survive and reproduce, these are expected to influence the local spatial variability of the bee community. In the first chapter, we explore how the spatial distribution of flower and nesting resources determines local spatial variability in the bee community. We found a clear geographical pattern of spatial variability in bee community composition. This pattern was partly driven by floral resources with a negligible contribution of nesting substrate availability, and was strongly related to body size: small bee species (<55mg) displayed strong spatial patterns, while large species (>70mg) tended to be more evenly distributed across the area. Bee spatial distribution may also be affected by perturbation regimes. The study area contains several apiaries, affording an opportunity to explore the potential effects of beekeeping on wild bee communities (Chapter 2). Honey bees (*Apis mellifera*) are highly efficient foragers and have the ability to recruit other foragers to the most rewarding flower patches, potentially establishing a competitive effect on other pollinators. We found that honey bees were the main contributors to pollen/nectar depletion of the two main flowering plants in the study area. We also found that the bee community was modified in areas close to apiaries, where honey bee densities were higher. Large-sized bee species, with greater energetic requirements, and therefore more likely to be affected by low food resource availability, were less abundant in areas close to apiaries. These spatial changes in community structure are bound to affect the identity and the network structure of the interactions bees establish with other organisms Consequently, in the third chapter, we study the local spatial variability of the community of cavity-nesting bees and wasps together with their nests associates (parasitoids, cleptoparasites, predators and scavengers), as well as their interactions. We analyze the relationship between community and interaction β-diversity, and explore the sources of the observed spatial variability.

Spatial variability of both communities (hosts and parasites) was high and mainly driven by species turnover, with a very low influence of nestedness, meaning that local communities were highly idiosyncratic. Interaction β-diversity was also very high and mostly due to the high species turnover, with a very low contribution of interaction rewiring. In other words, species tended to interact similarly across plots. Overall, this thesis demonstrates that bee communities and their antagonistic interaction networks may vary at a very small scale and are highly conditioned by local factors, and that bee communities may be affected by intensive beekeeping, a widespread human activity usually assumed to be beneficial.

Resum

Els organismes es troben distribuïts heterogèniament seguint patrons espacials, els quals són conseqüència de varis factors biòtics i abiòtics. Un dels principals objectius de l'ecologia és entendre com les interaccions de les espècies amb altres organismes i amb l'ambient determinen els seus patrons de distribució. En aquesta tesi analitzem els factors que expliquen la variabilitat espaial de les comunitats d'abelles i de les xarxes d'interaccions associades a escala local. L'estudi s'ha dut a terme al Parc del Garraf, en una àrea de 32km² amb una vegetació arbustiva mediterrània homogènia, sense barreres ecològiques ni gradients marcats. Atès que les abelles depenen dels recursos florals i de nidificació per tal de sobreviure i reproduir-se, s'espera que aquests influenciïn la variabilitat espacial local de la comunitat d'abelles. En el primer capítol explorem com la distribució espacial dels recursos florals i de nidificació determina la variabilitat espaial local de la comunitat d'abelles. Hem trobat un patró geogràfic clar de variabilitat espaial en la composició de la comunitat d'abelles. Aquest patró es deu parcialment als recursos florals, amb una contribució de la disponibilitat de substrats de nidificació quasi insignificant, i està estretament relacionada amb la mida corporal: les espècies d'abella petites (<55mg) mostren forts patrons espacials, mentre que les espècies grans (>70mg) tendeixen a trobar-se distribuïdes més uniformement en l'àrea d'estudi. La distribució espacial de les abelles també pot veure's afectada per règims de pertorbacions. L'àrea d'estudi conté varis apiaris, oferint-nos la oportunitat d'explorar els efectes potencials de l'apicultura en comunitats d'abelles salvatges (Capítol 2). L'abella de la mel (*Apis mellifera*) és una espècie molt eficient a l'hora de buscar menjar i, a més, té l'habilitat de reclutar altres abelles de la mel en els llocs amb més disponibilitat d'aliment, establint potencialment un efecte competitiu sobre els altres pol·linitzadors. Hem trobat que les abelles de la mel són les que contribueixen d'una manera més significativa a la disminució del pol·len i el nèctar de les dues plantes més importants a l'àrea d'estudi. També hem trobat que la comunitat d'abelles es veu modificada en àrees properes als apiaris, on les densitats d'abelles de la mel són més elevades. Les espècies d'abelles grans, amb majors requeriments energètics i, conseqüentment, amb més probabilitats de veure's afectades per una disponibilitat baixa de recursos alimentaris, són menys abundants en àrees properes als apiaris. Aquests canvis espacials en l'estructura de les comunitats afectaran la identitat i l'estructura de les interaccions de les xarxes que les abelles estableixen amb altres organismes. En conseqüència, en el tercer capítol, estudiem la

variabilitat espacial a escala local d'una comunitat d'abelles i vespes nidificants en cavitats preestablertes i de llur fauna associada (parasitoids, cleptoparàsits, predadors i carronyaires), així com de les seves interaccions. Analitzem la relació entre la β-diversitat de les comunitats i de les interaccions i explorem les causes de la variabilitat espacial observada. La variabilitat espacial d'ambdues comunitats (hostes i parasitoids) és elevada i deguda principalment al recanvi d'espècies, amb una influència molt baixa de l'aniuament, indicant que les comunitats locals són altament idiosincràtiques. La β-diversitat d'interaccions també és molt elevada i deguda principalment al recanvi d'espècies, amb una contribució molt baixa del recablejat d'interaccions. En altres paraules, les espècies tendeixen a interaccionar de manera similar en totes les parcel·les. En general, aquesta tesi demostra que les comunitats d'abelles i les seves xarxes d'interaccions antagonistes poden variar a una escala espaial molt petita i es veuen altament condicionades pels factors locals, i que les comunitats d'abelles poden veure's afectades per l'apicultura intensiva, una activitat humana generalitzada que habitualment és considerada com a beneficiosa.

General introduction

Introduction

Ecology can be defined as the study of organisms and their interactions with their environment and with other organisms that determine abundance and distribution of species (Krebs et al. 1994). Countless studies have been done to understand how such interactions shape the observed patterns of diversity and distribution of species (e.g. Darwin 1859; Hutchinson 1959; MacArthur 1972; May 1988). Nature is highly heterogeneous, meaning that animals and plants are distributed unevenly across the space, becoming systems of organized heterogeneity (Margalef 1968). The observed heterogeneity in the spatial distribution of species is a consequence of the spatial variability of abiotic factors and biotic interactions, in combination with processes of extinction and colonization (Strong et al. 1984, Ricklefs and Schluter 1993, Begon et al. 2006). Thus, spatial distribution patterns provide important information not only about current conditions but also about past processes at a given site.

It has been well established that environmental heterogeneity is crucial to maintain species diversity (e.g. MacArthur and MacArthur 1961, Pianka 1966, Weibull et al. 2000), and that homogenization of the environment can lead to biotic impoverishment and consequent homogenization of the biota (McKinney and Lockwood 2001, Rahel 2002, Thrush et al. 2006). Furthermore, increased biodiversity usually promotes functional diversity, which has been proven to ultimately guarantee ecosystem services (Díaz and Cabido 2001, Cadotte et al. 2011, Gagic et al. 2015). However, global biodiversity is threatened by multiple factors, potentially jeopardizing ecosystem services. Unfortunately, information about species distribution and spatial dynamics is lacking for most species and most regions of the world.

Local variability of community composition and structure

As defined by Margalef (1993), biosphere is not only composed by individuals, which are the cornerstones of ecosystems, but also by the physical environment. Populations need to find adequate abiotic and biotic resources to be able to sustain themselves at a given site. Seen from this perspective, a good-quality habitat should provide enough resources for survival and reproduction, and habitat quality is known to influence species diversity (Berg 1997, Wettstein and Schmid 1999, Thomas et al. 2001). Therefore, resource availability and

distribution are expected to play a very important role in determining species diversity and composition (MacArthur 1965, McKane et al. 2002, John et al. 2007).

Species functional traits are also expected to shape biodiversity patterns (Shipley et al. 2006, Green et al. 2008, Kraft et al. 2008). Especially important is the use of space by mobile organisms, which is usually related to body size (Swihart et al. 1988, Kelt and Vuren 1999, Greenleaf et al. 2007). Mobility is important not only in relation to foraging ranges, but also in terms of dispersal ability, which determines the capacity to colonize new areas (Willson 1993, Reed et al. 2000, Bullock et al. 2002, Plaisance et al. 2008).

Local community structure can also be affected by historical events (Ricklefs 1987, Fukami 2010). Consequently, current community assemblages are the result of species responses to past processes such as immigration/emigration and extinction events or historical population dynamics.

Effects of perturbations on local communities

Nature is constantly affected by natural and anthropogenic disturbances. As one of the main drivers of habitat modification, disturbances may have profound effects on community dynamics, sometimes leading to radical changes (Nyström et al. 2000, Jackson and Overpeck 2000, Cardoso et al. 2008). Such disturbances are considered to be a primary cause of spatial heterogeneity in natural communities (Sousa 1984). For instance, wildfire is known to play an important role in the structure of world ecosystems (Bond and Keeley 2005). While some species may be favored by fire, other species are negatively affected and may even undergo local extinction (Esque et al. 2003, Peres et al. 2003, Arnan et al. 2006). Spatial distribution of communities will be also determined by the heterogeneity of perturbation intensity and frequency and the heterogeneity of post-perturbation patterns (Turner et al. 1998, Rodrigo et al. 2004).

Anthropogenic perturbations, including climate change, land use change and many biotic changes such as biological invasions, are the major cause of habitat modification (Vitousek et al. 1997). Land use change, probably the most common alteration of anthropogenic origin, may entail profound changes in community composition and structure through biotic and abiotic factors (Downing et al. 1999, Bossio et al. 2005, Pereira et al. 2010), and can

potentially alter ecosystem services (Kremen et al. 2007). For example, urbanization has been proven to be the cause of several notorious extinctions of native species, leading to biotic homogenization, and strongly enhancing the establishment of nonnative species (Blair 2001, Marzluff 2001, McKinney 2006, Pauchard et al. 2006, Mcdonald et al. 2008, Shochat et al. 2010). Climate change is another important driver of community modification. One of its main general effects is poleward and altitudinal shift in species distributions (Parmesan et al. 1999, Hickling et al. 2006, Thomas 2010, Chen et al. 2011).

Finally, the introduction of exotic species is having important impacts on native community structures (Levine et al. 2003, Ehrenfeld 2010). Ecological invasions can carry important consequences for communities (Porter and Savignano 1990, Lodge 1993, Grosholz 2002) and have profound effects on interaction networks (Traveset and Richardson 2006, Bartomeus et al. 2008). Similarly to the introduction of invasive species, the introduction of large densities of domestic animals such as sheep and cattle may also have profound impacts on native communities through competition for food resources (Stewart et al. 2002, Baldi et al. 2004, Young et al. 2005).

Species and communities are, to a greater or lesser degree, sensible to all such habitat modifications, which lead to changes in species distributions and community structure and composition. Consequently, species and community spatial variability (β-diversity) can be a relevant measure for conservation purposes (Condit et al. 2002).

Local spatial variability of interactions

Species interact with each other, forming complex networks. Interactions play an essential role in species distribution trough, for example, competition, predation and different types of mutualism (Begon et al. 2006). Several models, such as the predator-prey Lotka-Volterra model, and the host-parasite Nicholson-Bailey model have been proposed to understand and predict the outcome of interactions on populations and communities. Other models have been proposed to explain the underlying mechanisms that ultimately cause species to interact. For instance, the neutrality hypothesis postulates that individuals interact randomly, so that the frequency of interactions and the number of interacting species depends on the abundance of each species (Blüthgen et al. 2008, Vázquez et al. 2009b).

Abundant species have a higher probability to experience a random encounter and therefore are more prone to interact. In antagonistic networks, host/prey abundance has also been proposed as a mechanism to explain network patterns through the learning abilities of parasites/predators (Ishii and Shimada 2012). Finally, trait matching (including morphological and phenological traits) between interacting species is an essential condition for the realization of an interaction (Jordano et al. 2003).

The heterogeneous distribution of organisms has an effect on the identity and strength of their interactions (Agrawal et al. 2006), leading to high variability in network structure and interaction composition across space (Olesen and Jordano 2002, Vázquez et al. 2009b). For instance, it has been demonstrated that the structure of plant-animal mutualistic networks is largely shaped by species distribution (Morales and Vázquez 2008, Vázquez et al. 2009a, Burkle and Alarcón 2011).

 Because local factors may change across space, the same species may interact differently at different sites. Importantly, some factors may differently affect the two trophic levels involved in an interaction, influencing not only the spatial distribution of a given species, but also its ability to interact with certain partners. As the trophic level increases, it is increasingly difficult is to predict species distribution because more factors intervene in shaping such distribution.

 In sum, interaction networks are particularly sensitive to habitat change because they depend on a combination of factors differently affecting the various trophic levels involved. For this reason, ecological networks can be regarded as potential bioindicators of ecological change. For instance, an impoverished network structure would reflect biotic homogenization (Albrecht et al. 2007). Interactions have been found to be more sensitive to habitat disturbance because they may be lost before communities show any signs of alteration (Tylianakis et al. 2007). Nonetheless, networks have seldom been used as bioindicators.

Changes in network organization may ultimately affect ecosystem services. For example, if extinction risk is associated to particular biological attributes, certain ecological functions may be lost in impoverished networks (Memmott et al. 2004, Fontaine et al. 2006). For this reason, the concept of interaction conservation has been proposed as an extension of species conservation (Kearns et al. 1998).

Ecological importance of bees

Approximately 87.5% of the existing angiosperms rely on animal pollination (Ollerton et al. 2011). Among the various groups of pollinators, bees are undoubtedly the most important, both in terms of flower visitation rates and pollinating efficiency, being the main pollinators in most ecosystems. Roughly, 20,000 bee species are currently described worldwide (Ascher and Pickering 2011). They are present in every continent except Antartica, in a wide range of different habitats (Michener 2000). As pollinators, they provide an essential ecosystem service (Losey and Vaughan 2006). Bee species diversity is therefore crucial for ecosystem functioning, both in natural and agricultural systems. .

Surveys based on historical data demonstrate that bee diversity is declining in several parts of the world (Biesmeijer et al. 2006, Potts et al. 2010, 2014, Cameron et al. 2011). In Europe, there are almost 2,000 wild bee species 9.2% of which are known to be threatened with extinction, and 56.7% cannot be evaluated due to lack of knowledge and funding (Nieto et al. 2014). Thus, the population status and viability of the vast majority of bee species in Europe are largely unknown.

Bee communities have a combination of characteristics that make them ideal for the study of spatial variability of communities and their interactions. First of all, bees have very contrasted functional traits, such as body size, proboscis length, and sociality (Michener 2000). Differences in body size imply differences in energetic requirements, foraging ranges and dispersal ability, and differences in proboscis length imply differences in accessibility to different types of flowers. Disparity in sociality in relation to colony size and thermoregulation also imply important differences in energetic requirements. Bee communities include various levels of feeding specialization, from narrow pollen specialists to wide generalists. Finally, bee communities display a range of nesting habits, including ground nesters, cavity nesters and species building exposed nests. In addition, some species require external materials, often of plant origin, to build their nests. Thus, different bee species within a community are likely to be sensitive to different factors, or respond differently to the same factors (Westrich 1996), and to interact with different flower species according to their functional traits.

In addition to interacting with flowers, bees also interact with a variety of natural enemies, including predators, parasitoids, cleptoparasites and nest scavangers. These various groups

of enemies may differ in their level of specialization, and may respond to a suite of additional factors in addition to bee community spatial distribution.

Objectives of this thesis

This dissertation studies the factors that influence the spatial variability of a bee community and its interaction network with natural enemies in the Garraf Natural Park (Barcelona). The study area is a continuous Mediterranean scrubland, with very similar vegetation type, geology and perturbation history. The lack of physical or ecological barriers and gradients in the study area affords us with an opportunity to analyze the intrinsic spatial variability of the community and its interaction network without noise factors due to changes in habitat type. The main objective of this thesis is to investigate how local resources and human perturbation may affect the spatial distribution of a bee community at a local scale, and how community spatial variability influences the spatial variability of its interaction network.

Chapter 1 analyses the spatial variability of a wild bee community at a local scale, and how the community responds to the spatial variability of floral and nesting resources. Because bees depend on both resource types to survive and reproduce, spatial distribution of bees is expected to follow spatial distribution of flowers and/or nesting sites (Ricklefs 1987). It has been well established that foraging ranges of bees are directly related to species body size (Gathmann and Tscharntke 2002, Greenleaf et al. 2007, Guédot et al. 2009, Zurbuchen et al. 2010). Thus, we additionally explore the role of body size on the spatial distribution of the bee community. While landscape-scale factors affecting bee communities have been amply studied, little is known about local-scale factors influencing them. Therefore, the first chapter of this thesis is intended to explore such relationships with three specific objectives: (1) To analyze floral and nesting resource composition heterogeneity at the habitat scale, (2) to analyze the distribution of bee composition across the habitat and (3) to establish whether species with different body sizes respond differently to local resource availability and show different patterns of spatial distribution.

Having seen the effect of flower and nesting resources on bee community composition and structure, **Chapter 2** studies the response of the same community to a human perturbation, namely beekeeping. Beekeeping activities result in the introduction of large amounts of

honey bee (*Apis mellifera)* individual workers foraging for nectar and pollen. Due to their highly efficient foraging behavior (von Frisch 1967, Seeley 1985, Richter and Keramaty 2003), managed honey bees may pose a problem to the native bee community when resources are scarce, lowering pollen and nectar availability. Wild bee species of large body size may be the most affected due to their higher feeding requirements (Müller et al. 2006). In Chapter 2, we measure pollen-nectar consumption in plots progressively distant from apiaries and relate this consumption to honey bee and wild bee foraging activity. Our objective is to understand the impact of honey bee flower visitation on pollen and nectar consumption and the effect of beekeeping on the abundance, richness and composition of the local wild bee community.

Finally, **Chapter 3** studies the local spatial variability of the cavity-nesting bee and wasp community, their nest-associated fauna (parasitoids, cleptoparasites, predators and scavengers), and the resulting interaction network. This chapter also addresses the relationship between spatial variation of the two trophic levels and with the spatial variation of the interactions. Species β-diversity patterns can be divided into two additive components: species turnover and species loss (Baselga 2010). Analogously, interaction β-diversity patterns may be divided into a component of species turnover and a component of rewiring (same species interacting differently in different localities) (Poisot et al. 2012). This last chapter evaluates these sources of variation and their contribution to the observed spatial patterns. In particular, we have the following objectives (1) to analyze the spatial variation of a community of cavity-nesting bees and wasps (henceforth hosts) and their nest associates (henceforth parasitoids) across a continuous habitat, (2) to study β-diversity of host-parasitoid interactions and (3) to explore the relationship between host, parasitoid and interaction β-diversity and to examine their distance decay.

ReferencesAgrawal, A. A. et al. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. - Q. Rev. Biol. 81: 349–376.

Albrecht, M. et al. 2007. Interaction diversity within quantified insect food webs in restored and adjacent intensively managed meadows. - J. Anim. Ecol. 76: 1015–1025.

Arnan, X. et al. 2006. Post-fire recovery of Mediterranean ground ant communities follows vegetation and dryness gradients. - J. Biogeogr. 33: 1246–1258.

Ascher, J. S. and Pickering, J. 2011. Bee species guide (Hymenoptera: Apoidea: Anthophora). - Discover Life: www.discoverlife.org.

Baldi, R. et al. 2004. High potential for competition between guanacos and sheep in Patagonia. - J. Wildl. Manage. 68: 924–938.

Bartomeus, I. et al. 2008. Contrasting effects of invasive plants in plant-pollinator networks. - Oecologia 155: 761–770.

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. - Glob. Ecol. Biogeogr. 19: 134–143.

Begon, M. et al. 2006. Ecology: from individuals to ecosystems. - Blackwell Publishing.

Berg, Å. 1997. Diversity and abundance of birds in relation to forest fragmentation, habitat quality and heterogeneity. - Bird Study 44: 355–366.

Biesmeijer, J. C. et al. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. - Science 313: 351–4.

Blair, R. 2001. Birds and butterflies along urban gradients in two ecoregions of the United States: is urbanization creating a homogeneous fauna? - In: Biotic homogenization. Spinger, pp. 33–56.

Blüthgen, N. et al. 2008. What do interaction network metrics tell us about specialization and biological traits? - Ecology 89: 3387–99.

Bond, W. J. and Keeley, J. E. 2005. Fire as a global "herbivore": The ecology and evolution of flammable ecosystems. - Trends Ecol. Evol. 20: 387–394.

Bossio, D. A. et al. 2005. Soil microbial community response to land use change in an agricultural landscape of western Kenya. - Microb. Ecol. 49: 50–62.

Bullock, J. M. et al. 2002. Plant dispersal and colonization processes at local and landscape scales. - In: Dispersal Ecology: 42nd Symposium of the British Ecological Society. pp. 279–302.

Burkle, L. A. and Alarcón, R. 2011. The future of plant-pollinator diversity: understanding interaction networks across time, space, and global change. - Am. J. Bot. 98: 528–38.

Cadotte, M. W. et al. 2011. Beyond species: Functional diversity and the maintenance of ecological processes and services. - J. Appl. Ecol. 48: 1079–1087.

Cameron, S. A. et al. 2011. Patterns of widespread decline in North American bumble bees. - Proc. Natl. Acad. Sci. U. S. A. 108: 662–667.

Cardoso, P. G. et al. 2008. The impact of extreme flooding events and anthropogenic stressors on the macrobenthic communities' dynamics. - Estuar. Coast. Shelf Sci. 76: 553–565.

Chen, I.-C. et al. 2011. Rapid range shifts of species of climate warming. - Science 333: 1024–1026.

Condit, R. et al. 2002. Beta-diversity in tropical forest trees. - Science 295: 666–669.

Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. - John Murray.

Díaz, S. and Cabido, M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. - Trends Ecol. Evol. 16: 646–655.

Downing, J. A. et al. 1999. The impact of accelerating land-use change on the N-Cycle of tropical aquatic ecosystems: Current conditions and projected changes. - Biogeochemistry 46: 109–148.

Ehrenfeld, J. G. 2010. Ecosystem consequences of biological invasions. - Annu. Rev. Ecol. Evol. Syst. 41: 59–80.

Esque, T. C. et al. 2003. Effects of desert wildfires on desert rortoise (*Gopherus agassizii*) and other small vertebrates. - Southwest. Nat. 48: 103–111.

Fontaine, C. et al. 2006. Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. - PloS Biol. 4: 129–35.

Fukami, T. 2010. Community assembly dynamics in space. - In: Verhoef, H. A. and Morin, P. J. (eds), Community ecology: processes, models, and applications. Oxford University Press, pp. 45–54.

Gagic, V. et al. 2015. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. - Proc. R. Soc. B Biol. Sci. 282: 1–8.

Gathmann, A. and Tscharntke, T. 2002. Foraging ranges of solitary bees. - J. Anim. Ecol. 71: 757–764.

Green, J. L. et al. 2008. Microbial biogeography: from taxonomy to traits. - Science 320: 1039–1043.

Greenleaf, S. S. et al. 2007. Bee foraging ranges and their relationship to body size. - Oecologia 153: 589–96.

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. - Trends Ecol. Evol. 17: 22–27.

Guédot, C. et al. 2009. Relationship between body size and homing ability in the genus Osmia (Hymenoptera; Megachilidae). - Ecol. Entomol. 34: 158–161.

Hickling, R. et al. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. - Glob. Chang. Biol. 12: 450–455.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so may kinds of animals? - Am. Nat. 93: 145–159.

Ishii, Y. and Shimada, M. 2012. Learning predator promotes coexistence of prey species in host – parasitoid systems. - Proc. Natl. Acad. Sci. U. S. A. 109: 5119.

Jackson, S. T. and Overpeck, J. T. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. - Paleobiology 26: 194–220.

John, R. et al. 2007. Soil nutrients influence spatial distributions of tropical tree species. - Proc. Natl. Acad. Sci. U. S. A. 104: 864–9.

Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant-animal interactions. - Ecol. Lett. 6: 69–81.

Kearns, C. A. et al. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. - Annu. Rev. Ecol. Syst. 29: 83–112.

Kelt, D. and Vuren, D. Van 1999. Energetic constraints and the relationship between body size and home range area in mammals. - Ecology 80: 337–340.

Kraft, N. J. B. et al. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. - Science 322: 580–582.

Krebs, C. et al. 1994. Ecology: the experimental analysis of distribution and abundance. - Harper Collins College Publishers.

Kremen, C. et al. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. - Ecol. Lett. 10: 299–314.

Levine, J. M. et al. 2003. Mechanisms underlying the impacts of exotic plant invasions. - Proc. Biol. Sci. 270: 775–81.

Lodge, D. M. 1993. Invasions: Lessons for Ecology. - Trends Ecol. Evol. 8: 133–137.

Losey, J. E. and Vaughan, M. 2006. The economic value of ecological services provided by insects. - Bioscience 56: 311.

MacArthur, R. 1965. Patterns of species diversity. - Biol. Rev. 40: 510–533.

MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. - Princeton University Press.

MacArthur, R. and MacArthur, J. 1961. On bird species diversity. - Ecology 42: 594–598.

Margalef, R. 1968. Perspectives in ecological theory. - University of Chicago Press.

Margalef, R. 1993. Teoría de los sistemas ecológicos. - Universitat de Barcelona.

Marzluff, J. 2001. Worldwide urbanization and its effects on birds. - In: Marzluff, J. M. et al. (eds), Avian ecology and conservation in an urbanizing world. Spinger, pp. 19–47.

May, R. M. 1988. How many species are there on Earth? - Science 241: 1441–1449.

Mcdonald, R. I. et al. 2008. The implications of current and future urbanization for global protected areas and biodiversity conservation. - Biol. Conserv. 141: 1695–1703.

McKane, R. B. et al. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. - Nature 415: 68–71.

McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. - Biol. Conserv. 127: 247–260.

McKinney, M. L. and Lockwood, J. L. 2001. Biotic homogenization: a sequential and selective process. - In: Lockwood, J. L. and McKinney, M. L. (eds), Biotic homogenization. Springer US, pp. 1–17.

Memmott, J. et al. 2004. Tolerance of pollination networks to species extinctions. - Proc. R. Soc. B Biol. Sci. 271: 2605–2611.

Michener, C. D. 2000. The bees of the world. - Johns Hopkins University Press.

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

Müller, A. et al. 2006. Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships. - Biol. Conserv. 130: 604–615.

Nieto, A. et al. 2014. European Red List of Bees. - Publication Office of the European Union.

Nyström, M. et al. 2000. Coral reef disturbance and resilience in a human-dominated environment. - Trends Ecol. Evol. 15: 413–417.

Olesen, J. M. and Jordano, P. 2002. Geographic patterns in plant-pollinator mutualistic networks. - Ecology 83: 2416–2424.

Ollerton, J. et al. 2011. How many flowering plants are pollinated by animals? - Oikos 120: 321–326.

Parmesan, C. et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. 399: 579–583.

Pauchard, A. et al. 2006. Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). - Biol. Conserv. 127: 272–281.

Pereira, H. M. et al. 2010. Scenarios for global biodiversity in the 21st century. - Science 330: 1496–1501.

Peres, C. A. et al. 2003. Vertebrate responses to surface wildfires in a central Amazonian forest. - Oryx 37: 97–109.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: a review of concepts. - Am. Nat. 100: 33–46.

Plaisance, L. et al. 2008. Colonization of Pacific islands by parasites of low dispersal ability: Phylogeography of two monogenean species parasitizing butterflyfishes in the South Pacific Ocean. - J. Biogeogr. 35: 76–87.

Poisot, T. et al. 2012. The dissimilarity of species interaction networks. - Ecol. Lett. 15: 1353–61.

Porter, S. D. and Savignano, D. A. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. - Ecology 71: 2095–2106.

Potts, S. G. et al. 2010. Global pollinator declines: trends, impacts and drivers. - Trends Ecol. Evol. 25: 345–53.

Potts, S. et al. 2014. Status and Trends of European Pollinators. Key findings of the STEP project. - Pensoft Publishers.

Rahel, F. J. 2002. Homogenization of freshwater faunas. - Annu. Rev. Ecol. Syst. 33: 291–315.

Reed, D. C. et al. 2000. The role of dispersal and disturbance in determining spatial heterogeneity in sedentary organisms. - Ecology 81: 2011–2026.

Richter, M. and Keramaty, J. 2003. Honey bee foraging behavior. - In: Plogger, B. J. and Yasukawa, K. (eds), Exploring animal behavior in laboratory and field: an hypothesis-testing approach to the development, causation, function, and evolution of animal behavior. Elsevier, pp. 133.

Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. - Science 235: 167–171.

Ricklefs, R. E. and Schluter, D. 1993. Species diversity in ecological communities: historical and geographical perspectives. - University of Chicago Press.

Rodrigo, A. et al. 2004. Direct regeneration is not the only response of Mediterranean forests to large fires. - Ecology 85: 716–729.

Seeley, T. D. 1985. Honeybee ecology: a study of adaptation in social life. - Princeton University Press.

Shipley, B. et al. 2006. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. - Science 314: 812–814.

Shochat, E. et al. 2010. Invasion, competition, and biodiversity loss in urban ecosystems. - Bioscience 60: 199–208.

Sousa, W. P. 1984. The role of disturbance. - Annu. Rev. Ecol. Syst. 15: 353–391.

Stewart, K. M. et al. 2002. Temporospatial distributions of elk, mule deer, and cattle: resource partitioning and competitive displacement. - J. Mammal. 83: 229–244.

Strong, D. R. et al. 1984. Insects on plants. Community patterns and mechanisms. - Blackwell Scientific Publicatons.

Swihart, R. et al. 1988. Relating body size to the rate of home range use in mammals. - Ecology: 393–399.

Thomas, C. D. 2010. Climate, climate change and range boundaries. - Divers. Distrib. 16: 488–495.

Thomas, J. A. et al. 2001. The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. - Proc. R. Soc. B Biol. Sci. 268: 1791–1796.

Thrush, S. F. et al. 2006. Predicting the effects of habitat homogenization on marine biodiversity. - Ecol. Appl. 16: 1636–1642.

Traveset, A. and Richardson, D. M. 2006. Biological invasions as disruptors of plant reproductive mutualisms. - Trends Ecol. Evol. 21: 208–16.

Turner, M. G. et al. 1998. Factors influencing succession: lessons from large, infrequent natural disturbances. - Ecosystems 1: 511–523.

Tylianakis, J. M. et al. 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. - Nature 445: 202–205.

Vázquez, D. P. et al. 2009a. Evaluating multiple determinants of the structure of plant-animal mutualistic networks. - Ecology 90: 2039–2046.

Vázquez, D. P. et al. 2009b. Uniting pattern and process in plant-animal mutualistic networks: a review. - Ann. Bot. 103: 1445–57.

Vitousek, P. M. et al. 1997. Human domination of Earth's ecosystems. - Science 277: 494–499.

Von Frisch, K. 1967. The dance language and orientation of bees. - Harvard University Press. Cambridge, MA.

Weibull, A. C. et al. 2000. Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. - Ecography (Cop.). 23: 743–750.

Westrich, P. 1996. Habitat requirements of central European bees and the problems of partial habitats. - In: Matheson, A. et al. (eds), Linnean Society Symposium Series. Vol. 18. Academic Press Limited, pp. 1–16.

Wettstein, W. and Schmid, B. 1999. Conservation of arthropod diversity in montane wetlands: Effect of altitude, habitat quality and habitat fragmentation on butterflies and grasshoppers. - J. Appl. Ecol. 36: 363–373.

Willson, M. F. 1993. Dispersal mode, seed shadows, and colonization patterns. - Vegetatio 107-108: 261–280.

Young, T. P. et al. 2005. Competition and compensation among cattle, zebras, and elephants in a semi-arid savanna in Laikipia, Kenya. - Biol. Conserv. 122: 351–359.

Zurbuchen, A. et al. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. - Biol. Conserv. 143: 669–676.

Chapter 1

Determinants of spatial distribution in a bee community: nesting resources, flower resources, and body size

Summary Understanding biodiversity distribution is a primary goal of community ecology. At a landscape scale, bee communities are affected by habitat composition, anthropogenic land use, and fragmentation. However, little information is available on local-scale spatial distribution of bee communities within habitats that are uniform at the landscape scale. We studied a bee community along with floral and nesting resources over a 32 km² area of uninterrupted Mediterranean scrubland. Our objectives were (i) to analyze floral and nesting resource composition at the habitat scale. We ask whether these resources follow a geographical pattern across the scrubland at bee-foraging relevant distances; (ii) to analyze the distribution of bee composition across the scrubland. Bees being highly mobile organisms, we ask whether bee composition shows a homogeneous distribution or else varies spatially. If so, we ask whether this variation is irregular or follows a geographical pattern and whether bees respond primarily to flower or to nesting resources; and (iii) to establish whether body size influences the response to local resource availability and ultimately spatial distribution. We obtained 6580 specimens belonging to 98 species. Despite bee mobility and the absence of environmental barriers, our bee community shows a clear geographical pattern. This pattern is mostly attributable to heterogeneous distribution of small (< 55 mg) species (with presumed smaller foraging ranges), and is mostly explained by flower resources rather than nesting substrates. Even then, a large proportion (54.8%) of spatial variability remains unexplained by flower or nesting resources. We conclude that bee communities are strongly conditioned by local effects and may exhibit spatial heterogeneity patterns at a scale as low as 500-1000 m in patches of homogeneous habitat. These results have important implications for local pollination dynamics and spatial variation of plant-pollinator networks.

1.1. Introduction

From a strictly theoretical perspective, a community may be defined as the assemblage of species occupying an area within which all individuals are equally likely to interact, thus hindering spatial heterogeneity in distribution or abundance (Holyoak et al. 2005). However, we live in a highly heterogeneous world, and even the most uniform habitats show important levels of spatial variability in environmental conditions at one scale or another. From a more deterministic perspective, species composition is expected to be closely related to this within-habitat heterogeneity, for example in resource availability (Ricklefs 1987). However, the effects of resource distribution on community composition may be difficult to predict for several reasons. First, different species may respond to resource distribution at different scales. Large species, with greater food requirements and greater

mobility are expected to respond to resource distribution at larger scales (Holling 1992). Small species, on the other hand, may be able to satisfy their needs within a small area and therefore be more sensitive to local scale factors. Second, a given species may depend on various resources with differing distribution patterns, and thus respond to each resource at a different scale (Westrich 1996). Local community structure is further shaped by species' functional traits, such as dispersal ability, and by interactions between species resulting in either avoidance or attraction (Resetarits et al. 2005). Finally, community structure may be historically contingent, so that even under similar environmental conditions, different species assemblages may arise as a result of different immigration history or disturbance events (Fukami 2010).

In this study we analyze the spatial distribution of a bee community as well as the distribution of the nesting and floral resources on which bees depend. Most bee species build nests and provision them with pollen and nectar as food for their larvae. Once a bee has established at a nesting site, it conducts repeated pollen-nectar foraging trips, thus becoming a central place forager. Because different species use different nesting substrates and favour different pollen sources, bee diversity is expected to be higher in areas hosting a variety of nesting and floral resources (Roulston and Goodell 2011). Pollen specialization in bees ranges from polylecty (species collecting pollen from many unrelated plant families), to oligolecty (collecting pollen from a single plant family), and monolecty (collecting pollen from a single plant genus). As for nesting substrates, most bee species excavate their nests underground, but some do so in dead wood or in soft-pith stems. Other species exploit different types of pre-existing cavities, and a smaller number build exposed nests attached to rocks or to the vegetation. Finally, some bee species are cleptoparasitic, laying their eggs in nests of other bee species, usually of a given genus. A number of studies have documented the influence of flower resources on the structure of bee communities (Gathmann et al. 1994, Potts et al. 2003, 2004, Schaffers et al. 2008, Grundel et al. 2010, Fründ et al. 2010, Castagneyrol and Jactel 2012, Ebeling et al. 2012). Fewer studies have addressed the role of nesting substrates (Eltz et al. 2003, Potts et al. 2003, 2005, Grundel et al. 2010, Murray et al. 2012), and establishing the relative importance of flower *versus* nesting resources has become a key topic in bee ecology research (Steffan-Dewenter and Westphal 2008). While attaching a greater weight to flower resources, the review of Roulston and Goodell (2011) emphasizes the need to consider both types of resources, partly because of the spatial

complexity of resource distribution and partly because nesting substrate diversity is often correlated with plant diversity.

Bees are able to fly long distances and therefore have the capacity to readily colonize suitable sites. Several studies have estimated bee foraging ranges through the use of various techniques, including measures of trip duration, experiments of homing ability, harmonic radar tracking, mark-recapture experiments and genetic analysis of foraging bees (Osborne et al. 1999, Walther-Hellwig and Frankl 2000, Gathmann and Tscharntke 2002, Westphal et al. 2006, Greenleaf et al. 2007, Guédot et al. 2009, Goulson 2010, Zurbuchen et al. 2010, Carvell et al. 2012). These studies indicate that most species forage within a few hundred meters from their nest but some may fly thousands of meters. These studies also show a consistent positive relationship between body size and estimated foraging distance. We may thus expect species of different body sizes to respond differently to spatial resource distribution.

Previous studies have shown differences in bee community composition at landscape scales and in relation to habitat composition, anthropogenic land use change and fragmentation (Steffan-Dewenter et al. 2002, Brosi et al. 2008, Ricketts et al. 2008, Winfree et al. 2009, Murray et al. 2012, Viana et al. 2012). However, we know of no studies exploring the distribution of an entire bee community at a local scale within a habitat that may be considered homogeneous at a landscape scale. This scale is important because most individual bee movements probably occur at this scale. Our study was conducted in an area covered by contiguous Mediterranean scrubland, with uniform climatic conditions and no ecological or physical barriers. Because bees are highly mobile, one might expect within-habitat differences in bee distribution to be small. However, a few studies have shown that pollinator assemblages visiting various plant-species may vary at scales of hundreds or even tens of meters (Herrera 1988, Minckley et al. 1999, Janovský et al. 2013).

Most models on community assembly dynamics assume that environmental conditions are homogeneous across a patch of uniform habitat, although this assumption is clearly not met in many systems (Fukami 2010). Our first objective is to analyze floral and nesting resource composition heterogeneity at the habitat scale. We ask whether this heterogeneity is irregular or else follows a geographical pattern across the scrubland at bee-foraging relevant distances. Our second objective is to analyze the distribution of bee composition

across the habitat. A homogeneous distribution would be in agreement with the above-mentioned theoretical definition of community (Holyoak et al. 2005), and would reflect high levels of connection among plots, either through foraging movements, through dispersal rates, or both. Given the size of the area sampled (5.4 km by 6.2 km) and the high degree of mobility displayed by bees, we assume that any bee species is able to colonize a suitable plot in our study area over one or a few generations. Alternatively, bee composition might show a heterogeneous distribution if bee foraging areas were small and bee distribution closely tracked spatial variation in resource availability at the local scale. If the latter, we ask whether bees respond primarily to flower or to nesting resource distribution. Our bee community is rich (98 species) and encompasses a wide range of body sizes and therefore presumed energetic requirements and mobility. Our third objective is to establish whether species with different body sizes respond differently to local resource availability and show different patterns of spatial distribution.

1.2. Materials and Methods

1.2.1. Study area

The study was conducted in the Natural Park of Garraf (Barcelona, NE Spain). We selected 21 plots of 40 m x 40 m distributed more or less regularly across the park, encompassing an overall area of 32 km^2. Distances between nearest plots ranged from 585 to 1354 m. The two most distant plots were 6.2 km apart. Plots ranged in altitude from 255 to 545 m, and their distance to the coast ranged from 1500 to 6800m. At a landscape scale, the study area can be considered homogeneous. The 21 selected plots share the same vegetation type, soil type and recent disturbance history. Physical or environmental barriers are lacking and there are no significant climatic gradients. The park is located on a karstic massif of limestone and dolostone. This soil type favours drainage, thus hindering water storage. Stream beds are lacking and none of the plots is located at the bottom of a valley. The area is occupied by a Mediterranean scrubland. Plant composition varies locally from plot to plot, but is always largely dominated by *Quercus coccifera*, *Pistacia lentiscus*, *Rosmarinus officinalis* and *Thymus vulgaris.*

1.2.2. Bee sampling

We conducted 8 surveys (one every two weeks) from mid March to late June 2010, thus encompassing the main flowering period of the scrubland (flowers are very scarce in July and August). To avoid the influence of weather conditions, surveys were conducted simultaneously in all plots. In each survey we placed 6 sampling stations in two parallel rows, with a distance of 10 m between stations. Following Westphal et al. (2008), each station was composed of a metal bar holding 3 pan traps (15-cm-diameter plastic bowls painted yellow, white and blue, respectively, with UV-reflecting paint). Traps were located at 20-40 cm above ground level and approximately 50 cm away from the nearest flowering plant. Before 9:30 on each sampling day, traps were filled with water containing a small amount of detergent and collected after 18:00, thus covering most of the daily activity period. Pan trapping has been shown to underestimate bee richness and to provide an incomplete measure of flower visitation compared to netting of flower visiting insects (Westphal et al. 2008, Popic et al. 2013). However, our main concerns were to sample all 21 plots simultaneously, to avoid collector bias, and to apply the same sampling effort to each plot. Our goal was to characterize the bee community, rather than sample bee-flower interactions.

Captured specimens were dried and pinned for identification in the laboratory. From these samples we obtained measures of species richness (number of species captured), abundance (number of individuals captured) and composition (abundance of each species) for each plot. Fresh body weights were obtained from netted specimens. All specimens were weighed a few hours after being captured and, inasmuch as possible, we measured more than one specimen per species (mean=7.4; range=1-52). We use female weight in all analyses.

1.2.3. Flower resources

To estimate flower richness we counted all flower species along two 40 m x 1 m transects arranged as an X centred in the centre of the sampling station grid. This was done three times, in mid April, mid May and mid June. In addition, we estimated flower density of the main flowering species (*R. officinalis*, *T. vulgaris*, *Dorycnium pentaphyllum, Cistus albidus*,

Cistus salvifolius and *Cistus monspeliensis*) in each plot. These species represent 70-90% of the flowers produced in the study area (unpublished data from weekly flower counts in transects at 12 different sites across the park). We first calculated the volume of each flower patch in the transects by measuring two perpendicular widths and the height. Then, to establish a relationship between patch volume and number of flowers, we counted all open flowers in a subsample of patches (n= 59 - 226 per species) at peak bloom (Linear regression: r^2= 0.36 - 0.63, p= 0.001 - 0.015). The three *Cistus* species were scarce compared to the other species and their blooming periods overlapped widely. Therefore, we lumped together the three species in a single variable (*Cistus* flowers). In an attempt to tease apart the effects of pollen and nectar we used measures of pollen and nectar production per flower of each species (unpublished data) to estimate pollen and nectar density in each plot. However, these two variables were highly correlated (r= 0.96, p< 0.0001), and they were also correlated to flower density (r= 0.82, p< 0.0001 and r= 0.77, p<0.0001, respectively). Therefore, we use flower density in all analyses.

1.2.4. Nesting substrates

We used the above-mentioned transects to measure availability of nesting substrates. On every m^2 of transect we placed a wire grid delimiting 32 cells (each measuring 0.031 m^2), and each cell was scored as containing one or no potential nesting substrates. We used the following nesting substrate variables: % bare soil, % bare soil with stones, presence of dead wood, number of holes in rocks, number of vacant snail shells, % *Quercus coccifera* cover, and % *Ampelodesmos mauritanica* cover. *Quercus coccifera* was included because we often observed *Bombus terrestris* bumblebees nesting at their base. *Ampelodesmos mauritanica* was included because it produces soft-pith and hollow stems that might be used by some bee species in the genera *Ceratina, Heriades, Protosmia* and *Hoplitis.*

1.2.5. Statistical analysis

All flower resource variables were square-root transformed to improve normality and homoscedasticity. Nesting resource variables were log transformed, except *Q. coccifera*

cover, which was square-root transformed. Bare soil and bare soil with stones were significantly correlated (r= 0.67, p= 0.001) and thus we lumped them together in a single variable (bare soil cover). The remaining resource variables were not significantly correlated.

We used Moran's I correlograms to explore spatial distribution of flower richness, flower density of each sampled species, overall flower abundance, cover of each nesting substrate, bee species richness, overall bee abundance, and bee abundance of each of the 19 most abundant species (those representing more than 0.5% of the total individuals captured). For the variable "presence of dead wood" we used the binary Join-Count correlogram. To explore spatial distribution of bee community composition, we used a Mantel's correlogram obtained from a matrix of geographical distances and a matrix of similarity (Sørensen's index) of bee species composition. The number of intervals in all correlograms was calculated based on Sturge's rule. Significance of each correlogram was tested through 300 permutations and p-values were applied a progressive Bonferroni correction. To further explore spatial distribution of bee composition, we run a cluster analysis to group plots according to bee composition similarity using UPGMA linkage rule and Euclidean distances, and represented the resulting groups on a map of the study area. These analyses were conducted with the statistical package Ape in R (R Development Core Team 2010) and the software SAM v.4.0 (Rangel et al. 2010).

The relationship between bee species richness and flower richness, between bee abundance and overall flower abundance, and between bee abundance and bee richness was analyzed with simple linear regression. The contribution of flower (flower density of *R. officinalis*, *T. vulgaris*, *D. pentaphyllum* and *Cistus*) and nesting resource (presence of dead wood, % bare soil, number of holes in rocks and number of vacant snail shells) variables to bee species richness and bee abundance was analyzed with general linear models. *Quercus coccifera* cover and *A. mauritanica* cover were not included in these analyses because our Redundancy Analysis (see below) could not find any species associated to these substrates. We selected the most parsimonious model based on Akaike's Information Criterion (AIC) using the step AIC function with forward and backward elimination implemented in the MASS library (Venables and Ripley 1999) of the R software (R Development Core Team 2010). Since neither bee species richness nor abundance were autocorrelated (see results), we did not include spatial variables in these analyses.

To establish the relationship between the spatial distribution of bee composition and flower and nesting resources we conducted an ordination analysis. We first run a detrended correspondence analysis (DCCA) to determine whether our data had a unimodal or a linear response (Lepš and Šmilauer 2003). The results of this analysis showed that our data were sufficiently homogeneous and conformed to a model with a linear response. We thus applied a Redundancy Analysis (RDA). We used the software Canoco v.4.5 to do these analyses (Ter Braak and Šmilauer 2002). Because body weight clearly conditioned bee spatial distribution, we run two RDAs, one including only small species (fresh body weight < 55 mg) and the other including only large species (> 70 mg). In both analysis, species abundance data were square-root transformed and centred. Because we did not want to attach too much weight to rare species (the majority) we did not standardize abundance data. In view of the results obtained in the cluster analysis, geographical coordinates were introduced as covariables. Resource variables were automatically selected with the *forward* option, and significance of each variable and significance of the overall model were tested with Monte Carlo simulations under reduced model (499 permutations).

1.3. Results

1.3.1. Bee community

We captured 6580 specimens corresponding to 98 species in five families: Apidae (27 species), Megachilidae (26), Andrenidae (23), Halictidae (18) and Colletidae (4) (Table A1). Nineteen species represented 93.2% of the specimens captured, and 30 of the remaining 79 species were singletons. *Lasioglossum subhirtum* was the most abundant species (27.1% of total specimens), followed by *Andrena djelfensis* (14.1%). Plot species richness ranged between 24 and 44, and abundance between 207 and 559. The relationship between bee species richness and abundance failed significance (r^2= 0.15; p= 0.09).

1.3.2. Spatial distribution of flower and nesting resources

Both flower density (27 to 265 flowers/m^2) and species richness (5 to 27) varied widely across plots (Table A2 and A3). Flower abundance and richness were not related (r^2= 0.07;

p>0.25). Flower abundance did not show spatial autocorrelation (I= -0.024, p= 0.51). Instead, flower species richness was significantly autocorrelated (I= 0.186, p<0.0001), with a gradient of positive autocorrelation at short distances (< 1000 m) progressively losing significance at longer distances. The only flower species with a significant Moran's I was *T. vulgaris* (I= 0.049, p= 0.015) (Fig. 1.1). The associated correlogram again showed a gradient of positive autocorrelation at short distance classes with a progressive loss of significance. *Rosmarinus officinalis* was more or less evenly distributed throughout the park, whereas *D. pentaphyllum* was most abundant in the north-western edge. *Cistus* spp. flower density was low compared to the other species, and varied from plot to plot showing no clear pattern (Fig. 1.1; Table A2).

Nesting substrate composition also varied widely across plots (Fig. 1.2). Bare soil and *Q. coccifera* cover were the only two nesting substrates present in all plots. However, all plots except one offered at least 4 of the 6 nesting resources. The spatial distribution of nesting substrates was highly heterogeneous (Fig. 1.2, Table A2). None of the nesting substrates showed a discernable spatial pattern, except for holes in rocks (I= 0.045, p= 0.02), again showing decreasing positive autocorrelation with increasing distance.

1.3.3. Bee spatial distribution

Neither bee abundance (I= -0.05, p= 0.99) nor species richness (I= 0.002, p= 0.17) showed spatial autocorrelation. Instead, bee composition did show significant autocorrelation (Mantel r= 0.27; p= 0.003). When we analyzed the 19 most abundant species separately, we found spatial autocorrelation for 9 of them (Table 1.1). Significant autocorrelation occurred mostly at distances < 950 m. Importantly, species showing significant autocorrelation had lower body weight (mean ± SD: 20 ± 13 mg; n= 9) than those with no significant autocorrelation (100 ± 72 mg; n= 10) (Table 1.1; Mann-Whitney U: Z= -2.858; p= 0.004). The cluster analysis of the plots based on bee composition similarity resulted in five groups and revealed a clear geographical pattern (Fig. 1.3). Interestingly, the two most abundant species, *Lasioglossum subhirtum* and *Andrena djelfensis*, showed partially segregated distributions. *Lasioglossum subhirtum* was dominant in the central and western areas of the park, whereas *A. djelfensis* was dominant on the eastern side. Abundance of these two
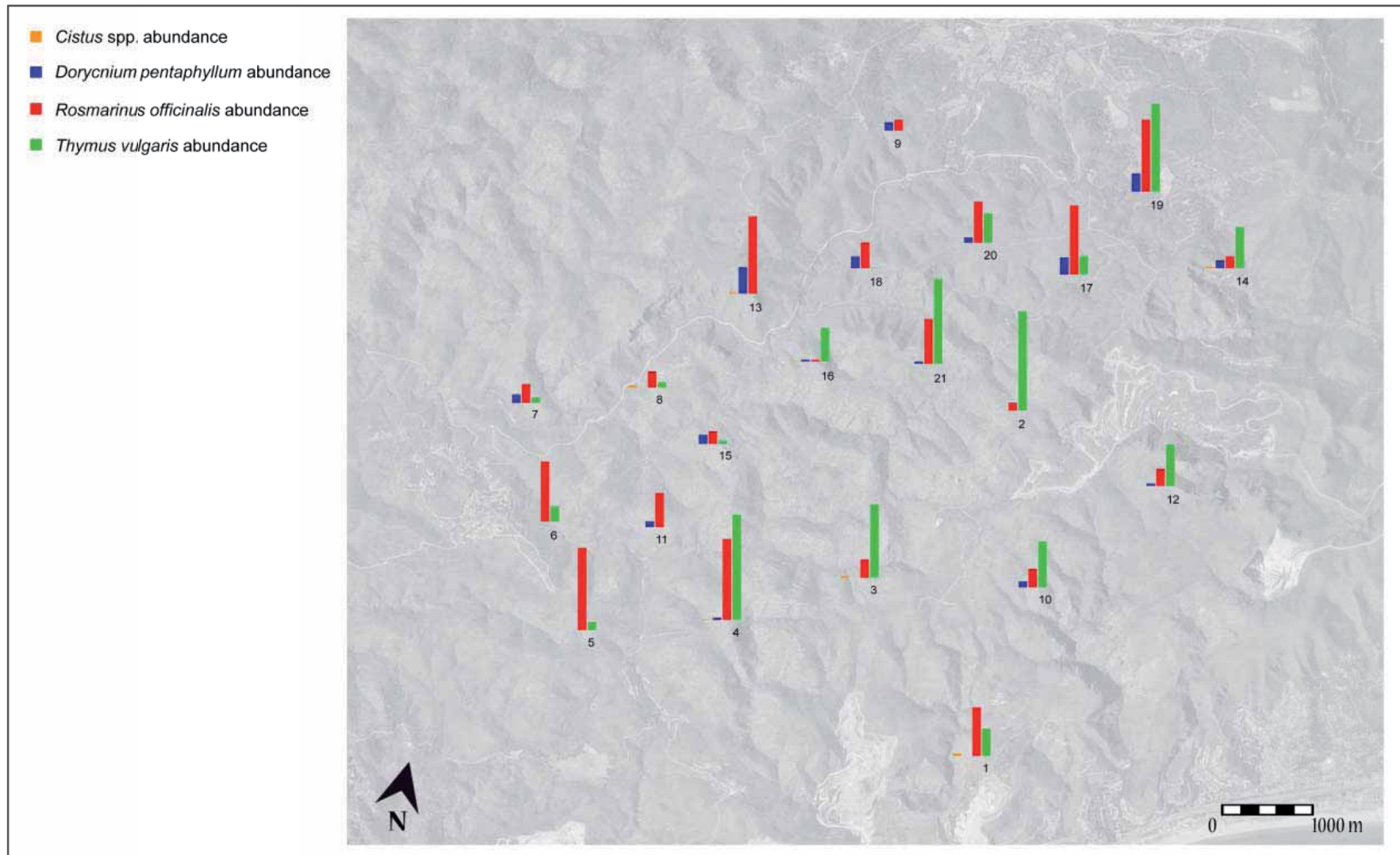
Figure 1.1 Map of the Garraf Park showing the density of flower resources (number of flowers/m^2) in each plot (n= 21).
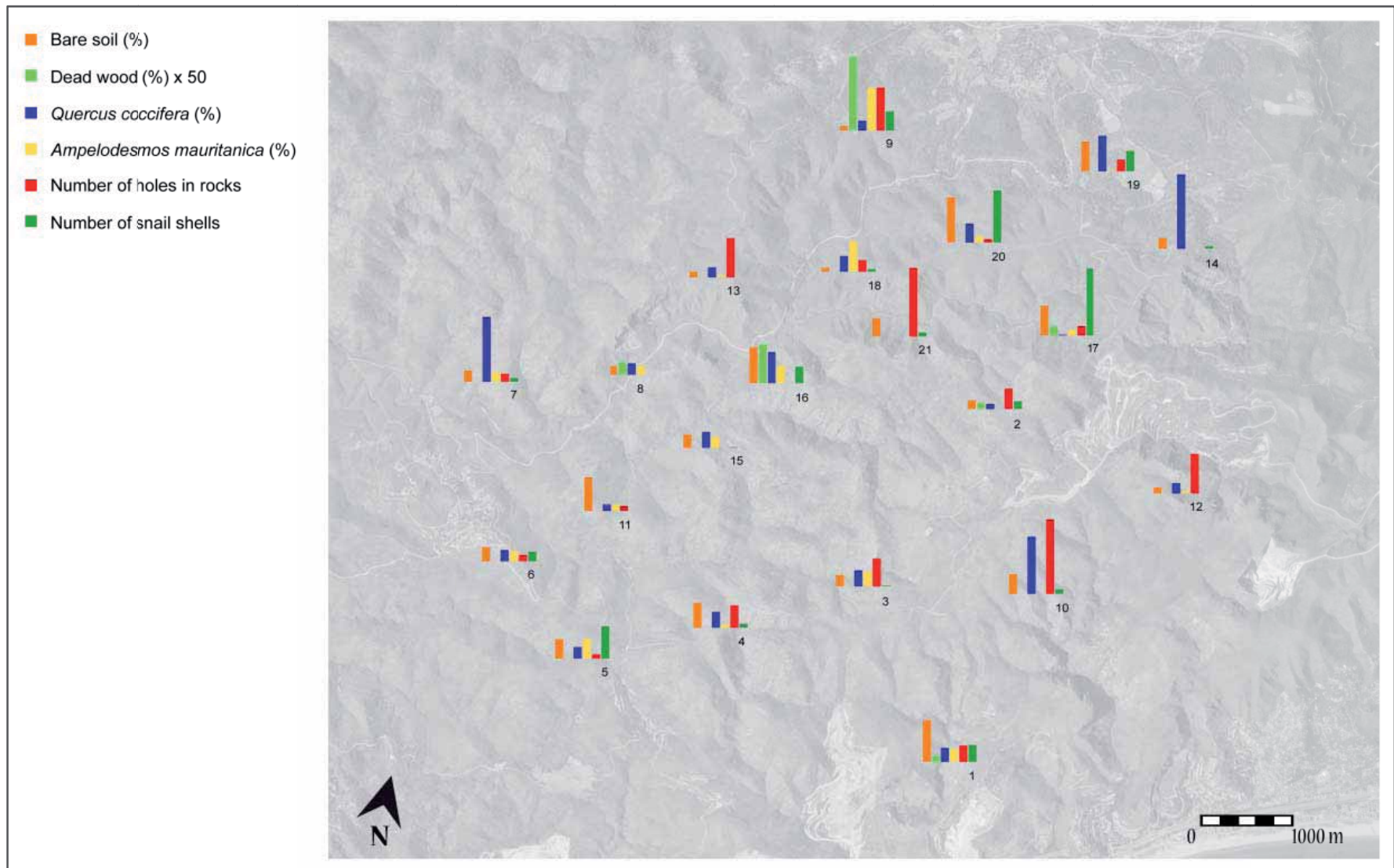
Figure 1.2 Map of the Garraf Park showing the abundance of nesting resources in each plot (n= 21).

species showed a significant negative correlation (r_s= -0.62; p= 0.003). Other species also showed a geographical pattern. *Lasioglossum malachurum* was most abundant in the NE side, *Lasioglossum bimaculatum* in the N and NW, and *Lasioglossum albocinctum* in the N. *Panurgus dentipes* was abundant only in plot 1, with a bee composition markedly different from that of all other plots. On the other hand, species such as *Rhodanthidium sticticum*, *Apis mellifera*, *Andrena nigroaenea* and *Bombus terrestris* showed a much more homogeneous distribution throughout the park. We calculated the coefficient of variation (n= 21 plots) of the abundance of the 19 main species as a measure of their degree of spatial heterogeneity. Species with higher coefficients of variation (>0.95) had lower body weight (mean ± SD: 27.4 ± 25.8mg; n= 11) than those with lower (<0.90) coefficients of variation (10.9± 76.4mg; n= 8) (Table 1.1; Mann-Whitney U: Z= 2.766; p= 0.006), corroborating the conclusion that the observed spatial pattern was mostly due to small species.

1.3.4. Relationship between resources and bee spatial distribution

Bee species richness was not related to flower species richness (r^2= 0.05; p= 0.32). However, this lack of relationship was caused by plot 1 (with the highest bee richness and a rather unique bee composition) strongly deviating from the general trend shown by the rest of the plots. Exclusion of this plot would cause the flower-bee richness relationship to become significant (r^2= 0.25; p= 0.02). The selected GLM explaining bee richness included no nesting substrate variables, and only one flower variable (*Cistus* flower abundance), but with a non-significant p-value (r^2=0.07, p=0.122; Table A4). Bee abundance was not related to overall flower abundance (r^2=0.05; p>0.3). The best model explaining bee abundance included abundance of *Cistus* and *T. vulgaris* flowers (r^2=0.32). However, only abundance of *Cistus* flowers was significant (p=0.013; abundance of *T. vulgaris* flowers, p=0.169). As with bee richness, bee abundance was not related to nesting substrate availability (Table A4).

The RDA of small species (< 55 mg) indicates that the spatial distribution of bee composition is clearly associated to flower resources and only weakly to nesting resources (Fig. 1.4). Two flower variables were significant in the model: *T. vulgaris* (Contribution to the model= 11.7%; p=0.01) and *Cistus* spp. (Contribution to the model= 9.8%; p=0.006). The model including all variables was significant (p=0.02) and explained 45.2% of the observed variance (Table 1.2). The first axis explained 25.4% of the variance and was defined by
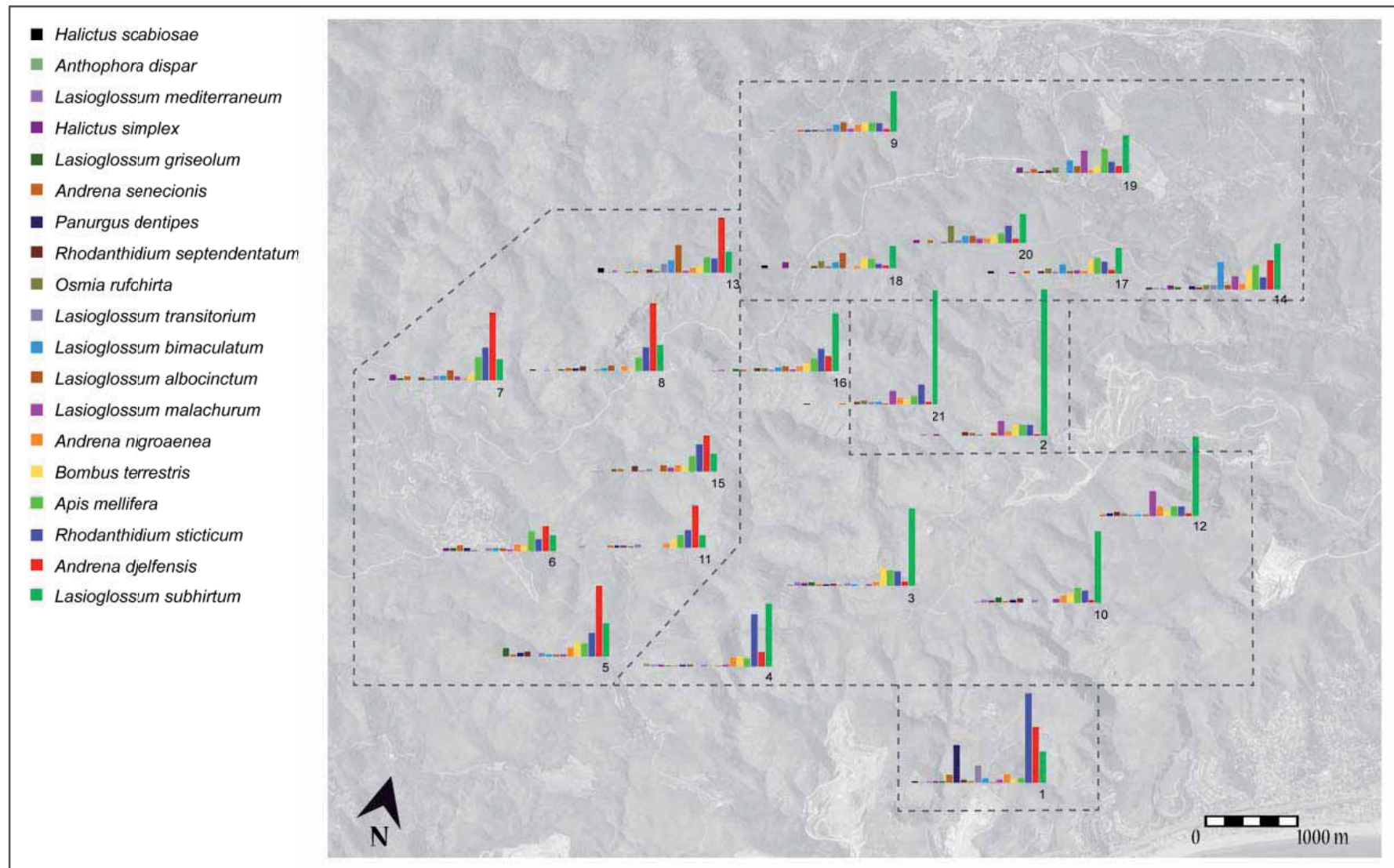
Figure 1.3 Map of the Garraf Park showing the abundance of the 19 most abundant bee species (representing more than 0.5 % of the specimens sampled) in each plot (n= 21). Plots grouped based on bee composition according to cluster analysis.

1 **Table 1.1** Parameters of the 19 most abundant bee species in the Garraf community.

	Abundance	Moran's I	P	CV of abundance	Body weight (mg)	Nesting substrate	Pollen specialization	Sociality
Lasioglossum griseolum	71	0.024	**0.04**	1.12	3.8	Soil	Polylectic	?
Lasioglossum transitorium	147	-0.05	0.9	0.99	7.6	Soil	Polylectic	?
Lasioglossum mediterraneum	46	0.034	**0.03**	1.24	12.4	Soil	Polylectic?	?
Lasioglossum malachurum	222	0.08	**0.001**	1.22	13.3	Soil	Polylectic	Social
Andrena djelfensis	926	0.131	**0**	1.03	15.0	Soil	Polylectic?	Solitary
Lasioglossum subhirtum	1780	0.045	**0.01**	0.73	16.4	Soil	Polylectic	?
Panurgus dentipes	117	-0.026	**0.03**	2.59	18.9	Soil	Oligolectic	Solitary
Lasioglossum bimaculatum	202	0.083	**0**	1.21	20.0	Soil	Polylectic?	Solitary
Osmia rufohirta	122	0.109	**0**	1.16	27.4	Snail shells	Polylectic	Solitary
Andrena senecionis	86	-0.038	0.7	0.89	40.0	Soil	Oligolectic?	Solitary
Halictus simplex	71	-0.077	0.5	1.13	40.0	Soil	Polylectic	Solitary?
Lasioglossum albocinctum	208	0.034	**0.01**	1.2	49.4	Soil	Polylectic	Solitary
Rhodanthidium septemdentatum	121	-0.006	0.3	0.5	85.9	Snail shells	Polylectic	Solitary
Andrena nigroaenea	228	-0.024	0.5	0.41	86.5	Soil	Polylectic	Solitary
Halictus scabiosae	38	-0.02	0. 3	1.64	93.6	Soil	Polylectic	Social
Apis mellifera	528	0.011	0.1	0.37	97.4	Large cavities	Polylectic	Social
Rhodanthidium sticticum	817	-0.011	0.2	0.87	103.2	Snail shells	Polylectic	Solitary
Anthophora dispar	46	-0.024	0. 5	0.8	188.4	Soil	Polylectic?	Solitary
Bombus terrestris	354	-0.015	0.4	0.47	250.5	Large cavities	Polylectic	Social

2 Abundance (number of specimens captured), Moran's I (significant *p*-values in bold), coefficient of variation of abundance (n= 21 plots), fresh female body weight, of the 19 most abundant species in the
3 Garraf bee community. Species ordered by increasing weight.

T. vulgaris flowers and number of holes in rocks on the one hand, and by *Cistus* spp. flowers on the other hand (Fig. 1.4). The second axis explained only 5.3% of the variance. On the other hand, the RDA model of large species was non-significant. The overall variance explained was lower (38.9%; Table 1.2), and no variables entered the model.

1.4. Discussion

The Garraf bee community shows a clear spatial pattern at the habitat scale, with different species dominating in different plots separated by as few as 500-1000 m. This pattern is due to small-sized species (< 55 mg), with larger species showing a more or less homogeneous distribution. A likely explanation for this outcome is that our inter-plot distance was sufficient to accommodate the foraging areas of small bees but not those of large species. A positive relationship between body size and foraging areas has been well established (Gathmann and Tscharntke 2002, Greenleaf et al. 2007, Guédot et al. 2009, Zurbuchen et al. 2010). The methods used in these and other related studies, however, tend to provide estimates of either minimum or maximum foraging ranges. Actual foraging distances have been shown to change in time and space based on resource availability (Visscher and Seeley 1982, Beekman and Ratnieks 2000, Vicens and Bosch 2000, Bhattacharya et al. 2002, Goulson 2010, Zurbuchen et al. 2010, Carvell et al. 2012, Smith et al. 2012). Our results provide indirect evidence that, in natural habitats with abundant flower resources, species smaller than 55 mg tend to forage within a radius of 250-500 m. Due to their low food requirements (Müller et al. 2006), small species may be able to obtain sufficient pollen-nectar resources within a small foraging radius. In parallel studies in our study area we have observed *Lasioglossum transitorium* females (body size: 7.6 mg) completing entire foraging bouts on single *R. officinalis* plants (which may display hundreds of open flowers) located within 50 cm of their nest.

In addition to foraging flights, our plots could be linked by dispersal movements. To our knowledge, information on bee dispersal distances is mostly lacking, but some evidence suggests dispersal distances of at least a few km. Marked *Osmia cornuta* females (a solitary species slightly larger than *Apis mellifera*) have been found nesting 2 km away from their release site (Bosch and Vicens 2006). There is also evidence that *Bombus* species may disperse as much as 3 -10 km (Goulson 2010). Even assuming smaller dispersal distances

for smaller bees, and given the lack of physical and environmental barriers in the Garraf scrubland, any species should be able to cover the limits of our study area over one or a few generations. Therefore, the fact that our bee community shows such a clear spatial pattern suggests a strong influence of environmental conditions at a very local scale, at least for small species.
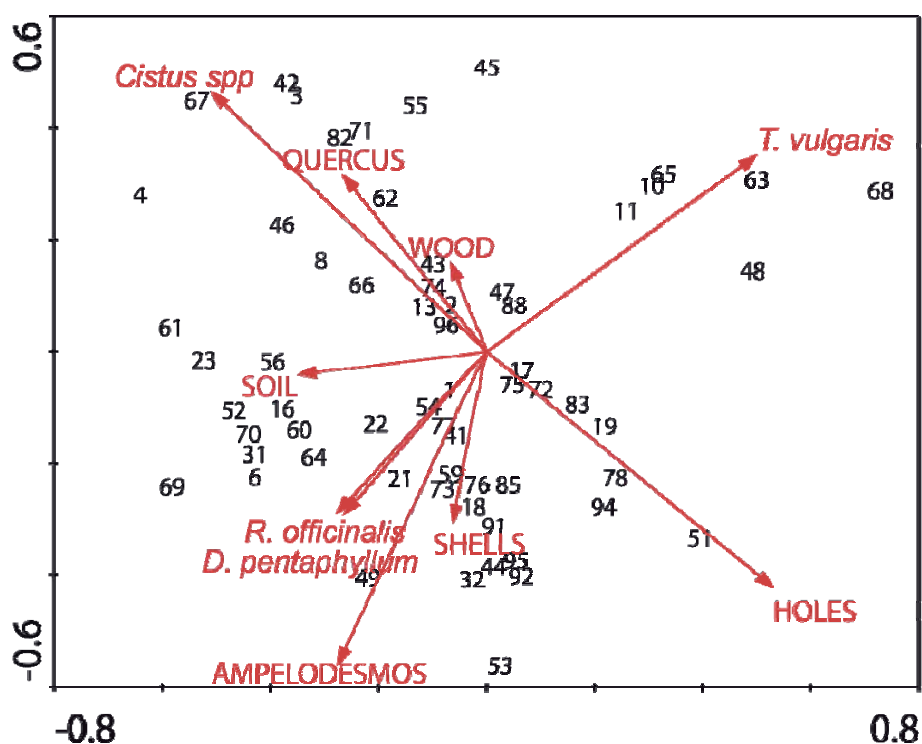
Figure 1.4 Biplot of RDA model relating small bee species (< 55 mg) to flower and nesting resources. Arrows represent resources (flowers in lowercase, nesting substrates in uppercase), and numbers bee species. For species names see Table S1.

Nesting resources show an irregular mosaic distribution across the park. They are not good predictors of bee abundance and richness, and only account for a small part of the explained variance of bee composition. In our community, most species (62%, including 13 of the 19 most abundant) nest underground or are cleptoparasitic on species nesting underground. At the same time, patches of bare soil are abundant and widely distributed across the park,

suggesting that they may not be a limiting resource. Species with more specialized nesting habits may be more conditioned by nesting substrate availability. For example, abundance of *O. rufohirta* was marginally associated to abundance of vacant snail shells (r= 0.41, p= 0.06).

Table 1.2 Cumulative variance explained by RDA models relating flower and nesting resources to bee species composition.

	Axis 1	Axis 2	Axis 3	Axis 4	Total Variance
Small species (n= 62)					
Cumulative percentage of species variance	38.2	46.3	52.0	56.5	
Cumulative percentage of species-environment variance	56.1	67.8	76.2	82.8	
Sum of canonical eigenvalues					0.452
Large species (n= 36)					
Cumulative percentage of species variance	15.3	23.8	31.6	36.8	
Cumulative percentage of species-environment variance	30.7	47.7	63.3	73.8	
Sum of canonical eigenvalues					0.389

Flower resources also show heterogeneity across the park, but in comparison to nesting resources, their distribution shows more of a geographical pattern. Flowers clearly play a greater role than nesting substrates in structuring the spatial distribution of our bee community, accounting for a good part of the explained variance in abundance and composition. This outcome is in agreement with the few studies considering both types of resources (Potts et al. 2003, Roulston and Goodell 2011). It is important to note that these results should not necessarily be interpreted in terms of evolutionary pollen specialization. For instance, abundance of *L. subhirtum*, the most abundant species, was positively correlated to *T. vulgaris* flower density (r= 0.74, p= 0.0001). However, *L. subhirtum* is clearly polylectic (Westrich 1990), and in Garraf we have observed females of this species (n= 45) foraging on 13 plant species belonging to 7 plant families. Other strong associations involving polylectic species include *Lasioglossum albocinctum* with *D. penthaphyllum* (r= 0.63, p= 0.002) and *L. transitorium* with *Cistus* spp. (r= 0.53, p= 0.01). Oligolectic species make up an important fraction of our bee community (21 oligolectic species, 45 polylectic, 13 cleptoparasitic, and 19 unknown), but only one positively known oligolege, the

Asteraceae specialist *Panurgus dentipes*, was among the 19 most abundant species. The remaining oligolectic species were rare, often represented by one or a few individuals, and mostly visiting non-abundant plants in the Asteraceae, Brassicaceae, Ranunculaceae and Boraginaceae. Several studies have found a positive relationship between flower and bee species richness (Potts et al. 2003, Ebeling et al. 2008, Kwaiser and Hendrix 2008, Grundel et al. 2010, Fründ et al. 2010). In Garraf, this relationship was non-significant but, as mentioned, this was caused by a single site (plot 1) displaying a unique bee composition and strongly deviating from the general trend.

Notwithstanding the significant effects of flower resources, as much as 54.8% of the variance in spatial distribution of the Garraf bee community remains unexplained. In addition to resource distribution, community assembly dynamics depend on immigration events and interactions between species. Immigration history (for example, the order of species arrival at a site) may strongly influence the final outcome in terms of species composition (Fukami 2010). Because our plots are located across an area of contiguous habitat it is fair to assume high levels of dispersal among patches, which would tend to homogenize bee distribution. However, immigration events from outside the habitat (Fukami 2010) and local differences in natural mortality factors such as predation and parasitism, as well as competitive interactions among bee species (Roulston and Goodell 2011) may contribute to the maintenance of local differences in community composition. We found a negative association between the two most abundant species, *Lasioglossum subhirtum* and *Andrena djelfensis*, whose flight periods overlap widely, but we do not have the necessary information to establish whether this pattern might be attributed to competition. Another factor that could partially explain the geographical pattern observed is philopatry. The tendency of females to nest at their natal nesting site has been shown in some bee species and could contribute to the increase of local bee density following colonization of a given patch (Yanega 1990, Antonini et al. 2000). Other unmeasured environmental factors such as topoclimatic variation could also contribute to the observed bee composition pattern. Daily maximum and minimum temperatures may vary as much as 8 ºC among microsites distant only few hundred meters from each other (Ackerly et al. 2010). Some studies have found pollinator composition of individual plants to be highly influenced by small-scale variation in microclimatic factors such as solar irradiance, shading and soil wetness (Herrera 1995, Janovský et al. 2013). In addition to trying to elucidate the factors responsible for the unexplained spatial variation observed, it would be important to

establish whether the observed pattern is stable in time. We do not expect nesting substrate availability to vary much from one year to the next, but blooming intensity is well known fluctuate widely from year to year (Ågren 1988, Arroyo 1990, Inouye and McGuire 1991), potentially affecting bee foraging areas.

Our study demonstrates that bee communities may display clear patterns of spatial heterogeneity at a relatively small scale (500-1000 m) in areas of contiguous suitable habitat and in the absence of local barriers. Importantly, the observed heterogeneity is not irregular, but follows a geographical pattern, and is only partly explained by flower availability. This result is remarkable because bees are highly mobile organisms (both in terms of foraging and dispersal), and therefore one might expect a more homogeneous distribution. Because different bee species have different flower preferences and differ in their pollinating abilities, our results have important implications for local pollination dynamics. Several studies have found differences in reproductive success among populations visited by different pollinators (Price et al. 2005, Brunet and Sweet 2006, Gómez et al. 2007). Our study suggests that differences in pollination levels may also occur within a plant population as a result of heterogeneous local pollinator distribution. Our results also have important consequences for the study of spatial variation of plant-pollinator networks (Morales and Vázquez 2008, Janovský et al. 2013), as overall pollinator community composition may be changing at smaller scales than previously thought.

References

Ackerly, D. D. et al. 2010. The geography of climate change: Implications for conservation biogeography. - Divers. Distrib. 16: 476–487.

Ågren, J. 1988. Between-year variation in flowering and fruit set in frost-prone and frost-sheltered populations of dioecious *Rubus chamaemorus*. - Oecologia 76: 175–183.

Antonini, Y. et al. 2000. Philopatry in the Neotropical ground-nesting solitary digger bee, *Diadasina distincta* (Holmberg, 1903) (Hymenoptera: Apidae) at a nesting site in southern Brazil. - Rev. Etol. 2: 111–119.

Arroyo, J. 1990. Ritmos climaticos y de floración en matorrales del SW de España. - Lagascalia 16: 25–50.

Beekman, M. and Ratnieks, F. L. W. 2000. Long-range foraging by the honey-bee, *Apis mellifera* L . - Funct. Ecol. 14: 490 – 496.

Bhattacharya, M. et al. 2002. Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? - Biol. Conserv. 109: 37–45.

Bosch, J. and Vicens, N. 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. - Behav. Ecol. Sociobiol. 60: 26–33.

Brosi, B. J. et al. 2008. The effects of forest fragmentation on bee communities in tropical countryside. - J. Appl. Ecol. 45: 773–783.

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

Carvell, C. et al. 2012. Molecular and spatial analyses reveal links between colony-specific foraging distance and landscape-level resource availability in two bumblebee species. - Oikos 121: 734–742.

Castagneyrol, B. and Jactel, H. 2012. Unraveling plant-animal diversity relationships: A meta-regression analysis. - Ecology 93: 2115–2124.

Ebeling, A. et al. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? - Oikos 117: 1808–1815.

Ebeling, A. et al. 2012. Multitrophic effects of experimental changes in plant diversity on cavity-nesting bees, wasps, and their parasitoids. - Oecologia 169: 453–465.

Eltz, T. et al. 2003. Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests in Sabah, Malaysia, with implications for forest management. - For. Ecol. Manage. 172: 301–313.

Fründ, J. et al. 2010. Pollinator diversity and specialization in relation to flower diversity. - Oikos 119: 1581–1590.

Fukami, T. 2010. Community assembly dynamics in space. - In: Verhoef, H. A. and Morin, P. J. (eds), Community ecology: processes, models, and applications. Oxford University Press, pp. 45–54.

Gathmann, A. and Tscharntke, T. 2002. Foraging ranges of solitary bees. - J. Anim. Ecol. 71: 757–764.

Gathmann, A. et al. 1994. Trap-nesting bees and wasps colonizing set-aside fields: succession and body size, management by cutting and sowing. - Oecologia 98: 8–14.

Gómez, J. M. et al. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. - Oecologia 153: 597–605.

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

Greenleaf, S. S. et al. 2007. Bee foraging ranges and their relationship to body size. - Oecologia 153: 589–96.

Grundel, R. et al. 2010. Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. - Ecol. Appl. 20: 1678–92.

Guédot, C. et al. 2009. Relationship between body size and homing ability in the genus Osmia (Hymenoptera; Megachilidae). - Ecol. Entomol. 34: 158–161.

Herrera, C. M. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. - Biol.J.Linn.Soc 35: 95–125.

Herrera, C. M. 1995. Microclimate and individual variation in pollinators: Flowering plants are more than their flowers. - Ecology 76: 1516–1524.

Holling, C. S. 1992. Cross-scale morphology, geometry, and dynamics of ecosystems. - Ecol. Monogr. 62: 447.

Holyoak, M. et al. 2005. Metacommunities: Spatial dynamics and ecological communities. - Chicago Press.

Inouye, D. W. and McGuire, A. D. 1991. Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change. - Am. J. Bot. 78: 997–1001.

Janovský, Z. et al. 2013. Conspecific and heterospecific plant densities at small-scale can drive plant-pollinator interactions. - PLoS One 8: e77361.

Kwaiser, K. S. and Hendrix, S. D. 2008. Diversity and abundance of bees (Hymenoptera: Apiformes) in native and ruderal grasslands of agriculturally dominated landscapes. - Agric. Ecosyst. Environ. 124: 200–204.

Lepš, J. and Šmilauer, P. 2003. Multivariate Analysis of Ecological Data using CANOCO. - Cambridge University Press.

Minckley, R. L. et al. 1999. Spatial predictability and resource specialization of bees (Hymenoptera: Apoidea) at a superabundant, widespread resource. - Biol. J. Linn. Soc.: 119–147.

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

Müller, A. et al. 2006. Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships. - Biol. Conserv. 130: 604–615.

Murray, T. E. et al. 2012. Local-scale factors structure wild bee communities in protected areas. - J. Appl. Ecol. 49: 998–1008.

Osborne, J. L. et al. 1999. A landscape-scale of bumble bee foraging study range and constancy using harmonic radar. - J. Appl. Ecol. 36: 519–533.

Popic, T. J. et al. 2013. Evaluation of common methods for sampling invertebrate pollinator assemblages: net sampling out-perform pan traps. - PLoS One 8: e66665.

Potts, S. G. et al. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? - Ecology 84: 2628–2642.

Potts, S. G. et al. 2004. Nectar resource diversity organises flower-visitor community structure. - Entomol. Exp. Appl. 113: 103–107.

Potts, S. G. et al. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. - Ecol. Entomol. 30: 78–85.

Price, M. V. et al. 2005. Temporal and spatial variation of a montane herb: a seven-year study. - Ecology 86: 2106–2116.

R Development Core Team 2010. R: A language and environment for statistical computing. in press.

Rangel, T. F. et al. 2010. SAM: a comprehensive application for Spatial Analysis in Macroecology. - Ecography (Cop.). 33: 46–50.

Resetarits, W. J. et al. 2005. Habitat selection, species interactions, and processes of community assembly in complex landscapes. - In: Holyoak, M. et al. (eds), Metacommunities: spatial dynamics and ecological communities. University of Chicago Press, pp. 374–398.

Ricketts, T. H. et al. 2008. Landscape effects on crop pollination services: are there general patterns? - Ecol. Lett. 11: 499–515.

Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. - Science 235: 167–171.

Roulston, T. H. and Goodell, K. 2011. The role of resources and risks in regulating wild bee populations. - Annu. Rev. Entomol. 56: 293–312.

Schaffers, A. P. et al. 2008. Arthropod assemblages are best predicted by plant species composition. - Ecology 89: 782–794.

Smith, A. R. et al. 2012. Pollen use by Megalopta sweat bees in relation to resource availability in a tropical forest. - Ecol. Entomol. 37: 309–317.

Steffan-Dewenter, I. and Westphal, C. 2008. The interplay of pollinator diversity, pollination services and landscape change. - J. Appl. Ecol. 45: 737–741.

Steffan-Dewenter, I. et al. 2002. Scale-dependent effects of landscape context on three pollinator guilds. - Ecology 83: 1421–1432.

Ter Braak, C. J. F. and Šmilauer, P. 2002. Canoco for Windows version 4.5. Biometris - Plant Research Interational.

Venables, W. N. and Ripley, B. D. 1999. Modern applied statistics with S-PLUS. - Springer, New York.

Viana, B. F. et al. 2012. How well do we understand landscape effects on pollinators and pollination services? - J. Pollinat. Ecol. 7: 31–41.

Vicens, N. and Bosch, J. 2000. Nest site orientation and relocation of populations of the orchard pollinator *Osmia cornuta* (Hymenoptera: Megachilidae). - Environ. Entomol. 29: 69–75.

Visscher, P. K. and Seeley, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. - Ecology 63: 1790–1801.

Walther-Hellwig, K. and Frankl, R. 2000. Foraging distances of *Bombus muscorum, Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). - J. Insect Behav. 13: 239–247.

Westphal, C. et al. 2006. Foraging trip duration of bumblebees in relation to landscape-wide resource availability. - Ecol. Entomol. 31: 389–394.

Westphal, C. et al. 2008. Measuring bee diversity in different European habitats and biogeographical regions. - Ecol. Monogr. 78: 653–671.

Westrich, P. 1990. Die Wildbienen Baden-Württembergs: die Gattungen und Arten. - E. Ulmer.

Westrich, P. 1996. Habitat requirements of central European bees and the problems of partial habitats. - In: Matheson, A. et al. (eds), Linnean Society Symposium Series. Vol. 18. Academic Press Limited, pp. 1–16.

Winfree, R. et al. 2009. A meta-analysis of bees' responses to anthropogenic disturbance. - Ecology 90: 2068–2076.

Yanega, D. 1990. Philopatry and nest founding in a primitively social bee, *Halictus rubicundus*. - Behav. Ecol. Sociobiol. 27: 37–42.

Zurbuchen, A. et al. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. - Biol. Conserv. 143: 669–676.

Chapter 2

Collateral effects of beekeeping: impacts on pollen-nectar resources and wild bee communities

Summary Due to the contribution of honey bees (Apis mellifera) to wild flower and crop pollination, beekeeping has traditionally been considered a sustainable practice. However, high honey bee densities may have an impact on local pollen and nectar availablity, which in turn may negatively affect other pollinators. This is exacerbated by the ability of honey bees to recruit foragers to highly rewarding flower patches. We measured floral resource consumption in rosemary (Rosmarinus officinalis) and thyme (Thymus vulgaris) in 21 plots located at different distances from apiaries in the scrubland of Garraf Natural Park (Barcelona), and related these measures to visitation rates of honey bees, bumblebees (Bombus terrestris) and other pollinators. In the same plots, we measured flower density and used pan traps to characterize the wild bee community. Flower resource consumption was largely explained by honey bee visitation and only marginally by bumblebee visitation. After accounting for flower density, plots close to apiaries had lower wild bee biomass. This was due to a lower abundance of large bee species, those more likely to be affected by honey bee competition. We conclude that honey bees are the main contributors to pollen/nectar consumption of the two main flowering plants in the scrubland, and that at the densities currently occurring in the park (3.5 hives / km2) the wild bee community is being affected. Our study supports the hypothesis that high honey bee densities may have an impact on other pollinators via competition for flower resources.

2.1. Introduction

The introduction of large populations of highly competitive species into a new area may affect resident populations ultimately resulting in changes in the structure of native communities (Levine et al. 2003, Ehrenfeld 2010). This may occur when exotic species, introduced either accidentally or intentionally, turn invasive and compete for limited resources with local species occupying a similar niche (Petren and Case 1996, Byers 2000). Paradigmatic examples of exotic animal species outcompeting local species are the Argentine ant (Human and Gordon 1996, Holway 1999) and the Asian carp (Irons et al. 2007, Sampson et al. 2008). In addition to exotic species, domesticated species may also affect resident species. A clear example is the presence of cattle or sheep in natural or semi-natural areas, potentially competing with large herbivores for pasture (Stewart et al. 2002, Young et al. 2005). Moreover, domesticated animals benefit from human assistance, including protection against predators and veterinary care.

Among domesticated animals, the European honey bee (*Apis mellifera*) is undoubtedly one of the most globally spread. Native to Eurasia and Africa it has been introduced into all

continents except Antarctica for the production of honey and other hive products (Crane 1990). Even if recent studies have shown wild pollinators to be more effective pollinators than honey bees (Garibaldi et al. 2013), honey bees are routinely reported to provide an important ecosystem service in terms of wild flower and crop pollination. Thus, beekeeping has traditionally been considered a beneficial practice, and its sustainability has been taken for granted. This is reflected in the current lack of specific legislation in most countries worldwide, whereby beekeeping is considered to be beneficial and is usually allowed in nature reserves and other types of protected areas, including some National Parks. In many cases, beekeeping in these areas is not only allowed but even promoted as a traditional, sustainable activity (information obtained from natural park and wildlife managers from 8 European countries, see acknowledgements). It is therefore not surprising that *A. mellifera* is routinely reported as one of the dominant species in plant-pollinator networks worldwide, even in studies conducted in natural habitats (e.g. Forup et al. 2008, Bosch et al. 2009, Kaiser-Bunbury et al. 2009, Valido et al. 2014; see Davila & Wardle 2008 for a rare exception). However, as in others kinds of animal husbandry, large apiaries resulting in high densities of foragers may have an impact on local food resources (pollen and nectar in this case), which in turn may negatively affect other flower-visiting insects. Honey bees have high energetic requirements because they live in large colonies comprising tens of thousands of individuals and because they maintain elevated hive temperatures even during the winter (Seeley 1985). Due to this high energetic requirements, their foraging ranges span several kilometres (Visscher and Seeley 1982). In addition, honey bees have the ability (unique to them and some stingless bees) to communicate the location of flower resources to nest mates, thus concentrating large numbers of foragers in highly rewarding patches (von Frisch 1967). Thus, honey bees are highly efficient pollen-nectar foragers and, when present in large densities, may potentially create a competition scenario with other pollinators.

Competition may take place through interference or through resource exploitation (Tilman 1982). Interference competition occurs directly between individuals through aggressive encounters (e.g., honey bees chasing other pollinators out of a flower or flower patch). Such aggressive interactions have sometimes been observed (e.g. Pinkus-Rendon et al. 2005), but the fact that most studies do not report aggressive encounters indicates that they are not common (e.g. Roubik 1978, Hudewenz and Klein 2013). After several years of field work, we can assert that such interactions are very rare in our study area. Exploitative competition

occurs indirectly between individuals through a limiting resource such as food or nesting sites. Competition for nesting resources can be ruled out because wild bees in temperate zones do not nest in the kind of large cavities used by honey bees, and because managed feral colonies are very rare in our study area, as in most of Europe (Jaffé et al. 2009). Competition for flower resources is much more likely to occur because honey bees are highly generalist in pollen and nectar use, and their diet widely overlaps with that of other flower-visiting species.

Various studies have explored potential adverse effects of honey bees on local pollinator communities. However, to demonstrate a competition scenario it is extremely difficult, on account of the large foraging ranges of honey bees (several km) (Seeley 1985, Goulson 2003), combined with their ability to communicate the location of rich flower patches, thus allowing colonies to adjust their foraging areas and flower choices as pollen-nectar standing crops vary through time (Visscher and Seeley 1982).

For this reason, most studies have so far focused on indirect evidences of competition between honey bees and wild bees, such as resource overlap (Steffan-Dewenter and Tscharntke 2000), and in changes in pollen-nectar resource use (Forup and Memmott 2005, Valido et al. 2014), foraging activity (Thomson 2004) and visitation rates (Roubik 1978, Hudewenz and Klein 2013) of wild pollinators confronted with different honey bee scenarios. Other studies have measured changes in population abundance and richness of wild bees under different honey bee densities (Roubik 1978, Steffan-Dewenter and Tscharntke 2000, Roubik and Wolda 2001, Forup and Memmott 2005). Fewer studies have looked for more direct evidence of competition, such as changes in reproductive success (Steffan-Dewenter and Tscharntke 2000, Thomson 2004, Goulson and Sparrow 2009, Elbgami et al. 2014), and the outcomes of these studies are not consistent. Some have found negative effects of honey bees (Thomson 2004, Goulson and Sparrow 2009) while others have not (Steffan-Dewenter and Tscharntke 2000, Roubik and Wolda 2001).

For exploitative competition to occur, floral resources should be limiting. Surprisingly, however, no study has hitherto measured the effects of honey bee abundance on pollen and nectar availability. This is important because we currently do not know the magnitude of the impact of honey bees on flower resources compared to resident pollinators. In this study we address the potential effects of beekeeping on wild bee communities in an environmentally

protected natural area. Our objective is to study the impact of honey bee flower visitation on pollen and nectar consumption and the effect of beekeeping on the abundance, richness and composition of the local wild bee community. Because honey bees are very abundant and given their ability to recruit large numbers of foragers to the most rewarding flower patches, we have three hypotheses: (1) Honey bees will be the main contributors to flower resource depletion. We therefore expect pollen and nectar availability to other pollinators to be lower in areas close to apiaries; (2) The structure of the wild bee community will be modified by high honey bee densities. We expect wild bee richness and abundance to be lower close to apiaries; (3) Among wild bees, we expect large species (with higher feeding requirements; Müller et al., 2006), to be most affected.

2.2. Materials and methods

2.2.1. Study area

This study was conducted in the Natural Park of el Garraf (Barcelona, Catalonia, NE Spain), a Mediterranean scrubland dominated by *Quercus coccifera*, *Pistacia lentiscus*, *Rosmarinus officinalis* and *Thymus vulgaris*. The Natural Park of el Garraf is classified as category V of the International Union for Conservation of Nature (IUCN) (Dudley 2008), which includes the majority (62%) of the environmentally protected land in the Mediterranean region (López Ornat et al. 2007). Category V parks are defined as protected areas with an important biological, ecological, cultural and picturesque value based on the interaction between human populations and the environment via traditional management practices. Thus, the protection of such people-nature interactions is the main conservation objective (Dudley 2008). In Catalonia, current policies regulating environmental impacts of human activities do not mention beekeeping (Law 20/2009, DOGC 5524). Rather, beekeeping is considered an innocuous activity and *A. melifera* is declared a "species of special interest" (Decree 110/2003, DOGC 3870).

Our study area is entirely located in the park, encompassing a surface of 32 km². We selected 21 plots of 40 m x 40 m distributed regularly across the study area. Distances between nearest plots ranged from 585 to 1354 m. Based on the information provided by the Autonomous Government and subsequently verified *in situ*, we pinpointed 21 apiaries close

to the study area for a total of 475 hives. Minimum distance of our plots to the nearest apiary ranged from 262 m to 5122 m.

2.2.2. Flower resource surveys

To study flower resource consumption, we worked on rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*). These two species are, by far, the most abundant entomophilous species in the study area, producing 70-90% of the flowers in the scrubland (Bosch et al. submitted, Flo et al. submitted; and unpublished data from 12 sites within the park). In addition, the two species are very attractive to honey bees and are considered highly desirable for honey production (Cambra 2008). All surveys were conducted in 2011 under fair weather.

Pollen

Rosemary pollen surveys were conducted in March, when the species was in full bloom. In each plot, we selected between 20 and 30 plants on which we marked 8 recently-opened flowers distributed randomly within the plant (with fresh, fully pollen-loaded stamens). Before the onset of pollinator activity (9:00), we collected 4 marked flowers per plant, and stored them together in a vial filled with ethanol 70%. After 18:00, when foraging activity had ceased, the remaining 4 flowers were collected and preserved following the same procedure.

Thyme pollen surveys were conducted in April, during peak bloom of this species. We selected between 20 and 30 thyme plants per plot and marked 4 recently-opened flowers in each of them, following the same criteria as for rosemary. Before 9:00 we collected the two stamens of one side (left or right) of each flower and stored them together in a vial filled with ethanol 70%. After 18:00 we collected the two remaining stamens of each flower.

In the laboratory, vials with stamens were sonicated for 10 minutes in an ultrasonic bath to dislodge pollen grains from the anthers. Afterwards, each anther was inspected under the stereomicroscope and pollen grains still adhering to the anthers were manually detached

with the aid of an insect pin. Later, we took 8 drops of 2.5 µl of the resulting pollen suspension and counted the number of pollen grains under a stereomicroscope. Previous trials showed that the number of drops necessary to stabilize pollen counts was 6. We then measured the remaining ethanol volume in the vial, and estimated the total number of pollen grains in each sample. From these data, we estimated the number of pollen grains per flower in the morning and in the evening, which we used to calculate pollen consumption. Overall, we sampled 4005 rosemary flowers and 2366 thyme flowers.

Nectar

Nectar consumption is difficult to measure because nectar secretion is a more or less continuous process (Pacini et al. 2003), so that consumption may be compensated by subsequent secretion. In some cases, secretion may be even stimulated by consumption (Castellanos et al. 2002, Ordano and Ornelas 2004). In addition, nectar secretion can be conditioned by weather conditions (Jakobsen and Kristjánsson 1994, Petanidou and Smets 1996). We therefore decided to measure nectar standing crops at the end of the day as a surrogate for nectar consumption.

Thyme flowers produce very small amounts of nectar (Arnan et al. 2014), which may become difficult to extract, especially in warm dry days. For this reason, nectar surveys were only conducted on rosemary. At the end of each sampling day, we used 1-µl capillary tubes to measure the volume of nectar remaining in the flowers. This was done on most of the flowers used in the evening pollen surveys. We measured nectar standing crops in 1628 rosemary flowers.

2.2.3. Pollinator visitation rates

To relate pollen and nectar consumption to pollinator activity, we conducted pollinator surveys between 9:00 and 18:00 in each plot on the same day in which pollen and nectar measures were taken. At each plot, we selected 10 rosemary and 10 thyme plants. These plants were not the same used in pollen/nectar surveys to avoid potential accidental contact with flowers marked for pollen-nectar measures. On each marked plant we conducted a

number of pollinator counts (mean= 10, range= 5-15) throughout the day. In each count the selected plant was observed for 2 minutes and all pollinators contacting flowers were recorded. Total observation time was 72 h 48 min for rosemary and 76 h 34 min for thyme. At the end of the day, we counted the number of open flowers in each plant. *Apis mellifera* and the bumblebee *Bombus terrestris* were, by far, the two most frequent species visiting the two plant species. Therefore, we grouped pollinators into three categories: *A. mellifera*, *B. terrestris*, and other pollinators (mostly other bees, along with some dipterans and a few lepidopterans and coleopterans). Visitation rates of each pollinator group were calculated as the number of contacts per minute and per 1000 flowers.

2.2.4. Bee community

To assess bee community structure and composition, we placed 6 sampling stations in each plot. Each sampling station consisted of a metal bar holding 3 pan traps painted yellow, white and blue respectively, one meter above the ground (Westphal et al. 2008). We conducted 8 biweekly surveys from mid March to late June 2010, in which traps were set up before 9:30 and collected after 18:00. All plots were sampled on the same 8 days (see Torné-Noguera et al. (2014) for details). We captured 6580 bee specimens, which were dried and pinned for identification. In addition, we netted and weighed a few individuals of each species/sex to obtain measures of fresh body weight (n=1-52 specimens per species). Species were subsequently classed as small (body weight <55 mg) or large (>70 mg) (see Torné-Noguera et al. (2014) for a more detailed explanation).

2.2.5. Flower abundance

To estimate flower abundance in each plot, we considered the main flowering species in the scrubland (*R. officinalis*, *T. vulgaris*, *Dorycnium pentaphyllum*, *Cistus albidus*, *Cistus salvifolius* and *Cistus monspeliensis*), which together account for >70% of the flowers in each plot. We measured two perpendicular widths and the height of each flower patch in two 40 x 1 m perpendicular transects centered in the middle of the plot. Then we estimated the number of flowers of each species based on previously established equations relating patch volume

and number of open flowers at peak bloom (r^2=0.36–0.63, p=0.001–0.015) (see Torné-Noguera et al., 2014). Because the three *Cistus* species were much less abundant than the other species, and their flowering periods largely overlap, we lumped together these three species into a single category (*Cistus* abundance).

2.2.6. Statistical analysis

Visitation rates and pollen/nectar consumption

Preliminary analyses showed no correlation between explanatory variables (visitation rates of the different pollinator groups). In pollen analysis, honey bee visitation rate and other pollinators visitation rate were log-transformed because there was a logarithmic relationship between these variables and pollen consumption. We initially fit a generalized linear model (GLM) assuming a binomial error distribution (adequate for proportion data such as pollen consumption), with *A. mellifera* visitation rate, *Bombus spp.* visitation rate and other pollinators visitation rate as predictive variables. However, the model showed overdispersion. Therefore, we finally opted for a quasibinomial GLM. We then compared the saturated model with the various non-saturated models and chose the best one using ANOVA (as AIC cannot be calculated for quasi model families). Finally, we checked for normality and homoscedasticity of the residuals. We use pseudo-R^2 as a measure of the goodness-of-fit.

In nectar analyses, we log-transformed the explanatory variable "other pollinators visitation rate" because it showed a logarithmic relationship with the response variable. We fit a generalized linear model (GLM) with a Gaussian error distribution, with nectar standing crop as the response variable and visitation rate of the various pollinator groups as predictive variables. We selected the best model using the second-order Akaike's Information Criterion (AICc), adequate for small samples.

Bee community

We used MiraMon software (Pons 2014) to establish the linear distance of each plot to the nearest apiary in the area. This measure, which is commonly used in honey bee studies (Steffan-Dewenter and Tscharntke 2000, Thomson 2004, Hudewenz and Klein 2013, Elbgami et al. 2014), was negatively correlated to honey bee visitation rates (r^2= 42.25, p=0.009), and to honey bee abundance in the pan traps (logarithmic relationship, r^2= 49.73, p=0.0004).

Figure 2.1 Partial regression plots showing the contribution of honey bee and bumblebee visitation rates to rosemary and thyme pollen consumption in 21 plots, once the effect of other explanatory variables entering the GLMs has been removed (bumblebee visitation rates in (A) and (C); honey bee visitation rates in (B) and (D)).

To evaluate the potential relationship between distance to the nearest apiary and wild bee community structure, we run GLM models for wild bee abundance, wild bee richness and

wild bee biomass. Because wild bee community structure may also be influenced by flower availability (Torné-Noguera et al. 2014), we included flower abundance of *T. vulgaris*, *R. officinalis*, *D. pentaphyllum* and *Cistus* as predictor variables. We did not include nesting substrate availability in the analysis because we know from previous studies that this is not a good predictor of bee community structure and composition in the study area (Torné-Noguera et al. 2014).

Bee biomass was analyzed with a GLM with a Gaussian distribution. For bee abundance and bee richness models, we chose a GLM with a Poisson error distribution, adequate for count data. However, both models showed overdispersion, and thus we opted for models with a negative binomial distribution. In all three analyses, we selected the best model with the AICc criterion. Best models were later checked for normality and homoscedasticity. Because large bees might respond differently from small bees due to their higher feeding requirements (Müller et al. 2006), we run additional analyses separately for small (<55 mg) and large (>70 mg) bees. The best model explaining wild bee richness showed heteroscedasticity. Thus, we used White's heteroscedasticity-corrected covariance matrices to make inference.

All analyses were computed with R (R Core Team 2014).

2.3. Results

2.3.1. Pollen and nectar consumption

Apis mellifera and *B. terrestris* accounted for the majority of visits to both rosemary (61.2 and 30.1%, respectively) and thyme (39.5 and 34.8%). Visits of other pollinators amounted to 8.7 and 25.7% of the visits to rosemary and thyme, respectively. Honey bee flower visits and bumblebee flower visits were not correlated (rosemary: r^2= 0.04, p= 0.27; thyme: r^2= 0.05, p= 0.19).

Mean ± SD number of pollen grains in newly-opened rosemary flowers was 5185 ± 1559, and these numbers decreased to 1831 ± 1517 by the end of the day. Pollen consumption in our plots ranged from 25.1% to 90.1% (mean ± SD = 65.6 ± 18.4). The best model for rosemary pollen consumption (pseudo-R^2= 0.54) included *A. mellifera* visitation rate

(p=0.004) and, marginally, *B. terrestris* visitation rate (p=0.06) (Fig. 2.1). The model including only *A. mellifera* visitation rate (p=0.001; pseudo-R^2= 0.44) was only marginally different from the saturated model (F= 4.19, p= 0.06).
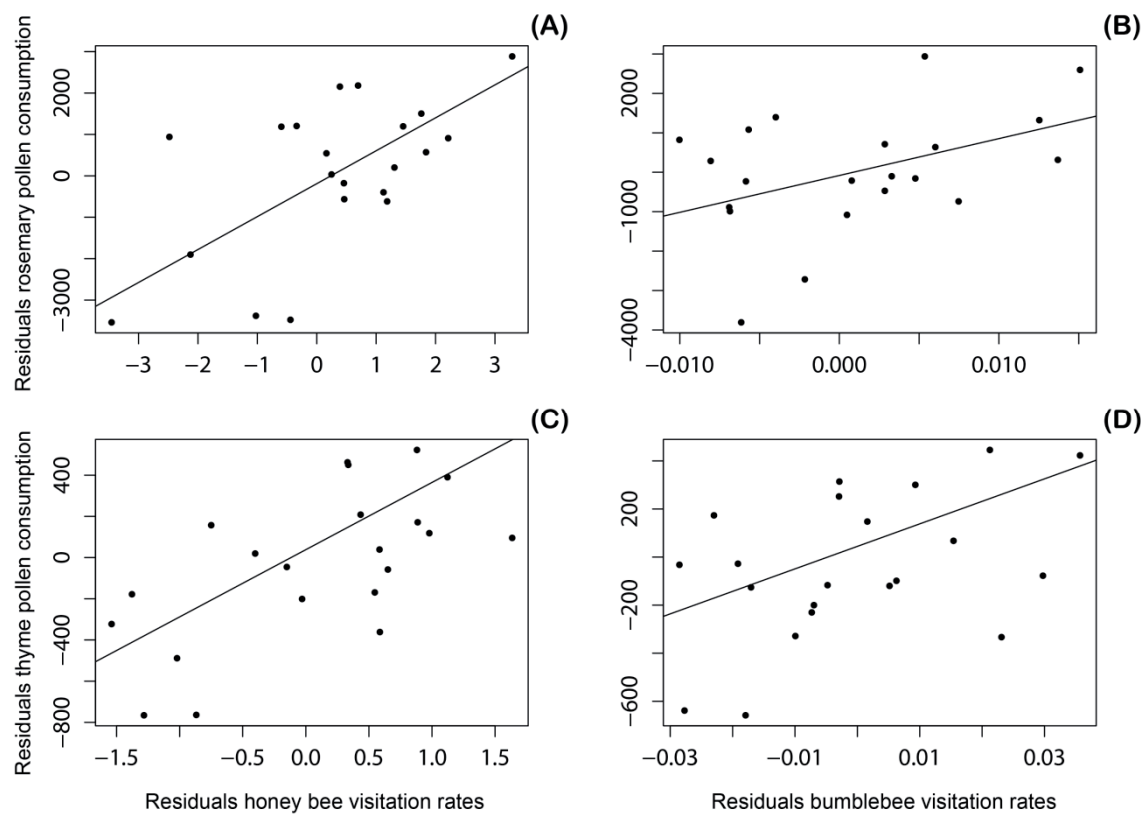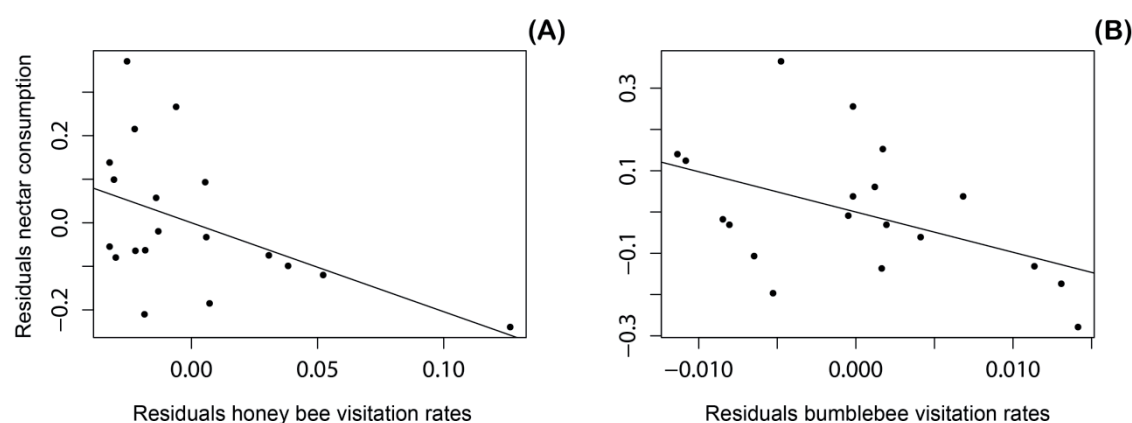

Figure 2.2 Partial regression plots showing the contribution of honey bee and bumblebee visitation rates to rosemary nectar standing crops in 21 plots, once the effect of other explanatory variables entering the GLMs has been removed (bumblebee visitation rates in (A); honey bee visitation rates in (B)).

Thyme flowers contained 1220 ± 737 pollen grains in the morning and 577 ± 439 at the end of the day. Thyme pollen consumption in the various plots ranged between 19.2% and 76.5% (mean ± SD = 54.3 ± 15.9). The best model for thyme pollen consumption (pseudo-R^2= 0.42) included *A. mellifera* visitation rate (p=0.002) and *B. terrestris* visitation rate (p=0.04) (Fig. 2.1).

Rosemary nectar standing crops in the 21 plots ranged from 0 to 6.31 μL/flower (0.26 ± 0.39). The best model explaining rosemary nectar levels (r^2= 0.42) included *A. mellifera* visitation rate (p=0.04) and, marginally, *B. terrestris* visitation rate (p=0.05) (Fig. 2.2). As with rosemary pollen consumption, the model including only *A. mellifera* visitation rate (p=0.027, r^2= 0.26) was only marginally less explicative than the saturated model (F= 4.19, p= 0.06).

2.3.2. Bee community

Pan trap surveys yielded 6580 bee specimens corresponding to 98 species. Sixty-tree of the non-*Apis* species were small (fresh body weight <55mg) and 34 were large (>70mg).
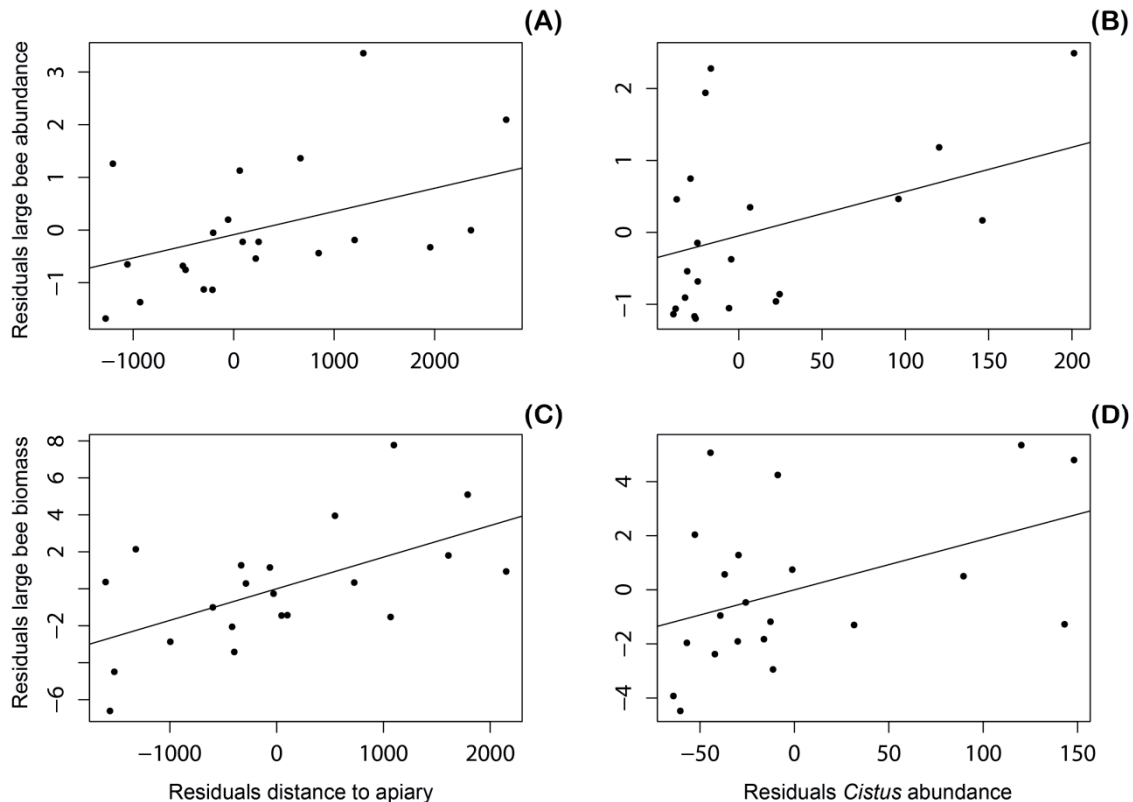
Figure 2.3 Partial regression plots showing the relationship between distance to the nearest apiary and large bee abundance and biomass in 21 plots, once the effect of other explanatory variables entering the GLMs has been removed (*Cistus* flower abundance in (A) and (C); distance to the nearest apiary in (B) and (D)).

No variables entered the model to explain wild bee richness (Table 2.1), and similar results were obtained when small and large bees were analyzed separately (Table 2.1). The best model for bee abundance (pseudo-R^2= 0.48) included *Cistus* flower abundance (p= 0.002) and *T. vulgaris* flower abundance (p= 0.004). Similar results were obtained when only small bees were taken into account (pseudo-R^2= 0.41; *Cistus* abundance (p= 0.008); *T. vulgaris*

abundance (p= 0.03)). Instead, the best fit model for large bees (pseudo-R^2= 0.50) included distance to the nearest apiary (p= 0.02) and, marginally, *Cistus* abundance (p= 0.06) (Fig. 2.3; Table 2.1). To be conservative, we re-run the latter analysis without 3 possible Leverage points (Cook's D = 0.5 to 1), and obtained similar results with a lower goodness-of-fit (pseudo-R^2= 0.28, distance to apiary p= 0.02, *Cistus* abundance p= 0.06). The best wild bee biomass model (pseudo-R^2= 0.56) included *Cistus* flower abundance (p= 0.002) along with distance to the nearest apiary (p= 0.02). The best model for small bees (pseudo-R^2= 0.27) included only *Cistus* abundance (p= 0.02) (Table 2.1). Conversely, the best model for large bees (pseudo-R^2= 0.54) included distance to the nearest apiary (p= 0.007) and, marginally, *Cistus* abundance (p= 0.06) (Fig. 2.3).

2.4. Discussion

Honeybees outnumbered the most frequent wild bee (the bumblebee *B. terrestris*) on rosemary and thyme flowers, the two main flowering plants in the study area. All workers of these two bee species collected nectar, and some of them also collected pollen. Our results demonstrate that honey bees were, by far, the main species contributing to pollen and nectar consumption. The contribution of *B. terrestris* was much lower, and other pollinators played a non-detectable role in flower resource consumption. Mean pollen consumption per plot was slightly higher for rosemary (mean= 65.6%, range= 25.1 - 90.1%) than for thyme (mean= 54.3%, range= 19.2 - 76.5%), but to a greater or lesser extent, all plots had considerable amounts of pollen and nectar available at the end of the day. This may suggest that flower resources were not a limiting factor for the bee community. However, the energetic gain obtained from flowers with pollen-nectar levels below a certain threshold may be insufficient to compensate foraging costs, especially for large bees, with higher energetic demands (Heinrich 1975). Bees have been shown to move away from less rewarding patches (Heinrich 1979). Our pollen-nectar surveys were conducted during peak bloom of the two main flower species in the study area. By the end of April, flower resources become much scarcer in the Park, and overall visitation rates are much higher (Bosch et al. 2009, Filella et al. 2013, Flo et al. submitted). Consequently, we expect the potential effects of intensive honey bee foraging to be greater late in late-spring and summer.

Table 2.1 Results of GLMs analyzing wild bee richness, abundance and biomass in 21 plots as a function of distance to the nearest apiary, and abundance of *Cistus*, *Thymus vulgaris*, *Rosmarinus officinalis* and *Dorycnium pentaphyllum* flowers. P-values are only given for variables entering the models. Pseudo-R^2 is provided for each model as a measure of goodness-of-fit.

Response variable		Explanatory variables					Pseudo-R^2
		Distance to apiary	*Cistus*	*T. vulgaris*	*R. officinalis*	*D. pentaphyllum*	
Wild bee richness	Large species[1]	ns	ns	ns	ns	ns	--
	Small species[2]	ns	ns	ns	ns	ns	--
	All species	ns	ns	ns	ns	ns	--
Wild bee abundance	Large species[1]	p=0.019	p= 0.061	ns	ns	ns	0.50
	Small species[2]	ns	p= 0.008	p= 0.030	ns	ns	0.41
	All species	ns	p= 0.002	p= 0.042	ns	ns	0.48
Wild bee biomass	Large species[1]	p= 0.007	p= 0.059	ns	ns	ns	0.54
	Small species[2]	ns	p= 0.016	ns	ns	ns	0.27
	All species	p= 0.017	p= 0.016	ns	ns	ns	0.56

[1] Body weight >70 mg: [2] Body weight <55 mg.

Our study also shows that wild bee community is affected and modified in areas close to apiaries, with a lower overall wild bee biomass mediated by a lower abundance of large bees. Small bees require less energy to fly and sustain foraging and nesting activities (Heinrich 1975). In addition, small bees require smaller pollen/nectar amounts to produce an offspring (Müller et al. 2006). Thus, pollen and nectar standing crops in areas close to the apiaries may be sufficient for small bees but not for large bees. If so, large bees may be forced to nest somewhere else or widen their foraging ranges, which are well known to be positively related to body size (Gathmann and Tscharntke 2002, Greenleaf et al. 2007). As for small bees, even if their abundance did not diminish close to apiaries, their fitness might still be affected by the lower pollen/nectar standing crops. At the intra-specific level, bee adult body size is directly related to the amount of pollen-nectar consumed by the larva (Ribeiro 1994, Bosch and Vicens 2002), and some studies have shown reductions in offspring body size in populations flying in areas with low levels of flower resources (Peterson and Roitberg 2006, Bosch 2008). Offspring allocated smaller pollen/nectar provisions are more likely to die during development (Bosch 2008) and during wintering (Tepedino and Torchio 1982, Bosch and Kemp 2004). Smaller females are also less likely to found a nest (Tepedino and Torchio 1982, Bosch and Vicens 2006). Other studies have found that bumblebee colonies produce smaller workers in areas with managed honey bees, probably due to pollen/nectar scarcity (Goulson and Sparrow 2009, Elbgami et al. 2014). Wild bees foraging in areas with low levels of resources may be forced to make longer foraging trips to gather a pollen/nectar load. In solitary bees, nests are left unguarded when the nesting female is foraging and long foraging trips (Seidelmann 2006) and low resource levels (Goodell 2003) have been shown to result in increased cleptoparasitism in solitary bees. Previous studies investigating the potential impact of honey bees on wild bee communities have also found wild bee abundance to be lower near apiaries (Forup and Memmott 2005, Thomson 2006), but others have not (Steffan-Dewenter and Tscharntke 2000, Roubik and Wolda 2001). On the other hand, and in agreement with other studies (Steffan-Dewenter and Tscharntke 2000, Roubik and Wolda 2001, Forup and Memmott 2005), bee richness was not influenced by proximity to apiaries in our study.

In addition to honey bee density, bee abundance and biomass may also be influenced by flower abundance and distribution. Our models show that *Cistus* flowers have an important role structuring the Garraf bee community. The three *Cistus* species occurring in the park bloom in April, at a time when wild bee abundance and diversity are high, and flower

resources show a strong decline after the blooming period of *R. officinalis* and *T. vulgaris* (Bosch et al. 2009, Filella et al. 2013, Flo et al. submitted). Other plants blooming at this time are either very scarce (*Gladiolus illyricus*, *Orobanche latisquama*), or produce smaller amounts of pollen and nectar (*D. penthaphyllum*) (Flo et al. submitted). Previous studies in the same area have shown that *C. albidus* and *C. salvifolius* constitute a hub in the Garraf pollination network, attracting higher numbers of pollinator species and receiving higher flower visitation rates than any other plant species (Bosch et al. 2009, and submitted).

Our study provides evidence to support the hypothesis that high densities of managed honey bees have a negative impact on wild bee communities. Our results point to pollen-nectar depletion as a mechanism explaining this negative impact. To our knowledge, this is the first time flower resource consumption has been measured in studies exploring the potential effects of managed honey bees on wild pollinators. To confirm or refute this hypothesis, future studies should include long-term monitoring of wild bee populations and direct measures of fitness. From an applied perspective, decisions on the number of hives allowed in an environmentally protected area should be based on the carrying capacity of the flower community at the landscape level. However, to provide a range of appropriate hive densities is extremely difficult for several reasons. First, even in a natural habitat such as the Garraf Natural Park, flower spatial distribution is far from homogeneous (Torné-Noguera et al. 2014). Second, availability of flower resources changes dramatically throughout the season and from year to year (Flo et al. submitted). Third, foraging ranges of honey bees span several kilometers (Visscher and Seeley 1982). Fourth, resource depletion may also depend on the abundance of resident pollinator populations. Nonetheless, our study suggests that at densities over 3.5 hives per km^2 (475 hives / 134 km^2), wild bee communities are likely to be affected in our study area.

Epilogue

The Garraf Natural Park is partially located in the municipality of Olivella. In May 2012, the city council discussed a petition to install 357 new honey bee hives in the Park. The council examined a report commissioned by the board of directors of the Park cautioning about the potential effects of intensive beekeeping on other pollinators. The council finally approved

the installation of the 357 hives based on current legislation considering beekeeping an "innocuous activity".

Aknowledgements

References

Arnan, X. et al. 2014. Female reproductive success in gynodioecious *Thymus vulgaris*: Pollen versus nutrient limitation and pollinator foraging behaviour. - Bot. J. Linn. Soc. 175: 395–408.

Bosch, J. 2008. Production of undersized offspring in a solitary bee. - Anim. Behav. 75: 809–816.

Bosch, J. and Vicens, N. 2002. Body size as an estimator of production costs in a solitary bee. - Ecol. Entomol. 27: 129–137.

Bosch, J. and Kemp, W. P. 2004. Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). - Apidologie 35: 469–479.

Bosch, J. and Vicens, N. 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. - Behav. Ecol. Sociobiol. 60: 26–33.

Bosch, J. et al. 2009. Plant-pollinator networks: adding the pollinator's perspective. - Ecol. Lett. 12: 409–19.

Byers, J. E. 2000. Competition between two estuarine snails: implications for invasions of exotic species. - Ecology 81: 1225–1239.

Cambra, J. 2008. Flora mel·lífera de Catalunya. - In: Apicultura i producció de mel a Catalunya. Dossier tècnic. Generalitat de Catalunya. Departament d'Agricultura, Alimentació i Acció Rural, pp. 20.

Castellanos, M. C. et al. 2002. Dynamic nectar replenishment in flowers of Penstemon (Scrophulariaceae). - Am. J. Bot. 89: 111–118.

Crane, E. 1990. Bees and beekeeping: science, practice and world resources. - Heinemann Newnes.

Davila, Y. C. and Wardle, G. M. 2008. Variation in native pollinators in the absence of honeybees: implications for reproductive success of an Australian generalist-pollinated herb *Trachymene incisa* (Apiaceae). - Bot. J. Linn. Soc. 156: 479–490.

Dudley, N. 2008. Guidelines for applying protected area management categories (N Dudley, Ed.). - IUCN.

Ehrenfeld, J. G. 2010. Ecosystem consequences of biological invasions. - Annu. Rev. Ecol. Evol. Syst. 41: 59–80.

Elbgami, T. et al. 2014. The effect of proximity to a honeybee apiary on bumblebee colony fitness, development, and performance. - Apidologie 45: 504–513.

Filella, I. et al. 2013. Floral advertisement scent in a changing plant-pollinators market. - Sci. Rep. 3: 3434.

Forup, M. L. and Memmott, J. 2005. The relationship between the abundances of bumblebees and honeybees in a native habitat. - Ecol. Entomol. 30: 47–57.

Forup, M. L. et al. 2008. The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. - J. Appl. Ecol. 45: 742–752.

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Garibaldi, L. A. et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. - Science 339: 1608–11.

Gathmann, A. and Tscharntke, T. 2002. Foraging ranges of solitary bees. - J. Anim. Ecol. 71: 757–764.

Goodell, K. 2003. Food availability affects *Osmia pumila* (Hymenoptera: Megachilidae) foraging, reproduction, and brood parasitism. - Oecologia 134: 518–27.

Goulson, D. 2003. Effects of introduced bees on native ecosystems. - Annu. Rev. Ecol. Evol. Syst. 34: 1–26.

Goulson, D. and Sparrow, K. R. 2009. Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. - J. Insect Conserv. 13: 177–181.

Greenleaf, S. S. et al. 2007. Bee foraging ranges and their relationship to body size. - Oecologia 153: 589–96.

Heinrich, B. 1975. Energetics of pollination. - Annu. Rev. Ecol. Syst. 6: 139–170.

Heinrich, B. 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. - Oecologia 40: 235–245.

Holway, D. A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. - Ecology 80: 238–251.

Hudewenz, A. and Klein, A.-M. 2013. Competition between honey bees and wild bees and the role of nesting resources in a nature reserve. - J. Insect Conserv. 17: 1275–1283.

Human, K. G. and Gordon, D. M. 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. - Oecologia 105: 405–412.

Irons, K. S. et al. 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? - J. Fish Biol. 71: 258–273.

Jaffé, R. et al. 2009. Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. - Conserv. Biol. 24: 583–93.

Jakobsen, H. B. and Kristjánsson, K. K. 1994. Influence of temperature and floret age on nectar secretion in *Trifolium repens* L. - Ann. Bot. 74: 327–334.

Kaiser-Bunbury, C. N. et al. 2009. Community structure of pollination webs of Mauritian heathland habitats. - Perspect. Plant Ecol. Evol. Syst. 11: 241–254.

Levine, J. M. et al. 2003. Mechanisms underlying the impacts of exotic plant invasions. - Proc. Biol. Sci. 270: 775–81.

López Ornat, A. et al. 2007. Use of IUCN protected areas management categories in the Mediterranean region (A López Ornat, A Pons Reynés, and M Noguera, Eds.). - Consejería de Medio Ambiente of Junta de Andalucía, Sevilla, Spain and IUCN, Gland, Switzerland and Malaga, Spain.

Müller, A. et al. 2006. Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships. - Biol. Conserv. 130: 604–615.

Ordano, M. and Ornelas, J. F. 2004. Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. - Oecologia 140: 495–505.

Pacini, E. et al. 2003. Nectar biodiversity: A short review. - Plant Syst. Evol. 238: 7–21.

Petanidou, T. and Smets, E. 1996. Does temperature stress induce nectar secretion in Mediterranean plants? - New Phytol. 133: 513–518.

Peterson, J. H. and Roitberg, B. D. 2006. Impacts of flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotundata*. - Behav. Ecol. Sociobiol. 59: 589–596.

Petren, K. and Case, T. J. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. - Ecology 77: 118–132.

Pinkus-Rendon, M. A. et al. 2005. Floral resource use and interactions between *Apis mellifera* and native bees in cucurbit crops in Yucatán, México. - Can. Entomol. 137: 441–449.

Pons, X. 2014. MiraMon. Geographic Information System and Remote Sensing Software. - Geogr. Inf. Syst. Remote Sens. Softw.

R Core Team 2014. R: A language and environment for statistical computing.

Ribeiro, M. F. 1994. Growth in bumble bee larvae: relation between development time, mass, and amount of pollen ingested. - Can. J. Zool. 72: 1978–1985.

Roubik, D. W. 1978. Competitive interactions between neotropical pollinators and africanized honey bees. - Science 201: 1030–1032.

Roubik, D. W. and Wolda, H. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. - Popul. Ecol. 43: 53–62.

Sampson, S. J. et al. 2008. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. - Biol. Invasions 11: 483–496.

Seeley, T. D. 1985. Honeybee ecology: a study of adaptation in social life. - Princeton University Press.

Seidelmann, K. 2006. Open-cell parasitism shapes maternal investment patterns in the Red Mason bee *Osmia rufa*. - Behav. Ecol. 17: 839–848.

Steffan-Dewenter, I. and Tscharntke, T. 2000. Resource overlap and possible competition between honey bees and wild bees in central Europe. - Oecologia 122: 288–296.

Stewart, K. M. et al. 2002. Temporospatial distributions of elk, mule deer, and cattle: resource partitioning and competitive displacement. - J. Mammal. 83: 229–244.

Tepedino, V. J. and Torchio, P. F. 1982. Phenotypic variability in nesting success among *Osmia lignaria* propinqua females in a glasshouse environment: (Hymenoptera: Megachilidae). - Ecol. Entomol. 7: 453–462.

Thomson, D. M. 2004. Competitive interactions between the invasive European honey bee and native bumble bees. - Ecology 85: 458–470.

Thomson, D. M. 2006. Detecting the effects of introduced species: a case study of competition between Apis and Bombus. - Oikos 114: 407–418.

Tilman, D. 1982. Resource competition and community structure. - Princeton University Press.

Torné-Noguera, A. et al. 2014. Determinants of spatial distribution in a bee community: nesting resources, flower resources, and body size. - PLoS One 9: e97255.

Valido, A. et al. 2014. Impacto de la introducción de la abeja doméstica (*Apis mellifera*, Apidae) en el Parque Nacional del Teide (Tenerife, Islas Canarias). - Ecosistemas 23: 58–66.

Visscher, P. K. and Seeley, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. - Ecology 63: 1790–1801.

Von Frisch, K. 1967. The dance language and orientation of bees. - Harvard University Press. Cambridge, MA.

Westphal, C. et al. 2008. Measuring bee diversity in different European habitats and biogeographical regions. - Ecol. Monogr. 78: 653–671.

Young, T. P. et al. 2005. Competition and compensation among cattle, zebras, and elephants in a semi-arid savanna in Laikipia, Kenya. - Biol. Conserv. 122: 351–359.

Chapter 3

Within habitat β-diversity in a host-parasitoid interaction network

Summary Species assemblages and their interactions vary through space, generating diversity patterns at different spatial scales. In ecology, β-diversity is a common measure of species turnover between localities, but it has rarely been used to explore interaction turnover. Recent development of β-diversity indexes provides a new framework to analyze spatial variation of ecological networks and to identify the sources of this variation. Here, we study the local-scale spatial variation of a cavity-nesting bee and wasp community (hosts), their nest associates (parasitoids), and the resulting antagonistic network over a homogeneous habitat. To obtain bee/wasp nests we placed nest-traps at 25 sites over a 32 km2 area. Sites were separated by 500-1000 m. Hosts and their parasitoids were reared in the laboratory. We obtained 1541 nests (4954 cells) belonging to 41 host species and containing 26 parasitoid species. The most abundant host species tended to have higher parasitism rate. β-diversity was high for both hosts and parasites, and the main driver of variability was species turnover, with a very minor contribution of ordered species loss (nestedness). That is, local species richness tended to be similar across the study area community composition tended to differ between sites. Interaction β-diversity was also high, and mostly due to species turnover with a low contribution of rewiring (same species interacting differently at different sites). In sum, although species composition was rather idiosyncratic to each site, species interacted similarly across different sites. Host β-diversity increased with geographical distance, but parasitoid and interaction β-diversity did not. Our results additionally indicate that interaction β-diversity is better explained by host β-diversity than by parasitoid β-diversity, probably due to the higher range of host β-diversity. We discuss the importance of identifying the sources of variation to understand the drivers of the observed heterogeneity. Because communities and their interactions may vary at a very local scale (this study), we also emphasize the need to sample a sufficiently large number of sites regardless of the cause of the observed variation.

3.1. Introduction

Diversity patterns we can currently observe in nature are the outcome of multiple biotic and abiotic factors, and a reflection of the interactions between them. To understand such patterns and their underlying processes is of major importance in community ecology. For many decades, ecologists have studied species diversity at scales ranging from several meters to many kilometers. Whittaker (1960) was the first to propose a partitioning of diversity across 3 different spatial scales: α-diversity, which measures the relationship between the number of species and individuals (i.e. diversity) in a particular locality (Fisher et al. 1943), γ-diversity, a measure of regional diversity, and β-diversity, a measure of diversity turnover between localities. β-diversity provides a measure of community spatial

variability, reflecting historical processes and revealing information on population dynamics and species responses to habitat modifications such as environmental gradients and perturbations. For this reason, β-diversity has become a relevant measure in biological conservation (Condit et al. 2002). β-diversity can be partitioned into two additive components: spatial turnover, that is, dissimilarity due to species replacement, and nestedness of assemblages, that is, dissimilarity due to ordered species loss (Baselga 2010). Knowing the relative importance of these two components is essential to understand the causes of the observed spatial variability.

Because species are not isolated but immersed in complex networks connecting them directly and indirectly with other species, spatial heterogeneity in species community structure is expected to profoundly affect network structure (Olesen and Jordano 2002, Vázquez et al. 2009b). Recently, some studies have either theoretically or empirically addressed such relationship (Burkle and Alarcón 2011). For example, spatial aggregation and identity of plants, together with animal mobility, has been shown to have a strong influence on identity, strength and distribution of interactions in plant-animal mutualistic networks (Morales and Vázquez 2008). It has also been demonstrated that network structural patterns are largely shaped by relative species abundance and spatiotemporal patterns of interacting species (Vázquez et al. 2009a). Finally, even if decay of similarity in species composition with distance has been well established (Nekola and White 1999, Soininen et al. 2007), little is known about decay of similarity of interactions between species, and how the two decay patterns are related. Because interactions are influenced by the variability of the two trophic levels plus their inherent variability (i.e. same species interacting differently), interactions are expected to display more spatial variation than species.

Although β-diversity is widely used among ecologists to explore spatial variation of communities, it has been seldom used to explore spatial variation of interactions. Poisot and colleagues (2012) proposed a new dissimilarity index to explore differences between interaction networks across space. To additionally delve into the source of variability between networks, they subdivided β-diversity of interactions into a component due to species turnover and a component due to interaction turnover between given species, that is, shared species interacting differently (rewiring). They also proposed comparing interactions between overlapping species of each local web to their counterparts in the

regional web to determine if interactions found at the regional level are also found at the local level, indicating that species interact similarly across the region. This approach allows establishing whether the regional web only reflects the regional, but not the local behavior of species interactions or, on the contrary, it reflects both the regional and the local behavior. The former outcome would apply if differences among local webs are mostly due to interaction rewiring, that is, if species interact differently across plots. Conversely, the second outcome would apply to situations in which spatial variability of interactions is mostly due to species turnover, and common species in different local webs tend to interact similarly.

Recently, some studies have empirically addressed β-diversity of interactions at individual- (Dupont et al. 2014) and community levels in natural (Poisot et al. 2012, Dáttilo et al. 2013, Carstensen et al. 2014, Simanonok and Burkle 2014, Trøjelsgaard et al. 2015) and agricultural (Norfolk et al. 2014) habitats. In some plant-pollinator networks, species turnover has been shown to be the main cause of interaction turnover (Simanonok and Burkle 2014, Trøjelsgaard et al. 2015), but others have yielded a slightly greater contribution of rewiring (Carstensen et al. 2014). In addition, some studies (Trøjelsgaard et al. 2015) have found pollinator turnover to be the major contributor to interaction turnover, while others (Simanonok and Burkle 2014) attribute a predominant role to plant turnover.

Here, we work with a community of cavity-nesting bees and wasps (henceforth hosts) and their nest associates, including parasitoids, cleptoparasites, predators and scavengers (henceforth parasitoids) and study the spatial variation of the two communities, as well as their interactions. Previous studies working with cavity-nesting bees and wasps analyze differences between habitats or along an environmental gradient (Albrecht et al. 2007, Tylianakis et al. 2007, Osorio et al. 2015). Conversely, our study was conducted across a continuous habitat and addresses spatial variability at a local scale (distance between plots ca. 1000 m). Notwithstanding the lack of environmental gradients or physical barriers, previous studies in the same study area have found marked geographical patterns of spatial heterogeneity in bee species composition (Torné-Noguera et al. 2014). Our aim is therefore to study the intrinsic variability of the host-parasitoid network, rather than to establish how different environmental factors may affect interaction identity and network structure.

We have the following objectives:

1- To analyze the spatial variation of the host and parasitoid communities across a continuous habitat. We ask how community composition varies locally and whether this variation follows a spatial pattern. We also ask how the two communities are related.

2- To study β-diversity of host-parasitoid interactions. We ask whether the main driver of interaction turnover is species turnover or rewiring.

3- To explore the relationship between host, parasitoid and interaction β-diversity and to examine their distance decay. We ask whether host and parasitoid β-diversity are good predictors of interaction β-diversity. We also ask whether distance decay patterns are similar for the three groups.

3.2. Methods and materials

3.2.1. Study area

The study was conducted in the Garraf Natural Park (Barcelona, NE Spain), a Mediterranean scrubland dominated by *Quercus coccifera*, *Pistacia lentiscus*, *Rosmarinus officinalis* and *Thymus vulgaris*. Our study area, entirely located in the park, encompasses a surface of 32 km^2. We selected 25 plots distributed more or less regularly across the study area. Distances between nearest plots ranged from 585 to 1354 m.

3.2.2. Surveys

In each plot we placed a trap-nesting station facing SE. Each station contained seven drilled wood blocks with inserted paper tubes. Each wood block accommodated 25 tubes of a given diameter (2, 3, 4, 5, 6, 7 or 8 mm), resulting in 175 nesting cavities per station. Paper tube length was 5 cm for the 2 and 3 mm diameters and 15 cm for the rest. Each nesting station had 7 trap-nests, one of each diameter. Nesting stations were checked every 2 weeks and tubes containing completed nests were pulled out and replaced with empty ones, so that there were nesting cavities of all diameters available at all times. Nesting stations remained

in the field from February to October, in 2011 and 2013. Data of the two years are pooled together in the analyses.

In the laboratory, nests were individually placed in test tubes and kept in a temperature chamber simulating monthly ambient thermoperiods of the study site. In spring and summer, nests were dissected and their contents recorded. Hosts and parasitoids were reared and identified.

3.2.3. Statistical analysis

Host and parasitoid communities

To characterize community structure at each nesting station, we used the variables host abundance (number of host cells), host richness (number of host species), parasitoid abundance (number of cells parasitized), parasitoid richness (number of parasitoid species) and parasitism rate (%host cells parasitized).

We used rarefaction curves to determine the completeness of our sampling of the host and parasitoid community. Rarefactions were conducted with vegan 2.0-10 (Oksanen et al. 2015) for R (R Core Team 2014).

We measured the correlation between host abundance and richness (for all hosts and for parasitized hosts separately), and between parasitoid abundance and richness. Using the same procedure, we also measured the correlation between host (all hosts) and parasitoid abundance and richness with a Spearman's rho.

We calculated the overall β-diversity (all-site comparison) based on Sørensen's dissimilarity index for the host (β_{SOR-H}), parasitized host (β_{SOR-PH}) and parasitoid (β_{SOR-P}) communities, and broke it down into the component of β-diversity due to species turnover (β_{SIM}), that is, species replacement, and the component of the β-diversity due to nestedness (β_{SNE}), that is, species loss (Baselga 2010). In addition, to have a measure of diversity for each site-to-site comparison, we calculated β-diversity of pairwise comparisons for all hosts (β_H), parasitized hosts (β_{PH}) and parasitoids (β_P). These analyses were conducted with betapair 1.3 (Baselga and Orme 2012) for R (R Core Team 2014). Correlations between host β-diversity (all hosts

(β_H) and parasitized hosts (β_{PH}) separately) and parasitoid β-diversity (β_P) were tested with Spearman's correlation. To analyze distance decay of the different components of β-diversity, we calculated correlations between geographical distance and host β-diversity (β_H), parasitized host β-diversity (β_{PH}) and parasitoid β-diversity (β_P) with Spearman's correlation.

Interactions

We tested whether parasitism rate was related to host abundance with a GLM model. A GLM model was also used to analyze the relationship between the abundance of each species and its parasitism rate.

As with host and parasitoid communities, we tested the quality of our interaction sampling with rarefaction curves using vegan 2.0-10 (Oksanen et al. 2015) for R (R Core Team 2014).

We built an interaction network for each plot (25 local webs), and a regional web pooling the data from all plots. For the regional web, we calculated generality (weighted mean number of hosts per parasitoid), vulnerability (weighted mean number of parasitoids per host), interaction evenness (Shannon's evenness) and H_2' (a measure of overall network specialization; Blüthgen et al. 2006). Then, to obtain a measure of significance for all these metrics we run a null model (1000 iterations) which maintains marginal totals and connectance of the original network (Vázquez et al. 2007). All network analysis were conducted with bipartite 2.02 (Dormann et al. 2008) for R (R Core Team 2014).

We explored local web dissimilarity by measuring β-diversity of interactions among local networks (β_{WN}). Following Poisot et al. (2012), this dissimilarity was subdivided into dissimilarity due to species turnover (β_{ST}) and dissimilarity due to interaction rewiring, that is, common host and parasitoid species interacting differently in different plots (β_{OS}). Sometimes it was not possible to calculate β_{OS} due to the lack of shared species between two plots. In these cases, all network variability (β_{WN}) was assigned to species turnover (β_{ST}). In addition, and again following Poisot et al. (2012), we calculated β'_{OS}, a measure of dissimilarity of interactions between local webs and their counterparts in the regional web. All these indexes were calculated with betalink package for R (R Core Team 2014).

The relationship of interaction β-diversity (β_{WN}) with parasitized host β-diversity (β_{PH}) and parasitoid β-diversity (β_P) was tested with a linear model. Since both explanatory variables were slightly correlated (r=0.37), we calculated the variance inflation factor to make sure colinearity was low (VIF= 1.17). Percentage of variance explained by the model was calculated with *pmvd* metric using relaimpo package (Grömping 2006) for R (R Core Team 2014). To analyze distance decay of the different components of network β-diversity, we made correlations between geographical distance and network β-diversity (β_{WN}), β-diversity due to species turnover (β_{ST}) and β-diversity due to rewiring (β_{OS}) using Spearman's correlation.

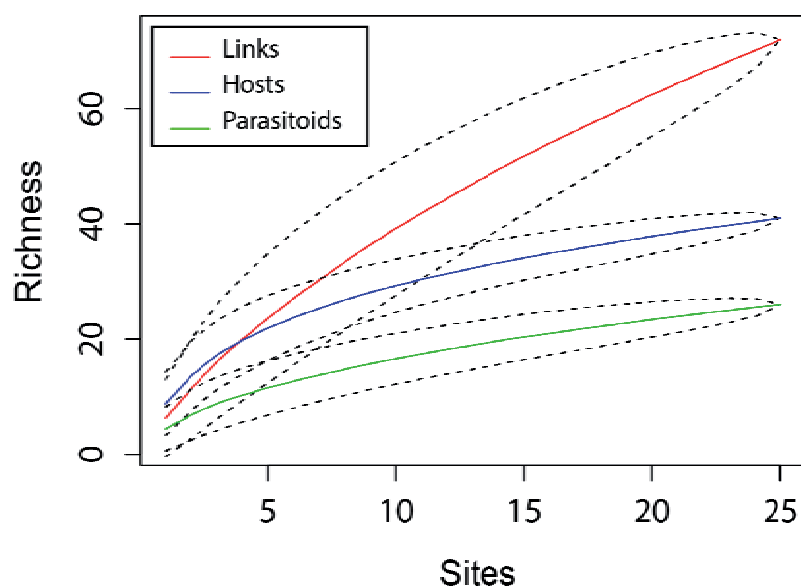**Figure 3**.1 Rarefaction curves for host and parasitoid species and their interactions. Dashed lines indicate 95% confidence intervals.

3.3. Results

We obtained 1541 nests (4954 cells) from 41 host species. Seventeen of these species were bees (Megachillidae – 15 sp., Colletidae – 2 sp.) and 24 were wasps (Crabronidae – 12 sp., Pompilidae – 5 sp., Vespidae – 5 sp., Sphecidae – 1 sp., Ampulicidae – 1 sp.). Twenty-four

host species had, at least, one individual parasitized. We found 26 parasitoid species (16 wasps, 5 flies, 3 beetles, 1 bee and 1 mite) associated to the nests, resulting in 654 parasitized cells. Of these 26 species, 20 were parasitoids, 4 were cleptoparasites, 1 was a predator and 1 was a scavenger. Overall parasitism rate was 13.2%.

3.3.1. Host community

Rarefaction curves indicate that our host community was relatively well sampled (Fig. 1). As expected, host abundance and richness showed a positive relationship (Spearman correlation: all hosts: rho= 0.59, p< 0.002; parasitized hosts: rho= 0.57, p< 0.003; n= 25 plots). Among the 11 most abundant host species (>100 cells), five showed spatial autocorrelation, while the remaining six did not.

Host β-diversity was high (β_{SOR-H}= 0.89), mostly due to species turnover (β_{SIM-H}= 0.85), with a poor contribution of nestedness (β_{SNE-H}= 0.04) (Table 1). Parasitized host β-diversity was equally high (β_{SOR-PH}= 0.91), again mostly due to species turnover (β_{SIM-PH}= 0.87), with a week contribution of nestedness (β_{SNE-PH}= 0.04). That is, local host communities differ in species composition, not due to species loss, but because there is a species replacement and thus the identity of the species differs among sites.

3.3.2. Parasitoid community

Rarefaction curves indicate that our parasitoid community was relatively well sampled, following a pattern parallel to the host community (Fig. 1). As expected, parasitoid abundance and richness showed a positive correlation (r= 0.57, p< 0.003). The parasitoid community was highly dependent on the host community: there was a significant positive correlation between parasitoid and host abundance (all hosts: rho= 0.62, p= 0.001, n=25 plots), and between parasitoid and host richness (all hosts: rho= 0.75, p< 0.0001).
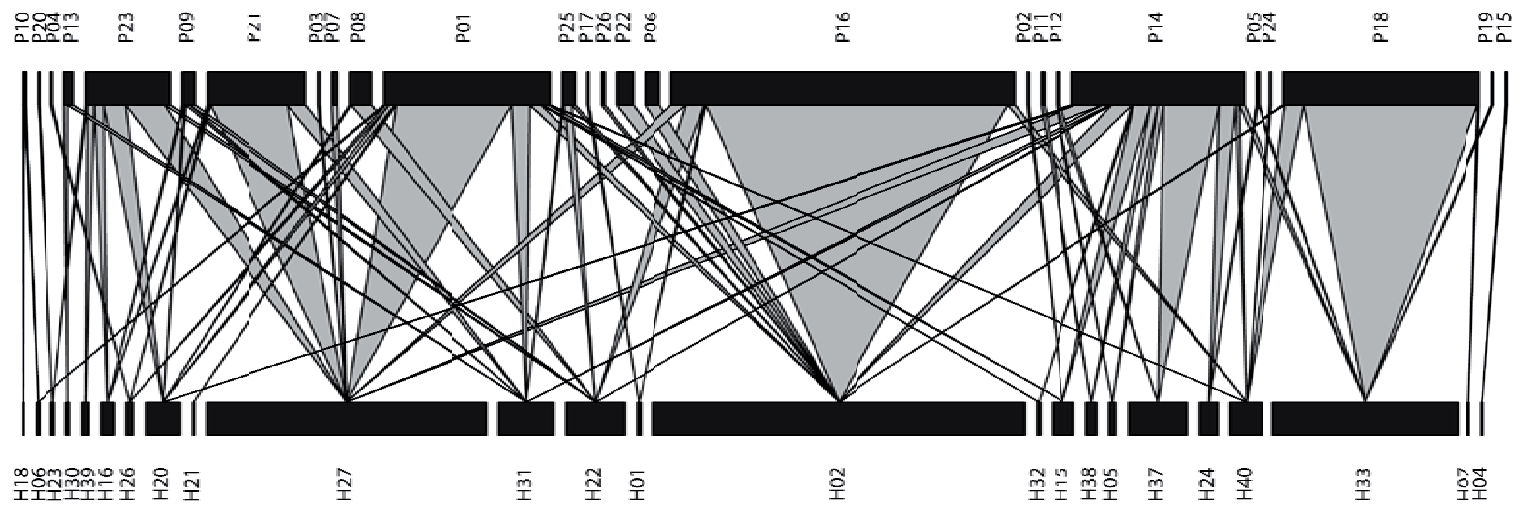
Figure 3.2 Regional host-parasitoids web (n= 25 plots). Species names can be found in Appendix A, Tables A1 and A2. Width of black bars denotes abundance of interacting species (only parasitized host individuals included).

Parasitoid β-diversity was again high (β_{SOR-P}= 0.91), and mostly attributable to species turnover (β_{SIM-P}= 0.85), rather than nestedness (β_{SNE-P}= 0.06) (Table 1). β-diversity of parasitoids (β_P) was significantly correlated to β-diversity of all hosts (β_H) (rho= 0.21, p= 0.0002) and β-diversity of parasitized hosts (β_{PH}) (rho= 0.37, p< 0.0001).
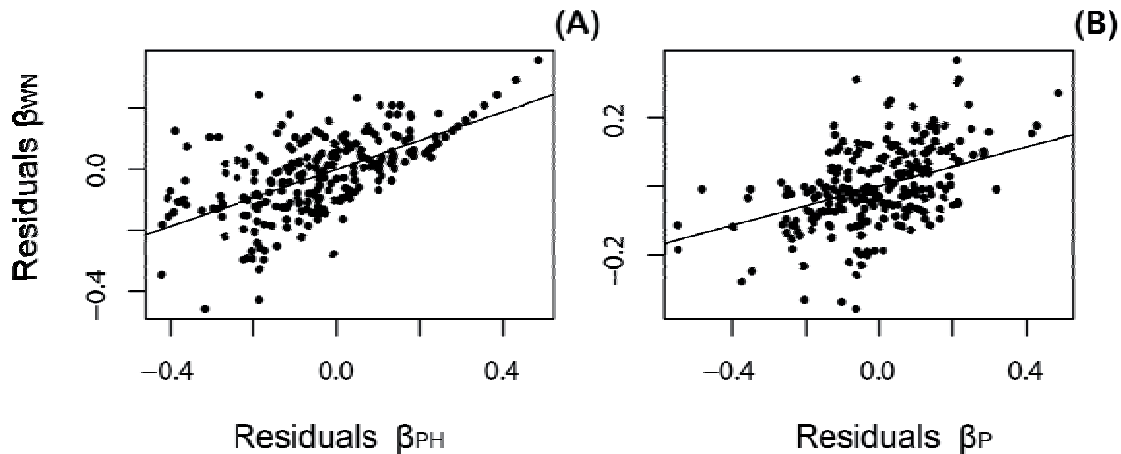
Figure 3.3 Relationship between network β-diversity and (a) parasitized host β-diversity and (b) parasitoid β-diversity. Partial regression plots obtained from a linear model when the effect of the other explanatory variable is removed.

3.3.3. Interactions

Rarefaction curves show that, contrary to host and parasitoid species, interactions were not sufficiently sampled (Fig. 1). Parasitism rate was not explained by host abundance at the plot level (GLM: pseudo-R^2= 0.005, p=0.75, n= 25 plots). However, at species level, parasitism rate of a given host species was partially explained by its abundance (GLM: pseudo-R^2= 0.13, p= 0.02, n=24 species).

We obtained a regional web with the data of all the plots sampled (Fig. 2). All network metrics calculated denote a highly specialized regional web (generality= 2.997, p<0.0001; vulnerability= 2.813, p< 0.0001; interaction evenness= 0.468, p< 0.0001; H_2'= 0.65, p< 0.0001).

We found 72 different specific interactions. However, most of them (63.9%) were found in just one plot, and 40.3% of them were found only once. Pairwise interaction dissimilarity between plots was high (β_{WN}, mean±SD: 0.83±0.16). Most of this dissimilarity was due to species turnover (β_{ST}, mean±SD: 0.60±0.32), with a much lesser contribution of interaction rewiring (β_{OS}, mean±SD: 0.23±0.32). β'_{OS} values were low (between 0 and 0.38), indicating that species interact similarly across local networks.

3.3.4. Relationship between species and interaction β-diversity

Parasitized host β-diversity (β_{PH}) and parasitoid β-diversity (β_P) were good predictors of network β-diversity (β_{WN}) (r^2= 0.63, p< 0.0001) (Fig. 3). However, while parasitized host β-diversity explained 49.1% of the variance, parasitoid β-diversity only explained 13.9%, indicating that parasitized host β-diversity is a better predictor of network β-diversity than parasitoid β-diversity.

Table 3.1 Host, parasitized host and parasitoid β-diversity based on Sørensen dissimilarity (β_{SOR}), partitioned into the component of β-diversity due to species turnover (β_{SIM}) and the component of β-diversity due to nestedness (β_{SNE}) (Baselga 2010)

	β_{SOR}	β_{SIM}	β_{SNE}
Hosts	0.89	0.85	0.04
Parasitized hosts	0.91	0.87	0.04
Parasitoids	0.91	0.85	0.06

3.3.5. Distance decay of β-diversity

Host β-diversity (β_H: rho= 0.29, p< 0.0001) and parasitized host β-diversity (β_{PH}: rho= 0.19, p= 0.001) were positively correlated to geographical distance. Conversely, parasitoid β-diversity (β_P: rho= 0.08, p= 0.18) (Fig. 4) was no related to distance. Similarly, network β-diversity (β_{WN}: rho=0.07, p= 0.20) and its two components, β-diversity due to species

turnover (β_{ST}: rho= 0.10, p= 0.08) and β-diversity due to rewiring (β_{OS}: rho= -0.06, p= 0.29) were not related to geographical distance (Fig. 4).
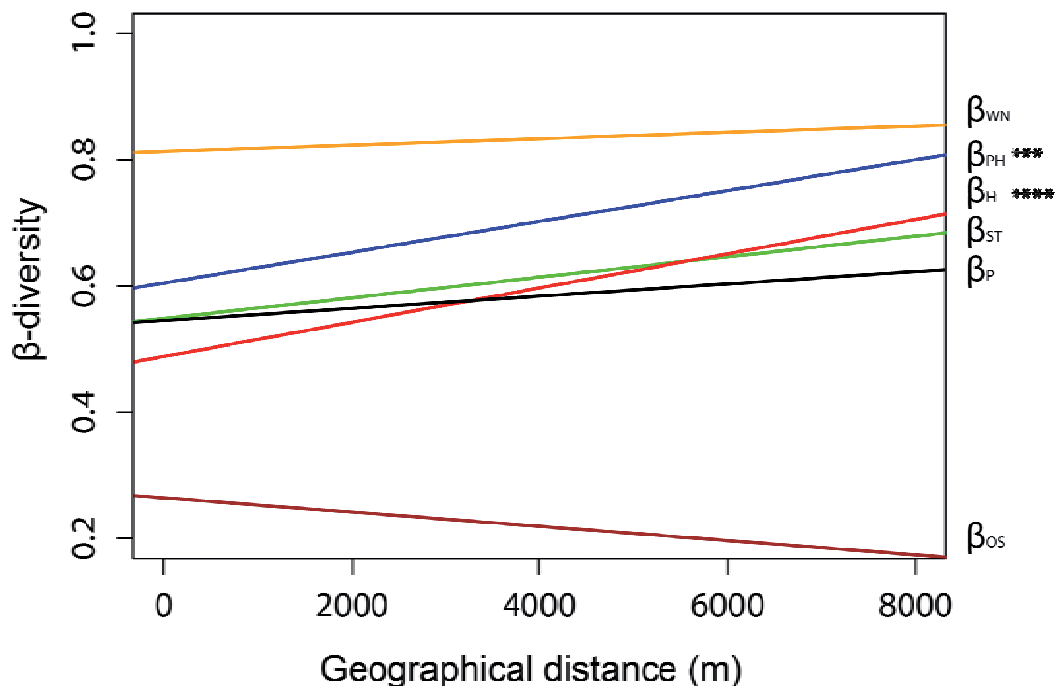
Figure 3.4 Distance decay of host β-diversity (β_H), parasitized host β-diversity (β_{PH}), parasitoid β-diversity (β_P), network β-diversity (β_{WN}), component of network β-diversity due to species turnover (β_{ST}), and component of β-diversity due to rewiring (β_{OS}). (Statistical significance; ***: p ≤ 0.001; ****: p ≤ 0.0001).

3.4. Discussion

Rarefaction results indicate that sampling 25 plots over an area of 32 km^2 provided a relatively adequate characterization of the host and parasitoid community in our study area. On the other hand, the rarefaction curve for interactions suggests that proper characterization of the interaction community would require a much greater sampling effort. Poisot et al.(2012) obtained similar results when they sampled mammal-ectoparasite interactions in 113 localities over Central Europe. However, rarefaction curves are probably ill-suited to assess sampling completeness of ecological interactions, as they assume all

species can interact with all species in the opposite trophic level, thus overlooking forbidden links resulting from different kinds of interaction constraints (Jordano et al. 2003). This is especially true in a system like ours, with high levels of specialization and low levels of rewiring.

In our community, parasitism rate was not influenced by host abundance at the community level. Other studies on cavity-nesting bees and wasps have found similar results (Tylianakis et al. 2006, Albrecht et al. 2007), yet others have found a positive correlation between them (Steffan-Dewenter 2003). However, in our community, more abundant host species had higher parasitism rate. Two mechanisms may explain this result. First, prey abundance has been demonstrated to influence prey choice, as predators with the ability to prey on different hosts obtain greater returns by learning how to handle the most abundant prey and focusing on it (Ishii and Shimada 2012). Second, for specialist antagonists, with a restricted range of potential preys/hosts, it may be difficult to build a stable population on a locally rare host.

Although the study was conducted in a continuous habitat, both the host and parasitoid communities, as well as their interactions, showed strong spatial variability at a local scale as indicated by the high β-diversity values. Given that bees and wasps are highly mobile organisms, one might have expected a more uniform host distribution. The local spatial variability found indicates a strong effect of local factors, as found for the entire bee community in a previous study in the same area (Torné-Noguera et al. 2014).

Host β-diversity (β_H) and parasitized host β-diversity (β_{PH}) increased with geographical distance, indicating that host species turnover increases with distance. On the other hand, parasitoid β-diversity (β_P) and interaction β-diversity (β_{WN}), as well as its two components (β-diversity due to species turnover (β_{ST}) and β-diversity due to rewiring (β_{OS})), did not vary with geographical distance. Even though few studies are available for comparison, distance decay of species composition of the lower trophic level and of interactions seems to be widespread (Novotny 2009, Carstensen et al. 2014, Trøjelsgaard et al. 2015). Composition of the higher trophic level, on the other hand, has been found to either increase (Trøjelsgaard et al. 2015), decrease (Novotny 2009) or not vary (Carstensen et al. 2014) with geographical distance. Discrepancies among these studies, including ours, may be due to differences in the biological systems researched (from more generalized plant-pollinator interactions to

more specialized host-parasite interactions) and in geographical distance (from 600 m to 500 km).

Our results additionally show that parasitoid community is highly dependent on host community, since abundance, richness and β-diversity of the two communities are correlated. Former studies conducted in different habitat types also showed a high dependency of the higher trophic level on the lower trophic level (Albrecht et al. 2007, Ebeling et al. 2012, Weiner et al. 2014, Osorio et al. 2015). Host-parasitoid relationships are, in general, less flexible (partner fidelity is often obligatory; Hawkins 1994) than plant-pollinator relationships (Waser et al. 1996), leaving little room to opportunistic interactions. Accordingly, our results yield a highly specialized network compared to other types of networks such as pollination, seed dispersal or ant-nectar plant networks (Blüthgen et al. 2007, Schleuning et al. 2012). Our values are analogous to those found for more specialized systems, such as host-parasitoid (Morris et al. 2014) and ant-myrmecophyte (Blüthgen et al. 2007) networks.

Results show that host and parasitoid communities had high values of β-diversity, mostly due to species turnover with almost irrelevant species loss. That is, local communities did not only differ in species richness, but tended to have species compositions idiosyncratic to each site. Analysis of β-diversity of local networks confirmed these results. Pairwise comparisons between local networks revealed high dissimilarity of interactions (β_{WN}, mean±SD: 0.83±0.16), and species turnover was the main factor contributing to such dissimilarity (β_{ST}, mean±SD: 0.60±0.32). Given the high level of specialization in our network, species turnover should be expected to be the major driver of interaction diversity (Olesen et al. 2011), and the effect of rewiring (shared species interacting differently) should be expected to be much lower (β_{OS}, mean±SD: 0.23±0.32, in this study). This result agrees with previous studies (Novotny 2009, Simanonok and Burkle 2014, Trøjelsgaard et al. 2015, but see Carstensen et al. 2014 for a similar contribution of both components of both components of interaction diversity). These studies, including ours, cover a variety of interaction systems (plant-pollinator, plant-caterpillar and host-parasitoid) and a range of geographical scales (from 400 m to 500 km). Thus, species turnover appears to be the main driver of network β-diversity across biological systems and spatial scales of observation.

Interaction similarity between local networks and their counterparts in the regional network is high, as reflected by the low β'_{OS} (mean$\pm$SD= 0.2$\pm$0.1). In other words, interactions in local networks are nested in the regional network. Interaction nestedness in our system results from a combination of a) species interacting similarly across plots, and b) large differences between plots in species composition. Because local communities are highly idiosyncratic (63.9% of interactions are only found in one plot), the contribution of each plot to the regional web is high. This interpretation differs from the explanation given by Poisot and collaborators in their analysis of a mammal-ectoparasite regional network (Poisot et al. 2012). These authors conclude that high values of β'_{OS} indicate that many interactions only occur in a few local webs, pointing that interactions are highly determined by local conditions. However, if there is a low proportion of shared species between local webs, and shared species interact similarly, β'_{OS} values will be low and it will still be true that there are a lot of interactions only occurring in a few local webs. In this case, which is our case, interactions are site-idiosyncratic not because interaction composition *per se* is determined by local conditions, but because species composition is determined by local conditions. Such results agree with previous work in the same area (Torné-Noguera et al. 2014), which found the overall bee community to be strongly conditioned by local effects. Our results are also supported by a study that found local factors to be much more important than landscape factors as determinants of community and network structure and composition in cavity-nesting bees and wasps (Osorio et al. 2015).

Our results additionally show that β-diversity of parasitized hosts is a better predictor of β-diversity of interactions than β-diversity of parasitoids. Mean value of pairwise community β-diversity is higher for parasitized hosts (β_{PH}: mean$\pm$SD= 0.68$\pm$0.21) than for parasitoids (β_P: mean$\pm$SD= 0.57$\pm$0.18). In other words, our host community turnover is higher and thus contributes more than parasitoid community turnover to interaction turnover. Greater turnover of the lower trophic level seems to be a feature common to other kinds of networks (Novotny 2009, Carstensen et al. 2014, Simanonok and Burkle 2014 but see Trøjelsgaard et al. 2015).

Overall, this study demonstrates that communities of mobile organisms and their interactions vary at a local scale ($\sim$500 m) even in the absence of ecological barriers or environmental gradients. Host spatial turnover is the major driver of the observed spatial heterogeneity, since the parasitoid community is highly dependent on the host community,

and interactions depend on both communities. Nonetheless, parasitoids and interactions are also subjected to their own intrinsic variability, resulting in different responses across space. Thus, it is important to take into account both levels of variability (communities and interactions) to adequately characterize ecological function. Our results also show the need to sample a large number of plots to adequately characterize a regional network, since networks may vary spatially at a very local scale even in a continuous habitat. Distinguishing between the two components of interaction β-diversity is essential to understand the drivers of such diversity. When network β-diversity is mostly due to interaction rewiring, the resulting network will reflect the regional, but not the local behavior of interacting species. In contrast, when network β-diversity is mostly due to species turnover, the regional network will reflect the local behavior of interacting species. With high levels of specialization and low levels or rewiring, our system falls within the latter scenario. However, even then it is necessary to sample a sufficiently large number of plots because species composition is highly dependent on local factors and subjected to variation at very small scales.

Aknowledgements

References

Albrecht, M. et al. 2007. Interaction diversity within quantified insect food webs in restored and adjacent intensively managed meadows. - J. Anim. Ecol. 76: 1015–1025.

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. - Glob. Ecol. Biogeogr. 19: 134–143.

Baselga, A. and Orme, C. D. L. 2012. betapart : an R package for the study of beta diversity. - Methods Ecol. Evol. 3: 808–812.

Blüthgen, N. et al. 2006. Measuring specialization in species interaction networks. - BMC Ecol. 6: 9.

Blüthgen, N. et al. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. - Curr. Biol. 17: 341–6.

Burkle, L. A. and Alarcón, R. 2011. The future of plant-pollinator diversity: understanding interaction networks across time, space, and global change. - Am. J. Bot. 98: 528–38.

Carstensen, D. W. et al. 2014. Beta diversity of plant-pollinator networks and the spatial turnover of pairwise interactions. - PLoS One 9: e112903.

Condit, R. et al. 2002. Beta-diversity in tropical forest trees. - Science 295: 666–669.

Dáttilo, W. et al. 2013. Spatial structure of ant-plant mutualistic networks. - Oikos 122: 1643–1648.

Dormann, C. F. et al. 2008. Introducing the bipartite Package : Analysing Ecological Networks. - R News 8: 8–11.

Dupont, Y. L. et al. 2014. Spatial structure of an individual-based plant-pollinator network. - Oikos 123: 1301–1310.

Ebeling, A. et al. 2012. Multitrophic effects of experimental changes in plant diversity on cavity-nesting bees, wasps, and their parasitoids. - Oecologia 169: 453–465.

Fisher, R. A. et al. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. - J. Anim. Ecol. 12: 42–58.

Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. - J. Stat. Softw. 17: 139–147.

Hawkins, B. A. 1994. Pattern and process in host-parasitoid interactions. - Cambridge University Press.

Ishii, Y. and Shimada, M. 2012. Learning predator promotes coexistence of prey species in host – parasitoid systems. - Proc. Natl. Acad. Sci. U. S. A. 109: 5119.

Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant-animal interactions. - Ecol. Lett. 6: 69–81.

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

Morris, R. J. et al. 2014. Antagonistic interaction networks are structured independently of latitude and host guild. - Ecol. Lett. 17: 340–9.

Nekola, J. C. and White, P. S. 1999. The distance decay of similarity in biogeography and ecology. - J. Biogeogr. 26: 867–878.

Norfolk, O. et al. 2014. Contrasting patterns of turnover between plants, pollinators and their interactions. - Divers. Distrib.: 1–11.

Novotny, V. 2009. Beta diversity of plant-insect food webs in tropical forests: A conceptual framework. - Insect Conserv. Divers. 2: 5–9.

Oksanen, J. et al. 2015. Vegan: Community Ecology Package.

Olesen, J. M. and Jordano, P. 2002. Geographic patterns in plant-pollinator mutualistic networks. - Ecology 83: 2416–2424.

Olesen, J. M. et al. 2011. Strong, long-term temporal dynamics of an ecological network. - PLoS One 6: 2–6.

Osorio, S. et al. 2015. Local and landscape effects in a host-parasitoid interaction network along a forest-cropland gradient. - Ecol. Appl. in press.

Poisot, T. et al. 2012. The dissimilarity of species interaction networks. - Ecol. Lett. 15: 1353–61.

R Core Team 2014. R: A language and environment for statistical computing.

Schleuning, M. et al. 2012. Specialization of mutualistic interaction networks decreases toward tropical latitudes. - Curr. Biol. 22: 1925–31.

Simanonok, M. P. and Burkle, L. A. 2014. Partitioning interaction turnover among alpine pollination networks : spatial , temporal , and environmental patterns. - Ecosphere 5: 1–17.

Soininen, J. et al. 2007. The distance decay of similarity in ecological communities. - Ecography 30: 3–12.

Steffan-Dewenter, I. 2003. Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. - Conserv. Biol. 17: 1036–1044.

Torné-Noguera, A. et al. 2014. Determinants of spatial distribution in a bee community: nesting resources, flower resources, and body size. - PLoS One 9: e97255.

Trøjelsgaard, K. et al. 2015. Geographical variation in mutualistic networks: similarity, turnover and partner fidelity. - Proc. R. Soc. B Biol. Sci. 282: 20142925.

Tylianakis, J. M. et al. 2006. Diversity, ecosystem function, and stability of parasitoid-host interactions across a tropical habitat gradient. - Ecology 87: 3047–3057.

Tylianakis, J. M. et al. 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. - Nature 445: 202–5.

Vázquez, D. P. et al. 2007. Species abundance and asymmetric interaction strength in ecological networks. - Oikos 116: 1120–1127.

Vázquez, D. P. et al. 2009a. Evaluating multiple determinants of the structure of plant-animal mutualistic networks. - Ecology 90: 2039–2046.

Vázquez, D. P. et al. 2009b. Uniting pattern and process in plant-animal mutualistic networks: a review. - Ann. Bot. 103: 1445–57.

Waser, N. M. et al. 1996. Generalization in pollination systems, and why it matters. - Ecology 77: 1043.

Weiner, C. N. et al. 2014. Land-use impacts on plant-pollinator networks: interaction strength and specialization predict pollinator declines. - Ecology 95: 466–74.

Whittaker, R. H. 1960. Vegetation of the Siskiyou mountains, Oregon and California. - Ecol. Monogr. 30: 279–338.

General conclusions

General conclusions

- Flowers and nesting substrates are unevenly distributed across a continuous habitat, but only flowers follow a spatial pattern at the scale considered. Despite bees being highly mobile organisms, bee species composition also follows a spatial pattern, demonstrating that bee communities may display strong heterogeneity at a local scale (500-1000m) in a continuous habitat (Chapter 1). Cavity-nesting bee and wasp community (hosts) and their associated fauna (parasitoids), as well as their interactions, also display high spatial variability at a local scale. However, only the host community shows a clear geographical pattern (Chapter 3).

- Resource availability has unequal influence in shaping the spatial distribution of the bee community. Floral resources partially shape spatial distribution of the community, while nesting resources are almost irrelevant. *Cistus* spp. flowers, in particular, play a foremost role in structuring the bee community at Garraf Natural Park, becoming an important pollen/nectar source at a time when other flower resources are scarce (Chapters 1 & 2).

- Species body size plays an important role determining the spatial patterns of the bee community. Small species show spatial structure, whereas large species tend to show a homogeneous distribution. Such relationship is probably due to the greatest foraging distances of large species, in relation to their greater feeding requirements (Chapter 1).

- Massive introduction of honey bee (*Apis mellifera*) populations resulting from beekeeping has significant effects on pollen/nectar availability. Honey bees are the main contributors to pollen/nectar consumption of the two main flowering species in the study area. The contribution of bumblebees (*Bombus terrestris*) is much lower, and that of other wild bees is irrelevant (Chapter 2).

- The wild bee community is modified in areas close to apiaries. Wild bee biomass decreases with proximity to apiaries due to a lower abundance of large bees (Chapter 2). Large bees are expected to be the most affected by potential exploitative competition with honey bees due to their higher feeding requirements (Chapter 2).

- Because flower availability is highly variable in space and time, it is difficult to provide estimates of the carrying capacity (sustainable hive density) of a habitat. Nonetheless, densities over 3.5 hives per km^2 seem to affect the wild bee community in our study area (Chapter 2).

- Parasitism rate of cavity-nesting bees and wasps is not explained by host abundance at the community level. However, abundant species tend to have higher parasitism rate. Probably, generalist parasitoids follow a frequency-dependent dynamic and have the ability to focus on the most abundant species. On the other hand, specialist parasitoids are probably specialized on abundant species, since it may be difficult to establish a stable population on a locally rare host (Chapter 3).

- The community of cavity-nesting bees and wasps (hosts) and that of its associated parasitoids, as well as their interactions, show high β-diversity at local scale. Host and parasitoid β-diversity patterns are mainly due to species turnover rather than species loss, meaning that while species richness does not differ among local communities, species composition is idiosyncratic to each site. Similarly, β-diversity of species interactions is mostly due to species turnover rather than link rewiring (shared species interacting differently). That is, even if local communities and networks differ from each other, species interact similarly across the study area (Chapter 3).

- β-diversity of the host community has a marked spatial structure, becoming more dissimilar with increasing geographical distance. However, β-diversity of the parasitoid community and β-diversity of the interactions do not change with geographical distance (Chapter 3).

- β-diversity of hosts (lower trophic level) is a better predictor of β-diversity of interactions than β-diversity of parasitoids (higher trophic level). Since host community turnover is greater than parasitoid community turnover, the contribution of host turnover to interaction turnover is greater (Chapter 3).

- Species composition is highly dependent on local factors (Chapters 1 & 3). This has implications on the spatial variability of interactions, which can vary at a very local scale (500 – 1000m), highlighting the need to sample a large number of plots to adequately characterize the regional network in a given area (Chapter 3).

- Twenty-five plots appear to be sufficient to sample a cavity-nesting bee and wasp community and its nest associates in a $32km^2$ area of uninterrupted habitat, but not to sample their interactions. However, rarefaction methods do not seem to be a good approach to evaluate sampling quality of interactions because they overlook forbidden links (Chapter 3).

Appendix A

Table A1 Bee species, their code numbers and body size category.

Family	Species	Code number	Body size
Andrenidae	*Andrena angustior*	1	Small
	Andrena cinerea	2	Small
	Andrena combinata	3	Small
	Andrena djelfensis	4	Small
	Andrena ferrugineicrus	5	Large
	Andrena fertoni	6	Small
	Andrena flavipes	7	Large
	Andrena hesperia	8	Small
	Andrena labialis	9	Large
	Andrena lagopus	10	Small
	Andrena lepida	11	Small
	Andrena limata	12	Large
	Andrena livens	13	Small
	Andrena nigroaenea	14	Large
	Andrena nigroolivacea	15	Large
	Andrena senecionis	16	Small
	Andrena similis	17	Small
	Andrena solenopalpa	18	Small
	Andrena sp.	19	Small
	Andrena trimmerana	20	Large
	Andrena verticalis	21	Small
	Andrena vulpecula	22	Small
	Panurgus dentipes	23	Small
Apidae	*Amegilla quadrifasciata*	24	Large
	Anthophora acervorum	25	Large
	Anthophora dispar	26	Large
	Apis mellifera	27	Large
	Bombus pascuorum	28	Large
	Bombus pratorum	29	Large
	Bombus terrestris	30	Large
	Ceratina cucurbitina	31	Small
	Ceratina cyanea	32	Small
	Eucera alternans	33	Large
	Eucera caspica	34	Large
	Eucera chrysopyga	35	Large
	Eucera collaris	36	Large
	Eucera elongatula	37	Large

	Eucera nigrilabris	38	Large
	Eucera taurica	39	Large
	Melecta luctuosa	40	Large
	Nomada connectens	41	Small
	Nomada dicrepans	42	Small
	Nomada discedens	43	Small
	Nomada flavoguttata	44	Small
	Nomada hispanica	45	Small
	Nomada integra	46	Small
	Nomada panurgina	47	Small
	Nomada serricornis	48	Small
	Nomada sheppardana	49	Small
	Xylocopa violacea	50	Large
Colletidae	*Hylaeus garrulus*	51	Small
	Hylaeus gibbus	52	Small
	Hylaeus hyalinatus	53	Small
	Hylaeus taeniolatus	54	Small
Halictidae	*Halictus fulvipes*	55	Small
	Halictus gemmeus	56	Small
	Halictus quadricinctus	57	Large
	Halictus scabiosae	58	Large
	Halictus simplex	59	Small
	Lasioglossum albocinctum	60	Small
	Lasioglossum bimaculatum	61	Small
	Lasioglossum griseolum	62	Small
	Lasioglossum ibericum	63	Small
	Lasioglossum interruptum	64	Small
	Lasioglossum malachurum	65	Small
	Lasioglossum mediterraneum	66	Small
	Lasioglossum morio	67	Small
	Lasioglossum subhirtum	68	Small
	Lasioglossum transitorium	69	Small
	Sphecodes pseudofasciatus	70	Small
	Sphecodes puncticeps	71	Small
	Sphecodes ruficrus	72	Small
Megachilidae	*Chelostoma florisomne*	73	Small
	Hoplitis (Anthocopa) sp.	74	Small
	Hoplitis adunca	75	Small
	Hoplitis anthocopoides	76	Small
	Hoplitis benoisti	77	Small

Hoplosmia ligurica	78	Small
Megachile baetica	79	Large
Megachile ericetorum	80	Large
Megachile pyrenaica	81	Large
Osmia aurulenta	82	Small
Osmia gallarum	83	Small
Osmia latreillei	84	Large
Osmia melanogaster/leaiana	85	Small
Osmia mustelina	86	Large
Osmia nasoproducta	87	Large
Osmia nasuta	88	Small
Osmia niveata	89	Large
Osmia niveocincta	90	Large
Osmia rufohirta	91	Small
Osmia submicans	92	Small
Osmia tricornis	93	Large
Protosmia (Nanosmia) sp.	94	Small
Protosmia capitata	95	Small
Protosmia exenterata	96	Small
Rhodanthidium septemdentatum	97	Large
Rhodanthidium sticticum	98	Large

Table A2 Mean and coefficient of variation (n= 21 plots) of flower and nesting resource variables.

	Mean	CV
Flowers		
Flower abundance (flowers / m^2)	110.58	0.61
Flower richness	15.57	0.34
Dorycnium pentaphyllum flowers / m^2	8.87	1.15
Rosmarinus officinalis flowers / m^2	52.01	0.74
Thymus vulgaris flowers / m^2	49.11	1.03
Cistus spp. flowers / m^2	0.59	1.54
Nesting resources		
% Bare soil	13.1	0.71
% Dead wood	0.1	2.54
% *Quercus coccifera* cover	12.9	1.08
% *Ampelodesmos mauritanica* cover	6.1	1.08
Number of holes in rocks / m^2	0.55	1.28
Number of snail shells / m^2	0.19	1.40

Table A3 List of flowering plant species.

Flower species
Allium roseum
Allium sphaerocephalon
Anagallis arvensis
Antirrhinum barrelieri
Aphyllanthes monspeliensis
Argyrolobium zanonii
Aristolochia pistolochia
Asperula cynanchia
Biscutella laevigata
Centaurea linifolia
Centaurea montana ssp. semidecurrens
Centaurium erythraea
Cistus albidus
Cistus monspeliensis
Cistus salviifolius
Clematis flammula
Convolvulus lanuginosus
Coris monspeliensis
Cytisophyllum sessilifolium
Dorycnium hirsutum
Dorycnium pentaphyllum
Echium vulgare
Erica multiflora
Euphorbia characias
Euphorbia flavicoma
Fumana ericifolia
Fumana ericoides
Fumana laevipes
Fumana laevis
Galium aparine
Galium palustre
Genista scorpius
Gladiolus illyricus
Helianthemum oelandicum spp.italicum
Helichrysum stoechas
Hippocrepis comosa
Hypericum perforatum

Lathyrus

Leuzea conifera

Linum strictum

Lithospermum fruticosum

Lonicera implexa

Muscari neglectum

Narcissus assoanus

Ononis minutissima

Orobanche latisquama

Phlomis lychnitis

Polygala rupestris

Potentilla sp.

Psoralera bituminosa

Ranunculus gramineus

Rosmarinus officinalis

Rubia peregrina

Scorpiurus muricatus

Sedum sediforme

Sideritis hirsuta

Teucrium chamaedrys

Thalictrum tuberosum

Thesium divaricatum

Thymus vulgaris

Torilis arvensis

Vicentoxicum hirundinaria

Vicentoxicum nigrum

Vicia cracca

Table A4 Model selection based on Akaike's Information Criterion (AIC).

Dependent variable	Independent variables									k	AIC	ΔAIC
	Intercept	Roff	Tvul	Dpen	Cis	Dw	Bs	Hinr	snail			
Species richness	x	x	x	x	x	x	x	x	x	9	137.84	10.45
	x	x	x	x	x	x		x	x	8	136.14	8.75
	x	x	x		x	x		x	x	7	134.46	7.07
	x	x	x		x			x	x	6	133.29	5.9
	x		x		x			x	x	5	132.07	4.68
	x		x		x			x		4	130.52	3.13
	x				x			x		3	129.07	1.68
	x				**x**					**2**	**127.39**	**0.00**
	x									1	128.10	0.71
Abundance	x	x	x	x	x	x	x	x	x	9	11.37	10.91
	x	x	x	x	x		x	x	x	8	9.38	8.92
	x		x	x	x		x	x	x	7	7.41	6.95
	x		x	x	x		x		x	6	5.46	5.00
	x		x	x	x				x	5	3.64	3.18
	x		x	x	x					4	1.76	1.30
	x		**x**		**x**					**3**	**0.46**	**0.00**
	x				x					2	0.73	0.27
	x									1	5.94	5.48

Analyses of the relationship of bee species richness and abundance (n=21 plots) with flower and nesting resource variables. Variables included in each model are marked with an x. The selected model is in bold, (k) is the number of parameters in the model and (ΔAIC) is the difference in AIC between the selected model and the given model. Independent variables are density of *R. officinalis* flowers –Roff-; *T. vulgaris* flowers –Tvul-, *D. pentaphyllum* flowers –Dpen- , *Cistus* spp. flowers –Cis-, presence of dead wood – Dw-, % of bare soil –Bs-, number of holes in rocks – Hinr- and number of vacant snail shells – snail-.

Appendix B

Table B1 Cavity-nesting bee and wasp species (hosts) with their codes.

Code	Species	Bee/wasp
H01	*Ampulex ruficollis*	Wasp
H02	*Ancistrocerus longispinosus*	Wasp
H03	*Anthidium florentinum*	Bee
H04	*Anthidium nigricolle*	Bee
H05	*Auplopus carbonarius*	Wasp
H06	*Chelostoma edentulum*	Bee
H07	*Diodontus sp.1*	Wasp
H08	*Dipogon sp.1*	Wasp
H09	*Dipogon sp.2*	Wasp
H10	*Dipogon sp.3*	Wasp
H11	*Eumenidae sp.1*	Wasp
H12	*Eumenidae sp.2*	Wasp
H13	*Eumenidae sp.3*	Wasp
H14	*Euodynerus sp.1*	Wasp
H15	*Heriades crenulatus*	Bee
H16	*Hoplitis adunca*	Bee
H17	*Hylaeus hyalinatus*	Bee
H18	*Hylaeus taeniolatus*	Bee
H19	*Isodontia mexicana*	Wasp
H20	*Megachile apicalis*	Bee
H21	*Megachile ericetorum*	Bee
H22	*Megachile rotundata*	Bee
H23	*Nitela fallax*	Wasp
H24	*Nitela sp.1*	Wasp
H25	*Nitela truncata*	Wasp
H26	*Osmia bicornis*	Bee
H27	*Osmia caerulescens*	Bee
H28	*Osmia latreillei*	Bee
H29	*Osmia melanogaster*	Bee
H30	*Osmia nasoproducta*	Bee
H31	*Osmia submicans*	Bee
H32	*Osmia tricornis*	Bee
H33	*Passaloecus gracilis*	Wasp
H34	*Passaloecus pictus*	Wasp
H35	*Pison atrum*	Wasp
H36	Pompilidae *Gen. sp.1*	Wasp
H37	*Psenulus fuscipennis*	Wasp
H38	*Solierella compedita*	Wasp

H39	*Solierella sp.1*	Wasp
H40	*Trypoxylon sp.1*	Wasp
H41	*Trypoxylon sp.2*	Wasp

Table B2 Parasite, parasitoid, cleptoparasite and scavenger species associated to trap-nesting hosts , their codes and type of interaction.

Code	Species	Order	Interaction type
P01	*Anthrax anthrax*	Diptera	Parasitoid
P02	*Anthrax sp.2*	Diptera	Parasitoid
P03	*Calliphoridae sp.1*	Diptera	Parasitoid
P04	*Chaetodactylus osmiae*	Sarcoptiforme	Cleptoparasite
P05	*Chalcis sp.1*	Hymenoptera	Parasitoid
P06	*Chrysis ignita*	Hymenoptera	Parasitoid
P07	*Chrysura sp.1*	Hymenoptera	Parasitoid
P08	*Coelioxys echinata*	Hymenoptera	Cleptoparasite
P09	*Cystomutilla sp.1*	Hymenoptera	Parasitoid
P10	*Gasteruption sp.1*	Hymenoptera	Parasitoid
P11	*Hedycridium sp.1*	Hymenoptera	Parasitoid
P12	*Hybomischos sp.1*	Hymenoptera	Parasitoid
P13	*Leucospis dorsigera*	Hymenoptera	Parasitoid
P14	*Melittobia acasta*	Hymenoptera	Parasitoid
P15	*Zonitis immactulata*	Coleoptera	Cleptoparasite
P16	*Miltogramma spp.*	Diptera	Parasitoid
P17	Mutillidae *Gen. sp.1*	Hymenoptera	Parasitoid
P18	*Omalus sp.1*	Hymenoptera	Parasitoid
P19	*Omalus sp.2*	Hymenoptera	Parasitoid
P20	Pteromalidae *Gen. sp.1*	Hymenoptera	Parasitoid
P21	*Sapyga quinquepunctata*	Hymenoptera	Cleptoparasite
P22	*Toxophora fasciculata*	Diptera	Parasitoid
P23	*Trichodes leucopsideus*	Coleoptera	Predator
P24	*Trichrysis cyanea*	Hymenoptera	Parasitoid
P25	*Trogoderma sp.1*	Coleoptera	Scavenger
P26	*Xorides sp.1*	Hymenoptera	Parasitoid