DISTINGUISHING POLLINATION FROM VISITATION: THE VALUE OF A POLLINATOR EFFECTIVENESS AND POLLINATOR IMPORTANCE NETWORK

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Distinguishing pollination from visitation:
The value of a pollinator effectiveness and pollinator importance network.

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The true biologist deals with life, with teeming boisterous life, and learns something from it, learns that the first rule of life is living.

John Steinbeck, The Log from The Sea of Cortez

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Abstract

For over twenty years, flower-visitation networks have been used to assess the effects of pollinator decline, linked to habitat loss, climate change and invasive species, on entire communities. However, most rely on flower visit frequency as a proxy for pollination; very few sample pollen from flower visitor's bodies or from stigmas and so do not include a quantitative measure of pollination success. Here, I add pollinator effectiveness (as single visit pollen deposition) into a traditional flower visitation network, creating a pollinator importance network that better evaluates the flower visitor community from the plant's perspective. Given recent interest in pollination in urban areas, I use an urban garden habitat, and compare visitation, pollen transport and pollinator importance networks, giving several novel conclusions.

Firstly, although there are similarities in the structure of my networks, interactions were most specialised in the pollinator importance network, with pollen transport proving to be a better proxy for pollinator importance than visitation alone. Secondly, the specialisation of individual plants and the role of individual flower visitors varied between the networks, suggesting that community-level patterns in simple visitation networks can mask important individual differences. Thirdly, the correlation between flower visit frequency and pollinator importance largely depends on bees, and may not hold in plant-pollinator communities that are not bee-dominated. Fourthly, heterospecific pollen deposition was relatively low, despite the unusually diverse plant community of a garden. Finally, bees (particularly *Bombus* and non-eusocial halictids) carried the largest pollen loads and were the most effective at depositing pollen on to the stigma during a single visit in this garden habitat.

The implications of this thesis highlight the strengths and limitations of each network for future studies, and raise important questions for the future of urban pollination studies.

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I take inspiration from you all.

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Chapter 1.

General Introduction

Pollination is one of the most widely studied mutualistic interactions, explored from evolutionary, ecological and economic perspectives. It is a vast topic and this study focuses on a single area of current research: the importance of distinguishing between flower visitors and true pollinators in a time of pollinator decline.

1.1 The importance of plant-pollinator interactions in the 21st Century

Interactions between plants and flower visitors date back to the time of the dinosaurs, preserved in amber as tiny thrips covered in pollen from 100 million years ago (Penalver *et al.* 2012). Today, flower visitors are represented by a wide variety of taxa, including many mammals (e.g. rodents and lemurs), birds (e.g. hummingbirds and sunbirds), reptiles and insects. By far the most common flower visitors are the invertebrates, especially bees (superfamily Apoidea) which are virtually the only insect to depend entirely on floral resources for the development of young (Thorp 2000). However, the total number of all species that exploit flowers is unknown (although estimates suggest 200,000 Buchmann & Nabhan, 1996) with many interactions undocumented, either because they are rare or do not result in pollination. From the plant's perspective, an estimated 87.5% of angiosperms rely on zoophilous pollination, representing about 300,000 species found on every continent except Antarctica (Ollerton *et al.* 2011). The reliance of many crop species on animal pollination, combined with declines in several pollinator taxa (Potts *et al.* 2010, Regan *et al.* 2015), make pollination one of the greatest, global ecological concerns.

Insects that visit flowers are of particular interest because of the widespread global declines reported in their populations. Following early publications (e.g. Buchmann & Nabhan 1996) and the seminal review by Potts *et al.* (2010) which demonstrated substantial losses in both bee and hoverfly species, the status of pollinator populations has been the subject of much research. The most studied groups include *Apis* and *Bombus*, particularly North American and European species, with declines reported in both groups (Oldroyd 2007, Williams & Osborne 2009). More recently, the role of dipteran visitors as pollinators has gained attention (Kearns 2001, Orford *et al.* 2015, Rader *et al.* 2016) with syrphids (hoverflies) now recognised as a major group of pollinators (e.g. Baldock *et al.* 2015). An excellent review of the major stresses facing flower visiting insect populations is provided by Winfree *et al.* (2009) and Winfree (2010), with recent studies supporting the evidence that several factors negatively affect wild and managed flower visitors, including anthropogenic habitat fragmentation (Darvill *et al.* 2006, Howell *et al.* 2017), mass monoculture (Kennedy *et al.* 2013), urban pollution (Fuentes *et al.* 2016), mismanagement of commercial *Apis* hives or invasive species (Hanna *et al.* 2014, Isaacs & Kirk 2010), disease (Fürst *et al.* 2014), climate change (Gallagher & Campbell 2017, Papanikolaou *et al.* 2017) and their effect when combined (Goulson *et al.* 2015).

Yet despite a wealth of research on the causes of pollinator decline, there is still a lack of information for many of the estimated 20,000 species of bee (many of which do not make large contributions to crop pollination, Kleijn *et al.* 2015), let alone for dipteran and lepidopteran visitors, and a paucity of standardised, long-term monitoring programs (although two examples are given in Winfree 2010) makes it difficult to confirm the IUCN conservation status of many species (Nieto *et al.* 2014) and to prove that a global 'pollinator crisis' exists (Ghazoul 2005, Winfree 2010). This crisis is a major area of current media and scientific interest, largely because of the dependency of many crop yields on pollination. With the world's human population expected to reach 9.7 billion by 2050 (UN DESA, 2015), insect pollination is known to increase the yield of at least 39 of the most important crops globally (n=57, Klein *et al.* 2007) and pollination 'gaps' threaten yields in several continents (e.g. in India by Pannure 2016 and in China by Teichroew *et al.* 2017). However, there are claims that this crisis has been over exaggerated, largely from agrochemical companies (e.g. Blacquière & van der Steen 2017) opposed to the growing body of evidence showing a negative effect of pesticides on pollinator populations documented by many studies (most recently Robinson *et al.* 2017, Tosi *et al.* 2017, Woodcock *et al.* 2017). Yet there is evidence that some pollinator populations are thriving (e.g. the expanding range of *Bombus hypnorum*, Crowther *et al.* 2014) and understanding why and how land management practices can support these populations (e.g. Burkle *et al.* 2017, Kovács-Hostyánszki *et al.* 2017) provides a promising, optimistic area for future research.

Flowering plants are also of major interest, not least because they account for 95% of the world's 390,000 plant species (RBG Kew 2016) and because a third of all land plants face extinction (Corlett 2016). These species link pollinators to many other organisms in different trophic levels (e.g. herbivores, Mothershead & Marquis 2010; below-ground soil communities, Barber & Soper Gorden 2015) and play key roles in the functioning of ecosystems. Aside from providing crops for food, fuel and building materials, flowering plants benefit soil quality (e.g. legumes fix nitrogen, while brassicas mitigate leaching, Dabney *et al.* 2001), provide pest regulation for other crops (e.g. Letourneau *et al.* 2011) and act as host plants for other beneficial insects (e.g. Lepidoptera, Cutting & Tallamy 2015; and domatia for ants, Heil 2008). One of the most promising areas of research is the use of wild taxa as gene sources for improvement of crops; traits that have previously been bred to promote high yields are not always those that are resistant to climate change, pests or pathogens, or favourable to pollinators, and wild relatives are increasingly used to reintroduce or improve these traits in commercial species (e.g. improving salt tolerance in citrus and tomato, Flowers 2004).

However, declines in pollinator populations have been mirrored by the loss of plant diversity (Biesmeijer *et al.* 2006), with many of the same stressors facing the plants on which pollinators depend. Anthropogenic habitat fragmentation can reduce gene flow between flowering plants, either by pollen limitation (Wilcock & Neiland 2002, Dick *et al.* 2003) or a reduction in flower visitation (Goverde *et al.* 2002, Lobo *et al.* 2016). Flower visitation may also be reduced by a shift in flowering phenology or abundance caused by climate change (Inouye 2008), the presence of invasive plant species (Bartomeus & Santamaria 2008) or even a reduction in nectar quality due to urbanisation (Wehner *et al.* 2017). Agricultural monocultures are the greatest global threat to plant diversity (RBG Kew 2016) and the loss of wildflowers poses serious threats to pollinators reliant on floral resources; recent work by Baude *et al.* (2016) revealed substantial declines in nectar provision in Britain, with just four species (*Trifolium repens*, *Calluna vulgaris*, *Cirsium palustre* and *Erica cinerea*) responsible for over 50% of national nectar availability. Studies such as these are desperately required to identify the most important plant species in lesser studied regions (e.g. Africa and the lowland tropics) and in high latitude biomes that are likely to be particularly affected by climate change (Wipf & Rixen 2010, Benadi *et al.* 2014).

1.2 A network approach for studying mutualistic interactions

Given the diversity of flower visitors that a single plant may receive (e.g. an extreme example being the 298 species in 84 genera recorded by Robertson, 1928) individual plant-pollinator partnerships are now rarely considered in isolation. Instead, all of the partnerships in a plant-pollinator community are often examined simultaneously by creating a ‘pollination network’. Derived from food-web theory, interest in mutualistic pollination networks began with Jordano (1987) and gradually became a popular tool for studying plant-pollinator interactions in a variety of habitats (Waser *et al.* 1996, Memmott 1999, Memmott & Waser 2002, Olesen & Jordano 2002, Olesen *et al.* 2002, Ollerton *et al.* 2003). These networks allow complex patterns of visitation to be visualised, permitting the stability of the interactions and likely persistence of biodiversity to be analysed (Montoya *et al.* 2006).

Unlike food webs, the interactions in plant-pollinator networks are bipartite, that is they only ever take place between two levels (i.e. plant and pollinator) rather than between individuals of the same level. Typically, plants are represented as nodes on the lower level, with flower visitors on top (**Fig 1.1**). In a traditional pollination network, the strength of the interaction is weighted by the frequency of flower visitation; the width of a species node represents the total number of visits recorded for a particular species, and the width of the connecting interactions represents the total number of visits between a specific visitor and plant. These interactions may also be illustrated as a matrix, where the depth of the shade indicates the relative strength of the interaction (**Fig. 1.2**).

Early on in the literature, several common patterns were noticed in the distribution of interactions in plant-pollinator networks: (i) interactions followed a truncated power-law distribution, where a small number of species have very many interactions, while many species have relatively few (Jordano *et al.* 2003); (ii) very few of all the possible links between species are actually realised (Jordano 1987); (iii) there is an asymmetry of dependence between partners, where a very rare visitor may visit a plant which receives many visits (Bascompte *et al.* 2006); and (iv) the interactions are often nested, such that rare visitors visit a subset of the plants that receive the most visitors (Bascompte *et al.* 2003). These properties are explained in excellent reviews by Bascompte and Jordano (2007) and Vázquez *et al.* (2009), and explored in more detail in Chapter 2. However, these patterns are the result of two important factors that shape interactions in all plant-pollinator networks: phenotypic trait matching between flowers and visitors, and interspecific variation in the abundance and distribution of both plant and flower visitor species (Vázquez *et al.* 2009).

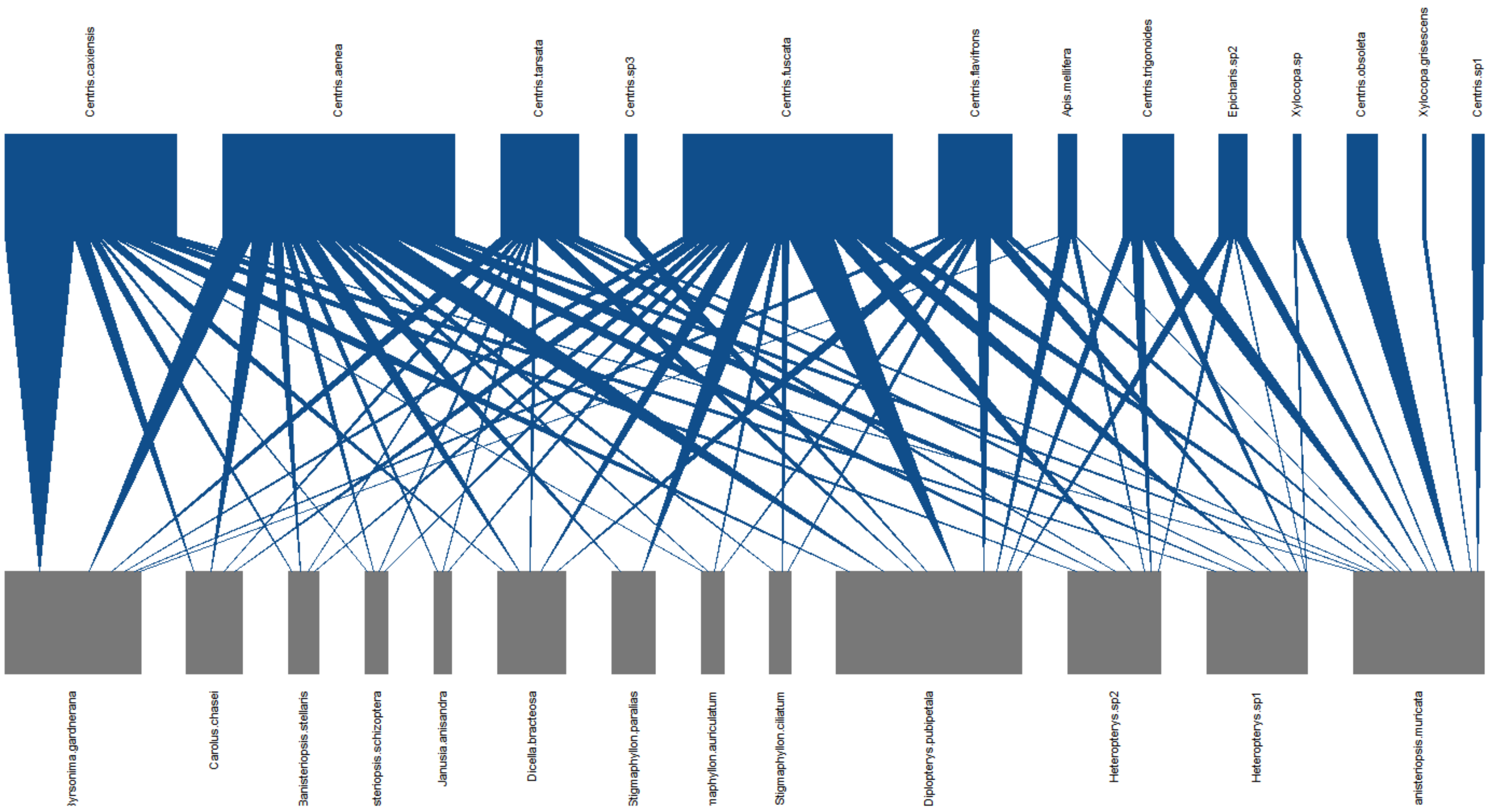


Figure 1.1 An example of a bipartite pollination network of 38 oil collecting bees visiting 13 oil producing flowering species (Malphighiaceae). Bipartite networks show the interactions between flower visitors (higher level nodes, here in blue) and plants (lower nodes, in grey). The width of the species' nodes reflects the total number of interactions recorded, and the width of interactions indicates the frequency of flower visitation between particular species. Data from Bezerra *et al.* (2009).

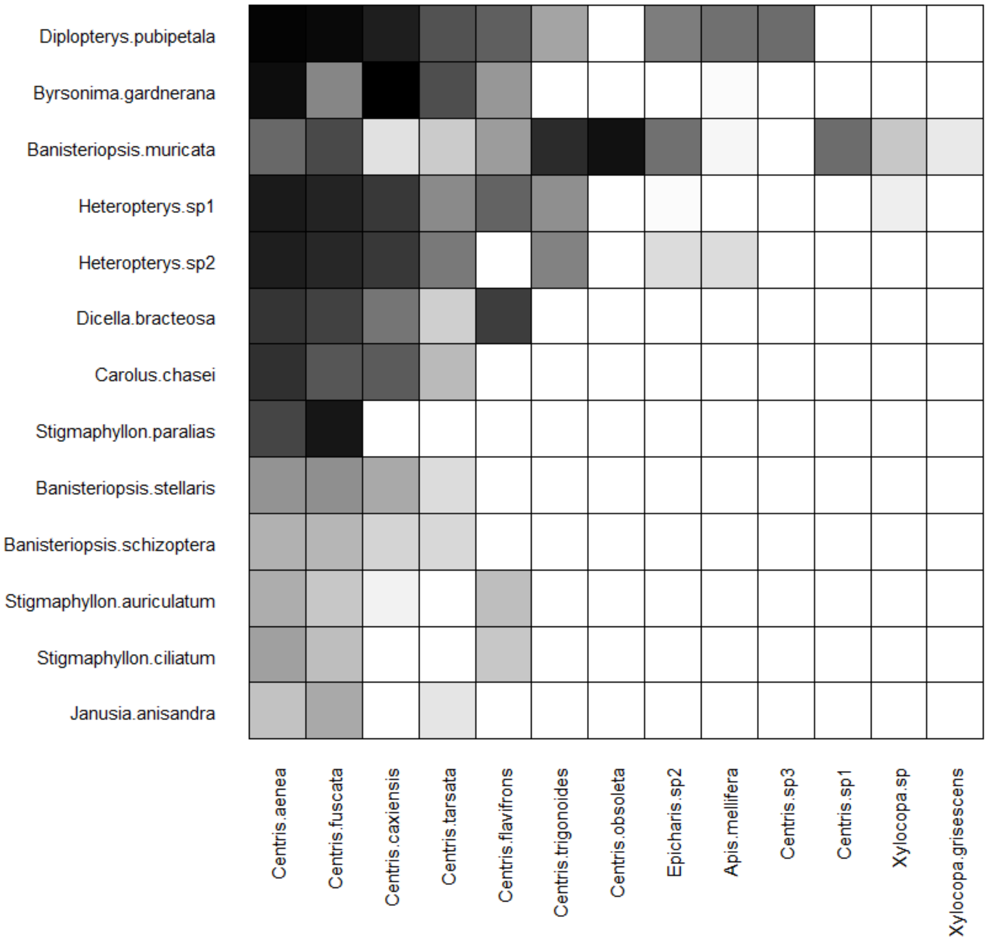


Figure 1.2 An example of a bipartite quantitative matrix of 38 oil collecting bees visiting 13 oil producing flowering species (Malphighiaceae). In this matrix, flower visitors are represented on the horizontal axis and plants on the vertical axis, in the order of most to least connected. The depth of the shading reflects the total number of interactions recorded, with darker boxes representing the most frequent interactions. Data from Bezerra *et al.* (2009).

Over the course of twenty years, plant-pollinator networks have been used to assess the impact of ‘alien’ invasions (Memmott & Waser 2002, Olesen *et al.* 2002, Lopezaraiza-Mikel *et al.* 2007, Aizen *et al.* 2008, Larson *et al.* 2016), habitat loss (Fortuna & Bascompte 2006), species extinctions (Memmott *et al.* 2004, Kaiser-Bunbury *et al.* 2010, Vanbergen *et al.* 2017) and climate change (Memmott *et al.* 2007, Hegland *et al.* 2009, Bartomeus *et al.* 2011) on flower visitation patterns. Using a network approach has also allowed the success of habitat restoration projects to be evaluated, for example in hay meadows (Forup & Memmott 2005, Kaiser-Bunbury *et al.* 2009), heathlands (Forup *et al.* 2008), pine forests (Devoto *et al.* 2012) and, most recently, following the removal of exotic shrubs from island mountaintops (Kaiser-Bunbury *et al.* 2017).

In many plant-pollinator communities, rare flower visitors account for a large proportion of the interactions (Williams *et al.* 2001, Minckley & Roulston 2006, Petanidou & Potts 2006, Gómez *et al.* 2007). This raises the intriguing question as to whether the diversity or abundance of flower visitors is more important for maintaining the structure of the network; it might be expected that rare species make a small contribution to the overall ecosystem (Vázquez *et al.* 2005), however recent work using a variety of non-insect taxa has shown that rare species can be crucial for maintaining the functional structure of the community (Leitão *et al.* 2016). Rare flower visitors are often the focus of conservation efforts, as these species are often the most vulnerable to disturbance (Winfree *et al.* 2014). However, very little is known about the value of these rare visitors from the perspective of the plants, and whether the restricted niche of these flower visitors is a true representation of their floral diet.

While networks can be used for large-scale comparisons, e.g. between island communities separated by hundreds to thousands of kilometres (Castro-Urgal & Traveset 2014, Traveset *et al.* 2016), there is a growing interest in smaller-scale networks, where individual visitors and plants are plotted to reveal intra-specific variation in foraging traits (Tur *et al.* 2014, Dupont *et al.* 2014, Lihoreau *et al.* 2016). This flexibility guarantees that plant-pollinator networks will continue to play a key role in pollination ecology, particularly for monitoring the persistence of species facing anthropogenic disturbance.

1.3 Generalisation, specialisation and pollination syndromes

In plant-pollinator networks, the density and distribution of the interactions allows differences in niche breadth between plant and flower visitor species to be visualised. This was illustrated in **Figure 1.2**, where the bee *Centris aenea* has a much wider niche than either

Xylocopa species, which is also true for the plant *Diplopterys pubipetala* compared to *Janusia anisandra*. These differences are referred to as ecological generalisation (in the case of *C.aenea* or *D. pubipetala*) or specialisation (*Xylocopa* or *J. anisandra*), an issue which has received considerable critical attention in plant-pollinator networks. In its simplest form, ecological generalisation is the use of several plant species by a flower visitor, or the use of several visitors by a plant (Waser *et al.* 1996). However, for a botanist, the term ‘generalist’ may also refer to a simple floral morphology, where the rewards are accessible to many visitors (Ollerton *et al.* 2007) and specialisation may not be as simple as counting the number of partners. For example, is specialisation classified at the species, family or genus level? Does it refer to the collection of pollen, nectar or both by visitors? (Waser *et al.* 1996); and over what time-scale does specialisation occur? (Alarcón *et al.* 2008, Brosi 2016).

In a community context (*sensu* Waser *et al.* 1996) specialisation is an important aspect of network analysis for two reasons. Firstly, niche breadth is closely linked to understanding interspecific competition (Blüthgen 2010) and how species’ traits evolve to be a balance between exploiting unique resources while remaining able to interact with a number of partners (Coux *et al.* 2016). Secondly, the level of specialisation may be used as an indicator of the biodiversity required to provide a complete ecosystem service; in communities that are highly specialised, many species are needed to maintain the stability of the interactions (Blüthgen 2010) and the loss of any pollinator will reduce pollen deposition and plant fitness (Wilcock & Neiland 2002). Interestingly, specialist species in plant-pollinator networks have not been found to be any more vulnerable to extinction than generalists in the long term (Vázquez & Simberloff 2002, Ashworth & Aizen 2004) although they may be more susceptible in the short term (Stang *et al.* 2007).

The issue of specialisation has also been a controversial and much disputed subject within the field of pollination ecology, largely because it connects network theory to the traditional concept of ‘pollination syndromes’. Pollination syndromes predict that the most effective pollinator (i.e. the flower visitor that makes the greatest contribution to plant fitness and therefore exerts the greatest selection pressure) can be predicted from the floral phenotype (e.g. size, symmetry, scent, colour, nectar traits, reviewed in detail by Willmer 2011). This theory played a large role in shaping the idea of co-evolution between floral morphology and flower-visitors to individual plant species in the literature during the 1980’s and 1990’s (e.g. Cruden & Hermann-Parker 1979, Rebelo 1985, Armstrong & Irvine 1989). However, the influential review by Waser *et al.* (1996) and the emergence of community-wide pollination networks challenged the extent to which specialisation was seen as the norm (Ollerton 1996, Memmott 1999, although see Fenster *et al.* 2004). Instead, cases where plants or flower visitors are highly specialised are relatively difficult to find in pollination networks, with most species interacting with multiple partners (Olesen & Jordano 2002). This makes sense from the plant's perspective, as generalisation is likely to be favoured in the long term if the most effective pollinator changes over plant generations, for example as a result of population fluctuations (Waser *et al.* 1996). On the other hand, the benefits of generalisation are relatively short-term for the visitor, who is likely to favour generalisation if the rewards are similar between plants and travel is costly, particularly if pollinator life-span exceeds that of individual plants (Waser *et al.* 1996).

Despite the pre-eminence of generalisation in pollination networks, the interactions often fall on a scale of generalisation (Ollerton *et al.* 2007) and some partnerships may still be very specialised (Johnson & Steiner 2000). After Ollerton *et al.* (2009) suggested that syndromes were redundant as they did not predict the most frequent flower visitors in a variety of habitats, more recent literature has emerged contradicting this. Using data from 417 studies where the effectiveness of pollinators was measured in several ways, including pollen deposition on to the stigma, Rosas-Guerrero *et al.* (2014) confirmed that floral evolution is largely driven by adaptation to the most effective pollinator – in accordance with syndrome theory. These findings, based on more robust measures of pollination, highlight an important problem that remains poorly understood: generalisation in flower visitation may not be equal to generalisation in pollination.

1.4 Flower visitation is not synonymous with pollination

The discrepancy between flower visitors and true pollinators has been well reported since flower visitation networks first started to appear, with Waser *et al.* (1996) clearly warning against the assumption that flower visitation is synonymous with pollination. In animal pollinated plants, pollination is defined as the movement and deposition of conspecific pollen from an anther to stigma, via an animal vector (Wilcock & Neiland 2002, Ne'eman *et al.* 2010, Willmer 2011). Wilcock and Neiland (2002) provide an excellent review of the many causes of pollination failure in plants, which can be classified into two groups: visits where no conspecific pollen is deposited on to the stigma, or those where pollen is deposited, but is not viable for fertilisation. In the first scenario, a visitor may fail to deposit any pollen if (i) it does not carry any pollen (Watts *et al.* 2013); (ii) all of the grains it carries are groomed or lost from its body during transport (Thomson 1986, Parker *et al.* 2015); (iii) it fails to contact the stigma of the flower, possibly during a larcenous visit (i.e. by robbing nectar or pollen, Inouye 1980, Irwin *et al.* 2010); or (iv) the stigma of the receiving flower is not receptive or already clogged with pollen (Cruden 2000, Larsson 2005). While the abundance of cheating flower visitors can vary between flower visitation networks, (e.g. from 28%-75% of all visitor species, Genini *et al.* 2010) these visitors are known to have a large impact on network structure (Genini *et al.* 2010). Secondly, even if pollen is deposited onto a stigma, several post-pollination factors may reduce the quality of the flower visit: the quantity of pollen may be insufficient for fertilisation (Knight *et al.* 2005), grains may have lost their viability in transport (Rader *et al.* 2011, Parker *et al.* 2015), or germination is inhibited by self-incompatibility mechanisms in the plant (Kawagoe & Suzuki 2005, Takayama & Isogai 2005, Allen *et al.* 2011). The presence of heterospecific species can also reduce the viability of pollen, either by allelopathy (Arceo-Gómez & Ashman 2011) or clogging of the stigma (Holland *et al.* 2007).

Despite the long success of pollination networks, almost all studies have acknowledged but failed to deal with the fact that flower visitation is only a proxy for pollination. Consequently, these networks measure interactions between flowers and visitors, rather than plants and pollinators. Recent studies have acknowledged this by replacing the term ‘pollination’ with ‘flower-visitation’ network (Castro-Urgal & Traveset 2014, Theodorou *et al.* 2017); however, these networks may still provide a biased view of the most important flower visitors from the plant’s perspective and, worryingly, the community’s tolerance to disturbance. Many agree that a true pollination network which evaluates flower visitors from the plant’s perspective, requires additional, quantitative, pollen-based measures alongside traditional visitation frequency. Although visitation frequency is undoubtedly an important aspect of pollination (Vázquez *et al.* 2005), the conclusions drawn from previous flower-visitation networks need to be tested by a direct comparison between a visitation network and a network that includes a measure of the pollen transported to stigmas by flower visitors.

1.5 Quantifying pollination effectiveness using pollen-based measures

‘Pollinator effectiveness’ is a familiar term in pollination ecology, defined as the quantity of pollen deposited by a flower visitor on to a stigma during a single visit (abbreviated to single visit deposition, SVD) as recommended by Inouye *et al.* (1994) and Ne’eman *et al.* (2010). SVD has been used to compare the effectiveness of pollinators in several studies, e.g. 105 flower visitors to 13 plant species (King *et al.* 2013) or several flower visitors to a single plant (Gómez & Zamora 1999, Mayfield *et al.* 2001, Sahli & Conner 2007). However, the term 'pollinator effectiveness' has also been used to describe pollen tube growth (Brittain *et al.* 2013), seed set (Olsen 1997), or as a blanket term for several aspects of pollinator behaviour (Fishbein & Venable 1996, Thomson & Goodell 2001, Ivey *et al.* 2003, Watts *et al.* 2012, Castro *et al.* 2013) and true measures of SVD have also been called pollinator intensity, efficiency or efficacy (reviewed in detail by Ne'eman *et al.* 2010). This interchangeable use of similar terminology has made it difficult to compare studies and evaluate the amount of data available for SVD; consequently, there is a real need for future studies to pay attention to previous work that promotes an unambiguous terminology (e.g. Ne'eman *et al.* 2010). Ne'eman *et al.* (2010) clearly differentiate between measures of pollination that are direct (SVD) or indirect (e.g. visit duration, quantity of pollen on the visitor's body) and show how the unit of the single visit can be scaled up to measure total pollinator importance in a certain number of visits or time frame.

To date, few studies have taken the measure of SVD and applied this to several plants. However, King *et al.* (2013) selected a variety of temperate and tropical plants and measured the SVD of all flower visitors, to show that the most effective pollinator always conformed to that expected from a syndrome approach. Similarly, when testing the SVD of all visitors to 76 plants in several habitats, Willmer *et al.* (2017) found that a syndrome approach matched the most effective visitors to most plants. This indicates the need to incorporate measures of SVD into community-wide flower-visitation networks, as generalised patterns in flower visitation are likely to mask more specialised patterns in pollen deposition. Previously published studies have incorporated indirect measures of SVD into flower-visitation networks, using the pollen loads on flower visitor bodies as a proxy for pollinator effectiveness (Forup & Memmott 2005, Alarcón 2010, Devoto *et al.* 2011, Popic *et al.* 2013, Tur *et al.* 2014). However, as flower visitors are known to vary in their effectiveness (Fenster *et al.* 2004, Watts *et al.* 2012) and not all of the pollen on the visitor's body is likely to make it to the stigma (Holmquist *et al.* 2012), SVD represents a promising measure for inclusion in the next generation of flower visitation networks.

Working in three diverse plant communities (Dorset heathland, Israeli garigue and Kenyan scrubland) Ballantyne *et al.* (2015, 2017) have presented the first pollinator effectiveness networks, where interactions are weighted by the quantity of pollen deposited on to the stigma. As previously published studies have hypothesised that flower visitation frequency is a suitable proxy for pollinator effectiveness (Vázquez *et al.* 2005), pollinator importance networks have also been created where the interactions are weighted by the mean SVD (pollinator effectiveness) multiplied by flower visitation frequency. These networks offer an insight into the plant-pollinator communities from the plant's perspective, suggesting that rare visitors may be important pollinators, and that specialisation in the interactions may be higher than predicted from visitation, with consequences for restoring and conserving plant-pollinator communities (Ballantyne *et al.* 2015, 2017). While this is a promising start, others have been put off by the sampling effort required for measuring SVD at the community level, although this may not be as high as expected (King *et al.* 2013, Ballantyne *et al.* 2015, Willmer *et al.* 2017). Instead, pollen loads from flower visitor bodies are seen as a more favourable measure to create pollen transport networks, as pollen loads are relatively easy to collect. However, to date no comparisons exist between a visitation, pollen transport and pollinator importance networks to test whether both proxies for pollination match the structure of the interactions in a pollinator importance network. A simultaneous comparison of each network type for a single community is therefore needed to provide a much stronger picture of the contributions of flower visitors to the fitness of plants, which is crucial at a time of both plant and visitor decline.

In conclusion, flower visitation networks are the most popular way to understand the structure of interactions between plants and their pollinators, and are set to continue to dominate the literature. Specialisation is an important pattern in these networks, although flower visitation appears to be largely generalised. However, flower visitation should not be confused with pollination, and the next step to achieving plant-pollinator networks based on quantitative measures of pollination is to continue to include SVD, whilst also comparing this to a pollen transport network.

1.6 Thesis aims

The specific objective of this study was to investigate how the structure of pollinator effectiveness and importance networks (particularly the level of specialisation) compare to that of (i) a traditional flower visitation network, (ii) a pollen load network and (iii) a pollen transport network. The data used for constructing each of these networks are summarised in **Figure 1.3**, with flower visitation frequency playing a central role in the calculation of pollen transport and pollinator importance. Therefore, while this study is designed to directly test the assumption that flower visitation frequency is a suitable proxy for pollination (Vázquez *et al.* 2005), I acknowledge its importance for determining the value of flower visitors as pollinators from the plant's perspective.

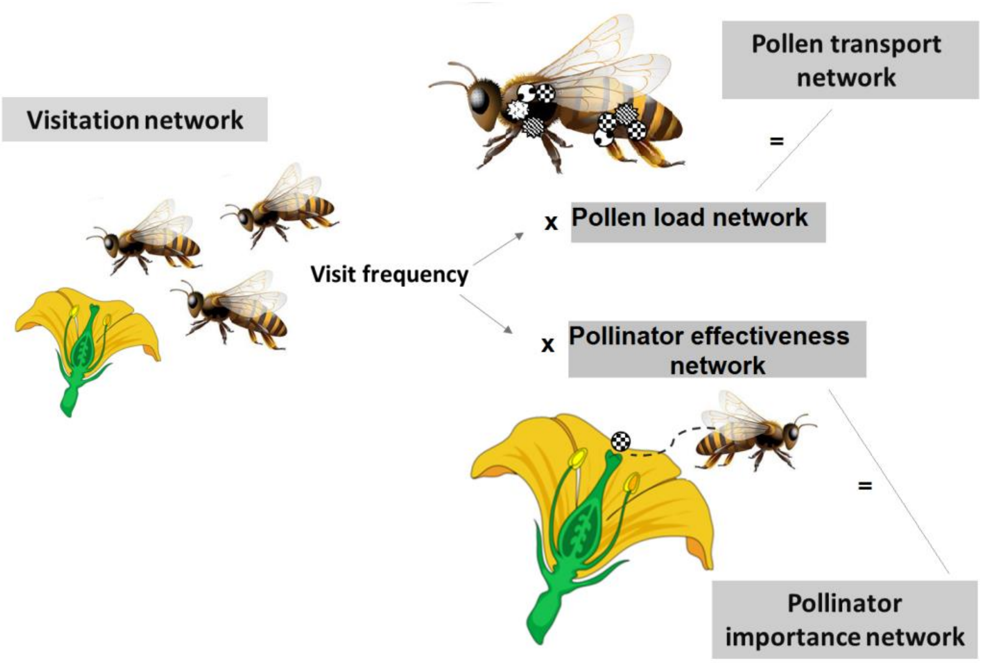


Figure 1.3 Visual representation of the data used to create five networks in this study: visitation, pollen load, pollen transport, pollinator effectiveness and pollinator importance. A traditional flower visitation network is constructed by recording all visitors to flowers. This forms the basis for creating a pollen transport network (visit frequency multiplied by the average pollen loads of flower visitors) and a pollinator importance network (visit frequency multiplied by the average single visit stigma deposition). A direct comparison between each type of network has never been recorded, and is required to assess the strengths and limitations of using proxies for pollination to assess the structure of the interactions in plant-pollinator communities. Images adapted from © Can Stock Photo / Merlinul.

This study also offers insights into the functioning of flower visitor communities in urban areas, as others are reporting the importance of these areas as refuges for insect pollinators (e.g. Baldock *et al.* 2015, Hall *et al.* 2017). Urban gardens have gained considerable attention from the media and scientific community, with recent studies reflecting a growing interest in how ‘pollinator-friendly’ gardening can benefit urban flower visitor populations (e.g. Garbuzov *et al.* 2015, Shackleton & Ratnieks 2016). A managed garden was selected as the study site so that a unique, diverse community could be used to test the conclusions of Ballantyne *et al.* (2015, 2017). As relatively little is known about the collection and movement of pollen loads by flower visitors in urban gardens, I use the data in this thesis to demonstrate the diversity of flower visitor interactions in a garden, to evaluate the importance of Diptera as pollinators, and to assess levels of heterospecific pollen receipt in diverse plant communities.

Thesis Outline

This research compares, for the first time, the structure of a pollinator importance network relative to a visitation and pollen transport network, making an important contribution to network theory where flower visitation frequency is used as a proxy for pollination. As the study was based in a garden and these sites have gained much recent interest, three chapters are dedicated to exploring the detail *within* these networks, before the networks are compared.

In this first chapter, I have reviewed the importance of pollinators and the plants they visit in the context of population declines, stressing the importance of understanding individual flower visitor interactions within a community context. I have introduced the importance of flower visitation networks, and in light of their limitations, explained how measures of pollen transport and deposition may be included to improve an understanding of these communities from the plant’s perspective.

In Chapter 2, I outline the study site and introduce the methods used for interpreting the structure of the networks, including quantitative measures of specialisation.

In Chapter 3, I introduce the diverse community of insects that exploit urban floral resources, and continue by exploring the causes of specialisation, temporal variation in visitation and differences in the foraging behaviour of flower visitor taxa.

In Chapter 4, I analyse the results of the pollen loads collected from a diverse range of flower visitors, to create pollen load and pollen transport networks. Pollen load diversity and variation between taxa are explored, including temporal differences in pollen load networks and the specialisation of individual flower visitors compared to the collective community. The extent to which flower visit frequency predicts pollen load is also assessed.

In Chapter 5, I use pollinator effectiveness (single visit deposition) data from the flower visitor community to compare the structure of a visitation network to that of pollinator effectiveness and pollinator importance networks. Methods for comparing control flowers to visited flowers are discussed, and the identity of the most effective and important pollinators revealed.

In Chapter 6, I draw together flower visitation, pollen transport and pollinator importance networks to illustrate how traditional visitation networks may be under-estimating the specialisation of flower-visitor communities, and ask whether pollen transport networks are better predictors of the structure of pollinator importance networks, compared to visitation alone. I end this chapter by outlining the strengths and limitations of a pollinator importance network, and the role of these networks in future studies.

In Chapter 7, my conclusions focus on how additional quantitative measures of pollination affect the interpretation of flower visitor communities. I offer ideas for the future construction of pollination networks and evaluate whether a network approach is the best option for evaluating plant-pollinator communities at a local or landscape scale.

Chapter 2.

Study site, species and network interpretation

2.1 Study site

All observations were made in a single managed, private garden in the city of Dover, Kent (51º7’ N, 1º18’ E), which lies on the South East coast of England. Flower visitor diversity is particularly high in the South of the UK (Falk 2015) and the district of Dover covers an area of 123 square miles, including 20 miles of chalk coastline. The garden was larger than a typical domestic garden (approximately 2 hectares) and was located in the centre of the city of Dover (making it an urban garden, **Fig. 2.1**). Outside the city, the surrounding area is largely rural, with expanses of calcareous grassland which provide important nesting sites for solitary bees (Falk 2015). The importance of urban areas for flower visitors is discussed in Chapter 3, and the site represented a unique community to add to the collection of pollinator effectiveness and importance networks created by Ballantyne *et al.* (2015, 2017).

As the site had never been studied before, three consecutive months of sampling in the summer of 2013 were used to gain familiarity with flowering plant phenology, insect taxonomy, identification of pollen types and to trial the methods for collecting pollen samples. Data were collected during two seasons: firstly 7th May – 16th August 2014 and then in 2015, during two periods spanning from the 30th March – 6th April and the 4th May – 9th August. These times covered peak British summertime flowering, with the earlier period of collection in 2015 to survey *Pulmonaria officinalis* only. A total of 227 hours was spent observing flower visitors in 2014 and a further 289 hours in 2015. Observations were restricted to fair weather conditions, typified by low winds and mild temperatures that were optimal for flower visitor activity. Dependent on weather conditions, sampling commenced at 07:30 and concluded at

18:30. Data were assigned to four diurnal time periods: 07:30-09:00, 09:00-12:00, 12:00-15:00 and 15:00-18:30 as in Baldock *et al.* (2011).

Figure 2.1 Location of the study site, a domestic garden in the grounds of Dover College, Dover, Kent, UK. The private garden (outlined) is approximately 2 hectares, surrounded by roads, residential gardens and the town's central railway station to the South West. Contains OS data © Crown copyright and database rights (2016).

2.1.1 Focal plant species

The garden contained a diverse range of flora typical of British gardens (at least 100 taxa flowering during the sampling months, **Appendix 2.1**) with peak flowering taking place in June-July. In total, 29 plant species flowering in the summer were selected for (i) the presence of more than one individual plant in the garden (ii) having pollen-producing flowers (iii) flowering phenology and (iv) for receiving a frequent number of visits per patch at peak flowering. As floral traits may be more important than species richness in determining the structure of flower visitor networks (Vázquez *et al.* 2009, Vizentin-Bugoni *et al.* 2015) a variety of floral morphologies were sampled (**Fig. 2.2, Table 2.1, Table 2.2**). The selection included a diversity of plants representative of a typical UK garden (listed by Loram *et al.* 2008, Owen 2010, Garbuzov & Ratnieks 2014) comprising plants from a garden-origin and wild plants that grew without management. Many of the plants studied were situated within herbaceous borders, although the non-managed species *Rubus, Smyrnium* and *Eupatorium* grew on large, unmanaged banks towards the North-West corner of the garden (**Fig. 2.1**). While the garden lawns contained plant taxa that were visited by *B.pascuorum* and *B.lapidarius* (e.g. *Trifolium pratense* and *Lotus corniculatus*) these were subject to regular mowing which made stationary observations difficult.

I acknowledge that this thesis does not provide an exhaustive flower visitation network in the garden, as some plants were excluded from data collection due to very low visitation rates (e.g. *Lysimachia*), were male-sterile (e.g. *Lavendula*), were only female (e.g. *Scabiosa, Thymus*) or were trees above 15m that could not be accessed (e.g. *Aesculus hippocastanum, Tilia* x *europea*). There were also small changes to the plant community studied between the two years; plants that were omitted after 2014 were *Weigela* and *Erysimum* (both garden hybrids that produced unpredictable quantities of pollen), *Verbascum* (a biennial) and *Lysimachia*. On the other hand, plants added in 2015 to encompass a wider diversity of flower visitors were the early-flowering *Pulmonaria, Crataegus* and *Smyrnium,* and the late-season *Eupatorium*. While these species undoubtedly contributed to the visitation network, the principal aim of this thesis was to compare a visitation, pollen transport and pollinator importance network which required reliable visitation and the presence of pollen.

Each plant was surveyed for an average of 19.9 (range 7.6-32.9) hours and 5.7 (range 2-11) days during peak flowering (n=29), defined as the period in which more than 50% of all flower buds were open (Dafni *et al.* 2005). The flowering phenology for each plant species are given in **Figure 2.3**, with the exact dates and timing of observations given in **Appendix 2.2.** I attempted to observe flower visitation to all plants at all time points throughout the day (i.e. 07:30-18:30) although this was limited by weather conditions at peak-flowering.

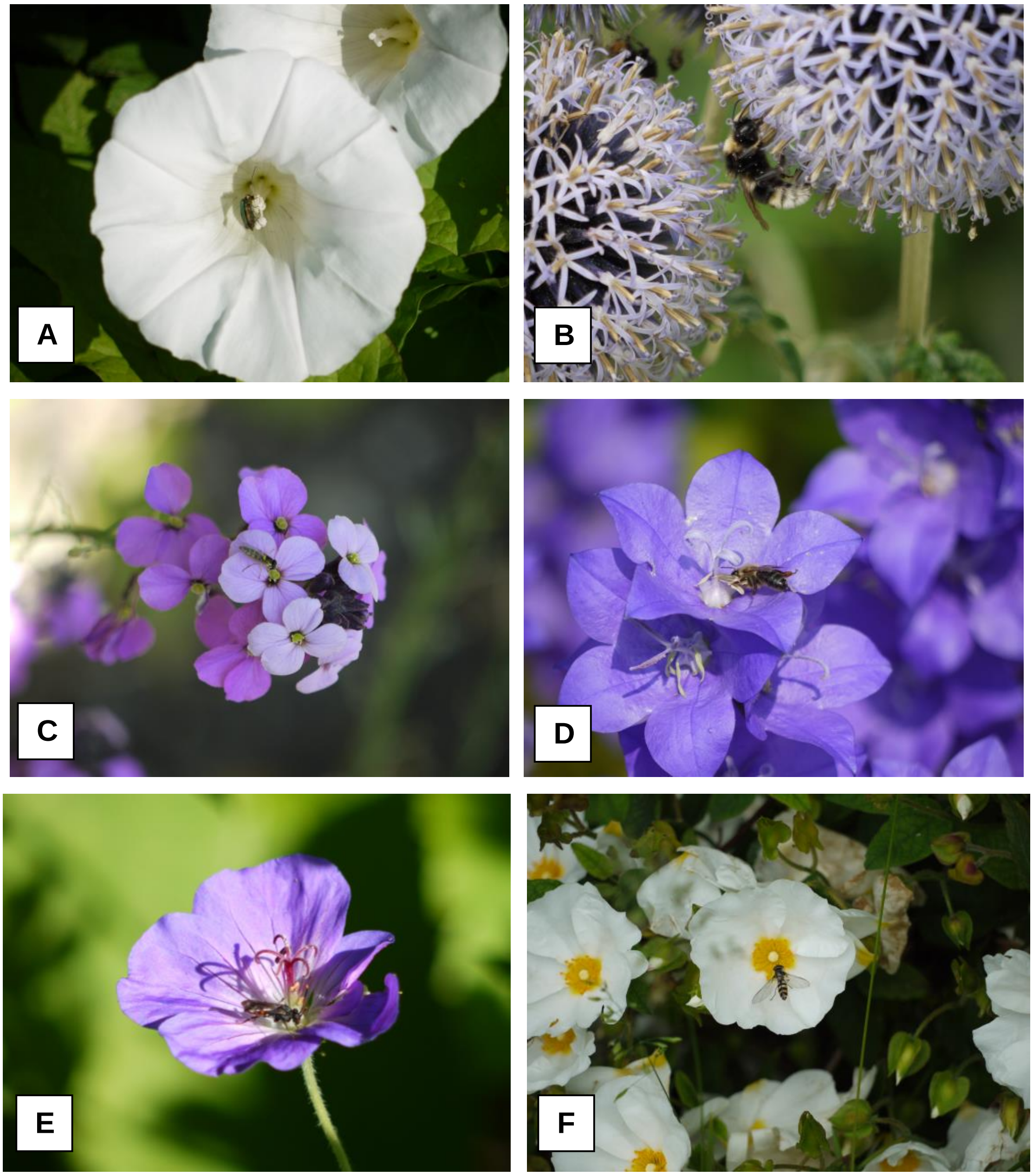


Figure 2.2 Examples of the focal plant flower morphology, showing variation in stigma position. From A to F: *Calystegia sylvatica*, *Echinops ritro* where stigmas protrude through the anther column, *Erysimum* ‘Bowle’s Mauve’, *Campanula persicifolia*, *Geranium x johnsonii* ‘Johnson’s Blue’, *Cistus salvifolius*.

Plant species	**Family**	**Plant type**	**Origin**	**Native range**	**Floral unit**	**Flower colour**	**Flower shape**	**Nectar site**
Smyrnium olusatrum	Apiaceae	Herbaceous perennial	Unmanaged	S Europe	Inflorescence	White	Umbellifer	Exposed
Polygonatum hybridum	Asparagaceae	Herbaceous perennial	Garden	Europe	Individual	White	Pendant	Concealed
Calendula officinalis	Asteraceae	Herbaceous perennial	Garden	S Europe	Composite inflorescence	Yellow/ Orange	Open disk	Exposed
Echinops ritro	Asteraceae	Herbaceous perennial	Garden	SE Europe	Inflorescence	Blue	Tubular	Concealed
Eupatorium cannabinum	Asteraceae	Herbaceous perennial	Unmanaged	Europe	Inflorescence	Pink	Raceme	Exposed
Leucanthemum x superbum	Asteraceae	Herbaceous perennial	Garden	Europe	Composite inflorescence	White/ Yellow	Open disk	Exposed
Echium vulgare	Boraginaceae	Herbaceous biennial	Garden	Europe	Individual	Blue	Tubular	Concealed
Pentaglottis sempervirens	Boraginaceae	Herbaceous perennial	Unmanaged	W Europe	Individual	Blue	Tubular	Concealed
Phacelia tanacetifolia	Boraginaceae	Herbaceous annual	Garden	SW America	Individual	Blue	Tubular	Concealed
Pulmonaria officinalis	Boraginaceae	Herbaceous perennial	Garden	Europe, W Asia	Individual	Pink/ Blue	Tubular	Concealed
Erysimum ‘Bowles’s Mauve’	Brassicaceae	Herbaceous perennial	Garden	Europe, SW Asia, Africa, N America	Individual	Purple	Tubular	Concealed
Buddleja davidii	Buddlejaceae	Shrub	Unmanaged	E Asia	Inflorescence	Lilac	Tubular	Concealed
Campanula persicifolia	Campanulaceae	Herbaceous perennial	Garden	W Eurasia, N Africa	Individual	Blue	Tubular	Concealed
Weigela ‘Florida variegata’	Caprifoliaceae	Shrub	Garden	E Asia	Individual	Pink	Tubular	Concealed
Cistus salvifolius	Cistaceae	Shrub	Garden	S Europe, W Asia, N Africa	Individual	White/ Yellow	Open disk	Exposed
Calystegia silvatica	Convolvulaceae	Climber	Unmanaged	S Europe, SW Asia	Individual	White	Tubular	Concealed
Geranium x johnsonii ‘Johnson’s Blue’	Geraniaceae	Herbaceous perennial	Garden	Europe, America, Asia	Individual	Blue	Open disk	Concealed

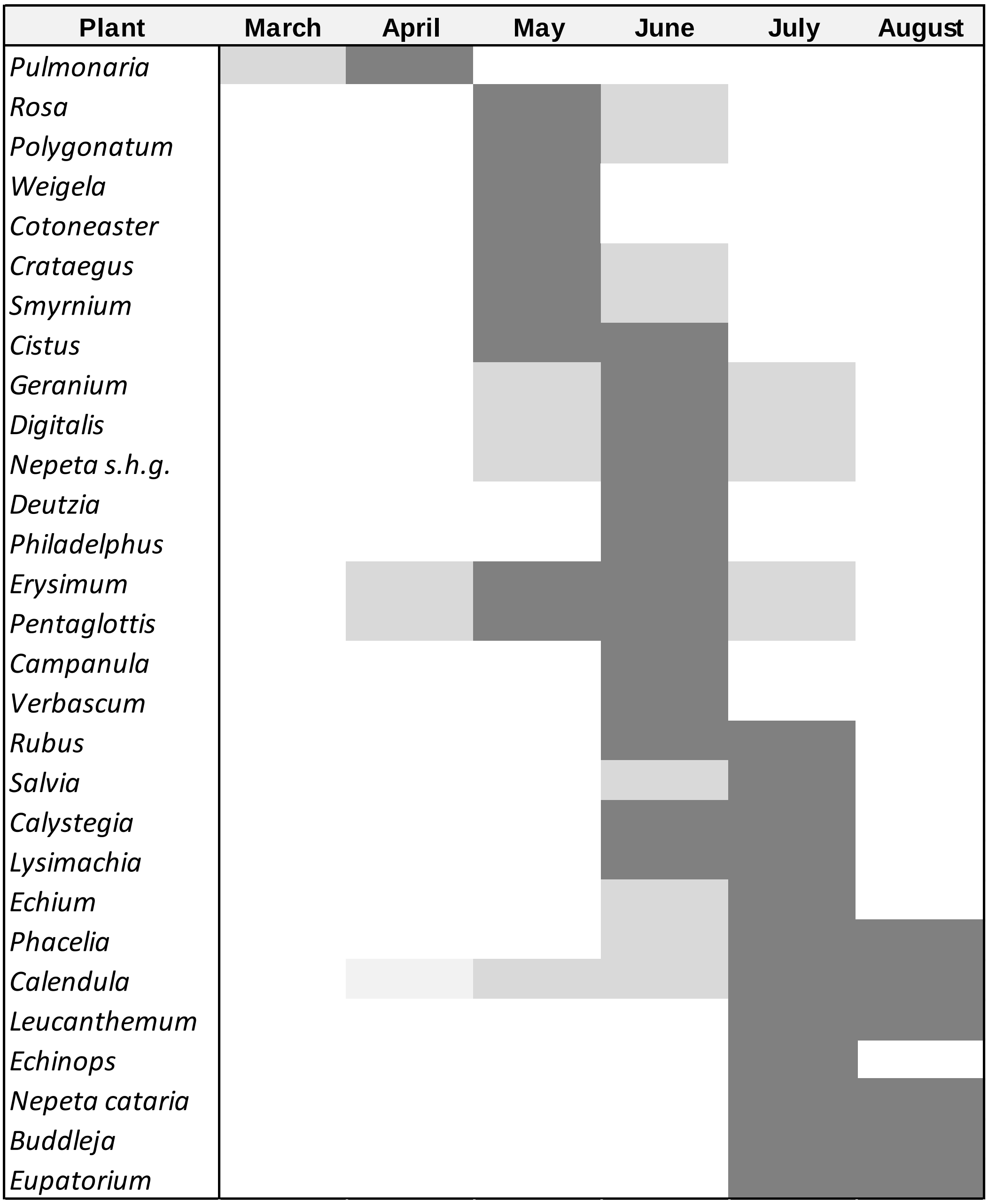
Plant species	Family	Plant type	Origin	Native range	Floral unit	Flower colour	Flower shape	Nectar site
Philadelphus coronarius	Hydrangeaceae	Shrub	Garden	SE Europe, N & C America, Asia	Individual	White	Open bowl	Exposed
Deutzia x *hybrida* ‘Mont Rose’	Hydrangeaece	Shrub	Garden	Asia, C America	Individual	Pink	Tubular	Concealed
Nepeta ‘Six Hills Giant’	Lamiaceae	Herbaceous perennial	Garden	SE Europe, SE & C Asia	Individual	Blue	Bilabiate	Concealed
Nepeta cataria	Lamiaceae	Herbaceous perennial	Garden	SE Europe, SE & C Asia	Individual	White	Bilabiate	Concealed
Salvia nemorosa ‘Pink Friesland’	Lamiaceae	Herbaceous perennial	Garden	C Europe, W Asia	Individual	Pink	Bilabiate	Concealed
Digitalis purpurea	Plantaginaceae	Herbaceous biennial	Garden	Europe	Individual	Purple	Tubular	Concealed
Lysimachia punctata	Primulaceae	Herbaceous perennial	Unmanaged	Europe, W Asia	Individual	Yellow	Open star	No nectary, oil-producing flowers
Cotoneaster horizontalis	Rosaceae	Shrub	Garden	E Asia	Individual	Pink	Closed bowl	Concealed
Crataegus monogyna	Rosaceae	Tree	Unmanaged	Europe, NW Africa, W Asia	Individual	White	Open bowl	Exposed
Rosa xanthina ‘Canary bird’	Rosaceae	Shrub	Garden	E Asia	Individual	Yellow	Open bowl	Exposed
Rubus fruticosus	Rosaceae	Shrub	Unmanaged	Europe	Individual	White	Open bowl	Exposed
Verbascum olympicum	Scrophulariaceae	Herbaceous biennial	Unmanaged	Europe, N Africa, Asia	Individual	Yellow	Open bowl	Concealed

Table 2.1 Details of the 29 garden plants selected for the study, ordered by plant family. The origin of cultivars and hybrids are marked as being of ‘garden origin’ and wild plants growing in the garden are marked as ‘unmanaged’. For plants of a garden origin, the native range is given for the species cross-bred to produce the hybrid. All flowers were hermaphroditic. The floral unit refers to the position of individual flowers.

Plant species	Flower size (mm)	Anther number	Anthers restricted	Pollen size	Pollen quantity	Stigma number	Total stigma width (mm)	Stigma restricted	Anther to stigma distance (mm)	Pollen present when stigma mature
Buddleja davidii	6.0	4	Y	Small	High	1	1	Y	1.2	Y
Calendula officinalis	Inflor.=40 Floret=1.9	1 per floret	N	Med	Med	1	1.3	N	*	Y
Calystegia silvatica	90.0	5	N	Large	Med	1	1.5	N	4.0	Y
Campanula persicifolia	43.0	5	N	Med	High	1	2	N	*	Y
Cistus salvifolius	43.0	75	N	Large	High	1	2.1	N	2.0	Y
Cotoneaster horizontalis	4.0	10	Y	Med	Med	3	0.8	Y	1.0	Y
Crataegus monogyna	17.0	20	N	Med	High	1	0.9	N	1.4	Y
Deutzia x *hybrida* 'Mont Rose'	24.0	10	N	Med	High	1	0.7	N	0.9	Y
Digitalis purpurea	30.0	4	Y	Med	High	1 bi-lobed	2.5	Y	2.0	Y
Echinops ritro	Inflor.=65.0 Floret=9.8	1	N	Large	Med	1	3.4	N	*	Y
Echium vulgare	13.0	5	N	Small	Med	1	1.2	N	5.0	Y
Erysimum 'Bowles's Mauve'	2.0	5	Y	Med	Variable	1	1.6	Y	1.2	Y
Eupatorium cannabinum	Raceme=45.0 Floret=0.7	1	N	Small	Med	2 stigma branches	0.4	N	*	Y
Geranium x johnsonii 'Johnson's Blue'	48.0	8	N	Large	Low	1, 5-lobed	3.3	N	3.5	N
Leucanthemum x superbum	Inflor.=74.0 Floret=1.7	1	N	Med	Med	1	1.3	N	*	Y
Lysimachia punctata	23.0	5	N	Small	Med	1	0.5	N	1.4	Y
Nepeta 'Six Hills Giant'	5.4	4	Y	Med	Med	1, bi-lobed	0.6	Y	1.2	Y
Nepeta cataria	5.2	4	Y	Med	Med	1, bi-lobed	0.5	Y	1	Y
Pentaglottis sempervirens	11.0	5	Y	Small	Med	1	0.5	Y	1	Y
Phacelia tanacetifolia	7.0	4	N	Small	Med	1	0.5	N	0.5-1.5	Y
Philadelphus coronarius	30.0	23	N	Small	High	1, 4-lobed	2	N	2	Y
Polygonatum hybridum	7.1	6	Y	Large	Med	1	0.5	Y	3	Y

Plant species	Flower size (mm)	Anther number	Anthers restricted	Pollen size	Pollen quantity	Stigma number	Total stigma width (mm)	Stigma restricted	Anther to stigma distance (mm)	Pollen present when stigma mature
Pulmonaria officinalis	6.0	5	Y	Med	Med	1	0.9	Y	0.8	Y
Rosa xanthina ‘Canary bird’	57.0	>50	N	Med	High	Multiple	5.0	N	6.5	Y
Rubus fruticosus	23.0	>50	N	Med	High	Multiple	3.5	N	1.5	Y
Salvia nemorosa ‘Pink Friesland’	5.0	2	Y	Med	Med	1, bi-lobed	1.5	Y	2.6	Y
Smyrnium olusatrum	Umbel=38-55 Floret=4.0	4	N	Med	Med	1, bi-lobed	0.5 (combined)	N	Not present	N
Verbascum olympicum	50.0	5	N	Small	High	1	2.0	N	3.0	Y
*Weigela ‘*Florida variegata*’*	30.0	5	N	Med	Low	1	2.4	N	2.0	Y

Table 2.2 Details of the floral morphology of each of the 29 garden plants, ordered alphabetically. Anthers and stigmas were classified as restricted if <2mm from the diameter of the perianth. Pollen size was grouped as small (<20μm), medium (25-50μm) or large (>50μm). The anther to stigma distance is shown as ***** if the stigma emerged above the anthers as it matured.



Plant	March	April	May	June	July	August
Pulmonaria	▒	■				
Rosa			■	▒		
Polygonatum			■	▒		
Weigela			■			
Cotoneaster			■			
Crataegus			■	▒		
Smyrnium			■	▒		
Cistus			■	■		
Geranium			▒	■	▒	
Digitalis			▒	■	▒	
Nepeta s.h.g.			▒	■	▒	
Deutzia				■		
Philadelphus				■		
Erysimum		▒	■	■	▒	
Pentaglottis		▒	■	■	▒	
Campanula				■		
Verbascum				■		
Rubus				■	■	
Salvia				▒	■	
Calystegia				■	■	
Lysimachia				■	■	
Echium				▒	■	
Phacelia				▒	■	■
Calendula		░	▒	▒	■	■
Leucanthemum					■	■
Echinops					■	
Nepeta cataria					■	■
Buddleja					■	■
Eupatorium					■	■

Figure 2.3 Flowering phenophase of the 29 garden plant species. The depth of shading indicates the number of open flowers. Observations occurred during peak flowering (i.e. when more than 50% of all flower buds were open). *Pulmonaria* did not overlap with any of the other focal plants, while *Calendula* had the longest flowering period, stretching from April-August.

2.2 Interpretation of bipartite networks

In Chapter 1, I introduced the concept of a bipartite pollination network. The following section introduces several indices that describe the structure of the interactions within a bipartite network, and expectations for how these might vary between visitation, pollen transport and pollinator importance networks.

2.2.1 Links versus interactions

These terms refer to the connections between flower visitors and plants, where individual visits are defined as ‘interactions’ and the partnership between a specific insect and plants as a ‘link’. For example, a network may contain 150 interactions that form 20 links, and the link ‘weight’ is the number of interactions it represents.

2.2.2 Network indices

All measures that report patterns in the networks are referred to as ‘indices’ (Dormann *et al.* 2009). Indices are divided into qualitative descriptors of the absence or presence of links, or quantitative descriptors which account for the number of species and the number of interactions between them (known as weighted indices). Quantitative indices are more meaningful and robust against variation in sampling effort (Banašek-Richter *et al.* 2004, Blüthgen *et al.* 2008, Vázquez *et al.* 2009) and in this thesis, I use weighted indices that either measure species niche breadth (e.g. specialisation and modularity) or the impact of different species in the network (e.g. interaction evenness and nestedness) both at the level of the entire community and individual species. All of the indices selected represent those widely reported in pollination networks, and are used here to compare the structure of the interactions in five networks (introduced in Section 1.6). Considerable differences in the values of indices between networks may be used to infer differences in the tolerance of the interactions to disturbance (Kaiser-Bunbury & Blüthgen 2015) and I am particularly interested in how descriptors of interaction specialisation (H_2' and d') vary between networks that distinguish flower visitors from pollinators.

2.2.3 Community-level indices

Six quantitative (weighted) indices are used to describe and compare the structure of the entire community between networks: (i) connectance, (ii) evenness, (iii) generality, (iv) specialisation H_2', (v) modularity, and (vi) nestedness. The predicted changes in the indices between the visitation, pollen transport and pollinator importance network are summarised in **Table 2.3** (page 30).

(i) Connectance

Weighted connectance describes the realised proportion of possible links in the network, divided by the total number of species in the network (Tylianakis *et al.* 2007). A highly connected community (value close to 1) is characterised by many connecting links between species, while a decline in connectance (to a minimum of 0) suggests a reduction in the number of connections between species. While connectance is often very low in flower visitation networks, particularly in larger, more species-rich networks (Olesen & Jordano 2002), it is often used to infer community stability; an increase in connectance may act as a buffer to any disturbance (Altena *et al.* 2016) or it may lead to a high threat of co-extinction (via a ‘snowball’ effect, Vieira & Almeida-Neto 2015). Consequently, how connectance is used to inform conservation efforts varies (Heleno *et al.* 2012). In this thesis, connectance is expected to increase in the pollen transport network if the number of links increases relative to the visitation network. On the other hand, connectance could decrease in the pollinator importance network, if many visitors are ineffective pollinators and their impact as pollinators (interaction strength) declines compared to their frequency as flower visitors.

(ii) Interaction evenness

A measure of the homogeneity of the link weights throughout the network (Tylianakis *et al.* 2007), in which differences exist due to the presence of strong links (representing a high number of flower visits) and those that are very weak (very few visits). A high interaction evenness (values close to 1) suggests an even network, where species have similar ecological impacts (Blüthgen 2010) and the community may be more robust to disturbance (Tylianakis *et al.* 2007). Consequently, a decline in interaction evenness between networks (to a minimum of 0) reflects a change in the proportion of strong versus weak interactions and greater difference in the ecological impact of the species involved; following a disturbance, generalist pollinators may strengthen existing links further (Aizen *et al.* 2008) while weak interactions can be lost entirely (Kaiser-Bunbury *et al.* 2011). Compared to the visitation network, it is unclear how interaction evenness will change with the incorporation of pollen data; some links may increase in strength (i.e. as rare flower visitors carry and/or deposit high quantities of pollen) whilst others may decline (i.e. as common flower visitors carry and/or deposit low quantities of pollen).

(iii) Generality

A measure of the mean diversity of interaction partners for either flower visitors or plants (sometimes called vulnerability for plants); i.e. an increase in plant generality indicates that on average, plants receive a greater diversity of flower visitors and insects visit a greater proportion of the plant community (Bersier *et al.* 2002). Low values of generality are synonymous with high levels of niche separation and specialisation, and may indicate communities that are at risk from disturbance (Kaiser-Bunbury & Blüthgen 2015). The generality of both guilds (plants and flower visitors) may increase in the pollen transport network, if many more interactions are revealed than observations of visitation alone. However, the opposite may be true if many flower visitors do not transport pollen. In the pollinator importance network, generality of both guilds is expected to be lower than in the visitation and pollen transport networks, if many flower visitors are not effective pollinators.

(iv) Interaction specialisation (H_2')

Specialisation (H_2') describes the extent to which the observed interactions vary from a random pattern determined by the frequency of interactions for each species (Blüthgen *et al.* 2006, Dormann *et al.* 2009). A high overall level of specialisation (value close to 1) suggests species engage in fewer partnerships than expected by their total abundance, indicative of high niche separation. If species interacts with many more partners than expected by their abundance (i.e. greater generalisation) this would give a H_2' closer to 0. High H_2' values may indicate greater vulnerability to disturbance, as many species depend on a limited number of partners (Kaiser-Bunbury & Blüthgen 2015). Importantly, H_2' is not biased by network size or the species' total observation frequencies (Blüthgen *et al.* 2006, 2010) and is therefore ideally suited to comparing networks. Any increase in H_2' in the pollinator importance network would suggest that flower visit frequency alone underestimates the specialisation of pollination, while H_2' may decrease in the pollen transport network if many hidden links are revealed.

(v) Modularity

Modules are formed in networks between species that interact frequently with one another, creating clusters of closely connected species (Olesen *et al.* 2007, Dormann & Strauss 2014). Modules help to visualise the topological roles of individual species, and a high modularity indicates a community with a complex structure of interactions, structured by species' phenology and morphology (Dicks *et al.* 2002, Vamosi *et al.* 2014); if complexity underlies stability, then a more modular community may be more robust to disturbance (Tylianakis *et al.* 2010, Altena *et al.* 2016). It is unclear how modularity may change between the networks, although a decline in the pollen transport network is expected if many visitors carry the pollen of flowers they are never observed to visit. Modularity may increase in the pollinator importance network if plants are more strongly connected to the most effective pollinators.

(vi) Weighted nestedness

Nestedness is one of the most widely reported patterns in bipartite networks, occurring as specialised species interact with a subset of the species connected to the most generalised species (Bascompte *et al.* 2003). When nestedness is high (values are not constrained on a scale) the most specialised interactions occur as a perfect subset of the most generalised interactions ('Perfect nestedness among columns' **Fig. 2.4**) although nestedness often appears to be more random ('Random', **Fig. 2.4**). Nested communities are assumed to be vulnerable to disturbance, as specialists are less vulnerable to co-extinctions (Burgos *et al.* 2007, Tylianakis *et al.* 2010) and a greater diversity of interactions are supported (Bastolla *et al.* 2009, Thébault & Fontaine 2010). In communities that are compartmentalised or interactions are divided into distinct subsets (**Fig. 2.4**) a nested pattern is not observed, as there are no links between subsets (or 'modules'). However, a modular community may still be nested if there is an overlap of interactions between modules or if a module contains both specialists and generalists (Fortuna *et al.* 2010).

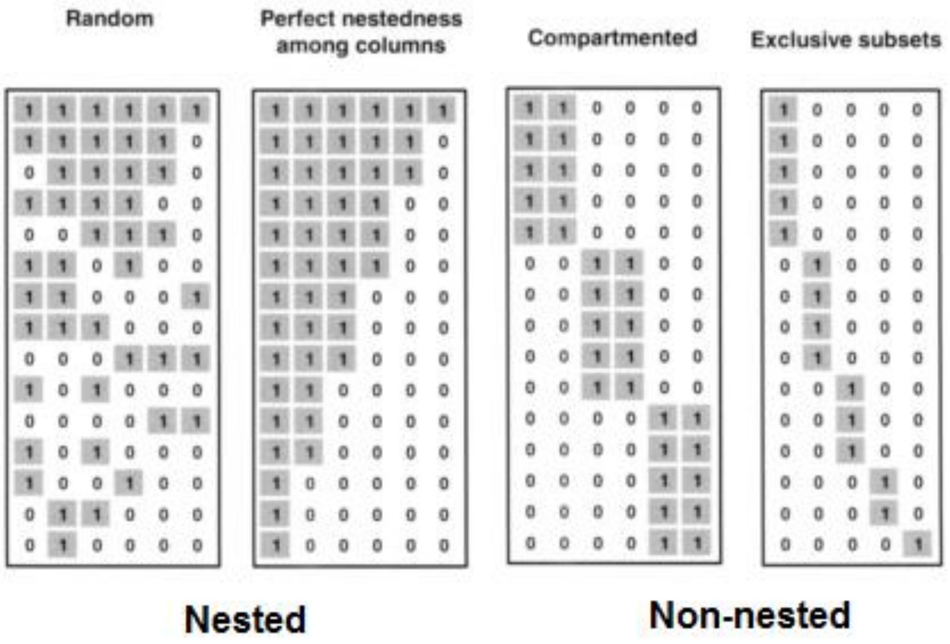


Figure 2.4 Examples of bipartite matrices illustrating the difference between nested and non-nested interactions. Species (i.e. plants and flower visitors) are listed along the top and left hand margins, with '1' indicating an interaction. In a perfectly nested matrix, the specialists interact with a subset of the most generalised species, whereas in an exclusively compartmented network, no interactions take place between specialists and generalists. (Adapted with permission from Almeida-Neto *et al.* 2008).

It is unclear how weighted nestedness (WNODF; Weighted Nestedness based on Overlap and Decreasing Fill; Almeida-Neto & Ulrich 2011) will change with the addition of pollen to the visitation network, as it will depend on the extent to which specialist and generalist species continue to overlap.

2.2.4 Species level indices

Two indices are used to describe and compare the position of individual species within a network: (i) species specialisation *d'* and (ii) species strength. The predicted changes in the indices between the visitation, pollen transport and pollinator importance network are summarised in **Table 2.4** (page 31).

(i) Species specialisation (*d')*

Analogous to H_2', *d'* describes the partner diversity (specialisation) of individual species relative to their abundance. The specialisation of individual species is most usefully compared within a network, and *d'* (like H_2') is not biased by species' total observation frequencies (Blüthgen *et al.* 2006). Values of *d'* close to 1 suggests a highly specialised species, interacting with fewer partners than expected from its abundance, possibly increasing the vulnerability to co-extinction (Blüthgen 2010). However, *d'* values should be interpreted with caution, as the specialisation of very rare visitors may be overestimated (Vázquez & Aizen 2004) while the generalisation of species with many links may be overestimated if all partners belong to a single taxon/genera (Blüthgen *et al.* 2006). If the pollen transport network reveals many hidden links, the *d'* of flower visitors and plants may decrease relative to the traditional visitation network. On the other hand, if many flower visitors are not effective pollinators, then *d'* is likely to increase in the pollinator importance network.

(ii) Species strength

Applicable to both plants and flower visitors, species strength is a measure of the total importance of a species for the alternative guild, in the context of all other interactions that take place (Bascompte & Jordano 2007). Species strength is useful for comparing the topological role of species within a network, e.g. a flower visitor with a high species strength indicates a high dependency of several plant species on these interactions. If the species strength of this visitor were to decrease in a pollinator importance network, this would then suggest a reduction in the value of the visitor as a pollinator. Alternatively, the species strength of flower visitors may increase in the pollen transport network, if they carry substantial pollen loads, or vice versa.

2.2.5 Network analysis

All network analyses and bipartite networks were created in the package *Bipartite* (Dormann *et al.* 2008) for RStudio Version 0.99.491 (R Development Core Team 2011). Community-level indices were calculated using the function *networklevel* (quoting weighted NODF for nestedness, Almeida-Neto & Ulrich 2011) with the exception of modularity which was calculated using the function *computeModules* (n=10,000,000 simulations). Species-level *d'* was calculated using the function *dfun* and species-strength using the function *specieslevel*.

Index		Description	Interpretation	Predicted change between networks
Community-level	**Connectance**	The proportion of realised links	Higher connectance (values closer to 1) indicates increased complexity	Greatest in the PT network, lowest in the PI network
	Interaction evenness	The uniformity of interactions between species	High evenness (values closer to 1) suggests similarities in the ecological impact of species	Uncertain
	Generality (of plants and visitors)	The average number of partners	Higher values indicate increased breadth of visitors' resource use	Greatest in the PT network, lowest in the PI network
	Interaction specialisation (H_2')	Specialisation of interactions at the community-level	High specialisation (values close to 1) indicate exclusive partnerships; low specialisation (values close to 0) indicates overlap in partnerships between many species	Lowest in the V network, highest in the PI network
	Modularity	Extent to which interactions are grouped into distinct modules	Increasing modularity (values close to 1) suggests complex partitioning of interactions	Uncertain
	Nestedness	Extent to which specialists interact with a subset of the most generalist interactions	Increased nestedness suggests greater tolerance of specialised interactions to disturbance	Uncertain

Table 2.3 Summary of the predicted changes in community-level indices between traditional visitation (V), pollen transport (PT) and pollinator importance (PI) networks. Predictions are based on the results of previously published pollen transport and pollinator importance networks (discussed in later chapters).

Index		Description	Interpretation	Predicted change between networks
Individual species	**Species specialisation (*d'*)**	The interaction specialisation of an individual species	Higher *d'* values (closer to 1) indicate species with partnerships that are more specialised than expected by total number of visits	Average *d'* lowest in the PT network, highest in the PI network. Direction of change will vary between species.
	Species strength	The importance of a species for all species in the alternative level	Higher values indicate species that are more important to all partners in the opposite group	Average species strength highest for visitors in the PI network. Direction of change will vary between species.

Table 2.4 Summary of the predicted changes in species-level indices between traditional visitation (V), pollen transport (PT) and pollinator importance (PI) networks. Predictions are based on the results of previously published pollen transport and pollinator importance networks (discussed in later chapters).

2.2.6 Limitations of networks and indices

Firstly, not all that is ecologically important about the interactions within the garden community may be explained by a network approach and the use of indices (e.g. the behaviour of insects between flower visits). However, the selected indices have been chosen to address the specific aims of this thesis. Secondly, all networks are only ever a snapshot of all the interactions that take place, and the indices measure only the visits recorded. It is also important to remember that network indices are not as static as they appear; for a single site, the structure of the interactions will vary temporally and spatially (explored in Chapters 3 and 4). Furthermore, not all of the interactions occur at the same time, and the impact of the interactions (e.g. effectiveness of a visitor as a pollinator) will depend on temporal differences (e.g. stigma receptivity, conspecific pollen availability) that are not shown.

2.2.7 Comparisons between networks using a null model approach

To claim that the interactions in a network are organised in a certain way (e.g. highly specialised or nested) the structure of the network must be compared to a null model network. Null models permit the observed interaction network to be compared to many randomly generated networks, which are most meaningful when the number of interactions in each row/column of the matrix are kept as similar to the observed network as possible (Vázquez *et al.* 2007). However, null models are not entirely fool-proof and must ensure that after randomisation the pattern of interactions are still meaningful (Heleno *et al.* 2014); flower visitors differ in how many species they exploit for a variety of reasons (Montoya *et al.* 2006) and the distribution of generalists versus specialists should match that observed (Joppa *et al.* 2010).

Null models are frequently used to compare visitation networks (e.g. Genini *et al.* 2010, Hanley *et al.* 2014, Miller-Struttmann & Galen 2014) however, no standard approach exists for comparing networks where the interactions are continuous data (i.e. weighted by the average number of pollen grains). Although some have created quantitative, pollen-weighted null networks (Popic *et al.* 2013, Tur *et al.* 2014) these have been used to compare a pollen-transport network to a null network, but not directly to compare a pollen-transport and pollinator-importance network. Therefore, I acknowledge the value of null models for confirming significant changes in network indices, but as no standard approach exists to do this for the continuous, proportional data (see below) used to build the networks in this thesis, I have not been able to use a null model approach; in particular, I wished to avoid randomly assigning pollen grains to each visitor at a rate determined by visitation frequency, which ignores ecologically important restrictions on the quantity of pollen involved.

2.2.8 Proportional networks

When measures of pollen quantity were incorporated into the networks (pollen transport and pollinator importance, Chapters 4 and 5) the variation in pollen grain production between plants was controlled for by creating ‘proportional’ networks. In these networks, the value of each link between a specific visitor and plant is a proportion of the value of all visitors to the plant (total=1). For example, if a plant received two visitors, the less important visitor might have a value of 0.2 compared to 0.8 for the more important visitor. At the community level, creating a proportional network increased the strength of the weakest links compared to the non-proportional network (**Fig. 2.5**); in terms of the network indices, creating proportional networks had the effect of increasing flower visitor generality (8.96 to 10.24), H_2’ (0.38 to 0.44) and modularity (0.41 to 0.44) slightly, while nestedness (NODF) declined (39.15 to 36.56). Very little difference was observed in weighted connectance (0.10 in both), plant generality (6.83 to 6.34) or interaction evenness (0.68 to 0.70). Therefore, I acknowledge that estimates of flower visitor generality, specialisation and modularity may be different if a non-proportional network were used, but the proportional data is not considered to have a large effect on other network metrics.

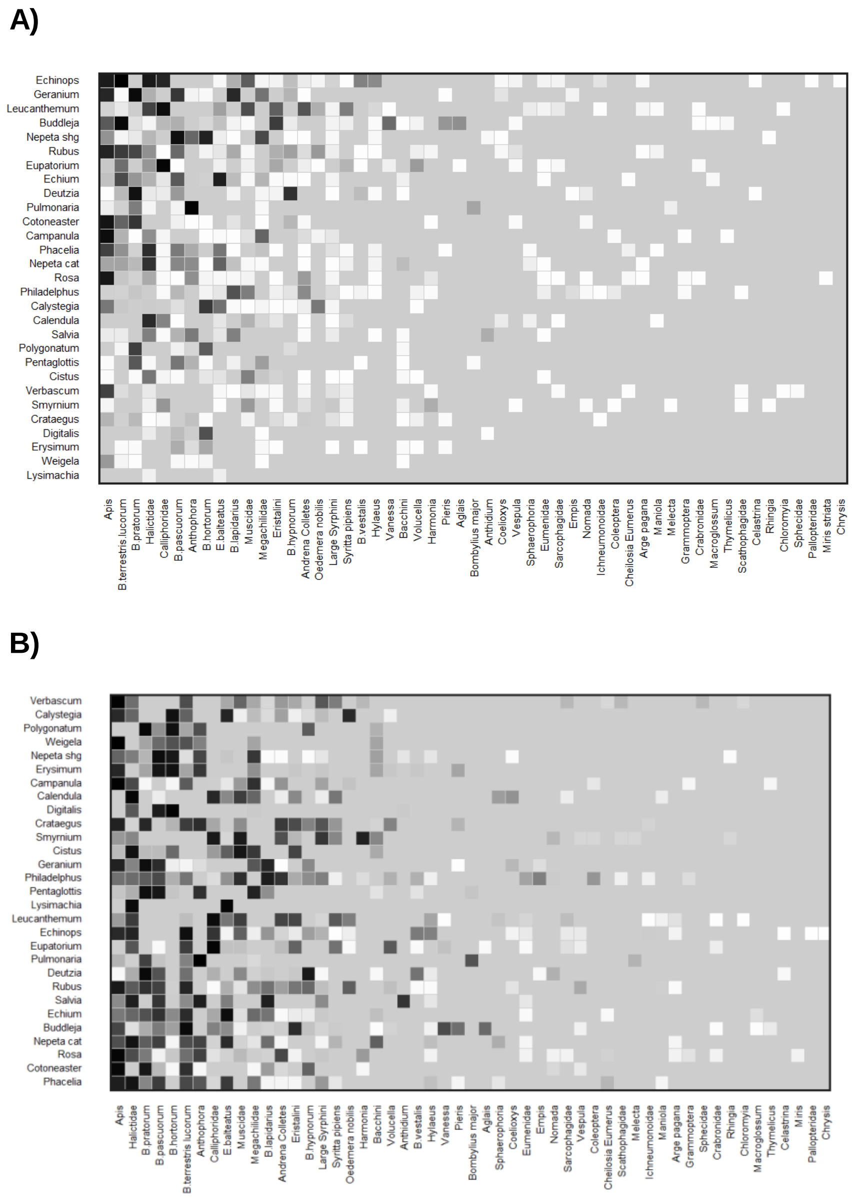


Figure 2.5 The effect of using proportional flower visitation in a bipartite matrix, where raw visitation values (A) are transformed into proportional values ranging from 0 to 1 (B). Proportional values remove the bias in interaction weights that are introduced when plants (left hand axis) produce different quantities of pollen that are carried or deposited by flower visitors (bottom axis). However, in a proportional network the strength of the weakest interactions in the original network are increased (illustrated by a greater proportion of darker boxes). Results are representative of 466 links recorded between 53 groups of flower visitors to 29 plants over the summer of 2014 and 2015. Species are organised according to decreasing linkage level (top to bottom, left to right) so that the order differs in each network.

Conclusion

In this chapter, I have outlined the study site and focal plant species included, plus the methods used for interpreting patterns in the flower visitation, pollen transport and pollinator importance networks. Before these three networks are compared simultaneously, I explore the detail contained within each, starting with the traditional visitation network.

Chapter 3.

Flower visitation, foraging behaviour and the dynamics of a traditional visitation network in a garden

Summary

1. Urban areas, particularly gardens, are increasingly recognised for their value to flower visitors, with further studies needed to understand which species thrive in these areas. However, very few flower visitation networks exist for these communities, and the level of specialisation and competition between these species is relatively unexplored.
2. All flower visits to 29 plant taxa were recorded over the course of two summers, representing a diverse range of visitors and floral morphologies. These visits were used to create the largest known flower visitation network for a single garden, including 53 taxa of flower visitors.
3. Overall, flower visitation was very generalised, similar to that reported in other urban studies. Larger, long-tongued bees demonstrated the highest levels of specialisation, while dipteran visitors were more specialised than expected. Temporal divisions of the network increased specialisation, although little change was observed in the other network metrics.
4. Interestingly, diurnal patterns in flower visitation suggested that visitors may have exploited the thermal micro-habitats of the garden to extend their foraging period. For the first time, floral resource collection was incorporated into the network analysis, to reveal differences in the importance of nectar and pollen for flower visitor taxa.
5. The diversity of interactions reinforces the value of these communities for flower visiting insects in urban areas, although it also highlights the difficulty in determining the value of flower visitors as pollinators.

3.1 Introduction

Flower visitation networks play a vital role in understanding the importance of different plant species for visitors, and which visitors may act as pollinators. In this chapter, I use a network approach to explore the interactions in a garden, and begin to question how differences in their behaviour may contribute to variation in their effectiveness as pollinators.

3.1.1 The impact of urbanisation and value of domestic gardens

The preservation of biodiversity in urban habitats has become a central issue for ecology; despite covering only 4% of the global land surface, 5 billion people are expected to live in urban environments by 2030 (UNFPA 2007). Recent research has exposed the negative effect urbanisation can have on arthropod species, via increased local temperatures (Youngsteadt *et al.* 2017), environmental contaminants (Lusebrink *et al.* 2015), invasive plant species (Jain *et al.* 2016), parasitism (Theodorou *et al.* 2016) and from the fragmentation of habitat (Banaszak-Cibicka *et al.* 2016). Winfree *et al.* (2015) reported an overwhelmingly negative impact of each of these factors on flower visitor populations, although the response varied between taxa. These issues are also experienced in non-urban habitats, making the study of species in urban environments relevant at a global scale (Harrison & Winfree 2015).

Typically, up to a quarter of urban green space is composed of managed, domestic gardens, (Loram *et al.* 2007) which after agriculture, represent one of the most anthropogenically altered habitats. Yet the role of these habitats as refugia for urban species is a topic of great importance, particularly for plant-pollinator communities (Hall *et al.* 2017). Focused scientific interest in the value of garden plants for flower visitors began over a decade ago (Comba *et al.* 1999a,1999b) and a burst of recent studies reflect the new emphasis on ‘pollinator-friendly’ gardening (Garbuzov *et al.* 2015, Salisbury *et al.* 2015, Kaluza *et al.* 2016). In the UK, gardens now contain a greater diversity of flowers than agricultural land (1,056 angiosperm species recorded in 267 gardens by Loram *et al.* 2008) and have been found to support 58% of the UK’s solitary bee genera (Sirohi *et al.* 2015). Elsewhere, 13% of native bee fauna were reported in gardens in New York City (Matteson *et al.* 2008) with a positive effect of urbanisation on certain bee taxa reported by Cane *et al.* (2006) and Carré *et al.* (2009). In an extensive survey of 36 sites, Baldock *et al.* (2015) found bee species richness to be greater in urban compared to rural areas, similar to that reported by Theodorou *et al.* (2016). However, other studies have reported a decline in bee species abundance and richness along rural-urban gradients (Bates *et* *al.* 2011). These conflicting findings highlight the need for more research, particularly as even less is known about the effect of urbanisation on non-bee visitors.

After Hymenoptera, Diptera are the second largest group of flower visiting insects (Larson *et al.* 2001), including both syrphids (hoverflies) and non-syrphids (here just referred to as 'flies' or other Diptera). Syrphids feed on pollen and nectar and are well-documented flower visitors in urban gardens (Owen 2010). However, a small collection of studies have reported a negative affect of urbanisation on syrphid abundance (Baldock *et al.* 2015) and species richness (Bates *et al.* 2011, Verboven *et al.* 2014), suggesting that the response of these visitors to urbanisation may be very different to that of bees. Even less information exists on the effect of urbanisation on non-syrphid Diptera (although see Gottschalk *et al.* 2007, Mulieri *et al.* 2011, Grimaldi *et al.* 2015). The plight of Lepidoptera in urban areas has received more attention, and the comprehensive review by Ramírez-Restrepo & MacGregor-Fors (2016) suggested a largely negative impact on these less-common flower visitors. Importantly, the authors of most studies have cited the lack of information as a major barrier to understanding the effect of urbanisation on flower visitors. These studies have called for future work to document the resources (including non-floral, such as nesting sites) provided by urban gardens, using these to assess how flower visitor populations vary along rural-urban gradients. This study seeks to obtain records of flower visitation by bee and non-bee taxa, using this to evaluate the importance of urban floral resources.

3.1.2 Flower visitation networks in urban areas

In 2009, Memmott highlighted the rarity of studies constructing interaction networks in urban areas. Since then, only a small collection of papers have applied these methods to the study of urban pollination (including Gelsin *et al.* 2013, Jędrzejewska-Szmek & Zych 2013, Emer *et al.* 2015,Theodorou *et al.* 2016) although others have constructed networks in rural gardens (Gotlieb *et al.* 2011). In the largest of these studies, Baldock *et al.* (2015), reported higher levels of interaction generalisation in UK urban sites compared to farmland, as urban visitors foraged from a larger number of plant species. Similar findings were reported by Theodorou *et al.* (2016), who suggested that higher levels of generalisation might increase heterospecific pollen transfer by urban visitors, reducing their effectiveness as pollinators. Generalisation was also greater in the desert gardens studied by Gotlieb *et al.* (2011). Interestingly, both urban studies reported an increase in the overall specialisation of the interactions in urban sites, as a greater proportion of the plant community received few or no visits. Little is known about the role of individual species in these networks, and this study examines how these methods can reveal competition between urban visitors.

3.1.3 Interpreting the structure of flower visitation networks requires an appreciation of flower visitor life histories

As networks emerge as powerful tools for studying urban flower visitor communities, it is important to recognise the biotic and abiotic factors that shape flower visitation rates, and how these vary between visitor taxa. The concept of pollination syndromes, where floral traits evolve to different flower visitors, has been extensively discussed elsewhere (see Willmer 2011) and to a certain degree explain differences in patterns of flower visitation between taxa. However, the frequency of flower visits also depends on the characteristics of the visitor's life history.

At a very basic level, flower visitors can be divided into taxa that depend entirely on nectar and pollen (bees, and the small masarid wasp family) and all other visitors, who to varying extents, supplement their diets with floral resources (including all other wasps, Diptera, Lepidoptera and Coleoptera). Consequently, in most habitats bees will be the most frequent flower visitors, and previous work has established the behavioural, physiological and environmental factors that influence their foraging patterns (Potts *et al.* 2003, Willmer & Stone 2004). Bees have specialised mouthparts for nectar collection (Michener 2007) and structures for pollen collection (Thorp 2000), which combined with a greater capacity for learning how to handle flowers (e.g. Hammer & Menzel 1995), enables them to extract rewards faster than other visitors (e.g. Couvillon *et al.* 2015). As a result, bees may be more likely to contact the floral reproductive parts of flowers, which has been used as a proxy for pollination in flower visitation networks (Memmott 1999, Alarcón *et al.* 2008). Flower visitors that are less dependent on nectar and pollen may be more generalised in their interactions (e.g. social wasps, Mello *et al.* 2011) although the plant taxa they visit will still depend on abiotic and biotic factors, including the structure of their mouthparts (Krenn *et al.* 2005). One major issue for future networks is the importance of different factors for driving patterns in the interactions, which are excellently reviewed in Vázquez *et al.* (2009), Kaiser-Bunbury *et al.* (2014) and Sazatornil *et al.* (2016). A full examination of these is beyond the scope of this thesis, although interactions will be interpreted in light of these constraints.

3.1.4 Temporal patterns in flower visitation allow niche separation

One of the major factors determining the structure of networks is temporal patterns in flower visitation. Networks can appear as static entities, although temporal differences in visitation alter the structure of networks divided throughout the day (Baldock *et al.* 2011), the season (Rasmussen *et al.* 2013) and between years (Petanidou *et al.* 2008, Dupont *et al.* 2009). At the diurnal scale, patterns in flower visitation for ectothermic insects are largely driven by temperature and the availability of floral rewards (Willmer 1983). Large bees often show a bi-modal pattern in flower visitation, with visits peaking in cooler periods (before and after mid-day) while smaller, non-eusocial species show a single peak usually in the middle of the day and related to the daily provisioning of the nest (Willmer & Stone 2004). The extent of daily variation in flower visitation will be interesting to explore in an urban garden, given that temperatures in urban areas are often higher and more stable than rural areas (Youngsteadt *et al.* 2017), and visitors are known to vary in their effectiveness as pollinators throughout the day as ambient temperatures change (Rader *et al.* 2013). Temporal variation over longer time-scales is driven by fluctuations in species richness and abundance (Alarcón *et al.* 2008, Petanidou *et al.* 2008) or mismatches in species phenology (Burkle *et al.* 2013, Vizentin-Bugoni *et al.* 2014), which may affect the estimates of network structured when different years are pooled.

This study seeks to use the temporal divisions that have been used in other communities in the urban garden to assess how specialisation and competition may arise over different time scales. To the best of my knowledge, no study has considered how the warmer temperature of urban areas affects patterns in flower visitation, or how the collection of floral rewards affects competition between flower visitor taxa. The answers to both questions will help to determine how urban gardens are utilised by flower visitors, and the importance of the rewards they provide.

3.1.5 Key questions

In this chapter I use quantitative visitation networks to record the diversity of flower visitors to a garden, and explore temporal patterns in flower visitation. Specifically, I ask the following questions:

1. How diverse and generalised is flower visitation in a garden? Are there patterns in the interactions that could suggest competition between visitors?

2. To what extent are flower visits structured by temporal patterns, and how does this affect the conclusions drawn from pooled networks?
3. How far does the importance of different floral rewards vary between visitors?
4. What do proxies for pollination (visit duration and contact with reproductive parts) suggest about the value of the flower visitor community as pollinators?

3.2 Methods

As flower visitation data were collected, simple details of foraging behaviour were noted, together with flower visitor tongue length and body size as measured from visitors sampled for pollen load analysis (Chapter 4). These measures are normally absent from visitation networks, although the results are often implied. However, as these measures were not the principal aim of the thesis, they are not expected to be interpreted as a comprehensive study of the specialisation of visitors nor the importance of plants in urban gardens.

3.2.1 Recording a flower visit

A visit was recorded whenever an insect entered a patch and made physical contact with a flower (Popic *et al.* 2013). In virtually all cases the insect then began to actively forage. The total number of visits recorded over two years represents all flower visitors that entered the patch and commenced foraging, plus those collected during pollen load sampling (Chapter 4) and pollinator effectiveness sampling (Chapter 5). As a result of stationary, focal plant-based sampling, the total number of visits is much larger than many published networks that are based on transect data. However, this number represents only the interactions that were observed, rather than all those that took place. Sampling during peak flowering attempted to record rare flower visitors, although visitors at the very beginning and end of flowering (which can span several weeks in some plants, e.g. *Calendula*) were not included.

Flower visitor identification

In most cases, flower visitors were identified to species as they foraged or from photographic evidence (for *Bombus*, Lepidoptera and the most common hoverflies). A reference collection of bees was confirmed by George Else, and a single specimen (*Bombus jonellus*) by Nikki Gammans. Bees outside of the genera *Apis* and *Bombus* are referred to as ‘non-eusocial’, although there are species of *Halictus* and *Lasioglossum* (Halictidae) that demonstrate eusociality (Michener 1974, Benton 2017). Pollen beetles (Nitidulidae*:* *Brassicogethes*) were observed in extremely large densities on the flowers of *Verbascum*, but were not included as they overwhelmed the visitation network, making it difficult to identify the value of other flower visitors. Previous studies have also eliminated these visitors based on their infrequent movement between flowers (Baldock *et al.* 2015). Unusually, no ants were observed visiting flowers. Visits from male *Bombus* species were also too infrequent to classify these as a separate visitor group.

Stationary sampling versus transect methods

All flower visits recorded in this study were made by stationary observations of focal plants, which allowed pollen loads and pollinator effectiveness (single visit deposition) data to be collected simultaneously. Stationary sampling was more suited to the distribution of plants in the garden (most of which were grouped together in distinct patches <10m^2) compared to traditional transect methods (e.g. Baldock *et al.* 2015) and increased the likelihood of recording very rare flower visitors (Edwards *et al.* 2015) and unique interactions (Gibson *et al.* 2011). However, stationary sampling provides a biased view of flower visitation from the plant's perspective and is more likely to be influenced by returning visitors; in the present study, these factors were moderated by collecting pollen loads to increase the resolution of flower visits from the insect's perspective, and the assumption that returning flower visitors still constituted potential pollinators.

3.2.2 Flower visitor morphology

The body size of flower-visitors was measured as the inter-tegular distance (mm) and classified into five categories: (i) <1mm (ii) 1.1-2mm (iii) 2.1-3mm (iv) 3.1-4mm and (v) >4mm. Tongue length (from face to tip) was classified into six categories: (i) <1mm (ii) 1.1-2mm (iii) 2.1-3mm (iv) 3.1-4mm (v) 4.1-5mm and (vi) >5mm. As exact data were not available for all flower visitors, grouping was based on a combination of taxonomic guides and measures collected in the field.

3.2.3 Flower visitor foraging behaviour

For a large proportion of visits, I recorded the type of floral reward collected (n=10,285, 71.8%) and the contact made between the visitor and plant reproductive structures (n=10,214, 71.3%). When multiple flower visits were made, the behaviour was pooled over all visits. This was not always possible, as some visits occurred so quickly I could not reliably identify the behaviour. Flower visit duration was also recorded for a subset of all visits (n=2,185, 15.3%)

with records of a single visitor to a specific plant, for up to 20 flowers or a maximum of 120 seconds.

3.2.4 Network analysis

Networks were created and interpreted as outlined in Chapter 2, with the weight of each interaction representing the number of times when a particular insect was observed to enter the flower patch and land on the flowers of a specific plant. The total number of visits is the simplest and most common value used in flower visitation networks (reviewed by Castro-Urgal *et al.* 2012). However, a very limited number of studies have adjusted the number of interactions to account for floral abundance (Kaiser-Bunbury *et al.* 2009, 2011) and when link weights accounted for total observation time and total flower abundance, Castro-Urgal *et al.* (2012) reported an increase in H_2', *d'* and decrease in generality and interaction evenness. Therefore, I acknowledge that the true level of specialisation in this study may be underestimated, as interactions are weighted by flower visitation only.

Community level indices for the entire network (all 2014 and 2015 visits pooled) were compared to a null model to assess whether the structure of the interactions differed from that expected by chance (with the exception of modularity). A null model based on 1,000 permutations of the original network was created using the function *nullmodel* and method *vaznull* in bipartite, so that the connectance of the null model matched the level of connectance in the original network (Vázquez *et al.* 2007). Statistical difference from the null model is quoted after each index.

3.2.5 Temporal comparisons

Annual variation was compared between all visits recorded in 2014 and 2015. Seasonal variation in visitation was compared by dividing the pooled records from 2014 and 2015 into four periods: Early (March – May), June, July and August. To make a fair comparison of diurnal variation, this analysis included only the visits to 7 plants in July (2014 and 2015 records were combined;) where sampling effort was similar for each of the plants throughout four diurnal time periods: 07:30-09:00, 09:00-12:00, 12:00-15:00 and 15:00-18:30. Although it would have been desirable to make daily comparisons for all plants throughout the season, variable weather conditions meant that the sampling effort at each time period varied by plant species, which may have influenced the visitor community.

3.2.6 Statistical analysis

All analysis in this thesis assumes a critical value of 5% (p=0.05). All average values are quoted as the mean±standard error (SE), unless otherwise stated. Most figures were made using *ggplot2* (Wickham, 2009) and additional RStudio packages are quoted where used.

(i) Flower visitor specialisation and plant species strength

Linear models were used to determine the relationship between flower visitor species specialisation (*d'*) and tongue length, body size (measured as the inter-tegular distance), flower visit frequency and taxa. As *d'* is sensitive to very low sample sizes, flower visitors with less than five visits were excluded from the data (n=12). Likewise, as *d'* values are proportional, all values were arcsine-square root transformed prior to testing. Given that linear models assume that all data are independent, and this is not strictly true when visitors within a group have a common phylogenetic history (e.g. *Bombus*) the relationship between *d'* and each measure was checked for differences between taxa by including taxa as an interaction effect (Stone *et al.* 2011). Linear models were fitted using the function *'lm'* for *R*.

The importance of sampling effort and patch size on estimates of plant species strength were tested using Spearman's Rank correlation, as the data could not be transformed to fit a normal distribution. Values for patch size and total hours of sampling for each of the 29 plants were log transformed prior to testing.

The specialisation of visitor and plants, and the species strength of plants were compared between annual networks using a generalised linear mixed model (GLMM, with a Gamma error distribution and identity link function) with species as a random effect to account for the non-independence of the data. Only species present in both years were included in the analysis. Visitors with 5 or less visits and plants with a species strength of 0 were excluded from comparisons. GLMMs were fitted using the function '*glmer'* in the package '*lme4'* for *R* (Bates *et al.* 2015).

(ii) Foraging behaviour

The foraging behaviour of visitors was compared between the four seasonal and diurnal time periods using a Chi-squared test on the proportional values. The proportion of visitors demonstrating each foraging behaviour at each time point was calculated as the mean±SE percentage of all plants studied during the time period.

(iii) Flower visit duration

The mean flower visit duration (s) was compared between bee and non-bee flower visitors using an unpaired 2-sample *t*-test, and between each group of flower visitors (*Apis*, *Bombus*, Other Bee, Lepidoptera, Hoverfly, Other Diptera, Coleoptera and Wasp) using a 1-way ANOVA with a Tukey multiple comparison of means test. Flower visit duration was log (x+1) transformed prior to testing to fit the assumptions of a parametric distribution.

(iv) Contact with floral reproductive parts

The proportion of all visits that resulted in contact with the floral reproductive structures (anthers, stigma, anthers and stigma or none) were compared between flower visitor taxa using a Chi-squared test, although differences in the sample sizes made it difficult to compare individual taxa.

3.3 Results

The structure of the flower visitor community was based on 14,317 interactions recorded between 53 taxa of insects and the flowers of 29 plant species (7,348 interactions in 2014, 6,969 interactions in 2015) representing 516 hours of observation.

3.3.1 Which insects visit flowers in a garden?

All flower visitors recorded and identified are given in **Table 3.1**, organised into eight groups for later analysis (*Apis, Bombus*, Other Bee, Lepidoptera, Hoverflies, Other Diptera, Coleoptera, Wasp and Other). Non-eusocial (other) bees formed the most speciose group, followed by the syrphid Diptera, *Bombus*, Lepidoptera, non-syrphid Diptera, Coleoptera, wasps and *Apis*. A note of caution is due here as the diversity of non-syrphid Diptera is much greater than that acknowledged by the two groups Calliphoridae and Muscidae, but visitors were grouped into these subdivisions due to the difficulties in their reliable taxonomic identification. Visits from *B.terrestris* and *B.lucorum* were grouped together given the difficulties in visually separating these species in the field (Wolf *et al.* 2010), which also applied to the small non-eusocial Halictidae (predominantly *Lasioglossum* species).

Group	**Genus (Family, Tribe)**	**Species**
Apis		
	Apis (Apidae)	*A. mellifera*
Bombus		
	Bombus (Apidae)	*B.hortorum* *B.hypnorum* *B.jonellus* *B.lapidarius* *B.pascuorum* *B.pratorum* *B.terrestris* *B.lucorum* *B.vestalis*
Other Bee		
	Andrena (Andrenidae)	*A.bicolor* *A.cineraria* *A.flavipes* *A.fulva* *A.minutula* *A.nigroaenea* *A.carontonica* *A.subopaca*
	Colletes (Colletidae)	*C.daviesanus*
	Anthidum (Megachilidae)	*A. manicatum*
	Anthophora (Apidae)	*A.plumipes* *A.furcata* *A.quadrimaculata*
	Coelioxys (Megachilidae)	*C.rufescens*
	Lasioglossum (Halictidae)	*L.calceatum* *L.morio* *L.pauxillum* *L.smeathmanellum*
	Halictus (Halictidae)	*H.rubicundus* *H.tumulorum*
	Hylaeus (Colletidae)	*H.hyalinatus*
	Megachile (Megachilidae)	*M.centuncularis* *M.willughbiella*
	Osmia (Megachilidae)	*O.bicornis* *O.caerulescens* *O.leaiana*
	Melecta (Apidae)	*M.albifrons*
	Nomada (Apidae)	*N.flava*
Lepidoptera		
	Aglais (Nymphalidae)	*A. urticae* *A. io*
	Celastrina (Lycaenidae)	*C.argiolus*
	Macroglossum (Sphingidae)	*M.stellatarum*
	Maniola (Nymphalidae)	*M.jurtina*
	Pieris (Pieridae)	*P.brassicae* *P.rapae*
	Thymelicus (Hesperiidae)	*T.sylvestris*
	Vanessa (Nymphalidae)	*V.atalanta* *V.cardui*

Group	Genus (Family, Tribe)	Species
Hoverflies		
	Baccha (Syrphidae)	*B.elongata*
	Platycheirus (Syrphidae)	*P. albimanus* *P.angustatus* *P.clypeatus* *P.manicatus* *P.peltatus* *P.scutatus*
	Episyrphus (Syrphidae, Syrphini)	*E.balteatus*
	Eristalis (Syrphidae, Eristalini)	*E.arbustorum* *E.pertinax* *E. tenax*
	Helophilus (Syrphidae, Eristalini)	*H.pendulus* *H.trivattus*
	Myathropa (Syrphidae)	*M.florea*
	Merodon (Syrphidae)	*M.equestris*
	Cheilosia (Syrphidae, Rhingiini)	*C.pagana* *C.variabilis*
	Eumerus (Syrphidae, Eumerini)	*E.funeralis*
	Eupeodes (Syrphidae, Syrphini)	*E.corollae* *E.luniger*
	Syrphus (Syrphidae, Syrphini)	*S.ribesii* *S.torvus* *S.vitripennis*
	Epistrophe (Syrphidae, Syrphini)	*E.eligans*
	Melangyna (Syrphidae, Syrphini)	*M.labiatarum*
	Meligramma (Syrphidae, Syrphini)	*M.trianguliferum*
	Scaeva (Syrphidae, Syrphini)	*S.pyrastri*
	Meliscaeva (Syrphidae, Syrphini)	*M.auricollis* *M.cinctella*
	Syritta (Syrphidae)	*S.pipiens*
	Sphaerophoria (Syrphidae)	*S.scripta*
	Rhingia (Syrphidae, Rhingiini)	*R.campestris*
	Volucella (Syrphidae, Volucellini)	*V.bombylans* *V.pellucens* *V.zonaria*
Other Diptera		
	Bombylius (Bombyliidae)	*B.major*
	Calliphora (Calliphoridae)	*C.vomitoria* *C.vicina*
	Pollenia (Calliphoridae)	*P.rudis*
	Lucilia (Calliphoridae)	*L.caesar*
	Chloromyia (Stratiomyidae)	*C.formosa*
	Empis (Empididae)	*E.tessellata*
	Musca (Muscidae)	*M.domestica*
	Fannia (Muscidae)	*F.canicularis*
	Palloptera (Pallopteridae)	*P.saltuum*
	Sarcophaga (Sarcophagidae)	*S.carnaria*
	Scathophaga (Scathophagidae)	*S. stercoraria*

Group	Genus (Family, Tribe)	Species
Coleoptera		
	Harmonia (Coccinellidae)	*H.axyridis*
	Grammoptera (Cerambycidae)	*G.ruficornis*
	Oedemera (Oedemeridae)	*O.nobilis*
Wasp		
	Ectemnius (Crabronidae)	*E.sexcinctus*
	Chrysis (Chrysididae)	*C.ignita*
	Ancistrocerus (Eumenidae)	*A.trifasciatus*
	Ichneumon (Ichneumonidae)	-
	Sphecid wasp (Sphecidae)	-
	Vespula (Vespidae)	*V.vulgaris*
Other		
	Arge (Argidae)	*A.pagana*
	Miris (Miridae)	*M.striatus*

Table 3.1 Details of the identification of all flower visiting species observed in the garden. Several genera included multiple species when visual identification of these visitors was not possible in the field, or flower visitors were rare. For *Andrena,* almost all visits were from *Andrena* species, although a single *Colletes daviesanus* specimen in the reference was identified by George Else; visual and size similarities between *C.daviesanus A.bimaculata,* combined with an overlap of the species exploited by these visitors (mostly *Asteraceae*) meant that rare *Colletes* visitors were therefore grouped with the *Andrena* (as in Ballantyne *et al.* 2015)*.* A reference collection of dipteran specimens held at The University of St Andrews remains to be identified and these genera (particularly calliphorids and muscids) are likely to be more speciose than recognised here. Results are representative of all flower visitors to 29 plants observed over two summers (2014 and 2015) in the garden of Dover College.

3.3.2 How are flower visits structured in a garden?

Over the course of two summers, 466 individual links were observed between plants and visitors to their flowers (**Fig. 3.1**). As in many flower visitation networks, the community of flower visitors included both rare and very common visitors. The majority of visits were made by *Apis* (n=2,202), *B.terrestris/lucorum* (n=1,543), *B.pratorum* (n=1,399) and *B.pascuorum* (n=1,049). Bumble bees were particularly frequent flower visitors, accounting for 39% (n=5,537) of all visits (n=14,317). Visits from Diptera were less frequent, with the exception of *E.balteatus* (n=595) and Calliphoridae (n=1,121), while coleopteran and lepidopteran visitors were poorly represented in the garden. Using stationary observations could explain why Hymenoptera constituted a greater proportion of flower visits, compared with Baldock *et al.* (2015) where Diptera were more frequent (67% of 7,412 records) as transect methods usually do not count return visitors (see Section 3.2.1).

The level of specialisation in the network was relatively low (H_2'=0.38) although this was higher than that predicted by the null model (p<0.001) and placed the community towards the lower end of H_2' values relative to the 22 visitation networks cited in Dormann and Strauss (2014). The generality of flower visitors (8.96) was greater than that for plants (6.80) with both values lower than that predicted by the null model (p<0.001). Interaction evenness was relatively high (0.68) and connectance low (0.10) with both values significantly lower than that predicted by the null model (p<0.001). The interactions had a low degree of nestedness (NODF=39.2, lower than null model p<0.001) and demonstrated a random pattern, rather than perfect nestedness between columns (**Fig. 3.2**).

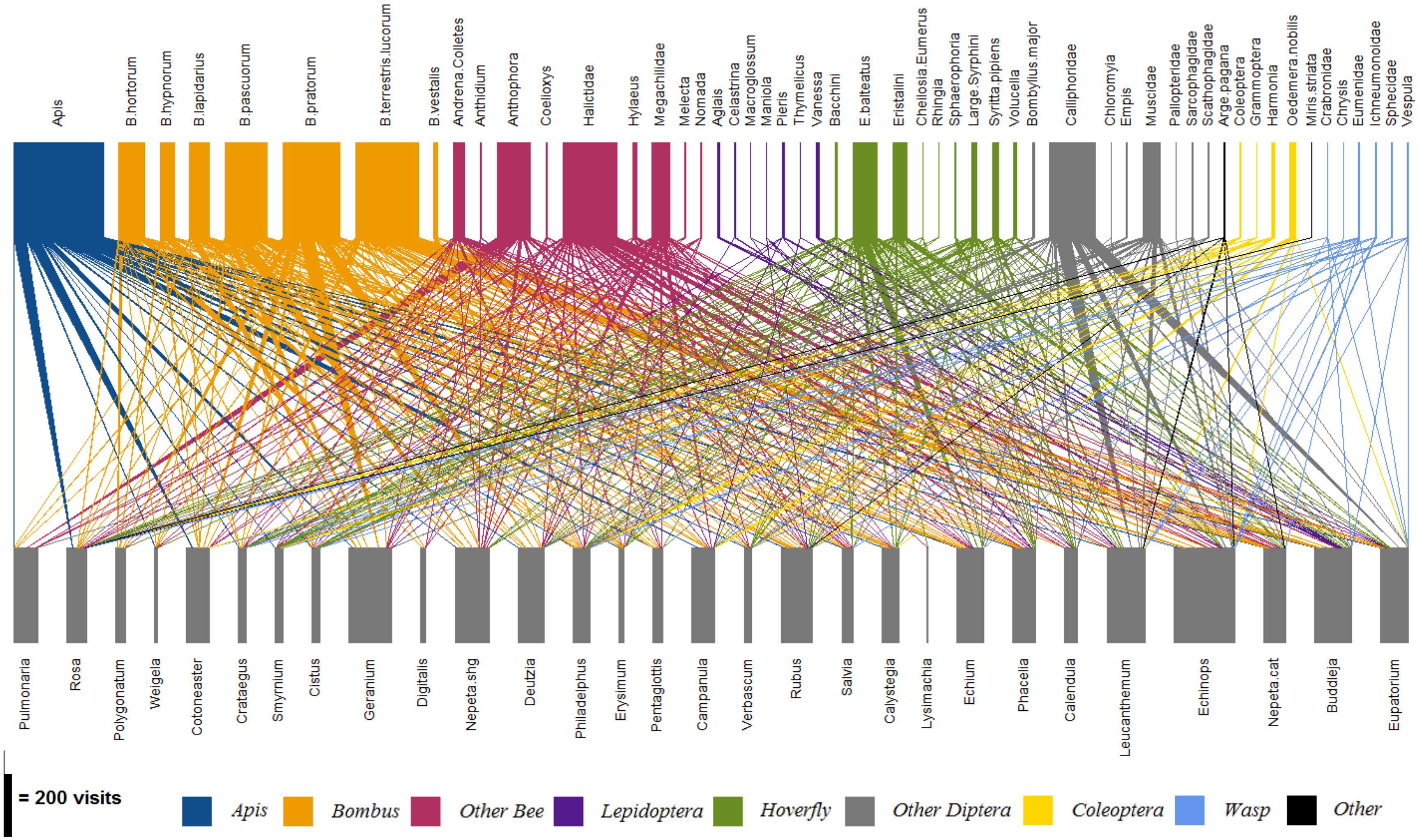


Figure 3.1 A traditional flower visitation network showing the flower visits recorded to 29 plants in a garden, UK. A bipartite network was constructed to show the individual flower visits from 53 groups of flower visitors, with the width of the species nodes and interactions representing the number of visits that were observed. Visits from honeybees, bumblebees and other bees dominated the network, while Lepidoptera, Diptera and Coleoptera were far less frequent flower visitors. Among the Diptera, the syrphids *E.balteatus* and Eristalini were particularly common, as were the calliphorid flies. Most groups of flower visitors visited several plants and hence the network appears relatively generalised. Results shown include all 14,317 individual visits recorded over the course of two summers (2014 and 2015) in the garden of Dover College.

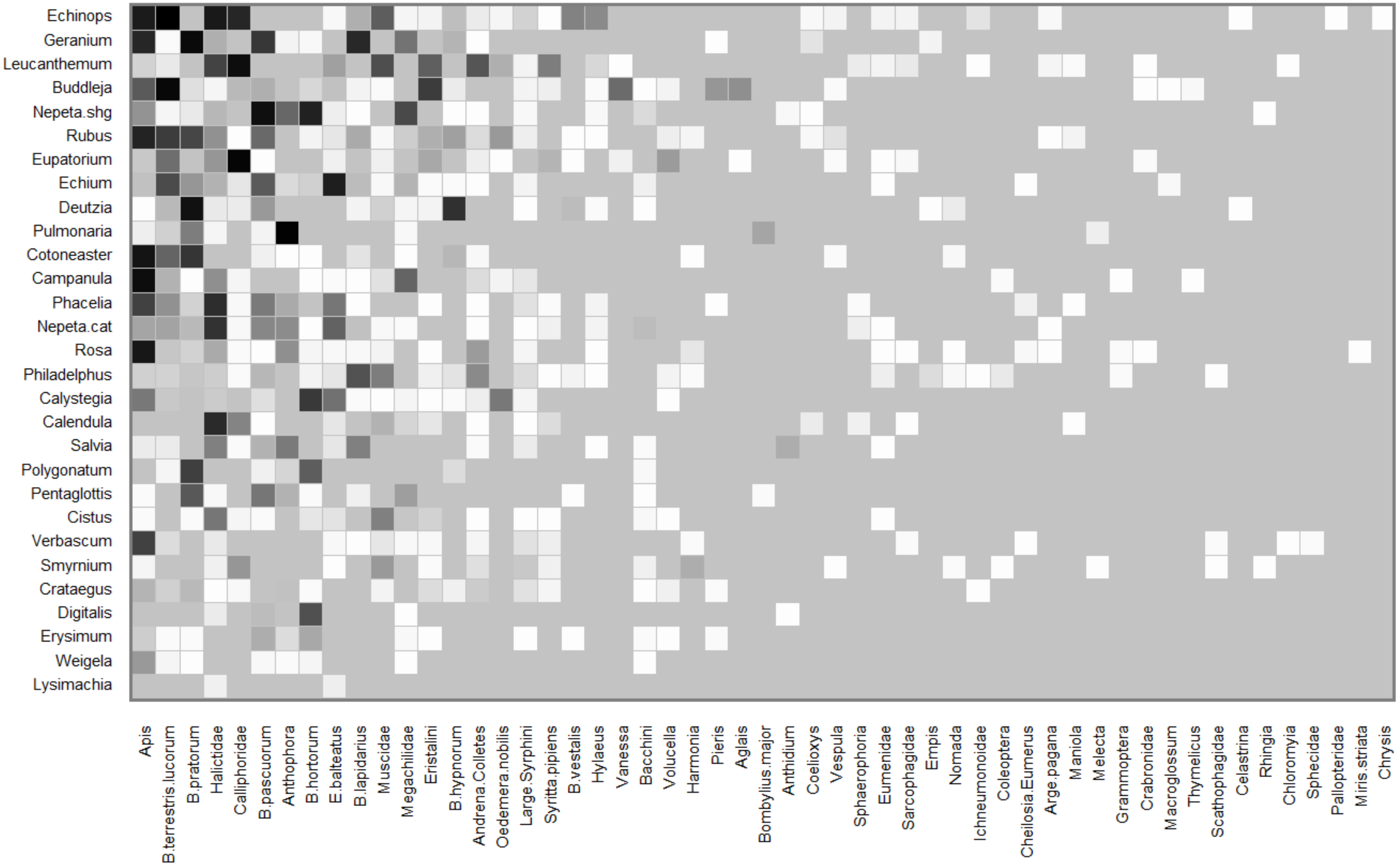


Figure 3.2 A bipartite matrix showing the flower visits recorded to 29 plants in a garden, UK. The matrix was constructed using the raw visitation values (as in Fig. 3.1) and organised from the most to least connected plants (top to bottom) and visitors (left to right). The number of visits varied considerably between flower visitors (where the darker shades indicate more visits), ranging from a maximum of 494 (*B.terrestris* to *Echinops*) to 1 (e.g. *Volucella* to *Calystegia*). Results include all 14,317 individual flower visits recorded over the course of two summers (2014 and 2015) in the garden of Dover College.

Interactions in the network were organised into distinct modules, with a value of modularity (0.41) slightly below that of the 29 networks tested for modularity in Olesen *et al.* (2007, mean modularity 0.52 ± 0.07). Flower visitors were organised into 6 modules of strongly connected species (**Fig. 3.3**) and each module contained an average of 14 species (5 plants and 9 flower visitors) with at least one species of bee. Interestingly, the module containing *Apis* did not contain another bee, suggesting some degree of niche partitioning between these visitors. The two long-tongued *Bombus* species (*B.hortorum* and *B.pascuorum*) were strongly connected, by the frequency of their visits to *Nepeta* ‘Six Hills Giant’. The foraging niche of the synanthropic *B.hypnorum* (introduced to the UK in 2001) was most like that of *B.pratorum* and *B.lapidarius*. The smallest module contained the early flowering *Pulmonaria* and three visitors, while the largest module (7 plants and 24 visitors) contained only one social bee (*B.terrestris/lucorum*) and many infrequent visitors, suggesting an importance in the role of *B.terrestris/lucorum* as a core that peripheral species connect to. Otherwise, there was no clear relationship between module composition and flowering phenology or floral morphology.

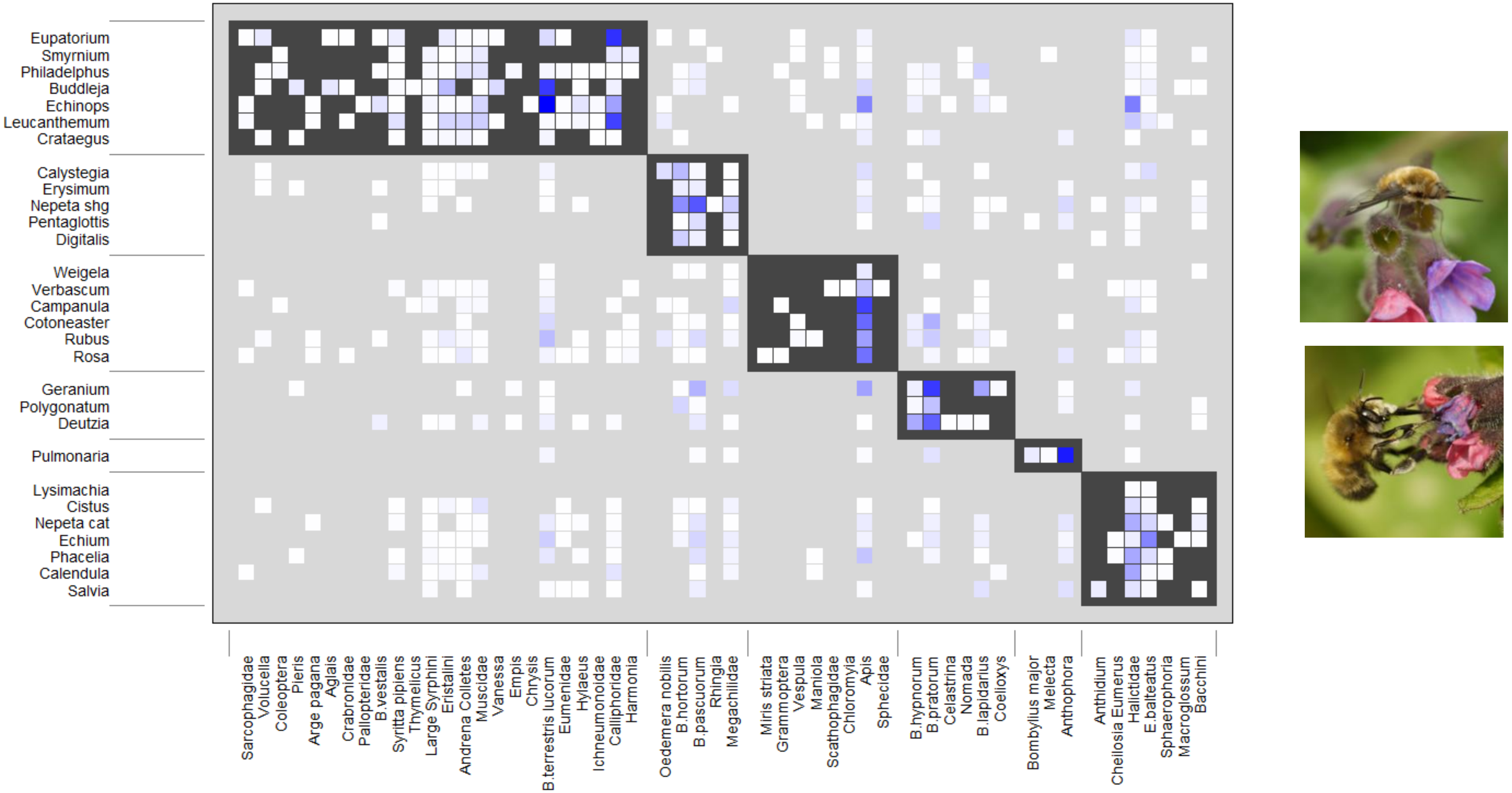


Figure 3.3 Modularity within the traditional flower visitation network, showing six distinct groups of highly connected species. A bipartite network was created with flower visitors and plants organised in modules with the other species they most frequently interacted with. The largest module contained mostly non-bee flower visitors, although *B.terrestris/lucorum* was the most frequent flower visitor within the module. The smallest module contained three visitors to the early flowering *Pulmonaria* (including *Bombylius major* and *Anthophora plumipes*, illustrated). Results shown include all 14,317 flower visits recorded over the course of two summers (2014 and 2015) in the garden of Dover College.

(i) Can insect morphological characteristics determine their specialisation as flower visitors?

The specialisation (*d'*) of flower visitors ranged from 0.11 to 0.58 (mean 0.33, n=41), although these values are limited to the subset of the plant community studied. The most specialised visitors were three of the large, long tongued bees *Anthidium*, *Anthophora* and *B.hortorum* (*d'*=0.58, 0.57, 0.54); although large bodied flower visitors may also have small tongues, longer tongues are always associated with larger bodies (Stang *et al.* 2006) so it was not surprising to find that the overall specialisation of visitors was significantly positively correlated with tongue length ($F_{(1,39)}$=6.65, p=0.01, r^2=0.12) and also with body size, although this was barely significant ($F_{(1,39)}$=3.07, p=0.09, r^2=0.05, **Fig. 3.4**). However, the data does not show how specialisation related to tongue length or body size within groups, e.g. Halictidae, and the results are limited to just two measures of morphology.

Although flies were not common visitors to many plants, calliphorids were exceptionally abundant on the flowers of *Leucanthemum* and *Eupatorium*. Surprisingly, these opportunistic generalists (Kearns 2001) were relatively specialised in their interactions with the garden plants, although these visitors are only facultative consumers of floral resources. No significant relationship was found between visitor specialisation and flower visit frequency ($F_{(1,39)}$=1.60, p=0.21, r^2=0.01) or taxa ($F_{(7,32)}$=1.00, p=0.45, r^2< -0.001) and the relationship between specialisation and each measure did not vary significantly between flower visitor groups. Categories for body size, tongue length and *d'* values are given in **Appendix 3.1**.

(ii) What determines the species strength of plants in a garden?

Plant species strength ranged from 0.02 to 6.87 (mean 1.83, n=29). The plants that were the most important for flower visitors in both years were *Echinops*, *Leucanthemum* and *Buddleja* (species strength=6.87, 5.08, 4.49), all three of which flowered towards the end of the season (July onwards) and whose flowers consisted of many florets. Plants often considered to be weeds in gardens (*Eupatorium*, *Rubus* and *Smyrnium*) were all within the top ten most important plants, which included both native and introduced taxa. The oil-producing flowers of *Lysimachia* were the least important to the flower visitor community, which was unsurprising given that the flowers produce oils that only *Macropis europea* is known to collect; however, these results should be interpreted with caution as plant species strength was significantly, positively correlated with the total hours of observation (r_s=0.52, p=0.005, n=29), but not the number of flowers available (r_s=0.28, p=0.14, n=29).

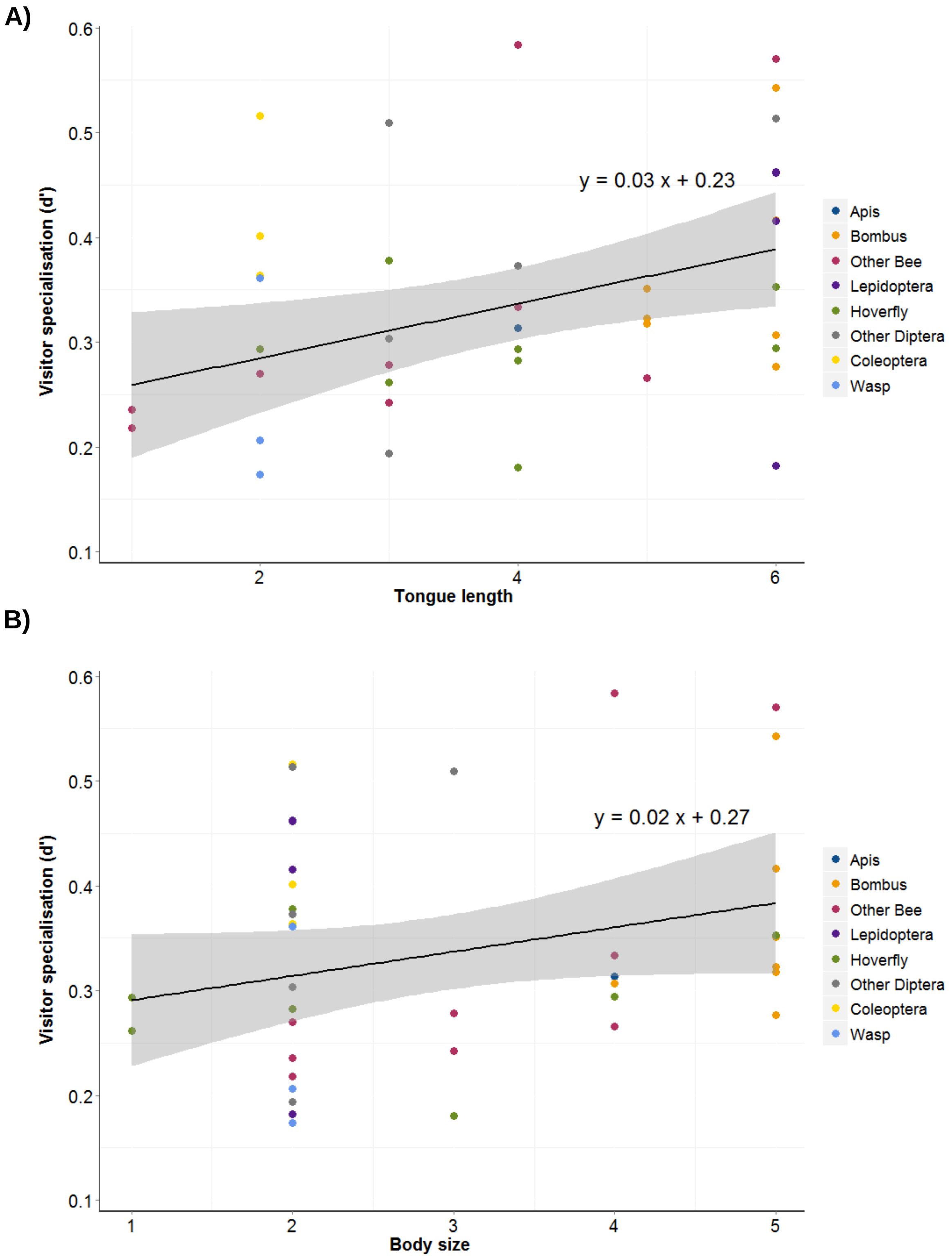


Figure 3.4 The relationship between the specialisation of flower visitors (measured using the species level index *d'*) and A) tongue length or B) body size (inter-tegular distance). The specialisation of individual visitors was calculated from the traditional visitation network, and plotted against categorical measures of tongue length and body size taken from individuals sampled for pollen load analysis. In both cases, the specialisation of flower visitors increased with tongue length and body size. Shading indicates the 95% confidence interval, with the linear equation for the non-transformed *d'* values. Results shown include 721 individual flower visitors collected over two summers.

3.3.2 To what extent does flower visitation vary temporally?

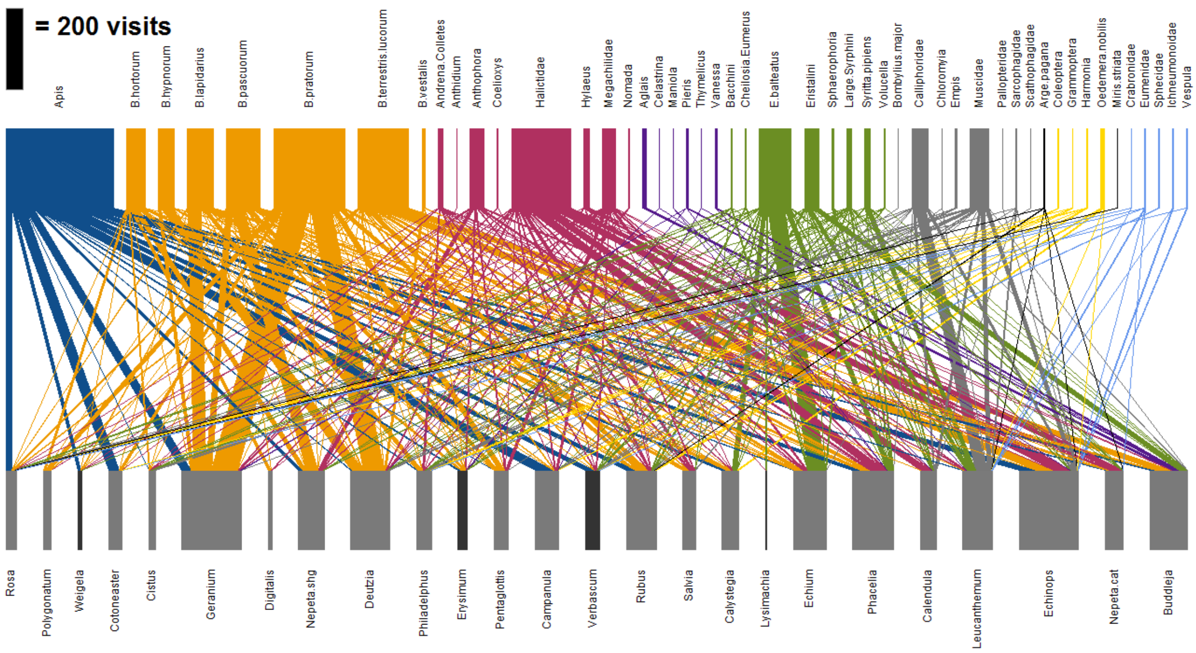
Dividing the pooled data into temporal networks (annual, seasonal and diurnal) can reveal variation in the structure of interactions that are otherwise masked.

(i) Differences between years

There was little annual variation in the structure of the visits to the focal plants (**Fig. 3.5**). Connectance and modularity were identical in both years (0.09 and 0.43) and the other community indices remained similar: interaction evenness (0.65, 0.67) generality of flower visitors (7.50, 7.07) and plants (5.08, 6.11), specialisation (H_2' 0.41, 0.43) and nestedness (33.89, 35.23). The interactions were slightly more specialised in the annual networks compared to the pooled network. Overall, the diversity of flower visitors was similar in either year, with only a small number of visitors unique to one summer: 8 visitors were exclusive to 2014, and 4 to 2015. Although *Apis* were the most abundant visitors in 2014 (n=1,467, 19% of all visits) they were less frequent in 2015 (n=735,11%). *E.balteatus* were also less frequent in 2015 with no migratory swarms observed. The inclusion of *Eupatorium* resulted in calliphorids becoming the most abundant visitor in 2015 (n=897, 13%) and observations of *Anthophora* increased by 29% with the inclusion of *Pulmonaria* (2014 n=187, 2015 n=634).

Overall, the average specialisation of flower visitors present in both years (n=40) did not differ between years (2014: d'=0.30±0.02, 2015: 0.31±0.02, GLMM t=-0.23, p=0.82) although the most specialised visitor changed from *B.hortorum* (d'=0.53) to *Anthidium* (0.73). The average species strength of 21 plants present in both years was slightly higher in the first year (2.13±0.36) compared to the second (1.52±0.27, GLMM t=-2.08, p=0.04) although *Echinops* remained the most important plant.

A) 2014



B) 2015

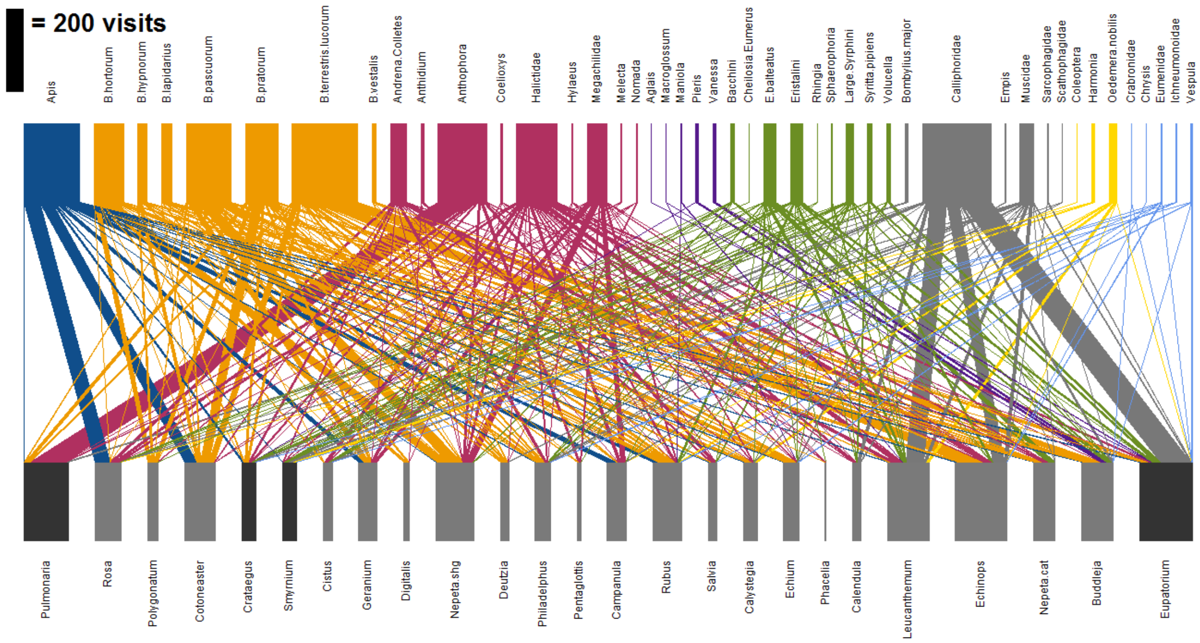


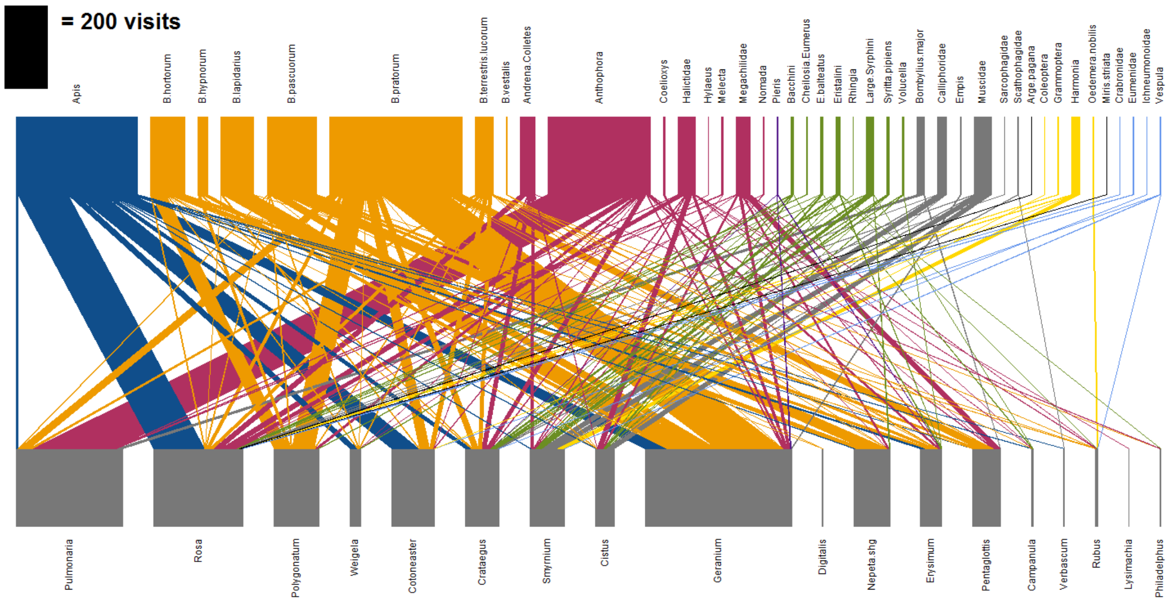
Figure 3.5 Two traditional flower visitation networks showing all of the visits recorded in the summer of A) 2014 and B) 2015 separately. Bees were the most frequent flower visitors in either year, although the proportion of visits from calliphorids was increased considerably with the inclusion of the plant *Eupatorium* in 2015. *E.balteatus* was a more frequent flower visitor to *Echium* in the summer of 2014, arriving to the garden late in the summer as a migratory swarm. Overall, the appearance of the networks was very similar between years. Results shown include all 7,214 flower visits recorded between May to August 2014, and 6,796 flower visits recorded between March to April and later May to August 2015. Plants shown in black indicate those that were only observed in a single year.

(ii) Monthly variation in interaction structure

Variation was greater between months than between years, revealing seasonal differences in the structure of flower-visitor interactions that were lost when all visits were pooled. Although connectance remained low throughout the summer (range 0.07 to 0.11), interaction evenness peaked in June and July (0.64 and 0.65, **Fig. 3.6**); however, this difference may reflect changes in the size of the networks. Specialisation (H_2') appeared to be greatest early and late in the summer (0.47 and 0.43) which was greater than for the pooled network (0.38). Modularity was highest early in the summer (0.50) and lowest in June (0.37). Nestedness was highest in June and August (44.45 and 43.37). The generality of flower visitors was lowest at the end of the season (August 2.69) and peaked in June (4.61). Interestingly, unlike in the pooled network, the generality of plants was higher compared to flower visitors, and lower early in the season (4.69) then peaked in July (7.51).

Flower visitors appeared to be most specialised early in the summer (*d'* 0.30±0.02, n=41) and least in August (0.22±0.02, n=32) and in each month the most specialised visitor was a bee (Early *Anthophora d'*=0.59; June: *B.hortorum* 0.54; July *Anthidium* 0.58; August *B.pascuorum* 0.47). The importance of plant species was lowest early in the summer (species strength 2.28±0.69, n=18) and greatest in August (4.00±1.21, n=8). The most important plant, *Echinops* (11.19), flowered in July.

A) Early



B) June


= 200 visits
Apis
B.hortorum
B.hypnorum
B.lapidarius
B.pascuorum
B.pratorum
B.terrestris.lucorum
B.vestalis
Andrena.Colletes
Anthidium
Anthophora
Coelioxys
Halictidae
Hylaeus
Megachilidae
Nomada
Celastrina.argiolus
Maniola
Thymelicus
Bacchini
Cheilosia.Eumerus
E.balteatus
Eristalini
Rhingia
Large.Syrphini
Syritta.pipiens
Volucella
Calliphoridae
Chloromyia
Empis
Muscidae
Sarcophagidae
Scathophagidae
Arge.pagana
Coleoptera
Grammoptera
Harmonia
Oedemera.nobilis
Eumenidae
Sphecidae
Ichneumonoidae
Vespula
Cotoneaster
Cistus
Geranium
Digitalis
Nepeta.shg
Deutzia
Philadelphus
Erysimum
Pentaglottis
Campanula
Verbascum
Rubus
Calystegia
Lysimachia

C) July


= 200 visits
Apis
B.hortorum
B.hypnorum
B.lapidarius
B.pascuorum
B.pratorum
B.terrestris.lucorum
B.vestalis
Andrena.Colletes
Anthidium
Anthophora
Coelioxys
Halictidae
Hylaeus
Megachilidae
Aglais
Celastrina.argiolus
Macroglossum
Maniola
Pieris
Thymelicus
Vanessa
Bacchini
Cheilosia.Eumerus
E.balteatus
Eristalini
Sphaerophoria
Large.Syrphini
Syritta.pipiens
Volucella
Calliphoridae
Chloromyia
Muscidae
Pallopteridae
Sarcophagidae
Arge.pagana
Oedemera.nobilis
Crabronidae
Chrysis
Eumenidae
Ichneumonoidae
Vespula
Digitalis
Nepeta.shg
Rubus
Salvia
Calystegia
Echium
Phacelia
Leucanthemum
Echinops
Calendula
Nepeta.cat
Buddleja
Eupatorium

Figure 3.6 Four traditional flower visitation networks showing the change in flower visitation patterns throughout the summer: A) Early; B) June; C) July; D) August. All of the observed flower visits were divided into four networks according to date. In each of the networks, bees remained the most frequent flower visitors, although the proportion of visits made by Diptera increased later in the summer. This was particularly due to the calliphorid flies who were frequent visitors to the late flowering *Eupatorium*. Results shown include all flower visits recorded over the course of two years, between A) March to May, n=3,384; B) n=4,073; C) n=5,258; D) n=1,602.

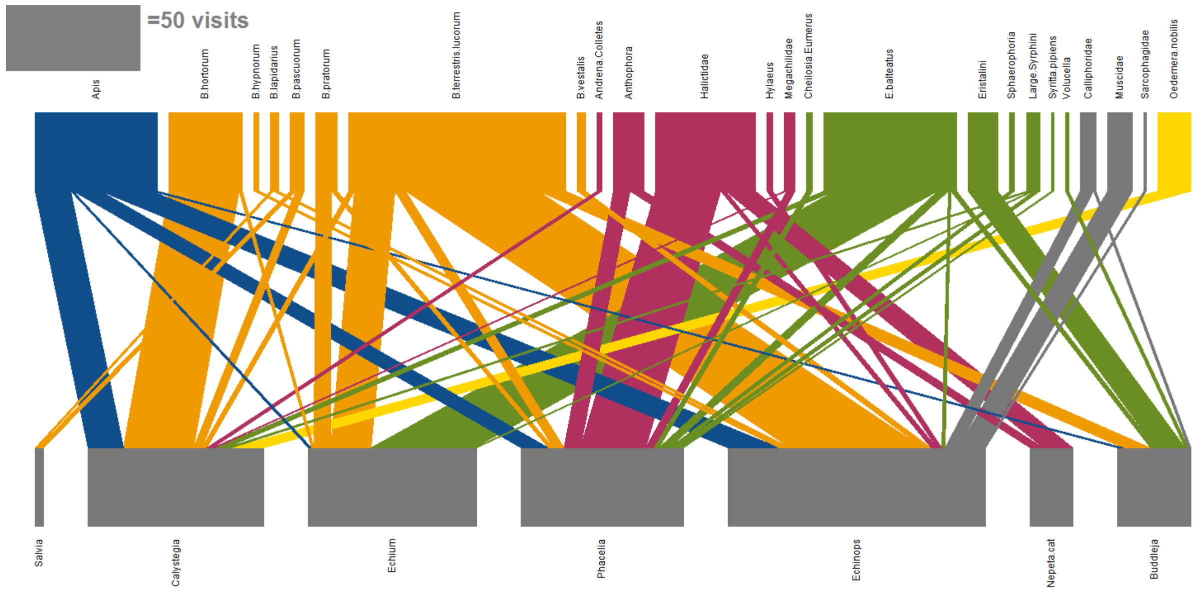
(iii) Diurnal variation

Diurnal variation in visitation to seven plant taxa (*Salvia, Calystegia, Echium, Phacelia, Echinops, Nepeta cataria, Buddleja,* Section 3.2.5) was compared in the peak of summer (July) when the sampling efforts between plants were most equal due to constant weather conditions (**Fig. 3.7**). Although the size of the networks varied and this is known to affect network indices (Blüthgen *et al.* 2006), connectance remained low throughout the day (range 0.11-0.12) and interaction evenness varied little (range 0.62-0.70). However, the interactions appeared more generalised for 09:00-12:00 (H_2'=0.38, n=1,743) and 12:00-15:00 (H_2'=0.34, n=1,411) compared to 07:30-09:00 (H_2'=0.54, n=328) and 15:00-18:00 (H_2'=0.55, n=673). Modularity followed changes in H_2', peaking in the early morning (0.52) and after 15:00 (0.46). Nestedness also peaked in the early-afternoon, before declining rapidly after 15:00 (from 39.27 to 22.08). Dividing the network into temporal periods resulted in higher estimates of plant generality compared to that of visitors. Generality for both groups was lowest early in the morning (plant=4.72, visitor=2.32) and while visitor generality peaked in the mid-morning (3.29) plant generality peaked later in the afternoon (7.31).

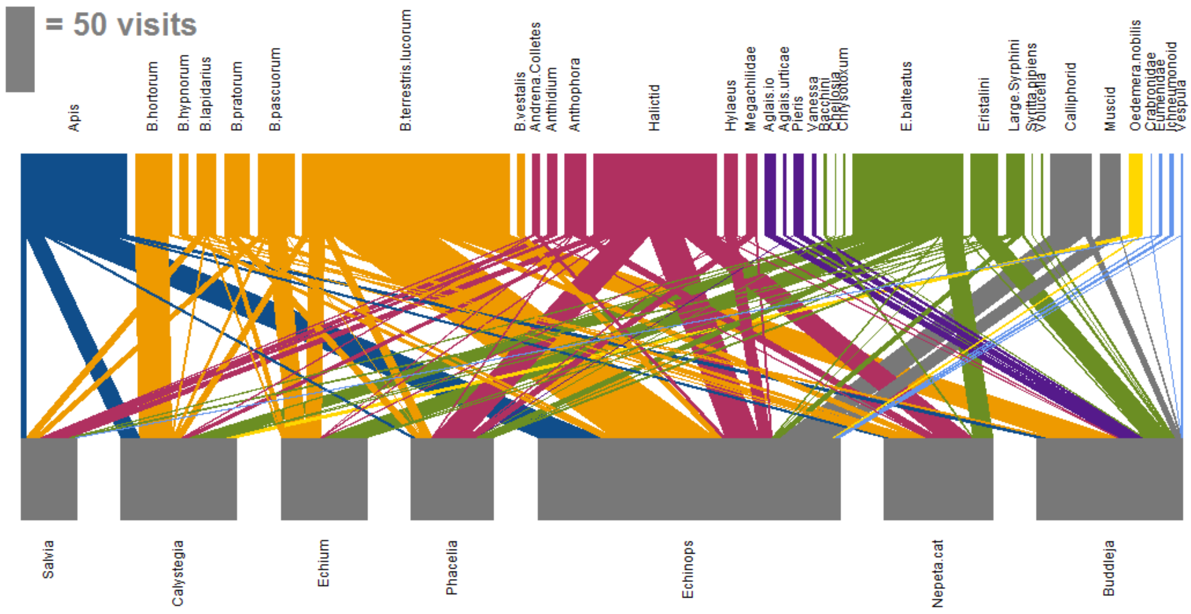
The activity of different taxa of flower visitors was surprisingly constant throughout the day, with no suggestions of large differences in flight temperature thresholds suggested for visitors; *Bombus* showed no decline in foraging in the warmest part of the day (12:00-15:00) and hoverflies were not uncommon visitors in the coolest parts (07:30-09:00, **Fig. 3.7**). A possible explanation for this might be the presence of shaded areas in the garden, providing cooler habitats for larger-bodied visitors, while smaller visitors may have benefited from greater average temperatures of urban areas compared to rural sites (the ‘heat island effect’, Meineke *et al.* 2013) although the extent to which this affects urban plant-pollinator populations is largely unknown.

The most specialised flower visitor varied throughout the day (07:30-09:00 Eristalini *d'* =0.73, 09:00-12:00 *Anthidium*=0.61, 12:00-15:00 *B.hortorum*=0.61, 15:00-18:00 *Anthidium*=0.61). The most important plant in July (*Echinops*) had the highest species strength throughout the day, although its comparative importance varied throughout the day, becoming most important in the early afternoon. A comparison of the indices for all temporal splits are given in a table in **Table 3.2**, with individual species details in **Table 3.3**.

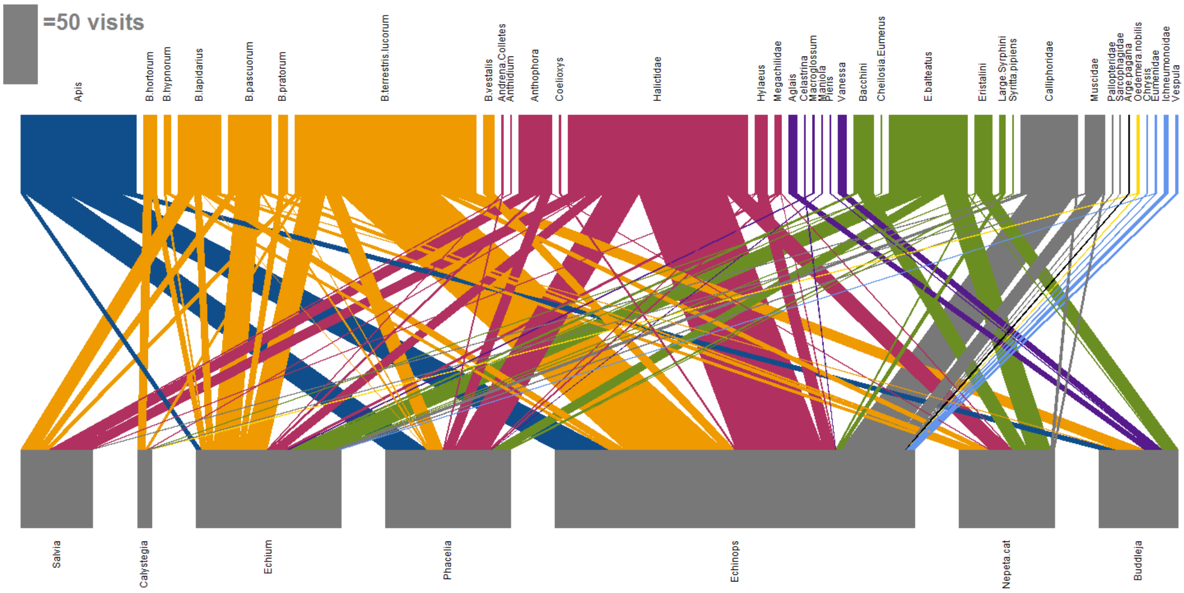
A) 07:30-09:00 (21.68±1.13°C)



B) 09:00-12:00 (22.70±0.46°C)



C) 12:00-15:00 (24.83±0.48°C)



D) 15:30-18:30 (24.59±0.49°C)

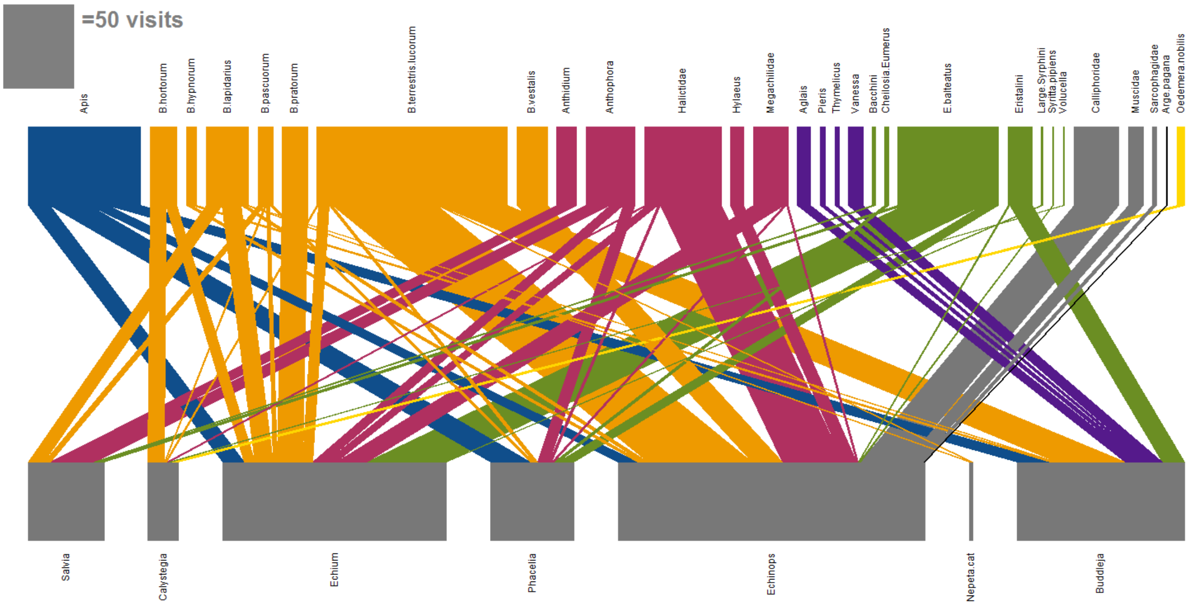


Figure 3.7 Four traditional flower visitation networks showing the change in flower visitation patterns throughout the day: A) 07:30 to 09:00; B) 09:00 to 12:00; C) 12:00 to 15:00; D) 15:00 to 18:30. All flower visits observed were separated by diurnal time period. There was surprisingly little variation in the proportion of flower visits made by different visitors, despite diurnal patterns in foraging behaviour linked to ambient temperatures reported by others (e.g. Willmer, 1983). Large-bodied bumblebees were frequent visitors in the hottest part of the day, while smaller-bodied *E.balteatus* may have benefited from the warmer temperatures in the urban garden compared to the surrounding habitats early in the morning. Results include all of the visits recorded to seven plants flowering in July over the course of two summers; A) n= 328; B) n=1,743; C) n=1,411; D) n=673. Temperatures indicate the mean±SE recorded in that period.

Network interactions		Species richness				Generality						
	Network	**Insects**	**Plants**	**Links**	**Visits**	**Insects**	**Plants**	**C**	**IE**	**NODF**	***H_2'***	***M* (n)**
2014 and 2015	**All visits**	53	29	466	14,317	8.96	6.83	0.10	0.68	39.15	0.38	0.41 (6)
Annual variation	**2014**	49	25	338	7,348	7.50	5.08	0.09	0.65	33.89	0.41	0.43 (6)
	2015	45	25	313	6,969	7.07	6.11	0.09	0.67	35.23	0.43	0.43 (5)
Monthly variation	**Early**	41	18	168	3,384	3.90	4.69	0.07	0.57	38.44	0.47	0.50 (5)
	June	42	14	174	4,073	4.61	6.65	0.10	0.64	44.45	0.38	0.41 (4)
	July	42	13	182	5,258	4.23	7.51	0.11	0.65	38.68	0.35	0.37 (4)
	August	32	8	86	1,602	2.69	5.89	0.11	0.60	43.37	0.43	0.42 (4)
Diurnal variation	**0730-0900**	24	7	47	328	2.32	4.72	0.11	0.62	27.50	0.54	0.52 (5)
	0900-1200	31		94	1,743	3.29	6.82	0.13	0.70	35.60	0.38	0.39 (4)
	1200-1500	37		89	1,411	3.15	7.31	0.12	0.65	39.27	0.34	0.32 (5)
	1500-1800	29		56	673	2.24	6.32	0.12	0.64	22.08	0.55	0.46 (5)
Foraging behaviour	**Nectar**	44	27	284	5,302	6.75	6.12	0.09	0.64	34.36	0.40	0.41 (5)
	Pollen	40	27	239	2,627	6.55	5.67	0.09	0.63	38.42	0.39	0.46 (4)
	N + P	31	24	157	2,231	5.66	4.19	0.09	0.64	26.37	0.50	0.52 (6)

Table 3.2 Community level indices for the traditional flower visitation network (2014 and 2015 pooled) compared to those divided by year, month, time of day or flower visitor foraging behaviour. All values are calculated using the function *networklevel* in the package bipartite, with the exception of modularity. Changes in the perceived level of specialisation (measured as *H_2'*) suggest that pooling the data can mask temporal changes in the pattern of interactions, particularly when visits throughout the day are pooled. All metrics are weighted as explained in Chapter 2; C= connectance; IE= interaction evenness; NODF= weighted nestedness; *H_2'*= interaction specialisation; M= modularity (n=number of modules). Results include all of the flower visits recorded over two summers, with diurnal visitation patterns limited to seven plants flowering in July.

Species interactions		**Plants**				**Flower visitors**			
	Network	**Most visitor groups (*n*)**	**Most visits (*n*)**	**Highest species strength**	**Average Species strength**	**Most plants visited (*n*)**	**Most visits (*n*)**	**Most specialised (*d'*)**	**Average *d'***
2014 and 2015	**All visits**	*Philadelphus* (26)	*Echinops* (1,491)	*Echinops* (6.87)	1.83 ± 0.30	*Apis* (25)	*Apis* (2,202)	*Anthidium* (0.58)	0.29 ± 0.03
Foraging behaviour	**Nectar**	*Echinops* (22)	*Echinops* (1,176)	*Echinops* (8.68)	-	*Apis, B.pascuorum, B.terr./luc.* (18)	*Apis* (962)	*Anthophora* (0.66)	-
	Pollen	*Rosa* (23)	*Leucanthemum* (447)	*Rosa* (8.73)	-	Halictidae (21)	Halictidae (502)	*Apis* (0.65)	-
	N + P	*Smyrnium* (15)	*Leucanthemum* (296)	*Smyrnium* (7.37)	-	*B.pascuorum,* Halictidae (16)	Calliphoridae (345)	*Anthidium* (0.70)	-
Annual variation	**2014**	*Echinops* (21)	*Echinops* (813)	*Echinops* (5.97)	1.96 ± 0.33	*Apis, B.terr./luc.,* Halictidae (20)	*Apis* (1,467)	*B.hortorum* (0.53)	0.30 ± 0.02
	2015	*Philadelphus* (21)	*Eupatorium* (684)	*Echinops* (4.25)	1.80 ± 0.28	Halictidae (22)	Calliphoridae (897)	*Anthidium* (0.73)	0.31 ± 0.03
Seasonal variation	**Early**	*Rosa* (24)	*Geranium* (801)	*Rosa* (10.71)	2.28 ± 0.69	*Apis* (13)	*B.pratorum* (724)	*Anthophora* (0.59)	0.30 ± 0.02
	June	*Philadelphus* (26)	*Nepeta* 'S.H.G.' (642)	*Philadelphus* (8.94)	3.00 ± 0.83	Halictidae, Megachilidae (11)	*Apis* (925)	*B.hortorum* (0.54)	0.23 ± 0.02
	July	*Echinops* (25)	*Echinops* (1,491)	*Echinops* (11.19)	3.23 ± 0.88	Halictidae (12)	*B.terr./luc.* (950)	*Anthidium* (0.58)	0.25 ± 0.02
	August	*Buddleja* (19)	*Eupatorium* (621)	*Eupatorium* (8.84)	4.00 ± 1.21	Halictidae (7)	Calliphorids (442)	*B.pascuorum* (0.47)	0.22 ± 0.02
Diurnal variation	**0730-0900**	*Phacelia/ Echinops* (11)	*Echinops* (95)	*Echinops* (7.61)	-	*Apis, B.terr/luc, E.balteatus* (5)	*B.terr./luc.* (80)	Eristalini (0.73)	-
	0900-1200	*Buddleja* (19)	*Echinops* (594)	*Echinops* (9.56)	-	*Apis,* Halictidae, *E.balteatus* (7)	*B.terr./luc.* (435)	*Anthidium* (0.61)	-
	1200-1500	*Echinops* (22)	*Echinops* (570)	*Echinops* (15.75)	-	*B.terr./luc.* (7)	Halictidae (393)	*B.hortorum* (0.61)	-
	1500-1800	*Echinops* (14)	*Echinops* (232)	*Echinops* (9.23)	-	*B.terr./luc.* (6)	*B.terr./luc.* (145)	*Anthidium* (0.61)	-

Table 3.3 Species level indices for the traditional flower visitation network (2014 and 2015 pooled) compared to those divided by year, month, time of day or flower visitor foraging behaviour. All values were calculated using the function *specieslevel* in the package bipartite. Only the species strength of plants is shown, as an indicator of their importance to the flower visitor community. Similarly, only the specialisation of flower visitors is given as the measure of flower visitation is interpreted here as being from the visitor's perspective. Changes in the identity of the most important plant and most specialised visitor indicates that the position of individual species varies temporally and depending on the resource collected. Results include all of the visits recorded over two summers, with diurnal visitation patterns limited to seven plants flowering in July.

3.3.3 How does floral resource collection vary temporally and between different flower visitors?

(i) Temporal foraging patterns

Over two years, half of flower visits were for nectar (51.6%, n=5,302) with the remaining visits divided between pollen-only visits (25.5%, n=2,627) and those collecting nectar and pollen together (21.7%, n=2,231). Only 1.2% (n=125) of all visitors exploited the flower as a site to groom, mate or rest without foraging. Nectar-only visits were more common early in the season (before June) and at each time point throughout the day (**Fig. 3.8**), although the proportion of visits for each reward did not differ signficiantly between months (χ^2 =20.46, df=12, p=0.06) or diurnal time periods (χ^2 =13.20, df=12, p=0.35). Nectar theft was exceptionally rare in the garden, with only four visits from early male bumble bees (*B.pratorum*) baseworking flowers of *Geranium*, by perching on the sepals behind the flower and extending the proboscis through the base of the petals, without contacting the floral reproductive structures. It was unclear why this behaviour occurred, as the nectaries were open and relatively accessible.

(ii) Foraging patterns of different flower visitors

Differences in the floral rewards sought by flower visitors were compared by constructing three networks based on reward type (nectar only, pollen only or both **Fig. 3.9**). Flower visits for nectar were dominated by bees (**Fig. 3.9a**) while Diptera (syrphid and non-syrphid) fed predominantly on pollen (**Fig. 3.9b**). If bees visited a flower to collect pollen, they often simultaneously collected nectar (**Fig. 3.9c**), which was particularly true for *Bombus* (**Fig. 3.9b** and **Fig. 3.9c** compared). Halictid bees appeared to make a greater proportion of pollen-only visits relative to all other bees (**Fig. 3.9b**). Specialisation was greatest in the nectar and pollen network (H_2'=0.50) compared to the nectar (0.40) and pollen (0.39) networks. *Echinops, Buddleja, Rubus, Philadelphus* and *Eupatorium* were the top five most important providers of nectar, as measured by species strength, while *Rosa, Leucanthemum* and *Nepeta cataria* were particularly important for providing pollen. All community-level indices and the details of species specialisation and importance are given in **Table 3.2** and **Table 3.3** (pages 62 and 63).

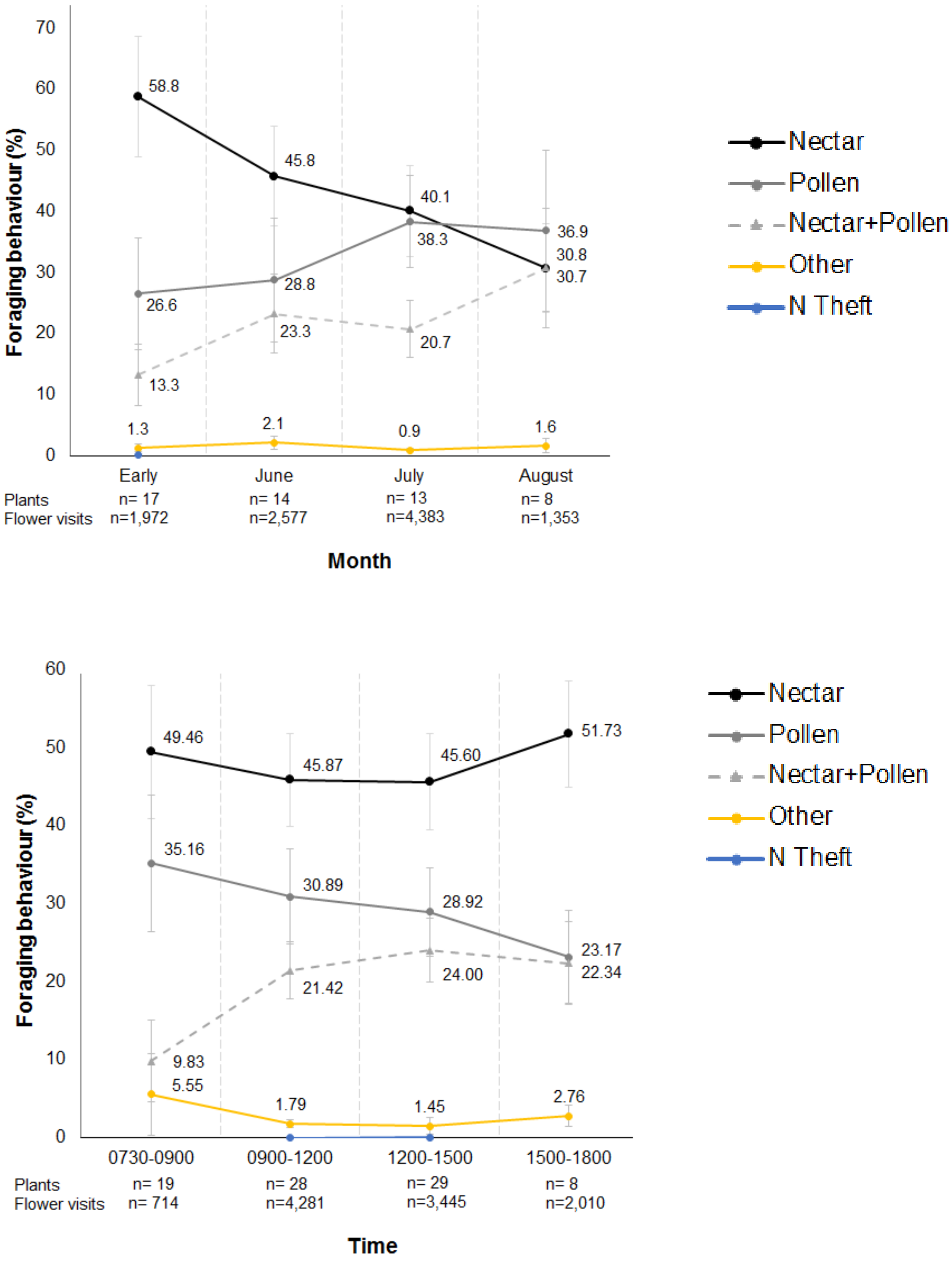
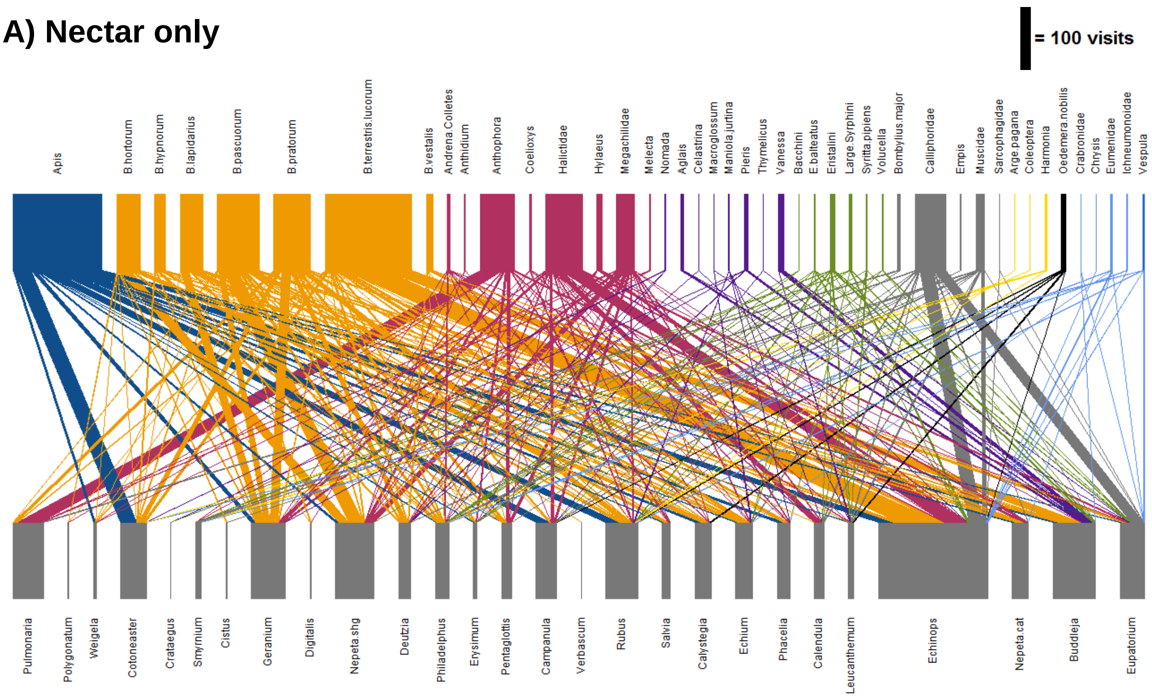
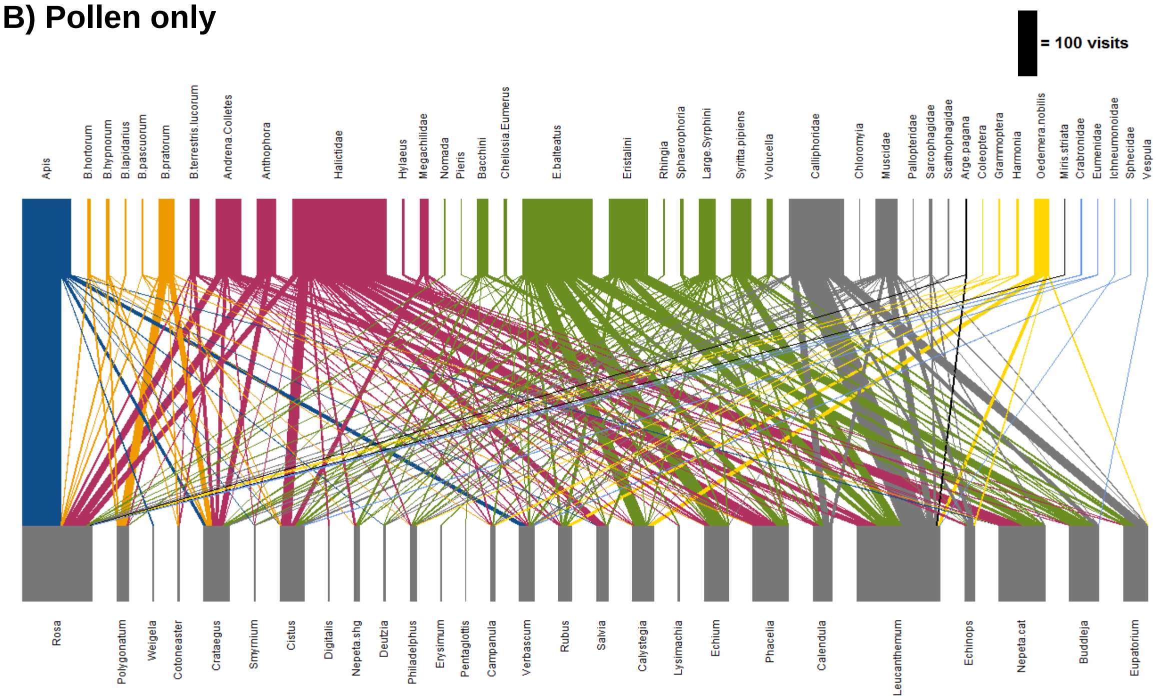


Figure 3.8 The proportion of visits made to flowers to collect a specific floral reward, divided by month and diurnal time period. When possible, the foraging behaviour and type of reward collected by all groups of flower visitors was recorded. At all times, flower visits for nectar were more common than those when pollen alone, or nectar and pollen, were actively collected. Results include all of the visits observed over the course of two summers to 29 plants when foraging behaviour could be noted. For clarity, standard error bars are shown which represent the variation in the proportion of visits made for the different plant species.



A) Nectar only



B) Pollen only

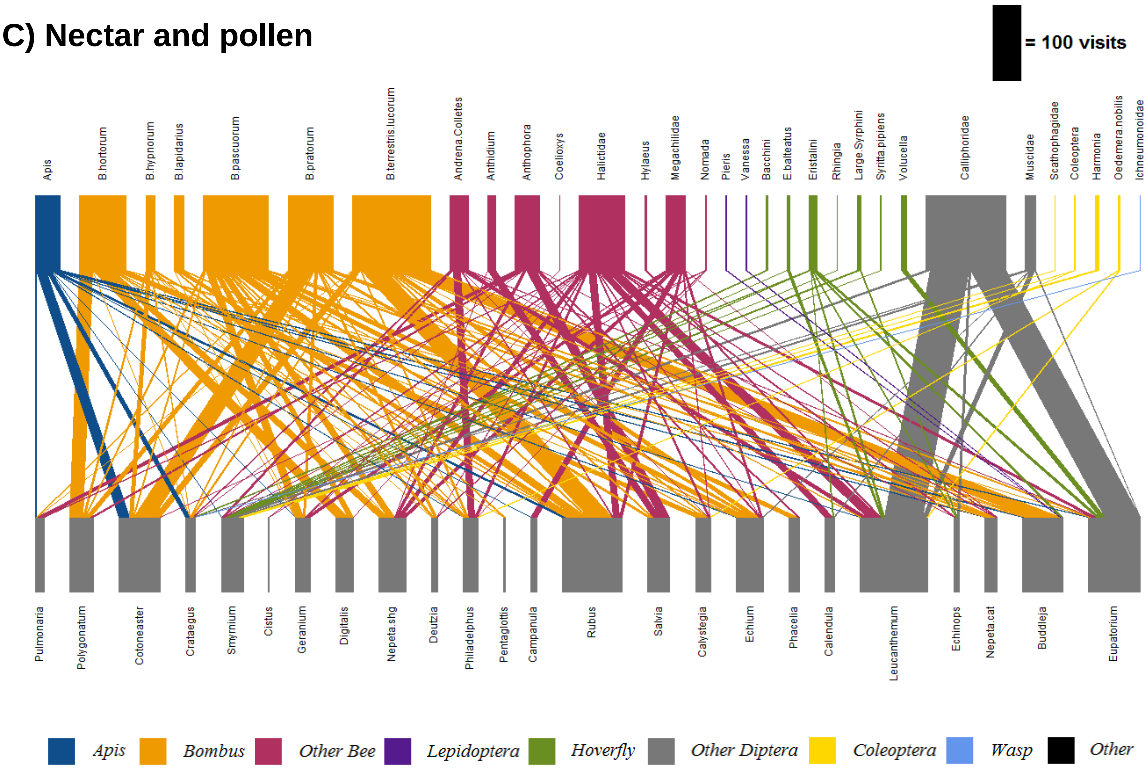

C) Nectar and pollen

Figure 3.9 Three traditional flower visitation networks where visits are divided into the type of floral reward collected: A) Nectar; B) Pollen; C) Nectar and pollen. Bumblebees very rarely visited flowers to only collect pollen, although this behaviour was common for the halictid bees. Despite the absence of specialised pollen carrying structures in Diptera, these visitors frequently visited flowers to feed on pollen grains. Results include flower visits to 29 plant taxa over two summers: A) n=5,302; B) n=2,627; C) n=2,231.

3.3.4 How long do visitors spend on flowers and do they contact the floral reproductive parts?

(i) Duration of single flower visits

The duration of flower visits was compared between bees and non-bees using a *t*-test and between all flower visitors using a 1-way ANOVA with a Tukey test used for multiple pairwise comparisons (Section 3.2.6), although caution is required when interpreting the results as the number of flower visits recorded for each taxon varied. On average, bees spent less time on a single flower (6.88±0.35s, n=1,553) compared to all other flower visitors (20.92±1.43s, *t*=-12.81, df=877.94, *p*<0.001, n=632, **Fig. 3.10**). There was a significant difference in flower visit duration between visitor groups ($F_{(7,2177)}$=86.14, *p*<0.001). Visits from hoverflies (16.02±1.60s, n=320) did not differ in duration to non-syrphid Diptera (16.38±2.30s, n=174, *p*=0.75), however both groups spent significantly longer on flowers than bees (*p*<0.001 with *Apis, Bombus* and other bees) and Lepidoptera (4.87±0.58s, n=61, *p*<0.001). The longest visits were made by Coleoptera, which spent significantly longer on flowers than all other visitors

(80.71±6.51s, n=60, *p*<0.001 in all pair-wise comparisons). Compared to honeybees (6.63±0.62s, n=182), bumble bees spent significantly less time per flower (4.72±0.24s, n=806, *p*<0.001). Solitary bees demonstrated the greatest variation in single flower visit duration (10.04±0.85s, n=565) reflecting the variation of species within this group. The duration of flower visits for individual species and pair-wise comparisons between taxa are given in **Appendix 3.2**.

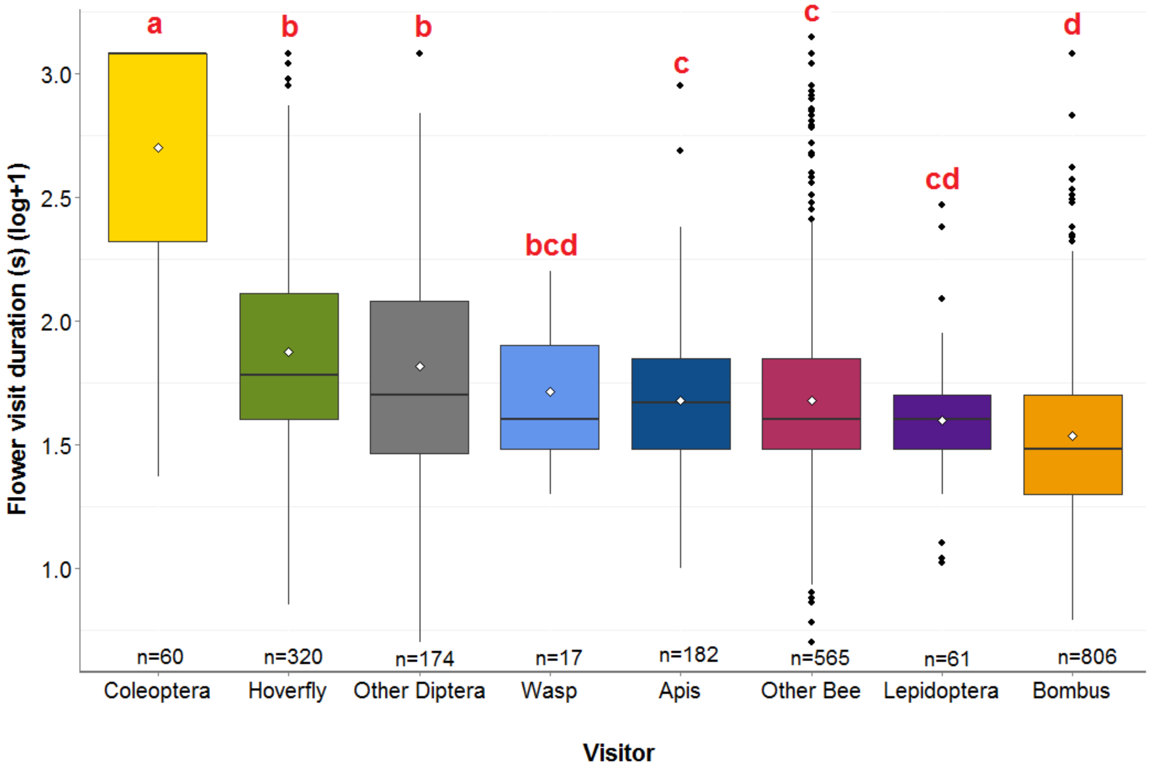


Figure 3.10 Variation between flower visitors in the mean duration of a single flower visit. When possible, the duration (s) of a flower visit was recorded for all groups of flower visitors. To reduce the variation in visit length for statistical testing, all values were log(*x*+1) transformed. On average, Coleoptera spent the longest on individual flowers, while bumblebees quickly moved between flowers. *Apis* spent significantly longer on flowers compared to *Bombus*. Results represent flower visits recorded over two summers to 29 plant taxa.The upper and lower box margins correspond to the first and third quartiles, with whiskers extending to 1.5*inter-quartile range. The mean visit duration is indicated by a white diamond, with shared letters indicating no statistical difference between groups (*p*>0.05).

(ii) Visitor contact with floral reproductive parts

Flower visitors frequently contacted the reproductive parts of the flower whilst foraging; 87% contacted both the anthers and the stigma (n=1,823), 5.8% contacted only the anthers (n=158) and 4.2% only the stigma (n=49). Only 3.0% of all flower visitors made no contact with the floral reproductive parts (n=65). Among the flower visitors, hoverflies and Coleoptera made the greatest proportion of visits that contacted only the anthers (24% and 28% respectively, χ^2=155.10, df=21, p<0.001, **Fig. 3.11**) as they often foraged for pollen without contacting the stigma.

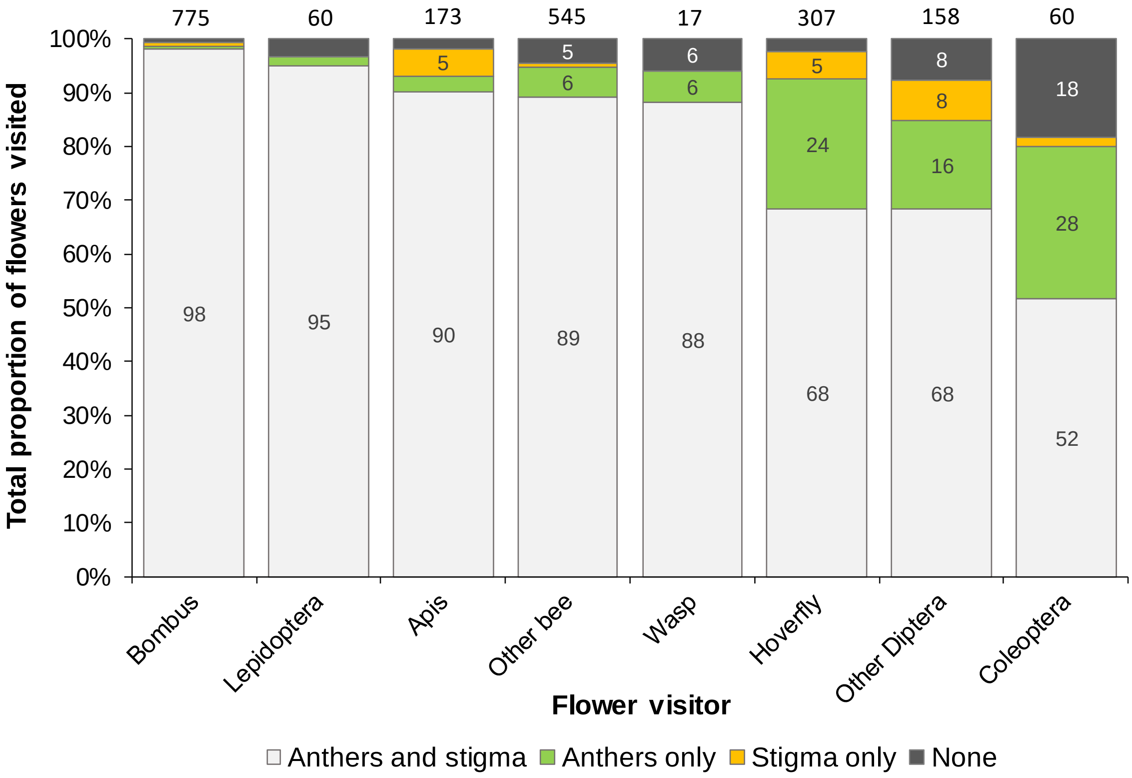


Figure 3.11 The proportion of flower visits resulting in contact between a flower visitor and the floral reproductive structures (anthers and/or stigma), shown for different groups of visitor. When possible, contact was recorded between any part of the visitor's body and the reproductive structures of the flower. In most cases a visitor did contact both the anther and stigma, although this was less common for Coleoptera, who also spent longer on individual flowers (Fig. 3.10). When only the anthers were contacted, this usually represented a visitor feeding on pollen (i.e. hoverflies). Results shown are from visits recorded to 29 plant taxa over two summers. Sample sizes for each group of visitor are given above the bars.

3.4 Discussion

This chapter sought to determine the structure of flower visitation in the garden community, identifying the extent of specialisation between plants and visitors, and temporal differences that are lost when temporal data are pooled. In doing so, differences in the foraging behaviour between flower visitors were also revealed.

3.4.1 A single urban garden supports a diverse flower visitor community

Several studies have shown the diversity of flower visitors supported by urban gardens (Matteson *et al.* 2008, Garbuzov *et al.* 2015, Lowenstein *et al.* 2015) although there is still much to be discovered about their value relative to other habitats (Baldock *et al.* 2015). This study is, to the best of my knowledge, the most detailed account of flower visitation in a single garden to date.

Non-eusocial bees were the most speciose taxa, with *Lasioglossum* particularly frequent flower visitors. These results are consistent with those found in other urban areas (Pardee & Philpott 2014), however the abundance of non-eusocial species on flowers did not exceed that of *Apis* or *Bombus* (as in Theodorou *et al.* 2016). The high abundance of *Bombus* spp. in this study supports previous research suggesting that gardens contribute to the maintenance of bumble bee populations (particularly *B.terrestris/lucorum*, Gunnarsson & Federsel 2014) and can reduce the negative effects of urbanisation (Hülsmann & Leonhardt 2015). It is interesting that *B.hortorum*, a specialist nectar forager (Goulson & Darvill 2004) tends to be less abundant than other *Bombus* species in urban sites (Garbuzov *et al.* 2015, Gunnarsson & Federsel 2014) which raises the possibility that urbanisation has a greater detrimental effect on these populations by including fewer long tubular flowers particularly visited by this long-tongued bee. Future conservation work must focus on increasing the diversity of flowers with deep corollas in urban areas, whose nectar is inaccessible to short-tongued visitors (e.g. *B.terrestris/lucorum* and *Apis*, Comba *et al.* 1999).

A recent review of the literature has reported the negative effect of urbanisation on Lepidoptera (Ramírez-Restrepo & MacGregor-Fors 2016) and in this study butterflies were infrequent flower visitors to all plants, despite the relatively large size of the garden. These results confirm the need for further studies to establish the importance of host plants for lepidopteran visitors in gardens, and the response of these visitors to habitat fragmentation.

The use of static observations in this study allowed the presence of very rare flower visitors (e.g. *Chrysis*) to be observed. While this method is labour intensive and the identification of these unusual visitors in citizen science projects may not be appropriate, the results respond to recent studies calling for all flower visitors to be recorded, to better understand how many insect species utilise floral resources (Wardhaugh 2015).

3.4.2 Flower visitors demonstrate moderate levels of generalisation

Previous studies have observed increasing levels of generalisation in urban compared to rural plant-pollinator communities (Gelsin *et al.* 2013, Baldock *et al.* 2015). In this study, the overall level of specialisation (measured by H_2') was low (0.38), although it fell within the range described by flower visitation networks in other communities (Dormann & Strauss 2014). While this study does not show how urbanisation impacts the generalisation of individual flower visitor taxa (as in Gelsin *et al.* 2013), it has shown that larger flower visitors, with longer tongues, are likely to be the most specialised (in terms of d') flower visitors in the garden. A possible explanation for this may be their ability to travel greater distances whilst foraging (Gathmann & Tscharntke 2002), allowing exploitation of higher quality flower patches in several gardens. These species should be a concern for future work, as recent studies have shown a negative correlation between climate warming and the abundance of flowers with deep corollas (Miller-Struttmann *et al.* 2015).

In all flower visitor networks, it is important to bear in mind that the levels of specialisation change with time and are only relevant to the plants studied; while urban flower visitors may visit a wider range of plant species, this may represent a smaller fraction of the total available plant community (Baldock *et al.* 2015). A high level of generalisation suggests potential for variation in the effectiveness of visitors as pollinators (explored in Chapters 4 and 5).

Little is known about the modularity of flower visitation in gardens, and an interesting finding of this study was that *Apis* was not strongly connected to other species of bee. Although data were not collected to specifically test this, it could be that other bees exploited the floral resources of different plant taxa to avoid competition with *Apis*. However, it could also be true that *Apis* demonstrates adaptive foraging to avoid competition with the most frequent *Bombus* species (Balfour *et al.* 2015) although this was not explicitly tested. Previous studies using species abundance and niche overlap as indicators of interspecific competition have found little evidence of competition between *Bombus* species (Goulson & Darvill 2004). In this study, the most frequent *Bombus* species (collectively *B.terrestris* and *B.lucorum*) were separated from the remaining short-tongued species (*B.hypnorum, B.pratorum* and *B.lapidarius*). As with *Apis*, *B.terrestris/lucorum* could cause competitive exclusion from certain floral resources however the evidence here is not concrete enough to confirm this. The success of *Apis* and *B.terrestris* in urban areas raises important questions for further research, especially as populations of both species are known to be supplemented by commercial nests from which workers may escape (Dafni 2010, Locke 2016). Experimental removal of these visitors could increase the generalisation of other bees (as in Brosi & Briggs 2013).

3.4.3 Temporal variation

A number of studies have shown temporal variation in plant-pollinator networks, and while the identity of species and interactions can fluctuate considerably between years, this has little effect on network parameters such as connectance (Petanidou *et al.* 2008, Dupont *et al.* 2009). In this study, the community of flower visitors remained remarkably similar over two years, with relatively small changes to the structure (including specialisation) of the network. In general, therefore, it seems that pooling two years of data is reasonable for comparisons between visitation, pollen transport and pollinator importance networks for a single site (Chapter 6). However, it is important to recognise that this finding may not apply to the entire plant community, where annual variation over the full flowering season might be greater.

Prior studies have also noted the importance of seasonal (Rasmussen *et al.* 2013) and diurnal variation (Baldock *et al.* 2011) in flower visitation networks. In this study, the estimates of interaction specialisation increased when visits were divided into diurnal and seasonal (monthly) networks. Although specialisation may be overestimated when observations are recorded over shorter time periods (Petanidou *et al.* 2008) and the time periods were not separated by two week gaps (as in Baldock *et al.* 2011), I found higher levels of specialisation early and late in the season, combined with increased specialisation at the start and end of each day. Neither of the previously mentioned studies measured H_2', making it difficult to extrapolate these findings to other communities. However, the higher levels of specialisation reported early and late in the season, plus at the start and end of each day, could reduce heterospecific pollen deposited onto flowers visited at these times. I also found that plant generality was underestimated in the pooled networks, suggesting these larger networks may overestimate their vulnerability to species loss.

3.4.4 Cheats and floral resource collection

Flower visitors which obtain a reward without contacting the floral reproductive parts are assumed to 'cheat' the plant, however recognition of these types of visits is often neglected from network studies. Genini *et al.* (2010) found that high levels of cheating contributed to the modularity of flower visits to the family Bignoniaceae, and suggested that cheats may play a larger role in the nestedness of networks than anticipated. Surprisingly, in this study very few visitors actively 'cheated' or did not contact the floral reproductive parts, suggesting that either most visits were legitimate, or that the real difference between 'cheats' and 'non-cheats' lies in the quantity of pollen deposited (Chapter 5).

In reviewing the literature, it became clear that flowers may be utilised by visitors for several purposes other than the collection of nectar or pollen. In my study, almost all visitors fed on nectar, pollen or both, making the use of active/non-active foraging a poor criterion for distinguishing between effective/non-effective pollinators. It was also clear from the literature that although the foraging preferences of flower visitors determine which interactions are realised, surprisingly few visitation networks differentiate between the collection of nectar and pollen. To the best of my knowledge, this study is the first to create separate resource based networks that revealed a degree of partitioning in the floral resources collected between bees (mostly nectar visits) and Diptera (mostly pollen visits). These results may help to explain how even a small plant community can support a wide range of flower visitors. While Diptera are known to feed on nectar and it is possible that nectar feeding was mistaken for pollen feeding when dipteran visitors fed on the surface nectaries of open plants (e.g. *Rubus* and *Leucanthemum*), care was taken to record pollen foraging only when feeding explicitly focused on the anther.

This study has also demonstrated differences in the collection of floral resources between bee taxa. Interestingly, Halictidae frequently exploited garden plants for the collection of pollen (and nectar to a lesser extent), which may explain why the species found in this group are able to thrive in urban gardens and are not negatively impacted from competitive exclusion by either *Apis* or *Bombus* species. Furthermore, if pollen collection is positively associated with an increase in the effectiveness of flower visitors as pollinators (Ballantyne *et al.* 2015) then these visitors could be particularly important pollinators in garden habitats (Chapter 5). One finding was that *Bombus* rarely exploited flowers just for pollen, in most cases simultaneously collecting nectar. These findings are in line with those of Goulson & Darvill (2004), who suggested that differences in tongue length counterintuitively led to niche partitioning in pollen collection in seven species of *Bombus*. An explanation for the paucity of pollen-only visits remains unclear, although Mayer *et al.* (2012) found that bumble bees abandoned pollen-only collection when plant populations became too small. Future studies comparing the foraging habits of flower visitor taxa in different habitats (e.g. rural and urban) are needed to determine whether these patterns are widespread or unique to this garden.

3.4.5 Visit length and contact with floral reproductive parts

Previous studies of flower visit duration have observed inconsistent results when compared to pollinator effectiveness; visit duration can be both positively (Ivey *et al.* 2003) and negatively (Boyd 2004) associated with pollen deposition. For a community of plants, my study found that bees were much faster visitors, spending significantly less time per flower than other visitors and were more likely to contact the floral reproductive parts. *Bombus* visitors were particularly fast and quicker than *Apis*, supporting previous findings for individual plants (Ivey *et al.* 2003, Balfour *et al.* 2013, Willmer *et al.* 2017). These results are consistent with evidence that bees exploit a variety of electrical (Clarke *et al.* 2013), visual (Muth *et al.* 2016) and scent signals (Roselino *et al.* 2016, Pearce *et al.* 2017) to quickly determine the quality of a flower, and learn how to rapidly manipulate flowers to gather the rewards (Balfour *et al.* 2013, Muth *et al.* 2015). However, faster and more frequent visits may be detrimental to the plant, if a greater proportion of geitonogamous pollen is deposited (Chapter 5).

The handling time of non-bee visitors has received less attention, making it difficult to explain why these visitors (Coleoptera, syrphid and non-syrphid Diptera) spent much longer on flowers. Learning constraints on flower handling by these visitors is a poor explanation of increased visit duration, as they often foraged for pollen which was easily accessible (Goulson & Wright 1998). The most likely explanation is that these visitors typically ate some of the pollen during collection, which is very rarely observed in bees (Nicholls & Hempel de Ibarra 2016). Interestingly, these visitors made a greater proportion of visits that did not contact the stigma, again suggesting a reduction in their values as pollinators (Chapter 5).

3.4.6 Limitations

(i) Sampling a single field site

The most important limitation of this thesis lies in the fact that only a single garden was sampled. While many studies create flower visitation networks at multiple sites (e.g. Kaiser-Bunbury *et al*. 2017) and it would have been ideal to extend data collection to several local gardens, the principal aim of this thesis was to compare a visitation, pollen transport and pollinator importance network for a single community (Chapter 6). This required a substantial sampling effort that would have been impossible for me to perform in several sites simultaneously, although this would be conceivable with a larger team of researchers. Therefore, I recognise that this thesis is not representative of all urban areas (e.g. roadsides, parks, cemeteries) and the results may not be representative of the interactions that occur in all urban gardens; the size of this garden (approximately 2 hectares) was considerably larger than the typical domestic urban garden in the UK (approximately 150m^2, Gaston *et al.* 2005) and only a subset of all flowering plants was sampled (Chapter 2).

However, the plants selected represent those typically found in UK gardens (e.g. Garbuzov & Ratnieks, 2014) and many notable previous studies have also reported results from a single community of plants and insects (e.g. Olesen *et al.* 2008, Petanidou *et al.* 2008, Owen 2010). The findings reported in this thesis should be interpreted with some caution as additional studies are needed to develop a full picture of plant-pollinator interactions in urban gardens, and when I use the term ‘urban’ I refer only to the location of this specific garden rather than results that are true of all gardens in urban areas.

(ii) Taxonomic identification of flower visitors

Flower visitors were identified using a combination of laboratory and field methods similar to those used in recent visitation networks (Baldock *et al.* 2011, Ballantyne *et al.* 2017). This introduces bias in the taxonomic resolution of ‘difficult’ and ‘easy’ visitors (e.g. Halictidae cf. *E.balteatus*) that could overestimate the generalisation of pooled groups, and thus reduces the quality of comparisons between taxa. Furthermore, the collection of some samples for laboratory identification reduces the size of the visitor population for later observation when only a single site is used. While this bias could have been removed by capturing all flower visitors, this was avoided as relatively little is known about the effect of fatal sampling on local populations.

(iii) Sampling a subset of the plant community

All networks are constrained by the decisions and efforts in sampling, and visitation networks that focus on a subset of the plant community should be interpreted with care, as estimates of specialisation are likely to be inflated. For example, the actual foraging niche of *B.hortorum* may not be as specialised as predicted if visitors exploited several plant taxa (with long corollas) in a neighbouring garden; difficulties in gaining access to private areas is a challenge for any study conducted in an urban habitat.

Estimates of specialisation will also be affected by restricting sampling to peak flowering, rather than the entire flowering duration which can span several months for individual plants (e.g. *Calendula*). Baldock *et al.* (2011) suggest that collection over a 2 week period is the minimum required for adequate sampling for each plant; although a longer sampling period would be ideal, unpredictable visitation rates made it impossible to do this whilst recording visitor pollen loads (Chapter 4) and single-visit deposition (Chapter 5) for 29 plants simultaneously. Consequently, the species strength of plants may be underestimated, while specialisation is often overestimated when sampling is restricted to short periods (Petanidou *et al.* 2008). Sampling was also restricted to diurnal flower visitors due to access agreements, eliminating visits from nocturnal moths, which can be up to 95% of the lepidopteran population in anthropogenic areas (Winfree *et al.* 2011). However, with an average of 19.9 hours of observations per plant, the total sampling effort of my study (516 hours) exceeded that of previously published visitation networks (e.g. Bosch *et al.* 2009 107 hours, Tur *et al.* 2013 69.4 hours) and those with less than 3 hours per plant (Philipp *et al.* 2006, Popic *et al.* 2013).

(iv) Pseudo-replication of flower visitors

Stationary observations of flower visitation can be heavily influenced by returning flower visitors, and marking of flower visitors in Chapter 4 revealed that some bees did return to the same flower patch to forage over the course of the day. While this could have biased the number of records of visits made by social bees, dipteran flower visitors have also been shown to demonstrate some degree of floral constancy (Goulson & Wright 1998) and the interactions recorded still represent interactions where visitors gained a reward and where flowers could be pollinated.

(v) Estimating plant species strength

Determining which plant taxa were most important for the visitor community was complicated by patch size (the number of open flowers), which ranged considerably between plants (average 30 in *Calystegia* to 963 in *Buddleja*) and was difficult to quantity for composite species. In this study, recording the diversity of flower visitor taxa was favoured over standardised patch size, although the number of observed flowers was recorded for each plant. From this, a network based on visitation per flower could be calculated, although this is rarely used in other visitation networks.

(vi) Application to flower visitation in other urban gardens

As with all flower visitation networks, the results are applicable only for the community in the time-frame studied. While the plant and flower visitor assembly is similar to that widely reported in UK urban gardens (Owen 2010) the abundance and interactions between species are likely to be very localised.

3.4.7 Directions for further work

In addition to the suggestions already made, further studies of flower visitation networks in urban areas should address the following areas:

(i) Invasive plants

Although the impact of invasive plants on flower visitation networks is well reported (e.g. Lopezaraiza-Mikel *et al.* 2007, Vilà *et al.* 2009, Stout & Casey 2014) relatively little is known about how invasive plant species alter the structure of visitation networks in urban areas; however Emer *et al.* (2015) recently reported that the negative effect of pollen transfer between *Impatiens glandulifera* was limited to a subset of the native plants growing in several habitats around the city of Bristol. Although invasive plant species are often assumed to have a negative effect on native plant communities, flower visitation networks could be used to assess their value to local pollinator populations; of particular interest is the importance of *Buddleja* for urban Lepidoptera, which were very rare in this study.

(ii) Tri-trophic interaction networks

Recently, tri-trophic networks have shown the interactions between plants, flower visitors and the crab spiders that prey upon them (Marrero *et al.* 2013). Tripartite networks could be applied in at least two ways in the garden: (i) tritrophic pollination-frugivory networks, illustrating the link between flower visitors and seed-set in plant taxa whose berries are important for garden bird populations (e.g. *Hedera helix, Pyrocantha, Berberis, Cotoneaster*) and (ii) tritrophic flower visitation-food webs, illustrating the relationship between the zoophagous larvae of flower-visiting syrphids and the predation of garden pests, e.g. aphids, which has applications in biocontrol.

(iii) Focal-visitor based networks

Creating focal-visitor based networks, rather than plant-based networks would improve understanding of the foraging patterns of flower visitors in urban gardens, although logistically this is quite difficult for fast-moving visitors with large foraging ranges. While visitor pollen loads can be used to identify flower visitation history (Chapter 4), these do not show the order in which plant taxa were visited, which has consequences for the value of visitors as pollinators (Chapter 5).

(iv) Scaling up from gardens to the level of urban landscapes

The species present in a single garden are not isolated from the surrounding landscape, and further work is needed to address how fragmented gardens are linked by flower visitation. Goddard *et al.* (2010) reviewed several important questions for future work, and visitation networks are a promising technique for illustrating the movement of visitors between isolated gardens. This is needed to test the true value of these habitats (especially if visitors demonstrate floral constancy) and for understanding pollen movement between urban plant populations. I anticipate that individual gardens do more to support smaller populations of visitors with short foraging ranges (e.g. syrphids) and a landscape level approach is needed to avoid overestimating the value of individual gardens to larger visitors with greater foraging ranges (e.g. *B.hortorum*).

3.5 Conclusion

Flower visitation networks have only recently been applied to gardens, and more are needed to understand these unique habitats. In this chapter, I have shown that a diverse community of insects utilise floral resources in the garden, and a network approach suggests competition between *Apis* and *Bombus*. Lepidoptera were poorly represented in the garden, and recommendations are made for future studies to concentrate on host plants and floral resources for these visitors. Dipteran visitors were also concentrated on particular plant taxa, which increased their specialisation. The study suggests that urban gardens may not be as beneficial for long-tongued flower visitors, and future work should evaluate how the fragmentation of urban gardens disproportionately affects foraging in these species. Specialisation increased when the networks were divided into temporal periods, although the overall effect on the network indices was small, similar to that reported elsewhere. Diurnal patterns in the abundance of flower visitor taxa revealed interesting possibilities that the foraging activity of these ectothermic visitors may be extended in urban areas, and in the first visitation networks to be divided by resource collection, *Bombus* were rarely found to collect only pollen from flowers, while halictids concentrated their visits on this resource.

In this study, visitation patterns to a subset of the community have been recorded, whereas future visitation networks will be very beneficial at the landscape level. However, to fully appreciate the value of the diverse community of visitors to garden plants, a closer inspection of the pollen loads carried by these visitors is required.

Chapter 4.

Flower visitor pollen loads in a garden

Summary

1. Evaluating the pollen loads of flower visitors is the obvious next step in distinguishing potential pollinators from simple visitors, but have only been incorporated into flower visitation networks in a small number of studies, with even less information available for garden visitors.
2. Using non-fatal methods, pollen was sampled from a diverse assembly of flower visitors in a garden, and the number of conspecific and heterospecific grains counted. A pollen load network (PL) was created to compare the proportion of pollen carried by flower visitors, and a pollen transport network (pollen load x visit frequency, PT) to show the total value of visitors as agents of pollen transport.
3. The first analysis of flower visitors' pollen loads from a garden revealed an unexpectedly high success rate in the number of visitors carrying pollen, although *Apis* carried smaller, less diverse loads compared to *Bombus* and non-eusocial bees, which were similar to those of the syrphid Diptera. While each group of flower visitors was collectively generalised, individual visitors were more specialised in terms of the diversity of pollen they carried.
4. Pollen load data substantially increased the number of links in the community, revealing a much larger network of interactions. This decreased the specialisation of the pollen load network (H_2'=0.38) compared to the visitation network (H_2'=0.47), although interestingly the specialisation of the pollen transport network increased (H_2'=0.55). Overall, flower visitation frequency explained only 18% of the variation in pollen loads, but was a much better predictor of pollen transport.
5. As the first pollen transport network in a garden, this study confirms that the inclusion of pollen loads increases community specialisation, and has implications for understanding how pollen is transported in urban flower visitor communities.

4.1 Introduction

In the previous chapter, the generalisation of the garden community suggested that flower visitors frequently exploit several plant taxa for the collection of floral rewards. This has been suggested to decrease their value as pollinators, as they may transfer higher quantities of heterospecific pollen between plants (Arceo-Gómez & Ashman, 2011). In this chapter, I measure the quantity and quality (diversity) of pollen from the bodies of flower visitors. These results reveal additional information about (i) flower visitor diet, and (ii) the potential value of these visitors as pollinators.

4.1.1 Visitor pollen loads provide a hidden history of flower visitation

Palynology (the study of pollen grains) is an important aspect of flower visitor interactions that surprisingly is often neglected from pollination studies. Quantitative analysis of pollen is of interest from both the plant and flower visitor perspective, because (i) it is the male gamete and its dispersal reflects male fitness, and (ii) it is offered as a protein- and lipid-rich reward to flower visitors. Either by active or passive collection, pollen grains adhere to the surface of flower visitors (particularly those that are very hairy) and may then be carried in the specialised structures present in bees (the corbicula, scopa or crop) and can remain on the body for several days (Courtney *et al.* 1982). From the perspective of flower visitors, pollen loads have been used to compare patterns in floral resource between species and over time (Kleijn & Raemakers 2008, Scheper *et al.* 2014, da Silva *et al.* 2017), the impact of introduced exotic plants (Lopezaraiza-Mikel *et al.* 2007, MacIvor *et al.* 2014) and in modelling flower visitor foraging behaviour (Marchand *et al.* 2015). Importantly, a palynological approach improves the resolution of flower visits in studies where observations are made to focal plants only, thereby increasing understanding of the importance of certain plant species (Bosch *et al.* 2009).

4.1.2 Pollen loads as a proxy for pollination

Previous studies have used the presence of conspecific pollen on a flower visitor as an indirect measure of pollination (e.g. Forup & Memmott 2005, Gibson *et al.* 2006), with the implicit assumption that greater pollen loads lead to more effective pollination. From the plant’s perspective, the size and heterogeneity of visitor pollen loads have been used to compare taxa in terms of their floral fidelity (Wilson *et al.* 2010, Rossi *et al.* 2015), the viability of the pollen carried (Rader *et al.* 2011) and, most importantly, to distinguish antagonistic from mutualistic visitors; for example Alarcón (2010) showed that many flower visitors carried no pollen, and were therefore deemed to be ‘cheaters’. Recently, pollen loads have been incorporated into flower visitation networks, so that the interactions represent total pollen transport (usually, a measure of pollen load x visit frequency). These studies have reported differences in the structure of pollen transport compared to visitation networks from communities in Mediterranean habitats (Bosch *et al.* 2009, Alarcón 2010), Arctic heathland (Olesen *et al.* 2011), arid grassland (Popic *et al.* 2013) and cloud forest (Ramírez-Burbano *et al.* 2017). Yet with the exception of Jędrzejewska-Szmek & Zych (2013) little is known about how pollen transport networks are structured in urban areas. Analysing the heterogeneity of pollen loads from the garden will shed light on the foraging preferences of visitors in communities where usually a large proportion of plants are exotics (Salisbury *et al.* 2015).

While pollen transport networks have become established as a more accurate measure of the value of flower visitors as pollinators, the effect on network specialisation has not been consistent between communities. In Alarcón (2010) and Popic *et al.* (2013), H_2' values increased by up to 94% in the pollen transport network, while Bosch *et al.* (2009) and Ramírez-Burbano *et al.* (2017) reported a decline in specialisation. To fully understand the factors driving specialisation of plant-pollinator interactions, more pollen transport networks are needed in different habitats and across varied spatial and temporal scales. In light of global pollinator declines, it is important to understand whether the presence of pollen on the bodies of many flower visitor taxa represents functional redundancy or complementarity in terms of their role as pollinators. Furthermore, determining why the proportion of flower visitors that carry pollen varies between habitats will be a key aspect of future conservation efforts.

Using pollen transport as a proxy for pollination is not without limitations, and assuming that larger pollen loads equate to more effective pollination is problematic for two reasons. Firstly, not all of the pollen on an insect body will make it to the stigma as some is lost to the environment, groomed into specialised pollen carrying structures or used to provision nests between flower visits. Adler & Irwin (2006) found the quantity of *Gelsemium sempervirens* pollen on visitor bodies to be a poor predictor of that transferred to the stigma, while Larsson (2005) estimated that only 0.10% of all pollen removed from *Knautia arvensis* flowers was subsequently deposited on to stigmas, as many of the pollen-collecting solitary bees avoided flowers in the stigmatic phase. Secondly, some visitors may remove and transfer pollen at a high cost to the plant, so that their overall effect on plant fitness is negative when flower visitors with smaller pollen loads are present (Thomson & Goodell 2001, Lau & Galloway 2004).

4.1.3 Downscaling from species to individuals in pollen load networks

In all networks, it is important to remember that each flower visitor node represents many individuals, with their own foraging preferences and behaviours. By recording visits made by individual *Apis* to *Cirsium* flower heads, Dupont *et al.* (2011) revealed that a small number of ‘scout’ bees visited many thistles, yet the majority of individual workers specialised on a much smaller number of flowers. Using a similar approach, Tur *et al.* (2013) analysed individual pollen loads from a diverse community of flower visitor taxa, and found downscaling substantially increased specialisation as many generalist species were composed of specialist individuals. What is not yet clear is whether this pattern holds true in gardens that are characterised by high and patchy floral diversity; to date, very little evidence has considered how planting in gardens should be shaped by the floral fidelity and opportunistic exploitation of floral resources by flower visitors. Although many plant varieties are recommended as ‘pollinator friendly’, it is unclear whether planting a larger diversity of plants in smaller patches is more beneficial than a reduced diversity in larger patches, and how this benefits different flower visitor taxa. From the plant’s perspective, high levels of individual specialisation are also likely to reduce heterospecific pollen transfer between flowers (Arceo-Gómez & Ashman 2011).

4.1.4 Flower visitor life history influences the quantity and quality of pollen loads

Using pollen load as a proxy for pollination implies that visitors carrying greater loads will be more effective pollinators. However, flower visitor life history is an important aspect of the quantity and quality of pollen carried, and shapes the structure of interactions in a network (Jordano *et al.* 2016); although many flower visitors feed on pollen, bees are the only insects that are entirely dependent on pollen as a source of protein for developing larvae (Thorp 2000). Consequently, most bees (>70% of species) are polylectic, actively collecting several species of pollen, and have evolved specialised structures for carrying pollen (Michener 2007). Pollen is usually collected on a pollen brush, located on the rear legs (e.g. *Apis*, *Bombus* and Halictidae) or beneath the abdomen (e.g. Megachilidae). *Andrena* species also collect pollen on the sides of the propodeum, while *Hylaeus* store pollen internally in their crops (Falk 2015). Although most bees collect dry pollen, *Apis* and *Bombus* can regurgitate nectar to moisten pollen so that it is stickier and easier to carry (Falk 2015). All these behaviours can greatly reduce the quantity of pollen reaching the stigma (Parker *et al.* 2015).

In comparison to bees, Diptera do not actively collect pollen for brood provision, however many families do have hairs that trap pollen on their bodies, e.g. hoverflies in the tribe Eristalini (Ball & Morris 2013) and the non-syrphid Muscidae (Orford *et al.* 2015). Lepidopteran visitors are also covered in a fine brush of scales hairs, however very little pollen is normally found on the body (although see Epps *et al.* 2015) adhering instead to the proboscis and face (Courtney *et al.* 1982). Therefore, while the pollen loads of non-bee visitors may be small in comparison, these visitors could carry larger 'free' pollen loads that increase pollen deposition on to the stigma. However, the frequency of grooming and of flower visitation may be limiting factors.

4.1.5 Key questions

In this chapter I use pollen load and transport networks to examine the variation between flower visitors in the quantity and diversity of pollen carried. Evaluating pollination from the perspective of the male function of flowers, I ask:

1. How diverse are flower visitor pollen loads in a garden and does this vary temporally?
2. Are pollen load and pollen transport networks more specialised than visitation networks?
3. Are flower visitor species less specialised in pollen transport than individuals of that species?
4. Is flower visitation frequency a good predictor of total pollen load and transport?
5. Overall, which flower visitors are the most important in terms of pollen load and transport?

4.2 Methods

To compare the pollen loads of flower visitors, a total of 1,003 individuals were sampled from 39 flower visitor taxa (representing 1,155,205 pollen grains). These included visitors from all of the major groups of flower visitors (*Apis, Bombus,* non-eusocial bees, Diptera, Coleoptera and wasps) although 72.4% of pollen loads were collected from bees (n=726). Comparisons between the visitation and pollen load/transport networks were made using networks that included only the interactions for which pollen load data were also collected.

4.2.1 Non-fatal removal of pollen loads

Pollen loads were collected from flower visitors as they entered a focal plant patch and began to forage. No *a priori* decisions were made about which flower visitor taxa were pollinators, so sampling attempted to maximise the diversity of visitors. Visitors were caught whilst on the flower in glass vials, then sealed with a foam plug. As in Adler & Irwin (2006), the number of flowers previously visited by an insect was not controlled for, so that the pollen loads represented that which was naturally available for pollen transfer. Individuals were then exposed to a small dose of carbon dioxide for approximately 30 seconds, which allowed pollen to be sampled from the body while the insect was briefly anaesthetised. Pollen was swabbed from the body (avoiding the corbiculae of *Apis* and *Bombus*) using a $3mm^3$ square of fuchsin gel mounted on a dissection needle for 1 minute, which was then melted onto a clean slide and sealed with a coverslip (Kearns & Inouye 1993). This provided a permanent record of conspecific and heterospecific pollen. For a subset of all individuals (n=721), body size was measured as the inter-tegular distance (mm) using digital calipers (Johnson, 0-150mm). Contamination of pollen samples was minimised by cleaning all equipment with ethanol between use. Although others have captured insects and washed all pollen from the body (e.g. Tur *et al.* 2013) this method was avoided as it was unclear how daily fatal sampling would affect the local visitor population. Also the collection of pollen from the surface of the body is more representative of that transferred to the stigma during a brief flower visit.

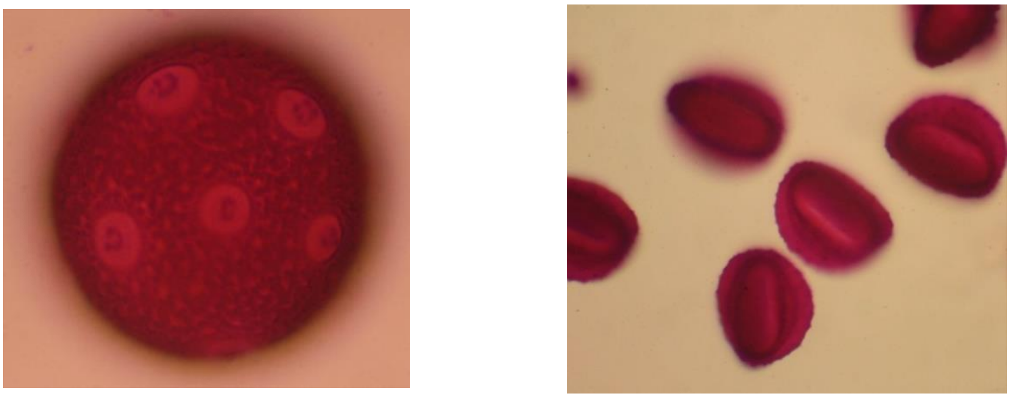
Given that a few studies have suggested that exposure to high carbon dioxide concentrations can negatively affect the behaviour and lifespan of bees (e.g. Czekońska 2009), a large proportion of captured individuals were marked using a queen marking kit or non-toxic paint. Marked individuals (mostly Hymenoptera) frequently returned to the garden to forage, suggesting that lifespan was not affected and no abnormal behaviour was observed in foraging behaviour, although no control group was used to confirm this. These findings suggest that brief exposure to carbon dioxide may be a reliable, non-fatal method for removing pollen loads in the field and may not not disrupt normal behaviour patterns.

4.2.2 Identification of conspecific and heterospecific pollen grains

All pollen slides were identified by a number, rather than visitor species, so that all grains were counted ‘blind’ to avoid visitor bias. Each slide was examined at x100-400 magnification using a light microscope, and all grains counted and identified as either conspecific, or belonging to a heterospecific pollen type. Palynological classification was based on the type and number of apertures, surface ornamentation and size (**Fig. 4.1**). A reference collection of all focal plant pollen and the remaining species in the garden was created, to which most pollen types could be identified to. ‘Alien’ pollen types that did not match any of the garden plants were classified by morphological characteristics. Pollen grains belonging to wind-pollinated species (e.g. *Pinus* and Poaceae) were excluded from counts, although they were very rarely found on slides. Pollen hydration status was not used to exclude grains from counts, as dehydrated grains may rehydrate once on the stigma surface (Edlund *et al.* 2004). The presence of pollen on the body of a visitor was considered to be an interaction, regardless of whether this grain was collected from a direct visit, or indirectly if heterospecific grains had been deposited on a floral surface by another visitor.

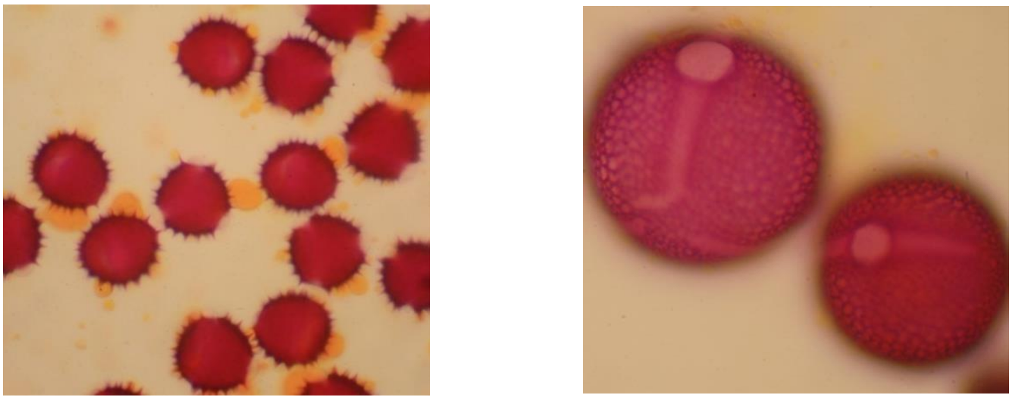
***Polygonatum*, 50µm**

***Nepeta* 'Six Hills Giant', 37.5µm**



***Calystegia*, 75µm**

***Echinops*, 60µm**



***Calendula*, 35µm**

***Cistus*, 50µm**

Figure 4.1 Pollen grains stained using fuchsin gel, viewed under x400 magnification with a light microscope. A reference collection of pollen belonging to all of the plants in the garden was created, with fuchsin gel proving to be a reliable method of identifying between grains based on their size, shape and surface morphology. Values indicate the mean length of *Polygonatum* and the mean diameter of all other pollen grains.

4.2.3 Construction of pollen load and pollen transport networks

Pollen loads were used to create two networks following the methods of Alarcón (2010) and Popic *et al.* (2013): (i) a pollen load network (PL), where interaction strength represented the mean number of pollen grains belonging to a plant species on individuals of a visitor species, and (ii) a pollen transport network (PT), where interactions in the PL network were multiplied by the number of visits between a particular insect and plant species as observed in the visitation network (Chapter 3). Following Popic *et al.* (2013) if a flower visitor carried pollen belonging to a plant species it was never seen to visit, these interactions were assigned a visit frequency of '1' in the pollen transport network.

As the number and size of pollen grains varied between plant species, all pollen load and transport networks were made proportional; that is, the value of the interaction between a particular insect and plant species is calculated as a proportional value (0-1) based on the value of the interactions of all other visitors to that plant (Section 2.2.8). In this way, the networks were not biased by variable production of pollen. Values in the proportional matrix had to be multiplied by 1,000 before calculating species specialisation (*d'*), as the function *dfun* cannot compute values smaller than 1. Two separate sets of networks were created, based on the data including (i) all pollen types recovered (including 'alien' species) or (ii) only the pollen from focal plants. Four plant species included in the visitation network in Chapter 3 were excluded from this analysis, due to high variation in the pollen produced by individual flowers (*Weigela* and *Erysimum*, both from a garden origin) or when insufficient pollen loads were collected (*Verbascum* and *Lysimachia*).

4.2.4 Statistical analysis

(i) Comparisons of pollen load size and diversity

The total number of pollen grains and species per pollen load were compared between flower visitor groups and diurnal time periods using a generalised linear model (GLM, negative binomial distribution and log link function) in the package *MASS* in *R* (Venables & Ripley 2002). Model validation was based on a parametric distribution of deviance residuals, heterogeneity in the spread of Pearson vs. Fitted Residuals and a theta overdispersion value of less than 1.5 (Thomas *et al.* 2013). Pairwise post-hoc Tukey HSD tests using the *multcomp* package and function *glht* (Hothorn *et al.* 2008) determined differences between flower visitors, months and diurnal periods. The overall significance of the model was determined using the function *anova(glm, test="Chisq")* with the model deviance reported as χ^2.

(ii) Flower visitor body size and pollen load size and diversity

Flower visitor body size was measured as the inter-tegular distance (mm) for a subset of the visitor community (n=721). A Spearman Rank correlation was used to test the relationship between body size and (i) pollen grains per pollen load (log transformed prior to testing) and (ii) pollen species per pollen load.

(iii) Temporal patterns in pollen load size and diversity

A GLM (negative binomial distribution, log link function) was used to determine whether the number of grains per pollen load, and number of species per pollen load, varied between (i) seasonal time period and (ii) diurnal time period. The overall significance of the model was determined using the function *anova(glm, test="Chisq")* with the model deviance reported as χ^2.

(iv) Comparisons between plant species *d'* and flower visitor species strength

Plant species specialisation (*d'*) values were calculated for each plant in each of the three networks. These values were then compared between the networks using a generalised linear mixed model (GLMM, with a Gaussian error distribution) using the function *glmer* in the package *lme4* (Bates *et al.* 2015) with plant species as a random effect. GLM analysis was selected as a normal distribution of the residuals could not be achieved using an ANOVA, and GLMM analysis was used to incorporate repeated measures from the same plants. As proportions (0-1), all *d'* values were arcsine square-root transformed before testing. Pairwise comparisons between networks were made using a post-hoc Tukey HSD using the *glht* function in the package *multcomp* (Hothorn *et al.* 2008).

Similarly, the species strengths of all flower visitors were compared between the networks using a GLMM (with a gamma error distribution) including visitor species as a random effect. Species strength values were x+1 transformed prior to testing to achieve a better model fit (e.g. the range of values in the PT network was transformed from 0.0003-3.9168 to 1.0003-4.9168). Pairwise comparisons between the networks were again made using a post-hoc Tukey HSD. For both species *d'* and strength, the models were validated by inspection of the distribution of the deviance residuals (for a parametric distribution) and the absence of any pattern in the fit between the Pearson and fitted residuals (Thomas *et al.* 2013).

(v) Correlations between flower visit frequency and pollen load/transport

Linear models were used to determine the strength of the relationship between visitation and measures of PL and PT. Firstly, the value of each visitor to the focal plants were calculated, as a proportional value relative to all other visitors to the same plant in the network (as in Vázquez 2005 and Alarcón 2010, see Section 4.2.3). As proportions (range from 0-1) all values were arcsine square-root transformed before testing. Using these values, correlations were performed between the value of a visitor to a plant in one network to its value in another (e.g. visitation compared to pollen load). A Spearman’s rank correlation (r_s) was used, as the data for each group did not fit a normal distribution.

As linear models assume independence of the data, and this is violated by the shared life histories within visitor groups (Stone *et al.* 2011), the relationships were tested for differences between groups using visitor group as an interaction effect. The significance of the interaction effect is reported from an ANOVA performed on the model, using the *R* function *anova(model, test=”F”)*.

4.3 Results

Almost all flower visitors had pollen on their bodies (96.1%, n=963) with the exception of 40 individuals. Flower visitors where pollen load data were not available (n=14 taxa) were excluded from the visitation network presented in Chapter 3, therefore the interactions are identical in both the visitation and pollen load/transport networks. This section combines plant-centred observations and visitor-centred pollen loads, to consider: (i) the diversity of pollen on flower visitors in a garden, (ii) the structure of pollen load (PL) and pollen transport networks (PT) compared to a visitation network, and (iii) which flower visitors made the greatest contribution in PL and PT at the community level. As interactions are weighted by the quantity of pollen carried, the networks are interpreted from the perspective of plant male fitness.

4.3.1 How diverse are the pollen loads of flower visitors in a garden?

On average, the number of pollen species per pollen load was relatively low (2.87±0.07, n=1,003) with over a quarter of visitors carrying pollen loads that were monospecific (29.1%, n=292). 46.3% of visitors carried between 2-4 pollen types (n=464) and 20.6% carried more than 5 pollen types (n=207, **Fig. 4.2**). For mixed pollen loads, the quantity of heterospecific pollen was very variable: it accounted for between 1 and 7,364 grains (average 425, n=671) which was between 0.02-100% of the total pollen load (average 42.6%, n=671). However, in over 70% of pollen loads (n=686) at least half of the total pollen was conspecific to the plant on which the visitor was foraging.

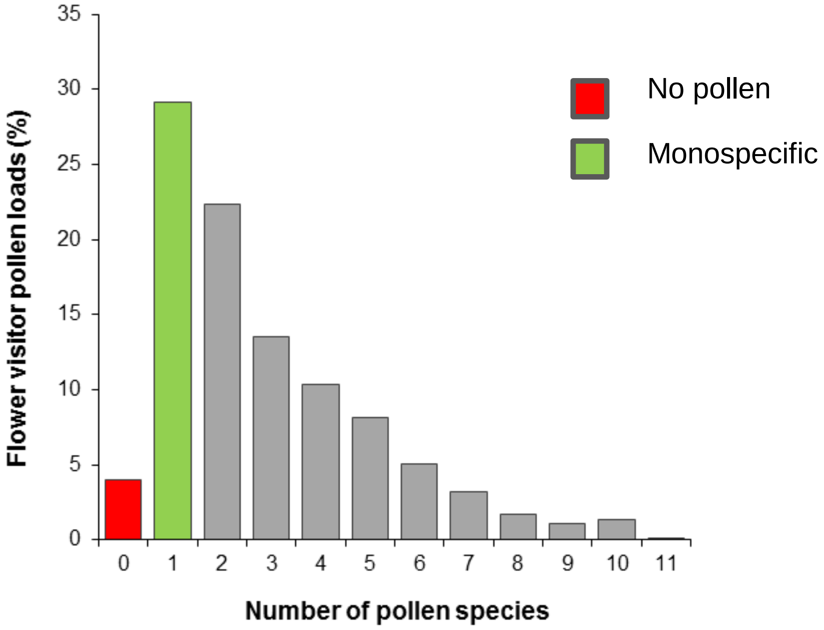


Figure 4.2 The frequency of monospecific pollen loads compared to those containing several species, as a proportion of all those collected. Pollen grains were classified as belonging to different species depending on their size, shape and surface morphology. The greatest proportion of pollen loads contained only a single species, with those that carried more than 4 species very rare. It was exceptionally rare for a flower visitor not to carry any pollen when they visited a flower. Results shown are based on 1,003 pollen loads collected over two summers from all groups of flower visitors.

(i) Variation between flower visitor pollen loads

The quantity of pollen recovered from flower visitor bodies varied significantly between flower visitor groups (GLM pollen load~visitor, df=7, χ^2=113.27 p<0.001). The largest pollen loads were recovered from non-eusocial bees (1,496.04±97.08 grains, n=318) and from *Bombus* (1,383.63±92.4, n=320), with several of these visitors carrying in excess of 5,000 pollen grains (**Fig. 4.3a**). Significantly less pollen was found on the bodies of *Apis* compared to the non-eusocial bees (906.88±97.17, p=0.03, n=88). On occasion, hoverflies carried large pollen loads (>2,000 pollen grains) but on average they carried significantly less pollen (735.95±85.07, n=146) compared to the non-eusocial bees (p=0.001) and *Bombus* (p=0.001). There was no difference between the number of grains carried by *Apis* and hoverflies (p=0.93). Non-syrphid Diptera (302.9±54.97, n=79) and Coleoptera (343.21±109.71, n=29) carried significantly less pollen than any of the bees (p<0.05, in all cases). However, these results do not show what proportion of the pollen load was conspecific.

The number of pollen species recovered from flower visitors varied significantly between groups (GLM pollen species~group, df=7, χ^2=125.69, p<0.001). The most diverse pollen loads were carried by *Bombus* (3.51±0.13 species, n=320) and non-eusocial bees (3.12±0.12, n=318, **Fig. 4.3b**). *Apis* carried less diverse loads compared to *Bombus* (2.66±0.27, p=0.02, n=88). Hoverflies carried a similar diversity of pollen compared to *Apis* (2.27±0.14, p=0.69, n=146), while non-syrphid Diptera (1.65±0.14, n=79) and Coleoptera (1.31±0.13, n=29) carried less diverse pollen loads compared to all bees (p<0.01). Small sample sizes for the less-common flower visitors (Lepidoptera and wasps) made it difficult to detect any differences between these and other taxa. Full pair-wise comparisons between taxa are given in **Appendix 4.1**.

(ii) Body size and pollen load

Previous studies have found the body size of visitors to be related to the total pollen load, although the largest loads can sometimes be carried by small (Pearce *et al.* 2012) or intermediate visitors (O'Neill & O'Neill 2011). Flower visitors to the garden varied considerably in size as measured by thorax width (0.4-10.0mm, average 3.04mm, n=721). The body sizes of all flower visitors are given in **Appendix 4.2**. A Spearman Rank correlation detected a significant relationship between body size and pollen load size (r_s=0.24, p<0.001) and pollen load diversity (r_s=0.29, p<0.001, **Fig. 4.4A and B**) although close inspection of **Figure 4.4** reveals a considerable scatter of size against either measure. The results do not show how pollen loads varied within groups of flower visitors (e.g. the smaller bodied halictids) and the size of the pollen grains is also likely to determine pollen load (grain size varied between 15-125μm). The exclusion of flower visitors that did not carry any pollen (n=29) did not alter either result. No significant relationships were found between body size and pollen load size/diversity for *Apis*, *Bombus* or the non-eusocial bees separately.

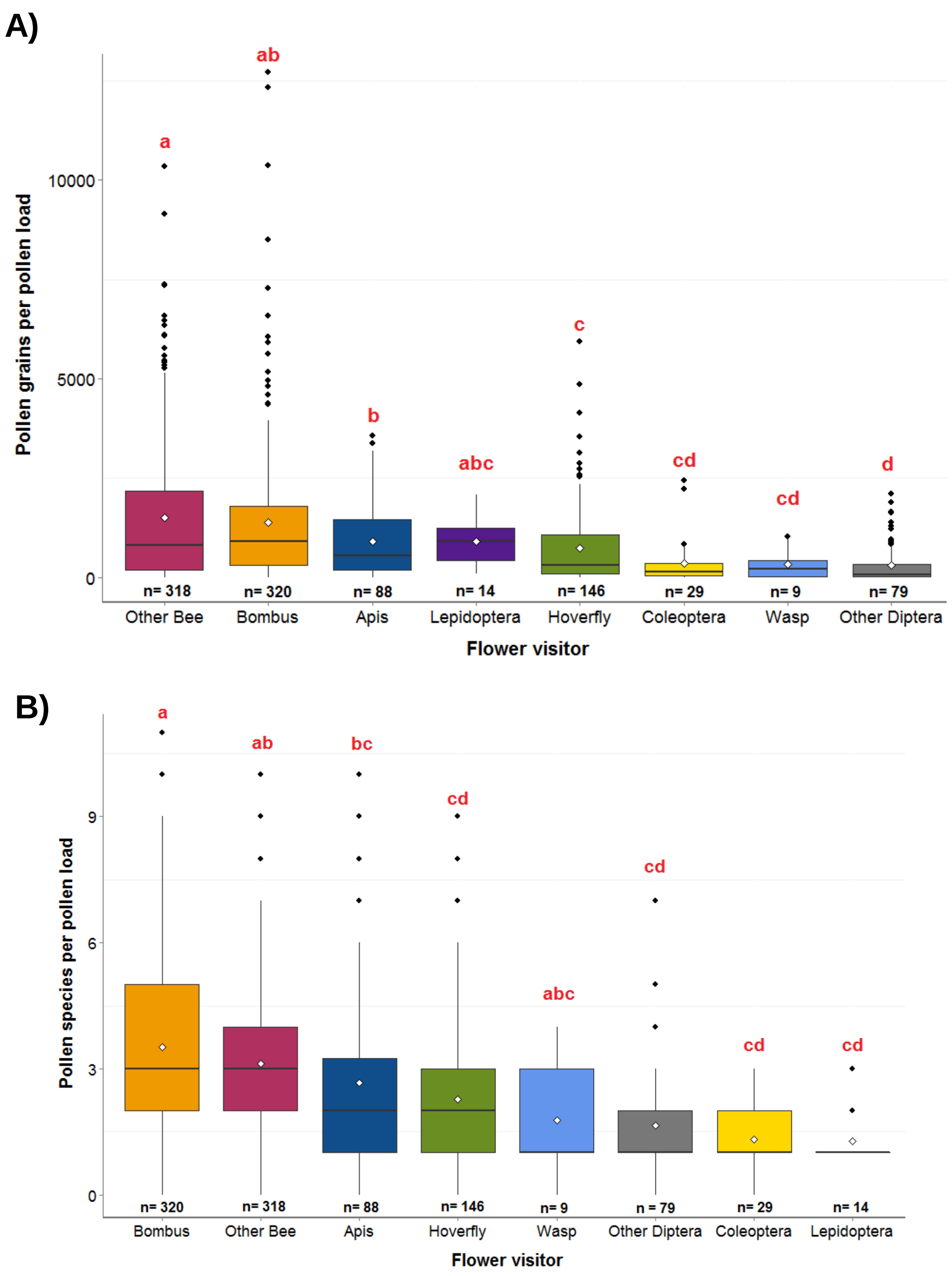


Figure 4.3 Mean numbers of pollen grains and pollen species in the pollen loads recovered from the bodies of several groups of flower visiting insects. All pollen grains recovered from the bodies of visitors using fuchsin gel were counted and grouped into species according to their morphology. Bees carried the highest pollen loads of any visitor, with those from other bees and *Bombus* higher on average than those from *Apis* (ns). However, bumblebees and other bees also carried the most diverse loads, with a mean of more than three species per individual. Shared letters indicate no significant difference between groups, which are organised according to decreasing mean (illustrated by the white diamond). Results shown include all of the pollen loads collected over the course of two summers (n=1,003) from visitors to 29 plant species.

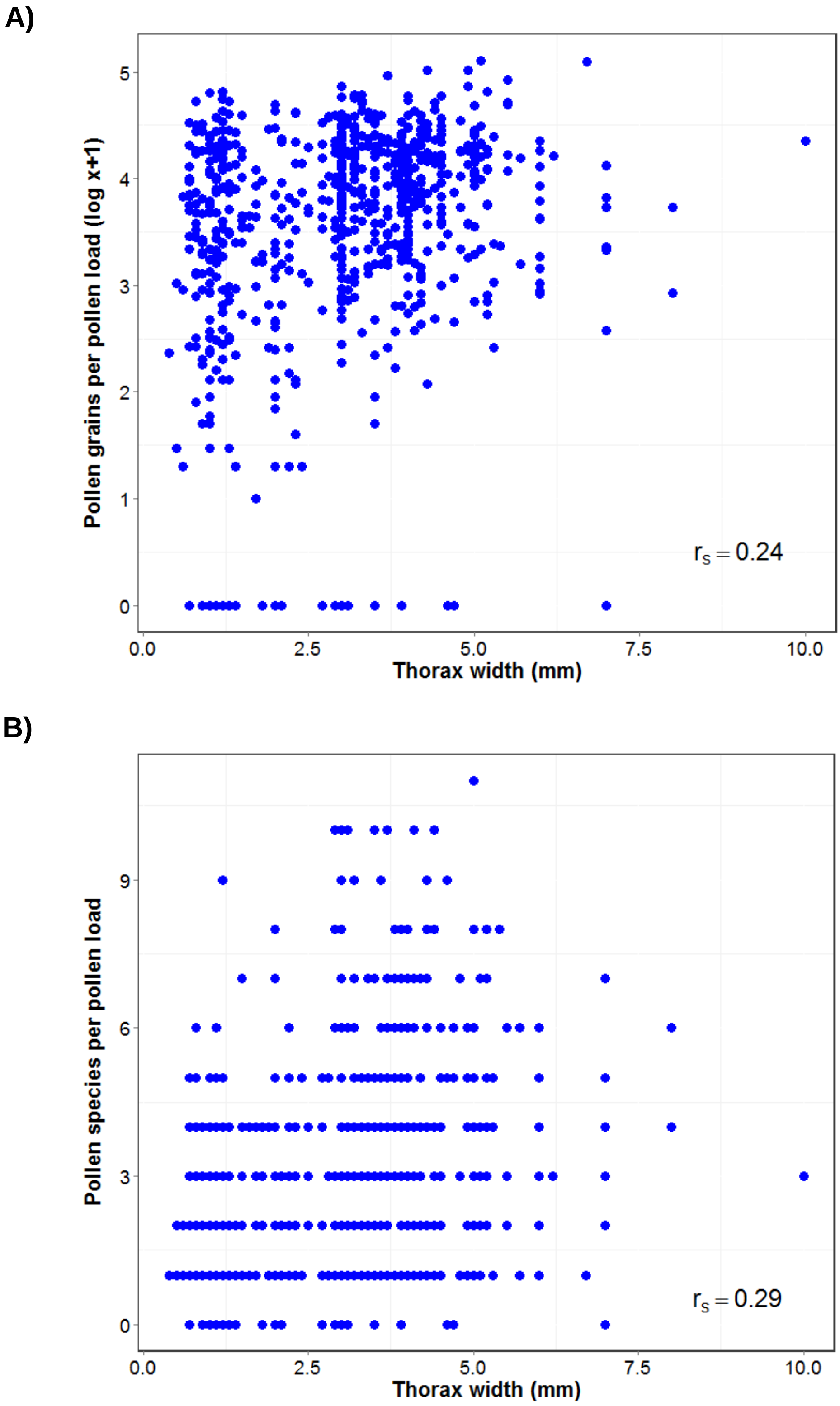


Figure 4.4 The relationship between the size of a flower visitor and the number of pollen grains and pollen species carried. The size of flower visitors was measured as the inter-tegular distance (mm) and all of the pollen grains sampled from the body counted and grouped into species according to morphology. Although Spearman Rank correlations detected a significant relationship between size and both measures of pollen loads, considerable spread existed in the data, making the overall trend difficult to identify. Results shown include all of the pollen loads collected from all groups of flower visitors to 29 plants over the course of two summers (n=721).

(iii) Temporal variation

The mean quantity of pollen carried by all visitors did not vary throughout the summer (GLM pollen load~seasonal period, df=3, χ^2=5.45, *p*=0.14), although the diversity of pollen loads did vary (GLM pollen load~seasonal period, df=3, χ^2=22.95, *p*<0.001). Pollen loads were more diverse in June (3.22±0.13 species, n=343) compared to July (2.71±0.11, *p*=0.01, n=366) and August (2.29±0.15, *p*<0.001, n=128). These results should be interpreted with caution, as the differences may reflect variations in the proportion of taxa sampled.

The size of pollen loads also varied throughout the day (GLM pollen load~time, df=3, χ^2=8.41, *p*=0.04); flower visitors carried less pollen early in the morning (07.30-09.00: 789.89±160.99 grains, n=72) compared to late in the afternoon (15.00-18.00: 1,325.80±112.27, *p*=0.03, n=201) which could relate to patterns of pollen and nectar collection, and to patterns of dehiscence, which may be weather-related (Willmer 2011). The diversity of pollen species did not vary throughout the day (GLM pollen load~time, df=3, χ^2=1.48, *p*=0.69). However, these results should be interpreted with caution as the total number of pollen loads collected varied between each temporal period. Full results of all GLMs are given in **Appendix 4.3**.

4.3.2 Pollen load networks in a garden

(i) Pooled network

Flower visitors in the garden were able to access plants outside the study area, and as a result 116 ‘alien’ (non-focal) pollen types were recovered from flower visitor pollen loads (n=1,003), although 81.4% of all the pollen belonged to the 29 focal plants. The additional pollen species revealed a much larger interaction network compared to the observed visits to the focal plants, and in these networks the number of plants outweighed the number of visitors (unlike Chapter 3). The identities of the ‘alien’ grains are given in **Appendix 4.4**. When all pollen loads were pooled, ignoring annual and seasonal variation (Chapter 3) a total of 869 links were observed between the 39 visitors and 141 plant taxa, substantially increasing the size of the focal plant visitation network (252 links, **Fig. 4.5, Table 4.1**). The additional 617 links more than doubled the generality of flower visitors (V=7.72, PL=16.05) and the generality of the focal plants also increased slightly (V=5.75, PL=7.67). Interactions in the pollen load network were more generalised (H_2' V=0.42, PL=0.37) and less modular (V=0.43, PL=0.22). Other network metrics remained similar: nested (NODF V=29.23, PL=28.90), connectance (V=0.11, PL=0.07) and interaction evenness (V=0.69, PL=0.65). In both the visitation and pollen load networks, halictid flower visitors had the greatest number of plant partners (V=21, PL=61).

Over the entire season, *Rubus* received the highest number of insect partners (n=17) although the pollen from *Taraxacum* (a non-focal plant) was found on the greatest diversity of flower visitors (n=29).

		Pollen Load	Visitation
	Pollen loads or visits (n)	1,003	13,262
Species richness	**Visitors**	39	39
	Plants	141	25
	Links	869	252
Generality	**Visitors**	16.05	7.72
	Plants	7.67	5.75
Distribution of interactions	**C**	0.07	0.11
	IE	0.65	0.69
	NODF	28.9	29.23
	H_2'	0.37	0.42
	M (n)	0.22 (6)	0.43 (7)

Table 4.1 Summary of the community level network indices for traditional visitation and pollen load networks. Pollen loads were collected from all groups of flower visitors and included ‘alien’ pollen grains belonging to plant species not included in the 29 focal plants. The revelation of many hidden links substantially increased the size of the pollen load network. An increase in link number reduced the specialisation of the interactions in the traditional visitation network. Results are representative of data collected over two summers.

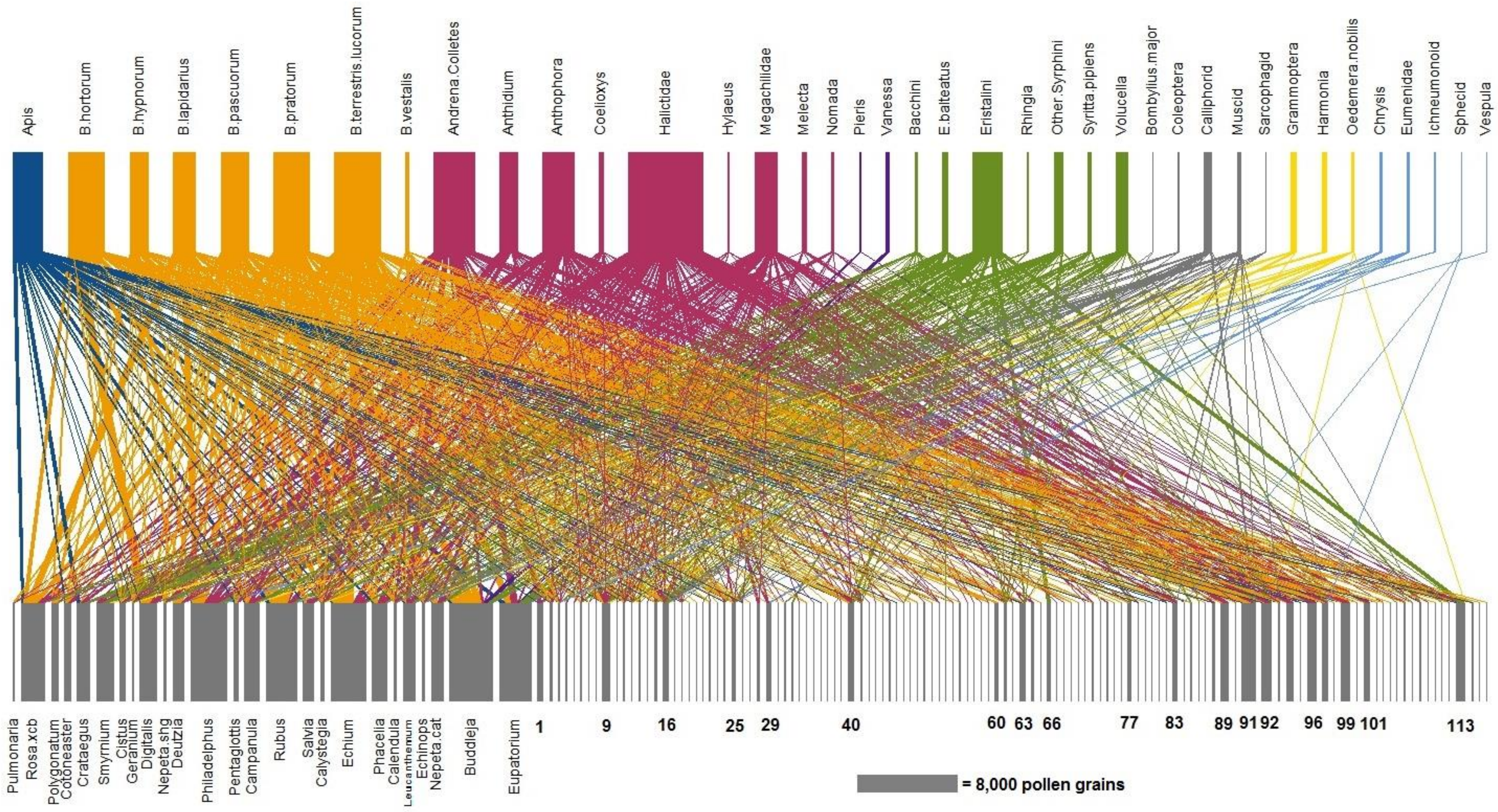


Fig 4.5 A pollen load network illustrating all of the pollen recovered from flower visitors, including links previously hidden from the visitation network and those to 'alien' plant species. Pollen loads from all flower visitors were sampled and the pollen belonging to 'alien' plants outside the 29 focal plant species identified to a reference collection where possible. The inclusion of pollen loads substantially increased the number of links in the visitation network, with a large proportion of hidden links revealed for bees. The weights of the nodes and interactions are directly proportional to the quantity of pollen grains carried. The identity of the most abundant 'alien' pollen grains (shown here as numbers) are given in Appendix 4.4.

(ii) Monthly pollen load networks

Pooled networks can mask temporal differences in visitation patterns (Chapter 3) and these differences have been neglected in pollen load networks. When data from both years were divided into four seasonal periods (Early, June, July and August, **Fig. 4.6**) pollen loads increased network size by an average of 199.75±50.38 (n=4) links and 54±10.12 (n=4) plant taxa per seasonal time period (**Table 4.2**). The pollen load network was also slightly more specialised in each time period (H_2' Early=0.52, June=0.45, July=0.41 and August=0.50), compared to the pooled network (H_2'=0.37). Similarly, the generality of the flower visitors was lower in each time period (Early=4.94, June=7.81, July=9.49, August=3.78) compared to the pooled network (16.05), which was also true for plants (Early=5.76, June=7.18, July=5.75, August=7.28, pooled network=7.67, **Table 4.2**). Throughout the season, the highest number of links per flower visitor taxa was much greater in the pollen load network compared to the number of links observed (**Table 4.3**) which was almost always true for plant link number. Interestingly, early in the summer, the plants with the most links were 'alien' species (*Taraxacum* and *Paeonia*, **Table 4.3**).

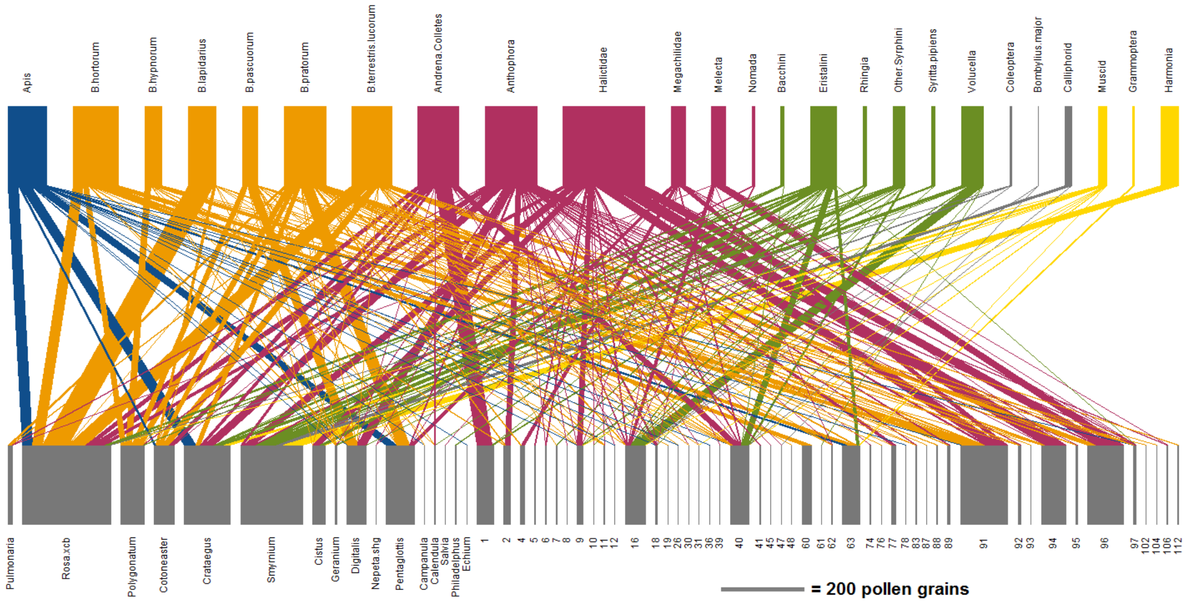
		Early		**June**		**July**		**August**	
		PL	**V**	**PL**	**V**	**PL**	**V**	**PL**	**V**
	Pollen loads or Visits (n)	166	3,023	343	3,697	366	5,016	128	1,526
Species richness	**Visitors**	25	29	28	28	29	31	20	23
	Plants	63	14	88	11	74	13	37	8
	Links	245	97	418	103	366	119	144	55
Generality	**Visitors**	4.94	2.94	7.81	4.08	9.49	4.07	3.78	2.52
	Plants	5.76	3.75	7.18	5.67	5.75	6.44	7.28	4.96
Distribution of interactions	**C**	0.06	0.08	0.06	0.12	0.07	0.12	0.1	0.12
	IE	0.59	0.56	0.6	0.66	0.61	0.66	0.56	0.6
	NODF	27.09	27.52	28.67	39.3	27.01	32.72	27.22	36.31
	H_2'	0.52	0.55	0.45	0.41	0.41	0.39	0.50	0.47

Table 4.2 Summary of the community indices for pollen load (PL) and traditional visitation (V) networks divided into four monthly time periods: early, June, July and August. All metrics were calculated using the function *networklevel* in bipartite. Variation in the network indices, particularly H_2' illustrates patterns in the pollen load networks that were hidden by pooling the data together. In each time period, pollen loads substantially increased the link number, suggesting that they provide a more complete record of flower visitation from the flower visitor's perspective. Data include all pollen loads collected over two years, including 'alien' pollen grains belonging to species not included in the list of 29 focal plants.

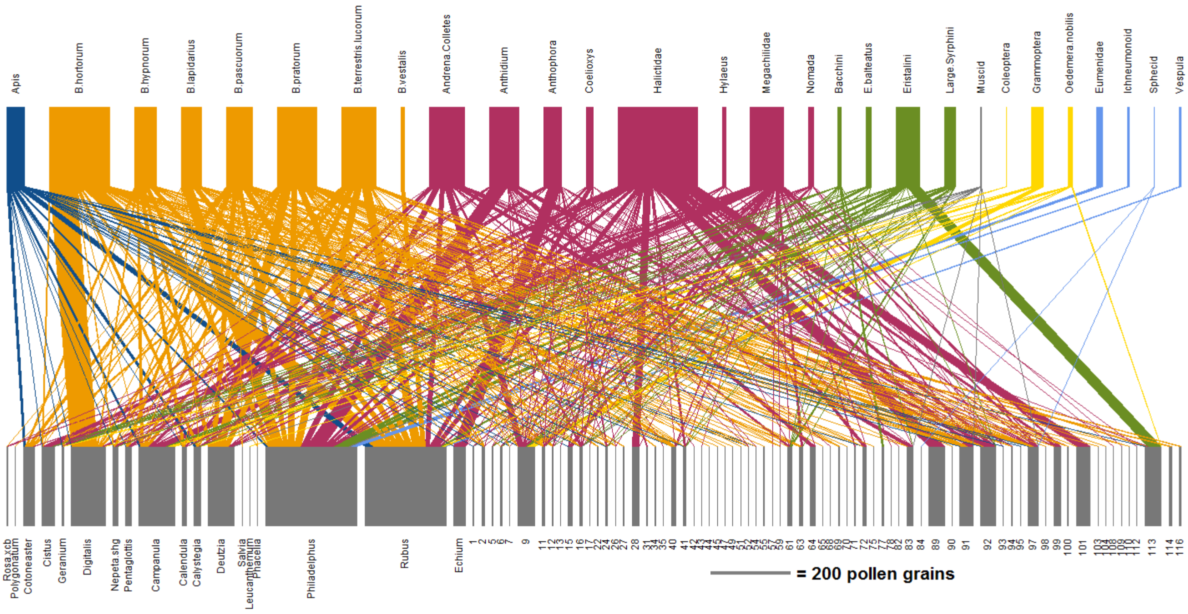
	Species link number (maximum)	
Network	**Flower visitor (n)**	**Plant (n)**
Early		
PL	*B.pratorum* (29)	*Taraxacum, Paeonia* (16)
V	Halictidae (8)	*Smyrnium* (12)
June		
PL	Megachilidae (42)	*Philadelphus* (20)
V	Halictidae, *B.pascuorum* (9)	*Rubus* (17)
July		
PL	Halictidae (38)	*Echinops* (18)
V	Halictidae (12)	*Echinops* (17)
August		
PL	*B.pascuorum,* Halictidae, Eristalini (17)	*Buddleja* (14)
V	Halictidae (7)	*Buddleja* (15)

Table 4.3 Identity of the flower visitor and plant species with the highest number of links in the pollen load (PL) and visitation (V) networks, divided into four monthly time periods: Early, June, July and August. Qualitative link numbers were calculated using the function *specieslevel* in bipartite. In almost all of the networks, the maximum link number in the visitation network was increased in the pollen load network, and the identity of the species with the highest number of links altered. In the Early network, pollen from two 'alien' plant species was found on the bodies of 16 flower visitors, compared to maximum number of visitors observed on the flowers of *Smyrnium*.

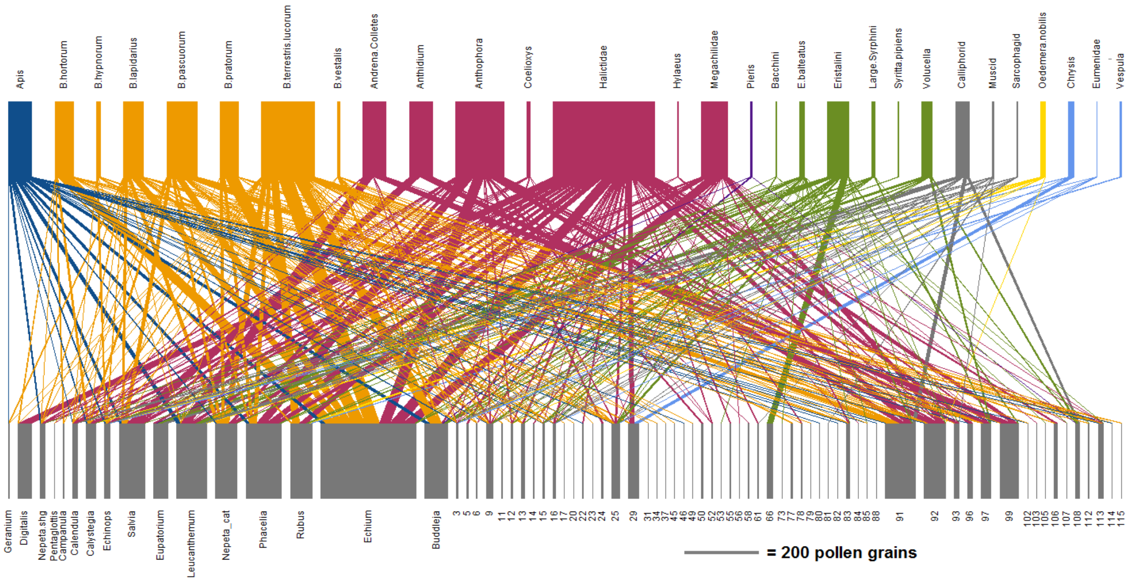
A) Early



B) June



C) July



D) August

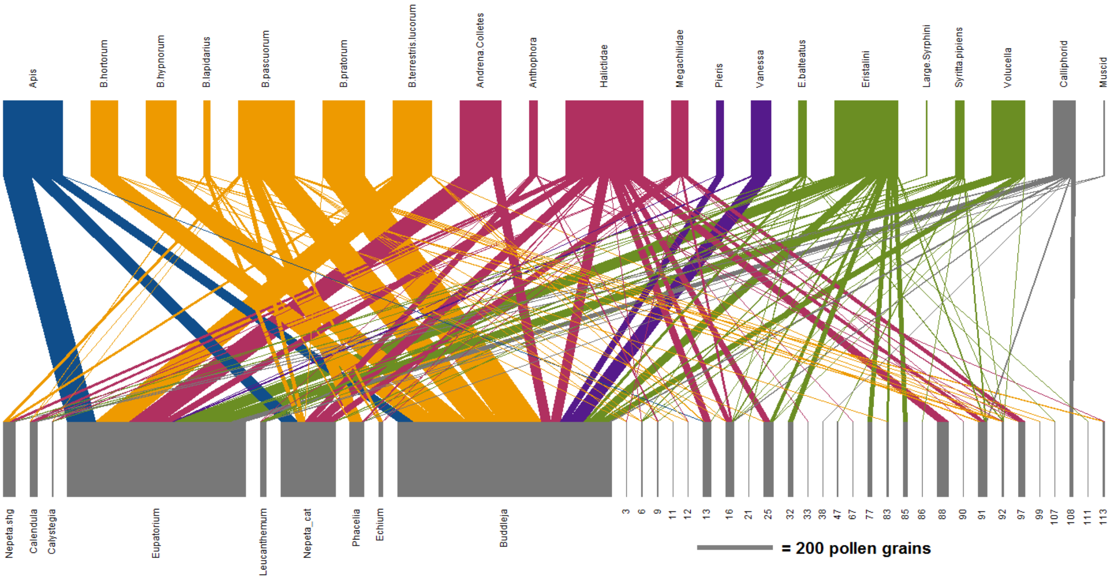


Figure 4.6 Four pollen load networks illustrating differences in the interactions between flower visitors and plants throughout the summer: A) Early; B) June; C) July; D) August. Bees carried the greatest proportion of pollen in each of the networks, with halictids carrying a particularly large proportion of all the pollen transported. The larger bodied hoverflies (Eristalini and *Volucella*) carried the greatest proportion of pollen from the dipteran visitors. Results include all of the pollen grains recovered from flower visitors over the course of two summers, with the width of the nodes and interactions indicating the total number of pollen grains counted. Pollen loads: A) n=166; B) n=343; C) n=366; D) n=128.

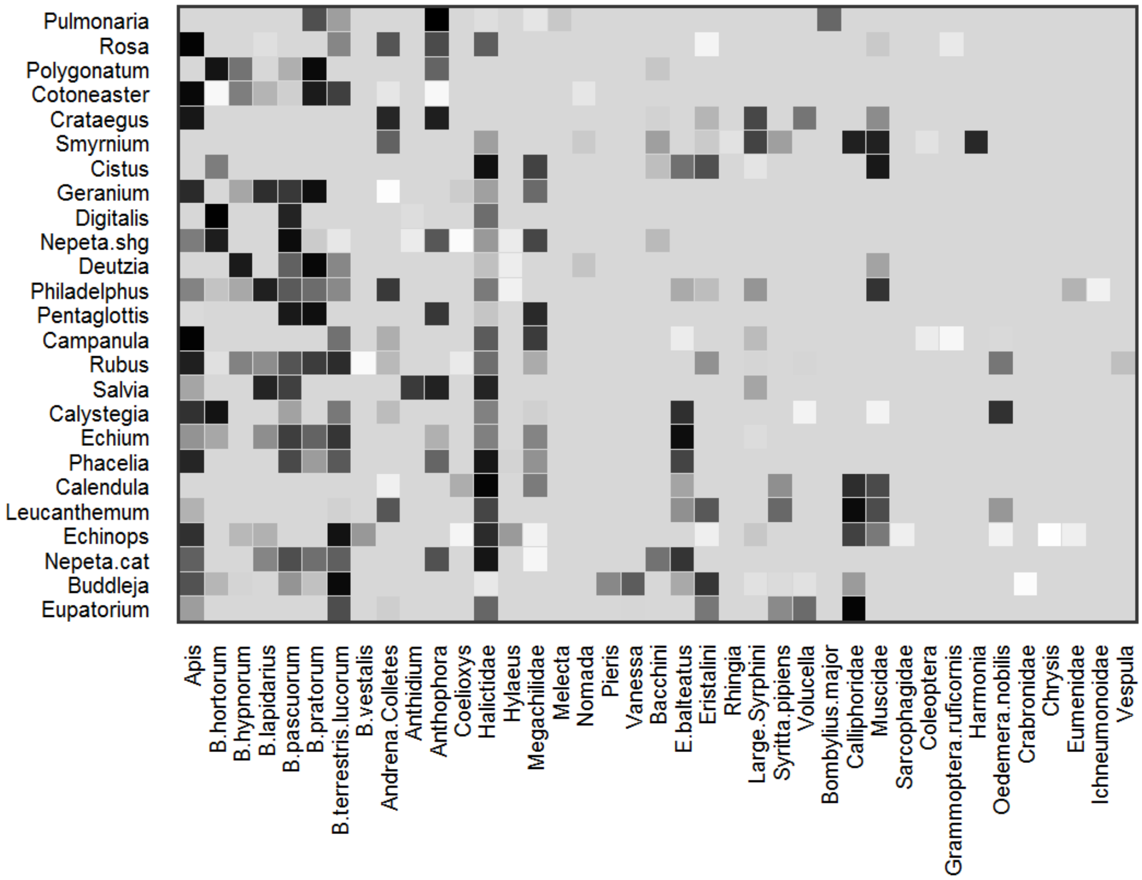
4.3.3 Comparisons between focal plant visitation, pollen load and pollen transport networks

(i) Community level

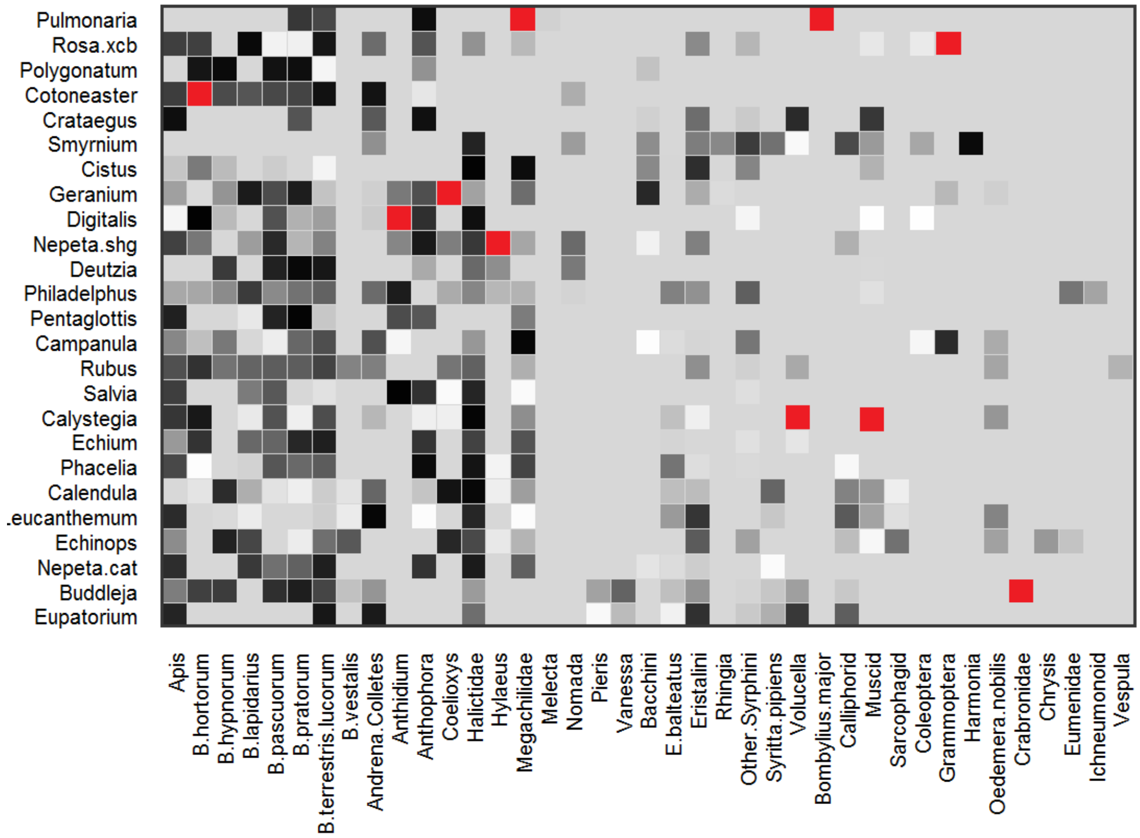
Pollen belonging to the focal plants revealed 77 additional ('hidden') links (V=252, PL=329) in the focal plant pollen load network. The majority of these additional links came from the pollen loads of bees (**Fig. 4.7**). Only 10 links were lost from the visitation network (V) in the pollen load network (PL), as most links involved flower visitors that carried pollen (**Fig. 4.7**). Two visitors (*Bombylius* and Crabronidae) were excluded from the pollen load network as they did not carry pollen. As a result of these additional links, the PL network was much larger than the V network (**Table 4.4**). This reduced the specialisation of the interactions (H_2' V=0.47, PL=0.38) and modularity (V=0.43, PL=0.31, **Table 4.4**) of the PL network. Nestedness in the PL network increased slightly (NODF V=27.61, PL=30.04) as did the generality of plants (V=7.83, PL=10.62) and of flower visitors (V=10.62, PL=8.21). Interaction evenness also increased (V=0.70, PL=0.76) as did connectance, although this remained low (V=0.11, PL=0.15).

Pollen loads were combined with visitation frequency to create a pollen transport network. In cases where visitors carried the pollen of flowers they were never observed to visit (n=24), the additional link was assumed to represent one visit (Section 4.2.3). Surprisingly, this increased the specialisation of the interactions (H_2'=0.55) and the modularity (0.50, **Table 4.4**). The generality of flower visitors (7.45) and plants (3.98) fell to below that of either the V or PL network, coupled with a decline in connectance (0.09) and interaction evenness (0.66, **Table 4.4**).

A) Visitation matrix



B) Pollen load matrix



C) Pollen transport matrix

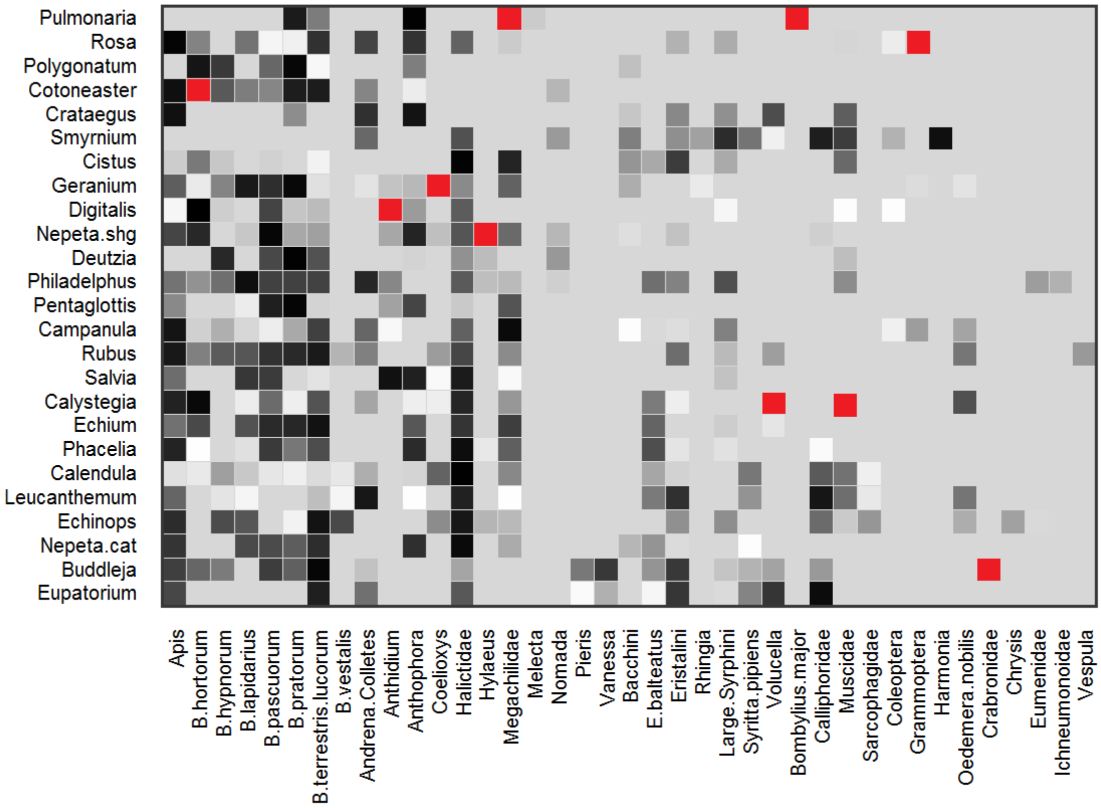


Figure 4.7 Bipartite matrices illustrating the differences in interaction strength between visitation, pollen load and pollen transport networks. In each network, the strength of the interaction is represented by the depth of the shade, with links where no pollen was found on a visitor’s body highlighted in red (n=8). Almost all flower visitors carried pollen, with many hidden links between the flower visitors and focal plants revealed in the pollen load network. The hidden links were particularly obvious for the bees (left hand side). Results are shown as the proportional data, so each interaction is weighted on a scale of 0 to 1. When flower visitors carried the pollen of plants they were not observed to visit, each interaction was assigned a visit frequency of ‘1’ in the pollen transport network. Only the pollen belonging to the 25 species of focal plants is included.

	Network	Visitation	Pollen Load	Pollen Transport
Species richness	**Visitors**	39	37	37
	Plants	25	25	25
	Links	252	329	329
	Visits	13,262	-	13,286
Generality	**Insects**	8.21	10.62	7.45
	Plants	5.45	7.83	3.98
Distribution of interactions	**C**	0.11	0.15	0.09
	IE	0.7	0.76	0.66
	NODF	27.61	30.04	33.31
	H_2'	0.47	0.38	0.55
	M (n)	0.43 (6)	0.31 (6)	0.50 (8)

Table 4.4 Community level indices for a visitation, pollen load and pollen transport network. The measure of specialisation (H_2') decreased in the pollen load network relative to the visitation network, possibly as a result of the increase in link number. However, the combination of flower visit frequency and pollen transport increased specialisation; as almost all flower visitors carried pollen, this increase in specialisation occurred as a result of the variation in the quantity of the pollen transported by visitors.

Removal of small quantities of pollen from the network

Previous studies have excluded small numbers of grains from pollen load analysis (less than 5 grains in Forup & Memmott 2005 and Banza *et al.* 2015, less than 10 in Bosch *et al.* 2009). When I removed pollen grains that totalled less than 10 on the body of visitors, the indices of the pollen load network remained similar to when all grains were included (**Table 4.5**). For example, specialisation in the pollen transport network with grains removed (H_2'=0.53) was only slightly reduced relative to the larger network (H_2'=0.55) and this was assumed to show that the inclusion of small numbering grains did not affect the overall level of specialisation. However, in the network with grains removed, insect generality decreased (from 7.45 to 4.14) as 38 links between plants and flower visitors were lost, yet this had the effect of increasing plant generality (from 3.98 to 7.79) as the strength of the remaining links was increased. No grains were removed from the data for this study, as even single grains on the bodies of visitors represent an interaction (direct or indirect) between a plant and the insect.

	Network	Pollen Load	Pollen Transport
Species richness	**Visitors**	36	36
	Plants	25	25
	Links	291	291
	Visits	-	13,234
Generality	**Insects**	10.94	4.14
	Plants	7.86	7.79
Distribution of interactions	**C**	0.15	0.1
	IE	0.77	0.67
	NODF	28.42	31.8
	H_2'	0.37	0.53
	M (n)	0.30 (6)	0.49 (7)

Table 4.5 Removal of pollen grains numbering 1 to 10 from visitor pollen load data had a negligible effect on the measure of H_2'. However, by removing the smallest grain numbers (i.e. the very weakest interactions) plant generality increased in the pollen transport network, while insect generality decreased. Results represent the loss of 38 unique links in the complete pollen load and pollen transport networks.

(ii) Comparisons between focal plant visitation, pollen load and pollen transport networks at the level of individual species

Although indices related to individual species are most usefully compared within networks, the average plant specialisation (*d'*) and flower visitor species strength for the V, PL and PT networks were compared using a GLMM (value~network+(1|species)), with pairwise post-hoc Tukey tests between networks (see Section 4.2.4, page 87). Full test results are given in **Appendix 4.5**.

The average specialisation of the plants in the visitation network (*d'* 0.37±0.02, n=25) was not significantly different to the pollen transport network (*d'* 0.41±0.02, *p*=0.10, n=25). However, plants in the pollen load network were significantly more generalised (*d'* 0.31 ± 0.02, n=25) than either the visitation or pollen transport networks (*p*<0.001 in both cases). Each network estimated *Rubus* to be the most generalised plant, although the identity of the most specialised plant changed between the networks (*Pulmonaria* or *Smyrnium*, **Table 4.6**). The species strength of the flower visitors in the visitation network (0.64±0.14, n=37) was not significantly different in either the pollen load network (0.64±0.13, *p*=0.99, n=37) or pollen transport network (0.64±0.17, *p*=0.99, n=37), although the identity of the least and most important visitor varied (**Table 4.6**). Interestingly, *Apis* was no longer the most important visitor in either the PL or PT networks, having been usurped by the small, solitary Halictidae.

Network	Plants		Flower visitors	
	Specialisation (*d'*)	Species range (min and max)	Species strength	Species range (min and max)
Visitation	0.37 ± 0.02 (n=25)[a]	*Rubus* (0.17) *Pulmonaria* (0.63)	0.64 ± 0.17 (n=39)[a]	*Chrysis* (0.001) *Apis* (3.46)
Pollen load	0.31 ± 0.02 (25)[b]	*Rubus* (0.13) *Smyrnium* (0.60)	0.64 ± 0.14 (39)[a]	*Melecta* (0.006) Halictidae (2.96)
Pollen transport	0.41 ± 0.03 (25)[a]	*Rubus* (0.16) *Smyrnium* (0.74)	0.64 ± 0.13 (39)[a]	*Melecta* (0.0003) Halictidae (3.92)

Table 4.6 Species level indices for a visitation, pollen load and pollen transport networks, showing the most specialised plant and flower visitor with the greatest species strength. The specialisation of plants and species strength of flower visitors is compared here as the pollen loads were interpreted from the perspective of the plant. While *Pulmonaria* was the most specialised plant in terms of visitation, *Smyrnium* was the most specialised in terms of pollen load and pollen transport. Likewise, *Apis* may have been the most important flower visitor, however in both the pollen load and transport networks, Halictidae had the greatest species strength. Results represent the proportional networks created for the 25 plant taxa. Shared letters indicate no significant difference between the mean value for the networks.

4.3.4 Are individual flower visitors more specialised in pollen collection than species collectively?

Pooling visitors by species or genera can exclude important information about individual foraging patterns. The pollen loads gathered from individual *Apis*, *Bombus* and four genera of non-eusocial bees were considerably more specialised than when individuals were pooled together (**Fig. 4.8**). *Apis* workers were collectively very generalised (*d'*=0.23) collecting 61 types of pollen (**Table 4.7**). However, when the pollen loads of workers were plotted individually, individual *Apis* were highly specialised in the pollen taxa they carried (H_2'=0.98, *d'*=0.64±0.02, n=88) with each worker having an estimated generality of just 1.16. Similar trends were observed for the other Apoidea listed (**Table 4.7**), although the proportion of *Apis* individuals carrying monospecific pollen loads (51.1%) was higher than that of the other bees (range 10.5% Megachilidae to 39.2% *B.terrestris/lucorum*). However, as the individual networks are much smaller caution must be applied as the increase in specialisation may be exaggerated.

Bee taxa (n)	**Community pollen load network**		**Individual pollen load network**		
	Pollen taxa	**Species *d'***	***H_2'***	***d'***	**generality**
Apis (88)	61	0.23	0.98	0.64 ± 0.02	1.16
Andrenidae (39)	28	0.41	0.84	0.58 ± 0.03	1.70
Anthidium (7)	19	0.62	0.80	0.62 ± 0.06	1.69
Anthophora (60)	51	0.40	0.73	0.54 ± 0.02	2.16
B.hortorum (45)	45	0.33	0.94	0.59 ± 0.04	1.20
B.hypnorum (29)	36	0.33	0.84	0.62 ± 0.03	1.67
B.lapidarius (33)	38	0.37	0.88	0.57 ± 0.05	1.40
B.terrestris/ lucorum (74)	56	0.20	0.91	0.61 ± 0.02	1.37
B.pascuorum (70)	54	0.22	0.89	0.60 ± 0.02	1.49
B.pratorum (57)	59	0.27	0.90	0.64 ± 0.02	1.47
Halictidae (120)	61	0.36	0.86	0.59 ± 0.01	1.87
Megachilidae (57)	56	0.40	0.85	0.57 ± 0.03	1.50

Table 4.7 Summary of the collective species and individual specialisation values of 12 groups of Apoidea, revealed by pollen loads. The pollen loads of all bees were used to create individual species pollen load networks, where each node represents an individual rather than several individuals belonging to a species. This revealed that while most bees were collectively generalised at the species-level, individuals were far more specialised, as shown in increases in both *H_2'* and *d'* values. Results include all of the pollen loads collected over two years and include pollen belonging to focal and ‘alien’ plant species.

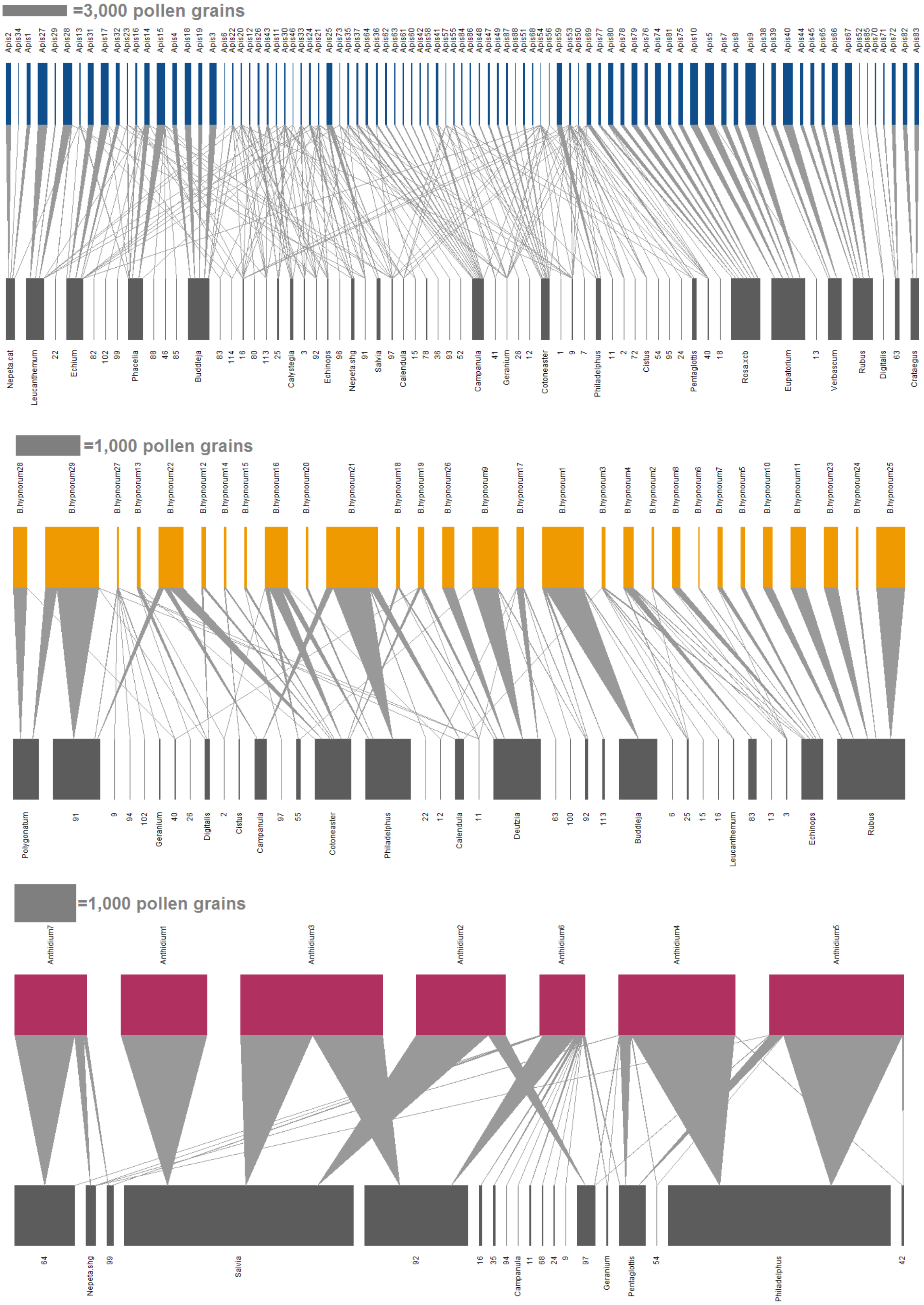


Figure 4.8 Downscaling pollen load networks to the individual level for three species of Apoidea: A) *Apis*; B) *Bombus hypnorum;* C) *Anthidium manicatum*. Individual flower visitors belonging to the same species were used to create a pollen load network, where the nodes represent individual foragers. In each example, individual flower visitors foraged from a smaller selection of the plants available than the collective species and the specialisation of the interactions (as measured by H_2' and d') was increased. However, the diversity of pollen species varied between individuals, with some carrying several pollen types and others only one. Results represent the data collected over two years.

4.3.5 Is flower visitation frequency a good predictor of the pollen loads and pollen transport of a diverse community?

Much debate concerns whether flower visitation frequency is a suitable proxy for pollination (Vázquez *et al.* 2005). A linear model was used to test the correlation between flower visit frequency and pollen load/transport, using the proportional values from the community data set (Section 4.2.4). Differences between visitors were tested for by including insect group as an interaction. Spearman's rank correlations were used to test the relationship for each group.

Flower visitation frequency was significantly, positively correlated with pollen load ($F_{(1,239)}$=52.02, $p<0.001$, r=0.42) and pollen transport ($F_{(1,239)}$=739.90, $p<0.001$, r=0.87). However, visitation frequency explained only 18% of the variation in PL (**Fig. 4.9**). Visit frequency explained 75% of the variation in PT, as PT is calculated using visit frequency. The strength of the relationship between flower visitation frequency and pollen load varied significantly between flower visitor groups (LM visit frequency~pollen load*group, F=9.45, df=7, $p<0.001$, **Fig. 4.10a**); visitation frequency was a reasonable predictor of the pollen loads of *Bombus* (r_s=0.38, p=0.001) and non-eusocial bees (r_s=0.58, $p<0.001$), but was a much poorer predictor of PL for all other flower visitors (**Fig. 4.10a**).

The relationship between pollen transport and visitation varied between flower visitor groups (LM visit frequency~pollen transport*group, F=9.63, df=7, $p<0.001$, **Fig. 4.10b**) although for most visitors, flower visitation was significantly correlated with the quantity of pollen transported. Full results of the linear models testing the relationship between visitation and pollen load/transport by visitor taxa are given in **Appendix 4.6**. No significant differences between plants were found in the relationship between (i) V and PL (LM visit frequency~pollen load*plant, F=0.38, df=24, p=1.00) or (ii) V and PT (LM visit frequency~pollen transport*plant, F=0.17, df=24, p=1.00).

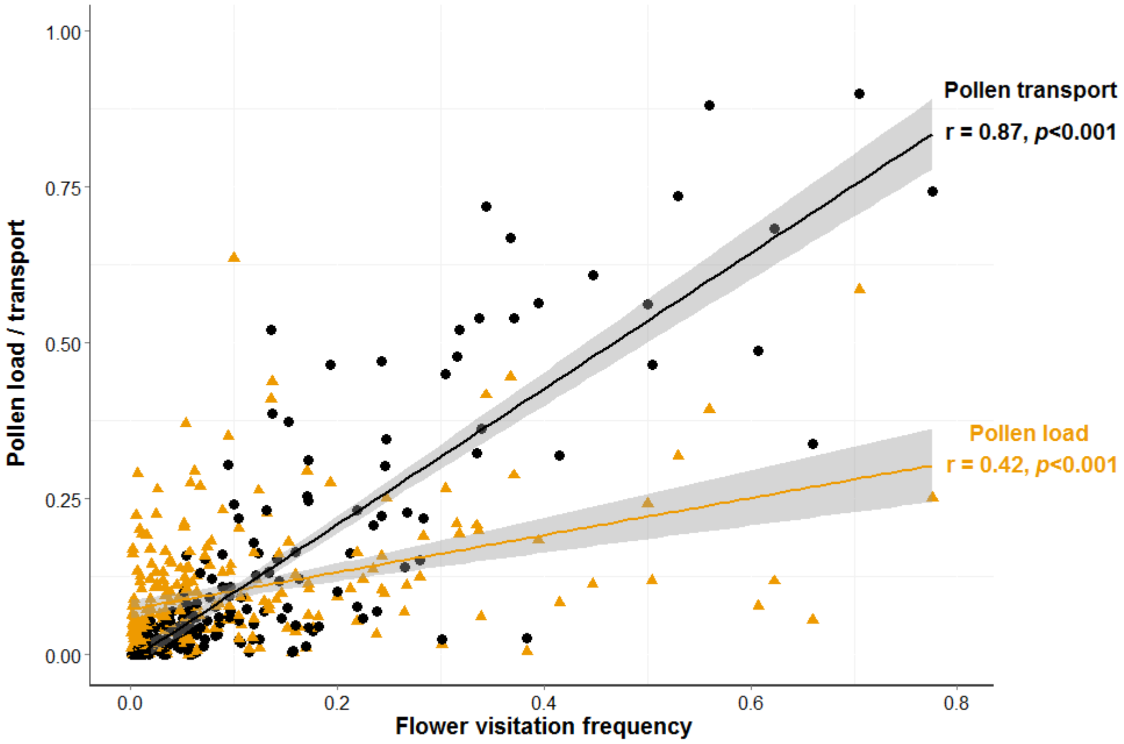
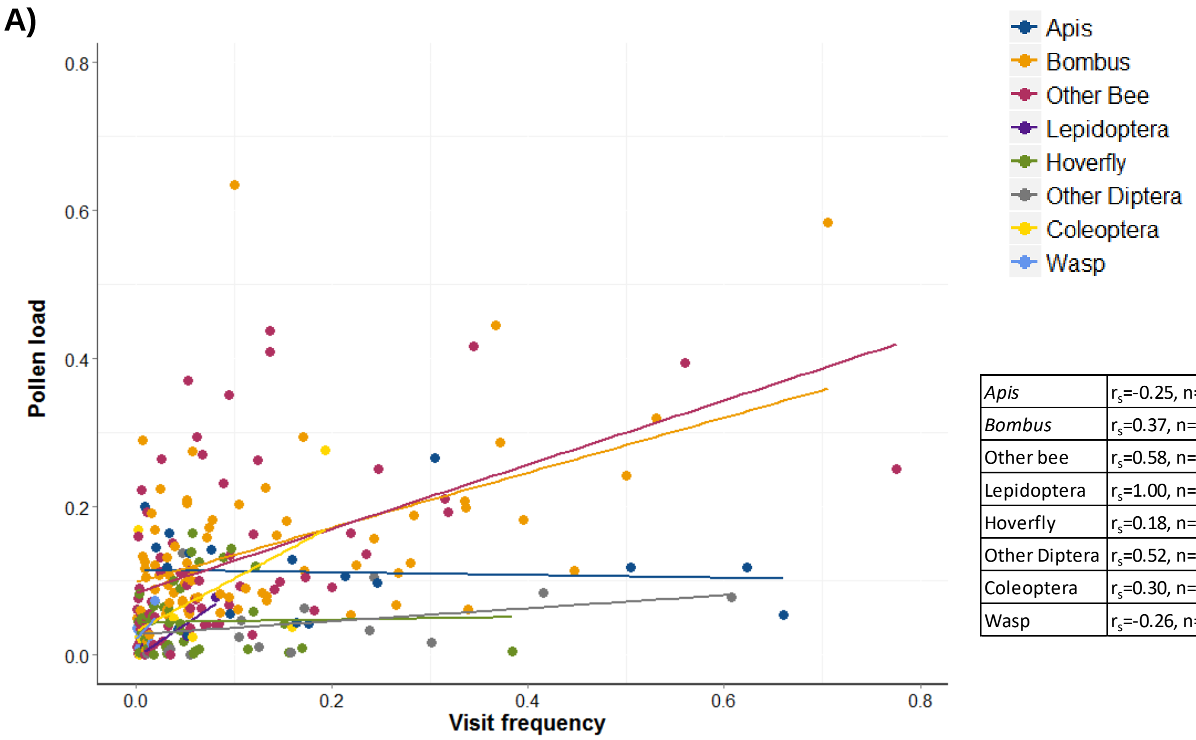
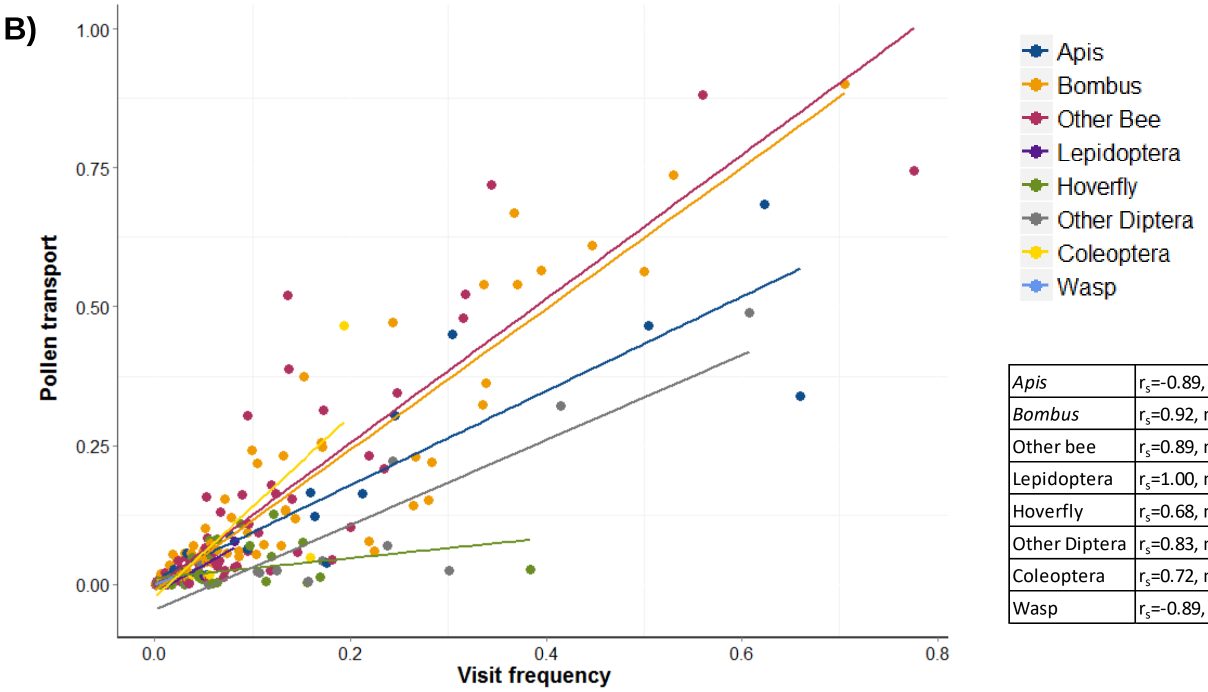


Figure 4.9 The relationships between flower visitation frequency and flower visitor pollen load or total pollen transport. For each plant, the proportional visitation value of each flower visitor was plotted against their corresponding proportional pollen load or pollen transport, e.g. each of the 14 visitors to *Buddleja* is represented by its own value. In both cases, flower visitation was positively correlated with the measure of flower visitor pollen load. However, flower visitation frequency did not always predict the size of the pollen load, shown in the scatter of points around the line. Shaded areas indicate the 95% confidence interval as fitted by a linear model.



Apis	r_s=-0.25, n=18, *p* =0.32
Bombus	r_s=0.37, n=74, *p* =0.01
Other bee	r_s=0.58, n=75, *p<0.001*
Lepidoptera	r_s=1.00, n=3, *p* =0.33
Hoverfly	r_s=0.18, n=48, *p* =0.23
Other Diptera	r_s=0.52, n=18, *p* =0.03
Coleoptera	r_s=0.30, n=10, *p* =0.41
Wasp	r_s=-0.26, n=6, *p* =0.66



Apis	r_s=-0.89, n=18, *p<0.001*
Bombus	r_s=0.92, n=74, *p<0.001*
Other bee	r_s=0.89, n=75, *p<0.001*
Lepidoptera	r_s=1.00, n=3, *p* =0.33
Hoverfly	r_s=0.68, n=48, *p<0.001*
Other Diptera	r_s=0.83, n=18, *p* <0.001
Coleoptera	r_s=0.72, n=10, *p* =0.02
Wasp	r_s=-0.89, n=6, *p* =0.03

Figure 4.10 The relationships between A) pollen load and flower visit frequency and B) pollen transport and flower visit frequency for 8 groups of flower visitors. In order to test whether the strength of the relationships varied between visitors, the relationships were plotted for each group separately. Although the relationship between the proxies for pollination appeared to be different for *Apis* compared to the other bees, no significant difference was found between visitors. Spearman Rank Correlations for each visitor group are given in the tables.

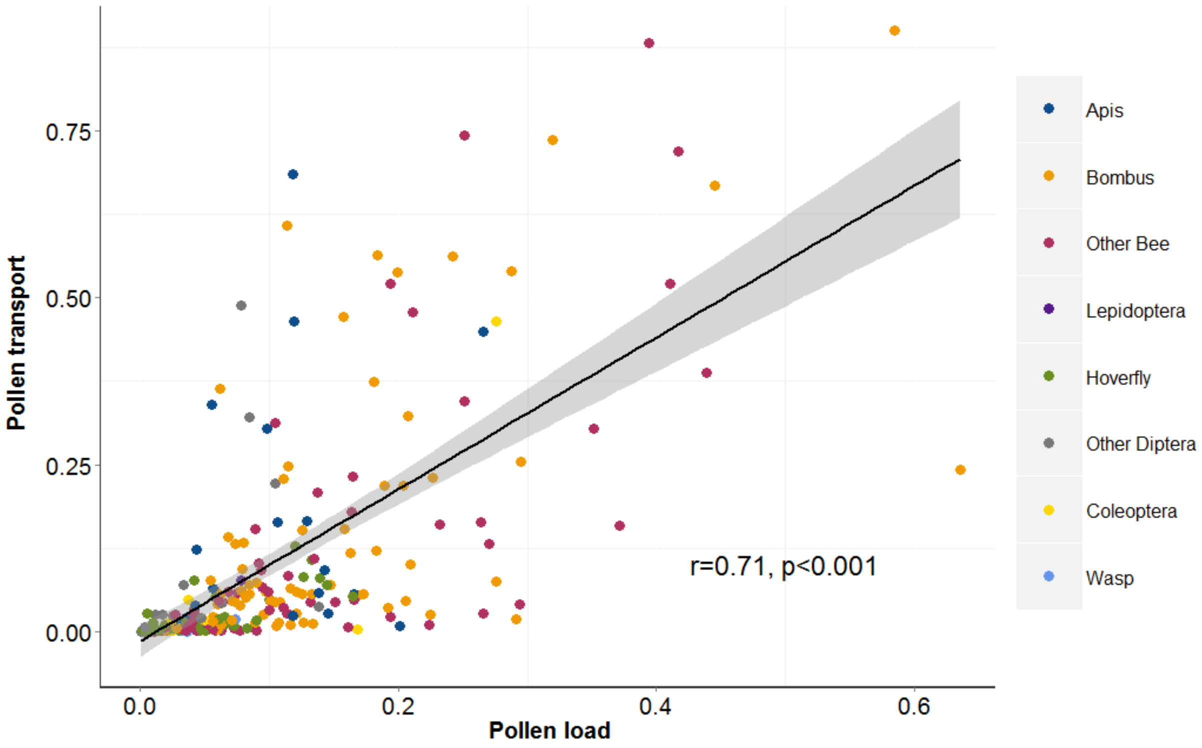


Figure 4.11 The relationships between flower visitor pollen transport and pollen load. The relationship between the proportional value of pollen transport and proportional pollen load for a visitor to a specific plant was plotted for all visitors. For the entire visitor community, the quantity of pollen on the insect bodies explained only 50% (r^2=0.50) of the variation in the total pollen transported. Although the scatter appeared to be greater for *Bombus*, there was no significant difference in the relationship between pollen transport and pollen load between visitors. Shaded area indicates the 95% confidence interval.

(ii) Overall pollen load and pollen transport for visitors to garden plants

A GLM was used to compare the proportional pollen load and pollen transport values of flower visitor groups. The proportional pollen load varied significantly between groups (GLM pollen load~group, F=11.09, df=7, p<0.001). *Bombus* had the highest proportional pollen loads per plant (0.15±0.13, n=74, **Fig. 4.12a**). Non-eusocial bees performed similarly to *Bombus* (0.12±0.14, p=0.38, n=74) as did *Apis* (0.11±0.15, p=0.91, n=18). However, hoverflies (0.45±0.07, n=48) and non-syrphid Diptera (0.43±0.11, n=18) had significantly smaller pollen loads compared to each group of bee (p<0.05 in each case) and were not different to one another (p=1.00).

The average contribution to community pollen transport varied significantly between groups (GLM pollen transport~group, F=7.95, df=7, p<0.001). When the frequency of flower visitation was accounted for, *Apis* had the highest average pollen transport per plant of all flower visitors (0.17±0.46, n=18) although this was not significantly greater than *Bombus* (0.15±0.24, *p*=1.00, n=74) or than non-eusocial bees (0.11±0.23, *p*=0.76, n=75, **Fig. 4.12b**). Hoverflies had significantly smaller pollen transport values (0.20±0.05, n=48) compared to each group of bee (*p*<0.05, in each case) although other Diptera (0.81±0.35, n=18) were no longer significantly different to any of the bees (*p*>0.05 in each case). The full results of all GLMs are given in **Appendix 4.7**.

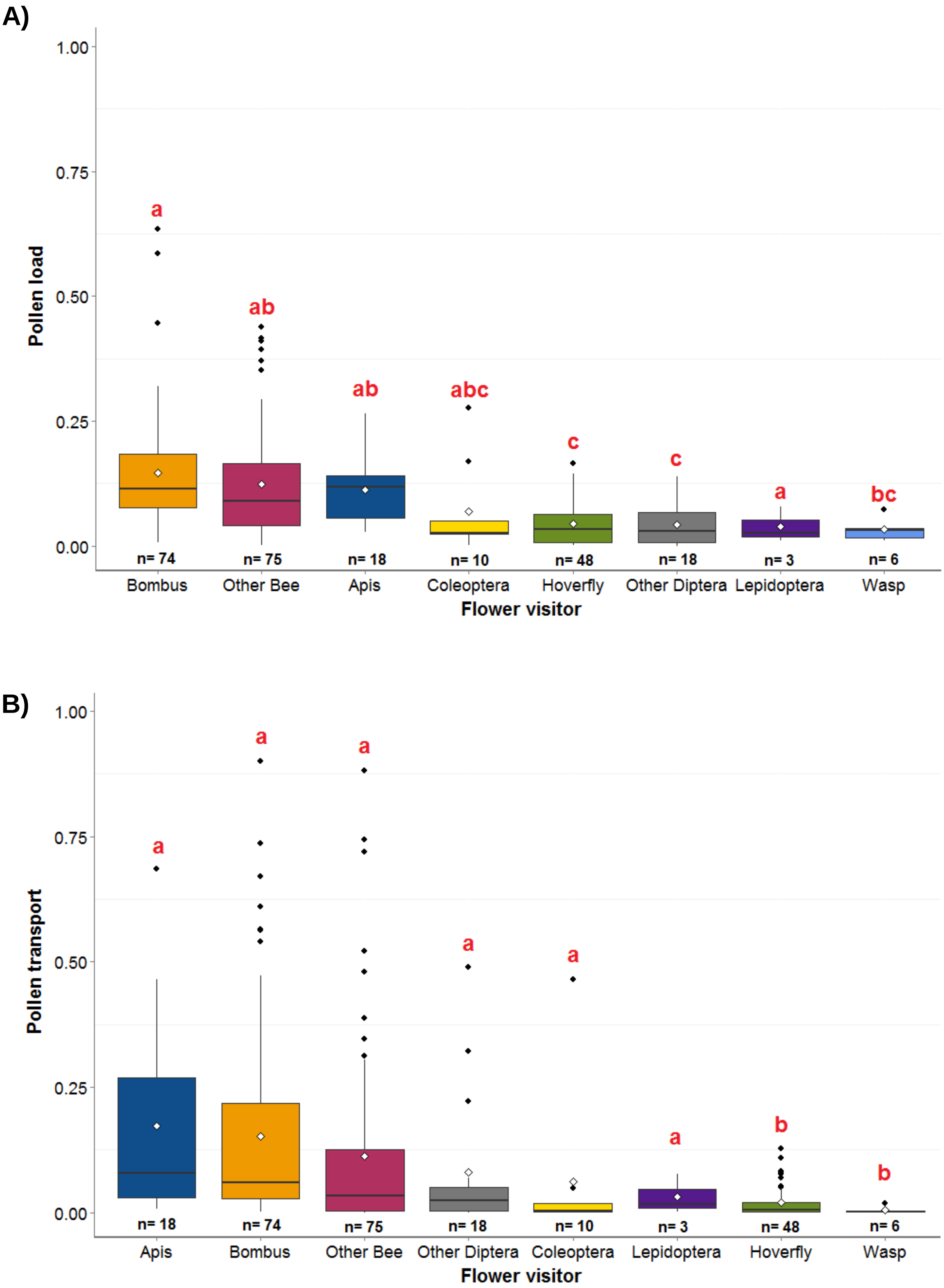


Figure 4.12 The variation between 8 groups of flower visitors in the overall value of A) pollen load and B) pollen transport to a community of plants. Proportional values for each of the plant species visited were compared between visitors to assess which visitors contributed most to pollen loads and transport at a community level. While *Bombus* and the other non-eusocial bees carried the largest pollen loads for the garden plants, *Apis* exceeded these bee visitors in terms of pollen transport (n.s.) due to its higher flower visit frequency. In both measures, bees outperformed the other groups of flower visitors. Results show the pollen collected from flower visitors over two years, including only the pollen to the focal plant species.

4.4. Discussion

Very few studies exist that measure the pollen loads of a diverse set of flower visitor taxa to a community of plants. This chapter used the third largest collection of pollen loads to date (n=1,003, compared to 1,745 in Alarcón 2010 and 1,245 in Olesen *et al.* 2011), to compare flower visitors and construct the first pollen load and transport networks in a single garden. Successful pollen transport by visitors is the first step in confirming their role as pollinators.

4.4.1 The diversity of flower visitor pollen loads in a garden

An initial objective of this study was to identify whether high levels of generalisation in flower visitation led to mixed pollen loads of flower visitors in a garden. However a quarter of pollen loads were monospecific, suggesting these visitors demonstrated pollen constancy (Ne'eman *et al.* 1999). Whether this also indicates floral constancy (visitors exploiting a single plant during a foraging bout, Chittka *et al.* 1999) is difficult to tell, as visitors may have also exploited non-pollen producing flowers, or avoided contact with the anthers during visits. These results raise intriguing questions regarding the foraging behaviour of flower visitors in gardens; others have already suggested that a large diversity of plants is less important than the abundance of the most rewarding species (Garbuzov & Ratnieks 2014), and these results suggest that some flower visitors may preferentially forage on a small selection of the plants available. For the remaining 60% of flower visitors that carried a mixed load (mostly 2-4 pollen species), a relaxation of flower constancy may indicate that visitors in gardens are actively seeking multiple resources (Marchand *et al.* 2015). It is difficult to predict the effect of this on plant fitness, as the proportion of heterospecific pollen in mixed loads varied considerably. In accordance with Forup & Memmott (2005), a fairly high degree of generalisation appeared to be the norm in this study, as all visitor taxa collectively carried more than one pollen species.

Mixed pollen loads containing 4-6 species have been commonly reported for solitary bees (Eckhardt *et al.* 2014) and bumble bees (Free 1970), and the results of this study support previous claims that these visitors carry more diverse pollen loads compared to *Apis* (Free 1970, Grüter *et al.* 2011, Leonhardt & Blüthgen 2012), although the difference between *Apis* and solitary bees was not significant. These findings are not entirely indicative of the diversity of floral resource use by bees, as pollen in the corbicula of *Apis* and *Bombus* was avoided and pollen packed into the leg corbiculae of solitary bees. However, the diversity of the 'free' pollen carried by bees was surprising, given that they are often cited as the most important pollinators, who by default should carry the ‘purest’ pollen loads (Alarcón 2010). Whether this translates to greater deposition of heterospecific pollen on to the stigma is explored in the next chapter.

The results of this study confirm that hymenopteran pollen loads are greater than those of other flower visitors (Forup & Memmott 2005), although *Apis* carried significantly less pollen than the solitary bees, potentially as a result of more frequent grooming. Very little is known about the size and diversity of pollen loads in dipteran flower visitors, although both syrphid and non-syrphid Diptera did, on occasion, carry substantial pollen loads in this study, similar to the finding of Orford *et al.* (2015). Although the amount of pollen on flower visitor bodies is not synonymous with that deposited on to the flower (Adler & Irwin 2006) and Diptera may often not be particularly effective pollinators, they still affect plant fitness via the removal of pollen grains and may transport pollen over larger distances (Rader *et al.* 2011).

4.4.2 ‘Alien’ pollen species in urban pollen load networks

Previous studies of pollen loads have found ‘alien’ grains belonging to non-focal plants (Popic *et al.* 2013, Marchand *et al.* 2015). The prevalence of these pollen types recorded from flower visitors in the garden was expected to be high, given the plant diversity and limited access to survey the surrounding floral landscape. The identification of 116 ‘alien’ pollen types from flower visitor bodies was larger than that reported elsewhere (e.g. 6 in Popic *et al.* 2013), although it accounted for <20% of the total pollen collected (similar to Wiesenborn *et al.* 2008). Consequently, pollen loads are a promising technique for improving the understanding of flower visitor pollen collection (but not nectar use) at the landscape level in urban areas, particularly given the recent advances in pollen identification offered by metabarcoding (Pornon *et al.* 2016). Given that *Bombus* were found to collect pollen and nectar simultaneously in Chapter 3, it is worth noting that pollen loads could be a better predictor of nectar use by these visitors. Future studies should preferably adopt a focal-visitor, rather than focal-plant method for assessing floral resource use in urban areas, as this greatly increased the number of links in this study.

4.4.3 Pollen load and transport networks alter the structure of the interactions

To the best of my knowledge, this study is one of only six to directly compare the structure of a visitation and pollen transport network (Forup & Memmott 2005, Bosch *et al.* 2009, Alarcón 2010, Popic *et al.* 2013 and Ramírez-Burbano *et al.* 2017). My pollen loads (including ‘alien’ and focal plant pollen) revealed an additional 617 links compared to my visitation network both within and beyond the focal plant community, confirming that many flower visitation networks are under sampled (Bosch *et al.* 2009). The additional 77 links to the focal plants substantially increased records of visitation for bees, suggesting that these highly mobile visitors are most disadvantaged by plant-based observations. A likely explanation for the increase in link number in the focal plant network, is that observations of flower visits were limited to peak flowering, while pollen loads also revealed visits to plants at the start and end of flowering. Interestingly, only 4% of visitors in this study did not carry pollen, which was considerably below that reported elsewhere (Bosch *et al.* 2009, Alarcón 2010, Popic *et al.* 2013). A possible explanation for this might be that pollen production in Mediterranean (Bosch *et al.* 2009) and desert communities (Popic *et al.* 2013) is limited by abiotic factors (e.g. rainfall) compared to the garden. In support of this, 82% of flower visitors were found to carry pollen in a UK hay meadow (Forup & Memmott 2005).

In my study, the generality of flower visitors and plants were reduced in the pollen transport network. Similar reductions in the generality of plants have been reported by Alarcón (2010) and Popic *et al.* (2013), suggesting that plants in PT networks consistently have their pollen transported by a less diverse cohort of visitors. This is surprising, given the increase in links revealed by pollen loads, and because generalisation increased in the PL network. A likely reason for this is that in the PT network the strength of the interactions between plants and rare visitors that carried high pollen loads was reduced.

In line with previous studies, specialisation (H_2') increased in PT networks, although the extent of the increase in this study (0.47 to 0.55) does not match that reported by Alarcón (2010, 0.42 to 0.81) or Popic *et al.* (2013, 0.49 to 0.81). These results may be explained by the inclusion of only bees by Popic *et al.* (2013) and the number of flower visitors that carried no pollen in both of these studies. However, more pollen transport networks are needed to identify trends in specialisation, as Bosch *et al.* (2009) found interactions in the PT network to become more specialised. Those authors did find that pollen data revealed additional modules in the interaction network, and in my study, modularity increased in the pollen transport network (0.47 to 0.55) with the addition of two modules. No differences in the species specialisation of plants or visitors were found in this study, although Alarcón (2010) and Popic *et al.* (2013) found *d'* to increase. However, this may have occurred if a subset of the garden species became more specialised, while others became more generalised, thus preventing any change in the average *d'* value. Interestingly, Popic *et al.* (2013) reported an increase in the importance of Halictidae in the pollen transport network, similar to that recorded in my garden study. The value of these small, but often abundant flower visitors as pollinators represents an important area for future research in urban areas.

4.4.4 Temporal variations in pollen loads

Very few studies have shown temporal variation in pollen loads, at either the level of species or the entire community. Although Alarcón (2010) reported variation in the structure of a pollen transport network over two years, low capture rates have made it difficult to construct seasonal or diurnal networks, while smaller networks can bias measures of specialisation (Dormann *et al.* 2009). With these problems in mind, my study has suggested that (i) pollen load diversity and network generality varies throughout the season, possibly as foraging preferences change depending on the phenologies of the flowering plants available (Ritchie *et al.* 2016), and (ii) pollen loads are smaller at the start of the day, as passive pollen collection increases later in the day with the number of flowers visited (Willmer 2011). In future investigations, this may be relevant for avoiding sampling bias. From the plant's perspective, it would be intriguing to determine whether the diversity and quantity of pollen deposited on to stigmas also varied seasonally and diurnally; native plants in gardens that flower when floral diversity is greatest (in this study, July) could be at a greater disadvantage from heterospecific pollen transfer. In contrast, plants whose stigmas are receptive later in the day, could benefit from receiving higher pollen loads.

4.4.5 Individual versus collective pollen specialisation

Recently, Tur *et al.* (2014) created the first individual-based pollen load networks, and found individual flower visitors to a Mediterranean montane site were more specialised in pollen collection than predicted by their species collective foraging patterns. Given the relatively high levels of generalisation found in the garden community, it was surprising to find that all bee taxa demonstrated an increase in specialisation (H_2' and d') similar to that predicted by Tur *et al.* (2014). This finding is also in line with that of Popic *et al.* (2013), who found 70.7% of individual bee pollen loads contained a single pollen species. These results do not mean that an individual only visits a single plant to collect pollen throughout its lifetime, but suggest that individual visitors reduce heterospecific pollen transfer by foraging from a single plant during a foraging bout. Although the downscaled networks suggest higher levels of floral constancy than predicted for the population sampled, inspection of the bipartite networks suggests that both social and solitary bee populations include specialists (individuals foraging on a single plant) and generalists (more than one pollen species present). However, these results should be interpreted with caution, as pollen was not sampled from the corbicula of bees, and the results only apply to pollen (not nectar) foraging.

Although creating individual networks is labour intensive, it would be interesting for further research to track the floral constancy of individual flower visitors throughout their lifetime, exploring the scale (hourly, daily or weekly) at which visitors switch between pollen sources. These results also raise intriguing possibilities for individual differences in pollinator effectiveness (single-visit deposition) networks, that are yet to be explored.

4.4.6 Using visit frequency as a proxy for pollen loads

Although pollen loads are themselves a proxy for pollination, they represent an intermediate step between visitation frequency and pollinator effectiveness (single-visit deposition, Chapter 5). In this study, visitation was positively associated with both pollen load and pollen transport, similar to the findings of Alarcón (2010). However, pollen transport was most strongly associated with visit frequency in the garden, rather than pollen load in the montane meadow (Alarcón 2010). One of the most important findings from my results was the difference in the strength of this relationship between taxa, and future community-wide studies should pay attention to this.

4.4.7 Flower visitors with the largest contribution to pollen transport

Previous pollen load networks have recorded rare flower visitors that carry high pollen loads, and frequent visitors with small loads. Consequently, visitors with high relative pollen loads can have very low pollen transport values (Alarcón 2010, Popic *et al.* 2013). In this study, only 50% of the variation in pollen transport could be explained by individual pollen loads. Visitors with high pollen loads, but low pollen transport, were either *Bombus* or solitary bees. *Apis* frequently had low pollen loads, but these were compensated for by their abundance on flowers, giving them relatively high pollen transport values (**Fig. 4.11**).

The sizeable pollen loads carried by Hymenoptera compared to other flower visitors have been well documented (e.g. Herrera 1987, Alarcón 2010, Orford *et al.* 2015), so it was not surprising to find that bees had the highest relative pollen loads and pollen transport values in the garden community (**Fig 4.12**). Despite honeybees carrying smaller pollen loads than *Bombus* and solitary bees (excluding the corbiculae), their abundance as visitors increased their total value in terms of pollen transport. While individual hoverflies were found to carry high pollen loads, their relative value in the comparisons between flower visitors were reduced, as other, and possibly more frequent visitors to the same plants carried even higher pollen loads. However, as these results did not consider the quality of the pollen on the insect bodies, the dipteran visitors should not be forgotten as potential pollinators (Orford *et al.* 2015), particularly as pollen loads do not predict single-visit deposition (Adler & Irwin 2006; and see Chapter 5). Comparisons between taxa must also be made with care, as individuals or species within different taxa can perform similarly (e.g. Gómez & Zamora 1999), and those in the same taxa can vary substantially (e.g. Adler & Irwin 2006).

4.4.8 Limitations

(i) Sampling effort and estimated specialisation

As with all studies of community pollen loads, the data are biased towards the most frequent flower visitors, while very rare visitors are under-represented. This may have inflated estimates of generalisation, as the most frequent visitors (bees) are known to usually have polylectic (at the level of plant species) pollen diets (Muller 1996, Falk 2015). Estimates of individual specialisation also depend on the stage during a foraging bout at which an insect is sampled, and this is almost impossible to control for.

(ii) Identification of pollen

Although a reference collection of pollen types for plants within the garden was created, the visual identification between similar grains is challenging. One particular ‘alien’ pollen (<15µm, psilate, tricolporate) was particularly frequent throughout the entire season, and could not be reliably identified to individual species. In this instance, generalisation may be overestimated. However, with the exception of the two *Nepeta* species, all focal plant pollen was easily differentiated by eye using the fuchsin-gel method (Kearns & Inouye 1993). The collection of pollen loads was relatively fast in the field (relative to stigma deposition, Chapter 5); however, the size and heterogeneity of these loads made the counting of pollen particularly labour-intensive compared to stigma pollen loads. While barcoding has the potential to improve the speed and accuracy of this, these methods do not provide an absolute total of the grains in a sample (Vamosi *et al.* 2016). The quantity of the pollen carried by visitors is also not synonymous with the quality (e.g. age, hydration status, Willmer 2011) which makes it difficult to determine the value of large loads from the plant’s perspective.

(iii) How much do differences in the network indices reflect differences in the size of the networks?

As mentioned in Chapter 3, differences in the size of the networks can influence network metrics, although indices that were less sensitive to network size were chosen in this study. However, as plant-pollinator networks continue to incorporate measures of pollen, there is a need for standardised methodology (null models) comparing networks with continuous data (currently unavailable in the *bipartite* package in *R*). An attempt to adapt the null model used by Popic *et al.* (2013) was unsuccessful as the code created unrealistic H_2' values when >1,000 flower visits were included. Furthermore, the networks do not account for the differences in the abundance of the plants and how this affects the total number of visits and the importance of a plant to flower visitors (see Chapter 3). However, given that the sampling effort for each plant is identical between the visitation and pollen transport networks (and later for pollinator importance) the comparison of specialisation from the plant's perspective between each is not affected.

(iv) Pooling of data

Low visitation rates made it difficult to compare diurnal and seasonal patterns in pollen loads for individual plants, so these data had to be pooled. In some instances, small sample sizes introduced the risk that the data were limited to under-performing individuals. Additionally, some flower visitors that may be important pollinators at the start or end of a plants flowering may have been excluded as sampling was focused during peak-flowering only. Finally, grouping flower-visitors into one genera (e.g. Halictidae) may reduce the estimated specialisation compared to each being treated as a species. It is also important to bear in mind the diversity of species included in groups such as *Bombus*, other bee or Diptera when compared to individual species, e.g. *Apis mellifera*.

(v) Sampling a subset of the plant community

In the pollen load network, four plants were excluded due to small pollen load sample sizes (*Verbascum* n=10, *Lysimachia* n=0) or inconsistent pollen production between flowers (*Erysimum* and *Weigela,* Section 4.2.3), while rare flower visitors had to be excluded if no pollen load data was available. These exclusions are likely to have increased the estimated specialisation of both the visitation network and pollen transport network, compared to the visitation network in Chapter 3. However, the H_2' value of the visitation network used for comparisons in this chapter was only slightly increased (0.38 to 0.42).

4.4.9 Future directions

In addition to those already suggested, the ideas raised in this chapter relate to four areas for further research. Firstly, very little is known about the quality of pollen provided by garden varieties of plants, this should be considered when suggesting ‘pollinator friendly’ plants, as pollen nutritive quality is known to vary between species (Roulston & Cane 2000) and affect the development of bee larvae (Vanderplanck *et al.* 2014). Secondly, pollen loads could also provide a way to record flower visitation by nocturnal lepidopterans in urban areas, similar to the methods used by Devoto *et al.* (2011). Thirdly, analysis of pollen loads along an urban-rural gradient could help to reveal which visitors are responsible for the movement of pollen between fragmented plant populations. Finally, pollen loads are likely to play a key role in assessing the diet of the 9.2% of European bee species currently threatened with extinction, and the 56.7% for which too little is known to reliably predict their extinction risk (Nieto *et al.* 2014).

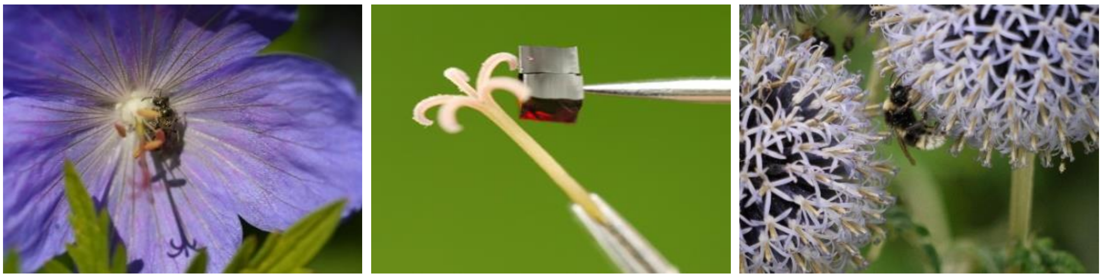
4.5 Conclusion

The inclusion of pollen loads into visitation networks is increasing, although it remains limited to a small collection of communities. In this chapter, it has been shown that almost all flower visitors in a garden carry pollen on their bodies, and in most cases, the pollen loads are dominated by the species on which the visitor is foraging. In general, bees carried the largest and most diverse pollen loads, and the findings of this research provide insights for differences between the pollen carried by *Apis* and other bees. The results of this study also support the idea that syrphid Diptera affect plant fitness via the removal of pollen. A key finding was that the pollen transport network became more specialised than the pollen load and visitation network, particularly when downscaled to the level of individuals, confirming the suggestions made by others that pollen analysis is not a substitute for visitation observations, but should be utilised as a complementary method. The data also highlighted the importance of exploring differences between visitor taxa in the correlation between visitation and measures of pollinator performance.

In this study pollen loads increased the diversity of the links observed, and hence are a valuable tool for understanding floral resource use by urban visitors. However, carrying conspecific pollen is only the first requirement for distinguishing pollinators from flower visitors; in the following chapters, the effectiveness of visitors at depositing pollen on to the receptive stigma of flowers is considered, and compared to measures of pollen transport. As many of the flower visitors were very effective at collecting pollen, it will be interesting to see whether this translates into a community of very effective pollinators – or if this pollen is lost between visits.

Chapter 5.

Pollinator effectiveness at the stigma level in a garden



Summary

1. The measure of pollen deposition by flower visitors on to the stigma has often been used to assess their effectiveness as pollinators, but only recently has this been performed for multiple plants at a community level.

2. Using a diverse community of plants in a garden, pollen deposited onto the stigma during the first visit by flower visitors (‘pollinator effectiveness’) was combined with flower visitation frequency to create a pollinator importance network. The specialisation of the pollinator importance network is expected to increase, if many visitors deposit little or no pollen.

3. Interestingly, many flower visitors successfully deposited pollen during the first visit, and the specialisation levels of the visitation (H_2'=0.49) and pollinator effectiveness (H_2'=0.48) network were remarkably similar. However, the combination of both visitation and pollinator effectiveness (‘pollinator importance’) created a network that was more specialised than either measure alone (H_2'=0.54).

4. For the garden plants, bees deposited the highest pollen loads although *Bombus* and other non-eusocial bees also deposited heterospecific pollen in approximately 20% of the flowers they visited. However, in general heterospecific pollen deposition was low with only 15.6% (n=442) of stigmas receiving an average of 2.94±0.37 heterospecific pollen grains.

5. This chapter adds to the small number of recent pollinator importance networks, illustrating the similarities and differences between these and visitation networks in a habitat that has received much recent interest: the garden.

5.1 Introduction

All hermaphroditic flowers contain a female stigma(s) that receives pollen; a flower can only be pollinated at the time when the stigma is mature and receptive for pollen germination, and visits outside of this window cannot be 'pollination events'. This chapter considers the plant-pollinator interactions in the garden solely from the plant's (maternal) perspective, measuring the pollen deposited by visitors on to the stigma.

5.1.1 Stigma conspecific pollen receipt as a measure of pollinator effectiveness

The importance of insects as agents of cross-pollination was reviewed in Chapter 1, and with many genera of flower visitors their effectiveness as pollinators varies. Assessing the value of flower visitors and identifying the 'most effective pollinator' has been popular since the phrase was coined by Stebbins in 1970. Since then, the 'quality' of pollinators has been measured using a plethora of methods (reviewed in Ne'eman *et al.* 2010) that determine how visitors influence plant fitness and the evolution of floral traits (e.g. Aigner 2004, Anton *et al.* 2013). One of the most common methods is to record the number and proportion of conspecific pollen grains deposited on to the stigma of a species during the first visit – a method which quickly distinguishes 'cheats' from genuine pollinators. Recently, this technique has identified bees as more effective pollinators than flies to a range of plants (Bischoff *et al.* 2013, Rader *et al.* 2013, Willmer *et al.* 2017), an insectivorous bat as a more effective pollinator compared to a specialised nectar-feeding bat (Frick *et al.* 2013), and confirmed the predictions from pollination syndromes regarding the most effective pollinator to several plant taxa (King *et al.* 2013).

Other measures such as pollen tube growth (e.g. Zhang *et al.* 2015, Maruyama *et al.* 2016) and total fruit or seed set (e.g. Jacobs *et al.* 2009, Lowenstein *et al.* 2015) provide greater detail but are more complex to collect for multiple plants, and include post-pollination factors (e.g. temperature or a lack of resources, Straka & Starzomski 2015) that complicate measuring a flower-visitor's contribution to pollination. Recent efforts to distinguish flower visitors from pollinators (King *et al.* 2013) and create more informative networks of pollinator effectiveness and pollinator importance (Ballantyne *et al.* 2015, 2017) have successfully used the measure of stigma deposition to answer their research questions.

Bees as the most effective pollinators

For many plants, Hymenoptera (particularly bees) are known to be the better pollinators, outperforming other visitors in the frequency of visitation (e.g. Theiss *et al.* 2007, Bischoff *et al.* 2013), quantity of pollen deposited (e.g. Rader *et al.* 2013, Willmer *et al.* 2017) and ultimately in seed-set (e.g. Sahli & Conner 2007) . However, this is not always the case which is related to the general phenomenon of floral syndromes. For example, heliconiid butterflies deposited similar loads to euglossine bees in *Psychotria suerrensis* (Stone 1996), hummingbirds were equally as effective as *Apis* at pollinating *Pitcarnia angustifolia* (Fumero-Cabán & Meléndez-Ackerman 2007) and an increase in the abundance of hawkmoths, but not bumble bees, increased outcrossing rates in *Aquilegia coerulea,* as these visitors travel larger distances between flowers (Brunet & Sweet 2006). Furthermore, dipteran visitors (syrphid and non-syrphid) have been shown to carry considerable pollen loads (Orford *et al.* 2015) and recent work has demonstrated their value as pollinators in particular plants (e.g. oilseed rape Stanley *et al.* 2013, and onion Howlett *et al.* 2017). Therefore, in this study, no *a priori* expectations were made about the identity of the most effective pollinators to the focal plants.

However, while measuring pollinator effectiveness at the level of individual plants is reasonably common, only recently has the effectiveness of a community of flower visitors to multiple plant species been compared. Studies in Dorset heathland (Ballantyne *et al.* (2015), Israeli shrub (Ballantyne *et al.* 2017) and Kenyan savannah communities (Ballantyne *et al.* in prep) are the first to include measures of pollinator effectiveness into traditional flower visitation networks. In the most diverse communities (Israel and Kenya), the inclusion of pollinator effectiveness data increased the specialisation of the interactions (measured using H_2', from 0.55 in the Israeli flower visitation network to 0.62 in the pollinator importance network, and from 0.53 to 0.62 in Kenya) and decreased plant generality (Israel 3.97 to 3.33, Kenya 6.87 to 5.33). From this, the authors concluded that visitation networks underestimate the specialisation of plant-pollinator communities and encouraged more detailed information of interaction quality to be collected in a variety of habitats. Continuing this work in a garden provides the opportunity to test the assumption that pollinator effectiveness might be reduced in gardens where the morphological traits of exotic garden plants have not developed over evolutionary timescales in association with their visitors (e.g. Betts *et al.* 2015) and whether stigma morphology is closely related to the most effective pollinator (ensuring precise contact with the body).

5.1.2 Heterospecific pollen receipt in a diverse plant community

Many flower visitors in the garden were shown to be generalists in Chapters 3 and 4, suggesting the potential for high levels of heterospecific pollen transfer between plants (HPT, as in the urban study by Irwin *et al.* 2014) and a reduction in pollinator effectiveness (Larsson 2005). Although the diversity of pollen species on the bodies of flower visitors was low in the garden (Chapter 4), any heterospecific pollen that reaches the stigma incurs both paternal costs for the parent plant ("pollen discounting", Harder & Barrett 1995), and maternal costs for the receiving flower, by preventing conspecific pollen from attaching to the stigma (Caruso & Alfaro 2000), interfering with pollen tube growth (Arceo-Gómez & Ashman 2011, Celaya *et al.* 2015, Bruckman & Campbell 2016) and misappropriating ovules (Burgess *et al.* 2008). Even at low levels, heterospecific deposition on stigmas can reduce viable seed production (e.g. Briggs *et al.* 2016) and acts as a major selective pressure on floral morphology, since restricted flowers with smaller stigma surfaces can reduce heterospecific pollen receipt (Montgomery & Rathcke 2012).

The proportion of HPT appears to vary considerably between studies; recent pollen transfer networks have reported HPT ranging from 0.07% to 74% of the total deposition for 29 plant species (Montgomery & Rathcke 2012) and 0 to 66.8% in two plant communities by Fang and Huang (2013, 2016). On the other hand, HPT accounted for <10% of the total stigma pollen load in Tscheulin & Petanidou (2013) and Tong & Huang (2016); and for the three large community studies conducted by Ballantyne and co-workers the HPT means varied between 3 and 24% for bees, and 3 and 32% for non-bees (Willmer *et al.* 2017). There is much interest in understanding the impact on HPT from invasive plant species (e.g. Moragues & Traveset 2005, Jakobsson *et al.* 2008, Tscheulin & Petanidou 2013) although in the recent pollen transfer networks produced by Emer *et al.* (2015) the inclusion of invasive *Impatiens glandulifera* pollen on the stigmas of 40 neighbouring species had little effect on the structure of the network. Similarly, Tur *et al.* (2016) concluded that the benefits of sharing pollinators outweighed the costs of HPT. Despite this, little is known about the extent of HPT in garden communities, where garden plants with little or no shared evolutionary history have not developed floral morphology or phenology that decreases heterospecific pollen receipt (Morales & Traveset 2008).

5.1.3 The importance of selfing and geitonogamy

Any study of pollen deposition must consider the potential for ‘self’ pollen to reach the stigmas. When flower visitation rates are low, facultative selfing can act as reproductive assurance (Fausto *et al.* 2001, Brys & Jacquemyn 2011) however it risks inbreeding depression that may lead to traits which can reduce flower visitation (e.g. a reduction in corolla size, flower number, pollen quantity and quality, Carr *et al.* 2014). To avoid this, at least half of all angiosperms demonstrate some degree of self-incompatibility (responses to prevent self-fertilisation, reviewed by Takayama & Isogai 2005) although this does not prevent self-pollen interfering with the growth of outcross pollen tubes (Kawagoe & Suzuki 2005).

Selfing is particularly relevant to the study of garden plant varieties, as characteristics that increase selfing (e.g. an increase in pollen production and anther number, or decreased anther-stigma proximity) can develop during artificial selection on floral characteristics (Lendvai & Levin 2003). Furthermore, the incidence of geitonogamy (transfer of pollen within flowers on the same plant) may be particularly high in garden plants, commonly selected for having a large number of open flowers and individuals are often dispersed sporadically in fragmented environments. For example, although little is known about garden plants selfing rates can be increased with the number of open flowers on the same plant (Eckert 2000) and visitors are known to probe more flowers on a single plant when the population size of a plant is small (Mustajarvi *et al.* 2001). Recent studies have also speculated on the possibility that insect-pollinated plants may evolve greater selfing rates in urban areas in response to human disturbance (Aguilar *et al.* 2006, Eckert *et al.* 2010) if the abundance and diversity of plants (and therefore mate availability) and of pollinators is reduced (Bates *et al.* 2011).

5.1.4 Managed versus wild bees

The differences in the total importance of managed and native bees as pollinators is often based on variation in flower visit frequency, with managed or invasive species outcompeting their native counterparts purely in terms of visit frequency (Madjidian *et al.* 2008, Rader *et al.* 2009, 2012, Aslan *et al.* 2016), despite native and non-managed bees depositing significantly more pollen during a single-visit (e.g. Thomson & Goodell 2001, Bruckman & Campbell 2014, Zhang *et al.* 2015). Given concerns that the role of honeybees can be overplayed (Ollerton *et al.* 2012) comparisons between the role of *Apis* and non-managed bees in visitation and pollinator effectiveness/importance networks (such as those by Ballantyne *et al.* 2015, 2017) are needed to test these predictions and assess whether traditional flower visitation networks over-exaggerate the importance of *Apis* as pollinators at a community-level.

5.1.5 Key questions

In this chapter, pollinator effectiveness and importance networks are constructed to examine the variation between flower visitors in the quantity and quality (proportion conspecific) of pollen deposited on to the stigma. Evaluating pollination from the perspective of the female function of flowers, the questions are:

1. What are the similarities and differences between a pollinator effectiveness and an importance network compared to a traditional visitation network?

2. Which flower visitors are the most effective and important pollinators?

3. How diverse are stigmatic pollen loads in a garden?

5.2 Methods

5.2.1 Recording single visit stigma deposition (pollinator effectiveness)

Unvisited flowers were bagged in thin mesh flower cages, supported by thin metal rods before the stigma became morphologically receptive (see **Appendix 5.1** for design). Although stigma receptivity can be measured in several ways (see Dafni *et al.* 2005) some are unreliable and morphological changes are most suitable for field studies. In most plants the flowers were bagged as buds. Once the stigma appeared receptive, the bag was removed and the flower observed until visited. Visitors were not disturbed whilst foraging and the stigmas were removed immediately after the insect had left the flower; the 'static' approach, according to Howlett *et al.* (2017). The stigma was swabbed with a $3mm^3$ square of fuchsin gel, which was melted onto a clean slide and the pollen grains counted as in Chapter 4 (see Kearns & Inouye 1993). Following the guidelines of Ne'eman *et al.* (2010) and methods of Ballantyne *et al.* (2015, 2017) the average number of conspecific grains deposited by an insect on to the stigma of a particular plant constituted the measure of pollinator effectiveness (PE), and total pollinator importance (PI) was calculated by multiplying PE by the total number of visits recorded between a flower visitor and plant.

5.2.2 Measuring pollen deposition in control flowers

While others have emasculated flowers to eliminate self-contamination (e.g. Castro *et al.* 2013, Bruckman & Campbell 2014, Howlett *et al.* 2017) this was avoided to ensure: (i) that data were collected for visitors foraging for pollen, (ii) that the behaviour of visitors on the flower represented the natural state (many visitors were observed to use the stamens for support whilst foraging) and (iii) that emasculation did not affect the position of the stigma (Richardson 2004). Consequently, in many bagged flowers the anthers were in full or partial dehiscence when sampled and self-pollen transfer was therefore an issue that had to be controlled for.

The average number of pollen grains per unvisited stigma was calculated as evidence of self-pollen transfer either naturally or by handling. For each of the focal plant species, flowers were bagged and handled in the same way as those used to calculate pollinator effectiveness, and then the stigmas were sampled prior to any insect visitation. The average pollen deposited onto virgin stigmas was calculated separately for each plant species.

5.2.3 Comparing methods for control values

To be classified as an 'effective pollinator', a flower visitor should deposit more pollen than found on unvisited control flowers. Initially, the effectiveness of each flower visitor was compared to control flowers using a general linear model (GLM, with control flowers as the intercept) for each plant species, and only flower visitors that deposited significantly more pollen than control flowers remained in the pollinator effectiveness network. Similar non-parametric, multiple comparisons have frequently been used to compare flower visitors to control flowers (e.g. Richardson 2004, Bischoff *et al.* 2013, Zych *et al.* 2013) as these account for considerable variation in the quantity of autogamous pollen found on control flowers of the same plant species (e.g. between 0-320 in *Chilopsis linearis*, Richardson 2004 and 340,000±40,300 in Zych *et al.* 2013) and variation in the pollen deposited by visitors.

However, when this method was used for the garden plants, it was very conservative and many flower visitors, especially those that were rare (and thus the sample size was small), did not deposit significantly more pollen than found on control stigmas, thus rendering them 'ineffective pollinators' to the focal plants. While this increased the differences between the visitation and PE network, it seemed to me to be biologically inappropriate to exclude flower visitors as pollinators based on small sample sizes alone. Others have excluded data from visitors that are only ever recorded once (e.g. Bischoff *et al.* 2013); however, this neglects the possibility that a very rare flower visitor may still be a pollinator.

Subsequently, in favour of biological relevance, the average numbers of conspecific grains found on the unvisited (control) flowers were subtracted from the total number found on visited flowers (as in King *et al.* 2013, Ballantyne *et al.* 2015, 2017). I acknowledge this is a more generous estimate of a flower visitor's effectiveness as a pollinator and may reduce the differences between the visitation and pollinator effectiveness networks. The results from the alternative GLM analysis are given and the implications discussed in Section 5.4.1.

5.2.4 Creating pollinator effectiveness and pollinator importance networks

Two networks were created from single visit stigma deposition measures and included only the pollen belonging to the 24 focal plants: (i) a pollinator effectiveness network (PE), where the interactions were weighted only by the average number of grains deposited during a single visit and (ii) a pollinator importance network (PI), where the average number of grains was multiplied by the total number of visits recorded. Both measures of PE and PI are in accordance with Ne'eman *et al.* (2010) and Ballantyne *et al.* (2015, 2017). As the PI network incorporates both measures, this is assumed to be the most reliable estimate of the value of flower visitors from the plant's perspective. The sampling effort and flower abundances varied between plants, outlined in Chapter 3. As in Chapter 4, the visitation network used for comparisons between the PE/PI networks includes only the visits for which PE/PI data were also available, therefore comparisons between the networks are made between an identical set of links.

5.2.5 Statistical analysis

(i) Comparing species-level indices in the networks

Plant species specialisation (*d'*) values were calculated for each plant in each of the three networks. These values were then compared between the networks using a generalised linear mixed model (GLMM, with a Gaussian error distribution) using the function *glmer* in the package *lme4* (Bates *et al.* 2015) with plant species as a random effect. GLM analysis was selected as a normal distribution of the residuals could not be achieved using an ANOVA, therefore a GLMM was used to incorporate repeated measures from the same plants. As proportions (0-1), all *d'* values were arcsine square-root transformed before testing. Pairwise comparisons between networks were made using a post-hoc Tukey HSD using the *glht* function in the package *multcomp* (Hothorn *et al.* 2008).

Similarly, the species strengths of all flower visitors were compared between the networks using a GLMM (with a gamma error distribution) including visitor species as a random effect. Species strength values were x+1 transformed prior to testing to achieve a better model fit.

Pairwise comparisons between the networks were again made using a post-hoc Tukey HSD. For both species *d'* and strength, the models were validated by inspection of the distribution of the deviance residuals (for a parametric distribution) and the absence of any pattern in the fit between the Pearson and fitted residuals (Thomas *et al.* 2013).

(ii) Correlation between pollinator effectiveness and pollinator importance

To determine the strength of the relationship between measures of pollination (PE and PI) the value of each visitor to the focal plants were calculated, as a proportional value relative to all other visitors to the same plant in the network (as in Vázquez 2005 and Alarcón 2010). As proportions all values were arcsine square-root transformed before testing. Using these values, a simple regression was performed between the PE and PI values per plant for each visitor using the function *lm* in *R*. Correlations were tested using a Pearson's rank correlation (r) as the data complied to a normal distribution.

To test whether the strength of the relationship between PE and PI varied between the 8 functional groups of flower visitors (*Apis, Bombus*, other bees, Lepidoptera, hoverfly, other Diptera, Coleoptera and wasps) flower visitor group was introduced as a factor in the regression (i.e. PE~PI*Visitor, Stone *et al.* 2011).

(iii) Comparing the effectiveness and importance of flower visitors

To determine which flower visitors were the most effective and important at the community level, the proportional PE and PI values for each visitor to all of the plants they visited were combined. The average proportional effectiveness and importance of flower visitors (arcsine square-root transformed prior to testing) were compared using a GLM (with a gamma error distribution and log link function) in the package *mass* (Venables & Ripley 2002). Comparisons between flower visitors were made using a pairwise post-hoc Tukey HSD.

(iv) Comparisons of heterospecific pollen deposition between plants and visitors

The number of heterospecific pollen transfer events and 'failed visits' was compared between plants and between flower visitors using a Chi-squared contingency test. However, the results should be interpreted with some caution, as the sample sizes differ considerably between plants and visitors.

5.3 Results

To compare the effectiveness of garden pollinators, 2,831 stigmas belonging to 24 species were sampled after visitation from 39 groups of flower visitors. Conspecific pollen was recovered from more than 85% of stigmas following the first visit (n=2,430) and a total of 678,799 pollen grains were counted. This section presents (i) how the structure of a PE and PI network compares to a visitation network, (ii) which flower visitors are the most effective and important pollinators and (iii) the diversity of pollen received on stigmas in a garden.

5.3.1 Comparisons between visitation, pollinator effectiveness and pollinator importance networks

(i) At the community level

In total, less than 10% of all links (n=11) and only a single visitor (the cleptoparasitic bee *Melecta*) were lost from the visitation network (V) in the pollinator effectiveness (PE) network, as the majority of visitors for which data were available transferred some pollen (**Fig. 5.1**). Consequently, the size of the V and PE networks were similar (link number V=188, PE=177) as was the specialisation of the interactions (H_2' V=0.49, PE=0.48). This was also true for connectance (V=0.12, PE=0.13), interaction evenness (V=0.71, PE=0.74) and modularity (V=0.45, PE=0.40), with no suggestion of the large differences in network structure expected if many flower visitors did not deposit pollen during the first visit. However, slight changes occurred in the nestedness of the interactions (V=23.42, PE=18.42); and in the PE network, the generality of both flower visitors (V=7.89, PE=8.20) and plants (V= 4.99, PE=5.67) increased despite the loss of 11 links. Greater equality in pollen deposition between rare and frequent visitors may explain this increase in generality.

However, in the pollinator importance (PI) network (when the PE interactions were weighted to include flower visitation frequency) the specialisation of the interactions increased (H_2'=0.54) and the generality of both visitors (7.17) and plants (4.20) declined (**Fig. 5.1**). This suggests that the inclusion of the additional measure of PE to visitation networks does alter the structure of the network; throughout the community, some interactions strengthened whilst others weakened, so that overall plant generality was reduced, and the estimated tolerance of plants to disturbance altered.

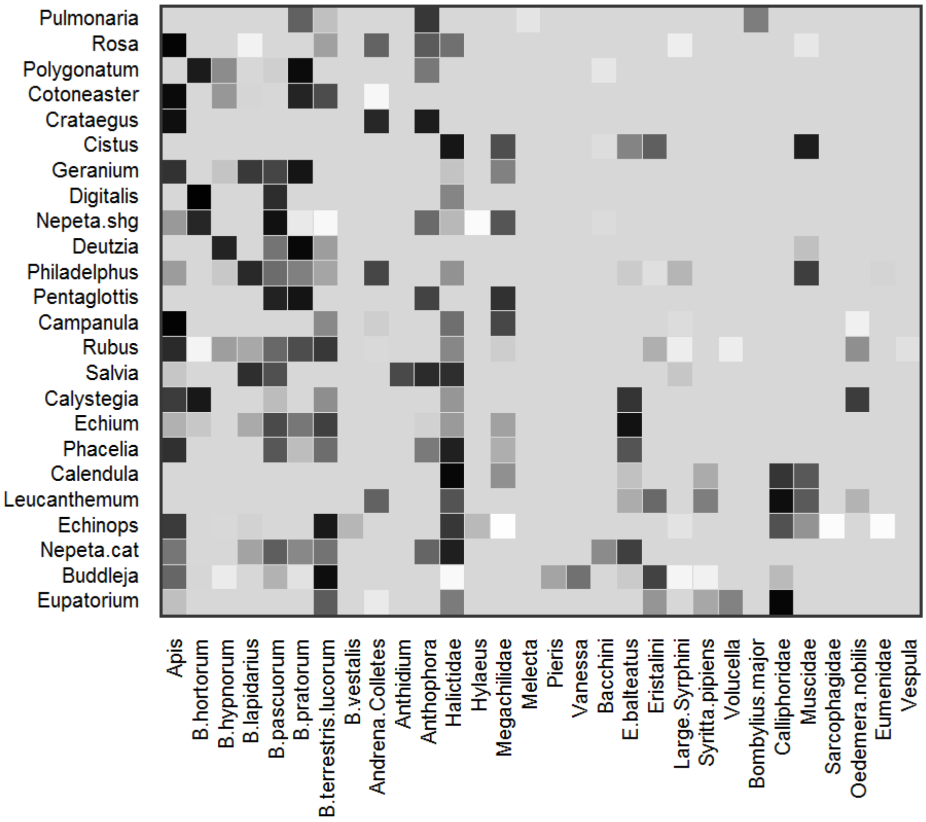
Yet the observed increase in specialisation and decrease in generality was smaller than expected, and at a community level the V and PI networks were remarkably similar;

connectance in the PI network remained similar to V and PE (0.11), nestedness in the PI network fell between estimates for the V and PE networks (23.03), interaction evenness decreased slightly (0.69) and modularity increased only slightly (0.47).

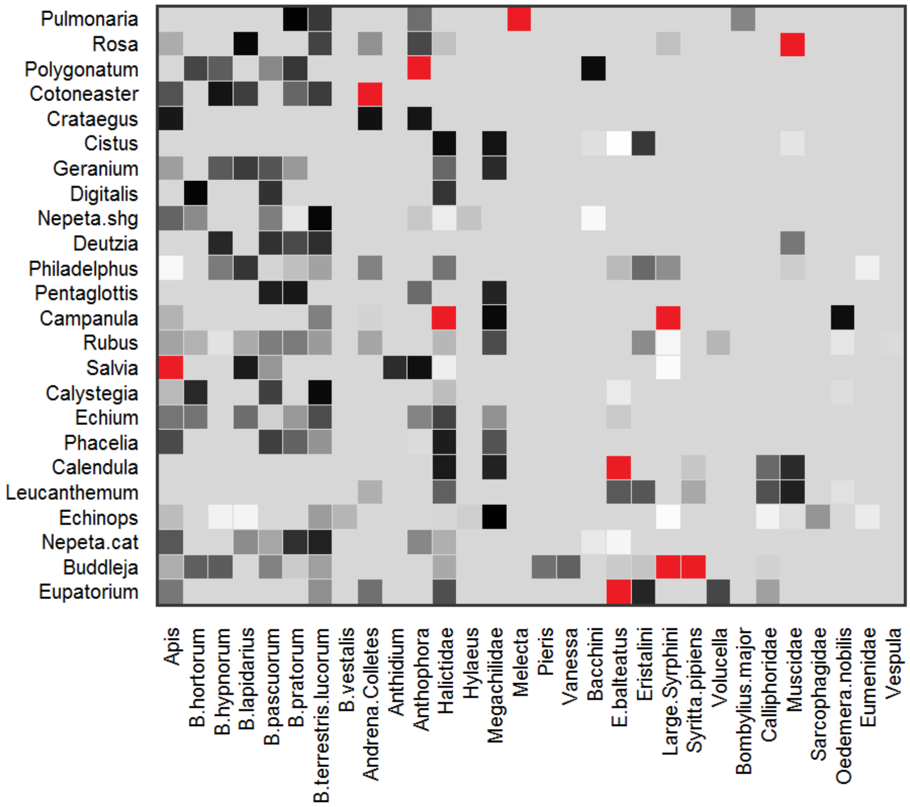
Alternative statistical methods for comparing control and visited flowers

As expected, greater differences between the visitation and pollinator importance networks were observed when the more conservative approach was used to compare visitor deposition to unvisited flowers (Section 5.2.3). When this approach was taken, 79 links were lost (3,284 visits) resulting in a much smaller pollinator importance network (links=109) where four visitors (*Vespula*, Eumenidae, Sarcophagidae and *Bombylius*) and one plant (*Polygonatum*) were lost completely (**Fig. 5.2, Table 5.1**). A reduction in size meant that the alternative pollinator importance network was substantially more specialised compared to the visitation network (H_2'=0.67). Nestedness also fell dramatically (11.51) and generality was considerably reduced, so that plants interacted with a weighted mean of 5.11 partners, and flower visitors only pollinated a weighted mean of 3.28 plants (**Table 5.1**). Connectance in the alternative pollinator importance network declined to a lesser extent (0.09). The considerable loss of links in this network (particularly in cases where visitation and therefore sample sizes were low) was deemed too extreme to be biologically meaningful, so the alternative pollinator importance network was not used in network comparisons.

A) Traditional visitation



B) Pollinator effectiveness



C) Pollinator importance

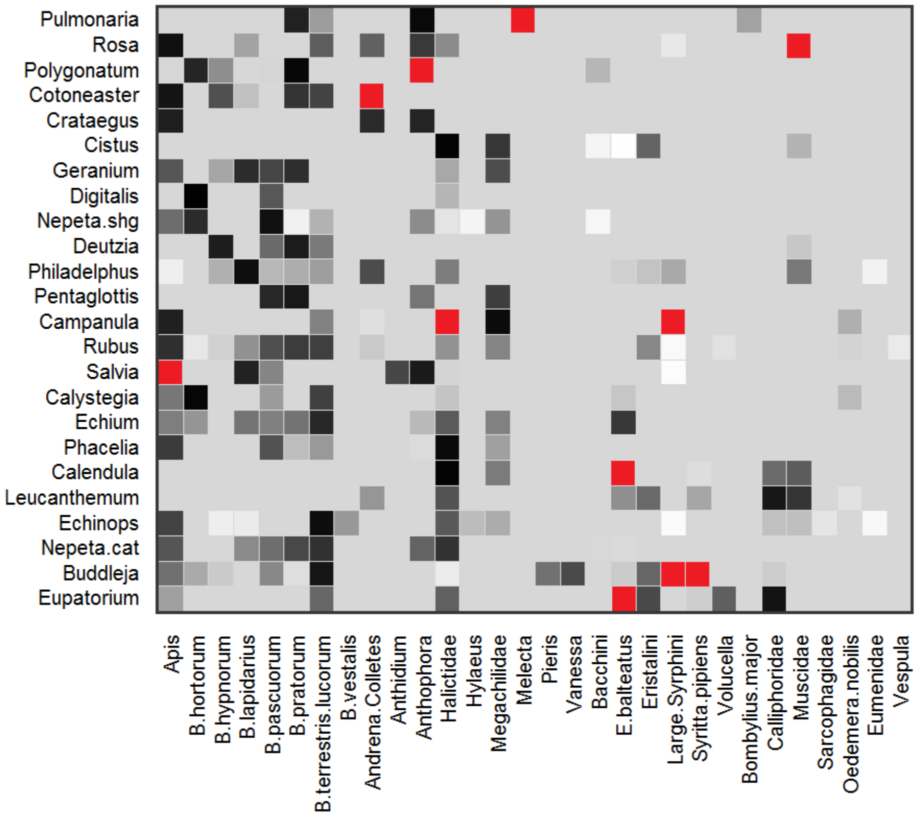


Figure 5.1 Bipartite matrices illustrating traditional visitation, pollinator effectiveness and pollinator importance networks. Flower visitation frequency and the number of pollen grains deposited onto a virgin stigma during a single visit were used to calculate the proportional values for visitation and pollinator effectiveness. When combined, the pollinator importance network indicates the total effect of the pollinator on plant fitness. Only 11 links were lost from the visitation network in the pollinator effectiveness network, as almost all visitors deposited some pollen on to the stigma. However, the proportional pollinator effectiveness of a visitor did not always match it’s proportional visitation; the strength of several interactions became weaker in the pollinator effectiveness network (shown by the shade of the interaction becoming lighter). An example of this was *Apis* for many of the plants visited. Results shown include all of the pollinator effectiveness data collected over two summers for 24 garden plants. Plants are organised in order of flowering time (early to late summer, top to bottom) with visitors organised in groups (left to right). Red squares indicate visitors that were ineffective pollinators, depositing less pollen, on average, than found on control stigmas. All interactions are proportional (the value of each visitor is relative to all other visitors to a plant).

Pollinator importance (more conservative)

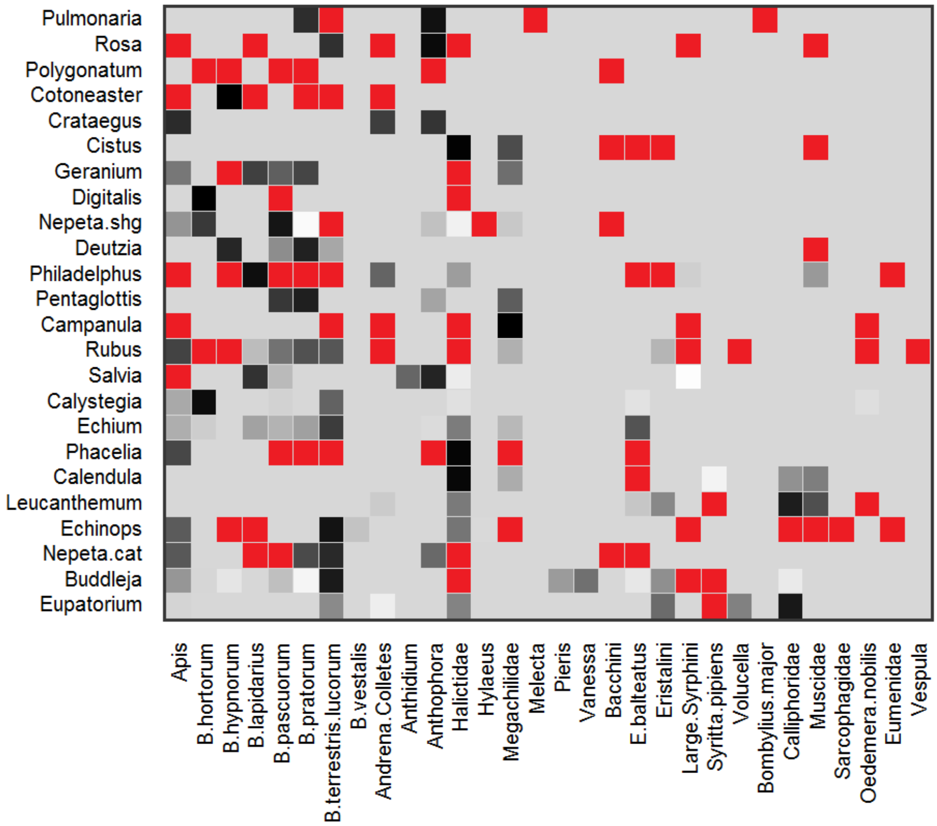


Figure 5.2 Bipartite matrix illustrating the results of the alternative pollinator importance network, where flower visitors were excluded as pollinators if they did not deposit significantly more pollen on average than found on control flowers. The mean pollinator effectiveness (single visit deposition) of a visitor was compared to the mean pollen recovered from unvisited stigmas belonging to a specific plant, using a GLM with control flowers as the intercept. In many cases, the difference was not significant due to high variation and relatively small sample sizes. This alternative pollinator importance network was much more conservative and resulted in the loss of 79 links compared to the less conservative pollinator importance network (Fig. 5.1). Consequently, the specialisation of this network was much higher than the original network. However, the differences were assumed not to be biologically meaningful, so all comparisons hereafter between visitation and pollinator effectiveness/importance networks used the method of subtracting the mean control value from the mean single visit deposition for each visitor. Plants are organised in order of flowering time (early to late summer, top to bottom) and flower visitors in groups (left to right).

		Visitation	Pollinator effectiveness	Pollinator importance	Alternative pollinator importance
Species richness	**Visitors**	30	29	29	25
	Plants	24	24	24	23
	Links	188	177	177	109
	Visits	12,877	-	12,694	9,593
Generality	**Visitors**	7.89	8.20	7.17	3.28
	Plants	4.99	5.67	4.20	5.11
Distribution of interactions	**C**	0.12	0.13	0.11	0.09
	IE	0.71	0.74	0.69	0.66
	NODF	23.42	18.42	23.03	11.51
	H_2'	0.49	0.48	0.54	0.67
	M	0.45	0.40	0.47	0.56

Table 5.1 Summary of the community-level indices for visitation, pollinator effectiveness, pollinator importance and alternative pollinator importance networks. Network metrics were calculated using the function *networklevel* in the package bipartite. Although the pollinator effectiveness network had little effect on network specialisation (H_2') the combination of both flower visit frequency and pollinator effectiveness increased the specialisation of the interactions in the pollinator importance network, although the change was relatively small. I deemed the differences between the traditional visitation network and the alternative pollinator importance network to be too extreme to be biologically meaningful, although variation in the quantity of pollen deposited by flower visitors is likely to increase the specialisation of individual flowers. Results are based on all pollinator effectiveness data collected over two summers for 24 focal plants, and all networks were calculated using proportional values.

(ii) At the level of individual species

Plant specialisation (d') and flower visitor species strength for the visitation, pollinator effectiveness and pollinator importance networks were compared using a GLMM, with pairwise post-hoc Tukey tests between networks (Section 5.2.5). Full test results are given in **Appendix 5.2.**

While H_2' increased in the PI network, the average specialisation of plants in the PI network was fairly low (d' 0.41±0.03, n=24) and this network was not significantly more specialised than in the V network (d' 0.38±0.02, n=24, p=0.32) nor the PE network (d' 0.38±0.03, n=24, p=0.93). However, plants in the alternative PI network were significantly more specialised (d' 0.51±0.03, n=23) than those in all aforementioned networks (p<0.001 in each case). Interestingly, although *Rubus* was the most generalised plant in many of the networks, the identity of the most specialised plant varied, with the greatest range in plant specialisation seen in the alternative PI network. The range of plant *d'* in each of the other networks remained similar (**Table 5.2**).

The average species strength of flower visitors in the PI network (0.80±0.19, n=30) was no different to that in the PE network (0.83±0.16, n=30, *p*=0.49) or the V network (0.80±0.18, n=30, *p*=0.83). Visitors in the alternative PI network (0.92±0.19, n=25) did not have a significantly greater species strength than in the PI network (*p*=1.00) PE network (*p*=0.54) or visitation network (*p*=0.85). However, the identity of the visitors with the smallest and greatest species strength did vary between the various networks, with the greatest range between visitors seen in the visitation network (**Table 5.2**). In both the V and PI network, *Apis* had the greatest species strength.

Network	**Plants**		**Visitors**	
	Specialisation (*d'*)	**Species range (min and max)**	**Species strength**	**Species range (min and max)**
Visitation	0.38 ± 0.02 (n=24)[a]	*Rubus* (0.16) *Pulmonaria* (0.63)	0.80 ± 0.18 (n=30)[a]	Sarcophagidae (0.003) *Apis* (3.57)
Pollinator effectiveness	0.38 ± 0.03 (24)[a]	*Rubus* (0.12) *Crataegus* (0.60)	0.83 ± 0.16 (30)[a]	*Vespula* (0.03) *B.terrestris/ lucorum* (2.76)
Pollinator importance	0.41 ± 0.03 (24)[a]	*Rubus* (0.13) *Digitalis* (0.60)	0.80 ± 0.19 (30) [a]	Eumenidae (0.003) *Apis* (2.90)
Alternative pollinator importance	0.51 ± 0.03 (23)[b]	*Echium* (0.23) *Cotoneaster* (0.89)	0.92 ± 0.19 (25) [a]	Eumenidae (0.001) Halictidae (2.80)

Table 5.2 Summary of the species-level indices for visitation, pollinator effectiveness, pollinator importance and alternative pollinator importance networks. Species level indices were calculated using the function *specieslevel* in the package bipartite. The specialisation (*d'*) of plants and species strength of visitors were used as the pollinator effectiveness data are used here to evaluate the strength of the interactions from the plant's perspective. Although the identity of most specialised plant changed between the networks, as did the flower visitor with the greatest species strength, no significant difference was found between the mean plant specialisation (*d'*) or flower visitor species strength. Results are based on all of the pollinator effectiveness data collected over two summers. All flower visitors including those with '0' species strength in the pollinator effectiveness network were compared between networks using a GLMM. Full results of the test are given in Appendix 5.2.

5.3.2 Which flower visitors are the most effective and important pollinators?

To compare the value of flower visitors in the garden, all insects were divided into 8 functional groups. These groups were compared using (i) the identity of the species contributing most to visitation, pollen loads and pollination, (ii) the strength of the correlation between PI and PE for each visitor, and (iii) the overall PE and PI for each visitor to the garden plants.

(i) The identity of the species contributing most to visitation, pollen loads and pollination

In almost all plants, a species or family of bee (e.g. *Bombus* spp. or Megachilidae) was the most frequent visitor, transported the most pollen and was the most important pollinator (**Table 5.3**). Exceptions to this were *Eupatorium*, *Leucanthemum* and *Echium* where dipteran visitors were the most frequent visitors, and in *Eupatorium* and *Leucanthemum,* Calliphoridae were also the most important pollinators (whereas the many flies visiting *Echium* deposited rather little pollen). In 22 of the focal plants, the visitor with the greatest pollen transport was also the most important pollinator; although this visitor did not always carry the greatest pollen load nor deposit the most during a single visit. For example, Calliphoridae exceeded all other visitors in terms of pollen transport and pollinator importance, purely on the basis of their frequency as visitors to *Eupatorium* flowers. Interestingly, two of the plants well-known for invading urban spaces (*Buddleja* and *Cotoneaster*) were most frequently visited and pollinated by two very common garden bees (*B.terrestris/lucorum* and *Apis* respectively).

Although it was rare that the most frequent visitor deposited the most pollen (only 7 plants, **Table 5.3**); nevertheless, the most frequent visitor was often the most important pollinator. In only four cases was this untrue: *Apis* was the most frequent visitor to *Campanula,* but rarely contacted the stigma unlike the larger but less frequent Megachilidae. For *Echium*, *E.balteatus* visited *en masse* yet often only contacted the anthers whilst consuming pollen, compared to the more effective visits by *B.terrestris/lucorum*. Likewise, halictid bees were frequent visitors to *Nepeta cataria,* but often failed to contact the stigma when collecting pollen from the anthers, such that *B.terrestris/lucorum* also exceeded them in terms of pollinator importance. Finally, *B.pratorum* was a frequent visitor to *Geranium* but deposited only about half (6.36±1.69, n=47) of the grains compared to the rarer *B.lapidarius* (14.45±9.26, n=11). Interestingly, *Apis* was never found to carry the single greatest pollen load nor to be the single most effective pollinator, despite being the most important pollinator to three plants with morphologically relatively open and ‘generalist’ flowers (*Cotoneaster, Rosa* and *Rubus*).

(ii) The strength of the relationship between PE and PI for visitors

To evaluate how well the measure of single visit pollen deposition (PE) predicted total pollinator importance (PI), a visitor’s proportional PI to a plant was correlated with their equivalent proportional PE (Section 5.2.5); pollinator effectiveness explained only 38% of the variation in pollinator importance (i.e. $r^2=0.38$, **Fig. 5.3**) as visitors from several taxa that were very effective pollinators at the single-visit level (high PE, >0.4) had their overall importance as pollinators reduced by low visitation rates (low PI, <0.3). On the other hand, several taxa, including *Apis*, were relatively ineffective pollinators (low PE, <0.2 **Fig. 5.3**) yet visited flowers frequently enough to increase their overall importance as pollinators (high PI, >0.4). As pollinators, the majority of Diptera (including hoverflies) had relatively low pollinator effectiveness and importance and were therefore well below the 95% confidence interval for other visitors (**Fig. 5.3**). Although the spread of the data was greater for some visitors (e.g. *Bombus*) there was no significant difference in the relationship between PE and PI between different flower visitors (for full results see **Appendix 5.3**).

	Visitation	Pollen load		Pollination	
Plant	**Most frequent visitor**	**Visitor with the greatest pollen load**	**Visitor with the greatest pollen transport**	**Most effective pollinator(s)**	**Most important pollinator**
Pulmonaria	*Anthophora*	*B.pratorum*	*Anthophora*	*B.pratorum*	*Anthophora*
Salvia	*Anthophora*	*Anthidium*	*Anthidium*	*B.lapidarius, Anthophora*	*Anthophora*
Campanula	*Apis*	Megachilidae	Megachilidae	Megachilidae	Megachilidae
Crataegus	*Apis*	*Andrena*	*Andrena*	*Apis, Andrena* and *Anthophora*	*Apis, Andrena* and *Anthophora*
Cotoneaster	*Apis*	*B.terrestris/lucorum, Andrena*	*Apis*	*B.hypnorum*	*Apis*
Rosa	*Apis*	*B.lapidarius*	*Apis*	*B.lapidarius*	*Apis*
Rubus	*Apis*	*Apis, B.hortorum, B.terrestris/lucorum*	*Apis*	Megachilidae, *B.pratorum, B.pascourum*	*Apis*
Calystegia	*B.hortorum*	Halictidae	*B.hortorum*	*B.terrestris/lucorum*	*B.hortorum*
Digitalis	*B.hortorum*	*B.hortorum*	*B.hortorum*	*B.hortorum*	*B.hortorum*
Philadelphus	*B.lapidarius*	*B.lapidarius*	*B.lapidarius*	*B.lapidarius*	*B.lapidarius*
Nepeta ‘S.H.G.’	*B.pascuorum*	*Anthophora*	*B.pascuorum*	*B.terrestris/lucorum*	*B.pascuorum*
Deutzia	*B.pratorum*	*B.pratorum*	*B.pratorum*	*B.hypnorum, B.terrestris/lucorum* and *B.pascuorum*	*B.pratorum* and *B.hypnorum*
Geranium	*B.pratorum*	*B.lapidarius, B.pratorum*	*B.pratorum*	*Megachilidae*	B.lapidarius
Pentaglottis	*B.pratorum*	*B.pratorum*	*B.pratorum*	*B.pascuorum, B.pratorum,* Megachilidae	*B.pratorum*
Polygonatum	*B.pratorum*	*B.hypnorum, B.pascuorum, B.pratorum*	*B.pratorum*	Bacchini	*B.pratorum*
Buddleja	*B.terrestris/lucorum*	*B.pascuorum*	*B.terrestris/lucorum*	*B.hortorum* and *B.hypnorum*	*B.terrestris/lucorum*
Echinops	*B.terrestris/lucorum*	*B.hypnorum*	*B.terrestris/lucorum*	*Megachilidae*	*B.terrestris/lucorum*
Leucanthemum	Calliphoridae	*Andrena*	*Andrena*, Calliphoridae	Muscidae	Calliphoridae
Eupatorium	Calliphoridae	*B.terrestris/lucorum, Andrena, Volucella, Apis*	Calliphoridae	*Eristalini*	Calliphoridae
Echium	*E.balteatus*	*B.hortorum, B.pratorum, B.terrestris/lucorum, Anthophora,* Halictidae	*B.terrestris/lucorum*	Halictidae, *B.terrestris/lucorum, B.lapidarius, B.hortorum, Apis*	*B.terrestris/lucorum, E.balteatus*
Calendula	Halictidae	Halictidae	Halictidae	Halictidae and Megachilidae	Halictidae
Cistus	Halictidae	Halictidae	Halictidae	Halictidae and Megachilidae	Halictidae
Nepeta cataria	Halictidae	*B.terrestris/lucorum,* Halictidae, *Apis, Anthophora*	Halictidae	*B.terrestris/lucorum, B.pratorum*	*B.terrestris/lucorum*
Phacelia	Halictidae	*Anthophora,* Halictidae, Megachilidae	Halictidae	Halictidae	Halictidae

Table 5.3 The identity of the flower visitor(s) with the greatest proportional contribution to flower visit frequency, pollen load, pollen transport, pollinator effectiveness and pollinator importance. In almost all cases, the flower visitor that made the greatest proportional contribution to either an indirect (visitation and pollen load) or direct measure of pollination was a bee, with the exception of visitors listed in red.

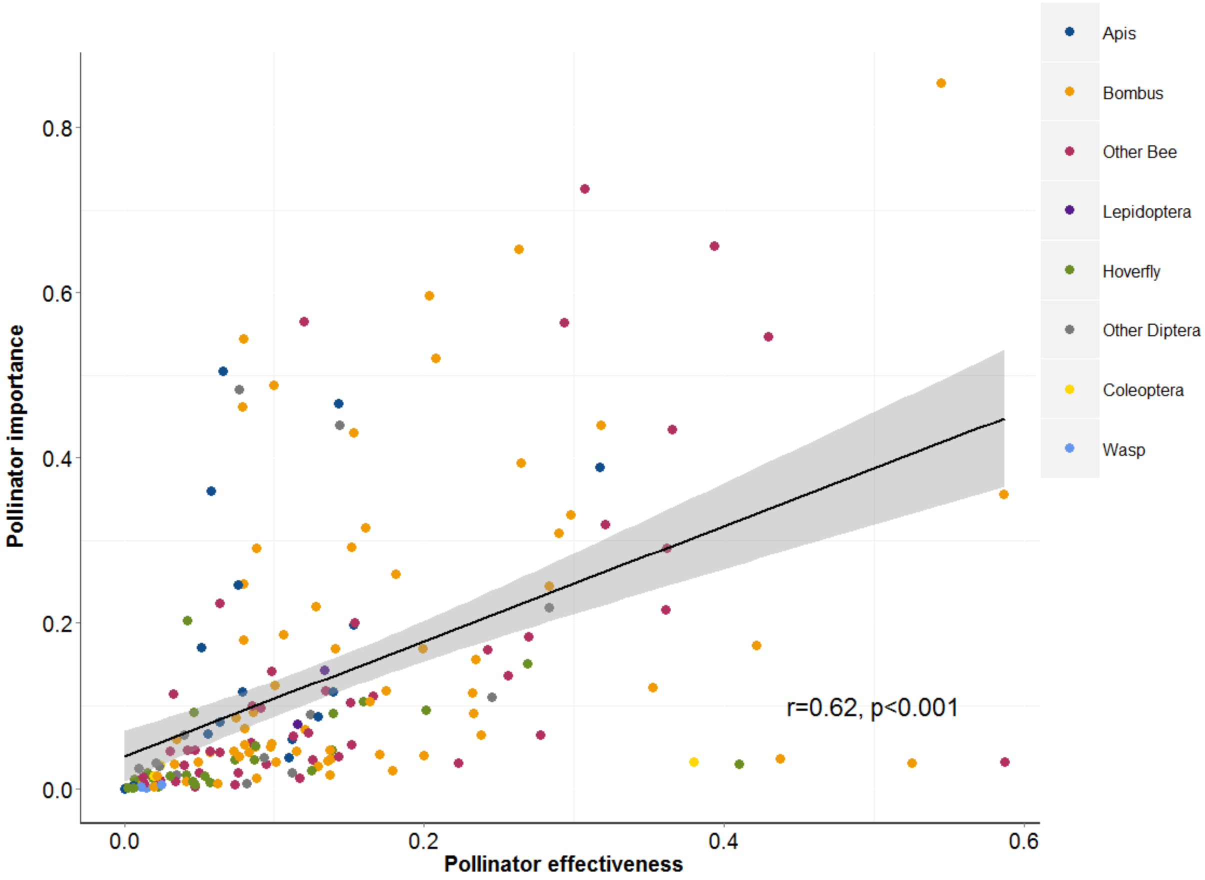


Figure 5.3 Relationship between pollinator importance and pollinator effectiveness. To compare the relationship between pollinator effectiveness (a measure of per visit effect) to pollinator importance (total effect) the proportional values for each visitor to a plant were plotted against each other and the relationship test with a Pearson's Rank correlation. In general, pollinator importance increased as pollinator effectiveness increased ($F_{(1,186)}=113.2$, $p<0.001$, $r^2=0.38$) however, values for some visitors fell outside the 95% confidence interval (shown as the shaded area). In these cases, visitors with high single visit deposition (pollinator effectiveness) were relatively infrequent and consequently had low pollinator importance. These visitors included *Bombus* and a non-eusocial bee. Results include all pollinator effectiveness data collected over two summers to 24 plants.

(iii) Overall PE and PI for visitors to the garden plants

To compare the 8 groups of flower visitors in terms of pollinator effectiveness and importance, the proportional PE and PI values were compared using a GLM with post-hoc tests to reveal differences between groups (Section 5.2.5).

On average, *Bombus* had the highest proportional pollinator effectiveness per plant (0.16±0.15, number of links=67) although this was not significantly greater than either the non-eusocial bees (0.15±0.19, n=50, *p*=0.99) nor *Apis* (0.09±0.19, n=16, *p*=0.68, **Fig. 5.4a**). All non-bee visitors performed similarly to at least one group of bees in terms of PE. However, hoverflies (0.07±0.16, n=32) and wasps (0.02±0.05, n=3) were significantly less effective pollinators at the community level compared to *Bombus* (*p*<0.01) and non-eusocial bees (*p*<0.01), although the number of interactions recorded from these groups was much lower.

Apis had marginally the highest average pollinator importance (0.18±0.41, n=16, **Fig. 5.4b**) although this was almost identical with *Bombus* visitors (0.17±0.23, n=67, *p*=1.00) and the non-eusocial bees (0.14±0.22, n=50, *p*=0.97). Again, only the hoverflies (0.34±0.09, n=32) and wasps (0.03±0.01, n=3) were significantly less important pollinators compared to bees (*p*<0.01 in each case, **Fig. 5.4b**). Although differences in the frequency of interactions between bees and non-bee visitors makes it difficult to compare PE and PI, these results do suggest that dipteran flower visitors are substantially less important than bees to a community-wide assembly of garden plants. Full test results are given in **Appendix 5.4**.

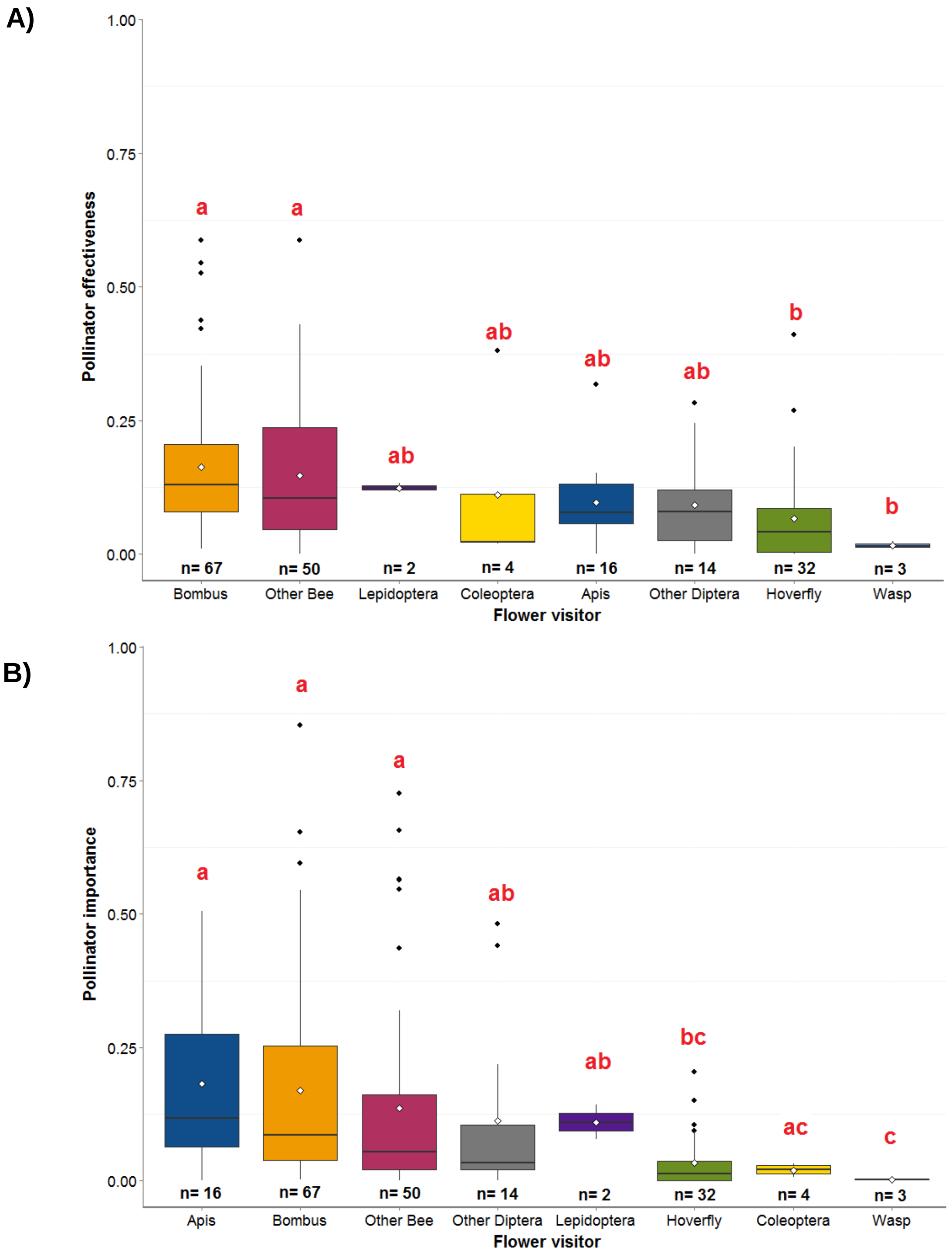


Figure 5.4 The variation between 8 groups of flower visitors in the overall contribution to A) pollinator effectiveness and B) pollinator importance to a community of plants. To compare the value of different flower visitor groups in terms of pollinator effectiveness and pollinator importance, the proportional values of visitors for all plants in the community were summed and the means compared. *Bombus* and non-eusocial bees had the highest values of pollinator effectiveness, although *Apis* exceeded all bees in terms of pollinator importance (the product of flower visit frequency) (ns). Bees exceeded the value of non-bee visitors in both cases, with no significant difference between the two groups of Diptera (syrphid and non-syrphid). Results include all of the pollinator effectiveness data collected over two summers to 24 plants. Shared characters indicate no significant difference between groups. The full results of both GLMs and pairwise comparisons are given in Appendix 5.6.

5.3.3 How diverse are stigmatic pollen loads in a garden?

Given the interest in heterospecific pollen transfer (HPT) in urban plant communities, all pollen samples from stigmas were inspected for HPT (including pollen belonging to plant species outside of the 24 focal plants).

Pollen received from insect visitation

In most cases, the pollen deposited on to the stigma during the first visit contained only conspecific grains (70.2%, n=1,988, **Fig. 5.5**). Only 15.6% (n=442) of stigmas received heterospecific pollen, despite 46.3% of flower visitors carrying between 2 and 4 pollen types (Chapter 4). Although 88 types of heterospecific pollen were recorded from these stigmas (72 of which were ‘alien’ and did not belong to any of the focal plant species) the number of heterospecific grains per stigma was typically low (2.94±0.37, n=442) and accounted for only 5.91±0.43% (n=442) of the average stigmatic pollen load. Stigmas that did not receive any pollen (12.5%, n=354) and those that received only heterospecific pollen (1.7%, n=47) were rare. This suggests that while unidentifiable ‘alien’ grains may be a difficulty for studying the movement of pollen in gardens (where many plants are non-native), stigma clogging and reduced fitness from heterospecific grains may be less of a problem than expected.

The proportion of stigmas receiving heterospecific pollen during the first visit are shown for individual genera of garden plant in **Figure 5.6a**, and ranged from less than 5% of all stigmas sampled (e.g. *Rubus, Eupatorium*, *Buddleja*, *Phacelia* and *Echium*) to over 50% (*Echinops* and *Geranium*). Although differences in the number of stigmas sampled per plant (range 37-214) make it difficult to statistically compare patterns in stigma deposition, **Figure 5.6a** illustrates the importance of recognising the wide difference in patterns of pollen deposition between plants in a single community.

Most interestingly, the proportion of flowers receiving mixed or pure pollen loads or failed visits differed between flower visitors (**Fig. 5.6b**). Again differences in the frequency of flower visitation make it difficult to statistically compare visitors (range 18-1,226). However, *Bombus* and the non-eusocial bees appeared more likely to transfer heterospecific pollen onto the stigma, depositing mixed pollen loads in >20% of all flowers visited; these visitors also carried the most diverse pollen loads (Chapter 4). Visits from hoverflies also resulted in a larger proportion of ‘failed’ visits, with >20% of stigmas receiving no pollen, despite these visitors carrying an average of 735.95±85.07 (n=146) pollen grains (Chapter 4).

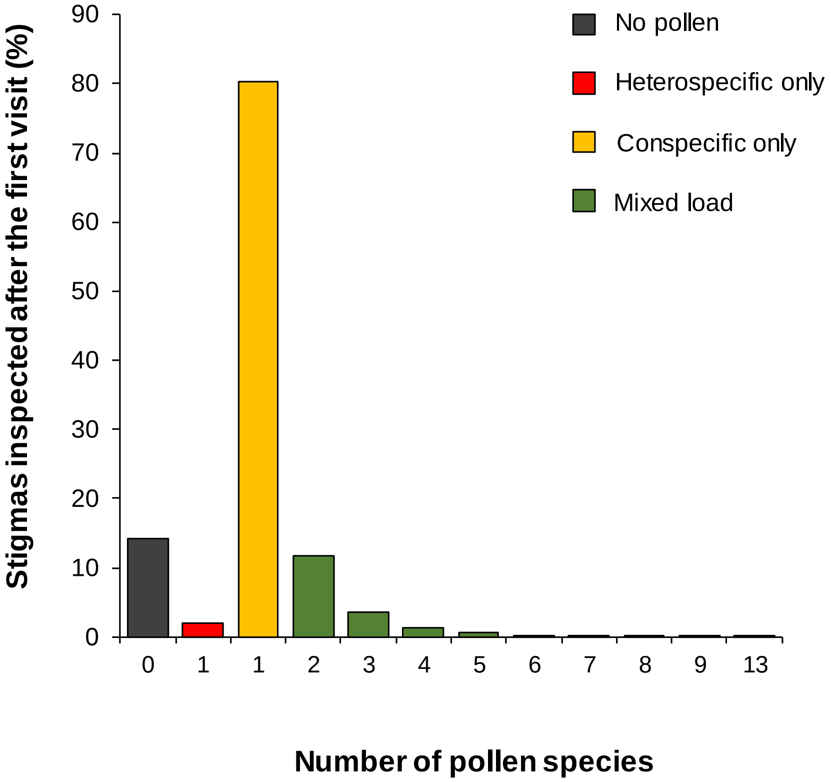


Figure 5.5 The number of pollen species deposited onto a virgin stigma during the first visit. All pollen grains recovered using a small cube of fuchsin gel were counted and identified as either conspecific (belonging to the same plant as the stigma) or heterospecific (from another focal plant or 'alien' plant species). The majority of stigmas received only conspecific pollen grains (n=1,988). 354 received no pollen at all, while only 47 stigmas received only heterospecific pollen. Stigmas with ≥2 pollen species were receiving both conspecific and heterospecific pollen (n=442). The most diverse stigma load (n=13 pollen species) was recorded from *Geranium x johnsonii* 'Johnson's Blue' after a visit from *Apis*. Results are representative of all the stigmas collected over two summers (n=2,831).

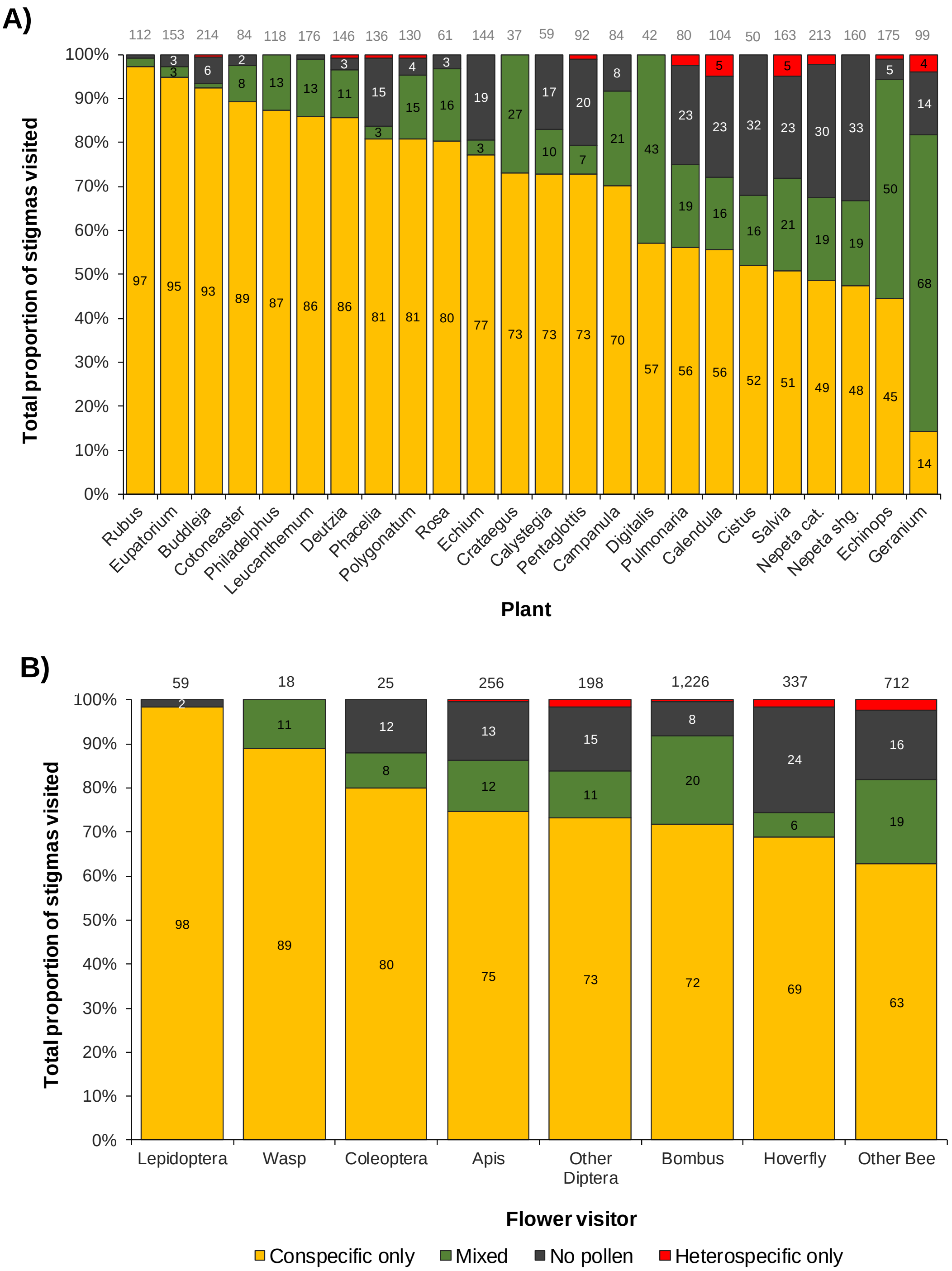


Figure 5.6 Comparison of the stigmatic pollen load between A) plants and B) flower visitors. For almost all garden plants, the first visit was most likely to result in the deposition of conspecific pollen only. However, some plants appeared more likely to receive heterogeneous pollen loads (e.g. *Echinops* and *Geranium*) while others were more prone to failed visits (no pollen, e.g. *Cistus* and *Nepeta* spp.). The frequency of conspecific pollen deposition was high amongst all flower visitors, although *Bombus* and non-eusocial bees appeared more likely to deposit heterogeneous pollen loads during the first visit, while hoverflies had the greatest proportion of failed visits. Variation in sample sizes made it difficult to test for statistical differences between plants and visitors, although overall the proportion of stigmas receiving particular types of pollen loads varied significantly between plants (χ^2=886.50, df=46, p<0.001) and flower visitors (χ^2=127.22, df=14, p<0.001). Sample sizes are given above each bar. Results are representative of all stigmas sampled over two summers (n=2,831).

Although restricted flowers (those with morphologically restricted access to the stigma and anthers) may receive less heterospecific pollen, there were no differences between plants with open or restricted flowers (Section 2.1.1) in the number of heterospecific pollen species received (open 11.71±2.98, n=14, restricted 8.60±1.39, n=10; W=67, *p*=0.88), despite some plant species receiving considerably more heterospecific pollen than others (e.g. *Geranium*, **Table 5.4**). Similarly, there was no difference between open or restricted flowers in the number of plant species to which their pollen was donated to (open 1.29±0.32, restricted 2.44±0.87; W=76, *p*=0.42) which was low in most cases; exceptions to this were *Digitalis* and the *Nepeta* species (6 and 7 recipient species respectively) for reasons that remain unclear.

Plant	**Heterospecific pollen species received** (number of stigmas sampled)	**Number of focal plant species pollen donated to**
Open flowers	**11.71 ± 2.98** (1,594)	**1.29 ± 0.32**
Rubus	2 (112)	0
Echium	4 (144)	0
Eupatorium	4 (153)	0
Crataegus	6 (37)	0
Phacelia	8 (136)	3
Philadelphus	8 (118)	2
Calendula	10 (104)	1
Cistus	10 (50)	1
Deutzia	10 (146)	0
Rosa	10 (61)	3
Campanula	11 (84)	2
Leucanthemum	11 (176)	2
Echinops	24 (175)	3
Geranium	46 (99)	1
Restricted flowers	**8.60 ± 1.39** (1,237)	**2.44 ± 0.87**
Buddleja	1 (214)	0
Calystegia	5 (59)	1
Pentaglottis	5 (92)	4
Cotoneaster	7 (84)	1
Pulmonaria	7 (80)	0
Digitalis	9 (42)	6
Nepeta cataria	11 (213)	7
Nepeta 'S.H.G.'	13 (160)	7
Salvia	13 (163)	2
Polygonatum	15 (130)	1

Table 5.4 Summary of heterospecific pollen receipt and transfer between the stigmas of 'open' and 'restricted' flowers. All heterospecific pollen grains were counted on the stigmas of the focal plants (88 heterospecific species, including 16 belonging to the focal plants) which were classified as restricted if the stigma was <1mm from the corolla. No difference in the mean heterospecific pollen receipt was found between flower types, although some plants (*Digitalis* and *Nepeta* spp.) appeared to receive higher heterospecific pollen loads than others. Results are representative of all stigmas collected over two summers.

5.3.4 Self-pollen receipt

Measuring autonomous self-pollen deposition in the focal plant species formed an important part of calculating the PE of visitors. Selfing in control flowers was common in the garden, with pollen deposited on to the virgin stigmas of almost all plants (**Table 5.5**). Selfing was particularly high in flowers where the anthers dehisced on to the style (*Campanula*) and those with open, disc-shaped flowers and many anthers (e.g. *Rosa, Deutzia, Philadelphus* and *Rubus*). On the other hand, the lack of selfing in certain plants may be explained by temporal dichogamy (*Calendula*) and spatial herkogamy (*Calystegia* and *Salvia*) (Barrett 2003). Despite this, the average quantity of pollen deposited following a visit was greater than that of control flowers in all species. Under natural conditions (i.e. not bagged), the quantity of self-pollen deposited on to the stigmas of protandrous flowers may have been much lower, as some would be removed by flower visitors prior to the stigma becoming receptive (Bischoff *et al.* 2013).

Plant	**Pollen grains per virgin stigma** (n)	**Pollen grains per visited stigma** (n)
Campanula	**501.57** ± 126.70 (21)	**539.36** ± 78.49 (84)
Rosa	**399.79** ± 79.38 (14)	**814.55** ± 77.59 (61)
Deutzia	**394.10** ± 73.55 (20)	**764.52** ± 36.95 (146)
Philadelphus	**373.50** ± 88.39 (18)	**778.04** ± 55.28 (118)
Rubus	**372.87** ± 98.23 (15)	**822.59** ± 53.11 (112)
Polygonatum	**347.67** ± 26.99 (18)	**402.26** ± 19.60 (130)
Digitalis	**115.29** ± 43.12 (14)	**269.74** ± 35.68 (42)
Eupatorium	**113.85** ± 10.17 (20)	**256.09** ± 15.23 (153)
Crataegus	**43.53** ± 9.10 (15)	**92.92** ± 11.74 (37)
Cotoneaster	**43.12** ± 12.43 (17)	**71.81** ± 9.22 (84)
Buddleja	**37.96** ± 14.87 (26)	**417.01** ± 26.00 (213)
Leucanthemum	**33.41** ± 6.81 (32)	**67.21** ± 4.50 (176)
Echinops	**22.85** ± 3.20 (34)	**55.21** ± 7.16 (175)
Cistus	**14.71** ± 6.19 (17)	**122.26** ± 29.26 (50)
Phacelia	**14.63** ± 7.11 (16)	**53.88** ± 12.85 (136)
Nepeta cataria	**6.63** ± 1.40 (16)	**23.08** ± 2.48 (213)
Pentaglottis	**7.16** ± 2.95 (19)	**101.93** ± 15.30 (92)
Echium	**4.00** ± 2.40 (14)	**58.06** ± 8.80 (144)
Pulmonaria	**2.27** ± 1.21 (15)	**25.14** ± 5.15 (80)
Geranium	**1.33** ± 0.45 (15)	**32.33** ± 7.40 (99)
Nepeta ‘S.H.G.’	**0.06** ± 0.06 (17)	**11.50** ± 1.50 (160)
Calendula	**0.00** ± 0.00 (15)	**27.01** ± 4.79 (104)
Calystegia	**0.00** ± 0.00 (12)	**94.75** ± 16.14 (59)
Salvia	**0.00** ± 0.00 (23)	**86.63** ± 11.40 (163)

Table 5.5 Mean pollen grain number on the stigma in unvisited (virgin) flowers and those counted after the first visit from an insect. Unvisited flowers were bagged and handled in the same way as visited flowers and the stigma sampled as soon as appeared morphologically receptive. Almost all plants demonstrated some degree of ‘selfing’, where pollen was transferred to the stigma prior to visitation. However, the mean number of grains following the first visit was significantly greater than that in control flowers for all plants (species specific results given in Appendix 6.1). Results are representative of all stigmas sampled over two years.

5.4 Discussion

Measuring the effectiveness of a community of flower visitors to multiple plants has been quoted as the ideal for studying plant-pollinator networks. This chapter has shown that it is possible to collect such data for a diverse section of a single garden community, creating pollinator effectiveness and importance networks that reveal more about the nature of the interactions from the plant's perspective.

5.4.1 Pollinator importance networks increase the specialisation of visitation networks

(i) At the community level

This study has created the first PE network for a single garden, and contributed to the small collection of PE networks by Ballantyne *et al.* (2015, 2017). Although the PE network did not reveal large differences in the structure of the interactions, the increase in generality of plants and visitors suggested that at the community-level visitors were more similar in terms of the quantity of pollen deposited, compared to their frequency as visitors (thus matching the predictions of Vázquez *et al.* 2005). While a PE network did not increase the specialisation (H_2') of a visitation network, the combination of both measures in the PI network resulted in a small increase in H_2', again similar to the findings of Ballantyne *et al.* (2015, 2017). The increases in H_2' reported from the Israeli (2017) and Kenyan (Ballantyne, pers. comm.) communities were slightly greater than that for my garden site. A possible explanation for this might be that many more visits to the garden plants were recorded, creating a much larger visitation network compared to Ballantyne *et al.* (2015, 2017), where only the visits resulting in PE data were included.

In neither this site, nor those of Ballantyne *et al.* (2015, 2017) did H_2' in the PI networks approach the levels of specialisation reported in recent studies of pollen-transfer networks (e.g. Emer *et al.* 2015, H_2'=0.89 and Banza *et al.* 2015, H_2'=0.79). However, pollen-transfer networks are inherently more specialised compared to PI networks, as they include only the interactions between plants (as in Emer *et al.* 2015) or those belonging to one group of flower visitors (e.g. Lepidoptera in Banza *et al.* 2015). In generalised plant-pollinator communities, the PI network could only approach these levels of specialisation if the majority of visitors were 'cheats', visiting flowers without depositing pollen. Although this study and Ballantyne *et al.* (2015, 2017) have shown that PI networks can be created for a large proportion of a community, some plants that received few visits (likely to be the most specialised) were excluded due to sampling constraints. Ideally, these species would be incorporated into PI networks, which would further increase estimates of H_2'.

(ii) At the level of individual species

Apart from the PI networks created by Ballantyne *et al.* (2015, 2017), it was unclear how the inclusion of pollinator effectiveness data would alter the recorded specialisation of individual plants and the strength of individual flower visitors. While the overall values for both measures showed no significant difference between the networks, it was interesting that the range of plant specialisation was similar in each of the V, PE and PI networks, while the species strength of visitors varied most in the V network. Therefore, rather than revealing large differences in the value of flower visitors as pollinators, measures of pollen deposition actually increased the similarity between visitors in terms of PE; all plants received one dominant visitor, which accounted for between 23 and 79% of all flower visits, while two visitors often accounted for similar proportions of the total pollinator importance (all plant specific V, PL, PT, PE and PI details are given later, in **Appendix 6.1**). These findings raise intriguing questions regarding the functional importance of flower visitors in garden communities, which are discussed later (Section 5.4.2).

Methods for comparing control and visited flowers

Creating PI networks in different habitats also highlights the issue of how single-visit deposition data are compared to control flowers. As yet, no standardised statistical method exists for this, and this study has highlighted the considerable difference between PI networks as a result of different statistical methods: the favoured PI network suggested a community of both generalists and specialists (H_2'=0.54) whilst the alternative PI network implied far more specialised pollinators (H_2'=0.67). These results are in agreement with those obtained by Ballantyne *et al.* (2017) who approached the same problem using a hurdle model. How to handle the data from control flowers, particularly when levels of selfing are highly variable between plants, has been raised by others (e.g. Frier *et al.* 2016) and if PI networks are to be used in future studies, represents an important area for consideration.

5.4.2 The value of bee and non-bee visitors as pollinators

Recent studies have highlighted the importance of urban plants for flower visitors (e.g. Baldock *et al.* 2015) and visitation networks linked to seed set in urban communities have noted the importance of *Lasioglossum*, *Halictus* (both non-eusocial bees) and *Bombus* species for a small collection of plants (Theodorou *et al.* 2017). However, as yet none have measured the pollinator effectiveness of specific visitors to a community of plants. This study provided the largest known comparison of the pollinator effectiveness of several groups of flower visitors in a single garden.

Whilst bees were collectively the most effective/important pollinators, comparisons between *Apis*, *Bombus* and the non-eusocial bees revealed differences in their performance (although these were not significant at the generic level). *Apis* rarely had the greatest pollinator effectiveness relative to all other visitors (similar to the findings of Wilson & Thomson 1991; Cane & Schiffhauer 2003; Adler & Irwin 2006) yet the abundance of *Apis* increased their importance as pollinators relative to other bees. This is similar to the results in Park *et al.* (2016) where wild bees had a higher PE, but the probability of seed and fruit set was the same as for *Apis*. Similarly, Rader *et al.* (2009) reported that the abundance of *Apis* made them equally as important pollinators of *Brassica rapa*, despite their reduced effectiveness compared to *Bombus terrestris*, *Leioproctus* and *Eristalis tenax*. In both Israel and Kenya, Ballantyne *et al.* (2015, 2017) found that, on average, *Apis* deposited less pollen per flower visit than all other bees; combined with my results, this supports the claim that *Apis* are less effective pollinators at the per visit level, which may be because they are less selective about visiting young and old flowers, forage over shorter periods of the day and visit less flowers per minute compared to other bees (e.g. *Bombus*, see Fig 3.10 page 68 and Fig. 4.3 page 91 in this thesis and Willmer *et al.* 1994).

This study set out with one of its aims being to assess the importance of dipteran visitors (both syrphid and non-syrphid) as pollinators in a garden, as these visitors are often neglected and recent studies have argued that this has been unfair. Although some have highlighted their value in transporting pollen (e.g. Forup & Memmott 2005, Orford *et al.* 2015), my study has been unable to demonstrate that dipteran visitors are important pollinators at the community level. While they did deposit pollen on to the stigmas in the majority of links, only two plants (the generalists *Leucanthemum* and *Eupatorium*) had non-syrphid Diptera as the most important pollinators. With the exception of *Echium*, where the sheer abundance of *E.balteatus* caused these visitors to be almost as important pollinators as *B.terrestris/lucorum*, syrphid visitors were never the most important pollinator. Caution must be applied when interpreting the combined pollinator effectiveness and importance values for the different groups, as bees visited far more plants in the community. It is possible that for a community with some sapromyophilous plants (those mimicking the odour of faeces or decay) the value of the non-syrphid visitors would increase. However, such plants were not present in the garden community.

To develop a full picture of the value of different visitors as pollinators, note that bees carried significantly higher pollen loads compared to Diptera (Chapter 4). This is important as a pollinator may function as a parasite if pollen removal (and possibly wastage) outweighs the quantity deposited on to a subsequent stigma, relative to other flower visitors (Thomson 2003, Parker *et al.* 2016). Therefore, the overall net worth of bees as pollinators may be lower than that demonstrated by pollinator effectiveness, if dipteran visitors deposit a greater proportion of the smaller pollen loads they collect, as it is likely given that they are not storing some of the pollen in scopae or corbiculae for deposition in a nest as larval food.

Functional redundancy or complementarity in a diverse assembly of pollinators

Recent studies have stressed the importance of non-bee visitors as pollinators (Rader *et al.* 2016), and the lack of flower visitors that failed to deposit any pollen on to the stigma raises questions regarding the functional redundancy of flower visitors in a garden. This study can only speculate whether the large number of visitors depositing pollen is a factor of functional redundancy (where successful pollination actually only depends on one or two species of pollinators, causing others to be redundant) or functional complementarity (where multiple pollinators contribute more to pollination than any do alone, Blüthgen & Klein 2011). Consequently, this study raises intriguing questions regarding the value a diverse community of flower visitors for the pollination of garden plants, which measures of pollinator effectiveness alone have not been able to answer.

5.4.3 Receipt of heterospecific pollen on stigmas is low in a diverse plant community

Recent studies have found heterospecific pollen transfer (HPT) in plant communities to be high enough to create pollen transfer networks (Fang & Huang 2013, Emer *et al.* 2015). Yet while the number of heterospecific pollen grains on a stigma can vary considerably between flowers and plant species, the overall levels of HPT tend to be low. Despite the diversity of flowering species in this study (including non-focal plants) HPT was minimal (although this varied by plant species), in line with the findings summarised in Willmer *et al.* (2017). Therefore, no evidence was detected for widespread ‘clogging’ from heterospecific grains for the plant community. This raises the possibility that flower visitors demonstrate significant ‘floral-fidelity’ in a garden, despite the diversity of plants available. It has already been shown in this chapter that all plants received a single most-common visitor, and a large proportion of visitor pollen loads were monospecific in Chapter 4. Future studies on the movement of pollinators between plants in gardens (i.e. the distance travelled between intraspecific flowers, and frequency of interspecific visits) are therefore recommended, particularly for *Bombus* and non-eusocial bees, whose visits resulted in the greatest levels of HPT.

5.4.4 Variation in single visit deposition

Prior studies that have measured single visit deposition have also found high levels of variation in the quantity of pollen deposited on to a particular plant by a specific visitor (e.g. Kawagoe & Suzuki 2005). In some plants, the duration and feeding behaviour of a visitor explain a small percentage of the variation in deposition (King *et al.* 2013); however, these observations have been inconsistent between plant species. The extent of intraspecific variation in pollen deposition was, in some plants, higher in this investigation compared to that of other studies, possibly as a result of differences in methodology (e.g. in King *et al.* 2013, where pollen was counted on the stigma using a lower magnification than in this study).

If stigma deposition is to be collected at a community level in future studies, then it is worth considering: (i) the timing of un-bagging, which may affect the quantity of floral rewards, duration of the visit, and contact with the stigma, (ii) the foraging behaviour of the visitor, (iii) differences in the likelihood of visitation for flowers at different locations on a plant (see Anderson, 1988), and (iv) the number of conspecific flowers previously visited, which will influence both the number of pollen grains adhering to the body and the chance of geitonogamy. Although data for points (i) and (ii) were collected in this study, it was not clear how to incorporate this into a network analysis.

5.4.5 Limitations

(i) PE does not measure pollen quality

The conclusions based on PE should be interpreted with caution for two reasons. Firstly, PE is a measure of pollen quantity, but not quality (although this could be interpreted in part as the proportion of conspecific grains). The quality of the pollen also refers to the genetic identity, compatibility and viability of the grains. While PE is a fundamental component of pollination, neither PE nor PI concludes whether the amount is high enough to result in seed-set (Ne’eman *et al.* 2010).

Secondly, PE assumes that plant female fitness increases linearly with pollen deposition, which is only true to a certain point. Excessive pollen deposition may delay fertilisation by overcrowding of the stigma surface (e.g. Cane & Schiffhauer 2003), may increase competition between pollen grains (Madjidian *et al.* 2012), or may result in pollen allelopathy (Murphy 2000, Roshchina *et al.* 2009). My study does not include data to address this, given inevitable constraints on time and sampling effort. However, the inclusion of PE data into plant-pollinator networks contributes a substantial improvement to the detail of these interactions.

Consequently, it is possible that true values of the most effective/important pollinators in this study were lower than predicted. In some of the focal plants (e.g. *Buddleja*), the importance of visitors may have been masked if they deposited smaller loads of a higher quality (e.g. Lepidoptera, as in Herrera 1987). In addition, measuring PE for the first visit to a flower, neglects the costs of secondary visitors that remove viable grains from previous visits (e.g. hoverflies ingesting pollen from the stigma, Holloway 1976).

(ii) Pooling of PE data

The pooling of PE data between and within flower visitor genera (e.g. *Lasioglossum, Halictus* and *Bombus*) is likely to have reduced estimates of species specialisation. Variation in flower visitation rates made it difficult to standardise the number of stigmas sampled for different plants and throughout the day, and small sample sizes forced PE to be pooled, excluding temporal patterns in PE. Small sample sizes also introduced the risk that PE measurements might be limited to poorly-performing individuals.

(iii) Sampling a subset of the plant community

Plants that did not produce pollen, or that demonstrated inconsistent pollen production between flowers (e.g. *Weigela*, *Erysimum*), or received too few visits to collect PE data (e.g. *Lysimachia*) all had to be excluded from the PE networks. Very rare flower visitors are also excluded from PE networks, due to a lack of data. Consequently, PE and PI networks are much more selective than either visitation or PL/PT networks, which limits the insights gathered from the community from the visitor’s perspective. However, this finding emphasises the need to consider V, PT and PI networks simultaneously, which is the focus of the next chapter.

(iv) The collection of visitation records compared to PE data

Pollinator effectiveness was multiplied by visit frequency to create the pollinator importance network. However, while records of flower visits were taken from flowers at all stages during their phenology, PE data were only collected from flowers during the female phase. Consequently, the pollinator importance of visitors that were more frequent during the flower's male phase (e.g. to exploit a plant for pollen) may have been inflated beyond their true value as pollinators.

5.4.6 Future directions

The creation of PE/PI networks is still in its infancy, and more plant-pollinator communities need to be studied in this way to test whether the results in my study are commonplace. Additional research questions raised by this chapter include:

(i) Experimentally manipulating species abundance

Only a small number of studies have manipulated plant-pollinator communities and documented the effect on network structure (e.g. Fontaine *et al.*, 2006; Lopezaraiza-Mikel *et al.*, 2007; Brosi & Briggs, 2013; Goldstein *et al.* 2016) and the collection of PE data represents an opportunity to expand this. By removing the most abundant *Bombus* from the flower visitor community, Brosi and Briggs (2013) found a reduction in the levels of floral fidelity (reduced specialisation) of the remaining visitors. This led to a reduction in the proportion of conspecific pollen carried and deposited, and ultimately seed set in the flowers of *Delphinium barbeyi*. One of the most interesting outcomes of these results is how small changes to the visitor community can have considerable effect on the fitness of individual plants, despite little effect on measures of network robustness. It would be intriguing to expand this to several plants, and test the effect of removing the most abundant pollinator in the garden (*Apis*) on the pollinator effectiveness of the remaining community.

(ii) Measuring pollen viability

The methods used in this study (fuchsin gel staining) could not distinguish between self and cross conspecific pollen. Consequently, it is possible that visitors which deposited large quantities of self-pollen were given greater importance than visitors that deposited small quantities of cross-pollen. To develop a full picture of the value of visitors as pollinators, future PE networks should attempt to take this into account by considering post-pollination events such as pollen germination, the growth of pollen tubes and ovule fertilisation. For self-incompatible species that demonstrate a clear response to self-pollen, e.g. the inhibition of self-pollen in Brassicaceae, this could be performed by counting the number of germinating (cross-) pollen tubes following the first visit, and using this data to create a more detailed PE network. Patchett & Willmer (in review) demonstrated that the number of pollen grains germinating on the stigma of *Brassica rapa* was two magnitudes lower than that deposited, which raises intriguing questions about the deposition values in this study. Similarly, Cresswell (1999) found pollen deposition by *Bombus* in flowers of *Brassica napus* was three times greater when pollen was not experimentally removed, in accordance with the difference in deposition on emasculated flowers reported by Delmas *et al.* (2016). However, measuring pollen tube growth is extremely time consuming and not appropriate for all plants especially those that lack self-incompatibility.

However there is a possibility that microsatellite genotyping, or AFLP-PCR, of the pollen collected from stigmas, could be used to clarify the importance of visitors as agents of cross-pollination in urban plant populations (Vamosi *et al.* 2016). While these methods are still in their infancy, there is potential to use them to determine the identity and distance travelled of pollen in urban environments. Bees are known to vary in their foraging ranges, with a maximum distance of 600m between nesting site and food patch for some solitary species (Gathmann & Tscharntke 2002) and up to 6km in *Apis* (Hagler *et al.* 2011). Documenting the genetic identity of pollen deposits along an urban-rural gradient would be fascinating, examining the extent of pollen mixing between urban and rural plant populations, and determining which species of bee (or other visitors) contributed most to the genetic diversity of urban plant gene pools. Furthermore, while the assessment of mixed pollen samples is still problematic (Keller *et al.* 2014), the low diversity of stigmatic pollen loads makes such data more attractive than processing pollen loads sampled from insect bodies.

5.5 Conclusion

The purpose of this chapter has been to show the similarities and differences between visitation, PE and PI networks, and evaluate the strengths and limitations of each approach.

Surprisingly, almost all flower visitors deposited pollen and none were found to be consistent ‘cheats’. While the number of grains varied between visitors to each plant, on the whole visitors were more equal in terms of their deposition than predicted by their visitation patterns. Consequently, the specialisation of the PE network was almost identical to the visitation network. However, the combination of both measures (as the PI network) did increase network specialisation slightly, although this did not approach the levels reported in recent pollen transfer networks. Measures of PE confirmed that the most frequent visitor does not always deposit the most pollen, but overall patterns of visitation confirmed that the most abundant visitors often are the most important pollinators.

The statistical methods used to evaluate community-wide pollen deposition were highlighted as an area that requires future attention, as these were shown to greatly influence network structure. The importance of bees as pollinators in gardens was highlighted, and although dipteran visitors had relatively low importance as pollinators, it remains unclear whether pollination in the garden represents a case of functional redundancy or complementarity.

Although the measurement of PE revealed intriguing insights into the interactions in the garden, it is still clear that measures of pollen quality are also needed to truly understand the value of different visitors as pollinators. This represents a considerable sampling effort for future studies at a community level, so it may be more useful to assess the quality of pollen transported along an urban-rural gradient by flower visitors, rather than use a network analysis.

While a small collection of PE and PI networks now exist, none have yet been compared to a pollen transport network, and this forms the basis of the next chapter.

Chapter 6.

A comparison between pollinator importance, pollen transport and visitation networks in a garden

“I always prefer to believe the best of everybody; it saves so much trouble”

– Rudyard Kipling

Summary

1. Although flower visitation networks have been improved by pollen transport networks, no pollen transport networks have been compared to networks that measure the quantity of pollen reaching the stigma.
2. For the first time, the structure of a visitation, pollen transport and pollinator importance network are compared for a single community, to reveal differences in the estimates of interaction specialisation by each.
3. The specialisation of interactions in the pollinator importance network (H_2'=0.54) was greater than the traditional visitation network (H_2'=0.49) although the extent of the differences between the networks was smaller than anticipated. When compared to the visitation network, the structure of the pollen transport network (H_2'=0.55) was more similar to the pollinator importance network.
4. Measures of pollinator effectiveness and importance were positively correlated with flower visitation frequency, although pollen transport, rather than visitation, explained the greatest proportion of the variation in pollinator importance (77%).
5. The comparison of all three networks suggests that measures of pollen are a valuable and relatively simple addition to traditional visitation networks, with implications for the future of plant-pollinator networks.

6.1 Introduction

While flower visitation networks are commonplace (Chapter 3) and more is being understood about the structure of pollen transport networks (Chapter 4), it is still unknown how these networks compare to the structure of a pollinator importance network (Chapter 5). This chapter sheds new light on how the structure and specialisation of all three network types compare in a single community.

6.1.1 Proxies for predicting pollinator effectiveness

(i) Flower visitation frequency

As very few studies of pollinator effectiveness and importance exist at a community level, comparisons of the quantity of pollen deposited by flower visitors has largely been based on the differences in flower visitation frequency. One of the most influential papers to support this was the meta-analysis published by Vázquez, Morris & Jordano (2005) in which visitation frequency was advocated as an appropriate proxy for pollination success, and this has been used to support the conclusions of many important visitation network analyses since (e.g. Blüthgen *et al.* 2007, Kaiser-Bunbury *et al.* 2010).

While in some plants the most frequent flower visitors deposit the most pollen during a single visit (Welsford & Johnson 2012) this is not always the case (e.g. Mayfield *et al.* 2001, Fumero-Cabán & Meléndez-Ackerman 2007, King *et al.* 2013, Barrios *et al.* 2016) and differences in the total effect of a flower visitor (pollinator importance) are often the result of differences in visitation rates, rather than per-visit performance (e.g. Sahli & Conner 2006). Vázquez *et al.* (2005) explained this by showing that the positive correlation between visit frequency and pollinator importance becomes stronger if the variation in flower visitation frequency exceeds that of single-visit deposition. These predictions were supported when the interaction strength of flower-visitors to five Argentinian plants (measured using pollen tube growth or fruit set) were found to be strongly positively correlated with visit frequency (Vázquez *et al.* 2012). Whether this holds true for a larger proportion of a community remains to be seen.

(ii) Using interaction frequency as an indicator of importance

Several areas of ecology discourage estimating a species' ecological importance based on its relative abundance, as many studies have shown that less abundant species can have a disproportionately large effect on the structure of the community (reviewed by Power *et al.*

1996). For example, the loss of rare species has had a disproportionately large influence on the functional structure of three tropical assemblages studied by Leitão *et al.* (2016), the loss of the least common plant species increased the establishment of an invasive grass (Lyons & Schwartz 2001), and the removal of rare species interacting with many partners caused considerable secondary extinctions in work by Christianou & Ebenman (2005). In a pollination network context, a direct comparison between a flower visitation and a pollinator importance network tests whether less-abundant visitors have disproportionately large effects as pollinators and whether this affects network structure.

(iii) Flower visitor pollen loads

As shown in Chapter 4, the measure of pollen loads from flower visitors to create pollen transport networks has improved traditional flower visitation networks (Bosch *et al.* 2009, Alarcón 2010, Popic *et al.* 2013). However, not all of the pollen on the flower visitors will reach the stigma, with pollen lost between flower visits during transport (Johnson *et al.* 2005) as a result of grooming (Thorp 2000) and in the provisioning of the brood sites (Michener 1974). As Adler and Irwin (2006) found, the amount or proportion of conspecific pollen on the body does not always reflect that reaching the stigma, and high quantities of particular pollen species found on flower visitor bodies may not be reflected in the quantity deposited on to stigmas (Emer *et al.* 2015). Equally, the most frequent flower visitors may not always carry the greatest pollen loads (see Chapter 4 and Watts *et al.* 2012). For this reason, even pollen load/transport networks may not reflect the structure of pollinator effectiveness/importance networks.

6.1.2 Expected similarities and differences between a pollinator importance, pollen transport and visitation network

The predicted changes in the structure of the pollinator effectiveness/importance networks were outlined in Chapter 2 (Section 2.2) and are briefly summarised as (i) an increase in the specialisation of the interactions (measured using H_2' and d'); (ii) a decrease in plant species generality and (iii) a decrease in tolerance to disturbance, implied by changes to several network indices. These changes would be seen if flower visitation overestimates the importance of non-pollinating visitors.

6.1.3 Key questions

In this chapter, pollinator effectiveness and importance networks are constructed to examine the variation between flower visitors in the quantity and quality (proportion conspecific) of pollen deposited on to the stigma. Evaluating pollination from the perspective of the female function of flowers, the questions are:

1. Is a pollinator importance network more specialised than pollen transport and visitation networks?

2. Which proxy for pollination (visitation or pollen transport) is the best predictor of a pollinator importance network?

6.2 Methods

6.2.1 Comparisons between the networks

To compare the different types of networks, traditional visitation networks, pollen transport (PT) and pollinator importance (PI) networks were created as outlined in previous chapters. However, these were modified to include only the visitors for which both PL and PE data were available (all plant specific V, PI and PT values for all visitors are given in **Appendix 6.1**) and are therefore smaller than those presented in Chapters 3 and 4. As in previous chapters, all of the networks are proportional (each interaction is weighted according to its value to a specific plant, Section 2.2.8) to standardise the quantity of pollen produced by different plant species, and only the pollen belonging to the 24 focal plant species is included. As outlined in Chapter 5, all comparisons between the V, PI and PT networks use the less conservative PI network.

6.2.2 Statistical analysis

(i) Comparisons between species-level indices in the networks

Plant species specialisation (*d’*) values were calculated for each plant in each of the three networks. These values were then compared between the networks using a generalised linear mixed model (GLMM, with a Gaussian error distribution) using the function *glmer* in the package *lme4* (Bates *et al.* 2015) with plant species as a random effect, as in Chapter 5. As proportions (0-1), all *d’* values were arcsine square-root transformed before testing. Pairwise comparisons between networks were made using a post-hoc Tukey HSD using the *glht* function in the package *multcomp* (Hothorn *et al.* 2008). Similarly, the species strengths of all flower visitors were compared between the networks using a GLMM (with a gamma error distribution) including visitor species as a random effect, as in Chapter 5.

(ii) Correlations between visitation, pollen load/transport and pollinator effectiveness/importance

Correlations were used to determine the strength of the relationship between explicit measures of pollination (PE and PI) and proxies for these (V, PL and PT) following the methods outlined in Section 5.2.5. Again, differences between the flower visitor groups were tested by including this as an interaction in the linear model (Section 5.2.5). As in Chapter 5, correlations were tested using a Pearson's rank correlation (r) as the data complied to a normal distribution. with Spearman rank correlations (r_s) used to test the correlation for individual groups of visitors, as these did not conform to a normal distribution.

6.3 Results

6.3.1 Is a pollinator importance network more specialised than a flower visitation and pollen transport network?

(i) Network appearance

There was little visible difference in the bipartite networks for the visitation, pollen transport and pollinator importance networks (**Fig. 6.1**). However, slight differences in the width of flower visitor nodes were observable (e.g. *E.balteatus*) and the density of interactions was much greater in the pollen transport network. Differences between the networks were more noticeable in an interaction matrix, shown in **Figure 6.2**. The majority of links were between plants and flower visitors that also transported and effectively deposited pollen onto the stigma (n=175, **Fig. 6.2**), while the additional 81 links revealed by pollen loads mostly involved bees. Cases where visitors transported pollen but did not deposit more pollen than control flowers were few (n=10, including both bee and dipteran visitors) and reflect small sample sizes. Only a single visitor neither transported nor pollinated the flower (Large Syrphini visiting *Buddleja*, again from a small sample size). In two instances, a visitor did not transport pollen but still deposited pollen on to the stigma (potentially by causing the flower to 'self'); these were *Bombylius*, which contacted the flowers of *Pulmonaria* only with its proboscis, and *Hylaeus* (bees which carry pollen in an internal crop) visiting *Nepeta*, although again these involve small samples sizes.

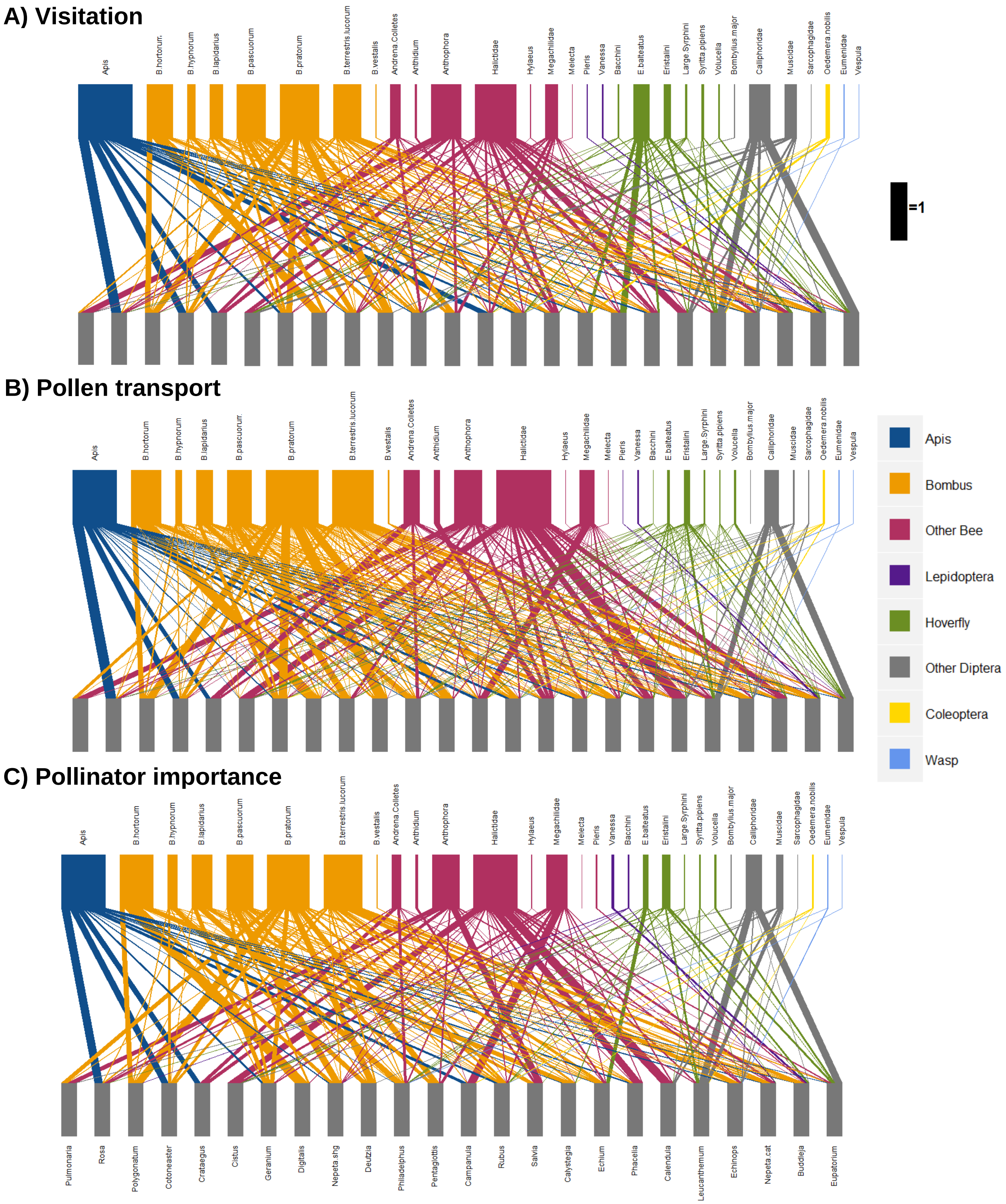


Figure 6.1 Bipartite networks showing the difference in interaction strengths between A) flower visitation, B) pollen transport and C) pollinator importance networks. The values of visitors in each of the networks are proportional and directly comparable. Bees dominated each of the networks, particularly *Bombus* and the Halictidae. Although some dipteran visitors were relatively frequent flower visitors (e.g. *E.balteatus*, calliphorids, muscids) the value of these visits decreased in the pollen transport and pollinator importance networks. Plant species are ordered according to flowering time (early to late summer, left to right) with all species remaining in the same order in each network. Results include of all pollinator effectiveness data collected over two summers for which pollen load data were also available.

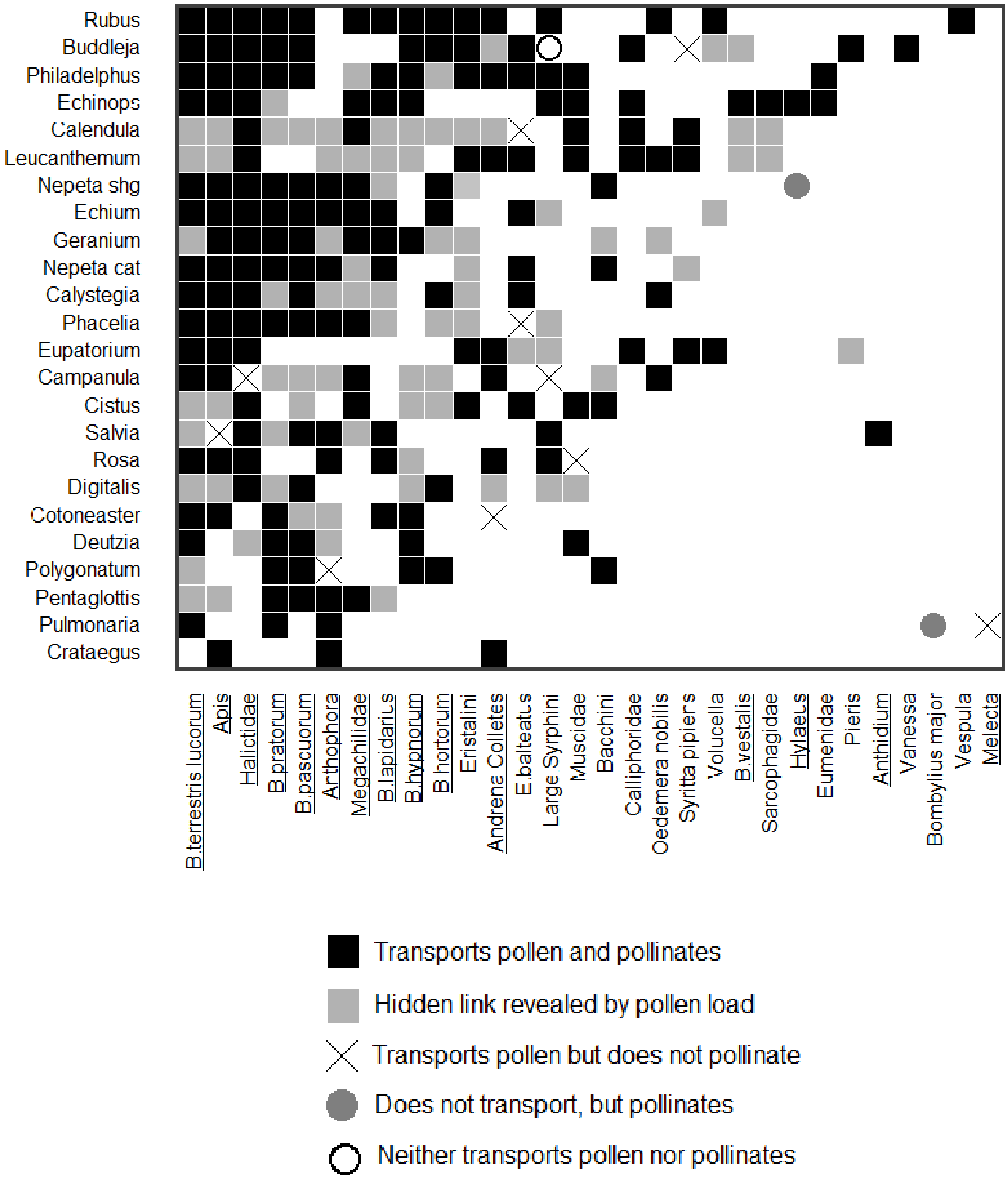


Figure 6.2 Qualitative interaction matrix revealing the presence/absence of visitation, pollen transport and pollinator importance interactions between 39 flower visitors and 24 plants. All interactions were assessed and assigned to one of the five categories listed. In most cases, flower visitors transported pollen on their bodies and deposited this on to the stigma during the first visit. Cases where visitors did not pollinate the flowers were relatively few, but included both bee (underlined) and non-bee visitors. Plants and flower visitor species are organised according to decreasing linkage level (top to bottom, left to right respectively). Results represent all of the links recorded over two summers, where both pollen load and pollinator effectiveness data were collected. ■=175, ■=81, ✕=10, ●=2, **O**=1.

(ii) Community level indices

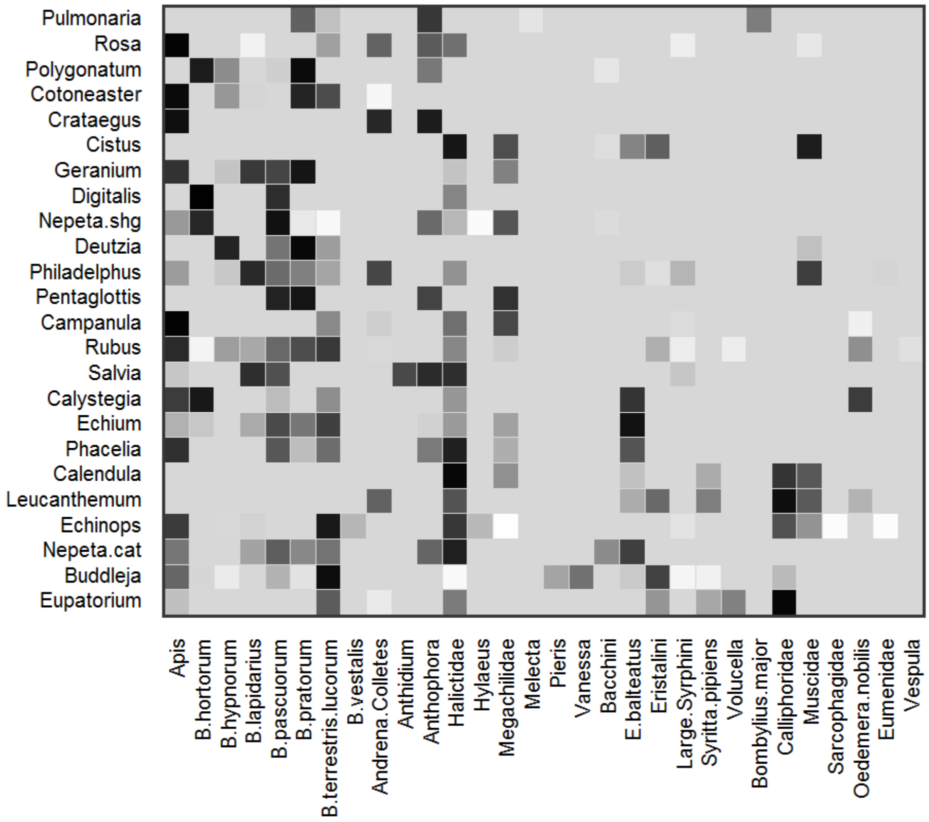
The specialisation of the interactions in the PI network (H_2’=0.54) was greater than that predicted by the V network (H_2’=0.49) and very similar to the PT network (H_2’=0.55, **Fig. 6.3, Table 6.1**), suggesting that the additional measure of pollen in both the PT and PI networks improves understanding of the specialisation of the plant-pollinator interactions from the plant’s perspective. As in the PT network, the generality of plants and visitors decreased in the PI network (4.20 and 7.17 respectively) although this was not as low as in the PT network (3.85 and 7.28). Species were also slightly less ‘even’ in terms of their ecological importance in the PI and PT networks (interaction evenness=0.69 and 0.68) compared to the V network (0.71).

The similarity in connectance between the networks was surprising given the difference in link number, and it remained low in each network (<0.13) in line with that of previous studies. Modularity increased slightly in the PI (0.47) and PT (0.50) networks (modules in each of the networks are illustrated in **Appendix 6.2**), while only nestedness was considerably greater in the PT network (33.01) compared to both V (23.42) and PI (23.03); nestedness in this network is likely to have increased as the hidden links increased the proportion of generalist species for specialists to interact with. Comparisons between the results and the predictions made in Chapter 2 are summarised in **Table 6.2**.

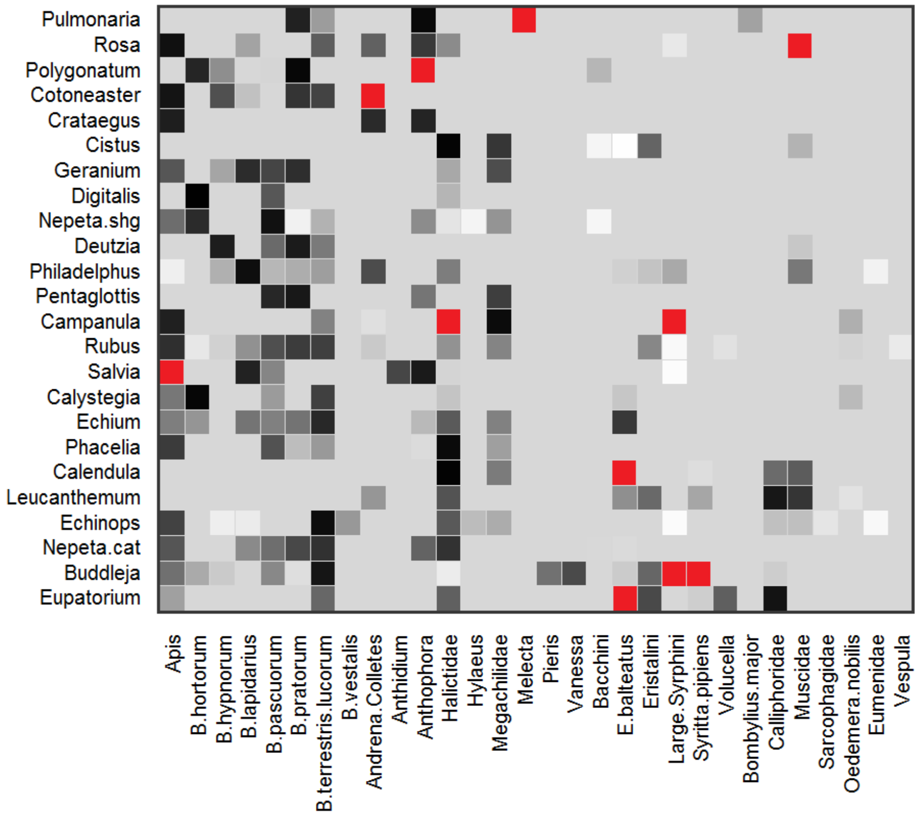
		Visitation	**Pollinator importance**	**Pollen transport**
Species richness	**Visitors**	30	29	29
	Plants	24	24	24
	Links	188	177	269
	Visits	12,877	12,694	12,958
Generality	**Visitors**	7.89	7.17	7.28
	Plants	4.99	4.20	3.85
Distribution of interactions	**C**	0.12	0.11	0.10
	IE	0.71	0.69	0.68
	NODF	23.42	23.03	33.01
	H_2’	0.49	0.54	0.55
	M	0.45	0.47	0.50

Table 6.1 Summary of the community-level indices for a visitation, pollinator importance and pollen transport network. All indices were calculated using the function *networklevel* in the package bipartite. The direct measure of pollination (pollinator importance) increased the specialisation of the interactions (as measured by H_2’) although the pollen transport network, based on an indirect measure of pollination was also more specialised than the traditional visitation network. Changes in the value of other community-level indices were minimal, although in both the pollinator importance and pollen transport networks plant generality declined. Details of the modules for each network are given in Appendix 6.2.

A) Traditional visitation



B) Pollinator importance



C) Pollen transport

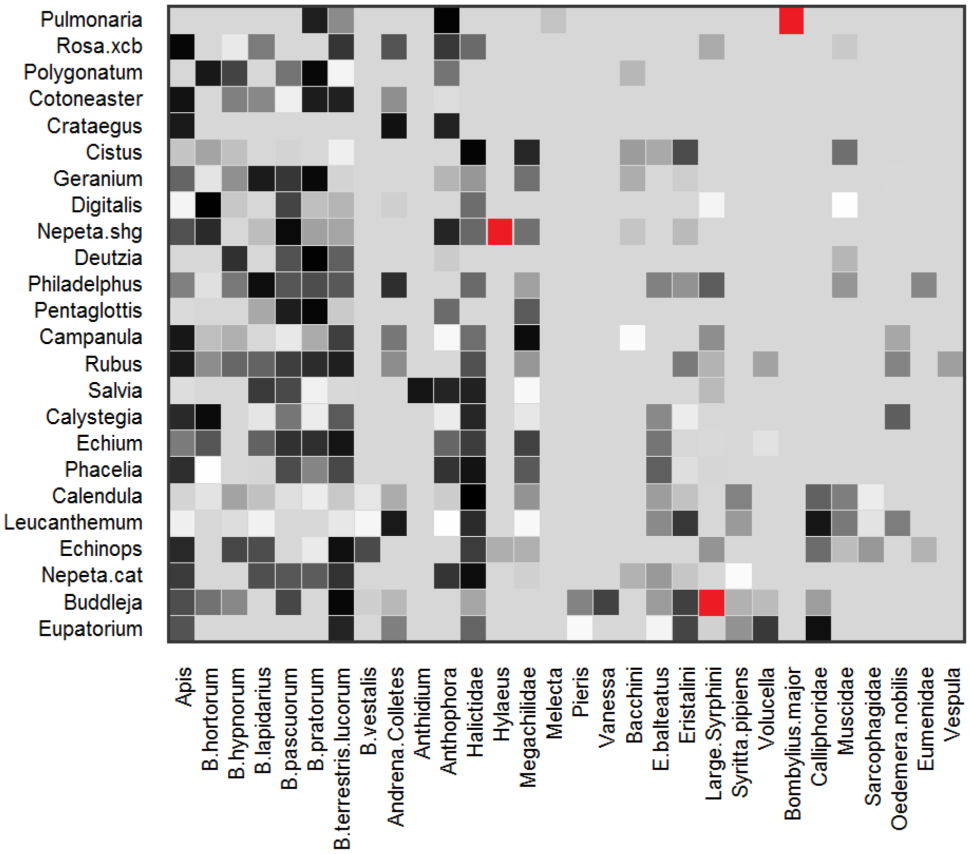


Figure 6.3 Bipartite interaction matrices illustrating the differences in interaction strength between traditional flower visitation, pollinator importance and pollen transport networks. The propotional values of each visitor to a plant were calculated for each of the networks, with only the measures of pollinator importance and pollen transport compared as these included visit frequency. Overall, the structure of the interactions appeared similar, although changes in the strength of individual visitors to plants did occur; for example, many interactions including *E.balteatus* became noticeably weaker in the pollinator importance network. The addition of hidden links is clearly shown in the pollen transport network. Plants are organised in order of flowering time (early to late summer, top to bottom) and visitors ordered in groups. Red squares indicate visitors that were ineffective pollinators depositing less pollen, on average, than found on control stigmas, or those that did not carry pollen on their bodies. Results include all of the interactions recorded over two summers, for which pollinator effectiveness and pollen load data were both available.

Index		Description	Predicted difference	Observed difference
Community-level	**Generality (of plants and visitors)**	The average number of partners.	Least partners in PI, most partners in V.	Less partners per visitor and plant in both the PT and PI networks compared to V.
	Connectance	The proportion of realised links.	Least connected in PI; most connected in PT.	Similar, although least connected in PT and most connected in V.
	Interaction evenness	The uniformity of interaction strength between species.	Uncertain.	Least even in PT, most even in V.
	Nestedness	Extent to which specialists interact with a subset of the most generalist interactions.	Uncertain.	Least nested in PI, most nested in PT.
	Interaction specialisation (H_2')	Specialisation of interactions at the community-level	Most generalised in V, most specialised in PI.	Most generalised in V, most specialised in PT.
	Modularity	Extent to which interactions are grouped into distinct modules	Uncertain.	Least modular in V, most modular in PT.

Table 6.2 Summary of the predicted and observed changes in several community-level indices between traditional visitation (V), pollinator importance (PI) and pollen transport (PT) networks. The predicted changes outlined in Chapter 2 were based upon the effect of incorporating measures of pollen loads and deposition into previously published pollen transport and pollinator importance networks, although this is the first time all three have been compared simultaneously. Although network specialisation (H_2') was expected to increase considerably in the pollinator importance network, the increase was relatively small and matched that in the pollinator importance network. Results are representative of the proportional networks for all interactions recorded over two summers, where pollinator effectiveness and pollen load data were both available.

(iii) The specialisation of individual plants and the species strength of individual visitors

Plant specialisation (*d'*) and flower visitor species strength for the V, PI and PT networks were compared using a GLMM with pairwise post-hoc Tukey tests between networks (Section 6.2.2). Full test results are given in **Appendix 6.3**.

Despite the overall increase in H_2' in the PI network, the average specialisation of plants in the PI network was fairly low (*d'* 0.41±0.03, n=24) and the network was not significantly more specialised than in the V network (*d'* 0.38±0.02, n=24, *p*=0.06). In the PT network, plants were no more specialised than in the V network (*d'* 0.40±0.03, n=24, *p*=0.20) or the PI network (*p*=0.83).

However, inspection of the *d'* of individual plants explained why no overall differences were found: while over half of the plants became more specialised in the PI and PT networks, this was counterbalanced by those that became more generalised (**Fig. 6.4**). *Salvia*, with small, zygomorphic flowers, demonstrated the largest increase in *d'* between the V and PI network (V=0.41, PI=0.58) and between the V and PT network (PT=0.51) as flower visitors (n=9, mostly bees) were relatively equal in terms of their visitation frequency, yet only two visitors (*Anthophora* and *B.lapidarius*, both relatively long-tongued) made substantial contributions as pollinators (**Appendix 6.1**). On the other hand, *Echium* became more generalised in both the PI and PT networks, as the inclusion of pollen data reduced the importance of *E.balteatus* (the most frequent visitor) which deposited and transported very little pollen in comparison to less common *Anthophora* (**Appendix 6.1**). The identity of the most specialised plant also changed between the networks; early-flowering *Pulmonaria*, visited predominantly by *Anthophora* were most specialised in the V network, compared to *Digitalis* in both the PT and PI networks. Therefore, similarities in the overall level of plant specialisation at the community level, did not accurately reflect changes in the specialisation of individual plants. For each of the networks, the individual *d'* values of each plant and species strength values of each visitor are given in **Appendix 6.4**.

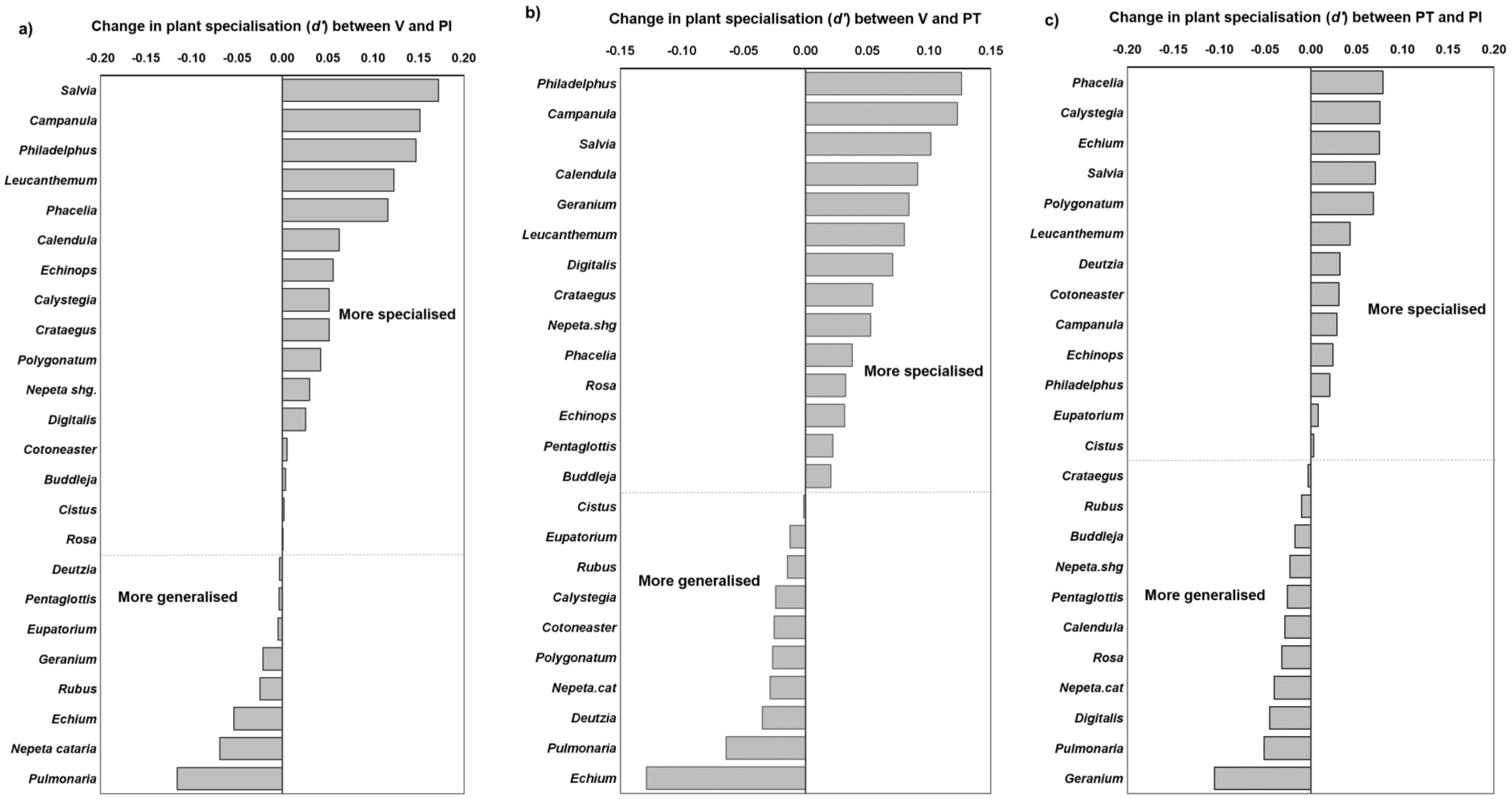


Figure 6.4 Observed changes in the specialisation (*d'*) of individual plant species between the visitation (V), pollen transport (PT) and pollinator importance (PI) networks. The change is calculated as the difference in an individual species' specialisation value (*d'* values range from 0 to 1) between the two networks (e.g. in a) the change is calculated as species' specialisation value in PI minus the value in V; a positive result indicates an increase in specialisation, and vice versa). Despite no significant increase in the overall specialisation of plants between the V and PT or V and PI networks, at least 50% of plants became more specialised in the PT and PI networks compared to the visitation network. Results include all of the interactions recorded over two summers, for which pollinator effectiveness and pollen load data were available.

Although the species strength of flower visitors was expected to increase in the pollinator importance network, no significant difference was found between the average species strength in the V network (0.80±0.18, n=30) and pollinators in the PI network (0.80±0.18, n=30, *p*=0.62). Although the species strength of visitors was greater in the alternative PI network, this was still not significantly different to the V network (0.92±0.19, n=25, *p*=0.83). Similarly, the average species strength of flower visitors in the PT network (0.80±0.20, n=30) was not significantly different to the pollinators in the PI network (*p*=0.69) nor to visitors in the V network (*p*=0.19). Interestingly, while *Apis* had the highest species strength in both the V and PI networks (3.57 and 2.90 respectively) the small non-eusocial Halictidae had the greatest species strength in the PT network (3.61), reflecting the substantial pollen loads recovered from these small, non-eusocial bees.

While there was no overall difference in the species strength of visitors between the networks, the strength of individual visitors did alter. **Figure 6.5** illustrates the changes in the species strength of each visitor between (a) the V and PI network (b) the V and PT network and (c) the PT and PI network. Overall, at least 40% of visitors became more important in both the PT and PI networks compared to the visitation network; in particular, the species strength of several *Bombus* species increased in the PI and PT networks relative to the V network, suggesting their relative transport and deposition of pollen was much greater than that predicted by the frequency of visits alone. Interestingly, the opposite was true for *Apis*, which became less important in both the PT and PI networks, relative to the V network.

All species-level indices presented are summarised in **Table 6.3**, with comparisons made between the results and the predictions made in Chapter 2 in **Table 6.4**. At the level of individual species, the networks differed in estimates of plant specialisation and visitor species strength; although this is important for an understanding of their role in the community, it did not result in a community-wide increase in species specialisation and strength in the PI network.

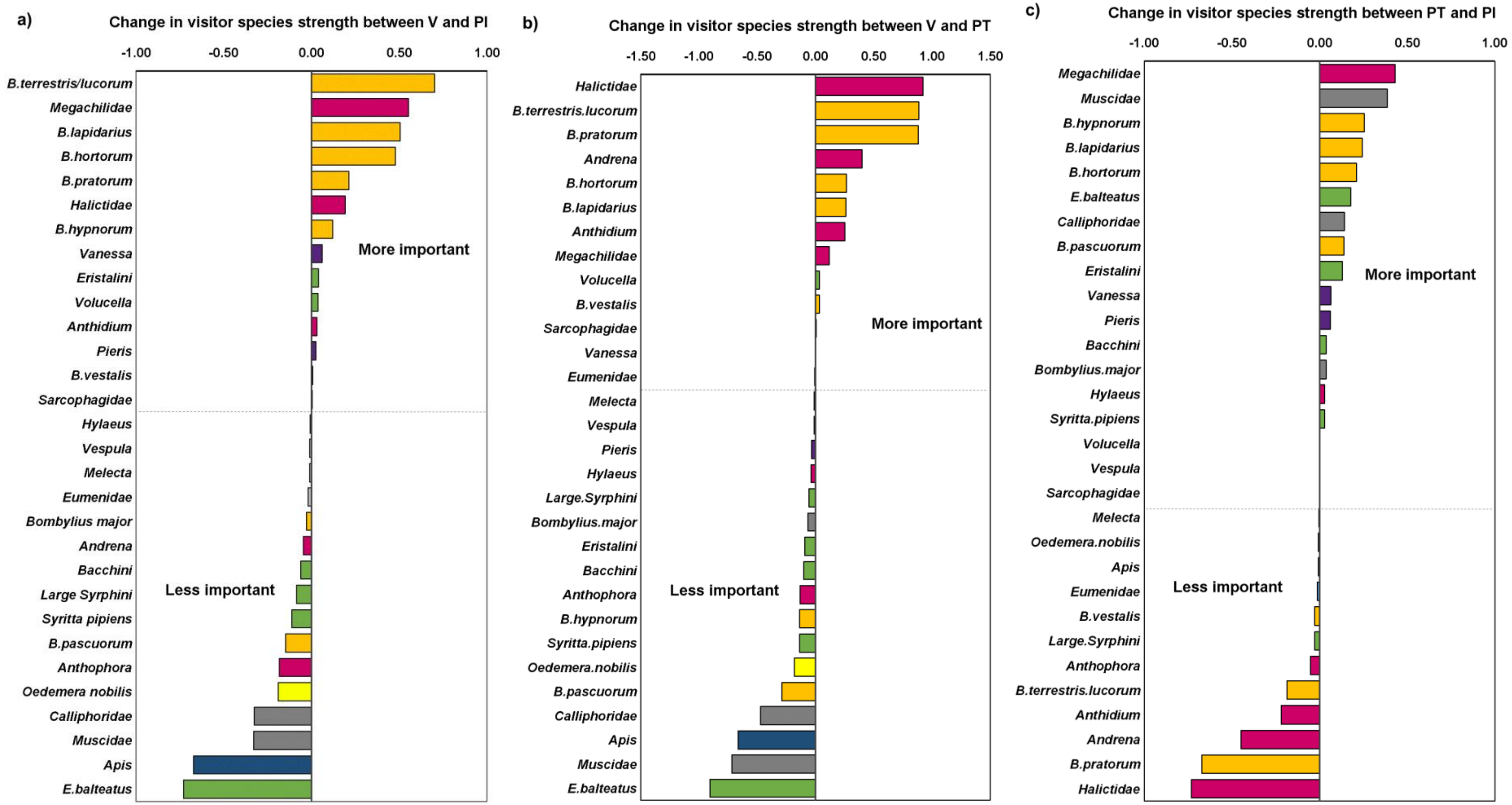


Figure 6.5 Observed changes in the species strength of individual flower visitors between the visitation (V), pollen transport (PT) and pollinator importance (PI) networks. The change is calculated as the difference in the species strength values between the two networks (e.g. in a) the change is calculated as species strength value in PI minus the value in V; a positive result indicates an increase in species strength, and vice versa).Despite no significant increase in the overall species strength between the V and PT or V and PI networks, at least 40% of flower visitor taxa became more important in the PT and PI networks, relative to the visitation network. Flower visitors are colour coded according to group: ■ *Apis* ■ *Bombus* ■ Other bee ■ Lepidoptera ■ Hoverfly ■ Other Diptera ■ Coleoptera ■ Wasp. Species strength values are not constrained to a range of values.

Network	**Plants**		**Flower visitors**	
	Specialisation (*d'*)	**Species range (min and max)**	**Species strength**	**Species range (min and max)**
Visitation	0.38 ± 0.02 (n=24)^{a}	*Rubus* (0.16) *Pulmonaria* (0.63)	0.80 ± 0.18 (n=30)^{a}	Sarcophagidae (0.003) *Apis* (3.57)
Pollinator importance	0.41 ± 0.03 (24)^{a}	*Rubus* (0.13) *Digitalis* (0.60)	0.80 ± 0.19 (30)^{a}	Eumenidae (0.003) *Apis* (2.90)
Pollen transport	0.40 ± 0.03 (24)^{a}	*Rubus* (0.14) *Digitalis* (0.65)	0.80 ± 0.20 (30)^{a}	*Melecta* (0.0002) Halictidae (3.61)

Table 6.3 Summary of the changes in species-level indices between visitation, pollinator importance and pollen transport networks. All indices calculated using the function *specieslevel* in bipartite. Plant specialisation (*d'*) and flower visitor strength are compared as the measure of pollinator importance and pollen transport is interpreted from the plant's perspective here. Although no significant difference was found in the mean specialisation (*d'*) of plants or species strength of flower visitors, the identity of the most specialised plant varied between the visitation and pollinator importance/pollen transport networks, while the identity of the flower visitor with the highest species strength varied between the visitation/pollinator importance and pollen transport networks. Despite carrying much greater pollen loads and consequently having the greatest species strength in the pollen transport network, halictid bees were usurped as the most important pollinators by *Apis*, which were more frequent visitors to flowers. Shared characters indicate no significant difference between networks.

Index		Description	Predicted difference	Observed difference
Individual species	**Species specialisation (*d’*)**	The interaction specialisation of an individual species.	Plants will be most generalised in the PT network, and most specialised in the PI network.	No overall significant difference in plant specialisation, as the change in specialisation varied between plant species. However, over 50% of plants became more specialised in PT and PI compared to in V.
	Species strength	The importance of a species for all species in the alternative level.	The species strength of flower visitors will be greatest in PI, and lowest in V.	No overall significant difference in flower visitor species strength, as the change in species strength varied between visitors. However, the species strength of more than 40% of visitors increased in PT and PI compared to in V.

Table 6.4 Summary of the predicted and observed changes in species-level indices between the traditional visitation (V), pollen transport (PT) and pollinator importance (PI) networks. Predictions are based on those in Chapter 2 and follow the results from previously published pollen transport and pollinator importance networks, although this is the first time all three have been compared together. Although no significant differences were observed in the mean values between networks, the results highlight the importance of considering changes in the specialisation and species strength of individual species when comparing networks using indirect or direct measures of pollination.

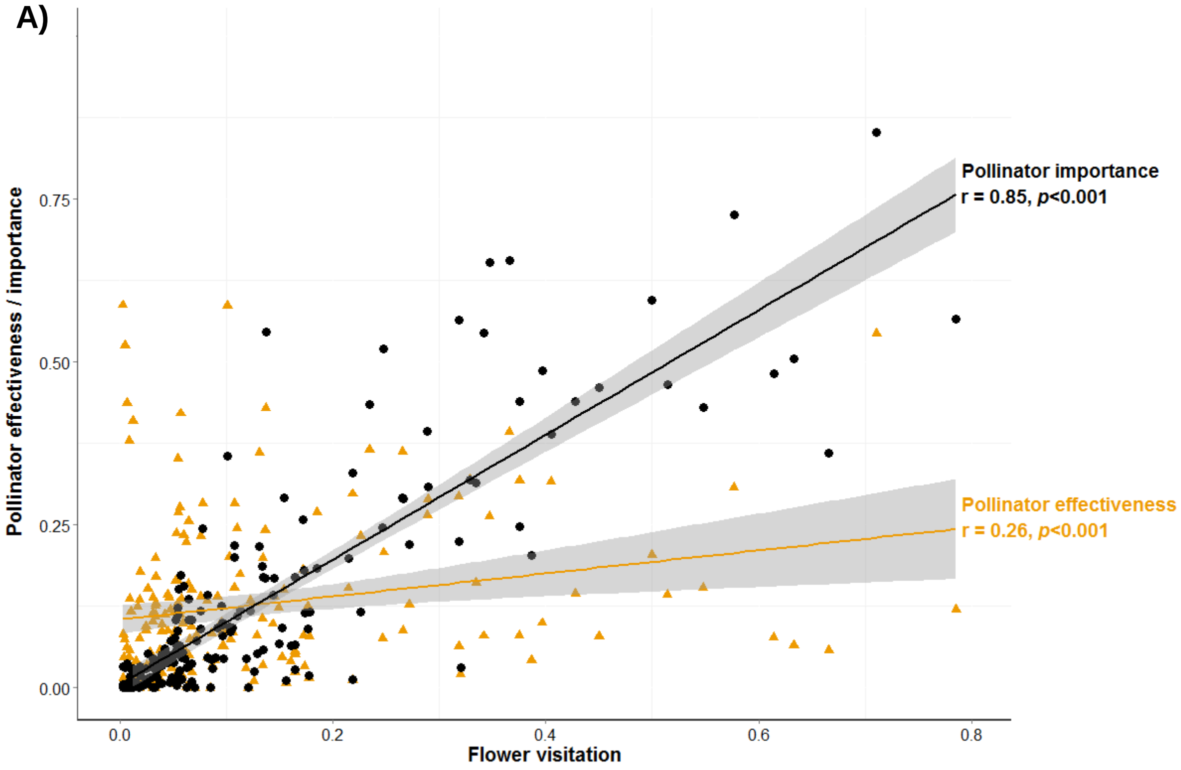
6.3.2 Which proxy for pollination is the best predictor of pollinator effectiveness and importance?

To determine which proxy for pollination (either visitation, pollen loads or pollen transport) was the best predictor of pollinator effectiveness and importance, correlations were used to compare the proportional value for each visitor to a plant in a network (values ranging from 0-1) with their corresponding value in an alternative network (Section 6.2.2).

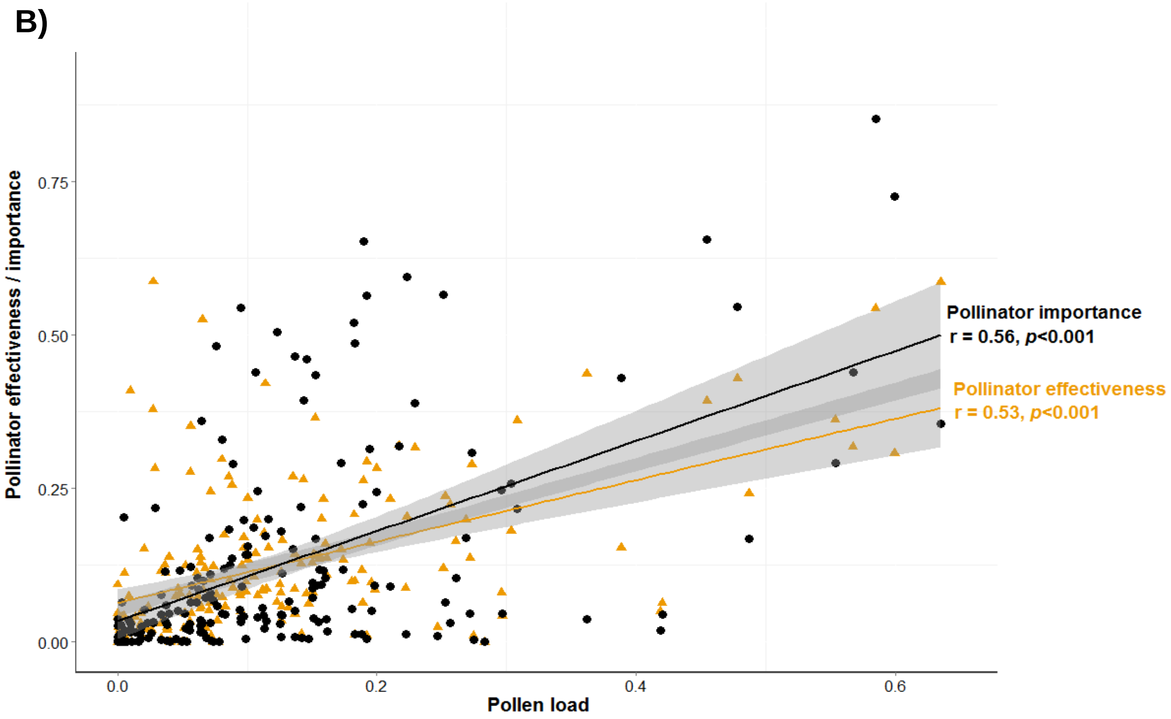
In accordance with Vázquez *et al.* (2005), flower visitation frequency was significantly positively correlated with pollinator effectiveness (PE) ($F_{(1,186)}$=13.91, p<0.001, r=0.26, **Fig. 6.6a**); however the relationship was weak, with some frequent visitors depositing relatively little pollen, and vice versa. As Vázquez *et al.* (2012) demonstrated with four plant species, the relationship between visitation frequency and pollinator importance (PI) (termed 'total effect' by Vázquez *et al.*) was much stronger ($F_{(1,186)}$=483.1, p<0.001, r=0.85, **Fig. 6.6a**); for 24 plant species, the most important pollinators were often the most frequent visitors. Consequently, the PE and PI of the flower visitors to the garden plants could, to varying extents, be predicted by the frequency of flower visitation.

Flower visitors pollen loads (the raw number of pollen grains on an insect's body) were also significantly positively correlated with pollinator effectiveness ($F_{(1,186)}$=71.44, p<0.001, r=0.53) and total pollinator importance ($F_{(1,186)}$=84.87, p<0.001, r=0.56, **Fig. 6.6b**), so that flower visitors with greater pollen loads tended to deposit more pollen on to the stigma. However, this was not always the case, and several visitors carrying large pollen loads contributed little to pollinator importance (**Fig. 6.6b**). Total pollen transport (pollen load x visit frequency) was also significantly positively correlated with PE ($F_{(1,186)}$=33.96, p<0.001, r=0.39) and unsurprisingly (as both measures are calculated using visit frequency) was strongly correlated with PI ($F_{(1,186)}$=639.70, p<0.001, r=0.88, **Fig. 6.6c**).

A)


Pollinator effectiveness / importance
0.00
0.25
0.50
0.75
0.0
0.2
0.4
0.6
0.8
Flower visitation
Pollinator importance
r = 0.85, p<0.001
Pollinator effectiveness
r = 0.26, p<0.001

B)


Pollinator effectiveness / importance
0.00
0.25
0.50
0.75
0.0
0.2
0.4
0.6
Pollen load
Pollinator importance
r = 0.56, p<0.001
Pollinator effectiveness
r = 0.53, p<0.001

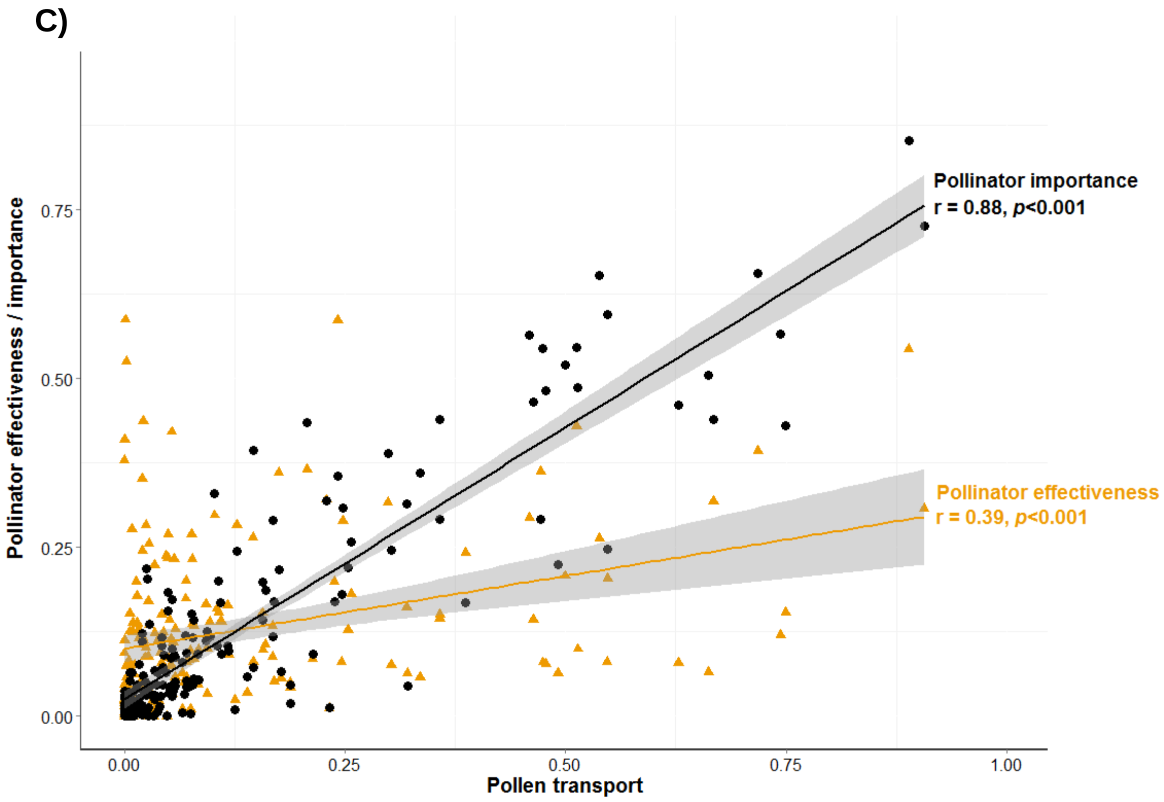


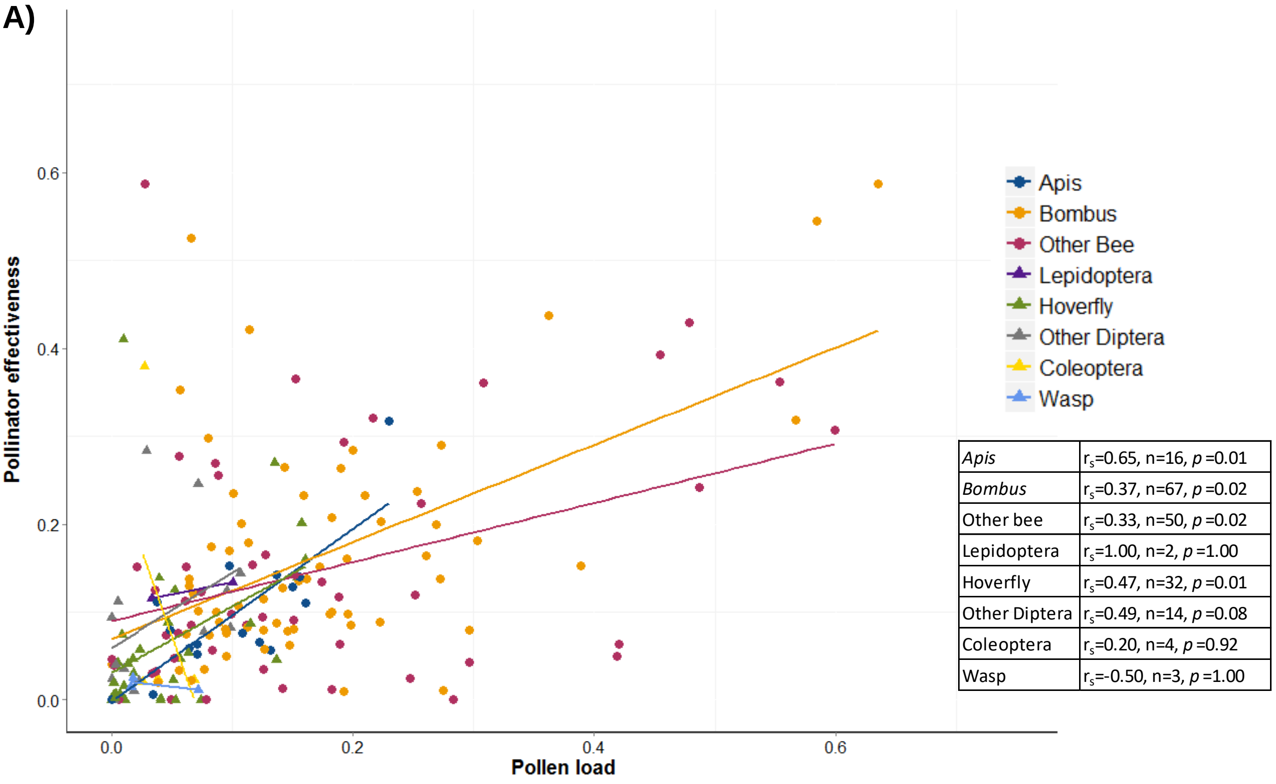
Figure 6.6 Relationships between two direct measures of pollination (pollinator effectiveness and importance) with three proxies for pollination: A) flower visitation frequency; B) flower visitor pollen loads; C) pollen transport. The proportional value for each visitor to a plant was plotted and a Pearson Rank Correlation used to test the strength of the relationship. Both direct measures of pollination (pollinator effectiveness and pollinator importance) were positively correlated with each proxy. The relationship between pollen transport and pollinator importance was slightly stronger than that between visitation and pollinator importance, suggesting pollen transport is a more accurate proxy for the total effect of a flower visitor as a pollinator. Shading indicates the 95% confidence interval. Full linear equations are given in Appendix 6.5. Results include all of the interactions recorded over two summers to 24 plants, for interactions where both pollinator effectiveness and pollen load data were available.

In summary, the pollen loads of flower visitors in the garden explained the greatest variation in PE (although only 27%) while pollen transport explained the greatest variation in PI (77%, **Table 6.5**). Pollen transport was confirmed to be, as expected, a slightly better predictor of PI than visitation frequency alone, as visitation frequency explained 72% of the variation in PI.

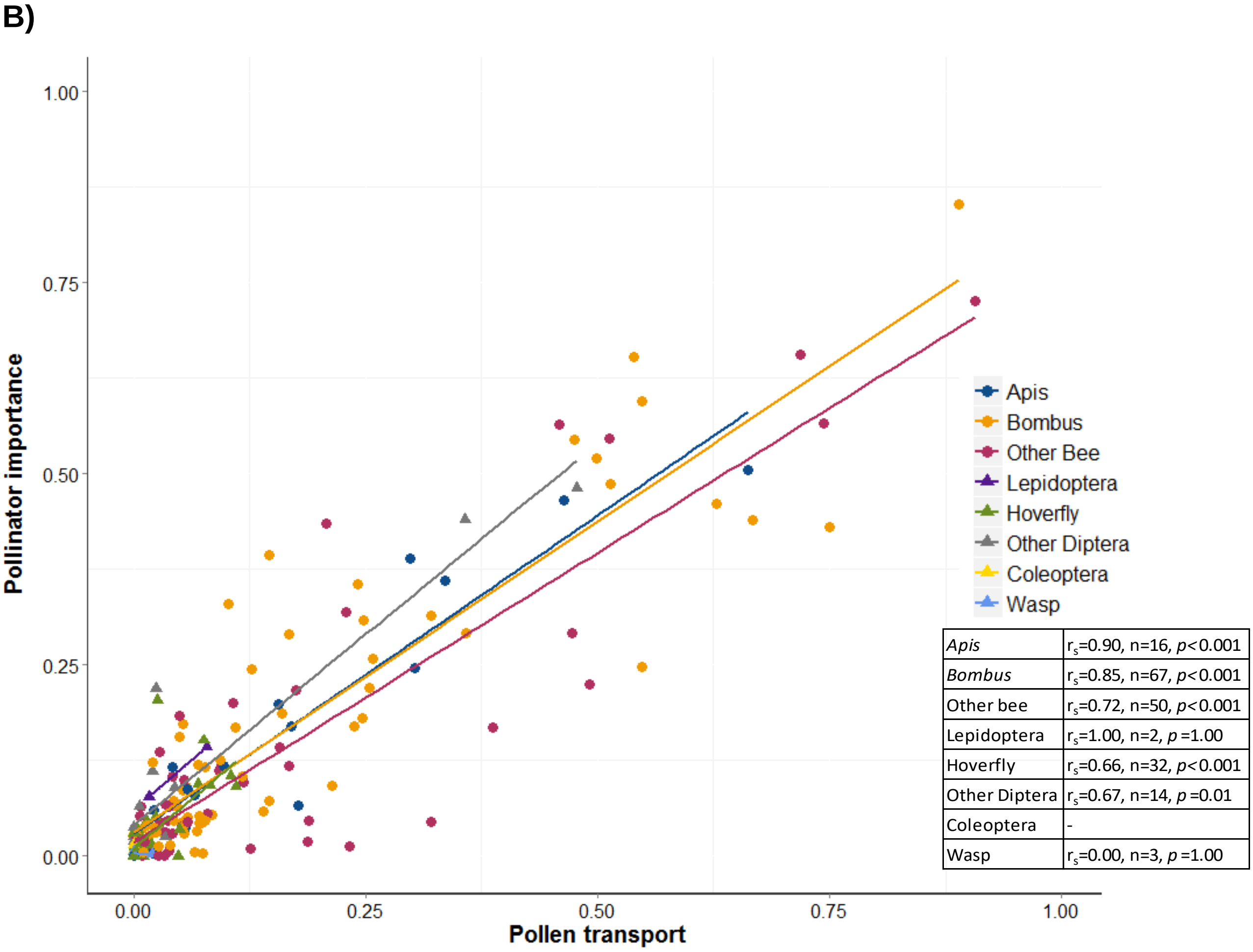
	Measure of pollination		
Proxy for pollination	**Pollinator effectiveness**	**Pollinator importance**	
Visitation	6%	72%	Fig. 6.6a
Pollen load	27%	31%	Fig. 6.6b
Pollen transport	15%	77%	Fig. 6.6c

Table 6.5 Summary of the percentage of variation in two explicit measures of pollination explained by three alternative (proxy) measures of pollinator performance (visit frequency, pollen load and pollen transport). No measure explained more than 30% of the variation in pollinator effectiveness, although pollen loads predicted the greatest percent of this variation. Both visitation and pollen transport explained a considerable proportion of the variation in pollinator importance. The percentage of variation (r^2) is calculated from the r values in Fig. 6.6.

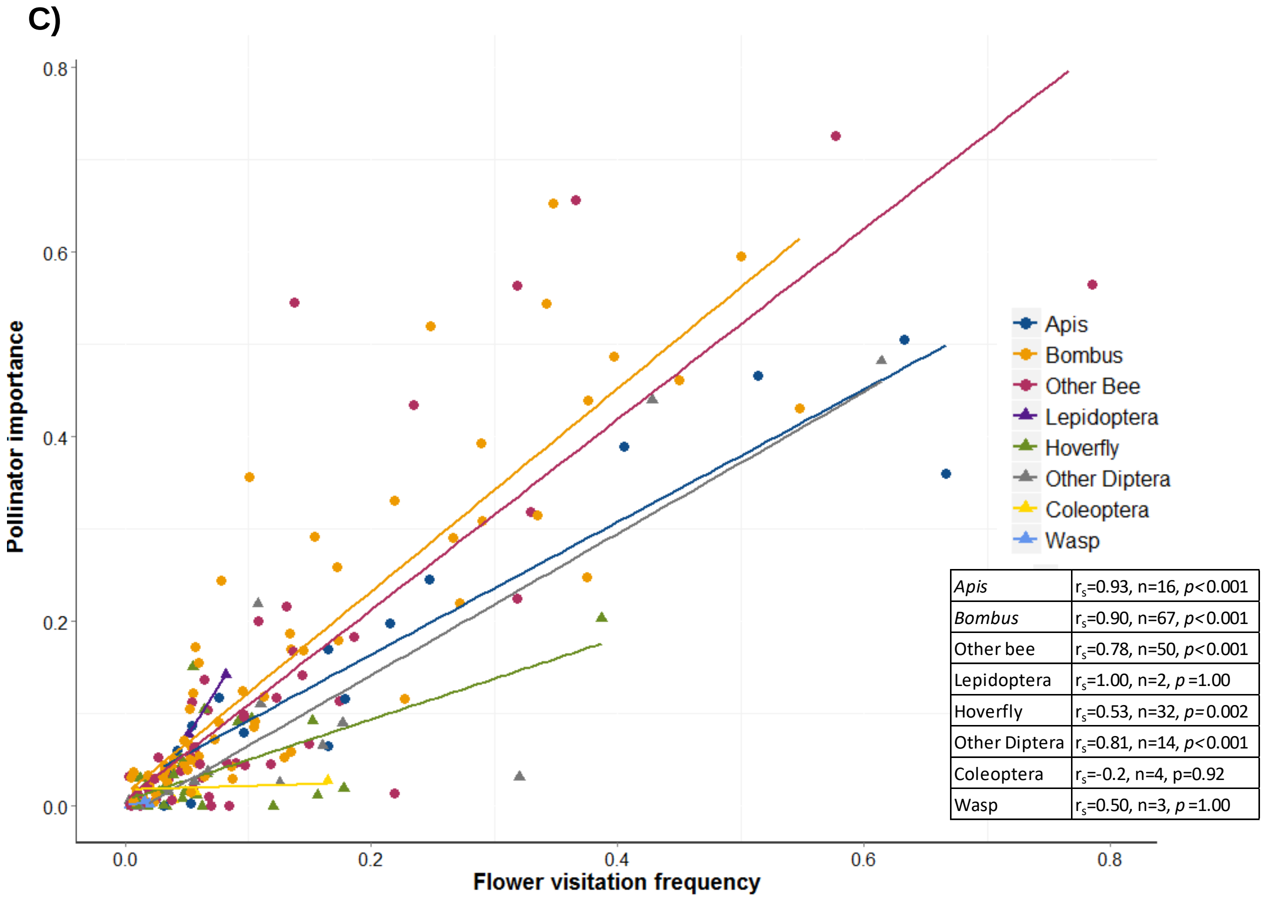
As flower visitor pollen loads (PL) explained the greatest variation in pollinator effectiveness (PE) (27%, **Table 6.5**) the relationship between PL and PE was examined for each group of flower visitors. I found that the correlation was only significant for *Apis* (r_s=0.65, p=0.01, n=16), *Bombus* (r_s=0.37, p=0.02, n=67), other bees (r_s=0.33, p=0.02, n=50) and hoverflies (r_s=0.47, p=0.01, n=32) (**Fig. 6.7a**). However, including flower visitor group as an interaction in the linear model (**Fig. 6.7a**) made no difference to the result (for full results, see **Appendix 6.5**). Despite this, it is interesting to note that in **Figure 6.7a** the values for PE and PL were much smaller for *Apis* when compared to *Bombus* and the other bees (although there were less data points for *Apis*). For almost all visitors the correlation between PI and PT (**Fig. 6.7b**) and PI and V (**Fig. 6.7c**) was significant; again, including flower visitor group as an interaction in the linear models made no significant difference to the results of **Figure 6.6b** and **6.6c** (for full results, see **Appendix 6.5**) although small sample sizes made these hard to detect.



Apis	r_s=0.65, n=16, p=0.01
Bombus	r_s=0.37, n=67, p=0.02
Other bee	r_s=0.33, n=50, p=0.02
Lepidoptera	r_s=1.00, n=2, p=1.00
Hoverfly	r_s=0.47, n=32, p=0.01
Other Diptera	r_s=0.49, n=14, p=0.08
Coleoptera	r_s=0.20, n=4, p=0.92
Wasp	r_s=-0.50, n=3, p=1.00



Apis	r_s=0.90, n=16, p<0.001
Bombus	r_s=0.85, n=67, p<0.001
Other bee	r_s=0.72, n=50, p<0.001
Lepidoptera	r_s=1.00, n=2, p=1.00
Hoverfly	r_s=0.66, n=32, p<0.001
Other Diptera	r_s=0.67, n=14, p=0.01
Coleoptera	-
Wasp	r_s=0.00, n=3, p=1.00



Apis	r_s=0.93, n=16, *p*<0.001
Bombus	r_s=0.90, n=67, *p*<0.001
Other bee	r_s=0.78, n=50, *p*<0.001
Lepidoptera	r_s=1.00, n=2, *p* =1.00
Hoverfly	r_s=0.53, n=32, *p*=0.002
Other Diptera	r_s=0.81, n=14, *p*<0.001
Coleoptera	r_s=-0.2, n=4, p=0.92
Wasp	r_s=0.50, n=3, *p* =1.00

Figure 6.7 Relationships between two explicit measures of pollination (pollinator effectiveness and pollinator importance) with three proxies for pollination (pollen loads, pollen transport and flower visitation frequency) for 8 groups of flower visitors. Each data point represents the proportional value of one visitor to one plant, e.g. *B.hortorum* to *Digitalis*, with regressions for each group of flower visitor calculated using a Spearman's Rank correlation. In each case, the strength of the relationships between the two measures of pollinator performance did not vary significantly between flower visitor groups, although the number of data points for each visitor varied as a result of the abundance of flower visitors to different plants. Full results of the linear models testing the relationships by visitor groups are given in Appendix 6.5. Results include all of the interactions recorded over two summers to 24 plants, for interactions where both pollinator effectiveness and pollen load data were available.

6.4 Discussion

Prior studies have noted the importance of the specialisation of interactions in plant-pollinator networks (as specialisation relates to the dependency of species and their resilience to disturbance) but have had to use proxies for pollination. The present study was designed to determine the similarities in specialisation of a pollinator importance network, compared to visitation and pollen transport networks.

6.4.1 Both pollinator importance and pollen transport networks increase the specialisation of a visitation network

(i) At the level of the community

As mentioned in Chapters 4 and 5, both pollinator importance and pollen transport networks are known to be more specialised than visitation networks. However, what remained unclear from the communities studied by Ballantyne *et al.* (2015, 2017) and from recent pollen transport networks (e.g. Alarcón 2010 and Popic 2013) was how well a PT network represented the specialisation of a PI network. Although many flower visitors transported pollen (Chapter 4) and most flower visits resulted in stigma deposition (Chapter 5), would the structure of these interactions remain similar in a PI network? The results of my study show H_2' in the PI network to be close to that of the PT network, with both estimates increasing the specialisation of interactions compared to visitation alone. However, the extent of this difference in specialisation (and other community-level indices) was less than expected, and despite an increase in the number of links in the PT network, the structures of the networks were remarkably similar. A possible explanation for this might be that, at a community level, the differences between flower visitors in visit frequency exceeded the differences in pollen loads and pollinator effectiveness, so that the structures of the PT and PI networks (incorporating visit frequency) were surprisingly similar to the original V network. This seems to be consistent with the expectation of Vázquez *et al.* (2005) and supports the idea that visitation is a suitable proxy for pollination under these circumstances. There may, however, still be communities where variation in pollinator effectiveness outweighs that of visitation, and this remains to be tested.

(ii) For individual species

Prior studies have noted the importance of species-level indices for specialisation (*d'*), and the present study hypothesised that plants would become more specialised in the PI network compared to both PT and V. The species strength of flower visitors was also predicted to increase in the PI network (Chapter 2). Surprisingly, no significant differences were found between the networks in either measure. This highlights how differences in the specialisation of individual plants and the species strength of individual visitors are lost when average values for all species are compared between the networks (e.g. the average plant *d'*). A note of caution is due here, since future comparisons of flower visitation at the community level with PT and PI networks could fail to recognise changes in the specialisation of individual plants and visitors.

In the current study, comparing the most specialised plant between the networks showed that *Digitalis* was in fact more specialised in terms of pollen transport and receipt, a point which was not recognised by visitation patterns alone. This result is in agreement with Verboven *et al.* (2012) who reported pollen limitation in urban populations of *Digitalis*.

A further important finding was that the species strength of *Apis* declined considerably in the PT and PI networks, further supporting the idea that *Apis* may not be as important a pollinator as species of *Bombus* and non-eusocial bees (Chapter 5). Although it could be argued that the increase in the importance of Halictidae in the PI and PT network is a result of the grouping together of several species, this argument cannot be applied to the increase in several distinct *Bombus* species (**Fig. 6.5)**.

6.4.2 Pollen transport is a better proxy for pollinator importance

This study sought to evaluate whether proxies for pollination, (visitation and pollen transport) are reasonably synonymous with true pollination (as defined by stigma deposition). The most interesting finding was that flower visitation frequency was only very weakly correlated with pollinator effectiveness, explaining just 6% of the variation in PE and supporting the conclusions of King *et al.* (2013) that visitation is a poor proxy for pollination in individual plants. However, the findings of the current study do not support the predictions made by King *et al.* (2013) that a PI network would be substantially more specialised at the community level. Unlike King *et al.* (2013), my study combined visit frequency and pollinator effectiveness to calculate pollinator importance for each plant (as in Ballantyne *et al.* 2015, 2017). Consequently, pollinator importance at the community level was strongly positively correlated with V; although visit frequency was a poor predictor of single-visit deposition, it explained 72% of the variation in the total importance of visitors as pollinators. Hence the predictions made by Vázquez *et al.* (2005) are found to be generally supported.

Previous studies using pollen loads (PL) to predict pollinator effectiveness have assumed this to be a more accurate predictor than visitation, although the weak correlation between PL and PE supports the conclusions of Adler and Irwin (2006) that the proportion of conspecific pollen on the bodies of visitors is a poor predictor of pollen deposition on to the stigma. However, pollen transport (PT, incorporating visit frequency) explained the greatest proportion of the variation in pollinator importance (77%), confirming that this measure (which requires less intensive fieldwork compared to PI) is a reasonable proxy for the importance of pollinators at a community level. While the ideal resolution would be to collect all three measures of pollinator performance, I acknowledge that the increase in sampling effort required for a PI network may be outweighed by the strong correlation between PT and PI.

While previous visitation and pollen transport networks have focused on a single order of flower visitors (Popic *et al.* 2013, Banza *et al.* 2015) my study considered how the suitability of proxies for pollination may vary between groups of flower visitors. Although no significant differences were found between the groups, the strength of the relationship between PI and PT/V was largely driven by bees. These findings raise the intriguing questions whether flower visitation is as suitable a proxy for pollination in communities where Hymenoptera are not the most frequent flower visitors; for example, in tropical montane forests at high altitudes where flower visitation networks are dominated by Coleoptera and Diptera and demonstrate relatively high levels of connectance and specialisation (e.g. Ramos-Jiliberto *et al.* 2010, Cuartas-Hernández & Medel 2015) or when nectavirous birds are the most frequent flower visitors (e.g. Gonzalez & Loiselle 2016). South African flower-visitor networks with exceptionally high levels of specialisation would be an excellent choice for testing this question, as many plants demonstrate adaptations for long-billed birds and long-proboscid flies (e.g. H_2'=0.83, Pauw & Stanway 2015).

6.4.3 Each network complements the others to provide a more complete picture of pollination

None of the three networks provide a complete picture of pollination. Pollen transport networks are valuable for revealing the hidden links between plants and their visitors, while pollinator importance networks are the only type to consider plant fecundity from the female perspective. In each of these networks, visitation frequency is a crucial component.

However, the additional measures included in the interactions in the PI network are solely from the plant’s perspective; they do not reveal any of the reciprocal benefits a flower visitor may gain from an interaction. Consequently, a PI network cannot replace a traditional visitation network entirely; it would be incorrect to assume only the plant's perspective is important for the continuation of the mutualism. Without an understanding of the importance of resources for the flower visitors, using a network to study a mutualism is partially redundant.

6.4.4 Limitations

It is important to bear in mind the limitations raised in previous chapters, including the differences in network size when comparing indices (Chapter 4) and the difference between pollen quality and quantity (Chapter 5). While the implications of studying a subset of the community for specialisation were discussed in Chapter 3, the sampling effort required to collect three simultaneous data sets is an important issue for future research: even for a subset of a community, the frequency of flower visitors (see Chapter 3) meant that the collection of pollen load and pollinator effectiveness data was not possible for all visitors, and the comparisons between the three networks include only those frequent enough so that all three measures were available. Therefore, while the PT and PI networks provide more detail to evaluate the flower visitors from the plant's perspective, the V network used in comparisons does not reflect the full extent of floral resource use by flower visitors.

The limitations of pooling pollen data were discussed in Chapters 4 and 5, and in an ideal situation sufficient data points for PL and PE at different temporal periods throughout the day (e.g. Baldock *et al.* 2011) would be collected; consequently, the differences between the V, PI and PT networks might be greater.

Finally, the net effect of a flower visitor on plant reproduction depends on the ratio between pollen removal and deposition, relative to all other visitors in the community. While PL and PE measure this, a comparison of networks does not account for it.

6.4.5 Future directions

While there are limitations to measuring pollinator effectiveness at the community-level, the results of this study raise even more questions for further investigation.

(i) Creating pollinator importance networks in different habitats

While similarities have been found in comparisons between visitation and pollinator importance networks in four habitats (the UK garden, Dorset, Israel and Kenya) the relationship between PE and PI with visitation frequency in this study was strongly influenced by bees. The

Arctic represents a community less-dominated by bee visitors, and the methods used in this thesis were applied in Zackenberg, Greenland, in the summer of 2016 (assisting Riikka Kaartinen, University of Edinburgh) by Gavin Ballantyne and myself. While the barcoding of pollen load and pollinator effectiveness data is still in progress, there were two immediately obvious issues. Firstly, visitation by *Bombus polaris* (the only social bee species) was very infrequent, thus creating large potential for differences as predicted in the strength of the relationships between measures of V, PT and PI. Secondly, the collection of PE data (in terms of the frequency of visits to bagged flowers) was considerably more difficult in the community where flowering was patchy throughout the habitat according to snow-melt compared to flowering in the garden. Very low visitation rates made it very difficult to simultaneously collect V, PL and PE data for several plants, and further studies, creating PT and PI networks will need to take this into account.

As this study is the first to create a visitation, pollen transport and pollinator importance network for a single community, further work is required to test the conclusions of these results in other habitats. There are still unanswered questions about the structure of V, PT and PI networks including particularly specialist plants, and those visited far less frequently; for example in tropical situations where visitors may be more varied, including vertebrate groups (Betts *et al.* 2015, Cárdenas *et al.* 2017), and where flowers may be highly specialised morphologically (Pauw & Stanway 2015) and very widely spaced (Caraballo-Ortiz *et al.* 2011) although this would be extremely challenging given the sampling effort required.

(ii) Experimentally manipulating species abundance

In future investigations, it might be possible to experimentally remove visitors with low PE/PL and high flower visit frequency, to establish whether this increases the differences between V, PT and PI networks. A candidate in this study would be *Apis*, which theoretically could be excluded by selecting habitats away from managed hives. The removal of these visitors might reduce the strength of the relationship between PI and V, although the visitation frequency of more effective pollinators could increase.

6.5 Conclusion

The purpose of this chapter has been to show how PI networks vary in structure and specialisation to V and PT networks, and assess the value of visitation and insect pollen loads as proxies for pollination.

Although visually the bipartite networks appeared similar (**Fig. 6.1**), interaction matrices (**Fig. 6.2, 6.3**) revealed interesting characteristics of the plant-pollinator relationships in the community, which were unseen from visitation alone and the first to be shown in a garden. Surprisingly, most observed visits resulted in both pollen transport and deposition on to the stigma. Consequently, the structures of the networks were more similar than expected; while the pollen transport network did predict the increased specialisation of the pollinator importance network, the extent of this was less than hypothesised at the start of the thesis. However, close inspection of the differences in the specialisation of individual plants, and of the strength of individual visitors, revealed that community level indices can conceal changes in the position of individual species between the networks. While analyses at this level contain a great deal of information about an entire community, it is important for the preservation of the interactions to consider the individual species.

Collecting the data for pollinator effectiveness requires a larger sampling effort than pollen loads, and certainly than visitation. Contamination from selfing or the deposition of unviable pollen also means it is not a ‘perfect’ measure of a visitor’s contribution to a plant’s reproductive success. However, the structure of the PI network for the garden, and the relationships between the proxies of visitation and pollen transport with PI, allow future studies to make informed choices and conclusions for the construction of plant-pollinator networks.

Chapter 7.

General Discussion

7.1 Thesis overview

The aim of this study was to examine how the structure of pollinator effectiveness and importance networks compared to pollen transport and visitation networks. This has been a longstanding question in pollination ecology, given that visitors are not always pollinators, that flower visitors may act as ‘cheats’ with varied effects on plant fitness (Irwin *et al.* 2010), and that pollen transport does not guarantee stigma deposition (Adler & Irwin 2006). Consequently, visitation and pollen transport networks may over-estimate the level of generalisation in plant-pollinator communities. While a small collection of pollinator effectiveness and pollinator importance networks have recently been published by Ballantyne *et al.* (2015, 2017), my study is the first to construct and compare all three for the same site which has been referred to as ‘the ideal resolution’ (Alarcón 2010) and represents a promising step forward (Willcox *et al.* 2017). In Chapters 1 and 2, I outlined the reasons why visitation is not equal to pollination, and why pollinator effectiveness and pollinator importance networks may be more specialised than either a visitation or pollen transport network. I begin this section by revisiting three of the most important findings of this thesis based on Chapters 3-6:

Firstly, by directly evaluating the relationship between flower visit frequency and pollinator effectiveness for a diverse community, I have confirmed the prediction made by Vázquez *et al.* (2005) that the most frequent flower visitors make the greatest overall contribution to pollen deposition, but that this is not always the case on a per-flower basis. In my study, the variation in flower visitation frequency exceeded that of pollen loads or deposition, so that the total effect of a visitor (pollen transport or pollinator importance) was largely determined by visit frequency. This finding is highly relevant to the large number of pollination networks that have relied upon this assumption.

Secondly, although the differences in the structure of three networks (particularly specialisation, H_2') were less than expected, I found strong evidence that the inclusion of pollen load and pollinator effectiveness data does affect the topological position of individual plants and visitors in the networks (i.e. their specialisation and species strength). This is one of the more significant findings to emerge from this study, as it highlights that patterns at the community level may not be reflected by all species within it, with implications for conservation. The data also suggested that the strength of the relationship between visit frequency and pollinator effectiveness may vary markedly between visitor taxa, and I advocate the need for caution in future studies that assume increased visitation frequency translates to increased pollinator importance for all flower visitors.

Thirdly, as the first study to directly compare flower visitor pollen loads to pollinator effectiveness, I have confirmed that at a community level, pollen loads provide a better predictor of stigma deposition than visitation alone and can be performed non-fatally. This finding is particularly valuable to future plant-pollinator networks, as pollen load data were relatively quick to collect (although lengthy to process) and can be interpreted from both the plant and visitor's perspectives. However, pollen load and pollinator effectiveness data are equally limited by the problem that pollen quantity does not measure quality. Although this was appreciated at the start of the fieldwork, the additional effort required to genotype pollen or follow deposition through to seed set exceeded that possible in this study. While in most studies of pollinator effectiveness visitors deposit some pollen on to the stigma (although the quantity can be very variable, e.g. Gómez & Zamora 1999, Javorek *et al.* 2002), surprisingly almost all flower visitors carried pollen, in contrast to some previously published studies (Bosch *et al.* 2009, Devoto *et al.* 2011, Popic *et al.* 2013). This is particularly valuable for future studies considering pollen deposition, as it highlights how the next step in pollinator importance networks should be to include a measure of the genetic identity and compatibility of the pollen carried by different visitors.

Despite finding limited differences between the network structures, I highly recommend the inclusion of pollinator effectiveness data into future network studies, as it provides a more informed measure of the value of flower visitors as pollinators, and the data from the present study contributes to several wider questions posed by Mayer *et al.* (2011), that are key areas for the future of pollination ecology. Just how incorporating pollinator effectiveness into network ecology addresses these questions is explored in the following section.

7.2 Is it practical to construct plant-flower visitor community networks from the plant’s perspective?

My data show clearly that it is possible to construct community networks from the plant’s perspective. Although some authors have suggested that flower visitation networks already provide the plant’s perspective (e.g. Bosch *et al.* 2009), many of the flower visitation networks referenced in earlier chapters have acknowledged that this is not the case. A limitation of pollinator effectiveness is the increased sampling effort involved, and I acknowledge that in this study and those by Ballantyne *et al.* (2015, 2017) data could not be collected for all plants in the community. However, I was able to sample 25 plants with no assistance, which shows that it is possible to collect these data – so long as visitation rates are reasonably high. This is a crucial point that became obvious when applying the methods in Greenland in the summer of 2016, and further research in different habitats is needed to explore how applicable pollinator effectiveness is to large proportions of different communities.

Recent studies have advocated methods for increasing the speed of pollinator effectiveness data collection, either by offering detached flowers to visitors (Howlett *et al.* 2017) or even by immobilising visitors before manually applying them to flowers (Park *et al.* 2016). While Howlett *et al.* (2017) found no difference in pollen deposition between the stigmas of detached and non-detached *Allium* flowers, these were emasculated which may affect visitor foraging and reduce pollen deposition (see Chapter 5). Furthermore, both studies used relatively simple flowers (*Allium* umbels and the open bowl-shaped flowers of *Malus pumila* 'Honeycrisp') which reduces the relevance of the findings to more complex flowers that require more experienced floral handling. Consequently, I propose that in communities where flower visitation is less frequent than in the garden, pollinator effectiveness (using a stationary approach) could be focused on a subset of plants that are also sampled for visitation and pollen transport data. This would allow the relationship between visit frequency and pollinator effectiveness/importance to continue to be tested, without requiring individuals or research teams to commit to a substantial increase in sampling effort.

After flower visitation frequency, an important complication in using pollinator effectiveness data is the variation in autonomous pollen deposition (‘selfing’) in unvisited individual flowers and plants (Chapter 5). The extent of this is likely to vary between communities, and this study has shown that the deposition of self-pollen on to unvisited stigmas can be quite high. Although a solution to this would be to emasculate all flowers prior to dehiscence, it will be interesting to assess how patterns in selfing vary between more plant communities, particularly when there are considerable anthropogenic selection pressures on floral morphology (e.g. in gardens). The effect of selfing on inbreeding depression and population viability has been identified as a major, unanswered question in pollination ecology by Mayer *et al.* (2011).

7.3 Insights into flower visitation in gardens

Although this study was based in a single urban garden the findings add to a growing body of literature on the importance of urban areas and gardens for flower visitors, and the pollination of plants in these highly modified habitats. These results have several meaningful implications for the study of pollination in gardens.

Firstly, heterospecific pollen transfer (HPT) can be of considerable cost to plants sharing generalist flower visitors in urban areas (Baldock *et al.* 2015, Tur *et al.* 2016). However, the extent to which this occurs between plants in gardens is largely unknown (although see Werrell *et al.* 2016). A major contribution of this study has been to show that heterospecific pollen transfer was on the whole uncommon (15.6% of all stigmas) and accounted for only 6% (n=442) of the average stigma load (Chapter 5). Although the level of HPT did vary between plants, as reported elsewhere (Fang & Huang 2013), this study has raised intriguing questions regarding the mechanisms that seem to reduce ‘stigma clogging’ in highly diverse plant communities with many ‘exotic’ species. However, stigmas may still be blocked by incompatible intraspecific pollen (e.g. from geitonogamous transfer) and the effect of habitat fragmentation (e.g. Noreen *et al.* 2016) and abiotic conditions (e.g. atmospheric pollution, Cuinica *et al.* 2013) on inbreeding and the viability of pollen in urban areas remains a key area for future studies.

One of the proposed explanations for low HPT is that flower visitors in gardens may demonstrate high levels of floral constancy. In Chapter 4, 51% of pollen loads (n=516) contained either 1 or 2 pollen types, suggesting that visitors frequently exploited a very limited number of plant species during a single foraging bout. This raises important questions regarding the best way to achieve planting for flower visitors in gardens, as a greater abundance of a lower diversity of species may be more beneficial than a large variety of plants planted in small patches. In Chapter 3, floral resource networks illustrated that *Bombus* rarely visited flowers that provided nectar only, while Halictidae largely exploited garden flowers for pollen. Findings such as these should be used to inform planting in ‘pollinator friendly’ gardens. Selecting plants with higher pollen viability can also improve the protein content for pollen-feeding visitors (Yeamans *et al.* 2014), and the quality of floral rewards strongly increases pollinator species richness and flower visitation frequency (Fornoff *et al.* 2017).

In Chapter 3, a diverse range of taxa was documented visiting the flowers in the garden. The use of stationary observations successfully recorded very rare flower visitors and equal attention was paid to dipteran and to hymenopteran visitors, given recent calls for more attention to focus on these as potential pollinators (Orford *et al.* 2015). One surprising result of the visitation network was the apparent specialisation of Diptera in the garden; although these species could be locally very abundant on some plants (e.g. *Leucanthemum, Eupatorium*) flies were opportunistic visitors that were uncommon on most plants. While hoverflies were observed on a wider variety of flowers, declines in species richness for both hoverflies and non-syrphid Diptera in urban areas have recently been reported by Baldock *et al.* (2015). Taken together, the impact of urbanisation on these species should be a priority for future studies of urban flower visitor biodiversity, and management to enhance populations might involve educating gardeners about the value of ‘weeds’ (such as *Eupatorium*). Similarly, Lepidoptera were very poorly represented in the garden, as reported in urban areas elsewhere in the UK (Baldock *et al.* 2015, Dennis *et al.* 2017), and continued efforts are needed to determine the causes for this.

Interestingly, both the pollen load (Chapter 4) and pollinator effectiveness data (Chapter 5) revealed differences in the behaviour of *Apis* and the other bee species in the garden. Although *Apis* were more frequent flower visitors to many plants, in general they carried smaller, less diverse pollen loads and deposited less pollen during a single visit (although this effect was not quite significant). However, the value of these visitors was largely increased by their visit frequency. This effect has been documented in other habitats (e.g. Thomson & Goodell 2001) and taken together, this study adds to a growing body of evidence confirming the importance of bee diversity for providing ecosystem services in urban areas (e.g. Lowenstein *et al.* 2015), and a need to avoid undue focus on honeybees. It also strengthens the need to assess whether urban beekeeping may reduce the pollen-nectar resources for wild bee populations (Torné-Noguera *et al.* 2016).

Data collected for the pollen transport network also revealed many extra hidden links, particularly for bees, suggesting that data on highly mobile visitors may be most distorted by focal plant observations. Based on the visitation patterns in the garden, a priority for further research should be the fitness of longer-tongued bees (e.g. *Bombus hortorum*) in gardens, as these visitors are known to be specialists (e.g. Hanley *et al.* 2014) that require flowers with long corollas.

7.4 Pollination at the stigma versus landscape level

Unfortunately, like many areas of conservation biology, the study of pollination faces difficult decisions regarding methods and sampling costs, which will determine the direction of future networks (see Hegland *et al.* 2010). In each chapter of this thesis, the specialisation of individual species versus that at the community level was a common theme, and maintaining pollination as an ecosystem service at the community level requires a very different approach to prioritising that of individual species (Vamosi *et al.* 2016). The appearance of pollinator importance networks (Ballantyne *et al.* 2015, 2017) and individual pollen load networks (Tur *et al.* 2014) alongside traditional flower visitation networks highlight the problem faced by pollination network ecologists: to go big or small? As the first garden study to include visitation and pollinator importance networks, I evaluate the strengths of each approach and suggest why both are needed.

I anticipate that traditional flower visitation networks will continue to dominate the field, as visitation remains the most efficient measure to use at a landscape level. Large scale visitation networks have already explored the effect of the surrounding landscape on the structure of interactions (e.g. agricultural management by Hagen & Kraemer 2010; localised grazing by Vanbergen *et al.* 2014) and how networks vary across gradients of urbanisation (Gelsin *et al.* 2013, Baldock *et al.* 2015), invasion (Bartomeus & Santamaria 2008), climate change (Devoto *et al.* 2007) and habitat restoration (Kaiser-Bunbury *et al.* 2017). The recent review by Senapathi *et al.* (2017) provides excellent suggestions for the future of landscape-level networks. As habitat loss and fragmentation continue, the strength of visitation networks continues to be their ability to include rare flower visitors, to cover the entire foraging range of very mobile visitors and to demonstrate the connectivity between populations. The results from landscape-level studies are also likely to be more readily generalised to different sites. However, visitation networks have also revealed differences in the structure of interactions at much smaller scales; Janovský *et al.* (2013) detected differences in the visitation networks over tens of metres at a single site, caused by subtle changes in the plant community. Consequently, landscape level networks may be misleading when visitation is averaged over large plots.

At the stigma level, pollinator importance networks differentiate between visitors by the quantity of pollen deposited. Although these differences may be exceeded by variation in flower visit frequency (as in Chapter 6), measuring pollinator effectiveness forces the researcher to consider the timing and duration of stigma viability, and in this area there is still much to be understood: particularly how stigma viability is assessed in the field (Mayer *et al.* 2011) and the negative effects of air pollution on stigma viability (Jaconis *et al.* 2017). Studying flower visitor interactions at the stigma level also benefits from considering plant mating systems (e.g. the occurence of ambophily, Duan *et al.* 2009), heterospecific pollen transfer (e.g. Emer *et al.* 2015) and the impact of a warming climate for phenological mismatch and the effect of this on pollinator effectiveness (Bartomeus *et al.* 2011, Rafferty & Ives 2012). These are factors which make the study of pollination so complex and intricate, and it would be a mistake to overlook them. Interestingly, Vamosi *et al.* (2016) have suggested that sampling pollen from the stigmas of herbarium samples could also be used to reveal trends in historical interactions between plants (pollen transfer networks). However, to be most informative, pollinator importance networks must proceed by measuring pollen viability.

An excellent example of how landscape and local data can be combined in future plant-pollinator networks is the recent study by Theodorou *et al.* (2017). These authors explicitly related network metrics (including specialisation) to seed set in four experimental plants along a rural to urban gradient, and concluded that metrics were a poor proxy for pollination.

I have already recommended that future visitation networks are supplemented with pollinator effectiveness for a subset of the plant community, and I encourage a certain degree of flexibility in the proportion of the plant community that can be studied in this way. This will permit a more robust evaluation of flower visitors from the plant's perspective, and a critical awareness of how relevant network metrics are for measuring pollination. While the use and sophistication of metrics increases, it is important not to lose sight of the ecology behind such interactions, selecting only those that most represent the questions asked.

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Appendices

Appendices embargoed at the request of the author