Spatial variation in plant-pollinator relationships: consequences on pollination function

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“When you talk, you are only repeating what you already know. But if you listen,
you may learn something new”

The Dalai Lama

Agraïments

És molt difícil resumir gairebé 5 anys de la vida d'una persona en un full d'agraïments. Les coses canvien, jo mateixa sóc una persona completament diferent de la jove innocent que va començar la tesi. Ha sigut un periode molt intens de creixement personal i aprenentatge continu. Massa gent ha tingut rellevància en la meva vida durant aquest període com per poder-los nombrar. A tots, gràcies.

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Abstract

Patterns of spatial variation in species communities have been usually studied at large spatial scales, comparing different habitats, where the distribution of species is mostly related to environmental factors. However, even within a habitat is pobible found important levels of spatial variability at the local scale. Variability in species occurrence and abundance across space will affect interaction structure because species differ in their functional traits and therefore in the number and identity of species with which they may interact. Furthermore, within-habitat spatial heterogeneity in species community structure may have far-reaching ecological and evolutionary consequences because these variability in the spatial distribution of the species affects the functioning of ecosystems. Pollination is a key function in terrestrial ecosystems, with almost 90% of the angiosperm species depending on animal pollinators for sexual reproduction. The general objective of this thesis is to analyze the relationship between plant-pollinator community structure, ecological function, and interaction patterns across space.

First, we aim to highlight the degree of spatial variation at the local scale in a plant-pollinator community across a uniform habitat and to understand the deterministic factors affecting this variation. For this purpose we analyse the spatial heterogeneity of a community of flowering plants and their pollinators within an uninterrupted Mediterranean scrubland. Also, since the study area is a honey producing area we want to know whether wild pollinator communities were affected by the massive presence of honey bees. We show that, in spite of the apparent physiognomic uniformity, both flowers and pollinators display high levels of heterogeneity, resulting in a mosaic of idiosyncratic local communities. Resources appear to be non-limiting, and honey bee visitation rate did not affect the distribution of the wild pollinator community. Overall, our results show that the spatial variation of the flower community is not the main predictor of the pollinator community, indicating that other factors besides flower community composition are important to explain pollinator distribution at the local scale.

Second, we take advantage of the spatial variability in local pollinator community across the same Mediterranean scrubland and analyse its effects on pollination function and reproductive success in the gynodioecious *Thymus vulgaris*. Homospecific pollen deposition in the female morph was positively related to pollinator richness and to the proportion of visits by honey bees and wild bees, while heterospecific pollen deposition was not affected by the structure or composition of the pollinator assemblage. Homospecific pollen deposition in the hermaphrodite morph was negatively influenced by pollinator visitation rate. We found no effect of the pollinator assemblage on plant reproductive success in either of the two morphs, showing pollination appears to be adequate in most of the populations sampled. Our study shows that local variation in pollinator composition may have important consequences on pollination function, especially on plants that are more dependent on pollinators.

Finally, we aim to establish whether there is a relationship between floral traits and pollinator visitation in natural communities, specifically focusing on floral colour. Although pollinators show innate colour preferences, the view that the colour of a flower may be considered an important predictor of its main pollinators is highly controversial because flower choice is highly context-dependent. We found consistent associations between pollinator groups and certain colours, and these associations matched predictions of the pollination syndrome theory. However, flowers with similar colours did not attract similar pollinator assemblages. The explanation for this paradoxical result is that most flower species are pollinator generalists. We conclude that although pollinator colour preferences seem to condition plant–pollinator interactions, the selective force behind these preferences has not been strong enough to mediate the appearance and maintenance of tight colour-based plant–pollinator associations.

Contents

Introduction 1

1.1 Spatial variability of ecological communities

The differences in the species present in each part of the world, with every territory having their own well-known and iconic species, is what makes this planet so fascinating. The first ecologists not only got trapped into the wonders of nature, but rather started asking questions about it. The main question was why the species were present only on certain places and not in others. They asked about the patterns and magniture of variability of species distribution through space and the mechanisms acting underneath. In their handbook of Ecology, Begon and collaborators (2006) state "This book is about the distribution and abundance of different types of organisms, and about the physical, chemical but especially the biological features and interactions that determines these distributions and abundances". These patterns have been usually studied at large spatial scales (biogeographical, regional), where the distribution of species is mostly related to environmental factors: temperature, rainfall, sunlight (Fernández-Palacios and de Nicolás, 1995; Lite et al., 2005). However, ecosystems worldwide have been shown to present a high degree of heterogeneity at different spatial scales (Levin, 1992), and even the most uniform habitats show important levels of spatial variability in environmental conditions at one scale or another. Therefore, local differences in species assemblages are expected even in continuous habitats.

Many factors, including microclimatic conditions, biotic interactions such as com¬petition and facilitation, dispersal capacity, historical events and disturbance regimes have been shown to be important in explaining heterogeneity in community composition at local scales (Fukami et al., 2010). The relative importance of the factors that determine species distribution may differ considerably between the group of organisms, depending on their degree of mobility or dispersion. Due to their sessile condition, plants are highly dependent on local environmental variables for their establishment. Plant distribution is variable at the scale of centimeters due to microsite differences in conditions, along with microenvironmental conditions limiting seedling survival and performance (Eriksson and Ehrlén, 1992; Jones and del Moral, 2005).

As opposed to plants, most animals are mobile organisms and are not clearly limited by microsite conditions. Nevertheless, their spatial distribution is far from homogeneous due to several factors, mostly to the spatial distribution of their feeding and nesting resources (Siemann et al., 1998; Schaffers et al., 2008; Ross et al., 2017; Potts et al., 2005). The quantity of resources present at the local scale will determine the size of the community that can be sustained at each specific site, and whether resources are limiting or not would determine if species have to compete for the resources. When resources are limited, consumers would compete for those scarce resources and they are expected to follow an ideal free distribution. In a connected landscape without barriers limiting the dispersion of individuals across space, the ideal free distribution hypothesis stipulates that consumers should distribute themselves according to the amount of resources (Fretwell and Lucas, 1969). Consumers in the poorer and more exploited areas would travel to the areas with a higher amount of resources, until the community reach an equilibrium where the ratio of resources per individual is the same across space. However, deviations from the ideal free distribution equilibrium have been found, where resource poor areas were overexploted and resource rich areas were underexploted (Kennedy and Gray, 1993). This hypothesis overlook species specificity in the use of resources, which can limit the potential distribution of species trhough space. Under competition for resources, generalist species may feed upon alternative resources, but specialist species depend on the distribution of their preferred resources which may be limited or patchy. If species don't find enough resources at certain sites, they may move elsewhere in order to forage.

1.1.1 Spatial variability of plant-pollinator communities

Pollinators are highly mobile herbivores, and therefore an appropriate group of organisms to study local spatial distri¬bution in relation to plant resource distribution. In a continuous habitat without any phisical barriers limiting dispersion of individuals, there is no expectation on any impediment against free movement of pollinators. Nevertheless, pollinator distribution would never be completely homogeneous, because they depend upon floral resources. Even when dealing with a homogeneous landscape flower spatial distribution is not homogeneous, promoting spatial differences in floral resource availability and the pollinator population that could be supported by those. It has been shown that there is spatial variation in the species composition of plant and pollinator communities across one habitat (Janovský et al., 2013; Carstensen et al., 2014; Torné-Noguera et al., 2014). Both plant and pollinator communities display heterogeneous patterns, and this variation is both due to differences in the species composition between sites and to differences in species' abundances.

Distribution of pollinator abundances across space depends on floral resources and nesting resources. First, quantity of resources is primordial, if resources are limiting or not. When floral resources are limiting, pollinator abundances will be distributed according an ideal free distribution. Pollinator abundance will be proportional to resource abundance, promoting a scenario under which competition between pollinators is minimum and resources per individual are maximized for all pollinators. This way, visitation rate will be constant all over the space because there will be always the same ratio of pollinators per available flower. Furthermore, pollinator community is known to be profoundly affected by floral composition, not only its abundance. A richer plant community will open up a greater range of different resources that could be exploited by a greater number of pollinator species differing in behavioural or morphological traits (Fründ et al., 2010; Dorchin et al., 2018; Weiner et al., 2011; Steffan-Dewenter and Tscharntke, 2001). The local floral composition will affect the number and identity of pollinator species present, and their behaviour: most pollinator species are generalist, but may show local specialization in flower choice which may differ among localities (Westrich and Schmidt, 1987; Petanidou et al., 2008; Fründ et al., 2010). However, resource availability is not the only factor influ¬encing the spatial distribution of pollinators. Some pollinator groups feed on floral resources only during the adult stage and their distribution may be strongly conditioned by the avail¬ability of dietary resources for their offspring. In bees and predatory wasps, nesting resources may also affect species distribution across space (Potts et al., 2005).

On the other hand, interactions between pollinator species is also an important force driving the structure and composition of the pollinator community and their behaviour. Interferences between pollinator species lead to a redistribution of the plant-pollinator interactions, through direct competition (aggressive encounters) or resource consumption (floral resources or nesting sites) (Inouye, 1978). Lázaro and Totland (2010) shown that the presence of other pollinators may affect individual behaviour, but the relationship between pollinator landscape and foraging behaviour is complex and dependent on the floral landscape. In Mediterranean areas beekeeping is a common practice, resulting in an overwhelming dominance of the honey bees over every wild pollinator species. This high abundance, together with the fact that honey bees are highly efficient collecting resources, may lead to the redistribution of the interactions of the wild pollinator species. Honey bees have actually been shown to modify foraging behaviour of wild pollinators (Walther-Hellwig et al., 2006). However, not all species are affected the same way by the honey bee presence. Some of them are more sensitive to resource availability and move to other places where honey bee density is lower, promoting a structured species change in communities as a function of the density of honey bees present. Honey bees have been previously shown to change bee species composition across an habitat (Herbertsson et al., 2016; Torné-Noguera et al., 2016), where the presence of most sensitive species is affected first.

1.1.2 Species traits and interaction patterns

Spatial variation in plant and pollinator communities has direct consequences over plant-pollinator interactions. Species identity and composition of an area will affect interaction structure because species differ in their functional traits and therefore in the number and identity of species with which they may interact (trait matching, Vázquez et al. 2009; Bartomeus et al. 2016). Spatial variation in the plant community leads to pollinators facing differences in foraging context across space, and the species composition of the sites determines plant-pollinator interaction patterns. A classical theory in pollination are pollination syndromes, a framework in which flowers adapt to their most efficient pollinators via the selective pressures exerted by them, and change their traits gradually to attract more efficiently their major pollinators (Faegri and Van der Pijl, 1979). The pollination syndrome theory is framed along general pollinator groups, such as bees, flies and beetles and states that all the pollinators from the same group visit preferentially flowers of the same type, and so all the flowers visited by them will be under the same selective pressures and converge on traits being similar to each other. Pollination syndromes have been under strong debate, principally since the publication of the seminal paper by (Waser et al., 1996). They confronted the most accepted vision that assumed most plant-pollinator interactions were specialist, with pollination syndromes as a framework, and demonstrated that many pollinators are generalist.

Most pollinator species have been shown to be generalist on their foraging habits, visiting multiple plant species for their floral resources (Herrera, 1996; Waser et al., 1996). However, most pollinators show floral preferences, which implies that pollinator species tend to visit some plant species more frequently than others. Flower preferences can be modified through learning and are flexible, so pollinator species may interact differently in different environments or community contexts (Tylianakis et al., 2007; Fründ et al., 2010; Janovský et al., 2013). At the very beginning these preferences act like a framework to facilitate the pollinators the location of flowers in the community (Lunau, 1992). Even though, most of the pollinator species try flowers beyond their innate preferences, because of the composition of the local community they are in or because there they cannot find their favourite resources. Then, depending on the rewards obtained on the different kinds of flowers, they learn to link some traits with energy obtained, and they overrun their innate flower preferences with new learnt flower preferences as a function of what was more profitable from every individual point of view (Gumbert, 2000; Goyret et al., 2008). Each individual then presents a different set of learnt preferences, depending on the learning and physiological limitations of the species, because every individual had interacted in a different way with the environment.

However, the ultimate mechanisms through which the flowering community is affecting pollinator communities are still unclear. Floral traits have been thought to be important in pollinator attraction, because these are the traits that plants use to advertise their pollen and nectar resources. Pollinators use a diversity of flower traits such as odour, shape, size and colour, as cues to locate pollen-nectar sources and discriminate between different flower species (Chittka and Raine, 2006). Ultimate flower choice undoubtedly depends on a combination of stimuli, but floral colour has been shown to be one of the most important determinants of pollinator attraction (Dafni et al., 1990; Heiling et al., 2003; Ômura and Honda, 2005; Dötterl et al., 2014).

1.2 Biodiversity-ecosystem function

Within-habitat spatial heterogeneity in species community structure may have far-reaching ecological and evolutionary consequences. Variability in species occurrence and abundance across space lead to changes in the interaction networks that are established in the different localities, ultimately affecting the functioning of ecosystems (Hooper et al., 2005).

There is a broad theoretical corpus about the effects of biodiversity on ecosystem functioning, with most evidence showing a positive relationship (Hooper et al., 2005; Balvanera et al., 2006). That is, richer and more complex communities can sustain higher levels of a number of ecosystem functions. This positive relationship can be explained by two not mutually exclusive mechanisms: functional complementarity and selection effect (Loreau and Hector, 2001; Loreau, 2001; Díaz and Cabido, 2001). Functional complementarity assumes that the species of a community differ in the use of resources, so multiple species would lead to a more efficient resource use though time and space (Loreau, 2001). The other mechanism, selection effect, assumes that not all species are equally efficient, and richer communities will increase their chances of incorporating highly efficient species which will have a strong effect on ecosystem function (Díaz and Cabido, 2001).

However, most of the studies to date encompass single trophic levels, mainly primary producers, but many ecosystem functions involve the interactions between two or more groups of organisms. Knowledge of biodiversity changes on ecosystem functions involving mutualistic relationships is still vague (Schleuning et al., 2015), and the responses of different ecosystem functions involving mutualistic relationships to increasing diversity are highly variable (Hooper et al., 2005; Balvanera et al., 2006; Woodward, 2009; van der Plas, 2019).

1.2.1 Pollination as a key ecosystem function

Pollination is a key function in terrestrial ecosystems, with almost 90% of the angiosperm species depending on animal pollinators for sexual reproduction (Ollerton et al., 2011). The pollination function is currently threatened following well-documented pollinator declines (Biesmeijer, 2006; Potts et al., 2010). Understanding how this impoverishment may affect pollination function is essential in Mediterranean areas, in which patches of natural and agricultural land are intermixed forming a mosaic landscape, and in which crop pollination is often mostly dependent on populations of wild pollinators.

The consequences of the spatial variation of pollinator species is that the sessile organisms, the different flower species, face different pollinator assemblages through space. A good number of studies have documented important levels of spatial variability in pollinator composition for a given plant species (Herrera, 1988, 2005; Price et al., 2005; Gómez et al., 2007). Different approaches (empirical, modelling and experimental) have shown a positive relationship between pollinator diversity and plant reproductive success (Fontaine et al., 2006; Gómez et al., 2007; Perfectti et al., 2009; Albrecht et al., 2012). This positive relationship indicates complementarity effects of the different pollinator species through niche partitioning. The different pollinator species differ in their morphological and behavioural traits, and the combination of several species usually leads to an improvement of the services (Blüthgen et al., 2007). Nevertheless, the positive relationship shown in previous studies is not lineal but saturating (Albrecht et al., 2012; Fründ et al., 2013). Since many pollinator species are generalist on their feeding habits, there exist some degree of overlapping on the functional niche of the different species and not all combinations of species necessarily increase linearly the pollination function. Using simplified experimental communities, Fontaine et al. (2006) found that plant reproductive success increased when bumblebees plus hoverflies were present compared to either group alone. The mechanisms explaining such synergistic effects are discussed by (Blüthgen and Klein, 2011), which show that the extent of redundancy and complementarity affects the shape of the relationship between plant-pollinator diversity and pollination levels. So far, gaining understanding on the relationship between community structure and composition and pollination function at the community level is an urgent task.

1.3 Objectives

The general objective of this thesis is to analyze the relationship between plant-pollinator community structure, ecological function, and interaction patterns across space. There are two modules within this thesis: in the first part, we studied within-habitat spatial variation in plant-pollinator communities, and evaluated the consequences of this spatial variation on ecological function by measuring pollination levels. In the second part, we evaluated the consequences of the spatial variation of species identity and traits on plant-pollinator interaction patterns, specifically focusing on the relationship between pollinator visitation patterns and floral colour. The results obtained are shown in the chapters two to four of this thesis:

The aim of the **Chapter two** is to highlight the degree of spatial variation at the local scale in a plant-pollinator community across a uniform habitat and to understand the deterministic factors affecting this variation. Also, since the study area is a honey producing area we want to know whether wild pollinator communities are affected by the massive presence of managed honey bees. We ask the following questions: 1) how does pollinator distribution vary across space? 2) to what extent does pollinator distribution follow plant distribution? 3) are honey bees affecting the spatial distribution of wild pollinators?

The aim of the **Chapter three** is to analyse the effects of spatial variability of pollinator communities in a continuous landscape of Mediterranean scrubland on pollination and reproductive success in the self-compatible gynodioecious plant species *Thymus vulgaris* L. We ask whether the spatial variability of pollination function (deposition of homospecific and heterospecific pollen) and reproductive success (fruit and seed set) of the two morphs (female and hermaphrodite) can be explained by the spatial variability of pollinator abundance (visitation rates), pollinator richness and pollinator composition (measured as proportion of visits by the major pollinator groups). Working with a gynodioecious species allows us to compare these effects on two morphs with similar pollinator composition but contrasting levels of reliance on pollination for reproductive success.

The aim of the **Chapter four** is to establish whether there is a relationship between floral traits and pollinator visitation in natural communities, specifically focusing on floral colour. We ask two questions: 1) Do different pollinator groups show preferences for certain flower colours? 2) Do plant species with similar colours attract similar pollinator assemblages? Although apparently similar, these two questions address colour-pollinator relationships from two different and complementary perspectives. The first question addresses plant-pollinator relationships from the pollinators' point of view, and the second from the plants' perspective. The distinction is important because pollination syndrome theory relies on the assumption that plants with similar traits have evolved to attract similar pollinators.

Spatial variability in a plant-pollinator community across a uniform landscape

2

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Abstract

Large-scale spatial variability in plant-pollinator communities (e.g., along geographic gradients, across different landscapes) is relatively well understood. However, we know much less about how these communities vary at small scales within a uniform landscape. Plants are sessile and highly sensitive to microhabitat conditions, whereas pollinators are highly mobile and, for the most part, display generalist feeding habits. Therefore, we expect plants to show greater spatial variability than pollinators. We analysed the spatial heterogeneity of a community of flowering plants and their pollinators in 40 plots across a 40 Km^2 area within an uninterrupted Mediterranean scrubland. We recorded 3577 pollinator visits to 49 plant species. The pollinator community (170 species) was strongly dominated by honey bees (71.8% of the visits recorded). Flower and pollinator communities showed similar beta-diversity, indicating that spatial variability was similar in the two groups. We used path analysis to establish the direct and indirect effects of flower community distribution and honey bee visitation rate (a measure of the use of floral resources by this species) on the spatial distribution of the pollinator community. Wild pollinator abundance was positively related to flower abundance. Wild pollinator visitation rate was negatively related to flower abundance, suggesting that floral resources were not limiting. Pollinator and flower richness were positively related. Pollinator species composition was weakly related to flower species composition, reflecting the generalist nature of flower-pollinator interactions and the opportunistic nature of pollinator flower choices. Honey bee visitation rate did not affect the distribution of the wild pollinator community. Overall, we show that, in spite of the apparent physiognomic uniformity, both flowers and pollinators display high levels of heterogeneity, resulting in a mosaic of idiosyncratic local communities. Our results provide a measure of the background of intrinsic heterogeneity within a uniform habitat, with potential consequences on low-scale ecosystem function and microevolutionary patterns.

Key words: spatial variation, beta-diversity, mosaic community, plant-pollinator interactions, local scale-networks, honey bees.

2.1 Introduction

Understanding how species and communities vary across space and the factors underlying spatial variability is a central topic in community ecology (Ricklefs, 1987; Levin, 1992). At large spatial scales (global, continental, regional), differences in community composition and structure are clear, mostly driven by climatic and productivity factors (e.g., along latitudinal or altitudinal gradients) and differences in land use resulting in contrasting landscapes (e.g., forests vs. grasslands vs. croplands) (Fernández-Palacios and de Nicolás, 1995; Lite et al., 2005; Batllori et al., 2009). Each landscape is expected to host a more or less singular community, defined as a group of populations of different species that coexist in space and time (Vellend, 2010), whereby all individuals have the same probability to interact (Holyoak et al., 2005). Therefore, within a landscape we would expect few differences in local community composition and structure across space, especially in highly homogeneous landscapes lacking physical or ecological barriers and therefore offering no obvious obstacles to the movement of individuals and species (Post et al., 2007). However, many factors, including microclimatic conditions, biotic interactions such as competition and facilitation, dispersal capacity, historical events, and disturbance regimes have been shown to be important in explaining heterogeneity in community composition at local scales (Fukami et al., 2010). The relative importance of the factors that determine species distribution may differ considerably between sessile (e.g., plants) and mobile organisms. Plants are highly sensitive to microhabitat conditions, so that differences at the scale of a few centimetres in soil depth or sunlight exposure may condition plant establishment and survival (Eriksson and Ehrlén, 1992; Jones and del Moral, 2005). For this reason, plant species composition is expected to be heterogeneous across space even at very small scales (Whittaker, 1960; Field et al., 2009; Grace et al., 2011). By contrast, most animals are highly mobile, which allows them to functionally occupy a larger area at the individual level, and to spread more rapidly at the population level, thus potentially displaying a more homogeneous spatial distribution at small scales (Strong et al., 1984).

Herbivorous animals are spatially linked to the plant species they depend on. Therefore, at the community level, a spatial correspondence between plant and herbivore community composition and richness is expected (Siemann et al., 1998; Blake et al., 2003; Schaffers et al., 2008; Ross et al., 2017). At the population level, if plant resources are limiting, we would expect herbivores to distribute themselves across space proportionally to resource availability, following an ideal free distribution of herbivory rate and thus minimizing competition for resources, promoting a homogeneous use of resources across space (Fretwell and Lucas, 1969). On the other hand, if plant resources are not limiting, we would expect no clear relationship between herbivore abundance and plant abundance, or even a decrease in herbivore frequency per plant resource unit, as increases in plant abundance would not be matched by increases in herbivore abundance (Kennedy and Gray, 1993). In any case, the extent to which the local spatial distribution of herbivores follows that of plants is expected to be greater for specialist than for generalist herbivores.

Pollinators are highly mobile herbivores, and therefore an appropriate group of organisms to study local spatial distribution in relation to plant resource distribution. On the one hand, most pollinators are considered to be generalist and opportunistic in their foraging habits (Waser et al., 1996), as exemplified by pollinator species foraging on different plant species in different floral contexts (Tylianakis et al., 2007; Fründ et al., 2010; Janovský et al., 2013). On the other hand, even generalist pollinators such as polylectic bee species (those collecting pollen from various plant families) have been shown to have certain flower preferences, and may locally restrict their visits to one or a few plants (Westrich and Schmidt, 1987; Petanidou et al., 2008; Fründ et al., 2010). The fact that some pollinators can be very generalist in their flower choice whereas others show strong flower preferences, makes predictions about the spatial distribution of pollinators complicated.

In this study we analyse the local heterogeneity of a community of pollinators in relation to that of flowering plants in a homogeneous habitat, defined as a habitat dominated by a vegetation type lacking clear abiotic gradients and/or obvious discontinuities. In addition to interactions with plants, the distribution of pollinator communities may also be influenced by interactions among pollinators. Dominant species in general, and managed species (which tend to occur at disproportionately high abundances) in particular, may affect the structure of communities through competitive pressure (Kunte, 2008; Cornelissen et al., 2013). In our study area, beekeeping is a common practice (Torné-Noguera et al., 2016). Due to their ability to recruit nest-mate foragers, honey bees (*Apis mellifera*) are able to exploit the most abundant floral resources very efficiently, showing local flower specialization (Lázaro and Totland, 2010), and potentially outcompeting other pollinator species (Torné-Noguera et al., 2016; Henry and Rodet, 2018; Valido et al., 2019). Competitive pressure may lead to niche differentiation between honey bees and wild pollinators, whereby honey bees monopolize the most highly-rewarding floral resources forcing wild pollinators to forage on less-preferred plant species (Walther-Hellwig et al., 2006; Geslin et al., 2017). In some cases, honey bee density has been shown to affect wild bee species composition, with special impact on certain, most sensitive, species (Herbertsson et al., 2016; Torné-Noguera et al., 2016).

The aim of this study is to understand the factors determining local variation in a plant-pollinator community across a uniform habitat. We ask the following questions: 1) how does pollinator distribution vary across space? 2) to what extent does pollinator distribution follow plant distribution? 3) are honey bees affecting the spatial distribution of wild pollinators? Because pollinators are highly mobile and, for the most part, can exploit a variety of floral resources, we expect greater heterogeneity in plant than in pollinator community distribution. We also expect pollinator abundance, richness and composition to follow plant community structure. Finally, we expect honey bee visitation rate to have a negative effect on wild pollinator visitation rate and to promote changes in wild pollinator composition across space.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in spring 2015 in the Garraf Natural Park near Barcelona (NE Spain, 41° 17’ 34” N, 1° 53’ 35.8” E). The Park is located in an area with mild topography (slopes < 20%) composed of small hills and valleys. Soils are poorly developed, derived from karstified limestone bedrocks (IUSS Working Group, 2015). Originally, the area was occupied by mixed pine (*Pinus halepensis*) and oak (*Quercus spp.*) Mediterranean forests (Folch, 1981; Riera-Mora and Esteban-Amat, 1994). However, a long history of recurrent wild fires has promoted a continuous and dense scrubland (< 2 m high) dominated by *Quercus coccifera*, *Pistacia lentiscus*, *Rosmarinus officinalis*, and *Thymus vulgaris* (Riera-Mora and Esteban-Amat, 1994; Riera-Mora, 1998; Lloret et al., 2003). The area can be considered uniform at the landscape scale, lacking clear discontinuities in topography, structural vegetation features or edaphic factors. The climate is Mediterranean, with a strong sea influence, and a mean annual temperature of 16-17ºC. Summers are dry and most precipitation (580-650 mm annually) occurs in spring (March-April) and autumn (September-November) (Ninyerola et al., 2000).

We selected 40 plots of 30 x 40 m across an area of nearly 40 Km^2 (Fig. A.2.1). Mean distance between nearest plots was 760 m (range: 520-1400 m). This distance is assumed to be representative of foraging distances of most pollinator species (Zurbuchen et al., 2010; Dorchin et al., 2013). Plot altitude ranged between 171 and 551 m. Exploratory analyses showed that flower and wild pollinator communities were not affected by altitude.

2.2.2 Field sampling

Each plot was surveyed on a single day. Although this sampling method does not account for variation in plant-pollinator interactions across time, it is a practical way to survey a high number of plots under comparable phenological conditions (Fründ et al., 2010; Weiner et al., 2011). We surveyed 2-4 plots per day between April 2nd and April 17th. This period corresponds to the flowering peak of the scrubland community (Flo et al., 2018). The field team was composed of 8 people trained to identify the pollinator fauna of the study area. We worked in pairs. Pair composition was changed every day to avoid potential observer bias.

Within each plot, we established four parallel 40 m x 1 m transects separated by 10 m. To characterize flower composition (number of flowers of each plant species), we counted the number of open flowers in the four transects at the beginning of each survey. To sample plant-pollinator interactions we conducted three surveys starting at 11 a.m., 1 p.m. and 3 p.m., respectively. Ambient temperature was registered at the beginning and at the end of each survey. During each survey, two observers walked slowly along the transects and recorded all pollinators observed contacting the reproductive organs of a flower. Some pollinators could be unmistakably identified on site. Others were captured for identification in the laboratory. Most individuals were identified to the species level using identification keys and an extensive reference collection from the study area. A few pollinators could only be identified to the morphospecies level. From these surveys, each plot was characterized based on its pollinator richness (number of species observed interacting with flowers), pollinator abundance (number of interactions recorded), pollinator composition (abundance of each pollinator species), and pollinator visitation rate (number of interactions recorded / 1000 open flowers). We use visitation rate as a measure of the use of floral resources by pollinators.

2.2.3 Spatial distribution of the flower and pollinator communities

Spatial variation in species abundance and richness of the flower and pollinator communities and of pollinator visitation rate was quantified using the coefficient of variation among plots. To check whether these variables were spatially autocorrelated we conducted Moran's I tests with the "ape" package (Paradis et al., 2004) in R version 3.4.4 statistical environment (R Core Team 2018). All analyses involving the pollinator community were conducted twice, once including all species and once excluding honey bees (which were, by far, the most frequent flower visitor, see Results).

Weather in general and temperature in particular affects pollinator activity (Vicens and Bosch, 2000; Willmer and Stone, 2004). In our dataset, maximum ambient temperature (highly correlated to mean temperature; Pearson $r = 0.96$, $p < 0.001$) of the survey day affected positively wild pollinator richness, wild pollinator visitation rate and honey bee visitation rate (general linear models; $n = 40$, $df = 38$, all $p < 0.04$). To extract the effect of temperature, Moran's I tests for these three variables were conducted using the residuals of these linear models. Wild pollinator abundance and honey bee abundance were not affected by temperature (general linear models; $n = 40$, $df = 38$, all $p > 0.1$).

To describe variation in flower and pollinator community composition across sites we calculated beta-diversity between plots with the Bray-Curtis quantitative dissimilarity index (Anderson et al., 2011) using the "betapart" package (Baselga et al., 2018) for R. As pollinator composition could also be affected by temperature, we performed Mantel tests between pollinator composition (including and excluding honey bees) and differences in maximum temperature between plots. None of these tests were significant (all $p > 0.1$). As a result, temperature was not accounted for in pollinator composition autocorrelation analyses. To check for spatial autocorrelation of flower and pollinator community composition, we performed Mantel correlograms with 9999 permutations using the beta-diversity indices and the geographical distance between plots implemented in the "vegan" package (Oksanen et al., 2018) for R. In these analyses, sample size was 780 possible combinations of pairs of plots.

2.2.4 Determinants of spatial variation of the pollinator community

Our aim was to understand to what extent the local wild pollinator community was affected by the local flower community and by local honey bee visitation rates. Since honey bee visitation rate may also be affected by the flower community, we used a path analysis approach, which provides the magnitude, significance and causality of relationships among variables. In our case, flower community descriptors (independent or exogenous variables) may have both a direct and an indirect effect (through honey bee visitation rate, intervening or mediating variable) on wild pollinator community descriptors (dependent or endogenous variables). We ran separate models for wild pollinator abundance, richness and visitation rate as dependent variables. The models for wild pollinator abundance and visitation rate included overall flower abundance and maximum ambient temperature (see above) as exogenous variables, and honey bee visitation rate as a mediating variable, which in turn could be directly affected by flower abundance and temperature. The model for wild pollinator richness was similar but also included flower richness as an exogenous variable. We performed these analyses using the "piecewiseSEM" package (Lefcheck, 2016) for R. All variables were log-transformed to ensure normality and homoscedasticity of the residuals.

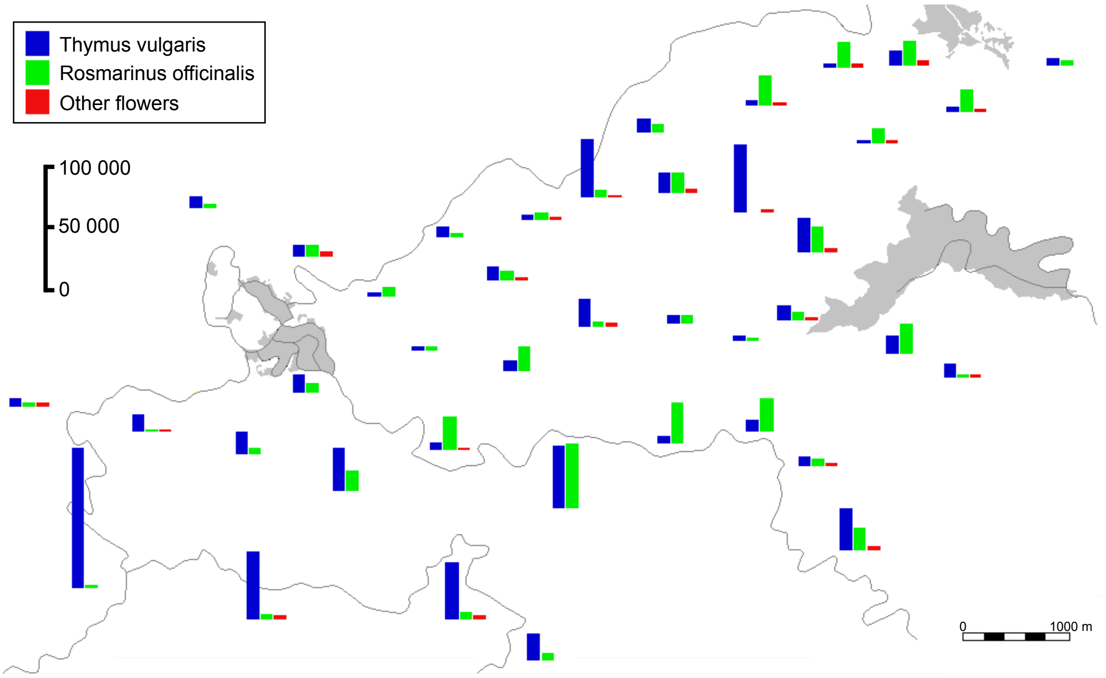

Figure 2.1: Flower community composition across the 40 plots surveyed in the Garraf Natural Park.

To analyse the effects of flower community composition on wild pollinator composition, we correlated the pairwise (plot x plot) dissimilarity (Bray-Curtis index) matrices of flower and pollinator composition. A strong relationship between the two matrices would indicate a high level of specialization in plant-pollinator interactions, whereas lack of relationship would indicate that pollinators act as generalists and adjust their floral choices to the local flower composition of each plot. As with the other descriptors of the pollinator community, honey bee visitation rate could affect wild pollinator composition. Certain pollinator species could be more affected by honey bee pressure than others (for example, large bees, which require larger amounts of floral resources, would be more likely to be affected by honey bee competition; Nielsen et al. 2012; Torné-Noguera et al. 2016). Likewise, temperature effects on pollinator foraging activity could be greater for some species than for others (Vicens and Bosch, 2000; Kühsel and Blüthgen, 2015). For these reasons, we generated a pairwise distance matrix of honey bee visitation rate and one of differences in maximum ambient temperature. Following previous studies facing the challenge of working with a path analysis approach with distance matrices, we used the methodology developed by Leduc and collaborators (Leduc et al., 1992; Paul and Anderson, 2013). We thus conducted partial Mantel tests between the sets of matrices involved in each analysis, and used partial Mantel correlation as a measure of the path coefficient and its significance. Because multiple tests are conducted in such analyses, we applied a Bonferroni correction.

2.3 Results

The flower community was composed of 49 species, and was strongly dominated by *Thymus vulgaris* (61.2% of the flowers recorded) and *Rosmarinus officinalis* (36.4%) (Fig. 2.1). We recorded 3577 individual pollinators visiting flowers across the 40 plots. The pollinator community comprised 170 species (38.2% dipterans, 23% bees, 17.7% coleopterans, 11.2% wasps, 5.9% lepidopterans and 4% other pollinators), and was clearly dominated by the honey bee (71.8% of the individual pollinators recorded) (Fig. 2.2). As many as 95 of the pollinator species were found in only one plot.

	Mean	CV (%)	Moran's I	p-value
Flower abundance	28277	83	-0.02	0.60
Flower richness	8	35	0.00	0.29
Pollinator abundance	89	65	0.01	0.17
Wild pollinators	25	64	-0.02	0.79
Honey bees	64	76	-0.01	0.44
Pollinator richness	14	41	-0.01	0.45
Pollinator visitation rate	3.96	58	-0.01	0.72
Wild pollinators	1.29	76	-0.01	0.56
Honey bees	2.68	69	0.01	0.12

Table 2.1: Mean, coefficient of variation and statistical outputs of Moran's I tests for flower and pollinator community descriptors in the Garraf Natural Park (n = 40 plots).

2.3.1 Spatial distribution of flower and pollinator communities

Flower abundance was highly variable across space, whereas flower richness showed much lower spatial variability (lower CV, Table 2.1). Flower composition was also highly variable across space. Beta-diversity of flower composition was 0.52 ± 0.21 (mean ± SD, Fig. 2.3). None of these community descriptors showed spatial autocorrelation (Moran's I, all $p > 0.1$, Table 2.1; Mantel correlogram, $p > 0.05$ for all distance classes, Fig. A.2.2a).

As with flowers, pollinator abundance (both wild pollinators and honey bees) showed greater spatial variation than pollinator richness (Table 2.1). Visitation rate (of both wild pollinators and honey bees) was also highly variable across space (Table 2.1). However, none of these variables showed spatial autocorrelation (Moran's I, all $p > 0.1$). Beta-diversity of pollinator composition was 0.56 ± 0.20 (similar to that of flower composition), but increased to 0.86 ± 0.09 when honey bees were excluded (Fig. 2.3). As mentioned, the honey bee was by far the most abundant pollinator species in the study area. Therefore the increase in homogeneity when this species was considered is not surprising. Pollinator composition did not show spatial autocorrelation at any distance class except at 900 m (the lowest distance class; including and excluding honey bees: $p = 0.009$ and 0.038, respectively, Fig. A.2.2b and Fig. A.2.2c). Even at that distance class,

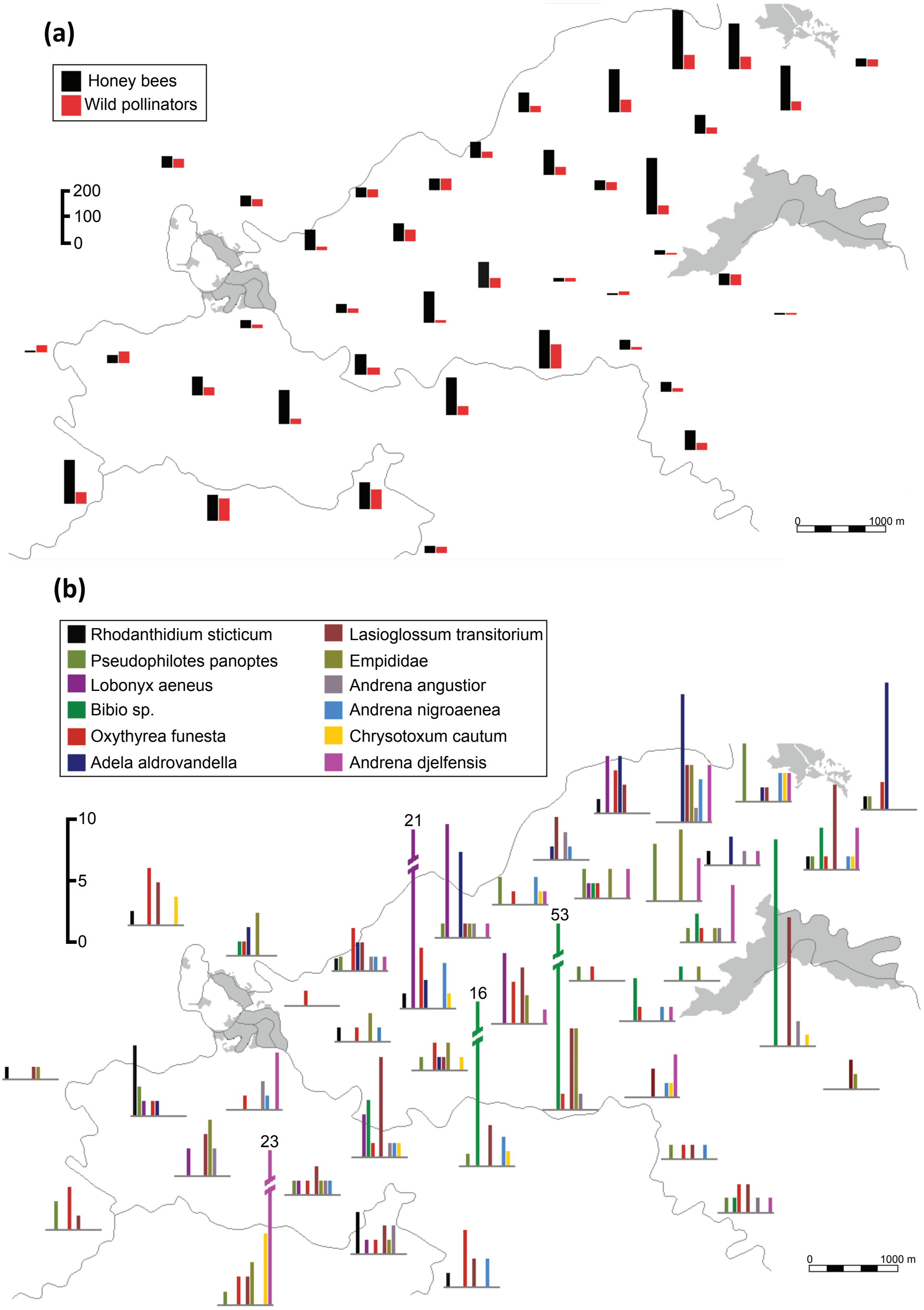

Figure 2.2: Pollinator community composition across the 40 surveyed plots in the Garraf Natural Park. (a) Honey bee and wild pollinator abundance. (b) Abundance of the 12 most abundant wild pollinator species.

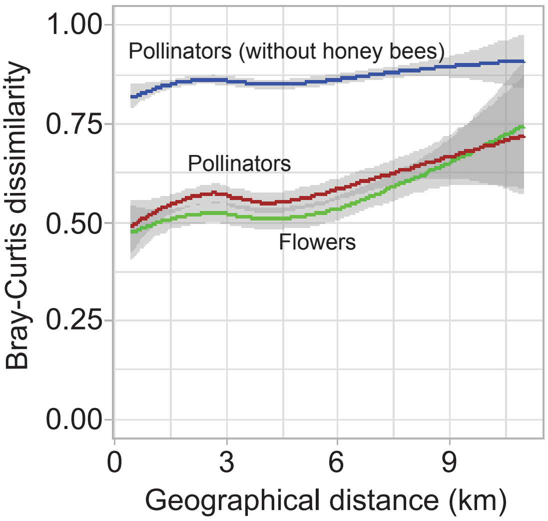

FIGURE 2.3: Relationship between geographical distance and differences in species composition, measured as beta-diversity (Bray–Curtis dissimilarity index) in the flower and pollinator (with and without honey bees) communities of the Garraf Natural Park. n = 780 plot pairs.

the correlation coefficients were low (Mantel correlation r, including honey bees = 0.10, excluding honey bees = 0.07).

2.3.2 Determinants of spatial variation of the pollinator community

Wild pollinator abundance was moderately related to both temperature and flower abundance (Fig. 2.4a). Honey bee visitation rate was strongly affected by temperature and less so by flower abundance. Wild pollinator visitation rate also increased with temperature, but displayed a moderate inverse association with flower abundance (Fig. 2.4b), indicating that, overall, floral resources were not a limiting factor for the wild pollinator community. Wild pollinator richness was positively and moderately related to flower richness, but was not related to flower abundance or temperature (Fig. 2.4c). Coefficient estimates of the relationships between wild pollinator abundance, wild pollinator visitation rate and honey bee visitation rate with temperature were high (Table A.2.1). Wild pollinator composition was positively related to flower composition (Fig. 2.4d), but the coefficient estimate was low. Honey bee visitation rate did not explain any wild pollinator variable, thereby indicating lack of competition between honey bees and wild pollinators at the community level (Table A.2.1).

2.4 Discussion

In this study we measured the variability of flower and pollinator communities across space in a continuous 40-Km^2 scrubland, thus providing a measure of the background of intrinsic heterogeneity within a uniform habitat. We found that variation of the pollinator community was at least as high as variation of the

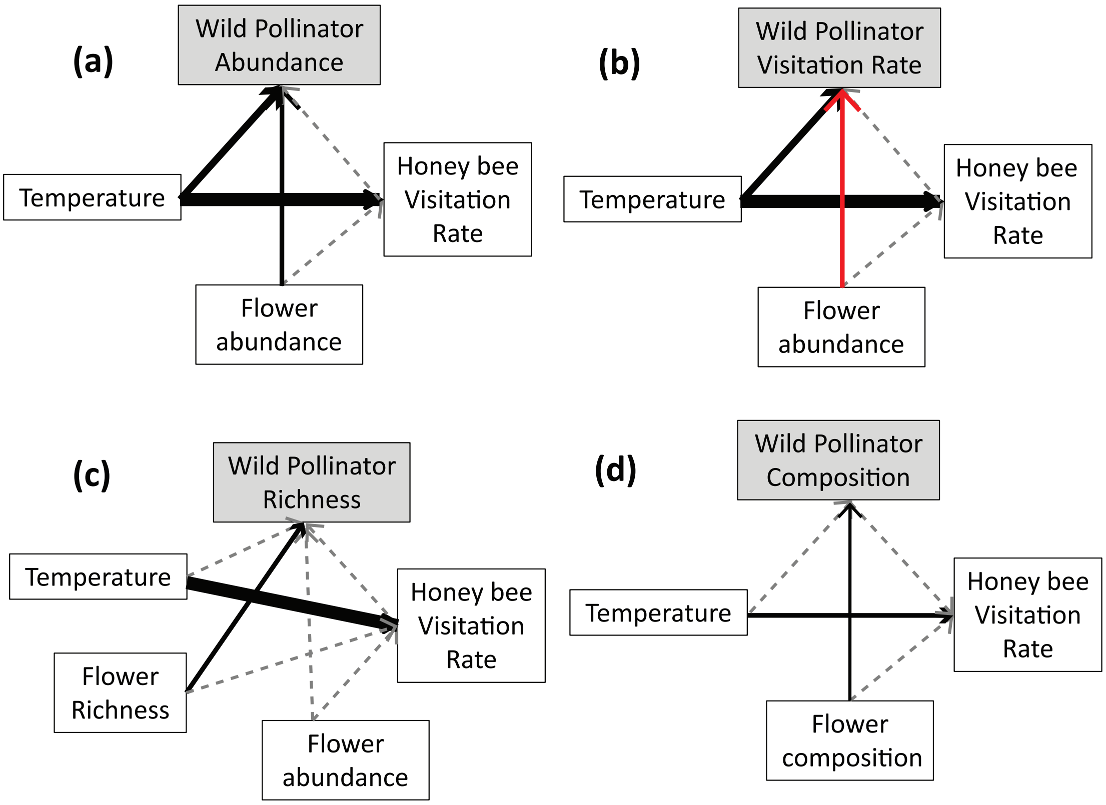

FIGURE 2.4: Graphical representation of path analyses measuring the effects of flower community structure and composition, ambient temperature and honey bee flower visitation rate on wild pollinator community structure and composition (response variables in grey boxes). The analysis of wild pollinator composition was performed with distance matrices following the methodology of Leduc et al. (1992). Dotted arrows denote non-significant relationships (after Bonferroni correction in (d)). Solid arrows represent significant positive (black) and negative (red) relationships. Arrow thickness denotes the magnitude of the effect.

flower community. At the same time, our results show that the spatial variation of the flower community is a poor predictor of the pollinator community, indicating that other factors besides flower community composition are important to explain pollinator distribution at the local scale. Although honey bees were by far the most abundant flower visitor, we found no indication of negative effects of honey bees on the wild pollinator community. Overall, these results are congruent with flower resources not being a limiting factor in our study area at the time the surveys were conducted.

Our first question addressed the extent to which pollinator distribution varies across space. We expected pollinator distribution to be more homogeneous than that of flowers for three reasons. First, pollinators are highly mobile organisms and therefore should be able to spread easily across the study area, blurring distribution patterns. Second, the study area was lacking physical barriers or strong environmental gradients limiting individual dispersion. Finally, because our pollinator community is mainly composed of flower-generalist species (Bosch et al., 2009), we did not expect large differences in local pollinator composition.

Instead, we found that both flower and pollinator communities displayed similar and highly heterogeneous patterns across space. We found strikingly different pollinator communities in different plots, and these differences did not follow any clear spatial pattern (Fig. 2.2b). The high variability in pollinator abundance, visitation rate, richness and composition across space together with the general lack of spatial autocorrelation, indicates that each plot hosts a rather unique pollinator community independent from its neighbouring plots. Some studies have found similar levels of variability in pollinator community distribution at spatial scales comparable to that of our study (from hundreds of meters to a few kilometres). However, these studies were conducted either in areas hosting a mosaic of different disturbance regimes and heterogeneous land uses (Potts et al., 2003*b*), or in separate patches of one type of habitat within a heterogeneous landscape matrix (Dorchin et al., 2018; Carstensen et al., 2014; Simanonok and Burkle, 2014). By contrast, our study was conducted in plots within a homogeneous continuous habitat at the landscape level. Phylopatry (or site fidelity), the tendency of individuals to stay at the site they were born rather than spreading to new areas (Dorchin et al., 2013), is a possible mechanism explaining the local idiosyncratic pollinator composition found in our study. The fact that about a quarter of the pollinator species in our study (non-parasitic bees and predatory wasps) are central place foragers, may also contribute to the establishment of local idiosyncratic assemblages. The super-abundant managed honey bee greatly contributed to the homogenization of the pollinator composition across space, as indicated by the increase in beta-diversity when this species was excluded from the analyses. Thus, the turnover of low-abundance wild pollinator species was the main driver of the differences in community composition across the park.

Our second question addressed to what extent pollinator distribution followed the distribution of food resources (flowers). We found a positive relationship between flower abundance and wild pollinator abundance. This relationship appears to be quite common in plant-pollinator communities (Dreisig, 1995; Steffan-Dewenter and Tscharntke, 2001; Westphal et al., 2003; Ebeling et al., 2008). However, increases in flower abundance were not paralleled by proportional increases in pollinator abundance, as reflected by the negative relationship between flower abundance and wild pollinator visitation rate. Therefore, pollinators did not follow an ideal free distribution, indicating that, at the community level, floral resources were not limiting. Other studies have also found deviations from the ideal free distribution. Kennedy and Gray (1993) showed that the distribution of various animal groups, from beetles to ducks, did not follow closely food resource distribution. Resource use rate was lowest in the resource-richest areas and highest in the poorest areas. Similarly, Janovský et al. (2013) found a decrease in flower visitation rate with increased flower abundance and suggested that pollinator saturation may be a commoner phenomenon than previously thought. If flowers were sufficiently abundant, pollinators could locally find the necessary floral resources to satisfy their energy requirements. This situation could lead to a local organization of the relationship between flowers and pollinator abundances, thus ultimately promoting independent communities between localities.

In line with other studies (Steffan-Dewenter and Tscharntke, 2001; Potts et al., 2003*a*,*b*; Fründ et al., 2010; Weiner et al., 2011; Dorchin et al., 2018), we found a clear relationship between flower and pollinator richness. Therefore, even though overall floral resources did not appear to be limiting, the distribution and abundance of certain pollinator species may have been conditioned by the availability of certain, less abundant, flower species. Of 170 pollinator species recorded, 127 (74.7%) visited mostly the two dominant plant species, *R. officinalis* and *T. vulgaris*, and these 127 species accounted for 95.2% of the visits recorded. The remaining 43 pollinator species were rare and visited mostly rare flower species. Thus, the positive relationship between pollinator and plant richness was mainly driven by interactions among rare species. On the other hand, wild pollinator composition was only moderately related to flower composition. A previous study conducted in the same study area with pan traps (rather than pollinator counts) also found that bee species composition was only weakly affected by flower composition (Torné-Noguera et al., 2014). This result is consistent with the generalist nature of plant-pollinator interactions (Waser et al., 1996) and the opportunistic flower choice displayed by pollinators (Fründ et al., 2010). The weak relationship between flower and pollinator composition also suggests that pollen/nectar availability is not the only (or the main) factor influencing the spatial distribution of pollinators. Distribution of some pollinator groups that only feed on floral resources during the adult stage (in our study area all pollinator groups except bees) may be more strongly conditioned by the availability of larval food than by flower availability. In bees and predatory wasps, nesting resources may also affect species distribution across space (Potts et al., 2005). However, previous studies conducted in our study area found that nesting resources were not a good predictor of bee community distribution (Torné-Noguera et al., 2014).

Finally, our third question addresses the potential competitive effects of honey bees on wild pollinator communities. As a managed species introduced in large numbers, honey bee abundance is frequently higher than the abundance of all other pollinator species together (Geslin et al., 2017). In our study, honey bees accounted for nearly 72% of the flower visits recorded, one of the highest proportions registered in natural habitats (range: 0-85%, Hung et al. 2018). Consequently, we expected that honey bees would have important effects on the spatial distribution of wild pollinator communities. However, we found no evidence of such effects. For honey bees to exert competitive pressure on other pollinators, floral resources should be limiting, but the negative relationship between flower abundance and wild pollinator visitation rate indicates that this was not the case in our study. Because as much as 99.1% of honey bee visits were on *R. officinalis* and *T. vulgaris*, it is unlikely that honey bees could have any competitive effects on pollinators visiting other, less abundant, flower species. This result may be related to the time of the year (early spring) in which surveys were conducted, corresponding to the peak of flower production in the park (Flo et al., 2018). At that time, flower visitation rates are low compared to late spring, when floral resources sharply decline and a greater number of pollinator species are active (Bosch et al., 2009; Filella et al., 2013). Therefore, floral resources are more likely to become limiting as the season progresses (Flo et al., 2018). A previous study in the same area encompassing the entire flowering season found evidence of displacement of wild pollinator communities (especially large bees) by honey bees (Torné-Noguera et al., 2016). Differences between habitats and/or seasons in floral resource abundance could explain discrepancies between studies finding negative effects of honey bees on wild pollinators (e.g. Thomson 2004; Walther-Hellwig et al. 2006; Henry and Rodet 2018) and studies not finding these effects (e.g. Steffan-Dewenter and Tscharntke 2000; Roubik and Wolda 2001; Goras et al. 2016).

Some studies have reported high levels of variation in plant-pollinator interactions across years (Petanidou et al., 2008; Dupont et al., 2009). We show that these interactions are also highly variable across space, even when comparing plots as close as 500 m within a uniform habitat. Overall, these results underscore a lack of consistency in plant-pollinator interactions. In our study, this lack of consistency is driven by high levels of heterogeneity in both the plants and pollinators, resulting in a mosaic of idiosyncratic local communities in spite of the apparent physiognomic uniformity of the study area. The pollinator community was as variable across space as the flower community. Nonetheless, flower distribution had only a moderate weight explaining the general trends of pollinator distribution across the landscape. The negative relationship between pollinator visitation rate and flower abundance indicates that floral resources are not limiting, and this result has a fundamental effect on the relationship between flowers and pollinators. When resources are not limiting and most pollinators are generalist, pollinators do not need to travel long distances to find food resources because they can find enough resources to satisfy their requirements at short distances, thus promoting local-scale organization of pollinator communities and strong spatial variation. Likewise, sufficient availability of floral resources reduces the potential impact of dominant species on the rest of the community. Ultimately, the spatial variation in flower and pollinator species composition recorded in our study may affect pollination and ecosystem functioning at the local level (Herrera, 2000) and may have far-reaching ecological and evolutionary consequences on ecological communities (Gómez et al., 2009). Beyond being structured by climatic gradients and differences in land use, both communities and interactions may show strong imprints of forces acting at a local scale within seemingly uniform tracts of habitat. Dissecting the reasons for, magnitude of and consequences of such small-scale variation is thus an urgent task for community ecologists.

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Variation in pollinator assemblage affects pollination function but not reproductive success in a gynodioecius plant

3

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Manuscript in preparation

Abstract

Most plant species show spatial variation in their pollinators, which may have important consequences on pollination function and reproductive success. In this study, we take advantage of the spatial variability in local pollinator community across a Mediterranean scrubland and analyse its effects on pollination function and reproductive success in the gynodioecious *Thymus vulgaris*. Our aim was to establish whether the variability of pollination function (deposition of homospecific and heterospecific pollen) and reproductive success (fruit and seed set) of the female and hermaphrodite morphs can be explained by the variability of pollinator visitation rates, pollinator richness and pollinator composition (proportion of visits by the major pollinator groups). We registered pollinators visiting both morphs in 40 plots and collected stigmas and fruits to analyze pollination function and reproductive success. The proportion of female flowers with homospecific pollen was positively related to pollinator richness, possibly explained by complementarity between pollinator species, and to the proportion of visits by honey bees and wild bees, which are the most efficient pollinators. The proportion of flowers with homospecific pollen in the hermaphrodite morph was influenced by pollinator visitation rate but this relationship was negative. Pollen deposited on the stigmas may be subjected to secondary mobilization, with this effect only being important in the hermaphrodite morph because of its lower stigmatic adherence. Heterospecific pollen deposition was higher in the female morph and was not affected by the pollinator assemblage, but had a close-to-significant positive relationship with the availability of heterospecific pollen. We found no effect of the pollinator assemblage on plant reproductive success in either of the two morphs, showing pollination appears to be adequate. Our study shows that local variation in pollinator composition may have important consequences on pollination function, especially on plants that are more dependent on pollinators.

Key words: gynodioecy, pollinator assemblage, pollen deposition, plant reproductive success, natural communities.

3.1 Introduction

Most plant species show high levels of spatial variation in their pollinator assemblage (Horvitz and Schemske, 1990; Price et al., 2005; Gómez et al., 2007; González-Varo et al., 2009), even at rather local scales (Herrera, 2005, 1995). This variation may have important consequences on pollination function and ultimately reproductive success because pollinator species differ in foraging behaviour and pollination effectiveness (Schemske and Horvitz, 1984; Herrera, 1987; Larsson, 2005; Castro et al., 2013). Different pollinator species also differ in their degree of complementarity (Albrecht et al., 2012; Fründ et al., 2013) and various studies have reported enhanced levels of pollination and/or reproductive success with increasing pollinator diversity (reviewed in van der Plas 2019). The relationship between pollinator community structure and composition and pollination function is especially important at a time when human activities are causing generalized pollinator declines and altering pollinator community composition in various parts of the world (Biesmeijer, 2006), potentially compromising pollination of wild (Ollerton et al., 2011) and cultivated plants (Klein et al., 2007).

An obvious factor affecting pollination function is pollinator abundance (usually measured in terms of visitation rate). High pollinator abundance is expected to result in a greater proportion of flowers receiving pollen and/or greater stigma pollen loads, and various studies have shown a positive relationship between visitation rates and pollen delivery (Mitchell and Waser, 1992; Niovi Jones and Reithel, 2001; Price et al., 2005) and plant reproductive success (Blitzer et al., 2016). However, this relationship is by no means universal. Several studies have found visitation rate not to be associated with plant reproductive success (Hoehn et al., 2008; Fulton et al., 2015; Ali et al., 2016), indicating that pollinator richness and/or identity may also play an important role. Pollinator richness may enhance pollination through complementarity. Each pollinator species has a set of morphological and behavioural traits that allow it to occupy a certain niche in the overall pollination function multidimensional space. For example, different pollinator species may have different flower handling behaviours, resulting in pollen deposition on different parts of the stigma. Similarly, different species may have different diel activity periods, resulting in pollen deposition at different times of the day (Herrera, 1990; Albrecht et al., 2012; Fründ et al., 2013). Therefore, pollinator richness may contribute to a wider coverage of the various pollination niches depending on the level of complementary of the species involved (Fontaine et al., 2006; Hoehn et al., 2008; Fründ et al., 2013), improving reproductive success (Albrecht et al., 2007, 2012; Hoehn et al., 2008; Blitzer et al., 2016; Ali et al., 2016). Finally, the relationship between pollinator assemblage and pollination function may also be affected by the strongly skewed pollinator abundance distribution, with a handful of very abundant species and a long tail of rare species (Herrera, 1989; Gómez et al., 2007). For this reason, several studies claim that pollination function is mostly driven by visitation rate of the most abundant species rather than pollinator richness (Sahli and Conner, 2007; Albrecht et al., 2012; Winfree et al., 2015). In other words, differences in abundance between common and rare species largely outweigh differences in pollinator efficiency (Vázquez et al., 2005). In addition to affecting deposition of homospecific pollen, differences in pollinator assemblage may also have potential consequences on the transfer of heterospecific pollen. High heterospecific pollen loads may have negative effects of plant reproductive success through stigma clogging, which may interfer with germination and pollen tube growth of homospecific pollen grains (Morales and Traveset, 2008).

The relationship between pollinator assemblage and plant reproductive success is less straightforward compared to that of pollen deposition for two reasons. First, the positive relationship between pollination and reproductive success may become saturated above certain pollen deposition thresholds (Young and Young, 1992; Alonso et al., 2012). Under these circumstances, factors different from pollination such as water and nutrient availability may limit fruit set or seed set (Ashman et al., 2004; Wesselingh, 2007). Second, differences among pollinators in pollinating effectiveness may be driven by differences in the quality of the pollen rather than the quantity of pollen grains deposited on the stigmas (Aizen and

Harder, 2007).

In this study, we take advantage of the spatial variability in local pollinator community across an area covered by a Mediterranean scrubland (Torné-Noguera et al., 2016; Reverté et al., 2019) to analyse the effects of spatial variability of pollinator assemblage structure and composition on spatial variability of pollination function and reproductive success in the self-compatible gynodioecious plant species *Thymus vulgaris* L. Working with a gynodioecious species allows us to compare these effects on two morphs with similar pollinator composition (Arnan et al., 2014) but contrasting levels of reliance on pollination for reproductive success. Our general aim is to establish whether the spatial variability of pollination function (deposition of homospecific and heterospecific pollen) and reproductive success (fruit and seed set) of the two morphs (female and hermaphrodite) can be explained by the spatial variability of pollinator abundance (visitation rates), pollinator richness and pollinator composition (measured as proportion of visits by the major pollinator groups). Studies on *Thymus vulgaris* have measured high levels of geitonogamy in the hermaphrodite morph (Valdeyron et al., 1977), a result that is consistent with the high numbers (ca. 20) of flowers per individual visited by the main pollinators (honey bees and bumblebees) (Arnan et al., 2014). Pollination of the female morph, on the other hand relies exclusively on allogamous pollen. For this reason, we expect a stronger relationship between variation in pollinator assemblage and variation in pollen deposition in the female morph. For the same reason, we expect heterospecific pollen deposition also to be higher in the female morph. *Thymus vulgaris* hermaphrodite flowers produce more seeds per fruit when cross- than when self-pollinated (Assouad et al., 1978), and a previous study conducted in our study area showed reproductive success to be pollen-limited in hermaphrodite but not in female individuals, suggesting low levels of cross-pollination in the hermaphrodite morph (Arnan et al., 2014). Therefore, we expect a stronger relationship between variation in pollinator assemblage and variation in reproductive success in the hermaphrodite morph.

3.2 Materials and Methods

3.2.1 Study system

The study was conducted in spring 2015 in a Mediterranean scrubland in the Garraf Natural Park near Barcelona (NE Spain, 41° 17’ 34” N, 1° 53 ’ 35.8” E), dominated by *Quercus coccifera* L., *Pistacia lentiscus* L., *Thymus vulgaris* L. and *Rosmarinus officinalis* L. The climate is typically Mediterranean, with a strong sea influence, and a mean annual temperature of 15.7^{o}. Summers are dry and most precipitation (580 mm annually) occurs in spring and autumn. Vegetation structure can be considered homogeneous at the landscape scale, but plant composition is highly variable at the local scale (hundreds of metres). The pollinator assemblage is also highly variable at the local scale, both in terms of abundance and species composition, but there is no association between plant and pollinator composition (Reverté et al., 2019). Due to intensive beekeeping, honey bees are the dominant pollinator species in the park (Torné-Noguera et al., 2016; Reverté et al., 2019).

We measured pollination function and reproductive success on the focal species *Thymus vulgaris* (Lamiaceae), a perennial self-compatible gynodioecious species. *Thymus vulgaris* individuals typically produce hundreds of small (4-6 mm) pale pink flowers with 4 ovules each.

3.2.2 Pollinator surveys

We selected 40 plots of 50 x 40 m across an area included in the Garraf scrubland of nearly 40 Km^2 (Fig. A.2.1). Mean distance between nearest plots was 0.76 km (range: 0.52 to 1.40 km). Within each plot, we established four parallel 40 m x 1 m transects separated by 10 m. Each plot was surveyed during a single day between April 2^{nd} and April 17^{th}. This period encompasses the flowering peak of the community (Flo et al., 2018). We surveyed 2-4 plots per day and avoided surveying nearby plots on the same day.

To obtain a measure of local flower community composition, we counted the open flowers of all plant species present in transects. To survey plant-pollinator interactions, we conducted three counts starting at 11 a.m., 1 and 3 p.m., respectively. During each count, two observers walked slowly along the four transects and recorded all pollinators observed contacting anthers or stigmas of flowers within the transects. Many pollinators were identified by sight, but some were captured for later identification in the laboratory.

From these surveys we extracted several variables of pollinator assemblage structure and composition visiting each *T. vulgaris* morph in each plot: visitation rate (number of visits / 1000 open flowers), pollinator richness, and the proportion of visits performed by the main pollinator groups (honey bees, wild bees, dipterans, coleopterans, lepidopterans, and wasps).

3.2.3 Pollination function

In each plot, we selected ten individuals of each morph more or less evenly spread across the plot. To avoid accidental contact with these individuals during the pollinator surveys, these plants were located outside of the transects. Early in the morning and prior to pollinator activity, we tagged eight newly-opened (unvisited) flowers in each selected plant. After the last pollinator survey (4 p.m.), we collected the stigmas of two of the marked flowers of each selected individual. The remaining six flowers were left for later fruit and seed counts. The stigmas were carefully removed from the flower with thin dissecting forceps and introduced into Eppendorf tubes containing a drop of fuchsine-stained gelatine at the bottom. Stigmas were placed over the gelatine avoiding contact with the walls of the tube.

In the laboratory, we prepared microscope slides with stigmas. Pollen grains were counted and identified at 300X by the Palynological Service of the Autonomous University of Barcelona (http://sct.uab.cat/lap/). Some flowers were lost in the field and some could not be processed. The number of pollen loads per individual and plot was 1.6 ± 0.15 (mean ± SE).

With these data we calculated: i) the proportion of flowers that had homospecific pollen, (comparable with fruit set); ii) the proportion of flowers that had heterospecific pollen; and iii) the mean number of homospecific pollen grains deposited per flower (stigmatic pollen loads) only considering the flowers with homospecific pollen (comparable with seed set). We first averaged the replicates per individual, and then averaged the 10 individuals per species to obtain the mean value of each variable per morph and plot.

3.2.4 Reproductive success

Fruits of the marked flowers were collected three weeks after the pollination surveys and brought to the laboratory where the number of seeds per fruit was counted. Sample size (number of fruits collected) for each individual plant and plot was 3.3 ± 0.8 (mean ± SE).

Reproductive success was then characterized by: i) the proportion of fruits that contained at least one seed (fruit set); and ii) the mean number of seeds per fruit that contained at least one seed (seed set). Again, we averaged the different replicates per individual, and then averaged the 10 individuals per plot to obtain the mean value of each variable per morph and plot.

3.2.5 Statistical analyses

We performed paired t-tests to compare the various pollinator assemblage descriptors and pollen deposition and reproductive success variables between the two *Thymus vulgaris* morphs. The female morph was present and received visits by pollinators in all 40 plots, while the hermaphrodite morph was present in 39 plots and received visits by pollinators in 36. The comparisons between morphs were performed using the 39 plots in which both morphs were present except for the values in the proportion of visits by the different pollinator groups, in which we used the 36 plots where both morphs received pollinator visits.

We used linear models to analyse the relationship of the assemblage of pollinators visiting each *T. vulgaris* morph with pollen deposition function and reproductive success in each plot. Sample size was 40 plots for the female morph, and for the hermaphrodite morph the 36 plots in which received pollinator visits. We used a separate model for each morph and response variable. The explanatory variables used were the proportion of *T. vulgaris* flowers that were hermaphrodite (a measure of homospecific pollen availability), along with the pollinator assemblage descriptors: visitation rate, pollinator richness, and the proportion of visits performed by the main pollinator groups (honey bees, wild bees, dipterans, coleopterans, lepidopterans, and wasps). Due to multicollinearity effects between the proportion of visits performed by the six pollinator groups, we only included the proportion of honey bees, wild bees and dipterans, which were the three most frequent pollinator groups in both morphs, amounting to 90% of the visits recorded.

We also used linear models to analyse the relationship between the assemblage of pollinators visiting each *T. vulgaris* morph and the proportion of flowers with heterospecific pollen in each plot. This analysis was only conducted for the female morph because the proportion of flowers of the hermaphrodite morph with heterospecific deposition was very low (7.9%). Six explanatory variables were used in this model: the five pollinator assemblage descriptors and the availability of heterospecific pollen (proportion of *Thymus vulgaris* pollen from the overall pollen available in each plot). The availability of heterospecific pollen was calculated by combining the data on flower abundance obtained in the four transects and data on pollen production per flower from previous studies in the study area (Primante, 2015; Salvador, 2016).

For the proportion of flowers with homospecific pollen, the proportion of flowers with heterospecific pollen and fruit set, we used generalized linear models with a binomial distribution and a logit link function. For stigmatic pollen loads and seed set we used generalized linear models with a Gaussian distribution and identity link function. Stigmatic pollen loads were log-transformed to achieve normality and homoscedasticity of model residuals.

We employed Akaike's information criterion with a correction for low sample sizes (AICc) to select the best-fit models (ΔAICc<2; Burnham and Anderson 2002); this approach reduces the problems associated with multiple testing, collinearity of explanatory variables, and small sample sizes (Burnham and Anderson, 2002). All initial models were full, including all the explanatory variables. We then applied model averaging to make inferences about how descriptors of pollinator assemblage influenced pollination function and reproductive success. First, for each variable, we averaged coefficients and 95% confidence intervals across the best-fit models. Second, we considered variables to be significant when the 95% confidence intervals did not include 0. Model selection and averaging were carried out using the functions 'dredge' and 'model.avg', respectively, in the "MuMIn" package (Bartoń, 2019) in R.

3.3 Results

We recorded 934 pollinator individuals visiting the flowers of *Thymus vulgaris*. Female and hermaphrodite flowers were visited by 62 and 55 pollinator species, respectively. Honey bees were the dominant flower visitor in both morphs (56.2% and 70.4% of the visits to female and hermaphrodite flowers, respectively), followed by wild bees (17.4% and 8.8%) and dipterans (13.9% and 10.9%), lepidopterans (7.3% and 8.8%), coleopterans (1.1% and 1.5%) and wasps (2.6% and 1.1%). Pollinator richness, visitation rate and proportion of visits by the different pollinator groups varied widely between plots (Table 3.1). Hermaphrodite flowers had higher visitation rates (paired t-test; t = -2.3, df = 38, *p* = 0.029), but female flowers had higher proportion of visits by wild bees (t = 2.7, df = 35, *p* = 0.011) and pollinator richness (t = 3.2, df = 38, *p* = 0.003). The proportion of visits by honey bees (t = -1.3, df = 35, *p* = 0.201), dipterans (t = -0.2, df = 35, *p* = 0.874), lepidopterans (t = 0.41, df = 35, *p* = 0.683), coleopterans (t = -1.4, df = 35, *p* = 0.161) and wasps (t = 0.14, df = 35, *p* = 0.873) did not vary between morphs. The two morphs had similar proportions of flowers with homospecific pollen (paired t-test; t = -0.1, df = 38, *p* = 0.910) (Fig. 3.1). On the other hand, the female morph had higher stigmatic pollen loads (t = 2.376, df = 38, *p* = 0.023), proportion of flowers

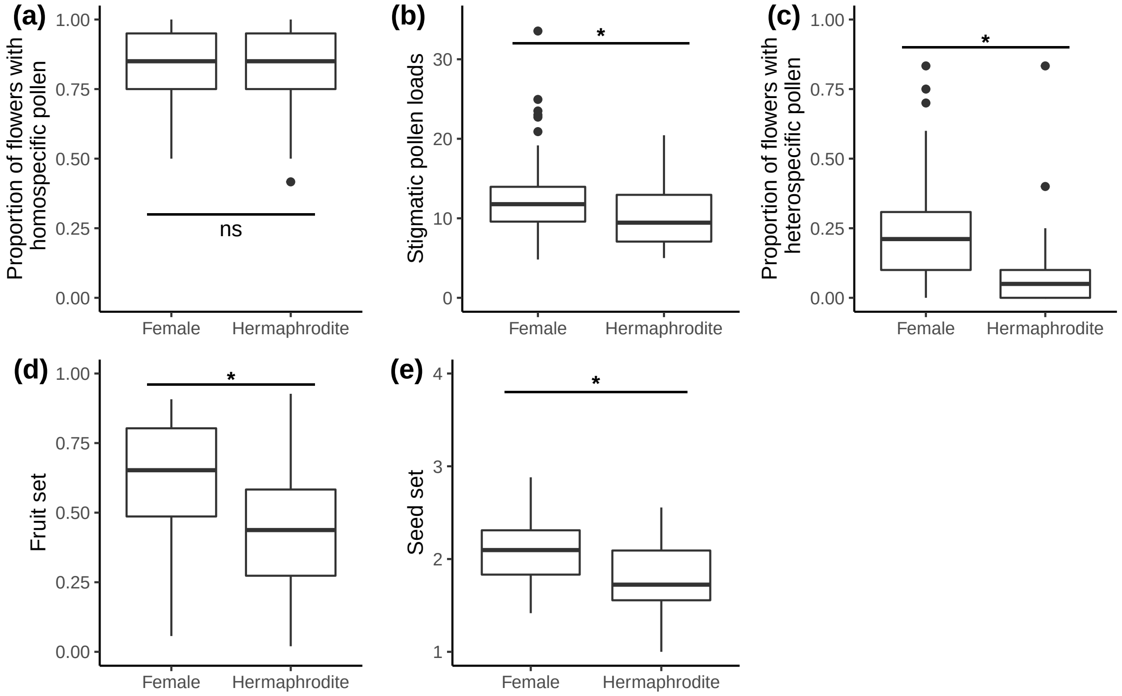

Figure 3.1: Boxplots of homospecific (a, b) and heterospecific (c) pollen deposition and reproductive success (d, e) in the two *Thymus vulgaris* morphs. Significant differences between morphs are indicated by an asterisk.

with heterospecific pollen (t = 5.0, df = 38, $p < 0.0001$) and fruit (t = 5.8, df = 38, $p < 0.0001$) and seed set (t = 3.5, df = 38, $p = 0.001$) (Fig. 3.1).

We expected a relationship between variation in the pollinator assemblage visiting each *T. vulgaris* morph and variation in pollination function, mainly on the female morph. This expectation was partially met. The proportion of female flowers with homospecific pollen loads was positively related to pollinator richness and to the proportion of visits by honey bees and wild bees (Table 3.2, Figure 3.2a-c, Table A.3.1). On the other hand, the proportion of flowers with homospecific pollen in the hermaphrodite morph was influenced by pollinator visitation rate but, surprisingly, this relationship was negative (Table 3.2, Figure 3.2d, Table A.3.2). Stigmatic pollen loads were not related to the pollinator assemblage in any of the two morphs (Table 3.2). At the same time, the availability of *T. vulgaris* pollen, measured as the proportion of hermaphrodite (pollen-producing) flowers did not affect homospecific pollen deposition (Table 3.2).

We also expected higher levels of heterospecific pollen deposition in the female morph. The proportion of female flowers was much higher (26.8%) than that of hermaphrodite flowers (7.9%). However, heterospecific pollen deposition in the female morph was not related to any of the pollinator assemblage descriptors (Table 3.2). The only explanatory variable that was selected and approached significance was the availability of heterospecific pollen, which tended to result in higher heterospecific pollen deposition (Table 3.2).

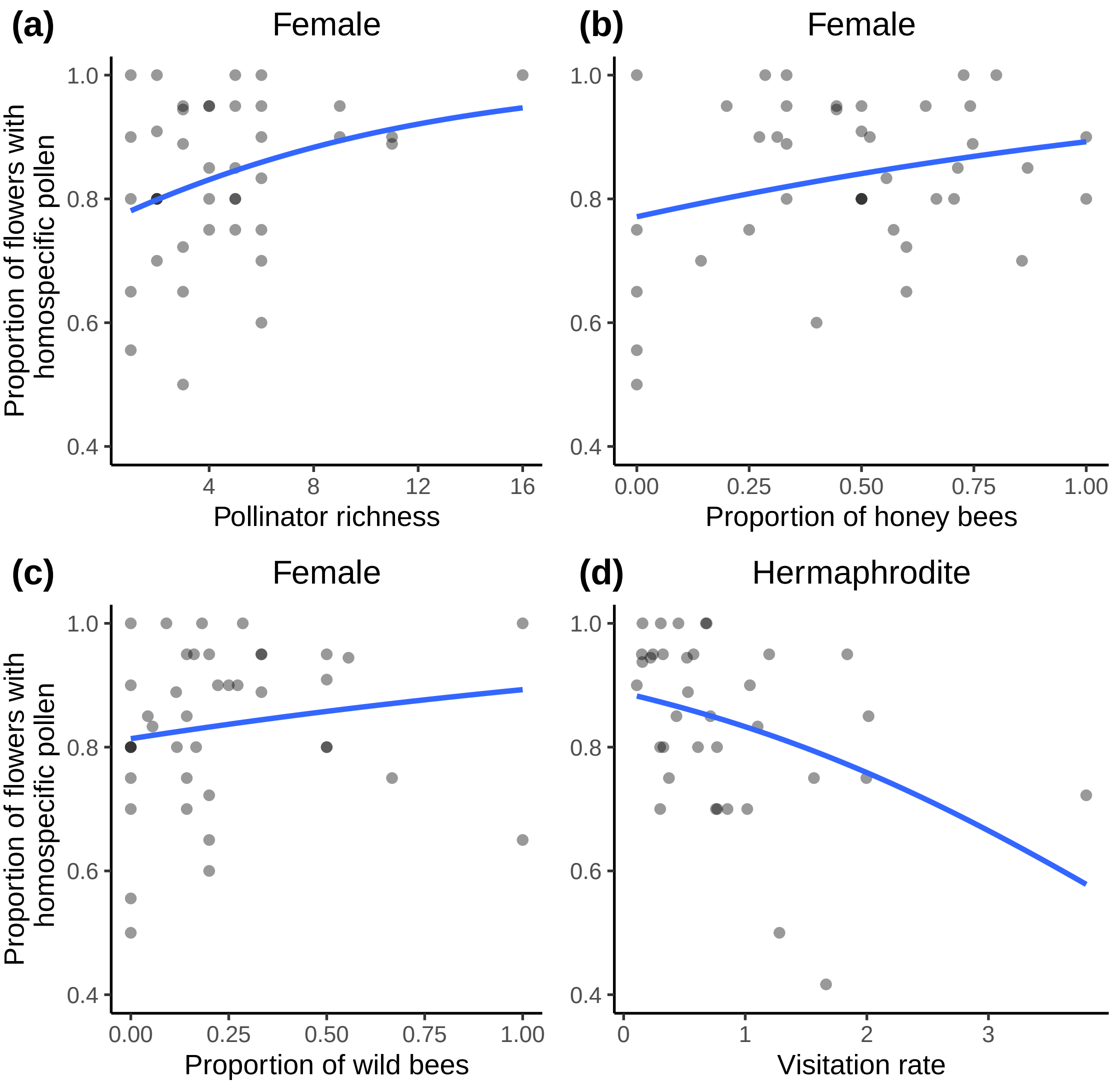

FIGURE 3.2: Graphical representation of the significant relationships between pollinator assemblage descriptors and pollen deposition obtained from model averaging procedures (see Table 3.2).

	Female		Hermaphrodite	
	Mean	Range	Mean	Range
Visitation rate	0.52	(0.07 - 1.52)	0.82	(0.11 - 3.8)
Pollinator richness	4.60	(1 - 16)	3.50	(1 - 12)
Proportion of honey bees	0.47	(0 - 1.00)	0.55	(0 - 1.00)
Proportion of wild bees	0.23	(0 - 1.00)	0.12	(0 - 1.00)
Proportion of dipterans	0.14	(0 - 0.71)	0.15	(0 - 1.00)
Proportion of lepidopterans	0.10	(0 - 1.00)	0.09	(0 - 0.75)
Proportion of coleopterans	0.01	(0 – 0.29)	0.06	(0 - 0.67)
Proportion of wasps	0.03	(0 – 0.33)	0.02	(0 – 0.50)

Table 3.1: Mean and range values of pollinator assemblage descriptors of both female and hermaphrodite *T. vulgaris* morphs: visitation rate (number of visits / 1000 open flowers), pollinator richness, and the proportion of visits performed by the main pollinator groups (honey bees, wild bees, dipterans, coleopterans, lepidopterans, and wasps).

		Female		Hermaphrodite	
Response	Explicative	Estimate	SE	Estimate	SE
Proportion of flowers with homospecific pollen	Visitation rate	0.08	0.26	**-0.55**	**0.19**
	Pollinator richness	**0.13**	**0.06**	-0.01	0.04
	Proportion of honey bees	**1.58**	**0.58**	-	-
	Proportion of wild bees	**1.57**	**0.62**	-0.12	0.57
	Proportion of dipterans	0.18	0.56	0.23	0.6
	Proportion of hermaphrodite *T. vulgaris*	-	-	-	-
Mean number of homospecific grains in flowers with homospecific pollen	Visitation rate	-0.02	0.09	-	-
	Pollinator richness	0.03	0.03	-	-
	Proportion of honey bees	-	-	-	-
	Proportion of wild bees	-	-	0.21	0.30
	Proportion of dipterans	-	-	-	-
	Proportion of hermaphrodite *T. vulgaris*	0.21	0.35	-0.10	0.24
Proportion of flowers with heterospecific pollen	Visitation rate	0.32	0.43	NA	NA
	Pollinator richness	-0.17	0.27	NA	NA
	Proportion of honey bees	-	-	NA	NA
	Proportion of wild bees	-	-	NA	NA
	Proportion of dipterans	0.70	0.74	NA	NA
	Proportion of heterospecific pollen in the community	1.45	0.81	NA	NA
Fruit set	Visitation rate	0.01	0.09	-	-
	Pollinator richness	0.03	0.04	0.01	0.02
	Proportion of honey bees	0.10	0.27	0.56	0.43
	Proportion of wild bees	-0.21	0.38	-	-
	Proportion of dipterans	0.09	0.35	0.12	0.32
	Proportion of hermaphrodite *T. vulgaris*	0.38	0.59	-	-
Seed set	Visitation rate	-	-	0.01	0.02
	Pollinator richness	-	-	-	-
	Proportion of honey bees	0.07	0.16	0.03	0.07
	Proportion of wild bees	-0.13	0.21	-0.02	0.09
	Proportion of dipterans	0.43	0.38	-	-
	Proportion of hermaphrodite *T. vulgaris*	0.33	0.34	-0.19	0.24

Table 3.2: Results of the best-fit models (ΔAICc<2) analysing the relationship of pollinator assemblage descriptors with pollen deposition and reproductive success. Values are model estimates and Standard errors obtained with a model averaging procedure. Numbers in bold denote significant results ($p < 0.05$). Hyphens indicate the best-fit models did not include this variable.

We also predicted that variation in the pollinator assemblage affected reproductive success, and we expected a greater effect on the hermaphrodite morph. However, neither fruit set nor seed set were related to any of the pollinator assemblage descriptors or to the availability of *T. vulgaris* pollen in any of the two morphs (Table 3.2,Table A.3.1, Table A.3.2).

3.4 Discussion

We found that variation in the pollinator assemblage at the local scale affected pollen deposition in the two *T. vulgaris* morphs. As predicted, this effect was greater in the female morph, which showed an increase in the proportion of flowers with homospecific pollen with increased pollinator richness and with increased visitation rates by the two main pollinator groups (honey bees and wild bees). Complementarity effects have been shown to explain the positive relationship between species richness and ecosystem function through niche differentiation (Loreau 2001). When different species occupy different pollination niches, richer communities are likely to include complementary species in terms of foraging times and/or behaviour (Herrera, 1987; Fontaine et al., 2006; Hoehn et al., 2008). Given that honey bees and wild bees visit the higher numbers of flowers per individual plant, in a female individual with no pollen production, a higher proportion of visits by both groups is likely to result in more flowers receiving pollen deposition. We demonstrate that small scale spatial variability in the species richness of the pollinator assemblage visiting *T. vulgaris* female individuals as well as the visitation rates of honey bees and wild bees to *T. vulgaris* females implies variability in the deposition of homospecific pollen across the plant population in a homogeneous landscape in terms of vegetation physiognomy. Pollen-producing hermaphrodite plants, on the other hand, will not be as dependent on pollinator richness and identity because even inefficient pollinators probably transfer self-pollen between flowers.

In principle, and in agreement with various studies (Mitchell and Waser, 1992; Niovi Jones and Reithel, 2001; Engel and Irwin, 2003), one would expect pollen deposition per flower to increase with visitation rate. However, other studies have not found this kind of relationship (Sakamoto and Morinaga, 2013; Kulbaba and Worley, 2014). Lack of relationship between visitation rate and pollen deposition may be explained by the large variability in pollinating effectiveness among pollinator species (Herrera, 1987; Young and Stanton, 1990; Wilson and Thomson, 1991; Pettersson, 1991), and even among individuals within a species (Herrera, 1987). In some plants, pollinator visitation rate has been found to be more strongly related to male function (pollen export) than female function (pollen deposition) (Fishbein and Venable, 1996). In our study, visitation rate did not influence pollen deposition in the female morph and, surprisingly, had a negative effect on pollination in the hermaphrodite morph. There are two possible explanations for this counterintuitive result. First, pollen deposited on the stigmas may be subjected to secondary mobilization and/or consumption (Inouye et al., 1994;

Thomson and Eisenhart, 2003; Hoyle and Cresswell, 2006). The fact that stigmatic adherence of *Thymus vulgaris* is greater in female than in hermaphrodite flowers (Assouad and Valdeyron, 1975), may contribute to explain why the negative relationship between visitation rate and pollen deposition was only found in the hermaphrodite morph. Second, because the vast majority of pollen grains produced by a flower are consumed and therefore lost to the pollination process (Inouye et al., 1994; Schlindwein et al., 2005), high levels of visitation rate may end up depleting pollen resources and consequently reducing the levels of pollen available for pollination. Visitation by pollinators that remove large amounts of pollen but deliver small amounts to the stigmas (so-called "ugly pollinators"; Thomson and Thomson 1992) may result in reduced overall pollen deposition (Wilson and Thomson, 1991; Hargreaves et al., 2009; Parker et al., 2016). This scenario is particularly plausible in protandrous flowers (such as hermaphrodite *T. vulgaris* flowers) if pollinators favour visitation to flowers in the male phase (Koski et al., 2018). Both secondary pollen mobilization and pollen depletion by "ugly" pollinators selectively visiting flowers in the male phase are congruent with the fact that hermaphrodite flowers showed lower levels of pollen deposition than female flowers, despite higher visitation rates in the hermaphrodite morph.

As predicted, heterospecific pollen deposition was higher in the female morph. A pollinator landing on a hermaphroditic individual is likely to pick up increasing amounts of *T. vulgaris* pollen as it visits a sequence of flowers, thus potentially masking heterospecific pollen grains that the pollinator could carry and hindering their transfer to the stigmas. Instead, a pollinator landing on a female individual will not acquire *T. vulgaris* pollen thus facilitating the deposition of any heterospecific pollen that the pollinator might carry. In contrast to homospecific pollen, the transfer of heterospecific pollen was not affected by the structure or composition of the pollinator assemblage, possibly because overall heterospecific pollen transfer was rather low. However, in agreement with other studies (Jakobsson et al., 2009), we detected a close-to-significant positive relationship between the availability of heterospecific pollen in the plant community and heterospecific pollen deposition. This result is congruent with pollinators decreasing their level of flower constancy in communities in which the relative abundance of the target species is lower (Delmas et al., 2016).

We found no effect of variability in the pollinator assemblage at the local scale on the variability of plant reproductive success in either of the two morphs. The flowers we examined for pollen deposition had been exposed to pollinators for a single day. Even with this short exposure, more than 80% of the flowers were pollinated and stigma pollen loads approached 10 pollen grains. Given that *T. vulgaris* flowers have only 4 ovules, and the flowers on which fruit and seed set was measured were exposed to pollinators for 3-4 days (duration of stigma receptivity in *T. vulgaris*; Assouad and Valdeyron 1975; Arnan et al. 2014 levels of pollination appear to be adequate in most of the populations sampled. A study applying pollen and resource supplementation to *Thymus vulgaris* in the same study area found seed set to be pollen limited in the hermaphrodite morph, suggesting low levels of cross-pollination (but not overall pollen deposition) in this morph (Arnan et al., 2014). Certain pollinators or pollinator compositions are known to favour allogamous over geitonogamous pollination (Karron et al., 2009; de Jong et al., 1993). For this reason, we expected that variation in pollinator assemblage compositions involved reproductive success variability in the hermaphrodite morph, but this expectation was not met. Another explanation for the lack of relationship between pollinator assemblage and pollinator composition is apomixis (production of seeds by unpollinated flowers). Apomixis has been described in the two morphs of *Thymus loscosii* (Orellana et al., 2005), but to our knowledge it has not been tested in *T. vulgaris*.

Our study shows that local variation in pollinator composition may have important consequences on pollination function. It is important to note that we worked with levels of spatial variation occurring naturally across a relatively small and uniform natural area. Changes in pollinator composition are likely to be much greater in current scenarios of pollinator diversity declines (Biesmeijer, 2006; Potts et al., 2010), introduction of exotic pollinator species (Aizen et al., 2019) and pollinator biotic homogenization (Bommarco et al., 2012). Under these circumstances, plants that are more dependent on pollinators (such as the female *T. vulgaris* morph in our study) are likely to be most affected.

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Pollinators show flower colour preferences but flowers with similar colours do not attract similar pollinators

4

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Abstract

Colour is one of the main floral traits used by pollinators to locate flowers. Although pollinators show innate colour preferences, the view that the colour of a flower may be considered an important predictor of its main pollinators is highly controversial because flower choice is highly context-dependent, and initial innate preferences may be overridden by subsequent associative learning. Our objective is to establish whether there is a relationship between flower colour and pollinator composition in natural communities. We measured the flower reflectance spectrum and pollinator composition in four plant communities (85 plant species represented by 109 populations, and 32,305 plant-pollinator interactions in total). Pollinators were divided into six taxonomic groups: bees, ants, wasps, coleopterans, dipterans and lepidopterans. We found consistent associations between pollinator groups and certain colours. These associations matched innate preferences experimentally established for several pollinators and predictions of the pollination syndrome theory. However, flowers with similar colours did not attract similar pollinator assemblages. The explanation for this paradoxical result is that most flower species are pollination generalists. We conclude that although pollinator colour preferences seem to condition plant-pollinator interactions, the selective force behind these preferences has not been strong enough to mediate the appearance and maintenance of tight colour-based plant-pollinator associations.

Key words: colour preferences, floral colour, floral reflectance spectra, generalization, floral traits, phylogenetic signal, plant-pollinator interactions, pollinator assemblage, pollination syndromes.

4.1 Introduction

Pollinators use flower traits, such as odour, shape, size and colour, as cues to locate pollen-nectar sources and discriminate between different flower species (Chittka and Raine, 2006). Although ultimate flower choice undoubtedly depends on a combination of stimuli, various studies have demonstrated that some pollinators rely strongly on colour to make their foraging decisions (Dafni et al., 1990; Heiling et al., 2003).

Diurnal pollinators have well-developed colour vision, which in most cases covers a wider range of the spectrum than human vision. The vast majority of pollinators in temperate regions are insects, and different visual models have been developed for different groups (Chittka, 1992; Troje, 1993; Ômura and Honda, 2005; Dötterl et al., 2014). The best studied species are the western honey bee, *Apis mellifera*, and the bumblebee *Bombus terrestris*, which share a similar trichromatic system. The visual system of the honey bee, which is usually used as a model for all bees, ranges between 300 and 700 nm, with three types of photoreceptors peaking in the UV, blue and green regions of the spectrum (corresponding to 344, 438 and 560 nm, respectively). Most other insects studied so far also have a trichromatic system, but there are also species with dichromatic (certain flies and coleopterans) and tetrachromatic (mostly butterflies) systems (Briscoe and Chittka, 2001). The trichromatic state is supposed to be ancestral, and the loss or gain of photoreceptor types would be secondary (Briscoe and Chittka, 2001). Insect colour vision appeared long before the emergence of the angiosperms (Chittka, 1997). For this reason, it is believed that flowers tuned their visual signals to the sensory system of pollinators, thus becoming as conspicuous and attractive as possible to them. In support of this view, some pollinator species have been found to show innate preferences for certain colours (Lunau and Maier, 1995; Raine et al., 2006; Raine and Chittka, 2007; Willmer, 2011).

To this extent, colour constitutes one of the main traits used in pollination syndrome theory (Faegri and Van der Pijl, 1979). According to this theory, unrelated plant species adapted to the same pollinators should show convergence of floral traits, including colour. For example, bee-visited flowers are expected to be blue or violet, and beetle-visited flowers are expected to be white or cream (Faegri and Van der Pijl, 1979). Accordingly, some studies have shown that colour changes among related plant species or between populations within a same species are sometimes accompanied by changes in pollinator composition (Bradshaw Jr and Schemske, 2003; Rodríguez-Gironés and Santamaría, 2004; Wolfe and Sowell, 2006). Other studies have shown pollinator colour preferences through experimental manipulations of flower colour (Campbell et al., 2010). Ultimately, flower colour could be considered an important predictor of pollinator group (Fenster et al., 2004). However, other studies do not support this view. Colour preferences predicted by pollination syndromes are not always matched by innate colour preferences (Lunau and Maier, 1995), which in many cases are displayed at a finer taxonomic level, such as genus or species, rather than functional group (Raine et al., 2006). Other studies report that changes in flower colour are not always associated with distinct pollinator assemblages (Cooley et al., 2008).

Even if pollinators show innate preferences for certain colours, actual flower choice in natural communities may not reflect these preferences for two reasons. First, in a natural setting, flower choice may be influenced by the presence and abundance of other co-pollinators potentially competing for flower resources (Waser, 1983; Lázaro et al., 2009). Second, colours may also be used by pollinators as signals of floral rewards, so that initial innate preferences may be modulated by subsequent associative learning (Gumbert, 2000; Goyret et al., 2008). Individual pollinators show remarkable plasticity and are known to switch plants in response to changes in pollen or nectar levels (Heinrich, 1979). Thus, the role of colour in determining ultimate flower choice will depend on the interaction between innate and learned preferences. A handful of studies have analysed the influence of floral colour on pollinator partitioning in plant communities, and most of them conclude that floral colour is an important cue (McCall and Primack, 1992; Bosch et al., 1997; Kingston and McQuillan, 2000; Wolfe and Sowell, 2006; Lázaro et al., 2008). However, colours in these studies were categorized as perceived by humans. Waser et al. 1996 characterized colour based on wavelength measures that were incorporated into the visual model of the honeybee to generate colour categories. In their study, the association between floral colour category and pollinator composition narrowly failed significance. Clearly, further research on this topic is needed.

The aim of this study is to establish whether there is a relationship between flower colour and pollinator visitation in natural communities. To do this, we studied the flower reflectance spectrum and flower visitor assemblages in four plant communities comprising 85 species and 109 plant populations. The four communities are located in the same geographic region, and therefore have similar climatic conditions and share the same regional pool of pollinators. Because the association between flower colour and pollinator composition may be affected by phylogeny, our analyses account for phylogenetic relatedness. We ask two questions: 1) Do different pollinator groups show preferences for certain flower colours? 2) Do plant species with similar colours attract similar pollinator assemblages? Although apparently similar, these two questions address colour-pollinator relationships from two different and complementary perspectives. The first question addresses plant-pollinator relationships from the pollinators' point of view, and the second from the plants' perspective. The existence of colour preferences by different pollinator groups does not necessarily imply that plants with the same colour attract similar pollinator assemblages. For example, dipterans may preferentially visit flowers with high levels of reflectance in the yellow region of the spectrum, but different yellow flowers may be visited by different pollinator assemblages. The distinction is important because pollination syndrome theory relies on the assumption that plants with similar traits have evolved to attract similar pollinators.

4.2 Materials and Methods

4.2.1 Study area

We conducted our study in four Mediterranean communities near Barcelona (NE Spain), whose geographic coordinates are given in Table 4.1. Distance between sites ranged from 10 to 66 km. The climate is Mediterranean, with a strong sea influence. Summers are dry and most precipitation occurs in spring and autumn. Weather conditions are very similar across the four sites (Table 4.1). We studied the vast majority of the entomophilous species in each community, without selecting species based on their flower traits or pollinator visitation patterns (Table 4.1). Therefore our plant sample was unbiased and representative of the flora of the region. Some species were present in more than one community. In these cases, we sampled flower colour and pollinator composition of each population separately (total: 85 species, 109 populations).

Community	Vegetation	Dominant species	Location	Elevation (m.a.s.l.)	Mean annual precipitation (mm)	Mean annual temperature (°C)	Plant species studied
CA	Grassland	*Hyparrhenia hirta*, *Brachypodium retusum*	Canet de Mar 41° 35’ N, 2° 34’ E	50	590	16.1	17
CO	Open scrubland	*Hyparrhenia hirta*, *Foeniculum vulgare*, *Cistus monspeliensis*	Collserola 41° 24’ N, 2° 6’ E	280	630	14.8	46
GA	Scrubland	*Quercus coccifera*, *Rosmarinus officinalis*, *Pistacia lentiscus*	Garraf 41° 16’ N, 1° 55’ E	330	580	15.7	25
PA	Scrubland	*Quercus coccifera*, *Ulex parviflorus*, *Pistacia lentiscus*	El Papiol 41° 27’ N, 2° 0’ E	150	628	15.4	21

Table 4.1: Descriptors of the four communities studied, including vegetation type, dominant species, location, elevation, mean annual precipitation, mean annual temperature, and number of species sampled.

4.2.2 Pollinator assemblages

Pollinator data were obtained throughout the main general flowering period in the study area (February-July). Very few species bloom in August. Each species was surveyed on several days covering its entire flowering period. In two communities (CA and CO), pollinator surveys were carried out by slowly walking along 25- or 50-m-long x 1-m-wide vegetation transects. This was done several times throughout the day, from early morning to evening. In the other two communities (GA and PA), selected individuals of each plant species in bloom were observed during 4- or 5-minute periods every 1-2 h. All insects observed contacting the reproductive organs of flowers were identified by sight and recorded. Pollinators were assigned to one of six taxonomic groups: bees, ants, wasps, coleopterans, dipterans and lepidopterans. From these surveys, we characterized the pollinator assemblage of each plant species in each of the four communities as the proportion of visits from each pollinator group.

4.2.3 Flower colour measurement

Flowers of each species were collected and transported with a portable cooler to the laboratory, where they were temporarily placed in a cold room at 4ºC. Flower reflectance spectra were measured using an USB4000 spectrometer with a USB-DT deuterium tungsten halogen source (Ocean Optics Inc., Dunedin, FL, USA) connected to a computer running SpectraSuite (Ocean Optics). The light spectrum analysed ranges from 300 to 700 nm divided into 0.22 nm intervals, and the spectrometer sensor was fixed at an angle of 45º from the measuring area. Petals were mounted on an adhesive tape to obtain a flat surface, thus minimizing reflectance variability due to uneven distances between the petals and the sensor. For small flowers, we had to use several petals from different flowers to cover the entire measuring area. Some species had corollas displaying two or more clearly different colours. When these different colours were largely separated (for example, some Fabaceae in which the keel is clearly differently coloured than the wings and the standard), we took colour measures of the different parts separately and calculated a weighted mean (according to the surface occupied by each part in the corolla), thus obtaining a single colour value. In corollas showing only small colour markings such as nectar guides or small dots, a single measure was taken.

Spectrometer readings incorporate a certain degree of noise. We used Avicol (Gomez, 2006) to clean this noise, thus smoothening the reflectance spectra. This correction (triangular smoothing) is based on a floating mean with weights and a window size of 15 nm. We measured 4-5 flowers per species except for three species for which we could only obtain one measure. Different measures were averaged to obtain a single value per species and community.

4.2.4 Colour characterization

We characterized flower colour using two sets of variables: colour descriptors and “colour composition”. Colour descriptors (brightness, chroma and hue) are physical properties of colours extracted directly from the reflectance spectrum

(Endler, 1990; Smith, 2014). Brightness is a measure of the total intensity of light reflected by a surface; chroma is a measure of purity or saturation of a colour, and it is a function of how rapidly intensity changes with wavelength; hue, represents the usual meaning of colour (such as red, pink, yellow, etc.), and it is a function of the shape of the spectrum. These three colour descriptors were calculated following Smith (2014). To obtain "colour composition", we divided the reflectance spectrum into 4 broad bands of 100 nm each (300-400, 400-500, 500-600, 600-700 nm; (Chittka et al., 1994)). Breakpoints delimiting bands were chosen assuming that the majority of the inflection points in floral reflectance spectra are located near 400, 500 and 600 nm Chittka and Menzel 1992. Roughly, the first band corresponds to the UV part of the spectrum, the second to the blue-violet, the third to the green-yellow and the fourth to the orange-red (henceforth U, B, Y and R bands). We calculated the proportion of the reflectance spectrum attributable to each band, obtained by dividing the brightness of each band by the total brightness of the sample. By using the proportion, instead of the raw values of brightness of each band, we avoid differences between chromatically equivalent spectra, i.e., spectra with the same shape but different brightness (Endler, 1990). It is important to note that flower colours result from the combination of reflectance levels across the spectrum. For example, white flowers reflect from 400 to 700 nm and yellow flowers from 500 to 700 nm. Lilac-pink flowers reflect in the blue and red regions with a variable proportion in the yellow region, and purple flowers reflect in the UV, blue and red regions of the spectrum (see Table A.4.1).

4.2.5 Data analysis

4.2.5.1 Phylogenetic signal of colour variables

To establish whether colour was phylogenetically constrained, we built a phylogenetic tree of the 85 species using Phylocom (Webb et al., 2008), with family names following the Angiosperm Phylogeny Group classification (Group, 2009). We used the "bladj" function in Phylocom to achieve an ultrametric rooted tree. Polytomies generated by the program were hand-resolved. All distances between families (assessed as millions of years of divergence) and some distances between genera were obtained from the database www.timetree.org (Hedges et al., 2006). Additional distances between genera and distances between species were extracted from the literature [Allan and Porter 2000,Lavin et al. 2005 (Fabaceae); Barres et al. 2013 (Cardueae); Bremer 2009 (Ericales); Guzmán et al. 2009 (Cistaceae); Mansion et al. 2009 (Boraginaceae); Couvreur et al. 2010 (Brassicaceae); Bendiksby et al. 2011, Drew and Sytsma 2012 (Lamiaceae); Koopman and Baum 2008 (Malvaceae); Ruiz-Sanchez et al. 2012 (Papaveraceae); Riina et al. 2013 (Euphorbiaceae); Banasiak et al. 2013 (Apiaceae)]. The resulting tree is shown in Figure Fig. A.4.1.

We tested for the presence of phylogenetic signal in brightness, chroma and hue with the Blomberg's *K* test (Blomberg et al., 2003) using the "phylosig" function of the R package "phytools" (Revell, 2012). This was done for each community separately and for the 85 species together. We also tested for the presence of phylogenetic signal in flower colour composition. To do this, we divided the colour spectrum into 40 bands of 10 nm each and obtained the mean reflectance value of each band. We used 40 bands instead of the 4 bands used to define colour composition to increase the resolution of this analysis. We then used the generalized K statistic described by Adams (2014), K_{mult}, specifically developed for high-dimensional multivariate data. This analysis was performed with the function "physignal" of the package "geomorph" for R (Adams and Otárola-Castillo, 2013). Again, we conducted this analysis for each community separately and for the 85 species together.

4.2.5.2 Association between pollinator groups and regions of the colour spectrum

We explored possible preferences of the different pollinators groups for certain regions of the colour spectrum by means of Canonical Correspondence Analyses (CCA) including the pollinator assemblage of each population and relative brightness of the four above-mentioned colour bands (U, B, Y and R). This was again done for the four communities separately and for all species together. CCAs were performed using the function "CCorA" in the R package "vegan" (Oksanen et al. 2015).

4.2.5.3 Relationship between flower colour and pollinator assemblages

To test whether flowers with similar colours had similar pollinator assemblages, we conducted a partial Mantel test between distance matrices of colour composition (combination of the proportion of the four colour bands) and pollinator assemblages, including the phylogenetic distance matrix as covariable. We then followed the same approach to test the association between pollinator assemblages and the other colour variables (brightness, chroma, hue). We used Bray-Curtis distances between pairs of species for pollinator composition and colour composition, and Euclidean distances between species for brightness, chroma and hue. These analyses were done for each community separately and for the four communities lumped together. In the latter case, because the same species may attract different pollinators in different communities, we maintained all 109 populations. For plant species present in more than one community, we assigned a very low value of divergence (1000 years) between conspecific populations. These analyses were performed with the function "mantel.partial" in the R package "vegan".

4.3 Results

We recorded 32,305 plant-pollinator interactions. The number of interactions recorded in each community ranged from 3505 (CA) to 13673 (CO) (Table Table A.4.2). Mean number of interactions per population was 206 in CA (range: 43 to 1454); 297 in CO (range: 42 to 1911); 364 in GA (range: 26 to 1730) and 307 in PA (range: 31 to 1359). Overall, 88.5% of the populations surveyed had more than 50 recorded interactions. Most of the pollinators recorded were bees, accounting for 48.9% of the flower visits. The second most frequent group was coleopterans (21.5% of the interactions), followed by ants (14.7%), dipterans (8.8%), lepidopterans (3.5%) and wasps (2.5%). Bees and coleopterans were the two most abundant groups in all four communities, except CA, which was largely dominated by ants (Table A.4.2).

The four communities showed a high degree of similarity in flower colours. The most common floral colour in the four communities was lilac-pink (30-50% of the species), followed by white (16-29%) (Table A.4.1). UV-yellow flowers were also well represented (14-24%), although they were lacking in GA. Yellow (12-15%), purple (4-9%) and green (4-6%) flowers were less frequent. The association between colour categories and pollinator composition is shown in Table A.4.3.

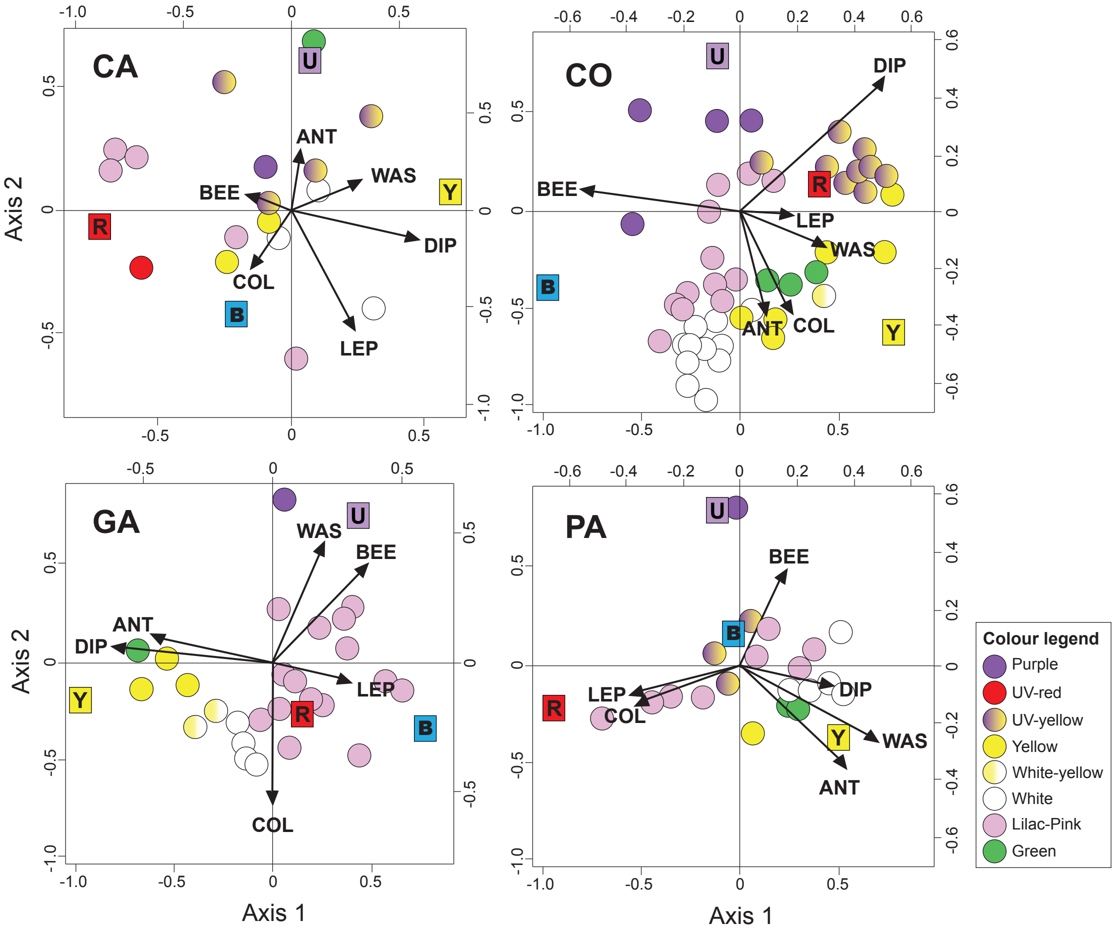

Figure 4.1: CCA biplots of pollinator groups and bands of the colour spectrum (coloured squares) corresponding to UV, blue, yellow, and red in each of the four communities (CA, CO, GA, PA). Each dot represents a plant population and dot colour corresponds to flower colour categories as shown in the legend (see Table A.4.1 for example spectra of each category). BEE = bees, ANT = ants, WAS = wasps, DIP = dipterans, COL = coleopterans, LEP = lepidopterans.

4.3.1 Phylogenetic signal of colour variables

All colour descriptors considered showed significant phylogenetic signal when the four communities were pooled together (Table 4.2). In most cases, however, significance was lost when the communities were analysed separately, possibly

Community	Number of species	Brightness	Chroma	Hue	Colour composition
CA	17	0.73	**0.78**	0.85	0.48
CO	46	0.35	**0.57**	**0.71**	0.33
GA	25	0.26	0.61	0.44	0.25
PA	21	0.34	**0.52**	0.76	0.26
CA+CO+GA+PA	85	**0.57**	**0.60**	**0.71**	**0.38**

Table 4.2: Analyses of phylogenetic signal for colour descriptors brightness, chroma, hue (Blomberg's K values) and colour composition (K_{mult} values) in the four study communities separately and lumped together. Significant results ($p < 0.05$) in bold.

Community	Brightness	Chroma	Hue	Colour composition
CA	-0.089	0.096	0.199	0.158
CO	-0.002	-0.031	0.015	-0.080
GA	-0.025	0.115	-0.082	-0.038
PA	-0.103	-0.063	-0.035	0.051
Overall	0.021	-0.023	0.019	0.006

Table 4.3: R values of phylogenetically-controlled partial Mantel tests between colour descriptors and pollinator composition in the four communities and overall (data of the four communities lumped together). All results are non-significant.

due to small sample sizes. In all cases, K and K_{mult} values were lower than 1, indicating that related species were less similar than expected under the Brownian motion evolution model.

4.3.2 Association between pollinator groups and regions of the colour spectrum

CCAs revealed clear associations between certain pollinator groups and certain colours (Figs. 4.1 and 4.2). Visual inspection of the resulting biplots revealed that some of these patterns were relatively consistent across the four communities (Fig. 4.1, Table 4.3).

Overall, bees were associated with purple flowers, and ants with UV-Yellow and green flowers. Wasps and dipterans were mostly associated with UV-yellow flowers. Coleopterans were associated with white and yellow flowers, and lepidopterans with pink flowers (Table 4.3, Fig. 4.2).

4.3.3 Relationship between flower colour and pollinator assemblages

Results from the partial Mantel test showed no significant association between flower colour and pollinator assemblages (Table 4.4). Plants with similar colour descriptors, including colour composition, did not attract similar pollinator assemblages in any of the communities, and similar results were obtained when data from the four communities were lumped together.

Community	Bees	Ants	Wasps	Coleopterans	Dipterans	Lepidopterans
CA	Purple UV-yellow	Purple UV-yellow	UV-yellow	White Yellow Pink	White UV-yellow	White Pink
CO	Purple	White Yellow	Yellow	Yellow Green White	Purple UV-yellow	Yellow UV-yellow
GA	Purple Pink	Yellow Green	Purple	White Pink	Yellow Green	Pink
PA	Purple Pink	Yellow Green White	White	Pink	White	Pink
Overall	Purple	UV-yellow Green	UV-yellow	White Yellow	Pink	Pink

Table 4.4: Relationships between pollinator groups and the different floral colours in the four communities and overall (data of the four communities lumped together) estimated visually from the CCA biplots of Fig. 4.1 for the four communities and Fig. 4.2 for the overall tendencies.

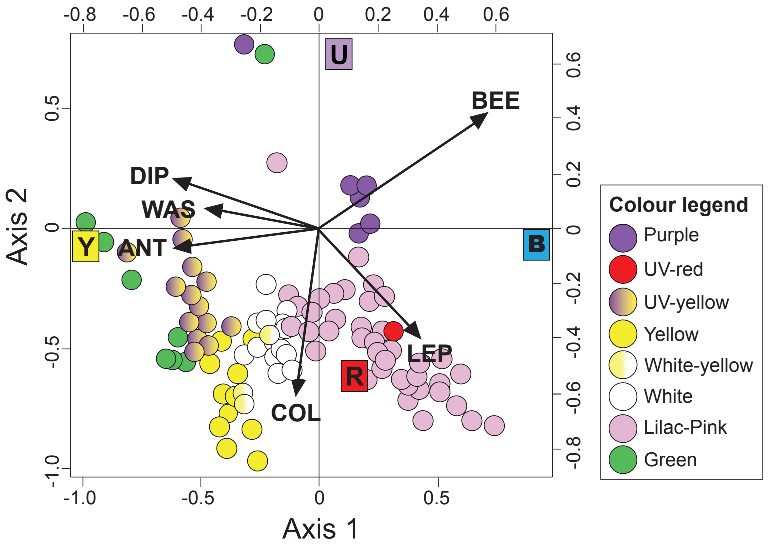

FIGURE 4.2: CCA biplot of pollinator groups and bands of the colour spectrum (coloured squares) corresponding to UV, blue, yellow, and red, respectively (data from the four communities lumped together). Each dot represents a plant population and dot colour corresponds to the flower colour categories as shown in the legend (see Table A.4.1 for example spectra of each category). BEE = bees, ANT = ants, WAS = wasps, DIP = dipterans, COL = coleopterans, LEP = lepidopterans.

4.4 Discussion

We traced the phylogenetic signal of floral colour under a neutral evolution model (Brownian motion). In this model, changes occur slowly and gradually along phylogenetic branches, and character similarity between species is related to phylogenetic proximity (Blomberg et al., 2003). Phylogenetic signal for flower colour was mostly lacking when we analysed the four communities separately, but when all species were lumped together all colour properties measured showed significant phylogenetic signal (related species were more similar in colour than expected from a random association between species and colours). Notwithstanding this influence of phylogeny on flower colour, the fact that all K values were lower than 1 indicates that similarity among related species tends to be lower than expected under Brownian motion. Most previous studies have found lack of phylogenetic signal in flower colour (Smith et al., 2008; Arnold et al., 2009; McEwen and Vamosi, 2010). However, one recent study on four Himalayan plant communities (Shrestha et al., 2014) obtained results similar to ours (significant phylogenetic signal with K values < 1). A corollary of our phylogenetic analysis is that the different colour categories considered are widely spread across the phylogenetic tree. This outcome is in agreement with reports showing important changes in flower colour as a result of relatively simple genetic changes (Rausher, 2008).

We analysed the colour preferences of the pollinator groups present in our communities. Results of the CCAs showed notable coincidences among communities and provided compelling evidence for the association between pollinator groups and certain colours. These associations were less clear in the communities with lower number of plant species, but were very apparent when the results of all 109 populations were lumped together (Fig. 4.2). Importantly, these associations are notably congruent with innate colour preferences found for specific pollinators (Lunau and Maier, 1995; Willmer, 2011). For example, inexperienced Eristalis tenax hover flies were found to land only on artificial flowers with yellow stimuli in the range of 520 to 600 nm (Lunau and Maier, 1995). Naive honey bees showed preference for stimuli reflecting between 410 and 530 nm (Giurfa et al., 1995), and bumblebees preferred artificial flowers reflecting in one or two of the three colour bands corresponding to their photoreceptor types (ultraviolet, blue, green) (Lunau, 1992). *Papilio demoleus* butterflies selected blue, green, and red colours while neglecting yellow (Ilse and Vaidya, 1956) and *Pieris brassicae* preferred artificial flowers reflecting at 450 and 600 nm (Scherer and Kolb, 1987). The hawkmoth *Macroglossum stellatarum* was shown to have a strong preference for wavelengths around 440 nm and a weaker preference for wavelengths of 540 nm (Kelber, 1997; Telles et al., 2014).

Importantly, the above-mentioned pollinator-colour associations found in our study are consistent with colour preferences described in pollination syndromes, according to which bees favour blue, flies yellow and white, lepidopterans pink and red, coleopterans white and cream and wasps favour brown and yellow flowers (Faegri and Van der Pijl, 1979; Willmer, 2011). Waser et al. (1996) explored the association between pollinator groups and bands of the colour spectrum in a natural community in Germany. Their results narrowly failed significance, but the observed trends were also consistent with associations predicted by pollination syndromes. Other community studies using more subjective (human-perceived) colour measures also found associations that were more or less consistent with pollination syndromes (McCall and Primack, 1992; Wolfe and Sowell, 2006; Lázaro et al., 2008).

However, our results cannot be interpreted as supporting pollination syndrome theory because flowers with similar colours did not attract similar pollinators (Table 4.4). That is, pollinator preferences are a necessary but not sufficient condition for the establishment of colour-based pollination syndromes. The lack of relationship between flower colour and pollinator composition can be explained by the influence of other traits, or trait combinations, on flower choices. Some studies have found phenology (Herrera, 1988), pollen/nectar rewards (Bosch et al., 1997), or corolla depth (Stang et al., 2007) to be the main drivers of pollinator partitioning in flower communities. A second factor contributing to the lack of relationship between flower colour and pollinator composition is pollinator generalisation. As in most temperate systems (Herrera, 1996; Waser et al., 1996), the majority of plant species in our study are pollinator generalists (with two or more pollinator groups each accounting for more than 10% of the observed visits), and therefore cannot be readily assigned to a given pollination syndrome. Even then, we could have found a correspondence between certain flower colours and pollinator assemblages (rather than single pollinator groups), but this was clearly not the case.

Our results are in agreement with the findings of Rezende et al. (2007), who found that plant-pollinator networks are more strongly associated to animal than to plant phylogeny. That is, related animal species are more likely to share host plants than related plant species are to share pollinator visitors. This result is attributed to pollinator mobility (Bascompte and Jordano, 2007), which allows pollinators to play a more active role in the definition of interaction identity.

Recently, Renoult et al. (2015) have used a novel approach to analyze the association between flower colour and generalization in pollinators. They argue that pollinators should preferentially visit flowers that are most conspicuous to them, and that colour perception should be analyzed as a measure of colour conspicuousness between emitters (flowers) and receivers (insects). They measure flower and background colour, and use evidence from various sources to assign one of eleven described visual systems to each pollinator species. Then they calculate 'colour matching' for each plant-pollinator pair as a measure of how a floral colour stands out from the environment for each visual system. They find that colour matching influences generalization in one of three regions studied. However, they also find that other factors (spatiotemporal overlap and co-abundance between plants and pollinators) play a greater role as determinants of plant generalization. The results of Renoult et al. (2015) in general, and the strong influence of these other factors in particular, support our conclusion that even though pollinator colour preferences seem to be conditioning plant-pollinator interactions, the selective force behind these preferences may not been strong enough to mediate the appearance and maintenance of tight colour-based plant-pollinator associations.

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Conclusions 5

Chapter two

- We highlighted the high heterogeneity in pollinator communities within a continuous habitat of Mediterranean scrubland. These differences did not follow any clear spatial pattern, resulting in a mosaic of idiosyncratic local communities. The turnover of rare species was the main driver of the variation in pollinator composition across space.

- Wild pollinator abundance was positively related to flower abundance. However, increases in flower abundance were not paralleled by proportional increases in pollinator abundance, as reflected by the negative relationship between flower abundance and wild pollinator visitation rate. Therefore, pollinators did not follow an ideal free distribution, suggesting that floral resources were not limiting at the community level.

- When resources are not limiting and most pollinators are generalist, pollinators can find enough resources to satisfy their requirements at short distances. This situation could lead to a local organization of the relationship between flowers and pollinator abundances, thus ultimately promoting independent communities between localities.

- We found a positive relationship between flower and pollinator richness. Therefore, even though overall floral resources did not appear to be limiting, the distribution and abundance of certain pollinator species may have been conditioned by the availability of certain, less abundant, flower species.

- Wild pollinator composition was only moderately related to flower composition. This result is consistent with the generalist nature of plant-pollinator interactions and the opportunistic flower choice displayed by pollinators.

- Honey bees accounted for nearly 72% of the flower visits recorded, one of the highest proportions registered in natural habitats. However, we found no evidence that honey bees had important effects on the spatial distribution of wild pollinator communities. For honey bees to exert competitive pressure on other pollinators, floral resources should be limiting, but this was not the case in our study.

- Overall, our results show that the spatial variation of the flower community is not the main predictor of the pollinator community, indicating that other factors besides flower community composition are important to explain pollinator distribution at the local scale.

Chapter three

- Our study shows that local variation in pollinator assemblage affected pollen deposition in the two *T. vulgar*is morphs, even in a relatively small and uniform natural area.
- The proportion of flowers with homospecific pollen in the female morph was positively related to pollinator richness, possibly explained by complementarity between pollinator species, and to the proportion of visits by honey bees and wild bees, which are the most efficient pollinators. On the other hand, visitation rate did not increase pollination function in the female morph.
- The proportion of flowers with homospecific pollen in the hermaphrodite morph was only influenced by pollinator visitation rate but, surprisingly, this relationship was negative. Pollen deposited on the stigmas may be subjected to secondary mobilization, with this effect only being important in the hermaphrodite morph because of its lower stigmatic adherence.
- Variation in species richness and composition of the pollinators visiting the hermaphrodite morph are not important to its pollination, probably because of the high levels of geitonogamy in this morph.
- Heterospecific pollen deposition was higher in the female morph, and was not affected by the structure or composition of the pollinator assemblage. However, the availability of heterospecific pollen had a close-to-significant positive relationship with heterospecific pollen deposition. This result is congruent with pollinators decreasing their level of flower constancy in communities in which the relative abundance of the target species is lower.
- We found no effect of the pollinator assemblage on plant reproductive success in either of the two morphs. Given that *T. vulgaris* flowers have only 4 ovules, levels of pollination appear to be adequate in most of the populations sampled.
- Changes in pollinator composition are likely to be much greater in current scenario of pollinator diversity declines. Under these circumstances, plants that are more dependent on pollinators (such as the female *T. vulgaris* morph in our study) are likely to be most affected.

Chapter four

- There is a phylogenetic signal for flower colour when all species were lumped together. Nevertheless, similarity among related species tends to be lower than expected under Brownian motion.
- We found notable coincidences among communities in colour preferences of the pollinator groups, showing compelling evidence for the association between pollinator groups and certain colours. These associations are very apparent when the results of all 109 populations were lumped together.
- Pollinator-colour associations found are notably congruent with innate colour preferences found for specific pollinators and consistent with colour preferences described in pollination syndromes, according to which bees favour blue, flies yellow and white, lepidopterans pink and red, coleopterans white and cream and wasps favour brown and yellow flowers.
- Despite the colour preferences of the pollinator groups, our results cannot be interpreted as supporting pollination syndrome theory because flowers with similar colours did not attract similar pollinators.
- We propose that pollinator preferences are a necessary but not sufficient condition for the establishment of colour-based pollination syndromes. Even though pollinator colour preferences seem to be conditioning plant-pollinator interactions, the selective force behind these preferences may not been strong enough to mediate the appearance and maintenance of tight colour-based plant-pollinator associations.

Appendix 2: Chapter 2 - Supplementary material

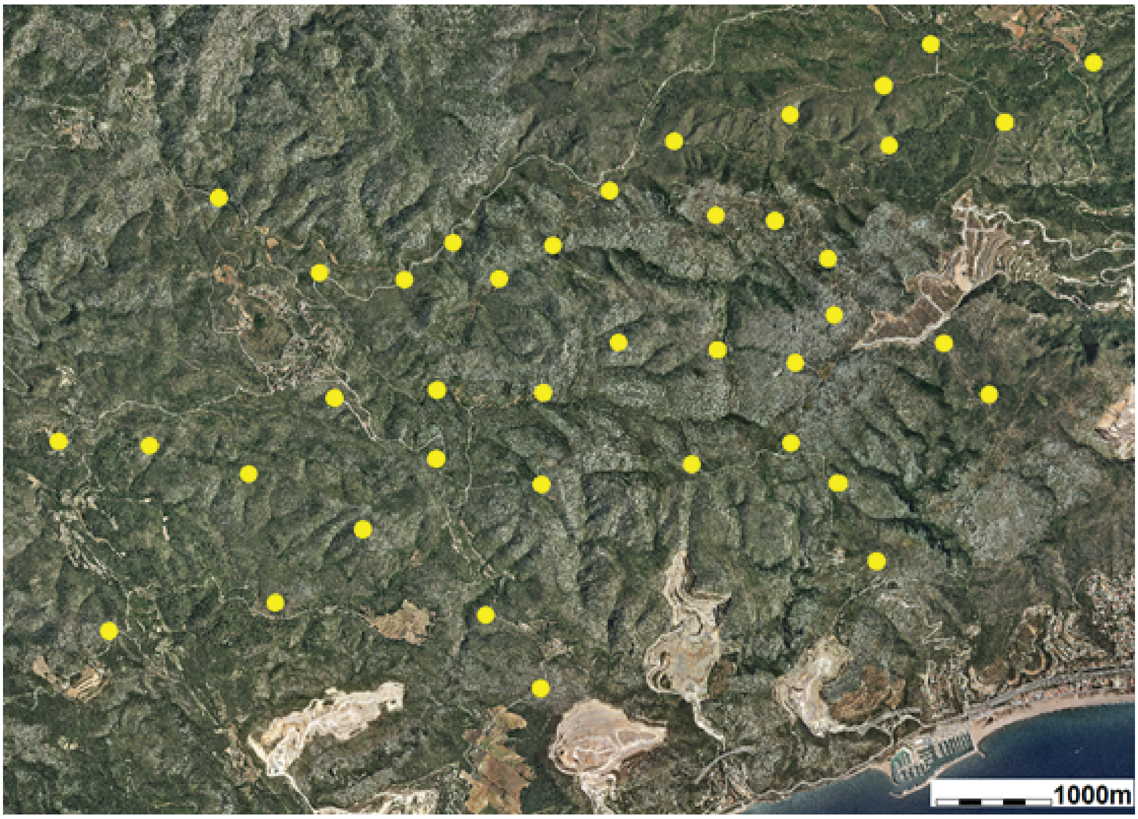


Figure A.2.1: Orthophoto map of the study area (Garraf Natural Park) indicating the 40 study plots (yellow dots).

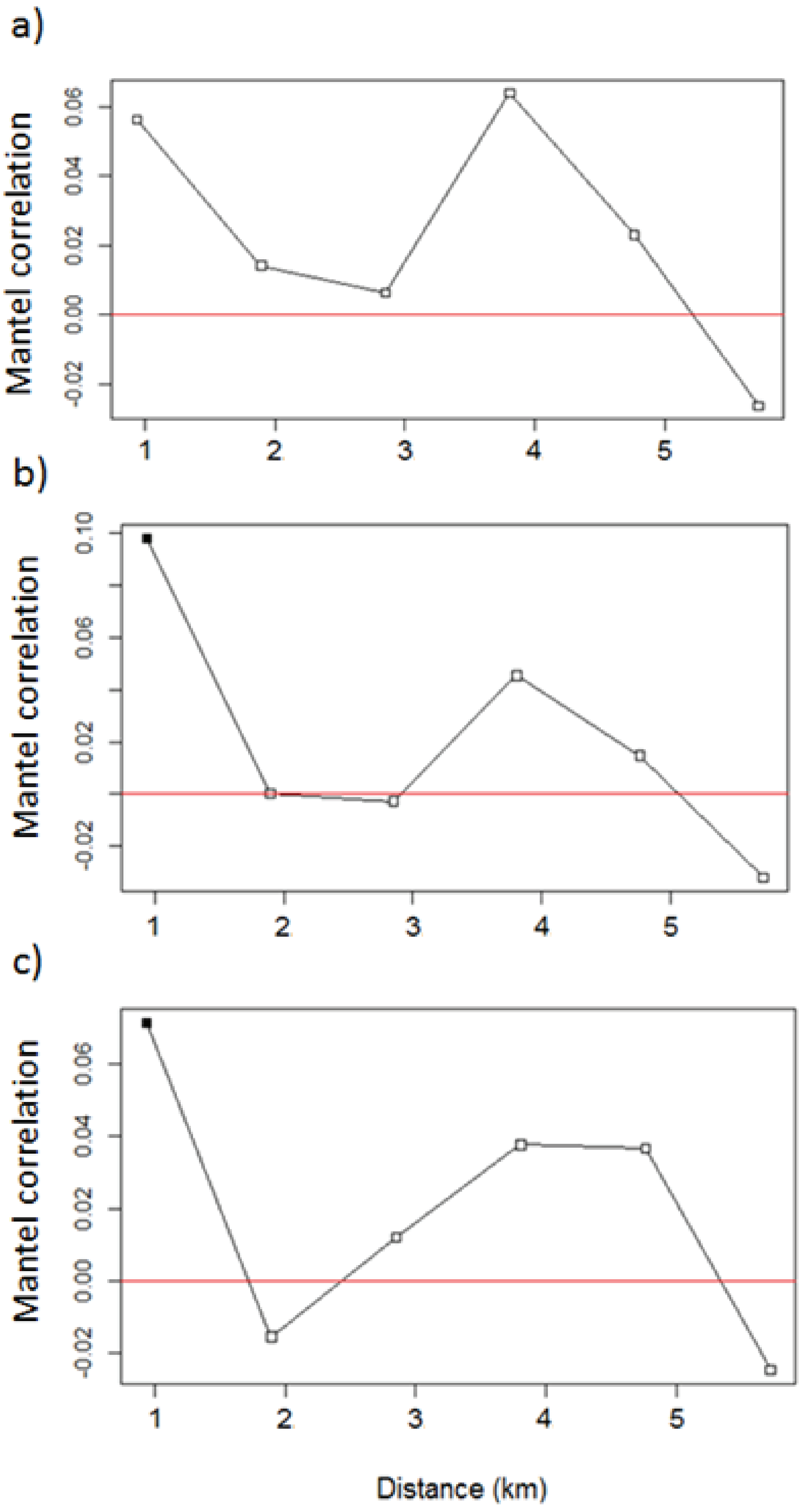


Figure A.2.2: Mantel correlogram of species community composition. Filled squares represent distance classes with a significant level ($p < 0.05$) of spatial autocorrelation. a) Flower community composition. b) Pollinator community composition (including honey bees). c) Wild pollinator community composition.

Model	Response	Predictor	Coefficient estimates	P-value
Wild Pollinator Abundance				
	Honey bee Visitation Rate	Temperature	2.805	0.000
	Honey bee Visitation Rate	Flower Abundance	0.016	0.900
	Wild Pollinator Abundance	Flower Abundance	0.436	0.001
	Wild Pollinator Abundance	Temperature	1.474	0.041
	Wild Pollinator Abundance	Honey bee Visitation Rate	0.104	0.506
Wild Pollinators Visitation Rate				
	Honey bee Visitation Rate	Temperature	2.805	0.000
	Honey bee Visitation Rate	Flower Abundance	0.016	0.900
	Wild Pollinator Visitation Rate	Flower Abundance	-0.564	0.000
	Wild Pollinator Visitation Rate	Temperature	1.474	0.041
	Wild Pollinator Visitation Rate	Honey bee Visitation Rate	0.104	0.506
Wild Pollinator Richness				
	Honey bee Visitation Rate	Temperature	2.852	0.000
	Honey bee Visitation Rate	Flower Richness	-0.066	0.822
	Honey bee Visitation Rate	Flower Abundance	0.025	0.850
	Pollinator Richness	Flower Richness	0.589	0.001
	Pollinator Richness	Temperature	0.588	0.179
	Pollinator Richness	Flower Abundance	0.098	0.181
	Pollinator Richness	Honey bee Visitation Rate	0.116	0.212
Wild Pollinator Composition				
	Honey bee Visitation Rate	Flower Composition	0.150	0.022
	Honey bee Visitation Rate	Temperature	0.330	0.007
	Wild Pollinator Composition	Flower Composition, controlling for Honey bee Visitation Rate	0.170	0.002
	Wild Pollinator Composition	Temperature, controlling for Honey bee Visitation Rate	0.170	0.012
	Wild Pollinator Composition	Honey bee Visitation Rate, controlling for Temperature	-0.090	0.880

Table A.2.1: Statistical outputs from path analyses measuring the effects of temperature, honey bee visitation rate and various flower community parameters on wild pollinator abundance, visitation rate, richness and composition. Coefficient estimates and p-values from the analysis of wild pollinator composition were extracted from partial Mantel tests (Leduc et al. 1992) and were subjected to a Bonferroni correction (alpha level of significance lowered from 0.05 to 0.01).

Appendix 3: Chapter 3 - Supplementary material

	(Int)	Visitation rate	Pollinator richness	Proportion of honey bees	Proportion of wild bees	Proportion of dipterans	Proportion of hermaphrodite *T. vulgaris*	Proportion of heterospecific pollen in the community	adjusted R^2	df	logLik	AICc	ΔAICc	weight
Proportion of flowers with homospecific pollen	0.015		0.140	1.471	1.458			NA	0.320	4	-59.2	127.5	0	0.546
	-0.177	0.347	0.120	1.611	1.610			NA	0.335	5	-58.7	129.2	1.72	0.231
	-0.311		0.131	1.800	1.820	0.816		NA	0.334	5	-58.7	129.2	1.79	0.223
Stigmatic pollen loads	2.276		0.040					NA	0.136	3	-20.3	47.3	0	0.315
	2.613		0.037				0.502	NA	0.198	4	-19.3	47.9	0.58	0.236
	2.840						0.593	NA	0.089	3	-21.0	48.6	1.37	0.159
	2.464							NA	0	2	-22.2	48.8	1.49	0.149
	2.323	-0.173	0.050					NA	0.165	4	-19.8	48.9	1.61	0.141
Proportion of flowers with heterospecific pollen	-2.591					0.994	NA	1.869	0.322	3	-83.0	172.7	0	0.225
	-2.469						NA	1.909	0.262	2	-84.7	173.7	1.04	0.134
	-2.527	0.378					NA	1.720	0.302	3	-83.6	173.8	1.14	0.127
	-0.888	0.971	-0.654			1.214	NA		0.345	4	-82.3	173.8	1.15	0.126
	-1.858	0.625	-0.425			1.113	NA	1.148	0.382	5	-81.2	174.1	1.47	0.108
	-2.610	0.242				0.826	NA	1.758	0.337	4	-82.6	174.3	1.62	0.100
	-2.182		-0.226			1.292	NA	1.646	0.335	4	-82.6	174.4	1.71	0.095
	-1.904	0.726	-0.338				NA	1.217	0.332	4	-82.7	174.6	1.93	0.086
Fruit set	0.203		0.057					NA	0.091	2	-84.6	173.5	0	0.118
	0.373		0.048		-0.548			NA	0.140	3	-83.5	173.7	0.13	0.111
	0.757		0.052				0.837	NA	0.136	3	-83.6	173.8	0.31	0.101
	0.622				-0.659			NA	0.080	2	-84.8	174.0	0.45	0.094
	0.027		0.059	0.351				NA	0.131	3	-83.7	174.1	0.55	0.090
	1.144				-0.593		0.843	NA	0.127	3	-83.8	174.2	0.71	0.083
	0.869		0.045		-0.495		0.770	NA	0.176	4	-82.6	174.5	0.96	0.073
	1.056						0.933	NA	0.065	2	-85.1	174.6	1.09	0.069
	0.577		0.054	0.298			0.789	NA	0.166	4	-82.9	174.9	1.39	0.059
	0.461							NA	0	1	-86.5	175.1	1.55	0.055
	-0.134		0.048	0.539		0.861		NA	0.161	4	-83.0	175.2	1.63	0.052
	1.007	0.303					1.103	NA	0.104	3	-84.3	175.3	1.77	0.049
	0.042			0.554		1.091		NA	0.100	3	-84.3	175.5	1.93	0.045
Seed set	2.297					0.561	0.531	NA	0.420	4	-7.8	24.9	0	0.171
	2.482				-0.376		0.550	NA	0.404	4	-8.0	25.3	0.36	0.143
	2.104			0.273		0.721	0.467	NA	0.517	5	-6.7	25.3	0.39	0.140
	1.770			0.323		0.814		NA	0.387	4	-8.2	25.6	0.73	0.119
	2.367				-0.270	0.424	0.507	NA	0.499	5	-6.9	25.7	0.80	0.114
	1.949					0.636		NA	0.249	3	-9.7	26.1	1.19	0.094
	2.429						0.610	NA	0.229	3	-9.9	26.5	1.60	0.077
	2.042				-0.295	0.482		NA	0.344	4	-8.7	26.6	1.68	0.074
	2.143				-0.419			NA	0.219	3	-10.0	26.7	1.80	0.069

Table A.3.1: Outputs from the model selection procedure for all response variables (Proportion of flowers with homospecific pollen, Stigmatic pollen loads, Proportion of flowers with heterospecific pollen, Fruit set and Seed set) for the female morph. Variables non used in the model are represented by “NA”.

	(Int)	log(Visitation rate)	Pollinator richness	Proportion of honey bees	Proportion of wild bees	Proportion of dipterans	Proportion of hermaphrodite *T. vulgaris*	adjusted R^2	df	logLik	AICc	ΔAICc	weight
Proportion of flowers with homospecific pollen	1.444	-0.545						0.268	2	-54.7	113.9	0	0.310
	1.636	-0.550	-0.050					0.304	3	-53.9	114.5	0.67	0.222
	1.250	-0.602				1.259		0.299	3	-54.0	114.8	0.91	0.197
	1.534	-0.580			-0.845			0.289	3	-54.2	115.2	1.36	0.157
	1.750	-0.588	-0.054		-0.927			0.328	4	-53.2	115.8	1.99	0.114
Stigmatic pollen loads	2.289							0	2	-14.0	32.5	0	0.372
	2.236				0.431			0.105	3	-13.0	32.8	0.27	0.324
	2.465						0.286	0.032	3	-13.7	34.3	1.75	0.155
	2.446				0.464		0.349	0.152	4	-12.5	34.4	1.82	0.149
Fruit set	-0.651			0.688				0.092	2	-72.8	150.0	0	0.394
	-0.830			0.854		0.557		0.124	3	-72.2	151.1	1.13	0.224
	-0.272							0	1	-74.5	151.2	1.19	0.217
	-0.750		0.036	0.633				0.108	3	-72.5	151.8	1.74	0.165
Seed set	1.820							0	2	-14.8	34.1	0	0.215
	2.162						0.560	0.113	3	-13.6	34.1	0.01	0.215
	1.686			0.241				0.091	3	-13.9	34.6	0.47	0.170
	1.858				-0.315			0.051	3	-14.3	35.4	1.32	0.111
	2.296	0.077					0.717	0.160	4	-13.1	35.6	1.51	0.101
	1.996			0.175			0.446	0.157	4	-13.2	35.7	1.59	0.097
	2.173		-0.010		-0.266		0.524	0.150	4	-13.2	35.9	1.75	0.090

Table A.3.2: Outputs from the model selection procedure for all response variables (Proportion of flowers with homospecific pollen, Stigmatic pollen loads, Fruit set and Seed set) for the hermaphrodite morph.

Appendix 4: Chapter 4 - Supplementary material

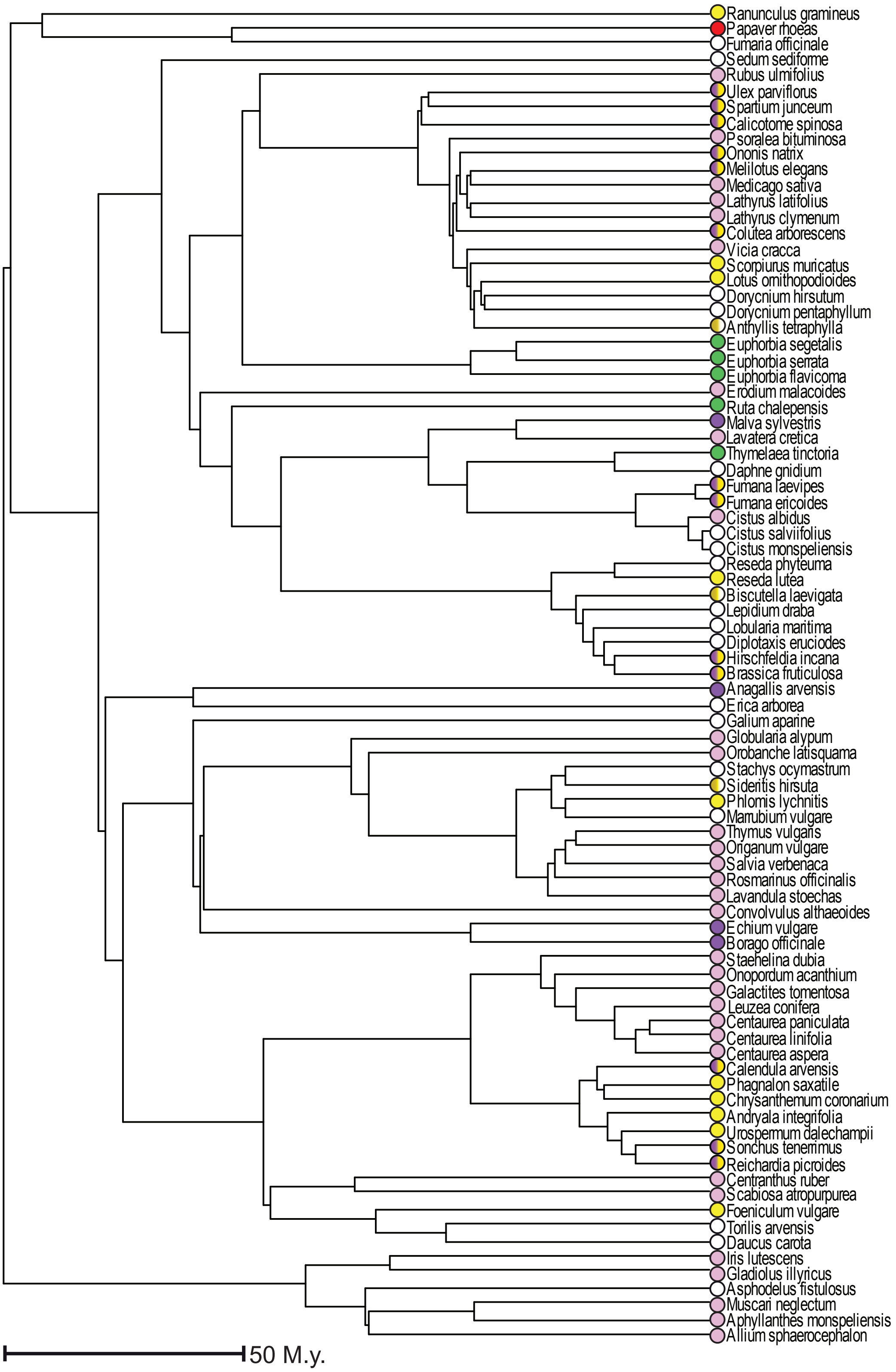


Figure A.4.1: Phylogenetic tree of the 85 species surveyed. Colour dots represent the colour category of each plant species (see colour legend in Fig. 4.1).

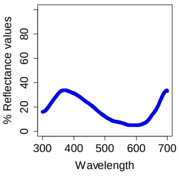
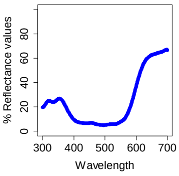
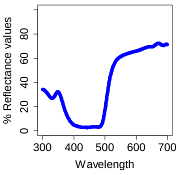
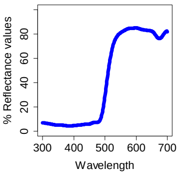
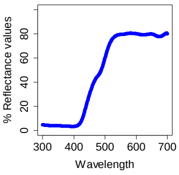
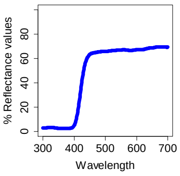
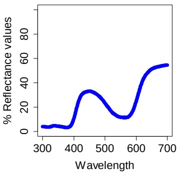
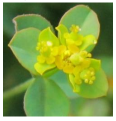
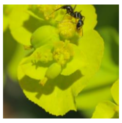
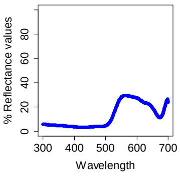
Colour category	Examples	Reflectance spectrum	CA	CO	GA	PA
Purple	*Borago officinalis*  *Malva sylvestris* 		6%	9%	4%	5%
UV-red	*Papaver rhoeas* 		6%	0%	0%	0%
UV-yellow	*Sonchus tenerrimus*  *Ulex parviflorus* 		24%	17%	0%	14%
Yellow	*Ranunculus gramineus*  *Scorpiurus muricatus* 		12%	15%	12%	14%
White-yellow	*Biscutella laevigata*  *Anthyllis tetraphylla* 		0%	2%	8%	0%
White	*Cistus salviifolius*  *Diplotaxis erucoides* 		18%	22%	16%	29%
Lilac-Pink	*Gladiolus illyricus*  *Cistus albidus* 		29%	30%	52%	38%
Green	*Euphorbia flavicoma*  *Euphorbia serrata* 		6%	4%	4%	0%

Table A.4.1: Percentage of species in each of the four communities (CA, CO, GA, PA) corresponding to the flower colour categories defined by Chittka et al. (1994) with example species of each category.

Community	Bees	Ants	Wasps	Coleopterans	Dipterans	Lepidopterans	Total
CA	721 (20.6%)	1592 (45.4%)	128 (3.7%)	831 (23.7%)	212 (6.0%)	21 (0.6%)	3505
CO	9170 (67.1%)	956 (6.9%)	173 (1.3%)	1755 (12.8%)	1401 (10.2%)	218 (1.6%)	13673
GA	2453 (28.3%)	2066 (23.8%)	109 (1.3%)	2845 (32.8%)	617 (7.1%)	576 (6.6%)	8666
PA	3464 (53.6%)	130 (2.0%)	413 (6.4%)	1512 (23.4%)	621 (9.6%)	321 (5.0%)	6461
Total	15808 (48.9%)	4744 (14.7%)	823 (2.5%)	6943 (21.5%)	2851 (8.8%)	1136 (3.5%)	32305

Table A.4.2: Number (and percentage) of pollinators of each pollinator group surveyed in each of the four communities (CA, CO, GA, PA).

Colour Category	N plant populations	Sample Size	Bees	Ants	Wasps	Coleopterans	Dipterans	Lepidopterans
Purple	7	2414	84.8	2.2	1.2	3.5	7.3	1.0
UV-red	1	43	14.0	0.0	7.0	79.1	0.0	0.0
UV-yellow	15	3611	47.9	5.5	1.8	24.3	15.5	4.9
Green	7	2016	27.3	21.8	5.3	11.4	33.2	1.0
Yellow	12	3380	43.6	24.6	3.7	17.5	10.1	0.6
White-yellow	3	1308	60.8	31.1	1.1	3.3	1.9	1.8
White	23	7175	47.7	8.6	3.1	28.1	8.1	4.3
Lilac-pink	41	12358	56.1	8.3	2.0	18.8	7.5	7.3

Table A.4.3: Mean percentage of visits of each pollinator group to each colour category (data of the four communities lumped together).

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