UNIVERSITY OF
BIRMINGHAM

An investigation into bee assemblage change along an urban-rural gradient

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A thesis submitted to the University of Birmingham for the degree of
DOCTOR OF PHILOSOPHY

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April 2014

Thesis Abstract

The supposed decline of bees is feared to impact on the pollination services they help to provide to 87% of angiosperms. Urban densification and a rise in human population call for greater investigation into how the urban-rural gradient can influence bees and their pollination services. This thesis aimed to further current knowledge by: (i) increasing site and regional replication by sampling bee assemblages along the urban-rural gradient in Birmingham, UK; (ii) increasing the current documentation of bee traits associated with pollen carryover; (iii) incorporating a trait-based analysis to bee assemblage change along the urban-rural gradient; (iv) testing whether the pollination services along the same gradient varied between land-use types and (v) investigating the provisioning and reproductive success of bees in the urban environment. This thesis supports previous evidence of species-specific variation by urbanisation and shows how trait composition and trait diversity are influenced by bee assemblage change along the urban-rural gradient. However, this recorded assemblage and trait variation appeared not to alter seed-set in Campanula glomerata *between urban and rural areas. However, this thesis lacked statistical power when analysing differences in seed-set. Further phytometer studies are essential if pollination services are to be assessed along the urban-rural gradient. I also found that nutritional quality could play an important role in provisioning rate in the solitary bee* Osmia bicornis*, with greater offspring produced in sites where more protein was found in provisioned pollen in urban areas. Furthermore, the inter-specific variation in pilosity (which could have an important role to play in pollen carryover and subsequent pollination) can be explained by body size and species' pollen transport adaptation. However, pilosity traits did not vary over the urban-rural gradient, giving no clear indication that pollen transfer was affected by urbanisation. This thesis helps to inform planning and conservation efforts to promote species diversity throughout urban-rural gradients. From this work, I suggest trait based analysis could be a common framework for future studies to measure bee assemblage change between urban and rural areas. Moreover, this research builds on previous work which suggests that particular traits affect pollen transport and subsequent pollination, and that further detailed study could give clues as to how changing bee assemblages could influence pollination.*

Acknowledgements

I would like to thank my supervisors Jonathan Sadler, Adam Bates and Mark Ledger for their time and patience in guiding me through this research. Thank you to James Hale for help with GIS and to Matt O'Callaghan for being a source of knowledge in ecological and entomological matters. Thank you to Mel Bickerton, Richard Johnson and Jamie Peart for assistance with field and laboratory work. For permission to access sites throughout Birmingham, I would like to thank Birmingham City Council, the Canal and River Trust (formerly known as British Waterways) and The University of Birmingham. Thank you to my family and friends who supported me through this exciting research project and for always taking an interest in the work I've undertaken. Thank you to Stuart Roberts for checking the identification of certain specimens and for the bee trait dataset. Thank you to the Bees, Wasps and Ants Recording Society (BWARS) for their extensive resources. Finally, thank you to the Engineering and Physical Sciences Research Council (EPSRC) who funded this research as part of the Urban Futures project.

"It is not the strongest or the most intelligent who will survive but those who can best manage change."
— *Charles Darwin*

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Definitions, Abbreviations and Physiological Terms

Cleptoparasite: A parasitic species of bee which has evolved to lay its eggs in the nests of non-cleptoparasitic bees and feed on the resources provisioned.

Corbicula/Corbiculae: The hind tibia on *Bombus* and *Apis* species which is flattened and has specialised hairs that enable pollen collected by bees to be mixed with nectar and kept in position whilst flying.

ITD: Intertegular distance (the distance between the wing bases on a bee's thorax).

Oligolectic: Pollen specialists using pollen collected from a restricted range of flowers, commonly but not always from one plant family only.

Pollen carryover: The transferral of pollen between conspecific flowers.

Pollination effectiveness: The extent to which the transfer of pollen between conspecific flowers leads to seed-set in plants.

Pollination services: The general term used to describe the whole process of pollen transferral and subsequent seed-set in plants, usually facilitated by a vector (including bees, wasps, bats, etc.)

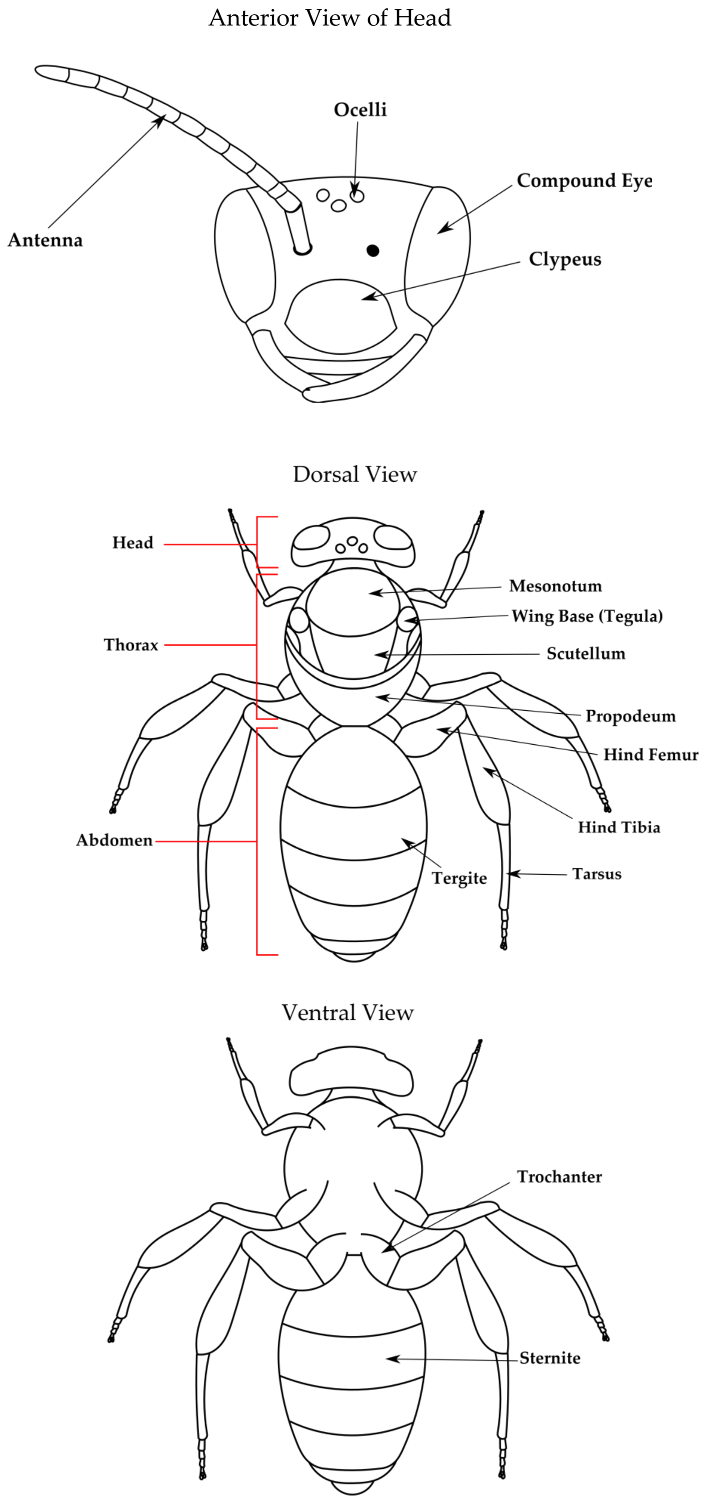
Pollination success: When pollen is moved from one flower to another con-specific and leads to pollination.

Polylectic: Pollen generalist able to forage on a range of plant species for their pollen resources.

PTG: Pollen transport groups are defined by Thorp (1979) . They represent groups bees can be placed into based on the types of morphological adaptations they have to transport pollen from flowers back to their nest.

Social Brood Parasite: Bees of the sub-genus *Psithyrus* of the genus *Bombus*. These are the equivalent of cleptoparasitic species for bumblebees. The female brood parasite usurps the queen of an active nest and lays its own eggs. Workers of the old queen provision and nurture the usurper's offspring.

Scopa: Specialised hairs adapted for transporting pollen.



Anterior View of Head

Dorsal View

Ventral View

Figure i.1: A labelled anatomical diagram of a bee, referring to terminology used throughout this thesis.

Chapter 1

The importance of pollination services across an urban-rural gradient

1.1 Introduction

The proportion of land utilised or disturbed by human populations has been estimated between 39 and 50% (Vitousek et al., 1997), but currently it is difficult to say whether any ecosystem is exempt from the influences of anthropogenic disturbance (Gaston, 2010). It is estimated that by 2030 over five billion people will reside within urban areas globally (United Nations, 2011). This will have exceeded 50% of the populace of the world in 2008 and is set to increase to 84% by 2030 (United Nations, 2008). It would appear most taxonomic groups show a negative trend towards increasing built space that is associated with urban density, caused by impervious surfaces limiting vegetation and suppressing potential habitat (McKinney, 2008). This leads to habitat reduction, isolation and ultimately fragmentation (Young & Jarvis, 2001) linked to adverse effects on bird, mammal and insect taxa (Andrén, 1994; Wood & Pullin, 2002). Maintaining habitat for taxa in urban areas is vital if human populations are to experience benefit (a.k.a. ecosystem services) from their presence within cities (Andersson et al., 2007; Dearborn & Kark, 2010). Although cities can support and enhance some ecosystem services, urbanisation can also limit certain services (Gaston et al., 2013). This in part is due to green space loss within cities (urban densification), a factor noted to inhibit the services ecosystems can provide to human populations (Tratalos et al., 2007; Dallimer et al., 2011).

An ecosystem service which is of direct benefit to human populations is pollination. The animals that provide pollination services to wild plants and crops include managed and unmanaged bees, beetles, butterflies, wasps, flies, bats, birds and lizards. Their pollination services are required to some extent by 87.5% of all angiosperms (Ollerton et al., 2011), plus 87 crop species that make up 35% of global food production (Klein et al., 2007). Bees are one taxonomic group highlighted as key in providing pollination services. Their greater abundance, diversity and trait complementarity lead to increased pollination efficiency and effectiveness (Kremen et al., 2002; Hoehn et al., 2008; Bommarco et al., 2012; Garibaldi et al., 2013; Klatt et al., 2014). It is believed there are ~20,000 species of bee worldwide (Michener, 2007), but concern is growing due to the recorded decline in range and loss of certain species in the UK, mainland Europe and North America (Biesmeijer et al., 2006; Potts et al., 2010). The most noted decline in bees has been observed in the honey bee (*Apis mellifera* L.), where

US beekeepers recorded the average loss of 42.2% of their total hives between 2009 and 2010 (Van Engelsdorp et al., 2011). The loss of honey bee hives has been attributed to a range of factors including pests and diseases (Genersch et al., 2010; Bacandritsos et al., 2010), pesticides (Johnson et al., 2010; Mullin et al., 2010) and habitat loss (Naug, 2009). As well as loss of bumblebee abundance in Europe, Asia and North America (Colla & Packer, 2008; Grixti et al., 2009; Williams & Osborne, 2009; Cameron et al., 2011), there is also evidence of range restrictions in UK bumblebees (Goulson et al., 2008). In general, wild bee decline is most closely linked to the simultaneous reduction in floral availability (Biesmeijer et al., 2006; Potts et al., 2010), and to the diminution in potential nesting and foraging resources at the landscape scale (Carré et al., 2009; Potts et al., 2010).

It is still too early to tell if pollinator loss will impair pollination services to crops and wildflowers (Ghazoul & Pin Koh, 2010; Ghazoul, 2013). However, if land-use conversion continues alongside human population increase, already intensively managed agricultural habitats may receive increasing pressure for greater crop output (Aizen & Harder, 2009). Currently, crops that bees pollinate represent ~20% of the agricultural crops grown in UK (Breeze et al., 2011). This is set to increase by 2020 however, with the UK requiring a further ~1.4 million ha of oilseed rape to be sown to meet the EU biodiesel target(Breeze et al., 2011). The crops bees pollinate make up a large proportion of human nutritional intake (Eilers et al., 2011) and hence bee loss has been emphasised as a concern for global food production (Allen-Wardell et al., 1998). Wild bee diversity is known as an important factor for increased crop yield (Bommarco et al., 2012), can constitute the majority of flower-visiting pollinators (Winfree et al., 2007) and increase the level of pollination even when honey bees are also present (Garibaldi et al., 2011). Equally, under threat to the limited availability of pollination services are those mutualisms that exist between species of bees and wild plants (Kearns et al., 1998; Kaiser-Bunbury et al., 2010). The conservation of bee assemblages which can maintain pollination services is therefore pivotal in alleviating potential pollination shortfalls in the future.

The literature implies that even with anthropogenic disturbances such as urban densification, an increase in the diversity of habitats in the landscape can supplement the resources bees require and increase their abundance and diversity (Steffan-Dewenter et al., 2002; Kleijn & Van Langevelde, 2006). There is increasing evidence that suburban areas may be able to provide a diversity of suitable nesting and foraging habitat for bees (Goulson et al., 2008; Osborne et al., 2008). Gardens have been highlighted as potentially vital habitat for a range of species within urban areas (Goddard et al., 2010), especially as the application of wildlife gardening has become widely accepted in urban households (Gaston et al., 2007). Urban areas can be beneficial to bees with fragments of grassland or parkland providing foraging opportunities (Chapman et al., 2003; Frankie et al., 2005; McFrederick & LeBuhn, 2006; Kearns & Oliveras, 2009). These sites can also offer nesting opportunities for cavity-nesting bee species (Cane et al., 2006; Bates et al., 2011) and provide foraging resources for winter-active bumblebees (Stelzer et al., 2010). From the current studies investigating bee assemblages along urban-rural gradients, generalist foraging species are found within urban areas, whilst foraging and to some extent nesting specialists are filtered out by increasing levels of built space (Cane et al., 2006; Bates et al., 2011; Banaszak-Cibicka & Żmihorski, 2012). That certain species can exploit urban areas is not restricted to bee assemblages (McIntyre, 2000; Kark et al., 2007). However, there is limited evidence as to the causes and implications of bee species-specific responses along urban-rural gradients, and even less is known regarding the implications these may have on the pollination services they provide.

Mostly, the research interest in bees and their pollination services has concentrated on agricultural areas. This is understandable as the economical and nutritional benefits to humans are directly affected by the pollination service bees provide to many crops (Gallai et al., 2009; Eilers et al., 2011). This has meant that the pollination service provided by bees over an urban-rural gradient has been researched to a lesser extent. However, there are several reasons why investigating the response of bees to urban-rural gradients should be continued further. First, bees are a valuable part of the wider biodiversity of the area and region. There are ~250 species of bee in the UK but there is still limited knowledge of how bees have and will be influenced by continuing land-use change. Although increasing in scientific attention, the interpretation of bee assemblage variation across the urban-rural gradient is restricted to specific study cities (Hernandez et al., 2009). In addition, comparable analysis between cities is limited by the scarce and sometimes unreliable of data on the regional presence of bee species (Vanbergen et al., 2013). Secondly, the value attributed to the pollination service of bees makes them economically important to maintain. How pollinator communities can persist along the urban-rural gradient will directly affect the pollination of amenity or garden plants and crops grown within urban areas (Garnett, 1996; Werrell et al., 2009) and potentially the crops of agricultural areas in close proximity to suburban and surrounding rural areas (Cussans et al., 2010; Goulson et al., 2010). However, to what extent pollination services are affected by change in bee assemblages along urban-rural gradients has attracted little attention (but see Pellissier et al., 2012; Verboven et al., 2012). Thirdly, by investigating how bee assemblages vary currently between the urban-rural gradient, it is possible to work towards mitigating the potential loss of bees and their pollination services as urban densification and urban population growth continues.

1.2 Thesis Aims

The general aim of this thesis was to improve the current understanding of how bee assemblages are altered along the urban-rural gradient and to gauge the impact this may have on the ecosystem service of pollination, whilst also improving the current documentation and understanding of bee traits important to the function of pollination. The thesis sets out specific objectives which need to be met for the general aim to be fulfilled. Through a series of chapters presented in the form of papers, the objectives of this thesis were to;

- improve the understanding of the effects of urbanisation on bees by characterising their assemblages across an urban-rural gradient (Chapter 2).
- explore the importance of pilosity as an important train in pollen carryover by bees and investigate whether two known functional traits explain the variation found in measurements of hair length, spacing, cover and type of UK bees (Chapter 3).
- investigate the explanatory importance of traits on species change across the urban-rural gradient and assess the implications this may have on trait diversity (Chapter 4).
- increase current understanding of the potential impact different land-use types have on pollinator visitation and subsequent seed-set (Chapter 5).
- investigate the resource provisioning potential and subsequent population dynamics of bees within an urban context (Chapter 6).

1.3 Thesis Summary

Initially, an extensive field experiment was undertaken in 2011 and 2012 to gain a comprehensive estimate of bee assemblages across the urban-rural gradient in Birmingham, UK. The aim of **Chapter 2** was to contribute to the growing data concerning the effects of urbanisation on bees by characterising their assemblage variation across an urban-rural gradient in canal side and parkland sites. This builds upon the previous investigation of Bates et al. (2011) which recorded bee assemblages in cemeteries and churchyards in Birmingham, UK.

Chapter 2 and previous literature illustrate the inter-specific response of bee assemblages across the urban-rural gradient. The implications this could have on pollination services of bees are still unclear as the importance of different bee species to ensure pollen carryover is unknown. To increase knowledge of how inter-specific variation in bees could influence pollination services, **Chapter 3** investigated pilosity variation between species and its association with other known traits.

Using the species composition dataset detailed in **Chapter 2** and a bee trait dataset (including **Chapter 3**'s pilosity analysis), **Chapter 4** investigated the explanatory importance of life-history traits on species change over the urban-rural gradient and explored the potential variation in trait diversity within cities.

To test if the variation in bee assemblages and the diversity of traits they have along the urban-rural gradient may influence pollination, pollination was evaluated between urban and rural areas. Through planting *Campanula glomerata* as a test species, **Chapter 5** measured seed-set and bee visitation abundance and diversity between the urban and rural areas of Birmingham, UK.

Finally, foraging and nesting resource availability is shown to be essential to influencing bee assemblages along the urban-rural gradient. How the urban environment influences the population dynamics of bee species is less clear however. Through the placement of red mason bee *Osmia bicornis* syn. *rufa (*Linnaeus) (Hymenoptera: Megachilidae) and artificial nests, **Chapter 6** investigated the provisioning and reproductive success of bees in an urban environment.

Chapter 2

The inter-specific variation of bee abundance and diversity along an urban-rural gradient in Birmingham, UK

Abstract

Urban densification in the UK is leading to the fragmentation and isolation of natural habitats in our cities. Current research investigating bee assemblage distribution along urban-rural gradients struggles to gain synthesis, as sites, cities and regions studied vary between studies. Since bees use a variety of areas for their nesting and foraging resources over the urban-rural gradient, increasing study of the types of sites sampled provides a firmer understanding of how bee assemblages respond to urbanisation. Bees were sampled at 33 canal side and parkland sites across Birmingham, UK, with sweep netting and pan trapping undertaken between April-September in 2011 and April-September in 2012. Abundance and diversity metrics were tested for variation between land-use types using ANOVA and rarefaction, and applied to mixed-models to test for relationships with landscape type (m^2 built space and agricultural land within 250 m, 500 m, 1 km, 1.5 km, 2 km, 2.5 km, 3 km, 3.5 km and 4 km of each site) and local site variables (floral availability, level of management and exposure). I found that in canal sides and parkland overall bee abundance showed a significant positive relationship with built space, whilst rarefaction indicated species richness was greater in rural areas. Furthermore, division of abundance metrics between genera and species showed increased abundance in several bumblebee species to greater built space, whilst Andrena *spp. showed a negative relationship with built space. This chapter presents further evidence that the variation in bee abundance between land-use types is species-specific, supporting recent studies that indicate highly disturbed urban environments 'filter' bee species and determine bee assemblages. This chapter also highlights the importance of site type replication within regions as these results contrast with a study of cemeteries and churchyards in the same region, underlining the importance of sampling all potential habitat sites along urban-rural gradients.*

2.1 Introduction

An ecosystem service which is of increasing scientific and economic interest is pollination., Bees (Apiforms, Apidae) and other vectors help to provide the transfer of pollen to approximately 87% of angiosperms worldwide (Ollerton et al., 2011). Nationally and globally, bees are exhibiting species loss and restriction in distribution (Biesmeijer et al., 2008; Potts et al., 2010). Integral to bee assemblage diversity throughout a landscape is the consistency of available foraging and nesting resources (Klein et al., 2007; Steffan-Dewenter & Westphal, 2008; Schüepp et al., 2011). The extent to which anthropogenic disturbances alter the availability of these resources at the landscape scale is currently a primary concern for mitigating bee species loss (Carré et al., 2009).

The anthropogenic disturbance caused by urbanisation not only negatively impacts on local biodiversity (McKinney, 2002) but also limits ecosystem services (Tratalos et al., 2007). Conversion of land through urban densification increases the level of impervious surface and habitat fragmentation (Young & Jarvis, 2001), resulting in green space loss (Dallimer et al., 2011). Through the loss of green space, essential resources become increasingly isolated for many taxa within urban areas (Andrén, 1994; Wood & Pullin, 2002), and most effectively explains the overall loss of biodiversity from urban areas (McKinney, 2008).

Currently, the literature that has investigated the effects of urbanisation on bee abundance and diversity has shown a loss of particular species towards higher levels of urbanisation (Zanette et al., 2005; Bates et al., 2011) even though some bees appear to show increased abundance within urban and suburban areas (Frankie et al., 2005; Fetridge et al., 2008; Bates et al., 2011). The bees that appear to cope with urbanisation tend to be social (Zanette et al., 2005), foraging generalists (Fetridge et al., 2008) and nest within cavities (Cane et al., 2006; Fetridge et al., 2008; Matteson et al., 2008). In particular, bumblebees appear to be able to use urban habitat patches for foraging due to their long foraging distances (Chapman et al., 2003; Greenleaf et al., 2007), and appear to have increased nest growth and reproductive success in suburban areas (Goulson et al., 2002). The variability in local factors such as management intensity and plant species richness are also known to influence the species recorded in urban habitats (Kearns & Oliveras, 2009).

However, a general synthesis as to the overall response of bee assemblages to urban-rural gradients is difficult to achieve as study city and region vary, whilst not all site types that occur along the urban-rural gradient have been tested (Bates et al., 2011). Previous investigations into bee species composition in cities have made use of suburban and urban gardens (Fetridge et al., 2008; Matteson et al., 2008), parkland (McFrederick & LeBuhn, 2006), and grassland or habitat fragments (Cane et al., 2006; Kearns & Oliveras, 2009). Limited in the literature however, are the recording and analysis of bee assemblage variation between site types within the same study region. It is therefore important that current research efforts concentrate on increasing regional replication and sample different aspects of the urban-rural gradient to understand bee assemblage change along an urban-rural gradient.

Understanding the variation in bee assemblages along the urban-rural gradient would allow further interpretation of how pollination services could be influenced by urbanisation. This ecosystem service is not only important for agricultural crops, but can also directly benefit urban populations by ensuring the fertilisation of flowers and improving the yield of crops grown within and adjacent to urban boundaries. These benefits are potentially widespread over a city such as Birmingham, UK that contains ~250,000 domestic gardens and ~7,000 allotments (Birmingham City Council, 2013). Even though the extent to which urban agriculture occurring in the UK is unknown, where it does occur there is evidence of local economic growth, provision for more sustainable ways to grow healthy food and the potential for greater levels of exercise and relaxation (Garnett, 1996).

Furthermore, an advantage of having viable populations of pollinating insects in an urban landscape includes their value as part of biodiversity throughout the wider environment. Suburban areas in particular have been identified as potentially providing greater bee abundance and pollination services (Cussans et al., 2010) which could spread out into agricultural areas. For species of bee that cannot adapt to the changes in the landscape resulting from urbanisation, the plant-pollinator inter-dependencies they represent could be lost (Kearns et al., 1998; Kaiser-Bunbury et al., 2010). By adding to the current literature in the types of site sampled when recording bee distributions along the urban-rural gradient, this chapter may help to focus urban planning and conservation efforts towards mitigating bee assemblage change.

2.1.1 Aims and Objectives

To further the current understanding of bee assemblage change along an urban-rural gradient, this chapter increases regional and site type sampling replication within Birmingham, UK. The research of Bates et al. (2011) which sampled churchyards and cemeteries in Birmingham, UK, was furthered by sampling canal sides and parkland sites throughout the same sample region. Using a dataset collected over two years of sweep-netting and pan-trapping sampling across Birmingham UK, the aim of this chapter was to improve understanding of the effects of urbanisation on bees by characterising their assemblages across an urban-rural gradient.

Specifically my objectives were to:

(i) record the variation in bee abundance, richness and composition across an urban-rural gradient;

(ii) test for significant relationships in bee abundance, richness and species composition between local and landscape variables;

(iii) record the observed variation in bee assemblages across the urban-rural gradient.

2.2. Methods

2.2.1 Study city and site selection

Four thousand hectares of Birmingham, UK's 27,000 consist of semi-improved grassland or ancient woodland. Eleven percent of Birmingham's land comprises green space or park land (Angold et al., 2006) and the city hosts ~250,000 domestic gardens (Birmingham City Council, 2013). Some 591 parks, allotment sites and public gardens make up a large proportion of the green infrastructure of Birmingham, and the 114 miles of canal side which run through Birmingham are important linear features of this city's natural landscape (Birmingham City Council, 2013) (Figure 2.1).

To ensure a balanced distribution of sites across the urban-rural gradient, areas of Birmingham were stratified by their relative land-use. Using previous categorisation of the West Midlands into 1km^2 blocks of land-use (Owen et al., 2006), three general classifications of Urban (made up of ‘urban’, ‘urban transport’ and ‘dense suburban’), Suburban (‘suburban’ and ‘light suburban’), and Rural (‘village/farms’) were created. As mentioned in the introduction, canal sides and parkland sites were chosen as sample sites. These sites were standardised by having either a canal or natural water course running through or alongside each sample area. All sites selected had at least 50% of their area exposed to sunlight rather than shaded and had a variety of vegetation including exposed tall ruderal grassland and a shrub layer and tree canopy.

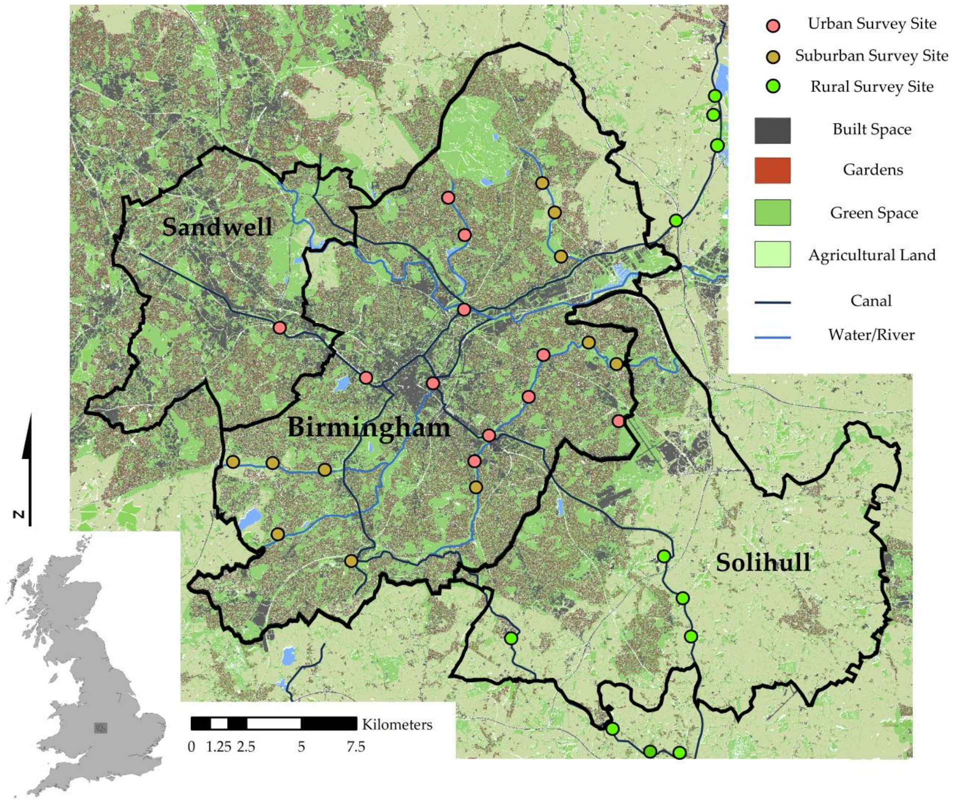


Figure 2.1: Map of sample sites across Birmingham, UK and wider area. Sample sites were selected following land-use classification set out by Owen et al. (2006). Eleven sample sites were selected from Urban (‘urban’, ‘urban transport’ and ‘dense suburban’ [Red dots]), Suburban (‘suburban’ and ‘light suburban’ [Yellow dots]) and Rural sites (‘village/farms’ [Green dots]) throughout Sandwell, Birmingham City and Solihul (Black lines). The landscape context variables used in this chapter analysis are shown with Built Space in grey, Gardens in orange, Green Space in green and agricultural land in light green. Canal sides are represented by dark blue lines, with bodies of water and rivers/streams shown as light blue.

2.2.2 Bee Sampling

Bees were sampled using sweep netting and pan trapping between April-September in 2011 and between April-August in 2012. Pan trapping is the best indicator of species richness in bees whilst minimising sampler bias, and is considered the best single method for data collection for bee abundance and diversity measurement (Westphal et al., 2008). However, some mining bee species can be missed with pan trapping alone, so sweet netting was also conducted.

Pan trapping was undertaken eight times for a period of ~48 hrs for each site over the survey period. Using a more intensive method than Bates et al. (2011), six sets of four different coloured pan traps were placed over an area of 100 m^2 in each site per sampling bout. Pan traps were rectangular plastic containers measuring 168 mm (L), 116 mm (W) and 52 mm (D), spray painted white (code 1109), pacific blue (code 1132), yellow (code 1115) and fluorescent yellow (code 655) using Plasti-kote® (Wokingham, UK) Projekt Paint Gloss Super. White, blue and yellow pan traps are considered to be sufficient to maximise richness of bee species captured (Leong & Thorp, 1999; Westphal et al., 2008; Wilson et al., 2008; Gonçalves & Oliveira, 2013). Each pan trap was half filled with water with ~1 ml of detergent as a surfactant. The position of pans was changed at each site for every pan trapping session, but pans were distant (>5m) from public rights of way when possible. Pan trapping bouts were conducted over 4 days; two days for placing all the pans and the subsequent two days for pan collection from all sites in the same order, providing an equal period of trapping exposure for each site. Site sequence was varied between bouts to vary time of day of pan trapping exposure. This method allowed all sites to be sampled within a short period of time, limiting the impact of variation in environmental conditions on the activity and subsequent capture of bees between sites. Pan traps were monitored throughout the survey to make sure sites were not subjected to high pan trap disturbance. Disturbance could be form animals eating collected samples, Birmingham City Council or British Waterways staff mowing over or moving the traps or members of the public emptying the contents of traps. To make sure this did not affect sampling design, a greater number of sites were initially sampled and removed if there was more than 20% of pan traps clearly interfered with throughout the sample period.

Sweep netting was undertaken once in spring and again during the summer months for each site in the 2011 sampling period, using an entomological kite net fixed upon a 6 foot carbon fibre fishing net pole. This added length and height, increasing the area swept, catch potential, allowed longer reach to collect early species of bee visiting floral rich trees (e.g. *Prunus* spp. and *Salix* spp.) and delayed bees reacting to experimenter presence. Sweep netting was conducted around flowering vegetation for 30 minutes per visit, making sure as much of each site's microhabitats were swept within the surveying time as possible. The sequence in which sites were sampled was varied to avoid variation in species' presence at different times of the day. All sweep netting visits were conducted between 11am and 4pm GMT in weather >15°C and <10mph wind speeds over the spring, and >18°C and <10mph wind speeds in the summer. Once bees were caught, smaller bees were collected from the net using a pooter. European honey bees and species of bumblebee that were easily identifiable in the field were placed into clear 50 ml centrifuge tubes, recorded and released. All other individuals were taken for specimen preparation and identification.

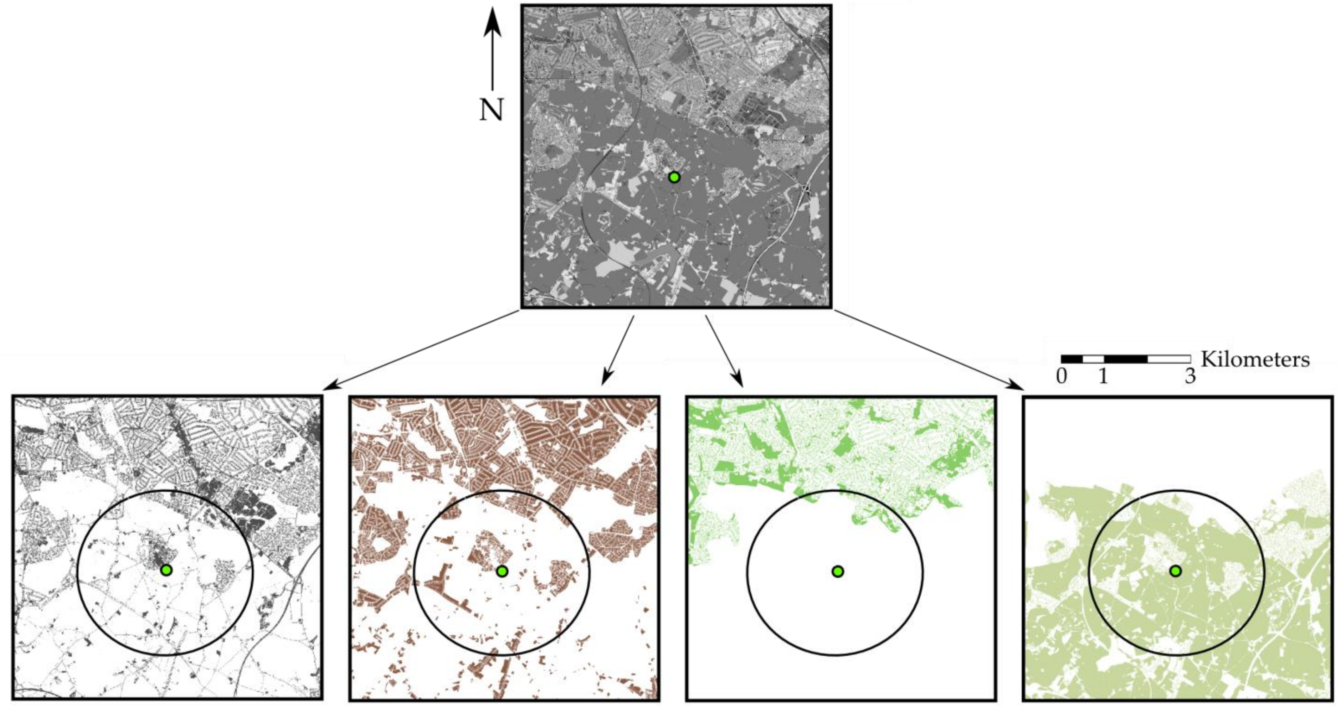
2.2.3 Explanatory variables

2.2.3.1 Local Variables

Floral richness was estimated by recording the number of different species of flowering plant at each survey site and floral availability by recording the number of flowers for each plant, shrub or tree in flower. Particularly for early bee species, trees such as *Salix* spp. and *Prunus* spp. can offer large foraging opportunities. To incorporate these into a measure of floral availability, the number of flowers present per flowering tree, shrub, or plant was estimated using a simplified system where it was allocated as having >10 flowers, >100 flowers, >1000 flowers and >10,000 flowers. These two metrics were recorded twice each year, one for the spring and one for the summer months. The total for spring and summer floral abundance and diversity were then averaged to gain a final measurement of each for each site. Mowing and cutting regimes were noted throughout the sampling period to gain a percentage estimate of Management Intensity at each site. Using a photogrammetric Digital Surface Model (DSM) (OS MasterMap, 2009) which took into account buildings and trees, and the 'hillshade' function in ArcGIS v10 (ESRI, 2011), a map of the relative sunlight exposure was created of the sample area. From this, all 2 × 2m cells within each site's boundaries were averaged to gain a mean value of exposure.

2.2.3.2 Landscape Context Variables

Also in ArcGIS v10, four main landscape context variables were derived from Ordnance Survey MasterMap datasets by grouping landscape metrics. Built space was made up of the metrics 'buildings', 'man-made structures', 'pavements', 'roads', 'man-made open space/landforms', 'railways' and 'developing land' (land to be built upon). Gardens were made up of 'gardens' of all sizes. Green space consisted of 'open natural habitat' (natural open space, natural habitat without trees, natural landforms, natural railway without trees and natural roadside without trees) and 'covered natural habitat' (natural roadside with trees, mixed natural habitat with trees and natural railway with trees) (Ordnance Survey, 2006). A layer of agricultural land was gained by overlaying the green space layer with a map of agricultural land-use (Natural England, 2009), giving a general measure of agriculture albeit no specifics on the type of agricultural practice performed in these areas. Concentric buffer zones of 250 m, 500 m, 1 km, 1.5 km, 2 km, 2.5 km, 3 km, 3.5 km and 4 km were then created around each sample site. The area (m^2) of each landscape metric raster within each buffer zone scale was then derived using Geospatial Modelling Environment (Bayer, 2012; Figure 2.2).



Built Space m^2
Buildings, man-made structures, pavements, roads, man-made openspace/landforms, rail and developing land from OS Mastermap

Gardens m^2
Gardens of all sizes from OS Mastermap

Green Space m^2
Open and covered natural habitat from OS Mastermap minus Agriculutral land map from Natural England (2013)

Agricultural Land m^2
Open and covered natural habitat in the OS Mastermap data set which also covers the Agricultural land map from Natural England (2013)

Figure 2.2: A diagram of landscape context layers for a rural survey site. Landscape context variables were extracted using two maps, OS Mastermap of landscape variables, and a map of agricultural land-use from Natural England (2013). Via these maps metrics of Built Space, Gardens, Green Space and Agricultural Land were generated in ArcGIS v10. Concentric circles with radii of 250 m, 500 m, 1 km, 1.5 km, 2 km, 2.5 km, 3 km, 3.5 km and 4 km were then created surrounding each site (example is 2 km). By extracting the density (m^2) for each landscape context variable layer for each site, a metric of each context was gained for each site.

2.2.4 Data analysis

The overall abundance and species richness for each site was calculated by totalling the number of individual bees and the number of different species recorded from pan trapping and sweep netting sample. To allow an analysis of variance in species abundance distribution which takes into consideration species richness and abundance, Inverse Simpson's Index (1 – Simpson's Dominance [D]) of each site was estimated (Magurran, 2004). This inferred the diversity of each site whilst taking into account the evenness of the species present:

(Equation 2.1)

$$\boldsymbol{D} = \sum_{i=1}^{s} \boldsymbol{p_i}^2$$

where Pi is the proportion of individuals in the ith species, and s is the total number of species. To ascertain if individual genera and species metrics also showed variation along the urban-rural gradient, overall abundance data were divided further to gain specific abundance metrics.

2.2.4.1 Rarefaction

The number of species found in sites can overlook the variation in species composition (Fleishman et al., 2006). Individual based rarefaction, which assumes all individuals caught are independent of each other, estimates the potential of encountering other species (Hurlbert, 1971). Through individual based rarefaction, a curve is fitted between the individuals and species recorded, giving a measure of species richness (Gotelli & Colwell, 2001; Colwell et al., 2012). Individual based rarefaction curves were generated for the bee assemblages recorded in urban, suburban and rural sites to allow comparison of species richness between land-use types (using R package Vegan; Oksanen et al., 2012).

2.2.4.2 ANOVA

Comparison of bee assemblage metrics between general classifications of urban, suburban and rural sites were undertaken using Analysis of Variance (ANOVA) if metrics were normally distributed, or Kruskall-Wallis if they were not normally distributed. Tukey-HSD and pairwise Wilcoxon tests were used alongside each analysis of variance, respectively, to record the relative differences between particular land-use types.

2.2.4.3 Generalised Linear Mixed-Models

Statistical modelling was used to test for significant relationships between explanatory variables and bee assemblage metrics (Table 2.1). Data exploration was undertaken following Zuur et al. (2010). All variables were checked for outliers using box plots and Cleveland dotplots. Local and landscape context explanatory variables were checked for co-linearity at each landscape scale prior to statistical modelling. At several scales, gardens and green space showed positive co-linearity to built space. To make sure the assumptions of independence in models were not violated, only built space and agricultural land landscape context variables were applied to GLMs (Table 2.1). Of the local site factors, floral richness was also shown to be positively co-linear with built space, and removed. This left exposure, management intensity and floral availability as site factors in the final models (Table 2.1). Several non-linear relationships were evident within the models, specifically with the abundance of bumblebees showing an asymptote with similar levels of abundance in medium and high levels of built space. These non-linear relationships were accounted for by adding quadratic terms to the built space variable in these particular models. A total of 10 bee assemblage metrics were identified as showing normality, independence and homogeneity of variance when statistically modelled, including overall bee abundance, species richness, *Bombus* spp. abundance, *Andrena* spp. abundance, *Bombus lapidarius* abundance, *Andrena bicolor* abundance, *Bombus pascuorum* abundance, *Bombus terrestris* abundance, and *Apis mellifera* abundance. Using the model scale with the lowest Akaike Information Criterion (AIC), the most parsimonious model scale for each response variable was identified.

Initial data exploration indicated bee assemblage metrics were nested between canal side and parkland sites. Following Zuur et al. (2009), this was accounted for by adding site type (parkland and canal sides) as a random factor in generalised linear mixed-effect models (GLMMs). Models were applied with Poisson or negative-binomial distributions to account for over-dispersed nature of count data (O'Hara et al., 2010).

Initial counts of abundance and species richness were applied to models, and then divided between genera and species for further modelling of inter-specific variation in bee species response to urbanisation. Only the genera and species abundance metrics that were recorded at a high enough frequency that allowed statistical models to show validity through normality, independence and homogeneity of variance at all explanatory variable scales were kept in this chapter's statistical modelling analysis. Normality of each model was tested using Q-Q plots, independence was tested by plotting model residuals against all explanatory variables (either included in the model or not), and homogeneity was checked by plotting the model residuals against the fitted values. If the GLMMs were unable to account for non-linear relationships between a response and explanatory variable, a quadratic term was added to that variable. Interaction terms between explanatory variables were checked for importance in models by testing significance compared to models without interaction terms using ANOVA. . Spatial auto-correlation was tested using a Mantel-T test for each response variable before applying to a model, as well as the residuals from each resulting model. This indicated that spatial auto-correlation was not evident in either response variable or the final model residuals.

Once a model was found suitable for each response variable at all explanatory variable scales, the most parsimonious model was found using an Information-Theoretic (IT) approach (following Burnham and Anderson, 2002) and presented as a final model. All analyses were undertaken using R v. 2.14.1 (R Core Team, 2012; www.r-project.org) within RStudio (RStudio, 2012). Modelling of GLMM models was untaken using 'glmmADMB' (Fournier et al., 2012).

2.2.4.4 Redundancy Analysis

All individual species' abundances were applied to ordination analysis to assess the association of local and landscape variables with the abundance of species of bee present. Using Detrended Correspondence Analysis (DCA), Redundancy Analysis (RDA) was selected as an appropriate ordination method due to total inertia of DCA remaining below 2 (Lepš & Šmilauer, 2003). The landscape scale most suited to describing the association of individual species' abundance was selected based on the scale which best explained the variance within the RDA. All local and landscape variables in the final models were applied as constrained variables in the RDA to assess their association to bee species' abundance. Significance values were found using Monte Carlo permutation tests. DCA and RDA were calculated using the Vegan package in R (Oksanen et al., 2012).

Table 2.1: The response and explanatory variables used in the final Generalised Linear Mixed Models (GLMMs) testing bee assemblage variation along the urban-rural gradient of Birmingham, UK.

Response Variables		**Response Variable Description**	
Overall Abundance		The total number of bees recorded at each sample site	
Species Richness		The total number of bee species recorded at each site	
Genera Abundance	*Bombus* spp. *Andrena* spp.	The total number of individual bees at each site within a particular genus (for *Bombus* spp. subgenus Psithyrus was excluded)	
Species Abundance	*Bombus lapidarius* *Andrena bicolor* *Bombus pascuorum* *Bombus terrestris* *Apis mellifera*	The total number of individual bees at each site of a particular species.	
Explanatory Variables		**Explanatory Variable Description**	
Landscape Context Variables	**Built Space**	Built Space (m^2) within a specified concentric buffer zone from the sample site.	Landscape context data were taken at intervals of 250 m, 500 m, 1 km, 1.5 km, 2 km, 2.5 km, 3 km, 3.5 km and 4 km.
	Agricultural Land	Agricultural Land (m^2) within a specified concentric buffer zone from the sample site.	
Site Variables	**Exposure**	The percentage of sunlight exposure each site offers. Derived from the Digital Surface Model, incorporating hills, trees and buildings.	
	Floral Availability	The number of flowers in a site. Rather than counting every flower, this metric was estimated per tree/shrub/flower. The classification of flower abundance was either >10, >100, >1000 or >10000.	
	Level of Management	A percentage of observed intensity of management in a site. Based on the mowing regime and the intensity of public occurrence at the site compared to other sites.	

2.3 Results

From eight pan trapping and two sweep netting surveys per site in 2011/2012, 3,309 individual bee specimens were recorded from 76 species within 18 genera across the urban-rural gradient of Birmingham, UK. Pan trapping surveys undertaken four times in 2011 and twice in 2012 caught the main proportion of bees, their catch making up 73.9% of all individuals recorded in this study. Sweep netting was conducted at each site once in the spring and again in the summer months of 2011, the bees recorded here making up 26.1% of all bees caught in this study. Individuals from the genus *Bombus* represented 41.2% of total bee abundance, whilst only 1.3% of individuals were from the subgenus *Bombus Psithyrus*. Solitary bees made up 49.4% of all individuals, and their cleptoparasites constituted 4.2% of total individuals caught. *Andrena* were by far the most abundant solitary bee genus represented by 20.1% of all bees recorded. The remaining 4% of bees caught were honey bees (*Apis mellifera*). Five species were dominant throughout the study area, making up 43.9% of the bees collected; *Bombus lapidarius* (385), *Andrena bicolor* (289), *B. pascuorum* (255), *B. terrestris* (247) and *B. lucorum* (279) (see Appendix I: Table AI.1 for abundance data between land-use types). Of the 76 species recorded, only 12 were not represented within urban or suburban sites (Appendix I: Figure A.I.1). The most variance in species' composition was explained in the RDA with landscape variables at the 2.5 km scale. The cumulative variance of species' data was 25.4% and all canonical axes were significant (F=1.84, p=<0.05; Figure 2.3).

Between land-use types, species richness (F=0.51, d.f.=2, p=0.60) and Simpsons Diversity Index (F=0.74, d.f.=2, p=0.74) showed no significant difference. In the mixed-models, species richness was also not significantly associated with either local or landscape variables. Overall abundance on the other hand, was significantly different between land-use types (F=5.17, d.f.=2, p=0.01), and showed a positive relationship with built space within a 1 km radius in mixed-models (Table 2.2). However, individual-based rarefaction revealed rural sites showed greater expected species richness than suburban and urban sites once more than 300 individuals are sampled in each land-use, based on confidence intervals not overlapping after this point (Figure 2.4).

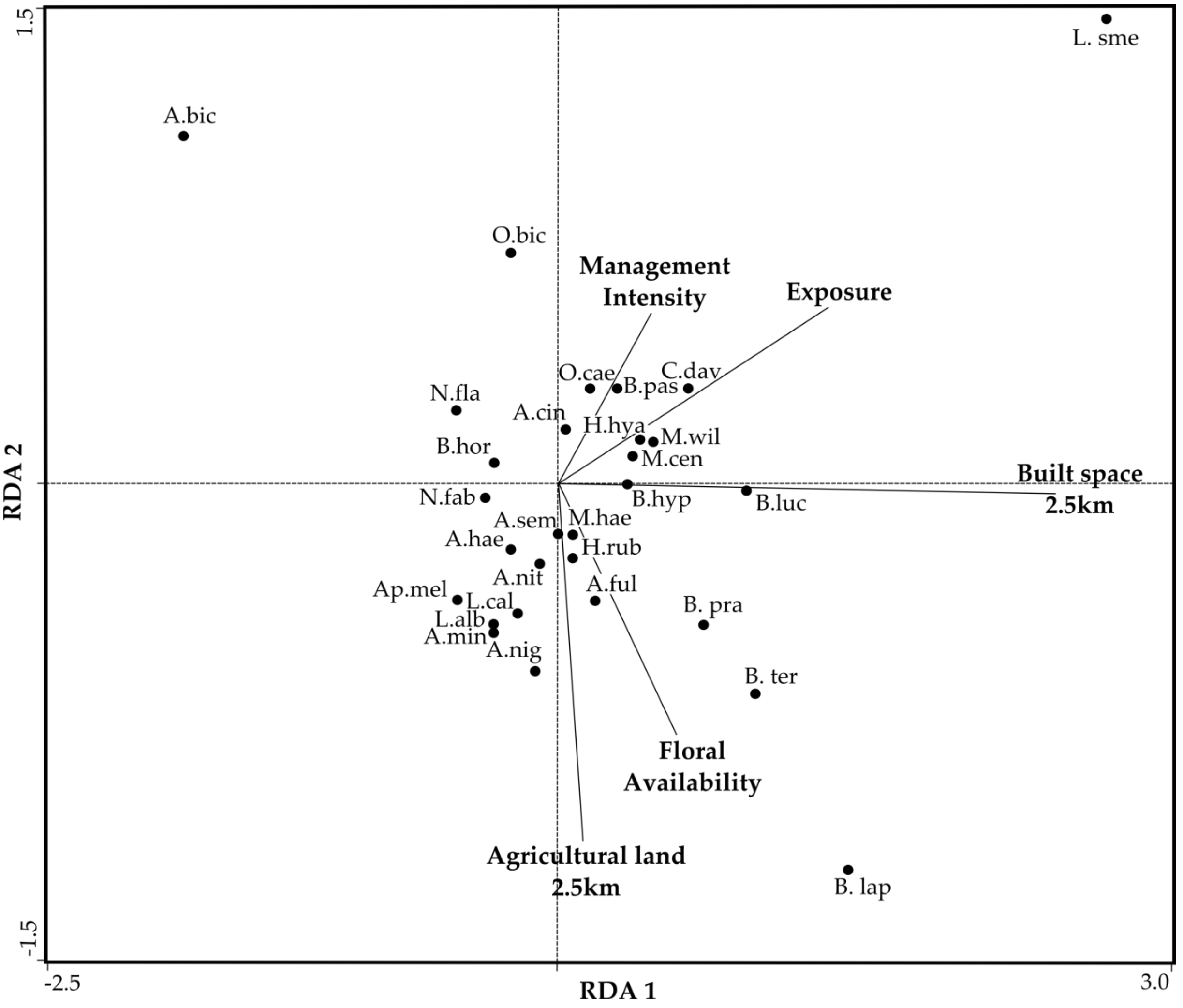


Figure 2.3: Redundancy Analysis (RDA) plot of species abundance as a response to local and landscape explanatory variables. The Landscape variable scale of 2.5 km was the model which explained the most variance compared to other scales, explaining 25.4% of the cumulative variance in the species data (*F*= 1.84, p=<0.05). Species with best model fit are represented. Species names were abbreviations follow A.bic = *Andrena bicolor*, A.cin = *A. cineraria*, A.ful = *A. fulva*, A.hae = *A. haemorrhoa*, A.nig = *A. nigroaenea*, A.min = *A. minutula*, A.nit = *A. nitida*, A.sem = *A. semilaevis*, Ap.mel = *Apis mellifera*, B.hor = *Bombus hortorum*, B.hyp = *B. hypnorum*, B.lap = *B. lapidarius*, B.pas = *B. pascuorum*, B.pra = *B. pratorum*, B.ter = *B. terrestris*, C.dav = *Colletes daviesanus*, H.rub = *Halictus rubicundus*, H. hya = *Hylaeus hyalinatus*, L. alb = *Lasioglossum albipes*, L.cal = *L. calceatum*, L.sme = *L. smeathmanellum*, M.lig = *Megachile ligniseca* , M.wil = *M. willughbiella*, M.hae = *Melitta haemorrhoidalis*, N.fab = *Nomada fabriciana*, N.fla = *N. flava*, O.cae = *Osmia caerulescens*, O.bic = *O. bicornis*.

Table 2.2: Model estimates and significance levels from the final GLMMs, testing for relationships of bee abundance and species richness metrics between landscape and local explanatory variables along the urban-rural gradient of Birmingham, UK.

Response Variable	**Model Scale**	**Explanatory Variables**					**Interactions**
		Built Space	Agricultural Land	Exposure	Floral Availability	Management Intensity	Floral Availability × Man. Intensity
Overall Abundance (3,309)	1km	0.000702*			-2.91e-07	0.00344	
Species Richness (76)[1]	4km	-5.73E-15	-0.0000174		3.10e-07	0.000282	
Bombus spp. (1,560)	1km	0.002***			-7.04e-07	0.00159	
Andrena spp. (663)	250m	-1.89e-09**		-0.00512	3.85e-06*	-0.00183	
Bombus lapidarius (385)	1km	2.52e-06**			1.37e-06	0.00356	
Andrena bicolor (289)	1km	-4.91e-06***			1.10e-05	2.56e-02***	-1.90e-07*
Bombus pascuorum (255)	1.5km	4.25e-07	-8.98e-09	0.00473	-1.35e-06	0.00703	
Bombus terrestris (247)	1km	3.25e-06**		-3.91e-03		-2.46e-03	
Apis mellifera (133)	1.5km	-2.1e-12	2.35e-07**	-0.00943	8.24e-11	0.000126	-1.94e-14*

Interactions in the data between Floral Availability and Management Intensity are shown in the column on the right. - indicates a negative relationship between the response and explanatory variables in question. Model scale represents the final model scale selected through Information-Theoretic (IT) following Burnham and Anderson (2002). The number of individuals recorded over the sampling effort is represented in brackets next to each species name, apart from Species Richness which presents the total number of species found in the study. Significant p-values of <0.05 shown as *, <0.01 as ** and <0.001 as ***.

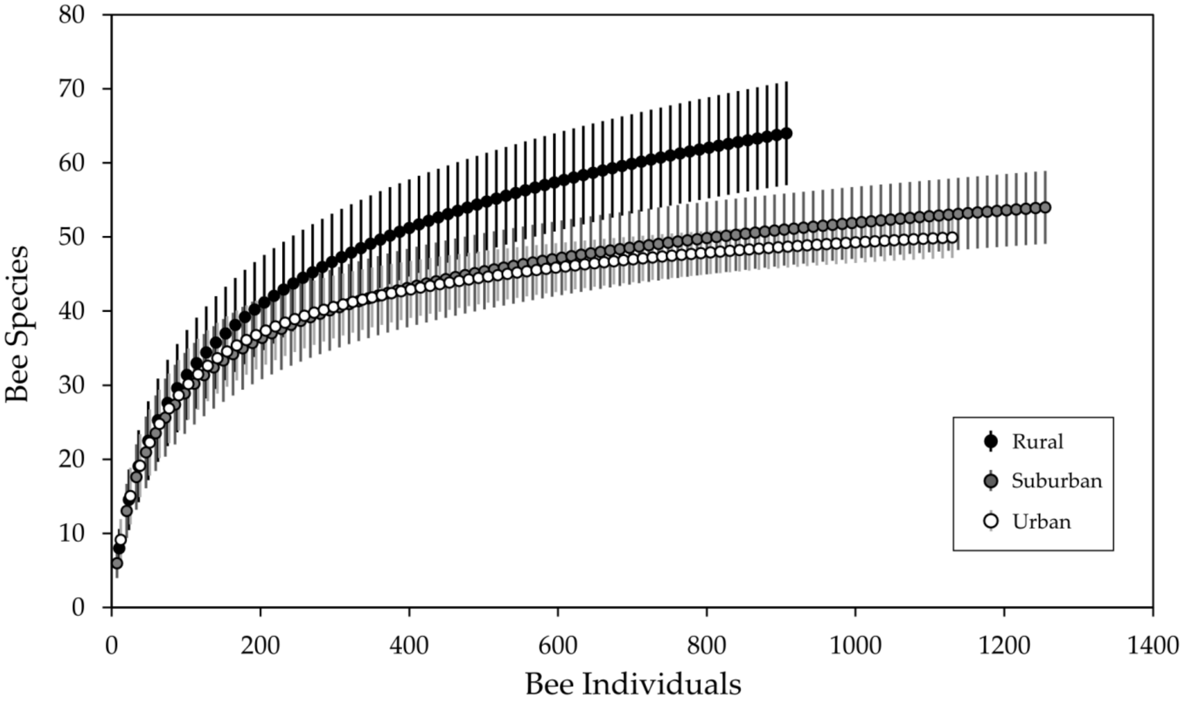


Figure 2.4: Individual-based interpolation (rarefaction) from urban (red), suburban (orange) and rural (green) sites with SE of the rarefaction iterations as error bars. This indicates rural sites had greater levels of species richness than both suburban and urban sites after 300 individuals were recorded. Urban and suburban sites show similar levels of species richness to each other until 1000 individuals sampled. Each line ends and represents the number of individuals recorded in that land-use type, indicating the greatest number of individuals in suburban areas.

The genus *Bombus* showed high levels of abundance in suburban areas (F=13.48, d.f.=2, p=<0.001; Table 2.3), caused by high abundance of *Bombus lapidarius* (H=15.37, d.f.=2, p=<0.001), *Bombus terrestris* (F=5.06, d.f.=2, p=<0.01) and *Bombus lucorum* (H=8.54, d.f.=2, p=0.01; Table 2.4). *Bombus* spp., *Bombus lapidarius*, *Bombus terrestris* and *Bombus lucorum* all showed positive relationships with built space (between 1 km and 1.5 km scale) in the mixed models (Table 2.2; Figure 2.6) and in RDA (Figure 2.3). *B. pascuorum* displayed no significant variation between land-use (H=3.34, d.f.=2, p=0.19), and showed not significant relationship with built space in mixed-models.

Table 2.3: Test statistics, test significance and between land-use significance from means tests and post-hoc tests of overall abundance, species richness and abundance metrics of bee genera between land-use types.

Analysis of Variance			Post-Hoc		
Test Response	**Test Stat**	**Test Sig.**	Urb <-> Sub	Urb <-> Rur	Sub <-> Rur
Overall Abundance (3,309)	F=5.17	0.01	0.57	0.09	0.01 (<)
Species Richness (from 76)	F=0.51	0.6	0.6	0.6	0.9
Simpson Diversity Index	H=0.59	0.74	1	1	1
Bombus spp. (1360)	F=13.48	<0.001	0.803	<0.001 (<)	<0.001 (<)
Andrena spp. (663)	H=3.8	0.14	0.3	0.32	1
Lasioglossum spp. *(340)*	H=1.466	0.48	1	1	0.97
Osmia spp. *(228)*	H=1.84	0.39	1	0.85	0.69
Halictus spp. *(140)*	H=1.52	0.46	1	1	0.86
Apis mellifera[1] *(133)*	H=4.29	0.11	0.45	0.15	1
Nomada spp. *(124)*	H=8.22	0.01	0.29	0.02 (>)	0.35
Megachile spp. *(98)*	H=8.74	0.01	1	0.02 (<)	0.07
Hylaeus spp. *(71)*	H=5.12	0.07	0.24	0.16	1
Bombus Psithyrus spp. *(42)*	H=1.91	0.35	1	0.59	1
Anthophora spp. *(44)*	H=6.40	0.04	0.94	0.04(<)	0.33
Colletes daviesanus[1] *(35)*	H=13.16	0.001	0.007(<)	0.03(<)	0.49

[1] These genera were only represented by one species. The number of individuals recorded over the sampling effort is represented in brackets next to each genus. *H* = Kruskal-Wallis non-parametric test statistic and post-hoc test as Wilcoxon tests, *F* = ANOVA test with a post-hoc Tukey-HSD test. Degrees of freedom were 2 for all tests. (<) and (>) represent the direction of the trend, with the arrow pointing towards the land-use type with a higher levels of abundance.

The abundance of *Andrena* spp. gave no indication of variation between land-use types, but was significantly negatively associated with built space in mixed-models. Species within the genus *Andrena* displayed contrasting results, with no significant differences in the abundance of *A. nigroaenea, A. minutula* or *A. haemorrhoa* between land-use types, yet *Andrena fulva* (H=9.74, d.f.=2, p=0.008) showed a greater abundance in suburban areas whilst *Andrena bicolor* (H=9.15, d.f.=2, p=0.01) was more abundant in rural sites. The only species of solitary bee that was found in sufficient abundance to be applied to mixed-models was *Andrena bicolor*, and showed a negative relationship with built space at a 1 km scale. In addition, *Apis mellifera* was positively associated with the level of agricultural land at a 1.5 km scale.

Table 2.4: Test statistics, test significance and between land-use significance from means tests and post-hoc tests between the most commonly recorded individual species abundance between land-use types.

Analysis of Variance			**Post-Hoc**		
Test Response	**Test Stat**	**Test Sig.**	Urb <-> Sub	Urb <-> Rur	Sub <-> Rur
Bombus lapidarius (385)	H=15.37	<0.001	1	0.002(<)	0.004(<)
Andrena bicolor (289)	H=9.15	0.01	0.05	0.03(>)	1
Bombus pascuorum (255)	H=3.34	0.19	1	0.97	0.24
Bombus terrestris (247)	F=5.06	0.01	0.99	0.02(<)	0.03(<)
Bombus lucorum (222)	H=8.54	0.01	0.96	0.02(<)	0.14
Osmia bicornis (191)	H=0.96	0.62	1	1	0.94
Bombus pratorum (122)	H=5.10	0.08	1	0.12	0.27
Lasioglossum smeathmanellum (102)	H=2.87	0.24	0.65	0.31	0.65
Andrena minutula (98)	H=1.14	0.57	1	0.95	1
Lasioglossum albipes (93)	H=2.29	0.32	0.4	0.92	0.92
Halictus tumulorum (91)	H=0.44	0.8	1	1	1
Lasioglossum calceatum (79)	H=0.96	0.62	1	1	1
Bombus hortorum (71)	H=5.86	0.05	0.13	0.08	0.62
Andrena nigroaenea (62)	H=2.05	0.36	0.6	0.76	0.6
Bombus hypnorum (58)	H=8.31	0.02	0.95	0.12	0.02(<)
Andrena haemorrhoa (51)	H=4.91	0.09	0.21	0.11	0.66
Halictus rubicundus (49)	H=0.45	0.8	1	1	1
Nomada fabriciana (47)	H=1.69	0.43	1	0.58	1
Hylaeus communis (45)	H=4.18	0.12	0.37	0.2	0.37
Andrena cineraria (38)	H=3.53	0.17	0.36	0.87	0.36
Megachile willughbiella (35)	H=7.79	0.02	0.25	0.03(<)	0.25
Anthophora plumipes (35)	H=4.22	0.12	0.68	0.15	0.21
Megachile ligniseca (35)	H=6.26	0.04	0.28	0.22	0.05
Andrena fulva (31)	H=9.74	0.008	0.4	0.1	0.003(<)

The number of individuals recorded over the sampling effort is represented in brackets next to each species name. *H* = Kruskal-Wallis non-parametric test statistic and post-hoc test as Wilcoxon tests, *F* = ANOVA test with a post-hoc Tukey-HSD test. Degrees of freedom were 2 for all tests. (<) and (>) represent the direction of the trend, with the arrow pointing towards the land-use type with a higher levels of abundance.

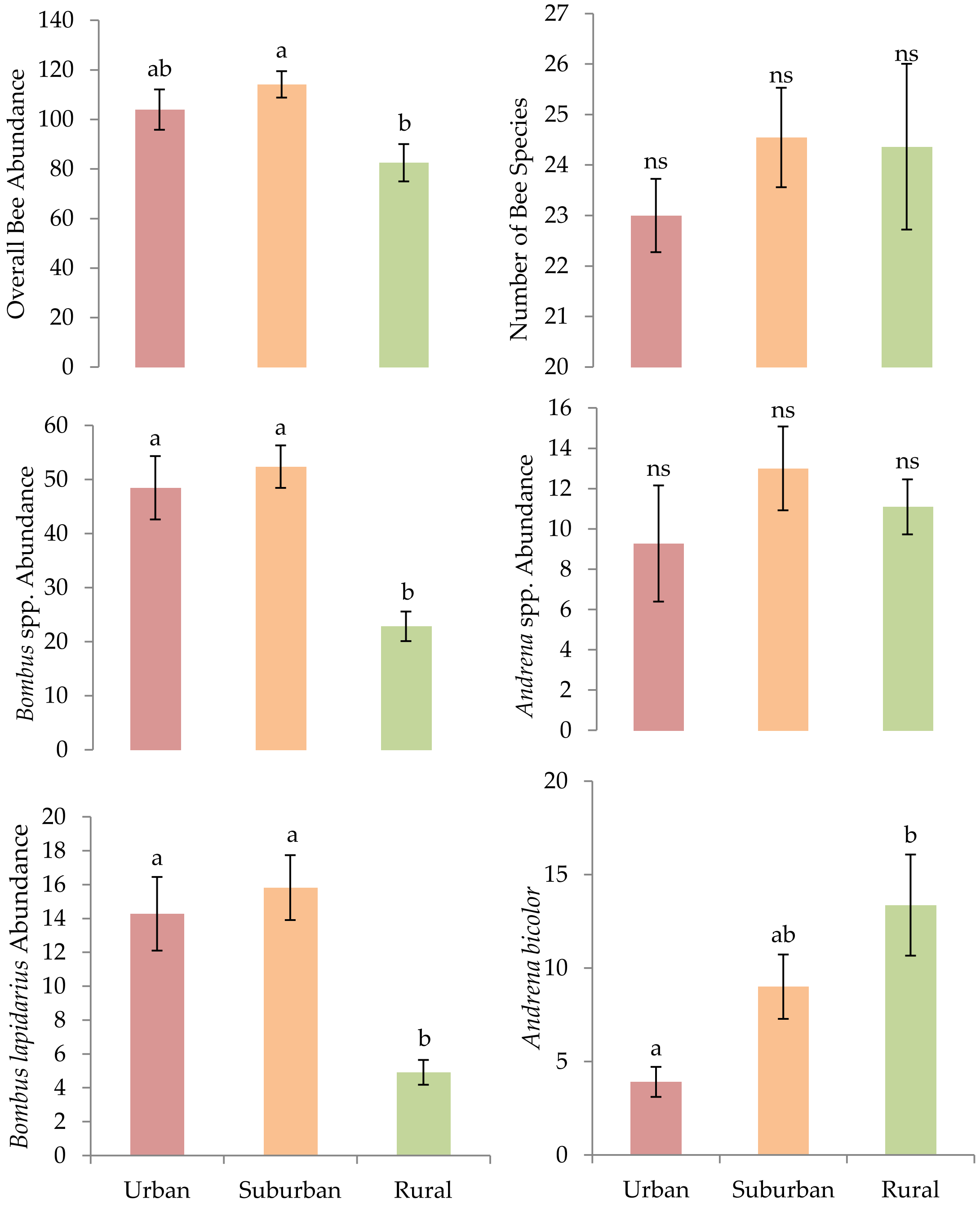


Figure 2.5: Mean bee abundance and diversity metrics between land-use types. (±1 SE). Bars with different letters represent significant differences, while 'ns' represents no significant difference between land-use.

The varied response of bee species to land-use was continually observed in other abundance metrics. *Megachile* spp. varied significantly between land-use types (F=8.74, d.f.=2, p=<0.01), and *Megachile willughbiella* (H=7.79, d.f.=2, p=0.02) and *Megachile ligniseca* (H=6.26, d.f.=2, p=0.04) both showing greater abundance in urban and suburban areas, respectively (Table 2.3). Similarly, *Anthophora* spp. and *Colletes* spp. both showed significant variation towards sites in more urbanised areas (F=6.40, d.f.=2, p=<0.04; F=13.16, d.f.=2, p=<0.001). However, several genera abundance metrics showed no significant variation with land-use, including the abundance of *Lasioglossum* spp., *Osmia* spp., *Halictus* spp., *Hylaeus* spp. and the subgenus *Psithyrus* (Table 2.3).Interestingly, the post-hoc analysis of variation between individual land-use types highlighted that the most significant differences between metrics were observed between urban to rural sites and suburban to rural sites.

GLMMs and RDA indicated associations between certain abundance metrics and local site factors. In GLMMs, there were significant interactions between floral availability and management intensity and the abundances of *Andrena bicolor* and *Apis mellifera* (Table 2.2; Figure 2.6). In RDA, *Osmia bicornis, O. caerulescens*, *Lasioglossum smeathmanellum* and several *Megachile* species were associated with measures of management intensity and exposure (Figure 2.3).

The cleptoparasitic genus *Nomada* was found to decrease in abundance significantly within urban areas (H=8.22, d.f.=2, p=0.01; Table 2.3). *Nomada* spp. host species are most commonly from the genus *Andrena* (Baldock, 2008), which as previously mentioned shows a negative relationship with built space. Further to this, *Nomada fabriciana* and its host species *Andrena bicolor* both showed a negative association with built space in RDA (Figure 2.3).

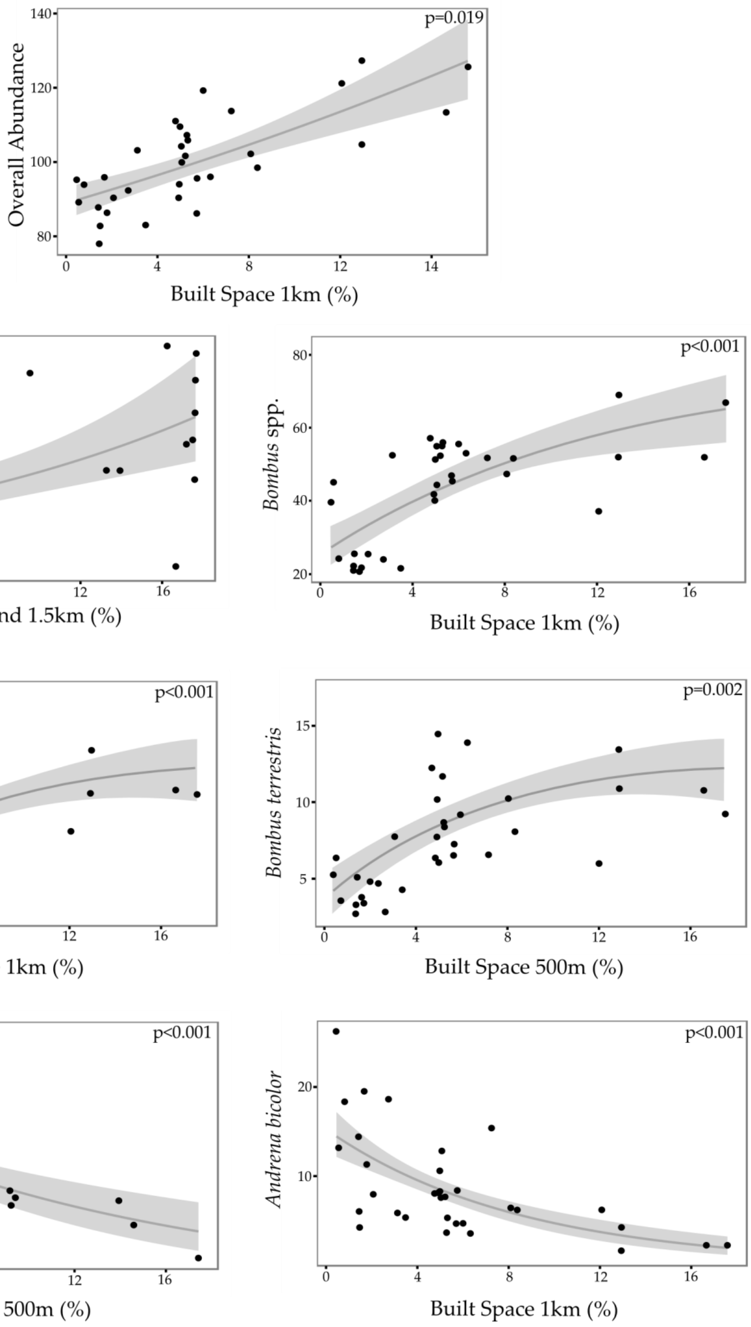


Figure 2.6: The significant relationships of bee abundance metrics and landscape variables from the final GLMMs recorded in canal side and parkland sites along the urban-rural gradient of Birmingham, UK (Table 2.5). The concentric scale at which each landscape variable was extracted and applied to the final model is represented within brackets (see 2.2 Methods for more detail). Overall Abundance shows a positive relationship with built space at a 1 km scale. Metrics of *Bombus* spp., *B. lapidarius* and *B. terrestris* abundance all illustrate a positive relationship with built space at a 1 km scale which appeared to reach an asymptote. In contrast, *Andrena* spp. and *Andrena bicolor* abundance showed the opposite trend with a negative association with built space at a 250 m and 1 km scale respectively. *Apis mellifera* abundance displayed a positive relationship to agricultural land. Grey lines indicate the trend of the relationship and grey areas represent 95% confidence intervals. R^2 represents the square of the correlation for each abundance metric model (also known as a proportion between 0-100 of model fit), p represents the significance of the relationship between abundance metric and

2.4 Discussion

Through sweep netting and pan trapping in canal and parkland sites, I recorded bee assemblages along the urban-rural gradient in Birmingham, UK. Using mixed-models, I found greater overall bee abundance in suburban compared to rural sites. Several species from the genus *Bombus* were highly abundant in these sites, and appear to be the main components of bee assemblages within urban and suburban areas. However, this overshadows the opposite trends found in *Andrena* spp. and *Andrena bicolor* which showed a negative relationship with increased built space density. By separating abundance data between genera and species, I present further evidence that the variation in bee abundance between land-use types is species-specific. These included interactions between local site variables in several mixed-models, supporting previous research on how site quality is an important component for bees in urban areas (Kearns & Oliveras, 2009; Bates et al., 2011).

In previous studies, bee assemblage variation along the urban-rural gradient was defined by a loss in species richness (Matterson et al., 2008; Kearns & Oliveras, 2009) or a change in community composition (Zanette et al., 2005; Cane et al., 2006; Wojcik & McBride, 2012). This chapter's analysis displays similar results with certain species demonstrating negative influences from increased urbanisation. Some studies however, have suggested that urban and suburban areas can maintain a bee fauna in terms of abundance and diversity to the same extent as their rural counterparts (Frankie et al., 2005; Fetridge et al., 2008; Frankie et al., 2009). My results support this whilst also revealing that the suburban areas I sampled show greater bee abundance than the rural sites .

There are certain elements of the urban and suburban landscape that could support greater abundances of particular bees. Fundamental to the diversity of bee species composition in any given habitat is the diversity of available foraging (Potts et al., 2003) and nesting opportunities (Potts et al., 2005). Habitat along the urban-rural gradient has been shown to provide such resources, most notably from sites such as suburban and urban gardens (Hostetler & McIntyre, 2001; Goulson et al., 2003; Matteson et al., 2008), allotments (Ahrné et al., 2009) and urban parks (McFrederick & LeBuhn, 2006). *Bombus lapidarius* and *B. terrestis* were two species that showed greater abundance in suburban areas and a positive relationship with built space (Figure 2.5). Bumblebees usually show less deleterious responses to habitat fragmentation than smaller solitary bees (Jauker et al., 2013), due to their body size and thus greater foraging range (Greenleaf et al., 2007). Bumblebees can forage longer distances in search of food in urban areas (Chapman et al., 2003) and utilise habitat corridors in their search for foraging resources (Cranmer et al., 2012). Furthermore, their reproductive success appears to be higher in suburban areas than it is in agricultural areas (Goulson et al., 2003). Hence, the higher abundance of bumblebees found in the suburban and urban sites in my study and others (McFrederick & LeBuhn, 2006; Matteson et al., 2008; Bates et al., 2011) is partially explained by their ability to forage much further and make use of the habitat patches not exploited by smaller bees.

However, this chapter also found contrasting relationships between land-use and abundance metrics of other species. For example, *Andrena bicolor* showed a negative relationship with built space and a positive relationship with management intensity, whilst there was also an interaction between the number of flowers on a site and its relative level of management. Several urban studies have shown that management regimes promoting native floral availability and nesting opportunities for ground nesters can offer bees consistent resources for survival and fecundity throughout the urban-rural gradient (McFrederick & LeBuhn, 2006; Kearns and Oliveras, 2009; Frankie et al., 2009). Therefore, sites that can maintain a high diversity of resources for different species of bee appear to support bees along the urban-rural gradient. Although this has been suggested and observed previously (Cane et al., 2006; Fetridge et al., 2008; Matterson et al., 2008; Bates et al., 2011; Banaszak-Cibicka & Żmihorski, 2011), this chapter adds to the growing concern that to gain a better understanding of bee assemblage variation along an urban-rural gradient, the inter-specific responses of bees must be considered. Limiting this chapter’s ability to infer the causes behind certain species’ change along the urban-rural gradient is the co-linearity between site and landscape variables, common to studies of the urban-rural gradient. Within this chapter, floral richness was positively associated with built space (and removed from further statistical analysis). Thus, the increase in bee abundance (specifically bumblebee abundance) observed in urban and suburban areas may be associated with floral diversity rather than built space *per se*.

Compared to Bates et al. (2011), it was apparent that there are differences in the bee assemblages recorded in different site types in this study region. Whilst Bates et al. (2011) showed bee abundance to be greater in rural compared to suburban areas when sampling cemeteries and churchyards, my study suggests the opposite on canal sides and in parkland with greater suburban abundance. *Lasioglossum smeathmanellum* was found in abundance in urban areas by Bates et al. (2011), potentially associated with the availability of crumbling mortar providing nesting resources for cavity nesters. In my chapter, 102 individuals of *L. smeathmanellum* were recorded with no significant difference between land-use types. These differences may be due to the foraging and nesting resource availability varying between site types, with some site types providing benefits which other sites do not. This underlines the need for all site types to be sampled before reaching firm conclusions about bee assemblages along urban-rural gradients.

There were some corresponding results however, with certain species showing greater abundance in urban and suburban sites of both studies, with *Bombus terrestris* and *Bombus hypnorum* both shown to be greater in urban or suburban areas. Bates et al. (2011) also showed a greater number of species in rural compared to suburban sites. My chapter found no difference in the number of species between land-use types, but with rarefaction a greater level of species richness was estimated for rural sites. This suggests that the main species observed in urban areas, and the general trend of species loss along the urban-rural gradient may be encapsulated by studies when sampling only one or two site types in a city. However, to assess the full scope of species-specific responses along urban-rural gradients a greater number of site types require sampling.

Potentially limiting the comparison between these two studies is this chapter's more intensive pan trapping sampling effort (greater number of pans placed at each site) than Bates et al. (2011). However together, this chapter and Bates et al. (2011) provide valuable insight into how the urban-rural gradient in Birmingham influences bee assemblages in multiple site types. Between these two studies within this study region, there is arguably one site type still remaining to be surveyed in this sample area; namely gardens. In order to gain a comprehensive assessment of bee assemblage variation, further analysis into the importance of gardens is necessary in Birmingham. As for different cities and regions, increasing regional and site type sampling is crucial in understanding the full impact the urban-rural gradient will have on bee assemblages. This could lead to a common sampling framework between cities, and bring current research closer to a widespread and definitive conclusion as to the response of pollinators to urbanisation.

The importance of an urban-rural gradient in defining bee assemblages has been highlighted in this chapter and in several studies (Cane et al., 2006; Fetridge et al., 2008; Matteson et al., 2008; Bates et al., 2011). Species variation in response to the urban-rural gradient is documented in previous literature (McIntyre, 2000; Kark et al., 2007). Usually these responses can be defined in three ways: (i) species present within rural areas show little or no presence in urban sites; (ii) species present in urban areas show a greater proportion of individuals or are only recorded in urban sites; and (iii) other species show no difference between the two (McIntyre, 2000; Kark et al., 2007). This is most clearly seen in my chapter's results when observing the proportion of species represented in each land-use type (Appendix I: Figure AI.1). It has been suggested that investigating the causes of species-specific variation is a key area for future research in urban ecology (McIntyre, 2000). One way species change along the urban-rural gradient may be assessed in the future is through evaluating assemblage data alongside the life-history traits of those species. This type of analysis has been shown before to explain the bee assemblage change observed in light of different aspects of anthropogenic disturbance (Moretti et al., 2008; Williams et al., 2010). In addition, as trait complementarity has been noted as an important component of pollination effectiveness (Hoehn et al., 2008: Albrecht et al., 2012; Fründ et al., 2013), a trait analysis may allow greater understanding of the implications bee assemblage variation could have on pollination services.

As to the influence of species change on pollination services provided by bees, there is still inadequate information to reach a firm conclusion. The species found within urban and suburban areas can represent a large proportion of the regional bee diversity (Frankie et al., 2009) helping to provide pollination services throughout urban landscapes (Tommasi et al., 2004). This is potentially the case in this chapter, with 64 species out of the total 76 species recorded found to be present within urban and suburban sites. However, before the full extent of how species change will (or will not) influence pollination services along a landscape gradient, the importance of certain species in providing adequate pollen carryover to facilitate pollination success in plants still needs to be assessed. Key areas of future research should include assessing how different species provide pollen-carryover, and whether or not the change in species observed along the urban-rural gradient can influence the pollination success of plants.

The first step towards applying a trait based analysis on bee assemblage composition along landscape gradients is to firstly assess and add to the databases of bee traits. This includes morphological traits that may be important in pollen carryover by bees, and thereby potentially influencing the pollination services bees provide to plants. The next chapter attempts this by measuring the inter-specific variation in bee pilosity (or the level and type of hair), a potentially important trait in pollen carryover and therefore pollination.

Chapter 3

An analysis of the inter-specific variation in the pilosity of UK bees and its relationship to body size and pollen transport adaptations

Abstract

Pollination success is provided to flowers by bees transferring pollen between conspecific flowers via floral visits (pollen carryover). The morphological attribute that enables bees to collect pollen passively is their hair (or pilosity). Although pollen transport adaptations are well documented in the literature, bee pilosity is lacking measurement and comparison between species and in reference to other functional traits such as body size and pollen transport adaptation. This will allow further investigation into how changes to bee assemblages may influence potential pollen carryover by bees. This chapter undertakes a pilosity analysis by measuring the hair length, hair spacing, hair cover and hair type at 10 pre-selected body regions of 122 species of bee (three female specimens per species) commonly found within the UK. Hair length, spacing, cover and branching in bee species measured showed significant genera- and species-specific variation. Hair length, spacing and cover were all significantly associated with bee body size Inter-Tegular Distance (ITD). Hair length on areas adapted for pollen transport was explained by the pollen transport adaptations that species have (i.e. corbicula), whereas the level of hair branching showed no association with body size, and only explained the variation in the genera Halictus *and* Lasioglossum. *It is therefore recommended that a comprehensive pilosity analysis is not required to assess the relative diversity of hair traits in bees, as body size and pollen transport specialisations explain most of the variation between species. I suggest future research into the importance of hair traits for pollen carryover, and the chapter supports the inclusion of pilosity measurements on the areas of incidental pollen transport into functional trait analysis.*

3.1 Introduction

In 87% of angiosperms, animal vectors need to deposit viable pollen on the correct part of a con-specific flower for pollination to be achieved or aided (Ollerton et al., 2011). There are many animal vectors including mammals, insects, birds and reptiles. Bees are one taxonomic group that has evolved to use pollen as a source of nutrition for their offspring (Roulston & Cane, 2000) and subsequently they are considered prolific pollen vectors. Traditionally, the phenotypic adaptations that lead to particular plants being pollinated by particular pollinators have been grouped into pollination 'syndromes' (reviewed by Waser, 2006). Pollination syndromes however, do not adequately take into account the full variability in the possible connections between plants and their pollinators (Ollerton et al., 2009). Pollination success is subject to the spatial and temporal disparity between plant species and potential pollinators, but how pollinators and flowers come into physical contact also determines the amount of pollen transferred between conspecifics (pollen carryover). For instance, bee species are likely to manipulate flowers differently, collect pollen in different ways, collect pollen on different parts of their body, collect pollen in different amounts, forage differently after collecting pollen, groom their bodies of pollen in different ways and to varying extents, and will come into contact with flowers in different ways (Mitchell et al., 2009). Hence, a great deal is still unknown regarding the potential pollen carryover between species of bees and plants, making it difficult to discern how pollination services will be affected by bee assemblage alteration (Mitchell et al., 2009).

Novel ways in which plant and pollinator traits are documented and compared to assess potential pollen carryover are required to allow a greater understanding of pollination outside of pollination syndromes. One way of doing this is through a more practical approach to assessing pollination relationships between different species of pollinators and different species of plant, with a bottom up approach which documents the traits that directly influence pollen carryover (Ollerton et al., 2009). Before a trait-based analysis however, it is necessary to catalogue inter-specific variation in traits specific to the function of bees and to compare them prior to a trait-based analysis (Wainwright, 2007). By cataloguing the inter-specific differences in pollen collection and transferral to flowers, further understanding of the variability of pollen carryover by bees could be achieved and applied to further research in pollination ecology.

There is evidence suggesting the extent to which bees facilitate pollen carryover between con-specific plants is partly due to variation in their pilosity (Rademaker et al., 1997; Thorp, 2000; Adler & Irwin, 2006). Alongside evolving to use pollen as a source of nutrition, bees have developed an array of morphological and behavioural adaptations which have aided in the collection and transportation of pollen grains (Thorp, 1979; Thorp, 2000; Schlindwein et al., 2005). Bees collect or accumulate pollen in two ways, through 'active' or 'passive' pollen collection (Figure 3.1). Active pollen collection refers to the specific behavioural or morphological traits enabling bees to remove pollen from particular flowers (e.g. sonication; Thorp, 1979, 2000), whilst passive pollen collection relates to the accumulation of pollen on the hairs of bees during flower visits (Thorp, 2000). After collection, pollen is transported in two ways: in designated transport adaptations (e.g. scopal hairs) or incidentally via body hair where passive pollen collection has occurred (Figure 3.1).

The hair present upon a bee is a primary factor in the level and type of pollen passively collected, the incidental transport of pollen on bees, and (although to a lesser extent as some bees use their crop to carry pollen) is part of the adaptations of bees to transport pollen. Bee hair is believed to aid passive pollen collection and incidental pollen transport as bees have evolved branched hairs that pollen can freely attach to through electrostatic force (Roberts & Vallespir, 1978; Vaknin et al., 2000). For areas adapted specially for pollen transport, scopal hairs are noted to vary between species based on the type of pollen collected (Roberts & Vallespir, 1978; Thorp, 1979). Scopal hair length, hair spacing and hair type (level of branching/plumosity) are all attributed to the type of pollen bees collect from particular flowers (Thorp, 1979; Roberts & Vallespir, 1978). However, previous research documenting the variation in the pilosity of bees has concentrated mainly on singular examples based on specialised plant-pollinator relationships (Thorp, 1979; Thorp, 2000). Currently, there has been no empirical comparison between pilosity in bee species as a group or in areas of incidental pollen transport.

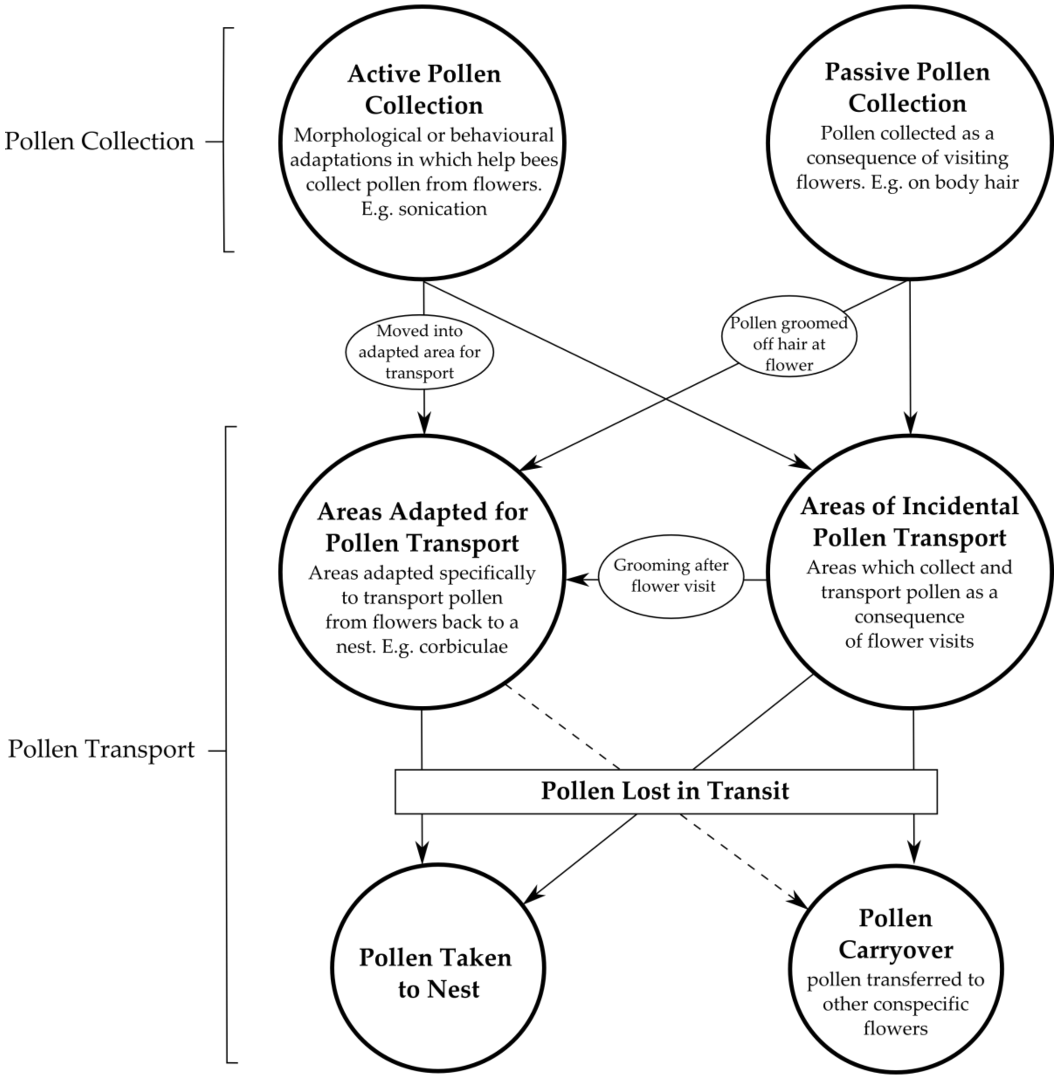


Figure 3.1: A diagram summarising the process of pollen collection and transportation by bees. Pollen is collected through actively manipulating flowers through sonication, biting, drumming or milking (Thorp, 2000; depending on anther position [Harder & Barrett, 1993]), or passively collecting pollen on hair whilst visiting flowers. Passively collected pollen, if grooming does not take place, is transported in the same position collected. If groomed, bees manipulate collected pollen via their relative morphological or behavioural adaption (i.e. carrying pollen in their crop or in scopal hairs). Pollen is then lost in transit, taken to a nest and used for nutrition, or becomes successfully carried over to a conspecific flower. Arrows show the movement of pollen in the process, with the dashed line representing the limitation of pollen carryover after placed in an area adapted for pollen transport (Thorp, 2000).

Trait complementarity is increasing in scientific interest towards pollination services provided by bees. Recent evidence suggests general measures of bee abundance or richness do not fully account for the variability in pollination success of plants, as the dissimilarity in traits between bees can complement each other to fulfil this service to a greater extent (Hoehn et al., 2008; Albrecht et al., 2008; Winfree & Kremen, 2009; Fründ et al., 2013). Specifically, Hoehn et al. (2008) has shown how the division of bee species through morphological and behavioural traits into functional guilds showed a greater increase in pollination services than bee abundance or richness. They further suggested this was due to larger bees transporting more pollen between conspecifics whilst smaller bees facilitated appropriate pollen movement within flowers. This implies the complexity of morphological and behavioural traits in bees complements each other and leads to greater pollination services of plants.

Increasingly, species diversity is a poor substitute for interpreting the how the loss of diversity impacts on ecosystem services as compared to trait-based frameworks (Diaz & Cabido, 2001; Flynn et al., 2009). With the incorporation of traits into ecological diversity analysis and research (Naeem & Wright, 2003; Petchey & Gaston, 2006), a connection between organisms and ecosystems can be applied empirically (Petchey et al., 2007). Therefore, the first step towards understanding the importance of pilosity towards pollination services is through its measurement and documentation. Secondly, through comparison between previously documented traits, the value of pilosity as a new trait towards assessing the variation in pollen carryover can be considered. There are traits which may be associated with pilosity that are already associated with function, including body size (Hoehn et al., 2008) and pollen transport adaptations (Thorp, 1979, 2000). This chapter outlines and undertakes a novel method for measuring the pilosity of bees. Using these data, I investigate the extent to which pilosity of UK bees varies between genera and species, and relates to species' body size and pollen transport adaptations.

3.1.1 Aims and Objectives

The main aim of this chapter was to measure and compare bee pilosity variation and investigate if pilosity is a valuable trait to include in analysis of pollination by assessing whether known functional traits explain the variation found in measurements of hair length, spacing, cover and structural type of UK bees.

Specifically the objectives of this chapter were to assess the implications of:

(i) body size in explaining the variation found in bee pilosity;

(ii) pollen transport adaptations in explaining the variation in bee pilosity

3.2 Methods

The measurements of pilosity were obtained from archived specimens at the Natural History Museum (London, UK). All measurements were made using an 8:1 Leica microscope and graticule. The inter-tegular distance (ITD) was also taken for each specimen as a measure of body size (Cane, 1987; Bullock, 1999). The species incorporated into the pilosity analysis were those classed as having ubiquitous, widespread and restricted UK distributions. However, some species defined as scarce were also incorporated but not all due to time constraints (Archer, 2007; Baldock, 2008; for species list see Appendix II). In total, 122 species from 23 genera were measured in the analysis of pilosity. Of these species, 72 were solitary, 25 cleptoparasitic, 15 eusocial (*Bombus* spp.), six social brood parasites (*Bombus*; *Psithyrus*) and the managed European Honey Bee (*Apis mellifera*).

3.2.1 Pilosity Analysis

The pilosity analysis consisted of four metrics measured on 10 areas from three female specimens of each species. Female bees were used as they are the gender which actively forages for pollen and have pollen transport even though males also transport pollen passively adaptations. The areas where pilosity metrics taken were determined by first including all regions most likely to come into contact with parts of flowers offering pollen, covering potential passive pollen collection. These included the front trochanter and clypeus associated with open flowers, and the mesonotum and gastral tergites for complex trigger flowers (Figure 3.2). Secondly, areas that are used for pollen transport, including the hind trochanter, hind femur, hind tibia and gastral sternites were included (Figure 3.2). Although technically part of active pollen transport morphology, these areas could still contribute to the transfer of pollen between flowers (Thorp, 2000) and will allow for comparison between pilosity and the respective pollen transportation adaptations in bees. Due to time constraints when collecting the pilosity data, it was not feasible to gain a complete measurement for all gastral tergites and sternites. Instead, the main plate and posterior marginal area (inter-marginal and marginal) of the second tergite and sternite were measured (Figure 3.2).

The four pilosity metrics included hair length, hair spacing, hair cover and hair type, each being measured for the 10 selected areas for all three specimens of each species (Figure 3.2). Hair length for each area was measured by aligning the graticule from base to tip of hair at the centre of the area or the patch of hair present. Measuring hair spacing as a number of hairs for the entirety of that particular area was not always possible, as in some areas the amount of hair was too prolific or dense to feasibly count. By selecting a transect most representative of the hair spacing in each area, hair spacing was measured by counting the number of hairs between a set distance of 250 μm on the microscope graticule in the centre of the measured area, then dividing 250 μm by the number of hairs counted. Percentage hair cover was estimated as a percentage of the amount of coverage hair represented for each area measured. Hair type was determined by assigning the hair in each area into one of five categories: smooth, scaled, branches <1 μm, branches 1-2 μm and branches >2 μm. More information on these measurements is given in Figure 3.3.

A protocol was undertaken for selecting which specimens of each test species were to be measured. First, worn or faded specimens were avoided by selecting the most unmarked and newly acquired specimens available from each species. Secondly, specimens collected from the British Isles were preferred for the analysis due to the possibility of phenotypic variation. Specimens from mainland Europe were only selected if no specimens from the UK were available. The intra-specific variation in body size (most notable in species of bumblebee) could have influenced the measurements of pilosity. To allow variation in pilosity to be measured within species while alleviating the influence of such large differences in body size, nine specimens from each species were chosen (following the above protocol), lined up in order of body size, with the three middle specimens used to measure pilosity for that species.

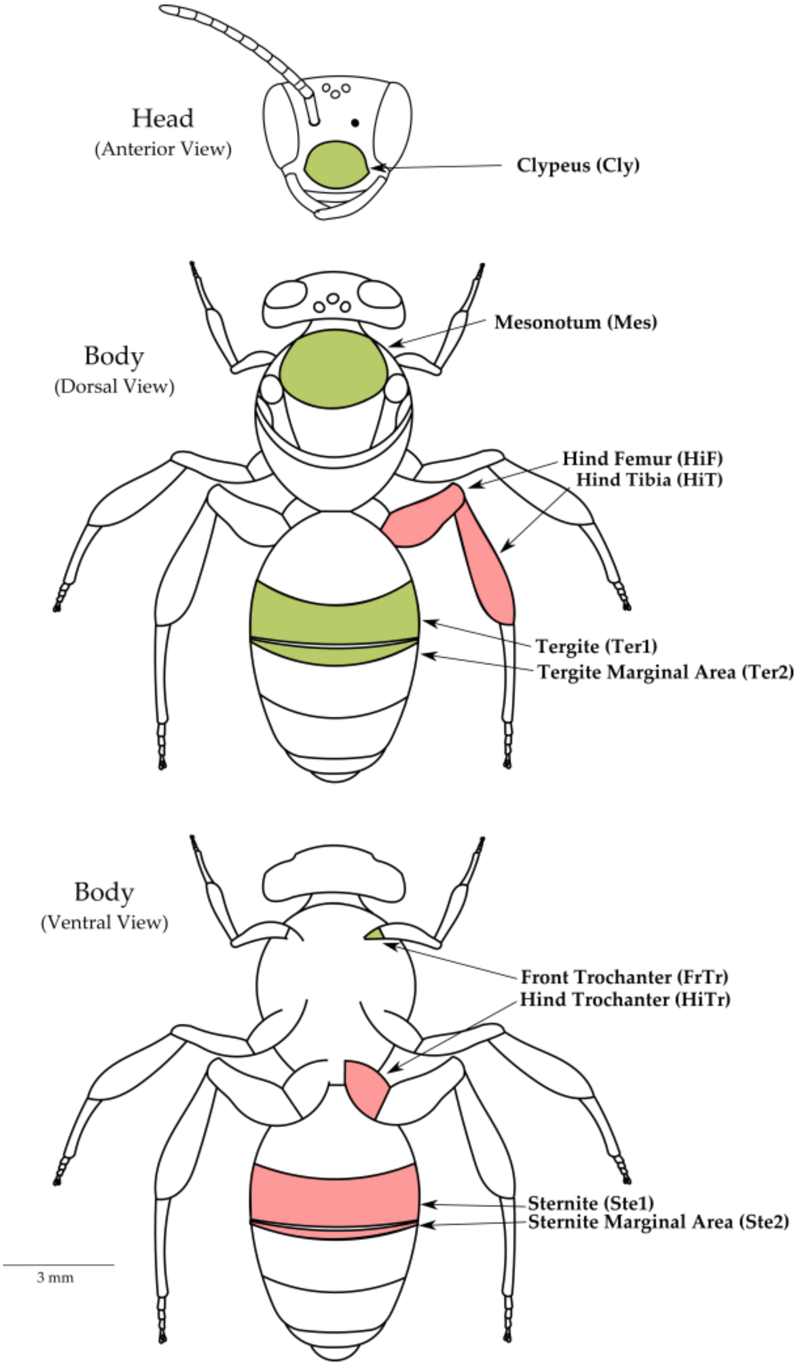
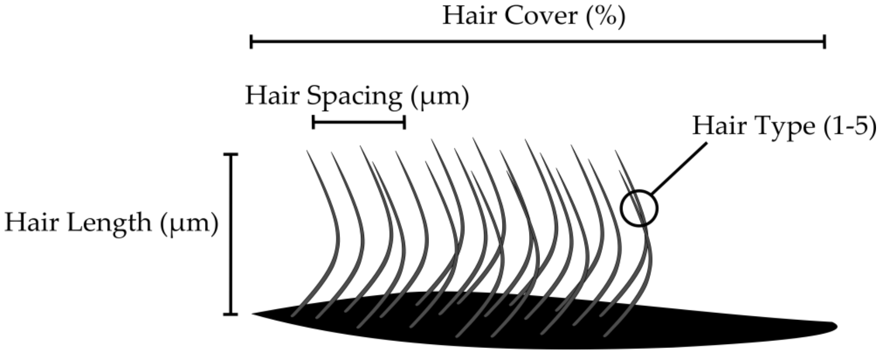


Figure 3.2: Schematic diagram of the dorsal and ventral views of a bee body, indicating areas where hair length, spacing, cover and type were measured as part of the pilosity analysis. Red shading indicates areas adapted for pollen transport and green shading indicates areas not adapted for pollen transport but still important for passively collected pollen and transporting pollen incidentally from flower visits. Codes of each area measured are in brackets.



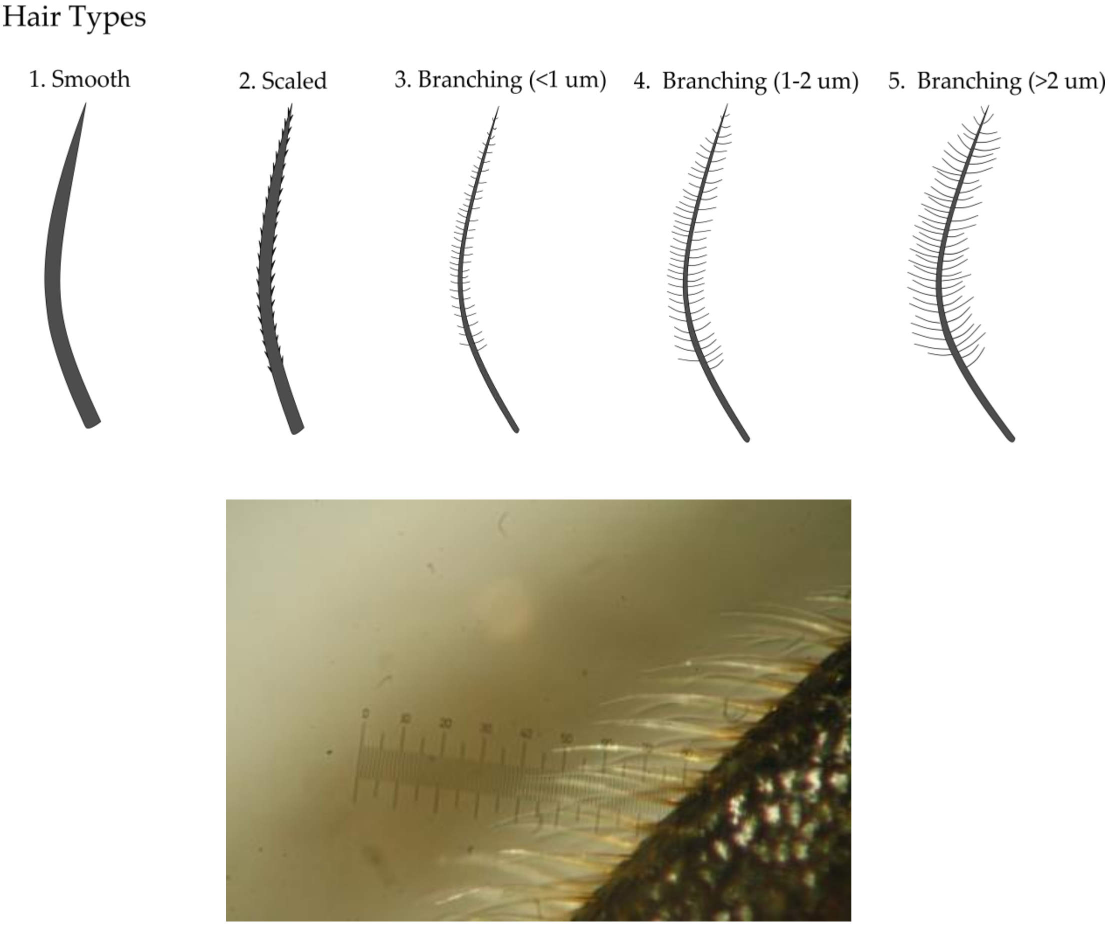


Figure 3.3: Outline of the four pilosity analysis measurements taken from 10 areas of three specimens for 122 species of bee. Hair length was a measure of the length of hair from the base to the tip of each area measured. Hair spacing was measured by counting the number of hairs in a set distance on the microscope graticule of 250 µm, and then divided 250 by the number of hairs. Hair cover was a measure of proportion of area covered by hair for each area measured. Hair type was categorised based on each area's hair type (categorised below). The photograph at the bottom shows the left hind tibia of an *Andrena bicolor*. Photo: R Fowler.

3.2.2 Pollen Transport Groups

Bees have developed a wide range of behavioural and morphological adaptations with which to transport pollen between flowers and their nests. The main ways in which bees are adapted to transport pollen are shown in Table 3.1, and briefly described here.

Species from the genera *Colletes* and *Andrena* tend to transport pollen on their hind femora and tibiae which have branched hairs, but also use their propodeum, hind trochanters and hind coxae. *Halictus* and *Lasioglossum* (Halictids) use the same adaptations as *Colletes* and *Andrena*, but can use their basitarsi and abdominal sternites as well (Thorp, 1979). All four of these genera are noted to have greater levels of branching on the hairs designated for pollen transport. Species from the family Megachilidae use long hardened smooth hairs on their sternites to transport pollen (Thorp, 1979, 2000). *Dasypoda* use their hind tibiae and basitarsi as well as sternite hairs. The hairs on *Dasypoda* hind tibiae and basitarsi are plumose and a great deal longer than other species of similar size (Baldock, 2008). *Panurgus*, *Anthophora*, *Eucera*, *Melitta* and *Macropis* all use hind tibiae and basitarsi hairs to transport pollen. *Bombus* and *Apis* have corbiculae which are bare flattened hind tibia, surrounded by long hard hairs which enable pollen to be clumped together using nectar and fixed in place during flight. *Hylaeus* transport pollen in their crops, regurgitating pollen with nectar when provisioning brood cells. *Hylaeus* is therefore similar to *Nomada*, *Sphecodes*, *Coelioxys*, *Epeolus* and *Melecta* which have no pollen transport adaptations due to their cleptoparasitism.

To assess the suitability of pilosity as an important functional trait the influence of pollen transport adaptations on the relative value of pilosity metrics will need to be assessed. In order to achieve this, I adapted approaches of Thorp (1979) and Roberts and Vallespir (1978) to place genera into nominal Pollen Transport Groups (PTGs, Table 3.1) based on their pollen transport adaptations. Throughout the analysis, species and genera are colour coded into these groups for interpretation of their potential importance in explaining bee pilosity.

Table 3.1: The relative importance of areas adapted for pollen transport between bee genera, areas of '1' being the most important and '3' the least important but still used. These are then used to place bees into Pollen Transport Groups (PTGs) to assess their comparability to other measures of pilosity. Adapted from Thorp (1979) and Roberts and Vallespir (1978).

Family	**Genera**	Crop[1]	Propodeum[2]	Hind Trochanter	Hind Femur	Hind Tibia	Hind Basitarsus[3]	Sternites	**Pollen Transport Group**
Colletidae	*Colletes*		2	2	1	1			**A**
Andrenidae	*Andrena*		2	2	1	1			**A**
Halictidae	*Halictus*		3	3	1	1	3	3 (no. 1-5)	**B**
Halictidae	*Lasioglossum*		3	3	1	1	3	3 (no. 1-5)	**B**
Megachilidae	*Megachile*							1 (no.2-6)	**C**
Megachilidae	*Osmia*							1 (no.2-6)	**C**
Megachilidae	*Chelostoma*							1 (no.2-6)	**C**
Megachilidae	*Anthidium*							1 (no.2-6)	**C**
Megachilidae	*Hoplitis*							1 (no.2-6)	**C**
Melittidae	*Dasypoda*					1	1	3 (no.2-6)	**D**
Andrenidae	*Panurgus*					1	3		**E**
Apidae	*Anthophora*					1	1		**E**
Apidae	*Eucera*					1	1		**E**
Melittidae	*Melitta*					1	1		**E**
Apidae	*Macropis*					1	1		**E**
Apidae	*Bombus*					1			**F**
Apidae	*Apis*					1			**F**
Collectidae	*Hylaeus*	1							**G**
Apidae	*Nomada*								**G**
Halictidae	*Sphecodes*								**G**
Megachilidae	*Coelioxys*								**G**
Apidae	*Epeolus*								**G**
Apidae	*Melecta*								**G**
Apidae	*Psithyrus*								**G**

[1] The stomach which bees use to collect nectar and break it down into honey. [2] The anterior dorsal lateral area of the thorax. [3] Basitarsi are enlarged parts of the tarsi which articulate with the tibiae proximally. See Figure 3.2 for other taxonomic terms.

3.2.3 Data Analysis

The analysis of pilosity data is outlined in Figure 3.4, and is outlined in the same order here. All analyses were carried out using R v. 3.0.2 (R Core Team, 2012; www.r-project.org) within RStudio v0.98.501 (RStudio, 2012). Principal Components Analysis (PCA) was calculated using the Vegan package in R (Oksanen et al., 2012).

3.2.3.1 Species and Genera Averages

Initially, this analysis assessed the level of variation in pilosity measurements between bee species and genera. By averaging the 10 measurements per species, an average of each pilosity measurement per species was gained. For genera with more than one species within this analysis, their species values were averaged again to gain a measurement for each genus. The error of means between genera was estimated as the standard error between species' measurements.

3.2.3.2 Pilosity and Body size

Using the original 10 measures of each pilosity measurement for each species (i.e. four pilosity measurements, at 10 areas, averaged for three female specimens per species), the importance of body size to pilosity was assessed (Figure 3.4). Common to physiological trait analysis is the co-linearity between trait measurement and body size (Zuur et al., 2007). In order to test if body size explained the variation in pilosity metrics, pilosity metrics were used in a Principals Component Analysis (PCA) on a covariance matrix. Using each set of 10 measurements per metric of pilosity, pilosity data were reduced allowing the first axis of each PCA (i.e. PC1) to be plotted against the species' ITD. If body size was an influence on morphological characteristics then a large percentage of the variance in the dataset will be explained by the first axis of a PCA (Zuur et al., 2007). Spearman Rank or Pearson Product Moment correlation tests were used depending on the normality of the data to test for significant correlations between PC1 scores and ITD (Figure 3.4). If the PC1 scores were strongly correlated with body size, data were double-centre standardised following Zuur et al. (2007) to allow pilosity metrics to be analysed further without the influence of body size.

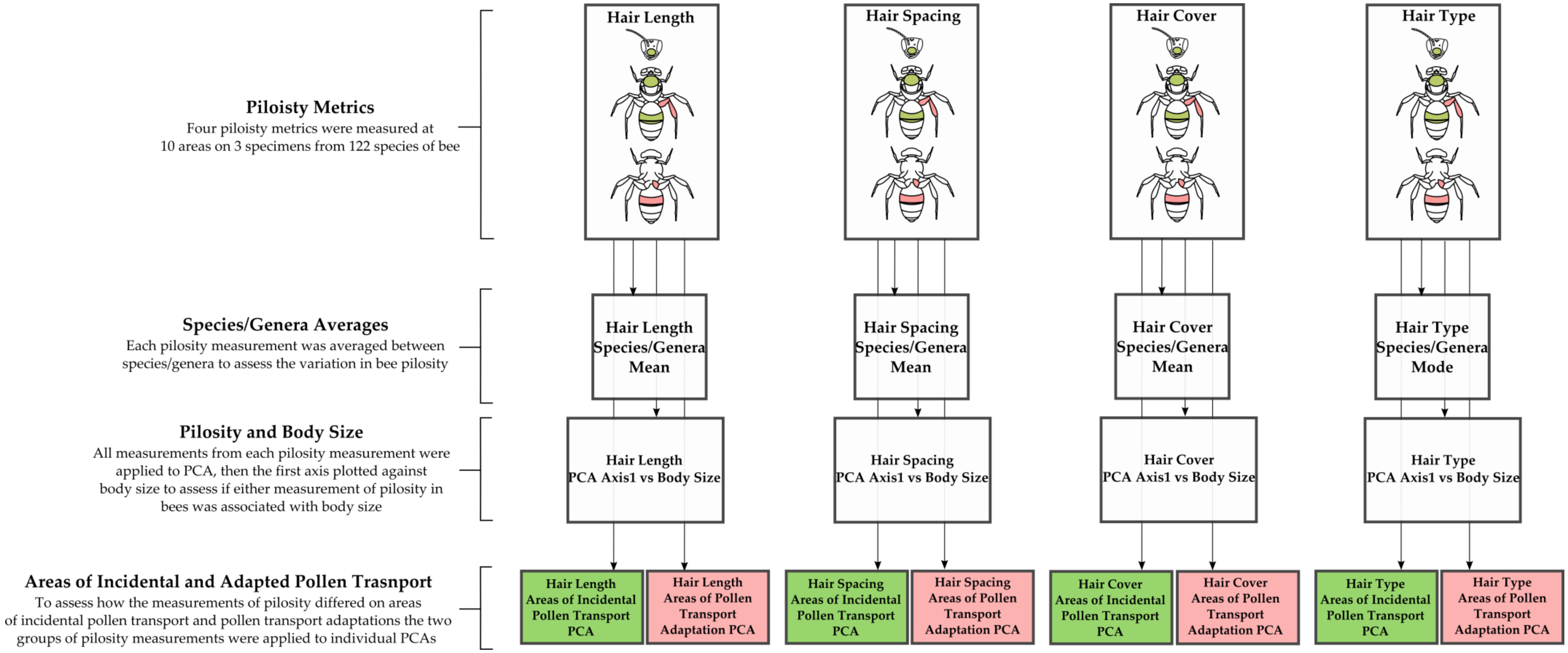


Figure 3.4: Diagram of the methods used to assess the importance of pilosity as a functional trait. Initially I measured hair length, spacing, cover and type at 10 areas on three specimens from 122 species. These 10 measurements were then averaged for genera (or species if only one species was measured from that genus) to compare each pilosity measurement type. Pilosity metrics were then each included in Principal Components Analysis (PCA) using a covariance matrix, with the loadings of the first axis of each PCA then plotted against ITD to assess the influence of bee size on pilosity. To assess whether pollen transport adaptations explained any variance in pilosity, pilosity measurements from incidental pollen transport areas and areas of pollen transport adaptation were included in individual PCAs.

3.2.3.3 Incidental Pollen Transport and Pollen Transport Adaptations

Once body size was accounted, it was possible to assess whether the differences in pollen transport adaptations accounted for bee pilosity variation. This was done by partitioning the pilosity metrics between the areas which contribute to incidental pollen transport and those that are part of adapted pollen transport structures. Incidental pollen transport areas included the clypeus, mesonotum, front trochanters, tergite and tergite marginal area. Areas that are adapted for pollen transport to at least some extent included the hind tibiae, hind femora, hind trochanters, sternite and sternite marginal areas. Each group per pilosity metrics were then included in PCAs, plotted in biplots and coloured based on their relative PTG to assess if their pilosity values grouped species by their transport adaptations. This allowed an assessment of how much the PTGs explained the variation in pilosity of bees on different areas of the bee's body (Figure 3.4).

3.3 Results

3.3.1 Species and Genera Pilosity Averages

The averages of each pilosity metrics between genera are represented in Figure 3.5 and explained in more detail in the following paragraphs.

3.3.1.1 Genera/Species Mean Hair Length

The social parasites *Bombus; Psithyrus* had the longest hair length (mean=1285 ± 84 µm, n=6) which was effectively the same as its host genus *Bombus* (mean=1229 ±50 µm, n=15). Hair length similarity between certain parasite species and their hosts was recorded again with *Anthophora* spp. (mean=657±119 µm, n=2) and their parasite *Melecta albifrons* (mean=632 µm). However, three of the four genera with the lowest average hair length were the cleptoparasitic genera *Sphecodes* spp. (mean=156 ±14 µm, n=8), *Nomada* spp. (mean=120±36 µm, n=11) and *Epeolus* spp. (mean=65±13 µm, n=2). *Hylaeus* spp. had the shortest hair length of all (mean=58±3 µm, n=4), a genera which has adapted to carry pollen within its crop (Thorp, 1979; Table 3.1). Averaged hair length showed significant variation between genera (H=105.77, d.f.=23, p=<0.001; Table 3.2) and PTGs (H=94.11, d.f.=6, p=<0.001; Table 3.2).

Table 3.2: Means tests of averaged species pilosity between genera and between Pollen Transport Groups (PTGs). Significance to p<0.001 = ***, p<0.01= ** and p<0.05 = *.

Grouping Variable	d.f.	Hair Length Test Statistic	Hair Spacing Test Statistic	Hair Cover Test Statistic
Genus	23	$H = 105.77^{***}$	$H = 68.21^{***}$	$H = 37.22^{*}$
Pollen Transport Group	6	$H = 94.11^{***}$	$H = 45.77^{***}$	$H = 26.51^{***}$

3.3.1.2 Genera/Species Mean Hair Spacing

The spacing between hairs was highest in genera that either do not need to collect pollen or collect it in their crop. The genera *Sphecodes, Nomada* and *Hylaeus* had hairs spaced on average every 74, 65 and 63 µm, respectively. This contrasts with the most densely spaced hairs recorded on *Eucera longicornis* and *Dasypoda hirtirpes* which were 24 and 29 µm, respectively. Hair spacing showed significant variation between genera (H=68.21, d.f.=23, p=<0.001; Table 3.2) and PTGs (H=45.77, d.f.=6, p=<0.001; Table 3.2).

3.3.1.3 Genera/Species Mean Hair Cover

Average hair cover varied to less of an extent than hair length, as many species presented all 10 areas fully covered in hair. However, several species showed <65% hair cover on average, including *Andrena tarsata, Lasioglossum fratellum, Nomada flavoguttata, N. goodeniana, Sphecodes geoffrellus* and *S. monilicornis*. The genera with the lowest levels of average percentage hair cover were *Epeolus, Sphecodes, Nomada* and *Hylaeus*. Hair cover showed significant variation between genera (H=37.22, d.f.=23, p=0.03; Table 3.2), and between PTGs (H=26.51, d.f.=6, p=<0.001; Table 3.2).

3.3.1.4 Genera/Species Mode Hair Type

Lasioglossum spp. and *Halictus* spp. were most commonly covered in hairs branched between 1-2 µm. Shorter hair branching (< 1 µm) was found in *Andrena* spp., *Colletes* spp., *Eucera longicornis,* and *Dasypoda hirtirpes*. The cleptoparasitic species *Melecta albifrons* also presents hairs branched < 1 µm, contrasting with other parasitic species that tended to have smooth hairs (*Nomada* and *Sphecodes*).

3.3.2 Pilosity and Body Size

To check for the influence of body size on the pilosity measurements of bees, PCAs of each pilosity measurement type were generated (Table 3.3). This showed that PC1 explained 79%, 42%, 45% and 44% of the variance in hair length, spacing, cover and type, respectively. The PC1 from all four pilosity metrics were deemed suitable to test their relationship to body size, and were plotted and checked for correlation with ITD (Figure 3.6). Hair length (Pearson: r=0.88, p<0.001), hair spacing (Spearman: r=-0.31, p<0.001) and hair cover (Spearman: r=-0.30, p<0.001) were all significantly correlated with body size, highlighting the influence body size has on bee pilosity (Figure 3.6). In contrast, hair type was not significantly correlated with body size (Spearman: r=-0.06, p=0.51). The explained variance for pilosity metric PCAs are presented in Table 3.3. In Figure 3.6, the association between hair length and hair spacing to body size is representative of the direction of the raw data, with increased hair length and decreased hair spacing alongside body size. With percentage hair cover however, when applied to ordination the first axis was reversed. Therefore, although the PC1 for hair cover and body size show a negative trend, the raw data shows that body size and hair cover were positively correlated. .

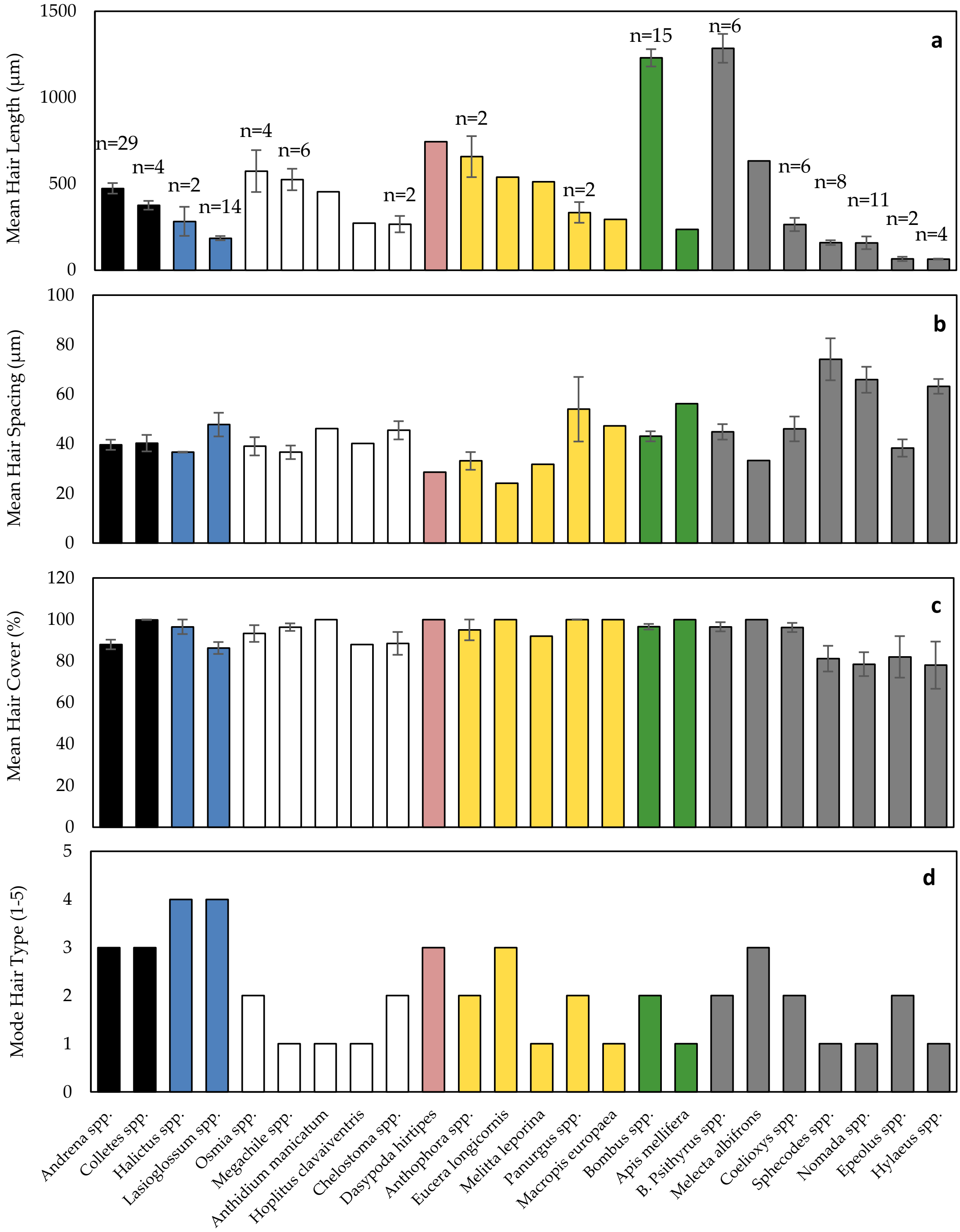


Figure 3.5: The variation in (a) mean hair length, (b) mean hair spacing, (c) mean hair cover and (d) mode hair type between genera (or individual species), averaged between 10 measurements taken from three specimens of 122 species. The number of species in each genus is displayed in plot a. Individual species therefore only represent the averaged value of their 10 measurements per pilosity metric. Error bars = ± 1 SE between the averaged species value for each pilosity measurement. Bee genera/species are coloured based on their pollen transport group (PTG; Table 3.1).

Table 3.3: Output of the Principal Component Analysis (PCA) for pilosity metrics hair length, spacing, cover and type, and also the double centre standardised hair length, spacing and cover. Included in each PCA were 10 measurements of each pilosity metric for 122 bee species.

PCA	Axis	Eigenvalue	Variance proportion	Cumulative variance proportion
Hair Length	1	1633000	0.79	
	2	163100	0.08	0.87
	3	93870	0.04	0.91
	4	57670	0.02	0.94
	5	37340	0.02	0.96
	6-10	79929	0.04	1.0
Hair Spacing	1	3707	0.42	
	2	1550	0.17	0.58
	3	1001	0.11	0.69
	4	854	0.09	0.78
	5	626	0.07	0.85
	6	470	0.05	0.90
	7	325	0.04	0.94
	8	291	0.03	0.97
	9-10	195	0.03	1.0
Hair Cover	1	2759	0.45	
	2	1208	0.20	0.65
	3	1057	0.17	0.82
	4	447	0.07	0.89
	5	301	0.05	0.94
	6	228	0.03	0.97
	7-10	132	0.03	1.0
Hair Type	1	7.09	0.44	
	2	2.49	0.15	0.59
	3	1.49	0.09	0.68
	4	1.42	0.09	0.77
	5	1.03	0.07	0.84
	6	0.71	0.04	0.88
	7	0.64	0.04	0.92
	8	0.51	0.03	0.95
	9-10	0.77	0.05	1.0

Hair Length (DC)	1	216000	0.43	
	2	107000	0.21	0.64
	3	60540	0.12	0.76
	4	37570	0.08	0.84
	5	33700	0.07	0.91
	6	16550	0.03	0.94
	7	12820	0.03	0.97
	8-9	17121	0.03	1.0
Hair Spacing (DC)	1	1689	0.27	
	2	1350	0.22	0.50
	3	852	0.14	0.64
	4	765	0.13	0.77
	5	583	0.10	0.87
	6	338	0.05	0.92
	7	292	0.05	0.97
	8-9	217	0.3	1.0
Hair Cover (DC)	1	6710	0.63	
	2	1311	0.12	0.75
	3	1183	0.10	0.85
	4	485	0.05	0.90
	5	431	0.04	0.94
	6	250	0.03	0.97
	7-10	290	0.03	1.0

DC = Pilosity metrics were double centre standardised to account for their relationship to body size.

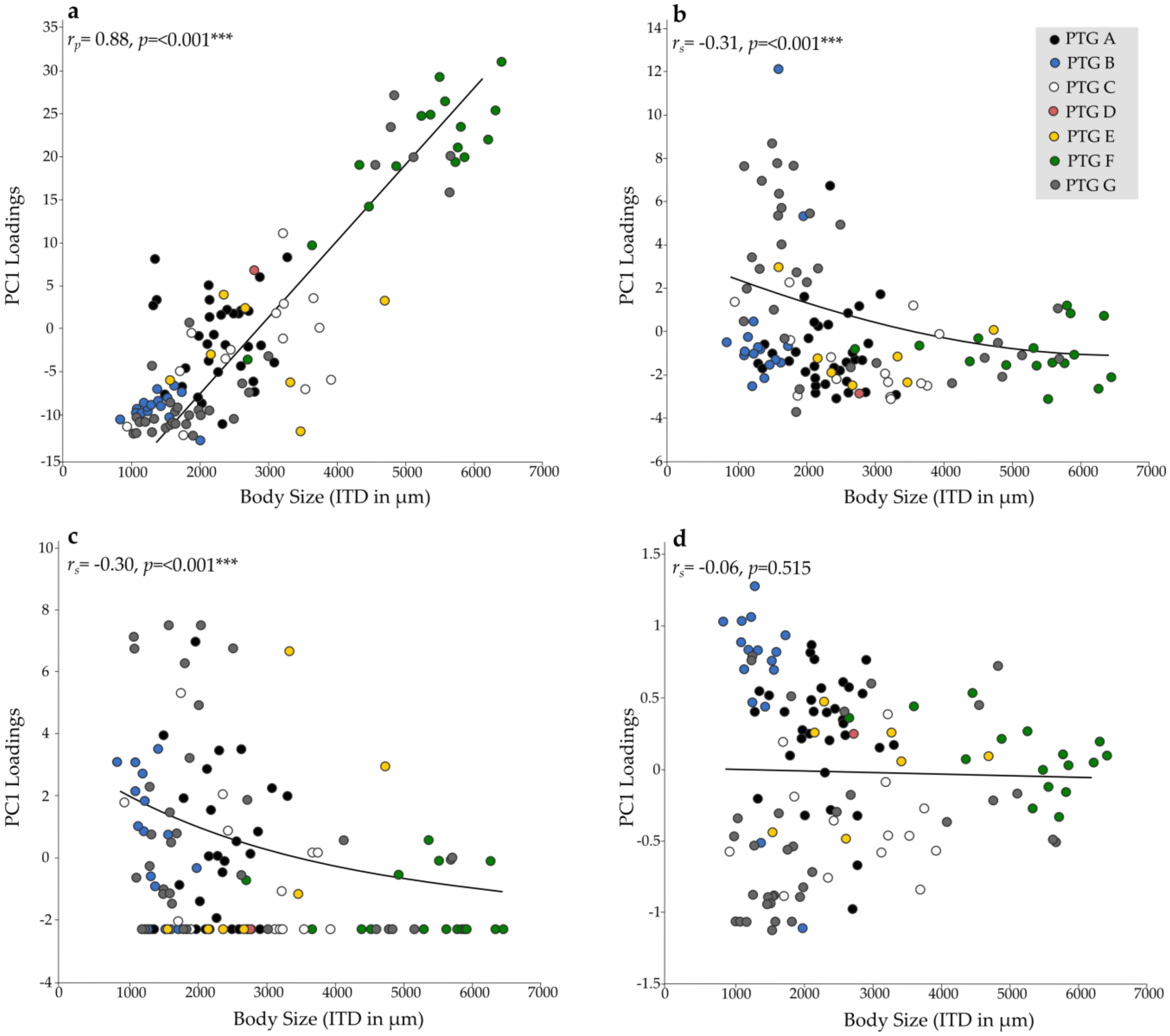


Figure 3.6: Scatter plots showing the relationship between body size and PC1 from PCAs for (a) hair length, (b) hair spacing, (c) hair cover and (d) hair type, to assess the variance in bee pilosity explained by body size. Points represent the averaged measurement of pilosity metrics for three specimens from 122 species of bee. Bee species are grouped by their pollen transport group (Table 3.1). r_s=Spearman Rank Order Correlation coefficient, r_p=Pearson Correlation coefficient. p=significance of correlation. For variance explained by each PCA, see Table 3.4. Although percentage cover shows a negative trend towards greater body size, this only shows the correlation between the loadings and body size. Therefore, although the hair cover PC1 and body size had a negative trend, with greater body size the hair coverage on bees actually increased. This was not the case for hair length and spacing, with the plots here representative of the nature of the association between length, spacing and body size.

3.3.3 Incidental Pollen Transport and Pollen Transport Adaptations

As bee body size was co-linear with hair length, spacing and cover, these pilosity metrics were double-centre standardised before being partitioned between areas of incidental pollen transport and areas of adapted pollen transport.

Through PCA, the length of hair for areas of incidental pollen transport appeared to group some bees based on their PTGs. This was most notable for the genera *Apis* and *Bombus*, with their greater tergite and tergite marginal area hair length grouping them away from other species. Other measures of pilosity on areas of incidental hair transport showed no clear grouping of species, suggesting these areas were not explained by the PTG to which they belonged.

For areas adapted to transport pollen, hair length appeared to group bee species by PTG. Species of *Apis* and *Bombus* (PTG: D) were grouped based on their hind tibial hair length; *Andrena* and *Colletes* (PTG: A) were grouped by hind femoral and hind trochanter hair length; and pollen-collecting species from the family Megachilidae (PTG: C) showed grouping based on their sternites' hair length. These relate to their pollen transport adaptations, showing hair length in areas of pollen transport can be explained to some extent by grouping bees by PTG. However, this was only obvious with hair length, as the only other pilosity metric which grouped bees based on their adapted areas for pollen transport was by the type of hair on their hind tibiae and femora on *Halictus* and *Lasioglossum* (PTG: B).

Table 3.4: Output of Principal Components Analysis (PCA) when pilosity measurements are separated between five areas of incidental pollen transport and five areas adapted for pollen transport.

PCA	Axis	Eigenvalue	Variance Proportion	Cumulative Variance Proportion
Areas of Incidental Pollen Transport				
Hair Length (DC)	1	95562	0.56	
	2	37311	0.22	0.78
	3	15689	0.09	0.87
	4	11350	0.07	0.94
	5	9467	0.06	1.0

Hair Spacing (DC)	1	1292	0.44	
	2	774	0.26	0.70
	3	362	0.12	0.82
	4	285	0.10	0.92
	5	249	0.08	1.0
Hair Cover (DC)	1	4504	0.69	
	2	1222	0.19	0.88
	3	430	0.07	0.95
	4	226	0.03	0.98
	5	127	0.02	1.0
Hair Type	1	3.48	0.51	
	2	1.22	0.17	0.69
	3	0.88	0.13	0.82
	4	0.77	0.11	0.93
	5	0.45	0.07	1.0
Areas Adapted for Pollen Transport				
Hair Length (DC)	1	173138	0.56	
	2	55503	0.18	0.74
	3	46055	0.15	0.89
	4	17804	0.06	0.95
	5	15337	0.05	1.0
Hair Spacing (DC)	1	1192	0.38	
	2	1082	0.35	0.73
	3	495	0.16	0.89
	4	216	0.07	0.96
	5	137	0.04	1.0
Hair Cover (DC)	1	3168	0.76	
	2	635	0.15	0.91
	3	283	0.07	0.98
	4-5	65	0.01	1.0
Hair Type	1	4.50	0.49	
	2	2.18	0.24	0.73
	3	1.32	0.14	0.86
	4	0.65	0.07	0.93
	5	0.58	0.07	1.0

DC = Pilosity metrics were double centre standardised to account for their relationship to body size.

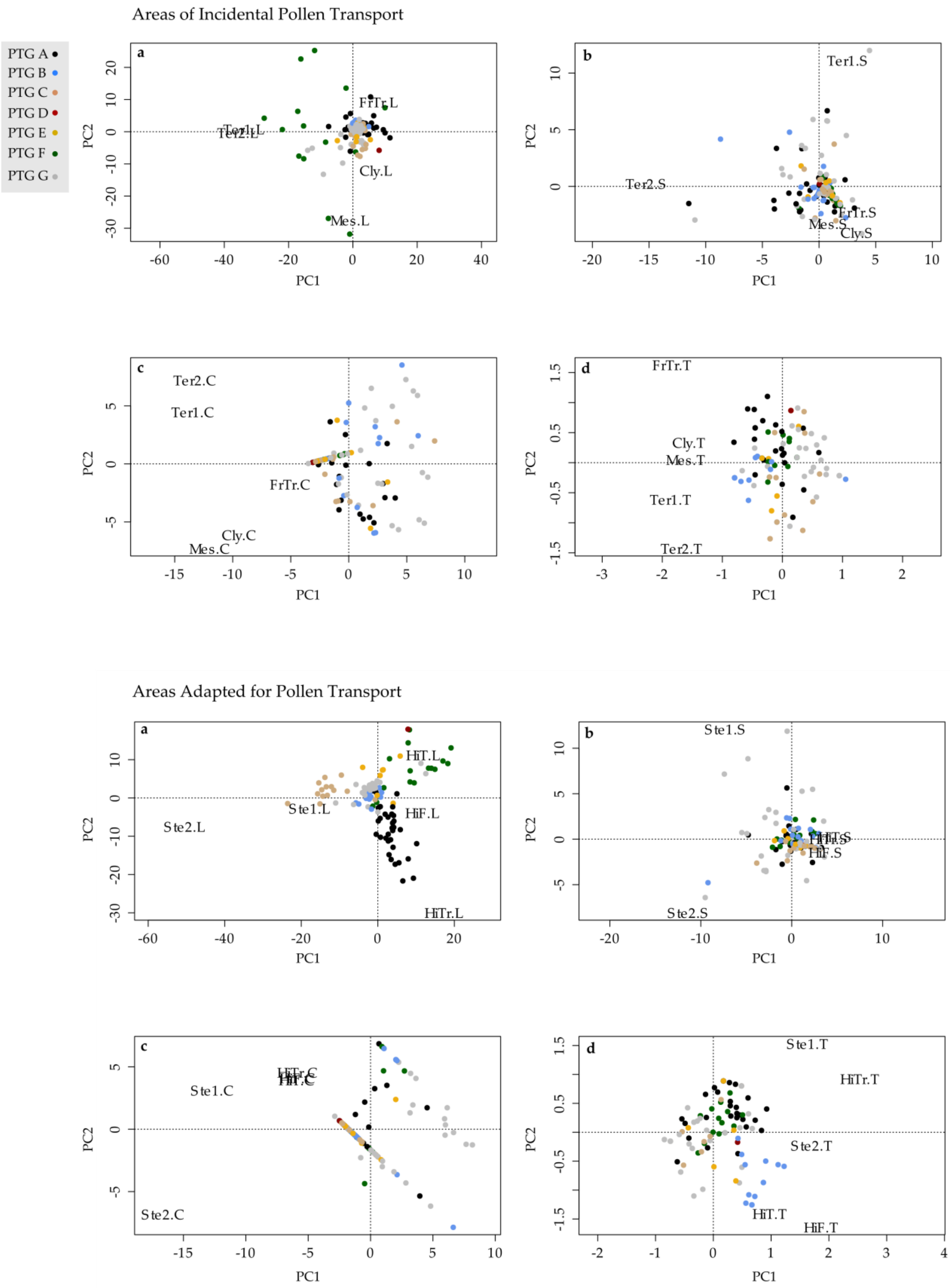


Figure 3.7: Principal Components Analysis of (a) hair length, (b) hair spacing, (c) hair cover and (d) hair type when separated between areas of incidental pollen transport and areas adapted for pollen transport. Hair length, spacing and cover metrics were double-centred to remove the influence of body size (Figure 3.6), whilst hair type was left unchanged. Measurement codes represent the area and type of pilosity metric. For measurement area codes see Figure 3.2; those ending in '.L' represent hair length, '.S' for hair spacing, '.C' for hair cover are '.T' for hair type.

3.4 Discussion

Previous literature has highlighted the diverse adaptations bees have developed to collect and transport pollen via physiological and behavioural traits (Thorp, 1979,2000). Through a standardised method of measuring bee hair length, spacing, cover and type this chapter found evidence of: (i) pilosity metrics varying between genera, and between the types of pollen transport adaptations they have; (ii) bee hair length, spacing and cover significantly correlating to body size; and (iii) for areas of incidental and adapted pollen transport, hair length (and to a lesser extent hair type) was explained by pollen transport groups (PTGs).

3.4.1 Genera and Species Variation

All measures of pilosity showed variation between the genera measured. There were some similarities however. The genus *Bombus* and the sub-genus Psithyrus (the social brood parasite of *Bombus*) had the longest hair length by far of any genera. This similarity between host and parasite was also evident for *Anthophora* spp. and their cleptoparasite *Melecta albifrons.* This highlights the potential importance that some cleptoparasites could provide in transferring pollen between flowers during their nectar foraging. However, this may be more dependent on body size, and is not consistent for all cleptoparasites with genera such as *Nomada* and *Sphecodes* having shorter and sparser hairs than their common hosts (*Andrena* spp.). The importance of hair length in pollen carryover is difficult to assess without more investigation into measurements of pollen collected and deposited by bees with varying hair lengths. It stands to reason that greater hair length results in more pollen collected and therefore potentially carried over to other flowers. However, there is evidence that with long hair pollen layers begin to accumulate on bees visiting flowers (Castellanos et al., 2003), which could restrict earlier collected pollen from being deposited on the correct flowers. More research is needed therefore to ascertain how hair length can influence pollen carryover in bees.

Nomada and *Sphecodes* were also found to have the sparsest hair in this analysis. Similar in hair spacing to these genera was *Hylaeus,* most likely due to their transporting pollen in their crop and not on hair. For bees that have hair adapted for pollen transport, spacing has been noted previously to vary between bees based on the species of pollen they forage for and collect (Roberts & Vallespir, 1978; Thorp, 1979). Therefore, these data may provide useful insights into pollen-collecting dynamics between species.

Some species were observed to have particular areas with <65% hair cover (see section 3.3.1.3 for species), but between species and genera hair cover did not decline ~80%. This suggests that percentage cover may be relatively consistent in all bees Therefore, percentage cover may not be the most important pilosity trait to incorporate into a pilosity analysis, as other hair measurements show more variation and most likely will lead to differences in how pollen carryover is facilitated.

Bees are said to have evolved branched hairs to help to facilitate pollen collection and transport (Thorp, 1979, 2000). In this analysis, I found *Halictus* and *Lasioglossum* were genera with the most consistent levels of greater hair branching. Bumblebees may not have branched hairs to the extent of solitary bees such as *Halictus* spp. and *Lasioglossum* spp., but they have long dense hairs which cover their entire bodies. However, as branching is arguably adapted to hold and transport pollen effectively its part in depositing pollen on subsequently visited flowers is difficult to discern. Further investigations should concentrate on the different ways in which hair types can both passively collect and deposit pollen to assess its relative importance in pollen carryover.

3.4.2 Pilosity and Body Size

The variation found in pilosity between bee species is interesting and useful. However, if pilosity metrics are used in future experiments there may be other ways of assessing differences between species. One of the most desirable ways is by attempting to understand how bee assemblage variation will influence the extent to which different types of pilosity are present in a community. One way pilosity variation may be accounted for within further research is body size. I found measurements of hair length, spacing and cover were significantly associated with body size.

Currently, the importance of different traits complementing (a.k.a. trait complementarity) pollination services is gaining momentum within the literature (Hoehn et al., 2008; Blüthgen & Klein, 2011; Albrecht et al., 2013; Fründ et al. 2013), with body size highlighted as an important component of trait complementary (Hoehn et al., 2008). This may be because larger bees are believed to be able to transport more pollen between con-specifics, whilst smaller bees facilitate the placement of pollen in the areas most conducive to pollination success (Hoehn et al., 2008). My results could have bearing on this relationship between pollen carryover and body size, most notably with larger bees having greater hair length. This could suggest large pollinators such as bumblebees may be able to transfer pollen to a greater extent as their capacity to collect pollen on their body hair can collect and carry more pollen between flowers. Furthermore, *Hylaeus* is one of the smallest genera recorded, which could allow them access to flowers or parts of flowers that other larger bees may find difficult, subsequently aiding in placing pollen on stigma (Hoehn et al., 2008). Therefore, the specific value of pilosity may not be as important as the complementarity differences in body size may offer to pollen carryover in bee assemblages as a whole. However, further investigation is needed as it is beyond this chapter to ascertain if greater hair length facilitates more pollen carried over to flowers, as hair length may mean pollen is less easily deposited on subsequently visited flowers.

Body size clearly described variation in three out of the four pilosity metrics measured, highlighting its use as a determinant of pilosity variation in bees. This may have applications in other studies investigating the pollen carryover of bees, and is an important component of trait complementarity that further studies should take into consideration. However, hair type showed no association with body size, suggesting there are elements of pilosity that go beyond body size in explaining variation in the pilosity of bees and may require incorporation into further analyses in their own right.

3.4.3 Pilosity and Pollen Transport Groups

Most of the literature documenting aspects of bee pilosity concentrates on the phylogeny of bees relating to their pollen transport adaptations (Thorp, 1979, 2000; Müller, 1996a). Within these adaptations bee scopal hairs show specialisation towards the pollen of host flowers. For example, bees which collect pollen from flowers with smaller pollen grains

(<40um) have more dense and branched scopal hairs, whilst bees which collect larger pollen grains (>100 um) show toughened and smoother scopal hairs (Roberts & Vallespir, 1978; Thorp, 1979). This chapter showed that bee hair length on areas adapted for pollen transport showed grouping based on their relative PTG. This is self-explanatory, with longer hairs having developed in areas relative to their specific pollen transport adaptation.

Although used in studies to a lesser extent than body size (but see Moretti et al., 2009), the inclusion of PTGs, or at least the types of adaptation each species has for transporting pollen, will allow some of the variation in hair length to be incorporated into analyses. There is evidence that the variation in pollen transport adaptations may influence pollen carryover by bees (Thorp, 1979, 2000). The pollen placed into hind leg morphological adaptations (corbiculae) found in *Apis* spp. and *Bombus* spp. or carried in the crops of Hylaeinae are thereafter considered unlikely to be transferred to another plant for pollination (Thorp, 1979, 2000). Conversely, bees of the Megachilidae family transport pollen via large smoothed scopa on the ventral mesonotal segments that come into contact with the stigma more readily when these bees visit open flowers (Thorp, 1979, 2000). Hence, it is important to factor these adaptations into further analyses of pollen carryover by bees. In terms of the areas that transport pollen incidentally, PTGs do not explain the variance observed after body size is accounted for. Although hair spacing and cover were associated with body size, future analyses of pilosity or pollen carryover should take these metrics into consideration.

3.4.4 Wider considerations of pilosity and pollen carryover

The natural selection of floral adaptation based on particular pollinator visitation (floral specialisation) is an important factor enabling pollen carryover for particular plant-pollinator relationships, and most likely dictated by the morphology and behaviour of pollinators (Ollerton et al., 2009). However, pollination 'syndromes' which give emphasis to specific individuals or the 'best' pollinators, may not encompass the whole range of ways pollen carryover is facilitated by pollinators (Ollerton et al., 2009). In *Hormathophylla spinosa* (Cruciferae), they can be pollinated effectively by all vectors visiting its flowers (Gomez and Zamora, 1999), and there is evidence that plants retain a level of generalism towards potential pollinators even when maintaining particular morphology towards specialised pollinators (Mayfield et al., 2001). For bees, a range of morphological adaptations allows the collecting of pollen from a variety of different types of flower (Thorp, 1979; Thorp, 2000). In terms of how bee species loss or assemblage change may affect pollination, there is a need for a greater level of interpretation in how particular vectors provide pollen carryover (Ollerton et al., 2009). This chapter provides a step in that direction by outlining how pilosity varies between bee taxa and its association with body size and PTGs. Not only will this allow further interpretation when incorporating these traits into analyses of function in bees, but it is hoped this chapter can give further interpretability in future research when assessing effectiveness of pollen carryover between bee species in relation to different species of plants.

The potential pollen carryover by bees is subject to a variety of constraints. First, disparity in spatial and temporal occurrence and activity between plants and bees will influence visitation potential. Secondly, even if both flower and plant are present at the same time and same location, if the morphological traits of flowers and pollinators are incompatible pollen carryover can be restricted (Padyšáková et al., 2013). Although there are examples of how particular bee species and genera collect and transport pollen (Thorp, 1979, 2000), there is still a great deal unknown regarding how bees can collect pollen, on which parts of their body it is collected and to what extent it is carried over to other plants (Adler & Irwin, 2006; Mitchell et al., 2009). This chapter adds to the current literature by recording the variation in pilosity metrics of bees in the UK, increasing the knowledge of what traits may differ between potential pollinators of plants.

Another factor in the potential pollen carryover of bees is plant morphology, specifically the placement of anthers and stigmas. Future analysis of pilosity should incorporate not only the implications of pilosity variation and where the pollen has been collected, but also how collected pollen will transfer to subsequent flowers. This is no easy feat and will require further investigations in controlled environments with different bee and plant species. However, with pilosity data such as those collected in this chapter, there is scope for a comparison between bee and flower morphological trait datasets. Through such research, it may be possible to test how certain individuals facilitate pollen carryover, and to what extent pilosity allows bee taxa to complement each other in pollinating different flowers. Through this, a great level of understanding as to the implications bee species loss and assemblage change may have on pollination services.

3.4.5 Study limitations

One factor that could limit the reliability of pilosity as a measure of pollen carryover in bees is their grooming behaviour. Bees undertake grooming at irregular intervals between flower visits to move passively collected pollen from their body to areas of pollen transport. Grooming is usually dependent on the placement of pollen prior to a flower visit (Thomson, 1986; Harder, 1990), and has been considered to remove pollen from possible transferral to con-specific flowers (Thorp, 2000). The potential rates of grooming are highly variable, with the levels of pollen lost from the potential total carryover to other flowers by bee grooming estimated at 6.1% (Rademaker et al., 1997). However, compared to vectors that do not groom pollen from their bodies between visiting flowers (e.g. Trochilidae), this behaviour does not necessarily lead to a reduction in pollen carryover (Castellanos et al., 2003).

There are other limitations, mostly associated with the methodology of measuring pilosity. First, not all areas of the bee body are included in the pilosity analysis. Second, what this chapter classes as an area of incidental pollen transport may also be part of active pollen-collecting structures. For example, certain species of *Panurgus* are known to use stiffened clypeal hairs to extract pollen from *Downingia cuspidata* (Campanulaceae, Lobelioideae) (Thorp, 2000). Third, I did not consider male bees even though they are still important to pollen carryover and pollination effectiveness (Canto-Aguilar & Parra-Tabla, 2000). It is however hoped that this chapter increases the level of academic interest in the pilosity of bees, but suggests that further investigations take into account the limitations in this analysis.

In the next chapter, I apply both these pilosity measurements and other morphological and ecological traits to an analysis of bee assemblage variation along the urban-rural gradient in Birmingham, UK. This will allow me to investigate whether functional diversity in bees changes along this landscape gradient.

Chapter 4

Bee assemblage individual trait mean and group trait diversity variation along an urban-rural gradient in Birmingham, UK

Abstract

Bee assemblages showed variation along urban-rural gradients. Important to pollination services is the level of trait complementarity in bee assemblages. Through estimating individual trait importance and group trait diversity, it was possible to investigate the causes and potential implications of species-specific variation in bee assemblages along the urban-rural gradient. By applying trait data to bee assemblage data recorded as part of Chapter 2, the Community Trait Mean (Com.T) and Group Trait Dissimilarity (Gr.FD) were measured between urban, suburban and rural land-use types. I found that traits related to the foraging, nesting, sociality and phenology of bees showed significant variation along the urban-rural gradient. Bee assemblages recorded in urban and suburban areas were represented more by bees which were social, foraging generalists, cavity nesters, carried pollen via corbiculae, were generally larger, had longer flight periods and were active in the spring and summer months. In contrast, bee assemblages in rural areas contained bees that were cleptoparasites, ground-excavating nesters, had two broods per year, carried pollen on their legs and body with branched hairs, and were active in the spring. This has significant implications on the trait diversity of bee assemblages along the urban-rural gradient. Urban and suburban sites had greater diversity of traits related to nesting strategy and the duration of activity, whilst rural areas had increased group trait diversity in lecty, tongue type and seasonal presence. This chapter discusses the importance of individual trait variation for future conservation and research, whilst also discussing the potential implications of variation in group trait diversity for trait complementarity for pollination services.

4.1 Introduction

Bee assemblage change along the urban-rural gradient is highly species-specific (Zanette et al., 2005; Cane et al., 2006; Banaszak-Cibicka & Żmihorski, 2011; Bates et al., 2011; Wojcik & McBride, 2012; Chapter 2). Particular species appear in abundance in urban areas comparable to the wider landscape (Cane et al., 2006; Fetridge et al., 2008) whilst others display a negative response to increased densities of built space (Bates et al., 2011; Chapter 2). This variation in bee species composition along the urban-rural gradient has been partially attributed to species' life-history traits (Cane et al., 2006; Banaszak-Cibicka & Żmihorski, 2011; Bates et al., 2011), specifically towards foraging and nesting preferences (Cane et al., 2006; Fetridge et al., 2008; Matterson et al., 2008; Bates et al., 2011).

In terms of foraging traits, generalist foragers are capable of using urban and suburban habitats for foraging, whilst foraging specialists are believed to be limited by the scarcity of their host plant within urban habitats (Fetridge et al., 2008; Matterson et al., 2008; Frankie et al., 2009). Previous literature emphasizes that cavity-nesting bees show particular association with urban areas (Cane et al., 2006), possibly due to the greater potential for crumbling mortar to offer more nesting opportunities in these habitats (Bates et al., 2011). In contrast, ground-nesting bees are shown to be negatively affected by greater porportion of impervious surfaces in urbanised areas (Neame et al., 2013). Furthermore, bee assemblages appear to shift to a greater abundance of social species with increasing urbanisation (Fetridge et al., 2008; Chapter 2). Although not specifically associated with urbanisation, bee assemblage change is shown to reduce the level of dietary and phenological breadth within a community (Bartomeus et al., 2013).

There is currently insufficient evidence to to support the idea that the change in bee assemblages along the urban-rural gradient will influence the pollination services provided by them. However recently, investigations into the plant-pollinator relationships along the urban-rural gradient show a reduction in the diversity of plant-bee interactions (Deguines et al., 2012; Geslin et al., 2013). For species of bee that cannot adapt to the changes in the landscape resulting from urbanisation, the plant-pollinator inter-dependencies they represent could be lost (Kearns et al., 1998; Kaiser-Bunbury et al., 2010). Furthermore, it is well established that the loss of individual species can lead to breakdowns in plant-pollinator relationships (Kearns et al., 1998; Memmott et al., 2004), creating foraging niche space which other species within that community are slow to occupy (Brosi & Briggs, 2013). Therefore, there is an urgent need to test whether pollination services along the urban-rural gradient are affected by bee assemblage change.

There is growing evidence to suggest that not only species loss, but also a reduction in trait diversity that species loss brings can influence pollination efficiency of bees (Hoehn et al., 2008: Albrecht et al., 2012; Fründ et al., 2013). This is because the traits and the diversity of traits bee assemblages possess can complement each other towards facilitating greater levels of pollination success (a.k.a. trait complementarity) (Blüthgen & Klein, 2011). Moreover, the composition of traits within a community is highlighted as an important factor in ecosystem functioning (Diaz and Cabido, 2001) and the level of dissimilarity between individual traits increasing ecosystem service provision (Garnier et al., 2004; Petchey et al., 2004; Mouillot et al., 2011). Therefore, investigating the implications of increased urbanisation on trait diversity in bees is integral to understanding further the implications of bee assemblage change on pollination services.

Current research into bee assemblage change lacks incorporation of life-history traits in analyses of bee assemblage variation along landscape gradients. This limits the interpretation of such research outputs and their further application. Usually particular traits that appear to explain observed variation in bee assemblages are only used post-hoc (but see Banaszak-Cibicka & Żmihorski, 2011). By measuring trait dominance and group trait dissimilarity, an analysis of bee assemblage change along an urban-rural gradient would allow for both greater definition of traits that show positive and negative associations with urbanisation, and what this means for the trait complementarity of bees. Furthermore, trait analysis poses a potential answer to the incompatibility of urban bee studies due to their regional disparity. If species are measured in the traits they possess rather than individual units of abundance and richness, there may be greater comparability between cities and regions. This could lead to better resolution in how the urban-rural gradient influences bee assemblages.

Locally, applying a trait-based analysis will allow greater inferential interpretation of variation in species' distributions compared to measures of abundance and diversity alone

(Diaz & Cabido, 2001; Flynn et al., 2009). This could increase the interpretation of bee assemblage variation along an urban-rural gradient and help to inform conservation efforts of what measures could be taken to manage urban habitats to promote bee assemblage diversity.

Therefore, a functional diversity analysis would allow an assessment of the importance of life history-traits along the urban-rural gradient, as well as gaining an understanding of how trait change may influence trait diversity. Through the application of traits (trait database from Stuart Roberts, including pilosity data from Chapter 3) alongside the composition of bee species across an urban-rural gradient (dataset from Chapter 2), the ecological cause of assemblage variation, as well as the extent of trait diversity change was estimated and tested empirically between land-use types.

4.1.1 Aims and Objectives

This chapter aimed to investigate the explanatory importance of traits on species change across the urban-rural gradient and assess the implications this may have on trait diversity.

Specifically my objectives include to:

(i) record and test the possible variation in community traits of bee assemblages across the urban-rural gradient;

(ii) record and test the possible variation in trait diversity in bees by assessing the dissimilarity of group traits across the urban-rural gradient;

(iii) characterize the possible implications of trait variation and trait diversity change along the urban-rural gradient for pollination services.

4.2 Methods

The bee assemblage dataset collected as part of Chapter 2 was used to assess the level of trait variation in bees between land-use types along the urban-rural gradient. Details on the collection of these data are outlined in greater detail in 2.2 Methods. The morphological and behavioural life-history traits of UK bees incorporated into this analysis were part of a larger database from Stuart Roberts (University of Reading). In light of Chapter 3's analysis of pilosity, hair length, spacing, cover and hair type from areas of bees deemed part of incidental pollen transport (see Section 3.2 for more details) were incorporated into the trait data for this chapter. Once bee assemblage and trait data were integrated, a total of 68 bee species and their traits were used in the analysis and this included species of parasitic bee (cleptoparasites and social brood parasites).

4.2.1 Functional Diversity Analysis

Based on previous literature concerning bee species variation along urban-rural gradients (Cane et al., 2006; Fetridge et al., 2008; Matterson et al., 2008; Frankie et al., 2009; Banaszak-Cibicka & Żmihorski, 2011; Bates et al., 2011), ecological traits were selected to be incorporated into this analysis based on four main categories. These categories were foraging traits, nesting traits, sociality and phenology (Table 4.1), and were both binary and continuous variables. Before analysis, the continuous traits were re-scaled between 0 and 1 prior to standardise trait range (Botta-Dukát, 2005).

There is a variety of measurements for testing functional diversity (Petchey & Gaston, 2006). As this chapter had both trait and species abundance data, it was possible to gain an estimate of both trait weight and group trait diversity at sites along the urban-rural gradient (Mouchet et al., 2007). These were used to measure trait variation and group trait diversity along the urban-rural gradient, respectively. The variation in these measures of community trait composition are shown to influence ecosystem services (Garnier et al., 2004; Petchey et al., 2004; Mouillot et al., 2011), and are outlined as a methodological framework for testing ecosystem services change in field experiments (Mouchet et al., 2007; Lavorel et al., 2011). These indices were calculated following Lepš et al., (2006) with the online macro http://botanika.bf.jcu.cz/suspa/FunctDiv.php.

Table 4.1: List and description of bee morphological and behavioural traits used to estimate the Community Trait Mean (Com.T), and Group Trait Dissimilarity (Gr.FD) index which were used to assess the functional diversity in bee assemblages along an urban-rural gradient.

Group Trait Dissimilarity (Gr.FD)		Community Trait Mean (Com.T)	Trait Type¶	Trait Description
Foraging Traits	**Lecty**[1]	**Oligolectic**	Binary	Foragers on one family of plants
		Polylectic	Binary	Forages on multiple families of plants
	Tongue Type	**Tongue Short**	Binary	Shorter tongue (genera *Andrena* and *Colletes)*
		Tongue Long	Binary	Long proboscis (usually from Apidae family)
	Pilosity (from incidental pollen transport areas)[2]	**Hair Length**	Cont.	The species' average hair length
		Hair Spacing	Cont.	The species' average hair spacing
		Hair Cover	Cont.	The species' average hair cover
		Hair Type	Cont.	Species' average of extent of branched hairs
	Pollen Transport	**Crop**	Binary	Bees that transport pollen in their crops
		No adaptations	Binary	Bees with no pollen adaptations
		Legs and body	Binary	Bees that transport pollen on their legs and bodies
		Legs	Binary	Bees that transport pollen only on their legs
		Abdomen	Binary	Bees that transport pollen on the gastral sternites
		Corbicula	Binary	Bees that transport pollen on modified hind tibiae
	ITD[3]	**ITD**	Cont.	Intertegular distance as a measure of body size
Nesting Traits	**Nesting Strategy**[1]	**Ground Excavators**	Binary	Bees that excavates nests in the ground
		Existing Cavities	Binary	Bees that nest in existing cavities
		Managed	Binary	Bees which are managed (European Honey Bee)
		Carder	Binary	Bees manipulate grass/moss to form a nest
Sociality	**Sociality**	**Solitary**	Binary	Reproduce individually
		Social	Binary	Form a colony or hive
		Variable Sociality	Binary	Alter their sociality on environmental conditions
		Cleptoparasite[1]	Binary	Bees which lay eggs in the nests of solitary bees
		Social-brood Parasite[1]	Binary	Parasitize bumblebee colonies (Psithyrus)
Phenology	**Duration of Activity**	**Months Active**	Cont.	The no. of months a bee is active over its season
	Seasonal Presence	**Spring**	Binary	Spring-active bees (Feb - June)
		Summer	Binary	Summer-active bees (July - Oct)
		Both Seasons	Binary	Active for spring and summer
	Voltinism	**Univoltine**	Binary	One brood of offspring per year
		Bivoltine	Binary	Two broods of offspring per year
		Multivoltine	Binary	Multiple broods of offspring per year

¶ The type of variable each trait applied to indices as, continuous variables were scaled between 0-1 prior to application to diversity indices. [1]Cleptoparasites and Social Brood Parasites although considered as components in measuring group traits (Gr.FD) within Lecty and Nesting Strategy, they are only presented in the results as individual trait (Com.T) within Sociality. [2] see 'Chapter 3' for detailed experimental protocol of pilosity analysis. [3] The distance between the two wing bases of a bee, a factor collinear with body size (Cane, 1987; Bullock, 1999).This was used as a foraging trait due to the positive association between body size and forage distance (Greenleaf et al., 2007).

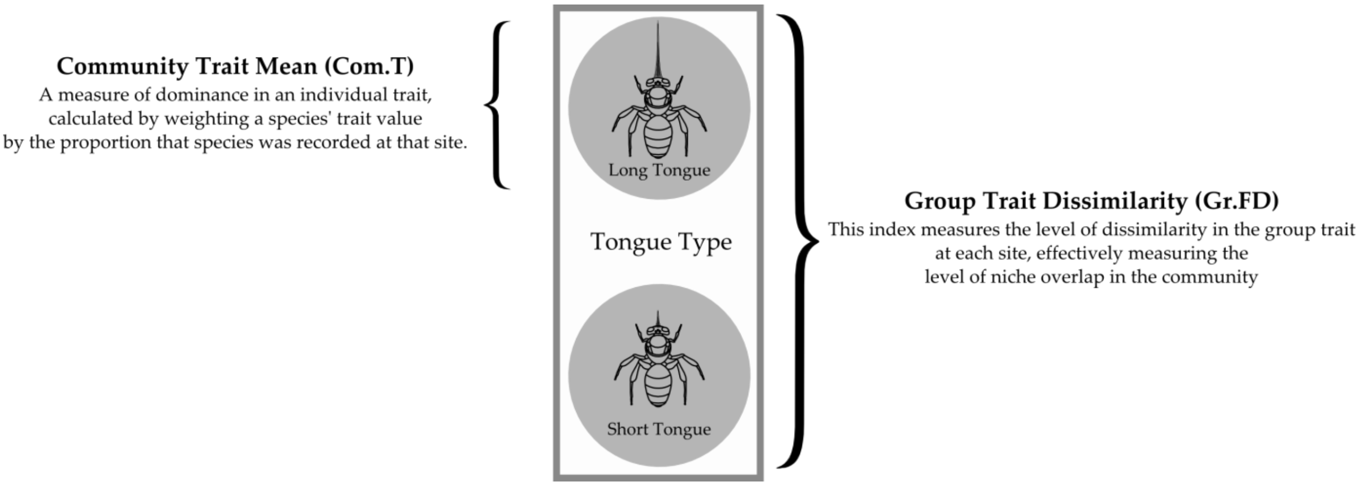


Figure 4.1: Diagram explaining the way in which Community Trait Mean (Com.T) and Group Trait Dissimilarity (Gr.FD) measurements are calculated and what they mean, using trait group Tongue Type as an example. See 4.2 Methods for more details.

4.2.1.1 *Community Trait Mean (Com.T)*

To assess the variation of individual traits along the urban-rural gradient, Community Trait Means (Com.T) were calculated for each trait at each site. Individual traits applied to Com.T are specified in Table 4.1. Com.T measures the level of dominance a trait has at a particular site, by weighting species trait value *i* by the proportion (p_i) of that species at a site;

(Equation 4.1)

$$\boldsymbol{Com.T = \sum_{i=1}^{s} p_i t_i}$$

where p_i is the proportion of individuals in the ith species, *s* is the total number of species, and *t* is the trait value (0 to 1) of the ith species (Garnier et al., 2004; Moretti et al., 2009; Roscher et al., 2013). This is effectively the averaged trait value within each site, based on the proportion of the community present which have that particular trait (for binary traits) or with a higher value of that trait (for continuous traits). The higher the Com.T value at each site, the more dominant that trait is within the community recorded to be present. With this estimate, it is also possible to look at the relative importance of each trait between land-use types, as well as to compare the importance of that trait within each land-use type relative to other individual traits.

4.2.1.2 *Group Trait Dissimilarity (Gr.FD)*

Understanding how species within a community occupy the available niche space is an important measure for ecosystem functioning (Mason et al., 2005; Diaz et al., 2007). To gain a measure of group trait diversity (the dissimilarity of species with different types of traits occupying the same site) across the urban-rural gradient, Rao's quadratic entropy (Rao, 1982; Botta-Dukát, 2005) was used to generate a Group Trait Dissimilarity index (Gr.FD). Group traits are specified in Table 4.1. Rao's quadratic entropy is a measure of both functional richness and functional divergence of species within a particular site (Mouchet et al., 2010). A generalisation of the Simpson Diversity Index, the Rao's quadratic entropy takes the proportion of individuals in a community and measures the weight of the traits by the dissimilarity of species, effectively calculating the trait complementarity observed in species for that community:

(Equation 4.2)

$$\boldsymbol{Gr.FD} = \sum_{i=1}^{s} \sum_{j=1}^{s} \boldsymbol{d_{ij}\, p_i\, p_j}$$

where d_{ij} is calculated as the difference in trait value between each pair of species $\boldsymbol{p}_i$ and $\boldsymbol{p}_j$. The distribution of d_{ij} ranges from 0 to 1: the higher the value the greater the dissimilarity in traits of species. For instance, if a pair of species both had the same trait value (i.e. both polylectic), then d_{ij} would be 0, but if they were different (i.e. polylectic and oligolectic) then d_{ij} would be higher than 0. Hence, a higher Gr.FD value suggests higher levels of trait diversity in that trait group at that particular site.

4.2.2 Data Analysis

4.2.2.1 ANOVA

All Com.T and Gr.FD values were selected to ANOVA to assess differences in individual traits and trait complementarity between urban, suburban and rural sites. Land-use type was defined following the 1 km^2 landscape classification of the West Midlands by Owen et al. (2006). Each land-use type contained 11 sites. Further details of land-use type allocation are provided in Section 2.1 of Methods. . ANOVA or Kruskal-Wallis tests were used to test for variation depending on the normality of the response variables tested. Post-hoc tests (i.e. Tukey-HSD and pair-wise Wilcoxon tests) were used to test differences between individual land-use types within each ANOVA. The variation between individual trait Com.T values within land-use types were not statistically tested as the main aim was to assess the importance of individual traits along the urban-rural gradient. However, the Com.T of individual traits was graphically represented in relation to the other individual traits within their allocated group trait when both Com.T and Gr.FD are compared between land-use types. This does not allow an ANOVA, but gives an indication of which individual traits are prevailing within those land-use types.

4.3 Results

4.3.1. Variation of Com.T and Gr.FD across the urban-rural gradient

There was evidence of variation in community trait mean (Com.T) and group trait dissimilarity (Gr.FD) between land-use types for a range of individual and group traits (Table 4.2). Details of this variation are presented in this section based on their trait categories.

4.3.1.1 Foraging traits

Across the study areas polylectic (generalist) foragers were greater in proportion compared to oligolectic (specialist) foragers (Figure 4.2). Between land-use types, polylecty showed greater preference for suburban compared to rural sites, whilst oligolecty was found more in urban sites compared to suburban sites (Table 4.2; Figure 4.2). In terms of Gr.FD, a significantly greater level of diversity in bee lecty was recorded in rural sites (Table 4.2; Figure 4.2).

Table 4.2: Results of ANOVA and post-hoc tests of Group Trait Dissimilarity (Gr.FD) and Community Trait Means (Com.T) in bee functional traits between urban, suburban and rural land-use types

	Group Trait Dissimilarity (Gr.FD)	Community Trait Means (Com.T)	**Test Statistic**	**Test Sig.**	**Urban - Suburban**	**Urban - Rural**	**Suburban - Rural**
Foraging Traits	**Lecty^{1}**		**F=6.92**	**0.03***			**Rural**
		Oligolectic	H=10.3	0.005**	Urban*		
		Polylectic	H=7.02	0.02*			Suburban
	Tongue Type		**H=6.31**	**0.04***		**Rural***	
		Short	F=2.84	ns			
		Long^{3}	-	-			
	Pilosity		**H=1.70**	**ns**			
	(from incidental pollen transport areas)^{2}	Hair length	F=3.2	ns			
		Hair spacing	F=1.55	ns			
		Hair cover	F=0.18	ns			
		Hair type	F=0.2	ns			
	Pollen Transport		**H=4.06**	**ns**			
		Crop	H=4.3	ns			
		No adaptations	H=11.1	0.003**		Rural*	Rural*
		Legs and body	H=3.78	0.03*		Rural*	
		Legs	F=3.82	ns			
		Abdomen	H=3.77	ns			
		Corbicula	F=7.32	0.002**		Urban*	Suburban*
	Inter-Tegula Distance		**H=2.60**	**ns**			
		ITD	F=4.18	0.02*		Urban*	
Nesting Traits	**Nesting Strategy^{1}**		**H=7.72**	**0.02***	**Suburban***		
		Ground excavators	F=5.78	0.007**		Rural**	
		Existing cavities	F=13.7	<0.001***		Urban***	Suburban*
		Managed	H=2.74	ns			
		Carder	F=0.41	ns			
Sociality	**Sociality**		**H=3.31**	**ns**			
		Solitary	F=2.22	ns			
		Social	F=6.05	0.006**		Urban*	Suburban*
		Variable sociality	H=0.56	ns			
		Cleptoparasite^{1}	H=9.54	0.008**		Rural*	
		Social brood parasite^{2}	H=3.29	ns			

Phenology	**Duration of Activity**		**F=4.71**	**0.01***		**Urban***	
		Months active	F=6.87	0.003**		Urban*	Suburban**
	Seasonal Presence		**H=11.5**	**0.003****		**Rural***	**Rural****
		Spring	F=16.8	<0.001***		Rural***	Rural**
		Summer	H=7.86	0.01*		Urban*	
		Both seasons	F=14.1	<0.001***		Urban***	Suburban***
	Voltinism		**H=1.17**	**ns**			
		Univoltine	H=1.21	ns			
		Bivoltine	H=7.05	0.02*		Rural*	
		Multivoltine	H=2.75	ns			

[1] For the estimation of Gr.FD, Cleptoparasites and Social Brood Parasites were incorporated within trait groups Lecty and Nesting Strategy, but only presented as individual traits within Sociality. [2] See 'Chapter 3' for a detailed experimental protocol on pilosity data collection. Variance between land-use types was tested using Kruskal-Wallis (H) for non-normally distributed response variables and ANOVA test (F) for normal distributed response variables. Post-hoc tests examined the variance between each pair of land-use types using Wilcoxon tests if a Kruskal-Wallis test was used or a Tukey-HSD test if ANOVA was used. All tests had 2 degrees of freedom and each land-use type consisted of 11 sites. The land-use type with the greater value is represented for each response. Significant p-values of <0.05 shown as * and <0.01 as **. <0.001 as ***. Where a means test was significant but the following post-hoc test was not, the land-use type which showed the greater value was only shown.

Bee tongue types were in greater diversity in rural sites (Table 4.2; Figure 4.2). Individual traits of tongue type, although showing no significant variation between land-use types in their Com.T, suggested long tongued species of bee were more abundant in urban sites than short tongue bees (Figure 4.2).

There was no difference in the group diversity of pollen transport adaptations presented between land-use types, but there was significant variation in several individual forms of pollen transport (Figure 4.2). Bees which carry pollen in corbiculae (an adaptation of the hind tibiae in which *Bombus* spp. and *Apis mellifera* affix pollen 'clumps' mixed with nectar) were significantly more abundant in urban areas. Bees with no adaptations to carry pollen and bees which carry pollen on the hairs of their legs and bodies (*Andrena* spp., *Halictus* spp. and *Lasioglossum* spp.) were more abundant in rural sites. There was no significant variation in the levels of Gr.FD pilosity or individual hair characteristics between land-use types (Table 4.2; Figure 4.2). Likewise, the diversity of bee body size did not change along the urban-rural gradient. Larger bees however, showed preferences for urban areas (Table 4.2; Figure 4.2).

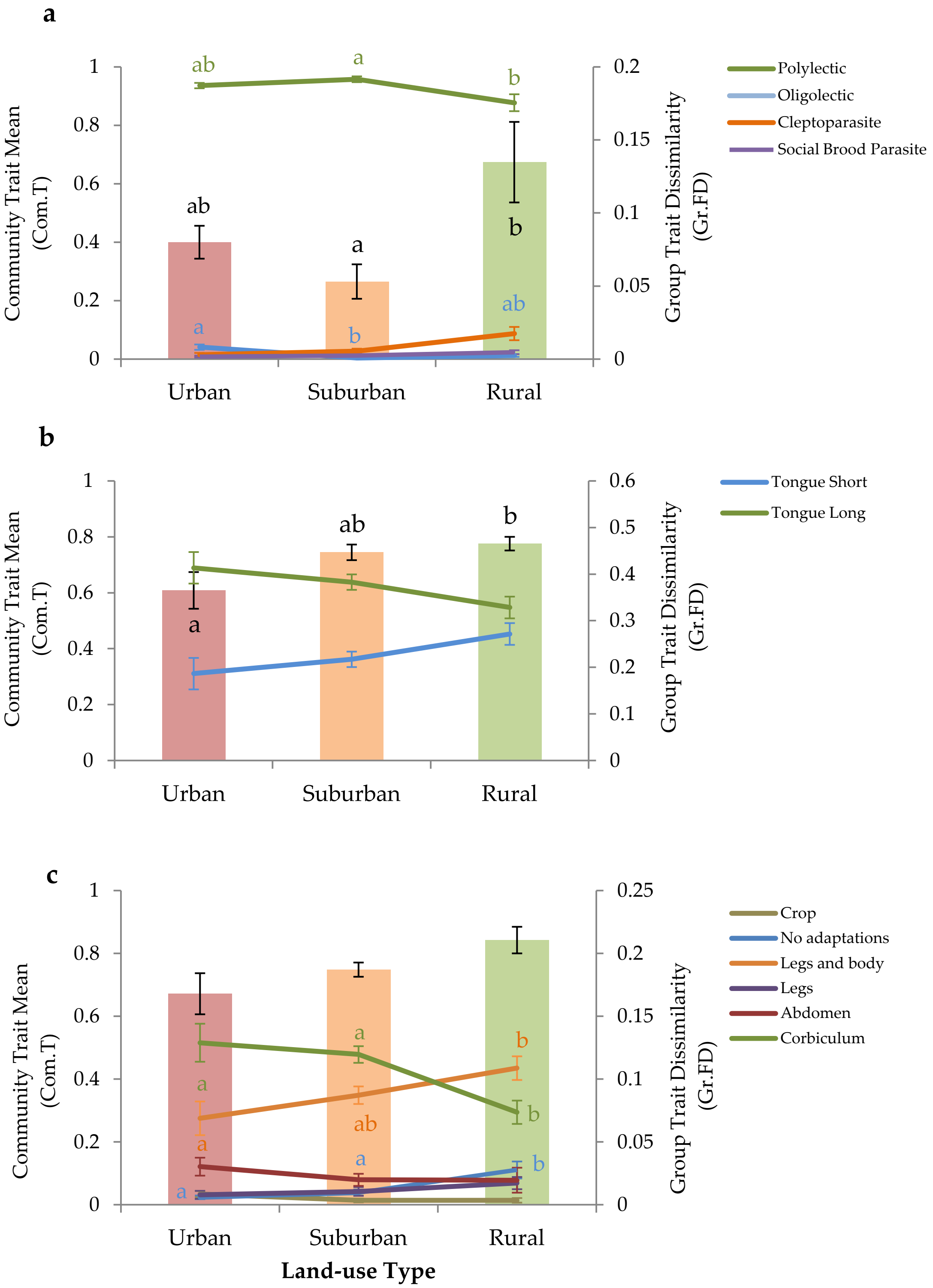


Figure 4.2: The variation found in the individual Community Trait Means (Com.T, Lines) and the Group Trait Dissimilarity (Gr.FD, Bars) of (a) lecty, (b) tongue type and (c) pollen transport of bees found between urban (red), suburban (yellow) and rural (green) sites. Com.T shows the relative dominance of individual traits, whilst Gr.FD is an index of how diverse the individual traits represented by bees in those land-use types are (see 4.2 Methods).. Error bars that do not share a letter represent significant differences (p<0.05) and no labels indicate that trait or group trait showed no significant difference. Black letters represent the Gr.FD and other colours correspond to their respective individual Com.T. Error bars = +/- 1 SE. N=11 sites per land-use type.

4.3.1.2 Nesting traits

The nesting strategies of bees were significantly more diverse within suburban areas than urban areas (Table 4.2; Figure 4.3). Bees that excavate their nests in the ground were found to be more numerous in rural sites compared to urban sites (Table 4.2; Figure 4.3), whilst cavity-nesting bees were significantly more abundant in urban and suburban compared to rural sites (Table 4.2; Figure 4.3). Managed bees and carder bees showed no variation along the urban-rural gradient, and were fewer proportionately throughout the study area compared to ground- and cavity-nesting bees (Figure 4.3).

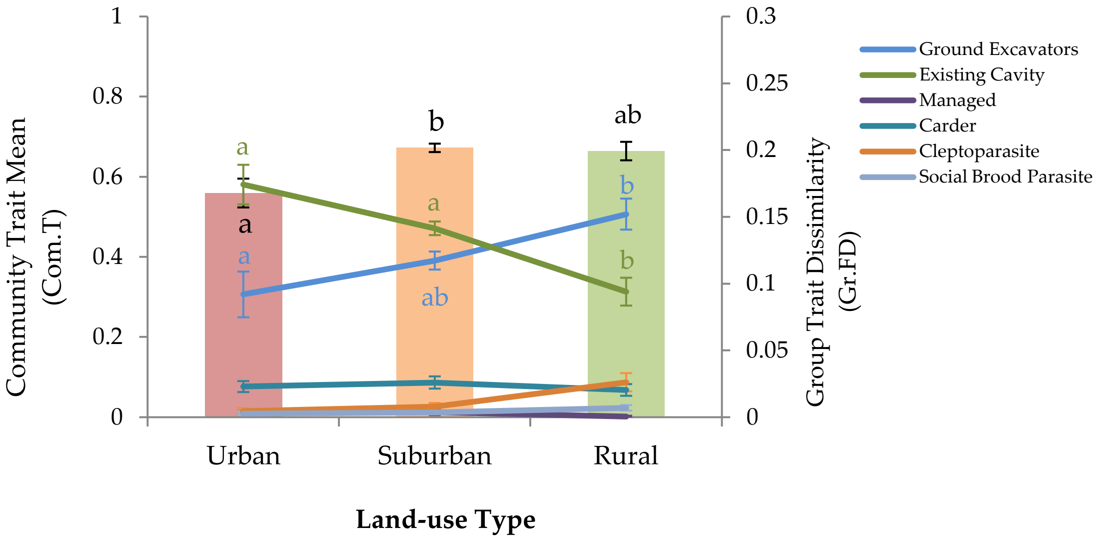


Figure 4.3: The variation found in the individual Community Trait Means (Com.T, Lines) and the Group Trait Dissimilarity (Gr.FD, Bars) of nesting traits in the bees found between urban (red), suburban (yellow) and rural (green) sites. Com.T shows the relative dominance of individual traits, whilst Gr.FD is an index of how diverse the individual traits represented by bees in those land-use types are (see 4.2 Methods).. Error bars that do not share a letter represent significant differences (p<0.05) and no labels indicate that trait or group trait showed no significant difference. Black letters represent the Gr.FD and other colours correspond to their respective individual Com.T. Error bars = +/- 1 SE. N=11 sites per land-use type.

4.3.1.3 Sociality

There was no significant variation in the diversity of sociality found between land-use types. Social bees increased in abundance towards urban and suburban areas compared to rural (Table 4.2; Figure 4.4). Also, cleptoparasitic species were significantly greater in abundance in rural sites compared to urban sites (Table 4.2; Figure 4.4). No significant variation was found in solitary, variable social or social brood parasitic sociality between land-use types.

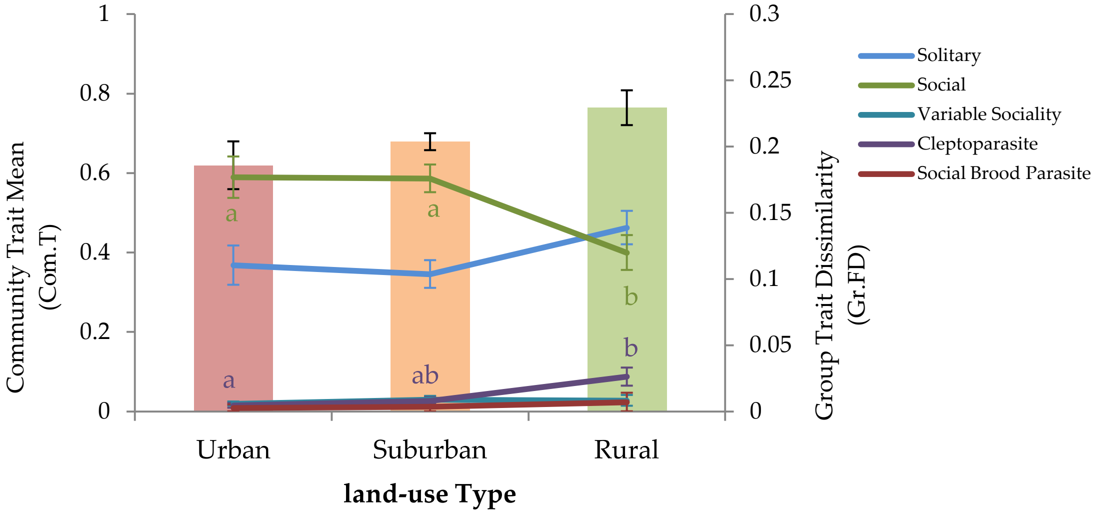


Figure 4.4: The variation found in the individual Community Trait Means (Com.T, Lines) and the Group Trait Dissimilarity (Gr.FD, Bars) of sociality in the bees found between urban (red), suburban (yellow) and rural (green) sites. Com.T shows the relative dominance of individual traits, whilst Gr.FD is an index of how diverse the individual traits represented by bees in those land-use types are (see 4.2 Methods).. Error bars that do not share a letter represent significant differences (p<0.05) and no labels indicate that trait or group trait showed no significant difference. Black letters represent the Gr.FD and other colours correspond to their respective individual Com.T. Error bars = +/- 1 SE. N=11 sites

4.3.1.4 Phenology

In terms of phenology, the difference in the length of time for which bee species are active was significantly greater in urban sites, whilst bee assemblages within the urban and suburban sites were significantly active for longer (Table 4.2; Figure 4.5). Bees active in both seasons and summer active bees showed a significant increase in urban and suburban sites land-use (Table 4.2; Figure 4.5). Bees active in the spring were found to be significantly more abundant in rural sites (Table 4.2; Figure 4.5). Even though bees active in summer and both seasons declined in number in rural areas, the greater Com.T of spring species in rural sites led seasonal activity Gr.FD to be significantly greater than in suburban and urban sites (Figure 4.5). Gr.FD voltinism showed no change in dissimilarity between land-use types, whereas Com.T of bivoltine (lay two broods per year) bees showed a significant increase in abundance in rural sites (Figure 4.5).

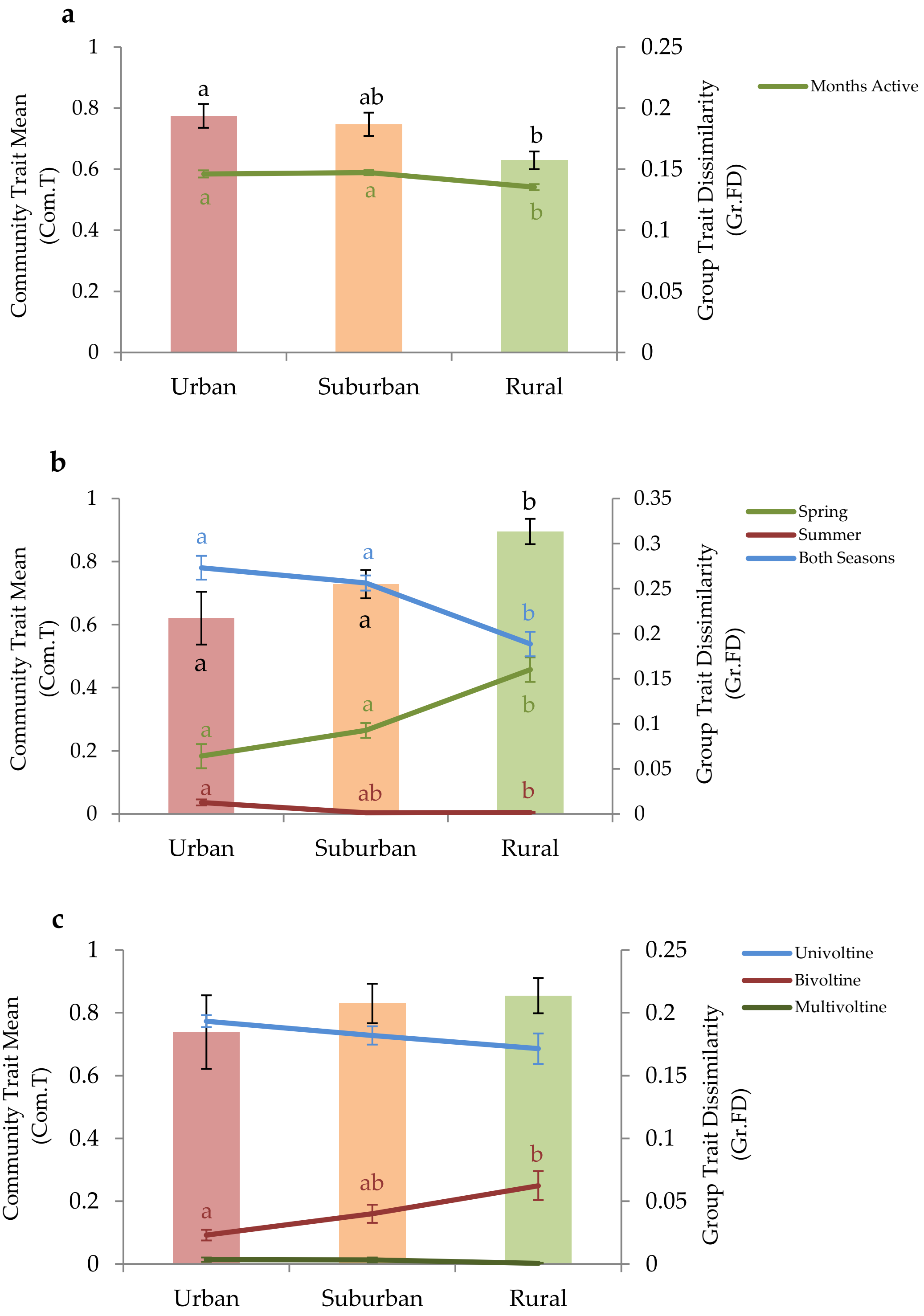


Figure 4.5: The variation found in the individual Community Trait Means (Com.T, Lines) and the Group Trait Dissimilarity (Gr.FD, Bars) of (a) duration of activity, (b) seasonal activity and (c) voltinism of bees found between urban (red), suburban (yellow) and rural (green) sites. Com.T shows the relative dominance of individual traits, whilst Gr.FD is an index of how diverse the individual traits represented by bees in those land-use types are (see 4.2 Methods). Error bars that do not share a letter represent significant differences ($p<0.05$) and no labels indicate that trait or group trait showed no significant difference. Black letters represent the Gr.FD and other colours correspond to their respective individual Com.T. Error bars = +/- 1 SE. N=11 sites per land-use type.

4.4 Discussion

The application of a trait-based analysis to assess the diversity of traits in bees is important in understanding the potential influence species and diversity loss could pose for pollination services of bees to plants (Blüthgen & Klein, 2011). This chapter applied a quantitative analysis of traits to bee assemblage data recorded along an urban-rural gradient in Birmingham, UK. I estimated community trait means (Com.T) and group trait dissimilarity (Gr.FD) for each site to assess the variation of bee traits along the urban-rural gradient and the subsequent result on group trait diversity. The results from this analysis demonstrate significant variation in Com.T and Gr.FD across the urban-rural gradient in terms of the foraging traits, nesting traits, sociality and phenology of bee species.

This chapter verifies that the inter-specific selection of bee assemblages along the urban-rural gradient observed previously (e.g. Cane et al., 2006; Fetridge et al., 2008; Matterson et al., 2008; Bates et al., 2011; Chapter 2) is defined by their respective life-history traits. Furthermore, I found that this trait-specific selection of bees along the urban-rural gradient subsequently influenced the diversity of traits, potentially impacting upon the trait complementarity of bee and the pollination services (Hoehn et al., 2008: Albrecht et al., 2012; Fründ et al., 2013). This variation in Com.T and Gr.FD not only allows for a greater interpretation of how bees vary between land-use types, but also highlights the potential influence of bee assemblage and subsequent trait variation on pollination services.

4.4.1 Bees, Traits and the Urban-Rural Gradient

Four key aspects of bee ecology relating to foraging traits, nesting traits, sociality and phenology of the bees recorded over the urban-rural gradient showed variation in Com.T and Gr.FD.

4.4.1.1 Foraging traits

I report greater dominance of generalist foragers in suburban areas, a trend most likely caused by the increased abundance of bumblebees at these sites (Chapter 2). Bumblebees have been recorded in great abundance in parkland and gardens in previous studies of urban and suburban habitats (McFrederick & LeBuhn, 2006; Fetridge et al., 2008; Matteson et al., 2008). However, the difference in Com.T of generalist foragers between suburban and rural is marginal (Figure 4.2), suggesting generalists were persistent throughout the entire survey area. However, the lack of specialist foragers, cleptoparsites and social brood parasites in suburban areas meant that the diversity of lecty was significantly lower in suburban areas as opposed to rural. Although suburbia has been noted as having beneficial foraging rewards for bees (Ahrné et al., 2010; Goddard et al., 2010; Samnegård et al., 2011), this chapter shows how this may only be evident for generalist foragers.

Interestingly, specialist foragers showed a slight, yet still significant, increase towards urban areas. This goes against previous trends which usually show specialist species to be under-represented or not found at all in urban sites (Cane et al., 2006; Fetridge et al., 2008; Matteson et al., 2008). Reduced floral diversity is known to decrease the level of specialisation and diversity of morphological adaptations in bees at the community level (Fenster et al., 2004; Ebeling et al., 2011) as their foraging niches are restricted (Winfree et al., 2011). Therefore, although urban habitats are associated with greater levels of disturbance causing instability in floral diversity and foraging resources (Sattler et al., 2010), this chapter illustrates that the urban areas sampled may have provided suitable resources for specialist foragers. The foraging specialists recorded within this chapter included *Osmia leaiana* and *Colletes daviesanus* which forage on species of Asteraceae, and *A. clarkella* that forage on species of the genus *Salix*. Both of these plant taxa were observed to flower in urban survey sites through the sampling season. Additionally, as mentioned in Chapter 2, floral diversity was positively co-linear with built space, suggesting that canal sides and parkland surveyed could support the foraging requirements of specialists to a greater extent than other land-use types.

It was also found that bee assemblages had greater tongue type diversity in rural areas. Preference for nectar foraging by bees on host flowers is subject (apart from nectar robbers) to the different morphological adaptations in their mouthparts. Hence, the diversity of foraging specialisation in bees appears to be lower in urban areas. This corresponds to previous research that reports a reduced level of bee-plant visitation complexity in urban areas (Deguines et al., 2012; Geslin et al., 2013). However, this chapter only divides bees into long and short tongued, even though there are various subgroups within these two classes. These two general tongue types are defined in previous research (Rodríguez-Gironés & Santamaría, 2006) and allow an assessment of morphological trait diversity associated with nectar foraging function (Fenster et al., 2004). However, this is a general classification and the reduction of its diversity in urban areas may not pose a threat to urban pollination as plant-pollinator relationships are likely to be more complex than classifying them into 'syndromes' (Ollerton et al., 2009).

The variation in morphological adaptations in bees for the collection and transportation of pollen could be important for the carryover of pollen by bees between plants (Thorp, 1979; Rademaker et al., 1997; Thorp, 2000; Adler & Irwin, 2006, Chapter 3). The group trait dissimilarity of pollen transport did not change across the urban-rural gradient, but several individual forms of pollen transported did, including 'no adaptations', 'corbicula' and 'legs and body'. Arguably, the most specialised anatomical modification for pollen transport is the corbiculae of *Bombus* spp. and *Apis mellifera* (Thorp, 2000). With flattened areas on their hind tibiae, species with corbiculae accumulate pollen mixed with nectar on these areas allowing easy pollen transport back to their colony or hive. This form of pollen transport effectively removes pollen from potential carryover to other plants (Thorp, 2000). Hence, the change in pollen transport adaptations within a community could impact upon pollen carryover. Bees which carry pollen on their legs, such as *Anthophora plumipes* and *A. furcata*, and bees that carry no pollen showed the opposite trend and were higher in rural areas. This could suggest that in the individual forms of pollen transport, there is deviation along the urban-rural gradient, which could also impact upon pollen carryover to particular plants.

In terms of pilosity, there was no observed variation in the hair traits or diversity of pilosity measurements across the urban-rural gradient. This suggests that although particular traits that could influence pollen carryover may vary across land-use types, there is still a level of trait diversity in the morphological adaptations of pollen collection and incidental transport along the urban-rural gradient.

4.4.1.2 Nesting traits

Nesting site limitation is shown as an important determinant of bee assemblage composition (Steffan-Dewenter & Sciele, 2008; Williams et al., 2010; Winfree et al., 2011). This chapter supports previous research which showed urban areas can support-cavity-nesting bees (e.g. Cane et al., 2006; Matteson et al., 2008; Bates et al., 2011). This chapter is limited by its lack of definition between types of cavity nesters, as bees can nest in dead wood, the ends of woody stems and masonry. However, it is still possible to highlight that cavity-nesting bees show an association with urbanisation not evident in other nesting types. This association between cavity nesters and increased urbanisation has been attributed to particular nesting opportunities present in urban and suburban habitats, including the greater level of masonry in built-up areas (Bates et al., 2011) and the diverse nesting potential of gardens (Neame et al., 2013). However, cavity nesters are likely affected by immediate climatic conditions within urban and suburban nesting sites (Everaars et al., 2011), so further understanding of the dynamics of cavity nesters within the urban habitat could allow for refined conservation efforts in ensuring the continuation of cavity nesters in urban and suburban areas.

Conversely, ground-nesting bees were more abundant in rural sites. Previous studies have shown high numbers of ground-nesting bees along urban-rural gradients, but only in well maintained florally diverse gardens (Fetridge et al., 2008) or in grassland with grazing regimes that increase flower abundance (Kearns & Oliveras, 2009). In my sampled area, floral diversity decreased towards rural sites, suggesting that floral diversity (although still an important factor) was not defining the observed trend in ground excavators. However, it has been suggested that ground excavation becomes more difficult for bees in urban areas due to increased impervious surfaces (Neame et al., 2013). This suggests that a lack of nesting opportunities is constraining the ability for ground excavators to persist in more urbanised areas.

Carder bees (those that use moss and dry grass to form a nest) showed no variation along the urban-rural gradient. The main carder species recorded was *Bombus pascuorum*, which showed no relationship to landscape or local site variables in mixed-models in Figure 2.2. The nesting requirements of *B. pascuorum* are mostly associated with the level of grassland in the immediate areas (Goulson et al., 2010). As with many bumblebee species however, queens are able to use various aspects of the local environment as nesting sites, including under hedges, holes in trees or even bird boxes. This highlights another limitation in my analysis, with some nesting behaviour of bees being difficult to define with a single trait. For further analysis, different grouping systems for nesting traits in bees may allow greater interpretation of bee assemblage change.

Another limitation in assessing the nesting behaviour of bees on certain land-use types is that there is no assurance that the bees found are using that site or even the same land-use type for nesting resources. Bees are central place foragers and foraging range from nesting sites is associated with the size of particular bee species (Greenleaf et al., 2007). As ITD as a measure of body size showed an increase towards urban areas, bees observed in urban areas may be those able to best forage further and are nesting at other sites. This should be taken into consideration in further research investigating where and how bees nest along the urban-rural gradient.

I found that trait diversity in nesting specialisation significantly decreased in urban areas compared to suburban areas and is therefore a vital element of the resources required to mitigate bee assemblage variation along the urban-rural gradient. Through a range of practices and products which promote the nesting resources of both cavity and ground-nesting bees, nesting resources can be increased throughout the urban-rural gradient. In terms of conservation and planning policy, providing nesting resources for a variety of species is an effective way of limiting species change along the urban-rural gradient. The loss of species due to nest limitation could very well lead to break downs in plant-pollinator relationships (Kaiser-Bunbury et al., 2010) as certain bee species are lost from certain land-use types. Hence, further investigation is required to understand the dynamics of nesting potential within the urban environment, and how the species loss it defines may influence pollination services along the urban-rural gradient.

4.4.1.3 Sociality

My analysis found social bees in greater abundance in urban and suburban sites, and has been found by several other studies (e.g. Fetridge et al., 2008; Matteson et al., 2008; Goulson et al., 2010). However, even with greater social species recorded in urban and suburban areas, there was no significant difference in diversity of bee sociality across land-use types.

Cleptoparasites were found in rural sites to a greater extent. This was expected as the original analysis of the data highlighted a negative association between cleptoparasitic species and built space (Chapter 2). The assumption that this is due to their host species being absent in urban areas is not entirely supported through this trait-based analysis, as solitary bees presented no variation between land-use types. However, it is known that the presence and level of parasitic bees are indicative of the health of their host community (Sheffield et al., 2013), and show negative association with fragmentation through host loss at the landscape scale (Schüepp et al., 2011). Due to the species-specific nature of host-parasite relationships however, similar trends between cleptoparasites and solitary bees are unlikely to be found between land-use in this kind of analysis. This limits interpretation of findings and is an instance where a different approach may be preferred. For example, to assess the influence of land-use type on the parasite-host relationships of solitary bees and their cleptoparasites, specific host-parasite relationships would need investigating.

4.4.1.3 Phenology

Along an urban-rural gradient, we found bees active in summer or in both seasons, and active for longer, were greater in number in urban and suburban areas. This has been found previously, with greater numbers of summer-active species found in urban areas in Poland (Banaszak-Cibicka & Żmihorski, 2011). In addition, the diversity of activity levels in bees was significantly increased in urban sites, suggesting bees are able to occupy wider phenological niches in urban areas. However, the diversity of bee seasonal activity, the trait mean of spring species and the trait mean of bivoltine species (bees which lay two broods per year) all increased in rural areas, suggesting that bees with particular phenology are negatively associated with urbanisation. When bees are active and how long they are active could be important for the pollination of flowers with particularly restrictive phenological niches. One extreme case of this is with winter active bumblebees (mainly *Bombus terrestris*), which visit winter-flowering shrubs such as the non-native *Mahonia* spp. (Stelzer et al., 2010). Maintaining a level of phenological diversity along the urban-rural gradient is therefore important in maintaining a level of trait complementarity towards pollination services.

4.4.2 Trait variation and urbanisation

Trait analysis has previously helped studies to achieve a greater understanding of bee assemblage responses to natural and anthropogenic disturbances (Moretti et al., 2009; Williams et al., 2010). Compared to analysis of only species richness and abundance metrics, incorporating traits is highly beneficial in interpreting bee assemblage variation along an urban-rural gradient in greater detail. Further to this, a trait based analysis gives a testable method of assessing which traits show change along this particular urban-rural gradient, which could offer a framework for future studies to follow. Through an analysis of multiple cities using this method, it may be possible to achieve greater comparability across geographic variation in current studies of urbanisation and bees.

Potentially limiting the results of this study is the lack of historical records as to the level of traits and the trait diversity of bees in Birmingham. Historical changes in species abundance and range can reduce the traits in bee communities (Bartomeus et al., 2013), and would support my findings if there was an observed trend in the change of bee species as land-use change occurred.

This chapter suggests the best way to alleviate future trait complementarity loss in the urban environment is through the management of habitats for a range of bee species at the site scale. Using the results of the trait analysis in this chapter, ways of mitigating trait loss along the urban-rural gradient may be undertaken. As bottom-up influences are most likely to affect bee populations, the provisioning of nesting site resources is highlighted as the main form conservation should take. However, providing floral resources suitable for a range of species will help to provide for bees along the urban-rural gradient.

Additionally, applying a trait analysis to bee composition data across urban-rural gradient is fundamental in future investigations of how urbanisation will influence pollination services of bees (Blüthgen & Klein, 2011; Brittain et al., 2013). Although evidence points towards the diversity of plant-pollination visitations being reduced in city centres (Geslin et al., 2013), there was still an abundance of generalist foragers and surprisingly an increase in specialist foragers in urban areas. Although arguably lower in species richness (Chapter 2), urban areas of Birmingham, UK do not show trait diversity loss across all traits, suggesting that pollination services may not be deleteriously affected by land-use change in this study region. This does not alleviate the need for conservation however, as the possible change in pollination services still needs to be supported with floral observation and seed-set experiments. The level to which different traits are represented within a particular habitat and the extent to which trait complementarity can remain in light of species loss (a.k.a. functional redundancy) could bolster ecosystem resilience and alleviate the risk of ecosystem service failure (Naeem, 1998; Hooper et al., 2005). By testing seed-set of bee pollinated plants along this urban-rural gradient, whether or not bee assemblage variation and the resulting change in some prevalence of functional traits affects pollination services could be tested.

Chapter 5

Does bee assemblage variation between urban and rural sites in Birmingham, UK influence the subsequent pollination service for *Campanula glomerata*?

Abstract

*Land-use change has been documented to cause change in bee assemblages and their trait diversity. The implications this could have on the pollination services of bees throughout the urban-rural gradient is of high importance in light of growing demand for ecosystem services both in agricultural and urbanised environments. This chapter tested the pollination service of bees between urban and rural areas of Birmingham, UK by measuring the seed-set of Clustered Bell Flower (*Campanula glomerata*) alongside bee observation sampling. This chapter found that although low in frequency, bee visitation abundance and species richness also showed no variation between urban and rural areas. I also found no significant difference in the seed-set of* C. glomerata *between urban and rural sites. These findings complement previous Chapters 2 and 4, showing that although species loss and some aspects of trait diversity are evident in this study area, there was no evidence of difference in pollination effectiveness. The extent to which test plants were subject to pollination limitation was unclear between land-use types in this study, and is recommended to be tested further. Although only one plant species, this suggests that species-specific responses of bees between urban and rural areas could sufficiently compensate for species loss in either land-use type. Further investigation with multiple plant species in different types of site (e.g. gardens) in the same area is required to assess the full implications of the urban-rural gradient on pollination services.*

5.1 Introduction

Urbanisation is an important form of landscape alteration that influences natural habitat in the UK (Antrop, 2000; Dallimer et al., 2011). Increasing areas of built space lead to habitat fragmentation and isolation of resources necessary for a range of taxa to survive and reproduce (Andrén, 1994: Young & Jarvis, 2001; Wood & Pullin, 2002; McKinney, 2008). This can lead to species change and subsequently ecosystem services can show high variability across urban-rural gradients (Tratalos et al., 2007). With over five billion people estimated to reside within urban areas globally by 2030 (United Nations, 2008), there is increasing demand for ecosystem services to remain resilient within our cities. The value of pollination has been mostly associated to the benefits in yield to agricultural crops. Their pollination contributes to 35% of global food production (Klein et al., 2007) and can influence seed-set, yield and the market price of many agricultural crops (Bommarco et al., 2012).

However, concern for pollination in urban areas is growing. Landscape alteration and conversion are underlined as influential in reducing pollination services due to reductions in area of natural habitat and subsequently of bee abundance and diversity (Steffan-Dewenter et al., 1999; Ricketts et al., 2008; Bommarco et al., 2012; Klein et al., 2012; Lentini et al., 2012). Currently, studies detail how urbanisation negatively influences bee assemblage diversity, with bee assemblage change species-specific along the urban-rural gradient (Bates et al., 2011; Matterson et al., 2011; Banaszak-Cibicka & Żmihorski, 2012; Chapter 2).

Pollination is a direct ecosystem service, and its continuation throughout the urban-rural gradient has several benefits to humans. First, if pollination services are sufficiently consistent throughout the urban-rural gradient, food crops grown in allotments, urban gardens and arable fields may benefit. Specifically, for allotments and community gardens, urban agriculture can boost local economic growth and provide sustainable and cheaper ways to grow healthier food (Garnett, 1996). For agricultural areas, maintaining diverse and abundant pollinator assemblages in areas adjacent to arable fields couldprovide pollination for crops (Goulson et al., 2003; Frankie et al., 2009; Cussans et al., 2010; Hennig & Ghazoul, 2011; Samnegård et al., 2011; Pellissier et al., 2012).

Pollen availability is the predominant limitation for seed-set in many angiosperms, with pollen transfer between con-specifics crucial in facilitating pollination success (Ashman et al., 2004; Ollerton et al., 2011). Between habitat fragments along an urban-rural gradient, pollen carryover of bees has been recorded for up to ~2 km (Van Rossum, 2010a). Although the further the distance between flowers, the less the amount of pollen transferred, it is proposed that pollen carryover in urban areas is equal to that of agricultural landscapes (Van Rossum, 2010b). In one study using the mainly bumblebee pollinated plant *Digitalis purpurea,* no clear variation in seed-set along an urban-rural gradient was reported (Verboven et al., 2012). In arable fields, bee visitation rates and resulting pollination services are greater in proximity to gardens (Cussans et al., 2010; Samnegård et al., 2011). This is most likely because farming practices can limit pollinator abundance by homogenising local habitats (Holzschuh et al., 2008,2011) whilst the floral availability of gardens and green space can increase bee visitation to plants in these habitats (Cussans et al., 2010; Hennig & Ghazoul, 2011). Only a few studies have aimed at examining seed-set in densely urbanised areas (Pellissier et al., 2012; Verboven et al., 2012), and further research in different cities and regions is still needed to gain a better understanding of how pollination services may vary between urban and rural areas.

Other studies have examined the visitation of bees to plants alone, not directly testing seed-set. These show plant-pollinator relationships tend to become more simplified within urbanised areas because bee-flower interactions in rural contexts are more complex (Deguines et al., 2012; Geslin et al., 2013). The loss of bee species that cannot adapt to the changes in land–use may cause breakdowns in certain plant-pollinator relationships (Kearns et al., 1998; Kaiser-Bunbury et al., 2010). Furthermore, bee communities take time to occupy the foraging niche vacated due to species loss, which in turn can influence the pollination success of flowers without pollinators (Brosi & Briggs, 2013). However, similar to bee assemblage studies along urban-rural gradients, it is difficult to draw conclusions until more scientific investigation has been undertaken.

In previous investigations, Birmingham, UK showed greater overall bee abundance in suburban sites and bumblebee abundance increases with area of built space (Chapter 2). This resulted in an observed variation in community trait weight and group trait diversity along the urban-rural gradient in Birmingham (Chapter 4). However, the conclusions these studies can draw relating to how the impact of bee assemblage and trait diversity change will ultimately affect pollination success, are limited. Initially, greater bee abundance may suggest species loss will not have a deleterious effect on pollination services, as the bees present will still be able to facilitate pollen carryover to the same extent (a.k.a. density compensation). However, this has not been found in crop pollination experiments (Winfree & Kremen, 2009). Furthermore, there has been growing emphasis on the importance of trait complementarity in bee assemblages towards pollination service function (Hoehm et al., 2008; Albrecht et al., 2012; Fründ et al., 2013), so how trait diversity changes in Birmingham, UK will influence pollination services between urban and rural sites is of interest to investigate. This suggests that even though there may be greater abundance of certain bees in urban areas, they may not provide equivalent pollination services to bee assemblages with, albeit lower abundance, greater species richness and trait complementarity. Hence, it is important not only to investigate bee assemblage and trait diversity change along the urban-rural gradient, but also to supplement those investigations with tests of pollination service itself.

With the abundance and trait diversity of Birmingham, UK well-documented in this thesis and in Bates et al. (2011), there is an opportunity for a greater level of interpretation from a test of seed-set in the same study region. There is very little research in this study area, and therefore I decided that rather than sampling the full urban-rural gradient, the differences at the end of the spectrum between urban and rural sites would be assessed. By undertaking a study of seed-set in the same geographical area of Chapters 2 and 4, greater insights into the how urbanisation may influence pollination services along an urban-rural gradient may be achieved.

5.1.1 Aims and Objectives

Therefore, this chapter aimed to increase current understanding of the potential impact of different land-use types on pollinator visitation and subsequent seed-set by planting *Campanula glomerata* in standardised habitat patches in both urban and rural sites in and around Birmingham.

Specifically the objectives were to;

(i) test the importance of bee species abundance and richness in influencing seed-set of *Campanula glomerata* between urban and rural sites in Birmingham, UK;

(ii) record the relative difference in seed-set of *Campanula glomerata* between urban and rural sites.

5.2 Methods

5.2.1 Study area and sample sites

Assessment of seed-set was undertaken between urban and rural areas sampled as part of Chapters 2 and Chapter 4 of this thesis. For further detail on this study area, refer to section 2.2 Methods.

Specific to this chapter's experimental design however, was the sole use of canal sides as opposed to a mix of canals and parkland sites. Birmingham canals have a long-standing history and are an important source of habitat for many taxa along the urban-rural gradient (Canal and River Trust, 2014). Canal sides offer potential foraging habitat to bees in the form of small florally diverse intermediately-managed clearings next to tow paths along Birmingham's urban-rural gradient. From the floral diversity and abundance surveys undertaken during the sampling effort for Chapter 2, canal sides showed similarity between urban and rural sites. It was therefore decided that canal sides could provide a basis for site standardisation between land-use types to test for seed-set. Canal side sites were narrowed down to those having an area of tall grassland that was less regularly mown than the tow paths, floral diversity in the local vicinity and their exposure to sunlight. Floral abundance and diversity were standardised between land-use types as the level of bee visitation to flowers in urban habitats can increase if flowers are also more abundant and diverse (Hennig & Ghazoul, 2011). To alleviate possible damage due to maintenance work or from members of the public along the canal sides, a protective area was created by surrounding each plot with 30 cm tall durable plastic lawn edging. Also, Canal and River Trust maintenance staff were informed of the experiment and asked not to mow over the plots.

Ten experimental sites (Figure 5.1) were selected along the canal sides of Birmingham, UK to test for pollination service, five sites in urban areas and five sites in rural areas. Urban and rural land-use types were singled out because they are on the either end of the urban-rural gradient, and there is still a lack of literature on how densely urbanised habitats differ in their pollination services from rural areas. Land-use types were derived from 1 km^2 classification of the West Midlands designated by Owen et al. (2006), with this chapter's urban sites classed as 'urban', 'urban transport' and 'dense suburban' and rural sites as 'village/farms'.

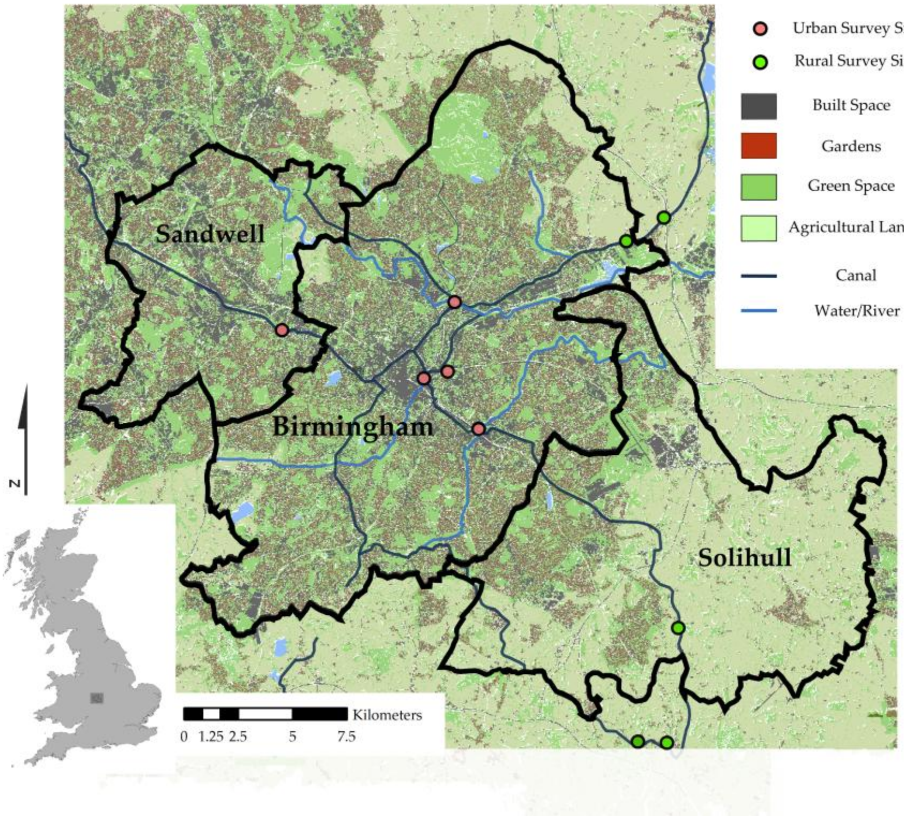


Figure 5.1: Map of sample sites across Birmingham, UK and throughout the wider area planted with *Campanula glomerata*. Sample sites were selected following the land-use classification of Owen et al. (2006). Six sample sites were selected from urban ('urban', 'urban transport' and 'dense suburban' [Red dots]), and rural sites ('village/farms' [Green dots]) in Sandwell, Birmingham City and Solihul (Black lines=council boundaries). The landscape context variables used in chapter 2's analysis are shown with Built Space in grey, Gardens in orange, Green Space in green and agricultural land in light green. Canal sides are represented by dark blue lines, with bodies of water and rivers/streams shown as light blue.

5.2.2 Study species

Clustered Bellflowers (*Campanula glomerata* L.) (Campanulaceae) was planted in sample sites, with their resulting seed-set a measure of pollination services provided to them in a particular site. The main pollinators of *C. glomerata* are bees, but are visited by a range of insect taxa (Bachmann & Hensen, 2007). Several bee species in the UK are pollen specialists of *Campanula* spp. (BWARS, 2014), but a range of other species including bumblebees are also important pollinators of species from this genus (Blionis & Vokou, 2005; Maad et al., 2013). In previous studies, *C. glomerata* specifically has been used to test pollination service with greater bee abundance and diversity increasing seed-set (Albrecht et al., 2007). *C. glomerata* is a perennial herbaceous plant that shows little to no self pollination when pollinators were excluded due to staggered anther and stamen maturity (Nyman, 1992; Blionis & Vokou, 2005, Albreckt et al., 2007, Buchmann & Hensen, 2007). No wild *C. glomerata* were recorded in the study area or were evident in the survey sites for Chapter 2. During site selection, *C. glomerata* were searched for, but none was found. This did not discount the presence of wild *C. glomerata* or other species of *Campanula* in the study area, but on a site scale there appeared to be no differences in presence of the test species.

Second year growth specimens of *C. glomerata* in 9cm pots were purchased from www.Wildflowers.co.uk. At each site, three specimens were planted into the ground at three areas cleared of tall grass. Miracle-Gro® Slow Release All Purpose Plant Food TM 25g of plant food was mixed into the soil of each plot. Each individual plot was chosen for its south facing aspect and distance from tall trees and footpaths.

5.2.3 Observation Sampling

Four observation surveys per site were undertaken between July and September in 2012, with each observation sampling effort lasting 45 minutes per site. This time was divided into 15 minute observations per plot, where visiting insects were observed and recorded. Per observation sampling effort, all sites were surveyed over a 2 day period. For each survey day, the same number of urban and rural sites was surveyed. The order of sites sampled was rotated between survey efforts. Surveys were only undertaken between 9:30 to 17:00 GMT when the temperature was above 18°C and wind speed was below 10 mph.

For each observed bee or other insect visit, initially the number of *C. glomerata* flowers visited by that individual during a foraging bout, the type of foraging behaviour and the time of visit were recorded. The behaviours of visitors were grouped into categories. Pollen collecting behaviour included bees landing on the flowers of test plants and actively rubbing or manipulating the anthers. Nectar collecting behaviour was defined by bees using their probosces to extract nectar from the base of the flowers. The other behaviour observed included <1 second investigation with full contact and <1 second investigation without full contact. Full contact was defined by a bee landing on a flower, compared to without full contact when bees were observed to hover above the flower and investigate it. If a bee visited more than one flower in a sample plot the behaviour that bee exhibited the most towards flowers was recorded for that individual. However, the behaviour of each bee tended to be consistent throughout the observation. Recording an individual twice was a low possibility due to short sampling times per site. If this did occur, as the same method was used in all sites the chance of re-recording an insect was equal throughout the study.

Upon observing a visitor, it was identified into a category of Fly, Hoverfly, Beetle, Wasp, Ant, Butterfly/Moth or Bee. If it was not easily identifiable or clearly a bee, it was caught when it tried to leave the plot. The bees caught that were identifiable in the field (using Edwards & Jenner, 2005) were recorded and released, while bees requiring microscopic identification were killed for preservation, pinned later that day and identified in the winter of 2012 to 2013.

5.2.4 *Campanula glomerata* Seed-set

Plots were checked continuously throughout the sampling period for ripened seed pods. Ten seed pods were taken at random from the three specimens per plot during the flowering season. From each plot, all 10 seed pods were cut open and all seed was extracted. The seeds were then dried in an oven at 65°C for 24 hrs. Once the seeds had dried, the seeds from each plot were weighed, and then averaged to gain a measure of seed-set per plot.

From the three plots per site, mean seed-set for each site was calculated. To gain a measure across land-use types, the seed-set for each site was averaged between urban and rural sites. In order to check if results could be dependent on individual plant vigour, the height in centimetres and mean number of flowers produced by plants at each site were measured and compared to seed-set. The growth of test plants was measured at each observation sample by measuring from the base of the plant to the tip of the tallest part of the plant. This was then averaged between plots to gain a mean height of test plants per site. The mean number of flowers produced by test flowers was estimated by counting the number of flowers each species produced and averaging across site plots. To account for possible loss of plants from inadvertent mowing, vandalism or natural mortality, more sites were originally planted with *C. glomerata*. Sites had a low rate of plant loss, although two sites early on had the majority of plants either fail or removed from sites. This reduced the original number of six sites per land-use type to five.

5.2.5 Local and Landscape variables

Floral availability was estimated by grouping the number of flowers present per flowering tree, shrub, or plant into either >10, >100, >1000 or >10,000 flowers. The number of different species presenting flowers was counted as a measure of floral richness. These were recorded at the same time for each observation sampling event, giving four measures of floral abundance and richness per site which were averaged to gain a measure of each per site.

Four main landscape context variables were extracted from an Ordnance Survey MasterMap dataset by grouping landscape metrics in ArcGIS v10 (ESRI, 2011). Built space consisted of 'buildings', 'man-made structures', 'pavements', 'roads', 'man-made open space/landforms', 'rail' and 'developing land'. The gardens layer was derived from 'gardens' of all sizes. Green space was made up of 'open natural habitat' (natural open space, natural habitat without trees, natural landforms, natural rail way without trees and natural roadside without trees) and 'covered natural habitat' (natural roadside with trees, mixed natural habitat with trees and natural railway with trees) (Ordnance Survey, 2006). An agricultural land layer was also obtained by separating the area covered by green space layer by a map of agricultural land-use (Natural England, 2009). Concentric buffer zones of 250 m, 500 m, 1.5 km and 2.5 km were then made for each sample site. Using Geospatial Modelling Environment (Bayer, 2012), the area (m^2) of each landscape metric raster was derived (See 2.2 Methods & Figure 2.2 for more detail).

5.2.6 Data analysis

To test for significant differences between metrics of observed visits, seed-set, plant vigour and site quality, independent t-tests were used. Due to the limited frequency in which individual bee genera visited *C. glomerata*, these metrics were not tested for significant differences. Only the overall abundance and species richness of bee visitors tested for differences between land-use types using t-tests. Due to the number of sites and frequency of bee visits, applying seed-set and observed visits to statistical models did not reliably contribute to the analysis. Instead, Pearson Product Moment or Spearman Rank correlations were applied to the seed-set, observed visits and landscape context variables of sites to look for potential co-linearity between these variables. These provided a complementary method to the simple test of means between urban and rural sites.

5.3 Results

In total, 111 insects were observed visiting test flowers in the observation sampling period, 37 individuals in urban areas and 74 in rural areas. The number of bees visiting flowers between urban and rural sites was 30 and 38, respectively. Prior to t-tests, all data were checked for normality. Flower visitors showed no significant differences in abundance (t=1.38, d.f.=8, p=0.12) or richness (t=0.42, d.f.=8, p=0.35), and neither did bee abundance (t=0.28, d.f.=8, p=0.39) and richness (t=0.40, d.f.=8, p= 0.34) between urban and rural sites (Figure 5.2). There was however significantly lower abundance (t=6.53, d.f.=8, p=0.003) and diversity of non-bee insects (t=2.78, d.f.=8, p=0.03) visits observed in urban sites. It was considered that site quality did not influence the occurrence of visitors of *C. glomerata* at each site as no significant difference was found between site floral availability (t=1.41, p=0.126), and richness(t=1.85, p=0.08) between land-use types.

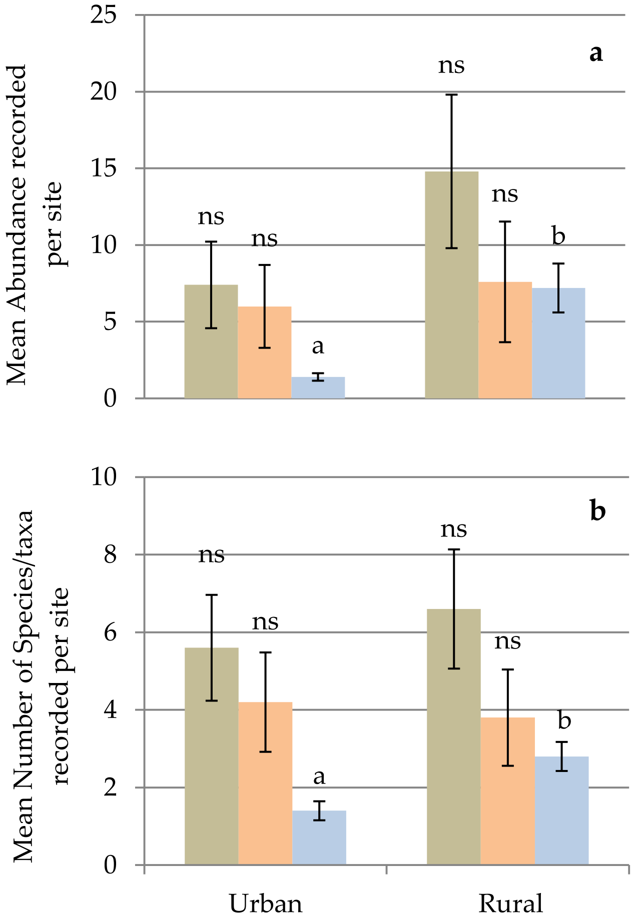


Figure 5.2: The abundance (a) and species/taxa richness (b) of all insect visits (Green), bee visits (Orange) and non-bee visits (Blue) recorded on *Campanula glomerata* flowers between five urban and five rural sites. Error bars = ± 1 SE. Bars with different letters represent significant differences, while 'ns' represents no significant difference between land-use type for each metric.

Andrena, *Bombus*, *Lasioglossum* and *Hylaeus* were the most frequently observed bee genera visiting *C. glomerata* across the land-use types. Although metrics of genera abundance were not tested statistically due to insufficient observed visits, there was no obvious difference between land-use types for bees visiting *C. glomerata* (Figure 5.3). Interestingly, the main genera of bees observed differed in their visiting behaviour of *C. glomerata* flowers. From observation sampling, it was recorded that once *Bombus* spp. visited a survey plot they tended to visit more *C. glomerata* flowers than the other genera (Figure 5.3) and tended to visit flowers briefly, for nectar only. In contrast, *Andrena*, *Hylaeus* and *Lasioglossum* were observed visiting only one or two flowers each time they were recorded, whilst also showing prolonged contact with flowers and actively collecting pollen from flowers (only *Lasioglossum* spp.; Figure 5.4). One species also observed during the sampling period was *Chelostoma campanularum*, a dietary specialist of C*ampanula* spp., but only found in two urban areas.

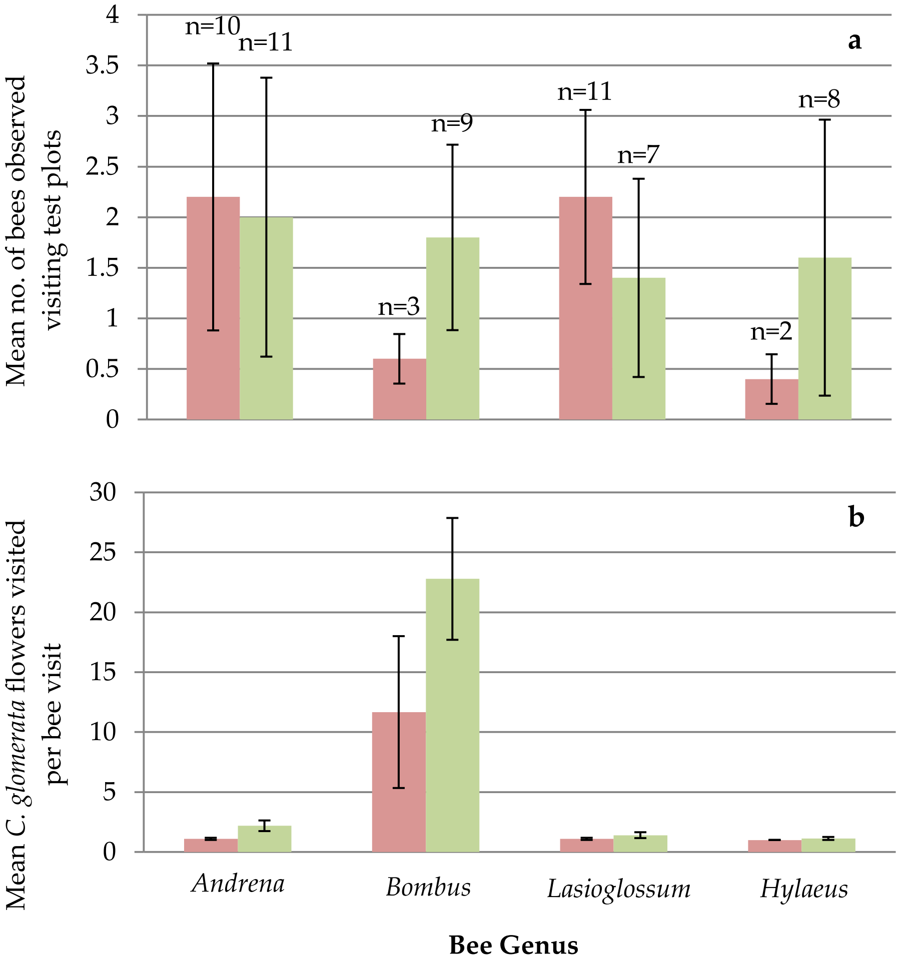


Figure 5.3: The mean number of the most abundant bee genera recorded visiting test plots (a), and the mean *C. glomerata* flowers visited by each visitor per plot (b) between urban (Red) and rural (Green) sites. Error bars = ± 1 SE. n=the number of bees from that genus recorded at each site type.

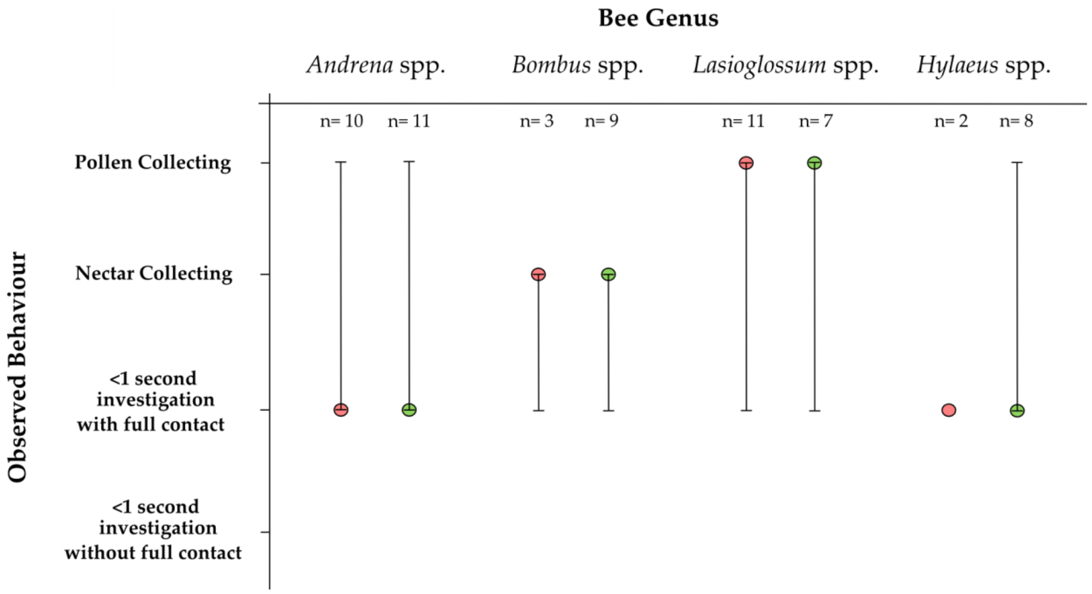


Figure 5.4: The mode (circles) and extent (error bars) of the type of visitation of the main bee genera visiting *Campanula glomerata* flowers between urban (Red) and rural (Green) sites. n=the number of bees from that genus recorded at each site type.

There was no evidence that plant vigour varied between land-use types, with the height of *C. glomerata* plants (t=0.69, d.f.=8, p=0.26, Figure 5.5a) and the mean flowers produced (t=0.26, d.f.=8, p=0.4, Figure 5.5b) not significantly different between urban and rural sites. In terms of *C. glomerata* seed-set, I found no significant difference between urban and rural sites (t=0.08, d.f.=8, p=0.46, Figure 5.6).

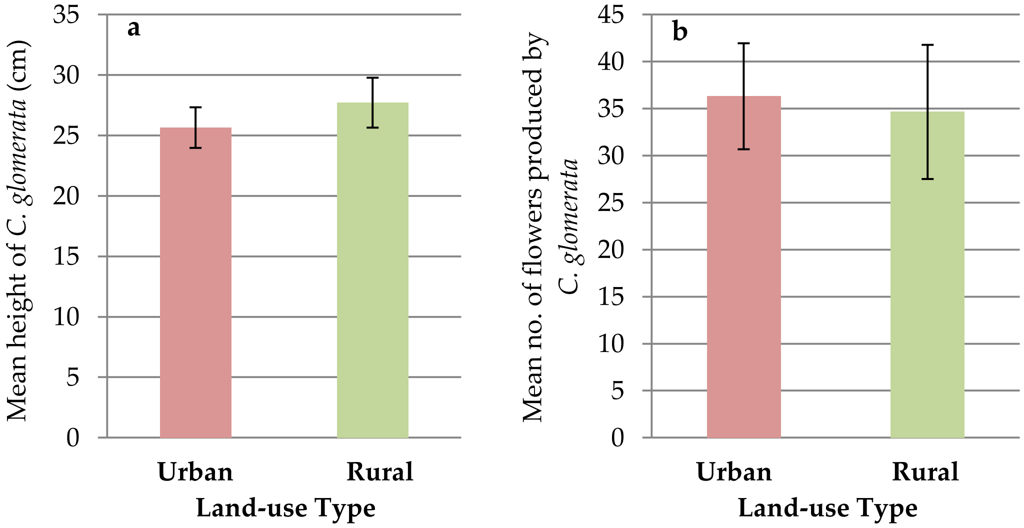


Figure 5.5: The mean (±1SE) height (a) and number of flowers (b) of test *Campanula glomerata* between five urban (Red bar) and five rural (Green bar) sites.

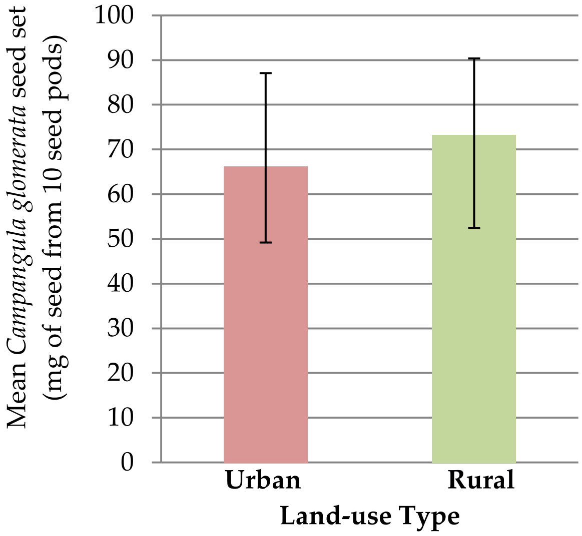


Figure 5.6: The mean (±1SE) weight (mg) of *Campanula glomerata* seed as a measure of seed-set between five urban (Red bar) and five rural (Green bar) sites.

Correlation analysis showed that seed-set in *C. glomerata* was not significantly correlated to any visitor abundance and species richness metrics, nor the site variables such as floral richness, floral availability or the number of *C. glomerata* flowers that were produced per sample site (Table 5.1). For landscape context variables, there was a significant negative correlation between the area (m^2) of gardens within 500 m (r=-0.81, p=0.03; Table 5.2) and 1,500 m (r=-0.83, p=0.02; Table 5.2). The only other significant correlation was with bee abundance increasing with the area of agricultural land within 2500m (r=0.73, p=0.04). Built space and green space showed no correlation to metrics of seed-set or bee abundance and richness. Proportionately, built space and agricultural land were the main landscape context variables that differed between land-use types, whilst the percentage of gardens and green space stayed relatively similar between land-use types (Figure 5.7).

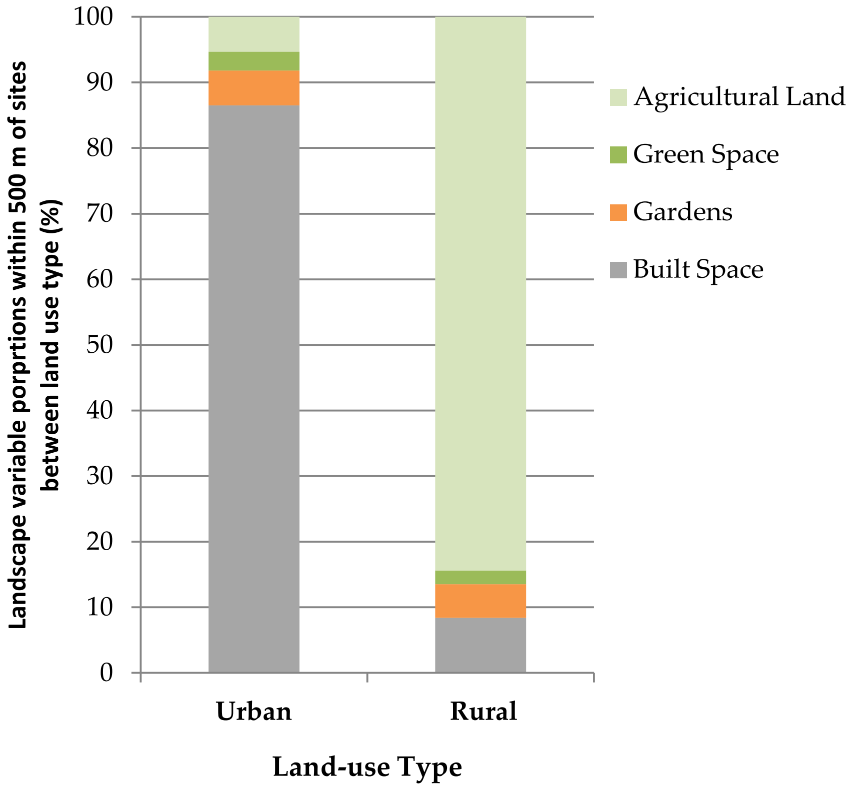


Figure 5.7: The percentage cover of landscape context variables within 500 m of five sites in each land-use type. Built space and agricultural land were the main varying components of the landscape variables between land-use types.

Table 5.1: Correlations between seed-set of *Campanula glomerata*, metrics of visitors to test flowers, site quality metrics of floral richness and availability, and the number of flowers produced from 10 sample sites where pollination service by insects was tested between urban and rural land-use

	C. glomerata seed-set (mg)	All Abundance	Bee Abundance	Other Insect Abundance	Bee Species Richness	All Species Richness	Floral Richness	Floral Availability
All Abundance	0.2							
Bee Abundance	0.35	0.96***						
Other Insect Abundance	-0.04	0.72**	0.59					
Bee Species Richness	0.34	0.8**	0.77**	0.21				
All Species Richness	0.36	0.9***	0.89***	0.43	0.97***			
Floral Richness	-0.22	0.05	0.12	0.13	-0.09	-0.06		
Floral Availability	-0.35	-0.5	-0.47	-0.43	-0.37	-0.44	0.74*	
C. glomerata flowers	-0.08	0.22	0.14	0.3	-0.06	0.01	0.49	0.43

* p<0.005; ** p<0.01; *** p<0.001

Table 5.2: Correlations between seed-set of *Campanula glomerata*, abundance and species richness of visitors to test plants and landscape context variables at scales of 250 m, 500 m, 1500 m and 2500 m from 10 sample sites where pollination service by insects was tested between urban and rural land-use

Landscape context variable	**Scale (m)**	*C. glomerata* seed-set (mg)	Bee Abundance	Bee Species Richness
Gardens	250	-0.56	0.07	0.14
	500	-0.81*	-0.06	-0.12
	1500	-0.83*	0.11	-0.09
	2500	-0.42	-0.05	-0.19
Built Space	250	0.01	-0.2	-0.19
	500	0.03	-0.1	-0.1
	1500	0.03	-0.1	-0.05
	2500	0.05	-0.03	-0.06
Green Space	250	-0.06	0.26	0.21
	500	-0.2	0.17	0.09
	1500	-0.17	0.16	0.05
	2500	0.03	0.22	0.16
Agricultural Land	250	-0.52	0.34	0.38
	500	-0.51	0.41	0.43
	1500	-0.39	0.64	0.58
	2500	-0.29	0.73*	0.68

* $p<0.05$

5.4 Discussion

Pollen limitation which leads to reduced seed-set in angiosperms (Ashman et al., 2004) is said to be potentially similar between urban and agricultural areas (Van Rossum, 2010b). This chapter set out to further the current literature of how urbanisation influences pollination services by testing the seed-set of *C. glomerata* between urban and rural areas of Birmingham, UK.

5.4.1 Observation sampling

Greater bee abundance and richness increases pollination services to flowers (Ricketts et al., 2008; Bommarco et al., 2012; Klein et al., 2012), specifically for *C. glomerata* (Albrecht et al., 2007). From observation surveys, I found that there was no significant difference between the mean abundance and richness of bees visiting *C. glomerata*. In previous literature, plant-pollinator relationships show simplification towards higher levels of urbanisation (Deguines et al., 2012; Geslin et al., 2013), most likely due to bee and plant species loss in urban areas (Wojcik et al., 2012). This was not observed here however, with similar bee abundance and number of species visiting test flowers. Factors that could have influenced the visitation rate of bees include the floral abundance and diversity at a site, which can increase bee visitation to plants in the urban environment (Hennig & Ghazoul, 2011). However, visitation rate was un-likely to have been influenced by site foraging quality, as floral richness and availability were found to not vary between land-use types or correlate to the number or species richness of visiting bees. However, the numbers of recorded bees visiting test flowers was low in frequency. This limited this chapter's ability to conclude the full variation in bee visits to test plants due to low statistical power. This could have been compensated for by pan trapping pairwise sites (Samnegård et al., 2011), but it was considered that a greater level of interpretability regarding how many insects directly visited test flowers and how they foraged on the test flowers was more worthwhile in meeting this chapter's objectives.

From observations of test plots, several differences were observed with regards to the type of visits recorded. Bumblebees on average visited test plots the same amount of times as other insects, but they visited more flowers on average once within test plots. This agrees with previous observations with bumblebee species that were found to be predominant visitors of flowers within urban habitats (Deguines et al., 2012; Geslin et al., 2013). This is in contrast to bees in the genera *Andrena*, *Lasioglossum* and *Hylaeus* which generally visited one or two flowers at a time. Furthermore, the most commonly observed behaviour of bees differed between genera, as *Lasioglossum* displayed active pollen collection throughout the study. Bumblebees, the most prolific genus of bees found along the urban-rural gradient and found in highest abundance in suburban and urban areas (Chapter 2), have been noted as important to pollination services due to their greater flower visitation rate (Albrecht et al., 2012). Surprisingly, I found lower occurrence of bumblebees visiting *C. glomerata* in urban areas which contrasts with earlier results (see Chapter 2). However, in this chapter bumblebees visited numerous test flowers when visiting a test plot, suggesting they could be important in pollinating *C. glomerata*. It has been shown before in studies of pollination services that bumblebees and their greater foraging ranges facilitate equivalent pollination services to *Digitalis purpurea* between urban and rural areas (Verboven et al., 2012).

One species found to visit test flowers in two urban sites was *Chelostoma campanularum,* a foraging specialist of flowers from the genus *Campanula*. Chapter 4 found that oligolectic bees were significantly greater in urban areas. This observation suggests that specialist species can still play a part in the pollination of flowers in urban areas. *Chelostoma* spp. are small, with foraging ranges generally within 500 m of their nests (Gathmann & Tscharntke, 2002). Their nests comprise existing cavities in dead wood which can be found on fence posts and garden sheds (BWARS, 2014). This also agrees with Chapter 4 which illustrated the preference of cavity-nesting bee species for urban habitats. This suggests that urban areas in Birmingham are not deleterious to flower visitation rates of *C. glomerata* and under some circumstances may promote specialist foraging bees as part of their pollinating fauna.

5.4.2 *Campanula glomerata* seed-set

C. glomerata did not vary in seed-set between urban and rural sites. There was also no variation in the height and flowering of test species between land-use types, nor did they correlate to seed-set. Hence, it is likely that plant health did not influence the seed-set results.

Few studies have aimed at examining seed-set towards densely urbanised areas, but for those that have there are contrasting results. Pellissier et al. (2012) found pollination services were reduced in more urbanised areas with greater impervious surfaces, suggesting more suburbanised areas were better at providing pollination to *Lotus corniculatus*. Verboven et al. (2012) on the other hand found pollination services remained consistent across the urban-rural gradient. This chapter supports Verboven et al. (2012) with seed-set showing no variation between urban and rural areas. Pollen carryover of bees has been recorded for up to ~2 km between urban habitat patches (Van Rossum, 2010a) and is equal to that of agricultural landscapes (Van Rossum, 2010b). This chapter suggests that this was the case in Birmingham, UK. Verboven et al., (2012) suggested that the abundance of bumblebees in their study region (Belgium) enabled pollination services to be provided across the urban-rural gradient. Similar to this, Birmingham, UK has bumblebees in abundance in both urban and suburban areas (Bates et al., 2011; Chapter 2). Although density compensation may not limit the reduction in pollination that results from bee species loss, species-specific variation may be able to (Winfree & Kremen, 2009). Therefore, if pollination services are negatively affected by loss of certain species in either urban or rural areas, the species-specific response of bees along the urban-rural gradient in my study area could have compensated for deleterious effects this had on pollination services (Chapter 2).

Further to this, Chapter 4 found some levels of trait diversity between urban and rural areas. Applying an analysis of trait diversity to the species recorded visiting test flowers in this study would not be recommended due to the low frequency in which bees were recorded. However, with the similarity in seed-set between land-use type there is evidence to suggest that the trait diversity variation shown in Chapter 4 may not be negatively influencing pollination services in Birmingham, UK.

These results however should be viewed with caution. The observed similarity in seed-set in this chapter does not necessarily suggest that pollination services are at their maximum in both urban and rural areas. One previous study suggests that flowers in urban environments were pollen-limited and that it could be increased with greater bee visitation rates (Verboven et al., 2012). This investigation did not test for pollen limitation in *C. glomerata*, which constrains the interpretation of the variation of pollination services between land-use types as these results may both be experiencing pollen limitation rather than 'high' or 'sufficient' levels of pollination.

In this chapter, there was evidence that other taxonomic groups other than bees varied significantly between land-use types. Previous studies have documented species-specific responses in the assemblages of other insect taxa, most notably in hoverflies along the urban-rural gradient (Bates et al., 2011) and in agricultural environments (Meyer et al., 2009). This may not have influenced seed-set in this chapter due to bees being the main pollinators of *C. glomerata* (Blionis & Vokou, 2005; Maad et al., 2013), but for flowers bees are less likely to help to pollinate and the presence of other insects may be crucial. Therefore, there is a need for further experimentation investigating the impact of the urban-rural gradient on other types of pollinators and the flowers they pollinate. Future research should not only concentrate on the abundance, richness and trait diversity of bees, but also on other pollinating taxa and their value in pollinating plants. One way of doing this would be to apply similar methodology as this chapter but with different plants, preferably varying in their morphological and phenological characteristics.

Suburban areas have shown increased pollination services compared to agricultural and urban areas (Goulson et al., 2003; Cussans et al., 2010; Hennig & Ghazoul, 2011; Samnegård et al., 2011; Pellissier et al., 2012). In this chapter, suburban areas were not incorporated as it was considered more important to use available resources to test rural and urban areas. In the sites selected, although varying in built space and agricultural land, gardens constituted similar proportions of the landscape within 500 m of all sites (Figure 5.7). Gardens specifically are shown to increase bee visitation rates, abundance and subsequent seed-set (Cussans et al., 2010; Samnegård et al., 2011). The presence of gardens within the local vicinity could have acted as a source for bees and caused equal numbers of bees in both site types, and subsequently limited seed-set variation. From the landscape context data however, greater percentage of gardens correlated with lower seed-set. This could suggest that the attraction of gardens for pollinators limits their pollination services to other sites in the surrounding area. Although beyond the scope of this chapter, the importance of site type for bee assemblages along the urban-rural gradient has been shown in Chapter 2 and Bates et al. (2011). Hence, it is important that other types of site are tested for pollination services along the urban-rural gradient to obtain a clearer picture of how particular sites differ and compare in their bee presence and subsequent pollination success. In summary, by increasing regional, site type and test plant replication, a firmer conclusion about how the urban-rural gradient may influence pollination services may be drawn.

Chapter 6

The brood cell provisioning and reproductive success of Red Mason Bees (*Osmia bicornis* syn. *rufa* [Linnaeus]) in an urbanised environment.

Abstract

*The response of bee assemblages to increased levels of urbanisation is species-specific, but detailed investigations into how species associated with urban areas can provision their young and reproduce successfully are scarce. To facilitate effective reproduction, bees require a source of nectar and pollen, and suitable nesting resources to sustain their offspring's growth and development. However, understanding the extent to which urban areas provide these resources and the subsequent population dynamics of solitary bees is integral to understanding the implications on pollination services. I tested the efficacy of urban habitats in their level of viable resources towards the foraging generalist solitary Red Mason Bee (*Osmia bicornis *syn.* rufa *Linnaeus), using pre-collected overwintering adults placed within 20 artificial nest sites throughout an urbanised area in Birmingham, UK. The quality of provisioned resources, as an indirect measure of habitat quality, was shown to be a main determinant of increased brood cell provisioning success in* O. bicornis. *Greater resource quality and provisioning success also correlated to increased offspring weight and the proportion of female offspring produced, indicating habitat quality can ultimately influence the population dynamics of* O. bicornis. *This chapter concludes that if nesting sites are in abundance, the foraging resource quality is integral in providing reproductive success for bees in the urban environment. This is only for one species and region however, and should be further investigated with different bee species in different areas.*

6.1 Introduction

Bees require both foraging and nesting resources to reproduce effectively. Once suitable nesting sites are found, the larvae of solitary bees (Hymenoptera, Apoidea) are provided with provisions to sustain their growth and development through metamorphosis into adults. It is the task of the parent bee to provision each brood cell with enough viable pollen to enable successful larval development (Michener, 2000). Restricting this is the distance to forage plants from nesting sites (Zurbuchen et al., 2010), as bees are central place foragers and spend valuable energy and time travelling from plants to their nest (De Vries & Biesmeijer, 1998; Dukas & Edelstein-Keshet, 1998; Heinrich, 2004). Urbanisation is known to fragment habitat for many taxa, leading to resource isolation (Andrén, 1994; Young & Jarvis, 2001; Wood & Pullin, 2002). Maintaining a mosaic of suitable habitat throughout the local landscape is an important factor that can increase the foraging ability of bees (Williams & Kremen, 2007). Although many species of bee are found within urban areas (Frankie et al., 2005; Matterson et al., 2008; Cane et al., 2006; Bates et al., 2011; Banaszak-Cibicka & Zmihorski, 2012; Chapters 2 & 4), how urbanisation influences the provisioning success of bees is relatively unknown.

An important part of the resources provisioned to larvae is protein. The amount of protein a larva eats in its early stages of life will have a significant effect on its subsequent adult body size (Roulston & Cane, 2002). Body size is an important factor in the reproductive fitness of female solitary bees as it determines her ability to compete for nesting space (Kim, 1997; Steffan-Dewenter & Schiele, 2004), be reproductively successful (Kim, 1997; Strohm et al., 2002), disperse and forage (Gathmann & Tscharntke 2002; Bosch & Vicens, 2006; Neff, 2008) as well as overwinter (Strohm et al., 2002; Bosch & Kemp, 2004). Male bee body size is also linked to ability to hold a mating territory (Alcock, 1994) and is an important factor for mate selection by females (Conrad et al., 2010). Other benefits of a protein-rich diet include increasing the ability of bees to cope with adverse conditions such as parasitism and disease (Alaux et al., 2010; Di Pasquale et al., 2013). Hence, bee survival and reproductive success are directly and indirectly affected by the quality of provisioned resources.

Bees provide pollination services to some of the flowers they visit, but to what extent they are successful in provisioning their young and how successful their offspring will subsequently be could impact the pollination services they provide. Therefore, how successful bees are in provisioning offspring in urban areas is integral to understanding how pollination services will be influenced by urbanisation.

Research into the quality of habitats relating to insect diversity should concentrate on the immature stages of individual development to estimate the capacity in which populations can be sustained (Thomas et al., 2001). Yet, complexity arises when determining the 'quality' of one patch over another, with many studies lacking feasible ways of measuring quality of habitats, or even knowing what to measure. Currently, studies investigating meta-population ecology either neglect the quantification of quality or simply dismiss it as a variable altogether (Thomas et al., 2001; Mortelliti et al., 2010). With central place foragers such as bees however, it is possible to measure the provisioned resources they have managed to gain from their surrounding habitat as a proxy for habitat quality. However, nesting limitation is considered a main bottom-up determinant of wild bee populations (Steffan-Dewenter & Sciele, 2008), and the microclimate surrounding nesting sites has a significant influence on provisioning and nesting of solitary bees (Everaars et al., 2011). It is therefore important to standardise certain aspects of the nesting sites of bees before differences in the provisioning and reproductive success of bees can be evaluated.

This chapter aimed to test the dynamics of provisioning and reproductive success in a cavity-nesting generalist bee species in urban areas. By standardising the nesting potential of bees and the presence of bees in an urban environment, it was possible to assess the variables other than nesting limitation that influence the breeding success of bees in the urban environment. This allowed an analysis of resource quality, resource quantity and landscape factors to be tested against the provisioning success of bees, and furthermore for the resulting variables of reproductive success to be assessed.

6.1.1 Aims and Objectives

Through the release of a standardised number and size of Red Mason Bees *Osmia bicornis* syn. *rufa* (Hymenoptera: Megachilidae) alongside artificial nests, the aim of this chapter was to investigate the resource provisioning potential of bees within an urban context.

Specifically our objectives were to;

(i) record the brood cell provisioning and reproductive success of *O. bicornis* within an urbanised habitat;

(ii) record the influence of resource quality and quantity as well as local and landscape variables on the brood cell provisioning of bees;

(iii) characterise the population dynamics of the Red Mason Bee in an urbanised environment.

6.2 Method

6.2.1 Study Area

The area designated for this study was the University of Birmingham's Edgbaston campus and surrounding area, situated 4.3 km south-west of Birmingham city centre (Figure 6.1). General maintenance of the campus consisted of annual pruning of shrubs and hedges, management of the trees, and regular mowing of grass areas throughout the spring and summer months. Surrounding the campus was a mixture of parkland, residential buildings, industrial buildings and a variety of infrastructure. The sample area has been classed as a mixture of suburban, dense suburban and urban transport (Owen et al., 2006). Through the sampling period, certain potential forage plants were observed on the campus and in the surrounding area, including *Cotoneaster* spp., *Pyracantha* spp. and *Berbris* spp., *Tilia x europea*., *Aesculus hippocastanum* and a variety of species from the genera *Prunus* and *Cretaegus.*

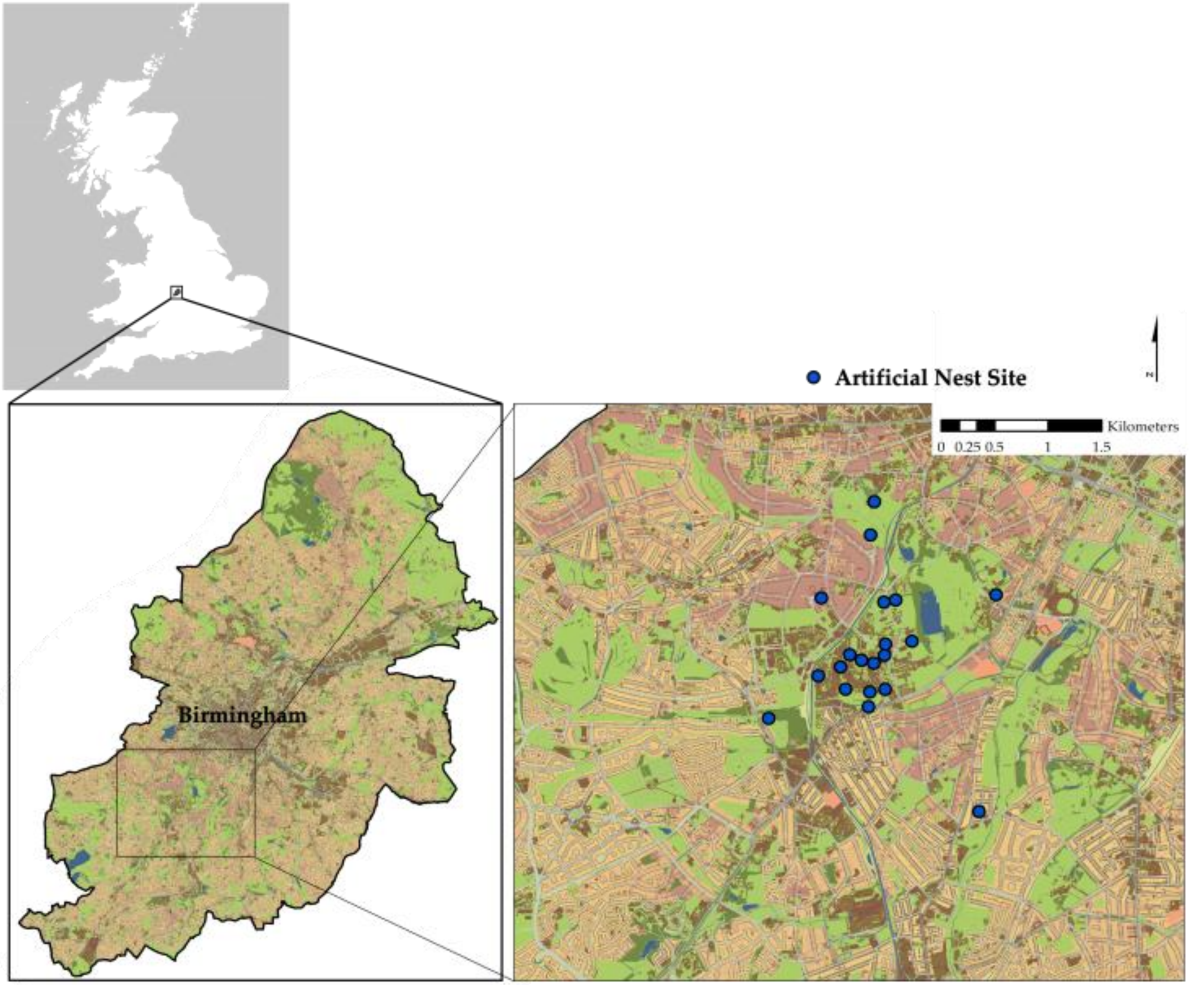


Figure 6.1: **Map of the sampling area and nesting site locations for the analysis of provisioning and reproductive success in *Osmia bicornis*.** Blue dots indicate sample sites, dark green indicates the vegetation layer, light green indicates green space, pink shows impervious surfaces, brown shows buildings, and taupe indicates domestic gardens.

6.2.2 Study species

Widely distributed throughout the UK and mainland Europe, the Red Mason Bee (*Osmia bicornis* syn. *rufa* Linnaeus) was selected as the study species. *O. bicornis* is univoltine, nesting in a range of pre-existing cavities by partitioning and capping the end of nests with soil. They provision brood cells (usually linearly) with pollen and nectar (>90% pollen), lay an egg on top of provisioned resources and cap the end of the cell with moistened soil. Bee larvae inside then eat the pollen and nectar and weave a cocoon where they eventually pupate over winter and emerge the following spring. *O. bicornis* show preference for artificial nesting tubes (Steffan-Dewenter & Schiele, 2008; Everarrs et al., 2011) and this makes them an ideal focal species for this study.

O. bicornis is a common pollinator of fruit and nut crops from the Roseaceae family with corresponding emergence and flowering periods between April and July. *O. bicornis* are dietary generalists in terms of both nectar and pollen, utilising a range of pollen sources for provisioning brood cells (Kraemer & Favi, 2010). Although generalists, there are records where the majority of pollen collected is from only one or two sources (Raw, 1974; *Qercus* spp. and *Ranunculus* spp.), with pollen collection varying depending on the plant species flowering in the immediate area surrounding a nest site (Rademacher & Strohm, 2010). The foraging range of *O. bicornis* is believed to be less than 500 m from their nesting site (Gathmann and Tscharntke, 2002) but individual foraging bouts of up to 900 m have been recorded when pollen and nectar are scarce within the local vicinity (Vicens & Bosch, 2000). Cleptoparasites are a top-down pressure on the reproductive success of *O. bicornis* (Steffan-Dewenter & Sciele, 2008). An example of such a parasite is *Cacoxenus indagator* (Diptera: Drosophilidae) which lays its eggs alongside the brood cell provisioning whilst the female *O. bicornis* is away from the nest gathering more resources.

6.2.3 Experimental Design

To gain a measure of total brood cells provisioned by *O. bicornis*, artificial nests, their placement, and the release of a set number of adult bees (in certain size categories ready to emerge) were standardised. This allowed this study to alleviate the potential influence of nest limitation (Steffan-Dewenter & Sciele, 2008), bee limitation and to some degree the microclimatic variability (Everarrs et al., 2011) on the brood cell provisioning potential of bees. Through this standardisation, it was possible for this study to evaluate what landscape and resource factors affect *O. bicornis* provisioning.

In 2011, the year prior to this experiment, artificial bee hotels were placed in several locations within Birmingham, UK to collect *O. bicornis* cocoons for this experiment. They were cleaned and stored outside in a secure location during the winter of 2011-2012. In early 2012 (January and February) whilst the bees were dormant, a small incision at the 'tip' of the cocoon was made to expose the head of each bee without waking them. Specimens that had not survived through the winter, those underdeveloped (usually with thin cocoon walls and a very small individual inside which may not be fully formed), and those that had been parasitized were removed from the study. The gender of each bee was checked by the assessing if the clypeal pilosity was cream coloured or black, separating individuals by male and female gender, respectively. To alleviate the potential bias bee body size could cause when attempting to analyse differences between bee hotels, *O. bicornis* cocoons in different weight classes were placed in an emergence tube at each artificial nesting site. The weight of each viable bee was recorded by measuring the bee within the cocoon. Each site had five females over the weight of 160 mg, seven females between the weight of 120-160 mg, and 10 males heavier than 80 mg, a total of 22 *O. bicornis* per site.

In the spring of 2012, artificial nests purchased from CJ Wildlife (www.birdfood.co.uk), were placed within and around the University of Birmingham's Edgbaston campus (Figure 6.2). Each hotel contained 29 cylindrical cardboard nesting tubes 8 mm wide and 15 cm long with a paper lining which could be removed and peeled away to reach the brood cells (Figure 6.3). Nests were placed in a south-facing position at each sample location from March-July in 2012. Filled and capped tubes were removed and replaced with new empty tubes throughout the sampling period.

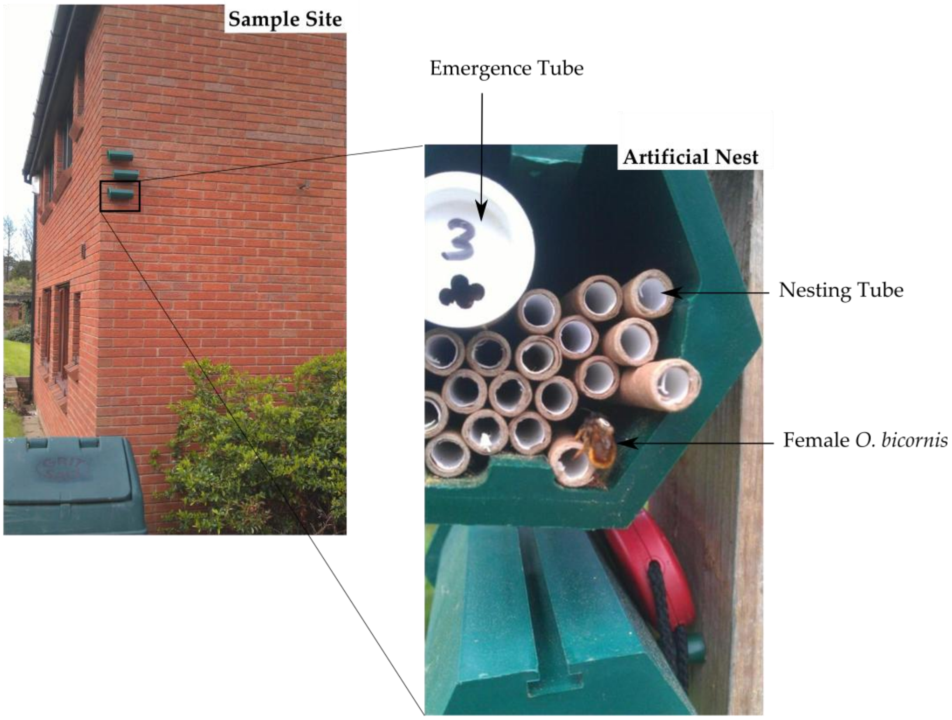


Figure 6.2: Example of *Osmia bicornis* artificial nesting sample site and artificial nest. Each sample site comprised three artificial nests (right). A set number of bees of standardised weights and gender were released in the emergence tubes for each sample site. Nesting tubes comprised an outer cardboard tube with a paper inner lining. These tubes were replaced throughout the sample period. Photos: R Fowler.

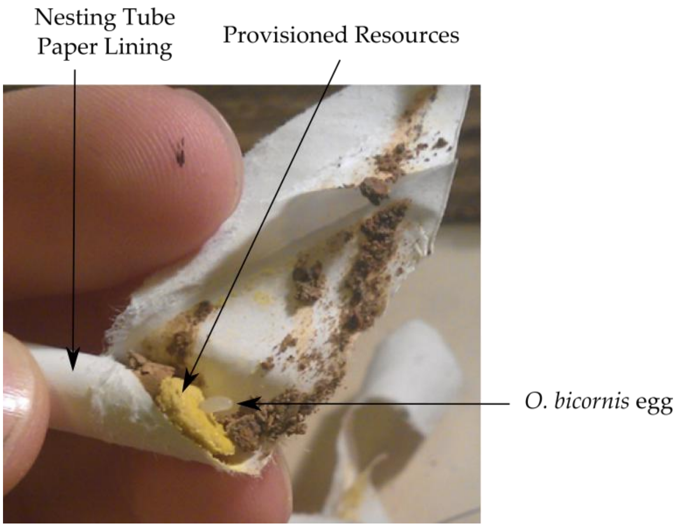


Figure 6.3: Example of the inner lining of the nesting tubes used to collect the resources provisioned by *Osmia bicornis*. Three tubes of *Osmia bicornis* provisioned brood cells were collected before the larvae had hatched and began consuming the provisioned resources (made up of >95% pollen). Paper tubes lining each nesting tube allowed for the easy removal and collection of provisioned resources for measuring quality and quantity. Photo: R Fowler.

6.2.3.1 Provisioning Success

After the sampling period, emergence tubes of the bees released at each artificial nesting site were checked to make sure they had not all failed to emerge and influenced the standardisation of the experiment. Of the newly provisioned cells, the nesting tubes capped in each site were removed, placed in a secure location and allowed to mature into adults over the summer. During the winter of 2012/13, after the offspring had pupated into adults, the nesting tubes were processed (as in Figure 6.3 but as cocoons), counted and the total provisioned brood cells recorded as a measure of provisioning success per site.

6.2.3.2 Provisioned Resource Quality and Quantity Analysis

Three capped nesting tubes were selected at random from each site during the sampling period to be used to gain estimates of the quality and quantity of provisioned resources. This was undertaken at the same time for all sites three weeks after adults had emerged. Tubes were placed in a -18ºC freezer to preserve the provisioned resources until analysis in the winter of 2012/13. For the analysis, the samples were removed from the freezer, the tubes were opened and provisioning resources were removed from each cell (Figure 6.3). Care was taken to remove the loam partitioning from each cell and limit its mixing with the resources provisioned. All resources from each cell were placed in a freeze drier for 24 hrs at -80ºC and weighed to the nearest mg as a measure of resource quantity.

To alleviate the potential influence of resource variation between cells in different positions of the nesting tubes, a standard protocol was used for cell selection. This protocol was to select six cells from the tubes collected, the first and last cell from tube 1, the second from first and second from last from tube 2 and the third from first and third from last cells from tube 3. For a comparison of resource quality between sites, an estimate of protein was determined. Full protein extraction of pollen is possible through acid hydrolysis but for this study, it was decided a light extraction would be adequate to dissolve the exine (outer layer) of the pollen grains and gain a measure of protein content without such an intensive method. From each of the six cells selected 50 mg of pollen was added to 5 ml ethanol (70%) with 0.1 ml of hydrochloric acid (0.1 mol/L). Then the sample was placed in an ultrasonic bath for 5 minutes to ensure homogenisation of samples before being centrifuged (5430, Eppendorf) at

2,500 rpm for 5 minutes. After centrifuging, 4 ml of supernatant was removed and used to test for protein content analysis following the Bradford Assay procedure (Bradford, 1976). This involved the binding of protein to Coomassie Brilliant Blue G-250 dye which is then tested for absorbance at 595 nm against a known protein standard to generate a standard curve for which the protein content of unknown samples can be estimated.

6.2.3.3 Landscape Context and Vegetation

In ArcGIS v10 (ESRI, 2011) a measure of exposure was extracted for each artificial nesting site location by creating a 'Hillshade' layer using a photogrammetrically derived Digital Surface Model (DSM) of the sample area (OS MasterMap, 2009). OS MasterMap data of landscape metrics were then separated into three categories: built space was defined by 'buildings', 'man-made structures', 'pavements', 'roads', 'manmade open space/landforms', 'railway' and 'developing land'. Gardens were classed as 'gardens' of all sizes. Green space consisted of 'open natural habitat' (natural open space, natural habitat without trees, natural landforms, natural railway no trees and natural roadside no trees) and 'covered natural habitat' (natural roadside with trees, mixed natural habitat with trees and natural railway with trees) (Ordinance Survey, 2006).

As estimating potential foraging availability for *O. bicornis* is made difficult by the nature of the sampling environment and variability of foraging preferences in *O. bicornis*, a measure of vegetation cover in the vicinity to artificial nesting sites was used as a proxy for viable habitat. Using the same concentric buffer zones as for the landscape context metrics, a measure of vegetation density in m^2 for each site was extracted using aerial near-infrared and colour photography at 2 × 2 m resolution (Bluesky International Limited, Leicestershire, 2007). With the foraging range of *O. bicornis* being on average 500 m from nesting site (Gathmann and Tscharntke, 2002), yet potentially reaching up to 900 m (Vicens & Bosch, 2000), the total area (m^2) of each landscape context metric and the vegetation metric was extrapolated within concentric circles from 100 m up to 1 km. This was undertaken by using concentric buffer zones surrounding each artificial nesting site in 100 m sequential increments, using the Geospatial Modelling Environment (GME) 'Polygon Intersect' tool (Beyer, 2012). See section 2.2 Methods for more details of this procedure.

Table 6.1: Description and sampling method of response and explanatory variables applied to the Generalised Linear Models (GLMs) to test the importance of resource quantity, quality and landscape context on the provisioning rate of *Osmia bicornis*.

	Metric Name	Description of Variables	Sampling Method
Response	**Total Provisioned Brood Cells**	The net total brood cells provisioned at each artificial nesting site.	The number cells produced at sites, with three artificial nests with 29 tubes in each
Explanatory Variables	**Resource Protein Estimate (mg)**	An estimate of protein content of provisioned resources per artificial nesting site	Mean taken from 6 × cells per site
	Resource Quantity (mg)	The amount of provisioned resources per artificial nesting site	Mean taken from 6 × cells per site
	Built Space (m^2)	Buildings, man-made structures, pavements, roads, manmade open space/landforms, rail and developing land.	Derived from OS Mastermap maps, m^2 of area within buffer zones ranging in 100 m intervals from 100 m to 1,000 m.
	Gardens (m^2)	Gardens of all sizes.	Derived from OS Mastermap maps, m^2 of area within buffer zones ranging in 100 m intervals from 100 m to 1,000 m.
	Green Space (m^2)	Open natural habitat (natural open space, natural habitat without trees, natural landforms, natural railway no trees and natural roadside no trees) and covered natural habitat (natural roadside with trees, mixed natural habitat with trees and natural railway with trees).	Derived from OS Mastermap maps, m^2 of area within buffer zones ranging in 100 m intervals from 100 m to 1,000 m.
	Exposure	The level of exposure each site is subject to throughout the day (including shade from buildings and trees)	Measurement derived from ArcGIS "Hillshade" tool using a height map photogrammetrically derived Digital Surface Model (DSM) (OS MasterMap, 2009)
	Vegetation (m^2)	The amount of vegetation for each sample site.	Derived from height and aerial near-infrared and colour photography (Bluesky International Limited, Leicestershire, 2007), area (m^2) within buffer zones ranging from 100 m to 1,000 m.

6.2.3.4 Reproductive Success

To measure how successful the provisioned brood cells were in each site, following pupation a sub-sample of 30 cocoons was chosen at random from each test site. These 30 cocoons were weighed and averaged per site to gain a measure of mean offspring weight. Bees were then placed in a ventilated container and kept until they emerged in the spring of 2013. Upon emergence, the ratio of successfully emerged females to males was recorded as a measure of the proportion of females produced.

6.2.4 Data Analysis

Variables were compared for correlations using either Pearson's Product Moment Correlation for parametric data or Spearman's Rank Correlation for non-parametric data.

6.2.4.1 Generalised Linear Models (GLMs)

Response and explanatory variables are detailed in Table 6.1. To assess the provisioning rate of *O. bicornis*, the total number of provisioned brood cells per site was modelled as the response variable in a GLM. Following Zuur et al. (2010), data exploration began with all variables subjected to box plots and Cleveland dot plots to check for outliers. Explanatory variables were checked for co-linearity prior to running models, to make sure assumptions of independence were not violated. As with over-dispersed ecological count data, alleviating the need to log response variables can be important in maintaining the variation in the data, and hence negative-binomial errors were applied to models (O'Hara et al., 2010). As there were 10 potential scales in which the landscape context and vegetation variables could be applied to a GLM, the most parsimonious model was selected using the Information-Theoretic (IT) approach following Burnham and Anderson (2002).

Q-Q plots were generated for each model to test for normality, as well as plotting model residuals against the fitted values to check the heterogeneity of the model. Cook's distance and Leverage plots were tested to make sure that individual sites did not have a disproportionate influence on the model. Correlograms were generated from model residuals to test for spatial auto-correlation in the data. Statistical analysis was undertaken using the free software R v. 2.14.1 (R Core Team, 2012; www.r-project.org) within RStudio (RStudio, 2012).

6.3 Results

Emergence tubes with the loose pre-collected *O. bicornis* cocoons were checked after the survey period and all individuals had emerged successfully from their cocoons. This was most likely due to the cleaning of cocoons and removal of any dead, parasitized or unhealthy bees prior to release in each sample site. It was therefore considered that each sample site was standardised in nesting potential and initial bee emergence.

6.3.1 Provisioning Success

A total of 2,157 brood cells were provisioned from across all sample sites. A mean of 107±11(SE) brood cells were provisioned for each sample site, ranging from 35 to 212. Per site, a mean of 13±2(SE) cells failed outright as no egg was laid or the egg died before eating the provisioned resources. This appeared to increase with the number of cells provisioned (r=0.49, n=20, p=0.02). The rate of parasitism on the provisioned cells was small with an average of 3.6 cells showing signs of parasitism and causing an average of 2.4 larval fatalities between sites, equating to 3.3% of the total brood cells produced. This left 1% cocoons which simply failed at either the pupation or cocoon weaving stages of development, and 1,849 (85.7% of total) provisioned brood cells that survived the summer, pupated and had woven a cocoon successfully, ready to overwinter as adults. Across the sample area, this equated to an average recruitment at each site of 4 bees for each adult bee released, ranging from 1.3 to 9.1.

6.3.2 Resource Quality and Quantity

On average 261.1±8.5 mg of resources were provisioned for each cell. The quantity of resources appeared to differ based on the position of each cell in the nest tube (Figure 6.4) with greater provisioned resources found towards the cells at the back of the tube (the first ones provisioned). This trend was not found for the quality of resources. The quality of resources was determined as 1.65±0.004 mg of protein across the sample sites (Figure 6.4).

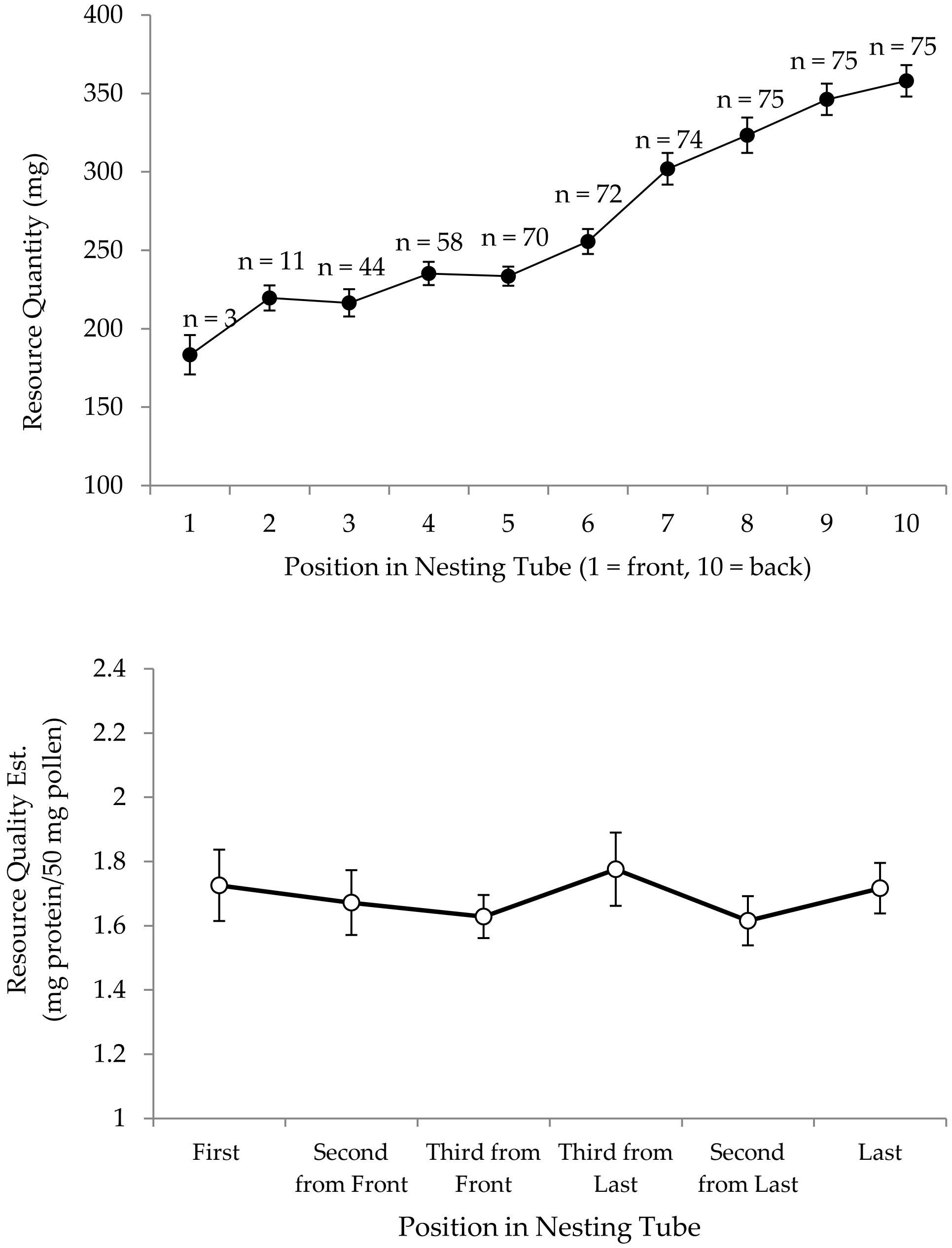


Figure 6.4: The quantity and resource quality for each cell relative to its position in the nesting tube. Resource weight was taken from all cells in three tubes removed from sites, and protein was taken from six cells of these tubes in set positions (see 6.2 Methods for more details). the number of provisioned cells recorded in that position from collected tubes is represented by n for resource quantity, whilst n=20 for each sample site for resource quality. Error bars = ±1 SE.

6.3.3 Provisioning Success

Prior to statistical modelling, no differences between the types of nesting site were found. Several landscape context variables were found to be co-linear with each other and the vegetation variable. This left gardens and vegetation in the models (Figure 6.5). The IT method used to select the scale of variables for the landscape context model is shown in Table 6.2. The small differences in w_i (rounded Akaike weights) of model scales shows there is weak support for selecting one model scale from another. The significant positive correlation between total provisioned brood cells to resource quality was evident in all models, and was the only significant relationship found (Table 6.3: Figure 6.6)

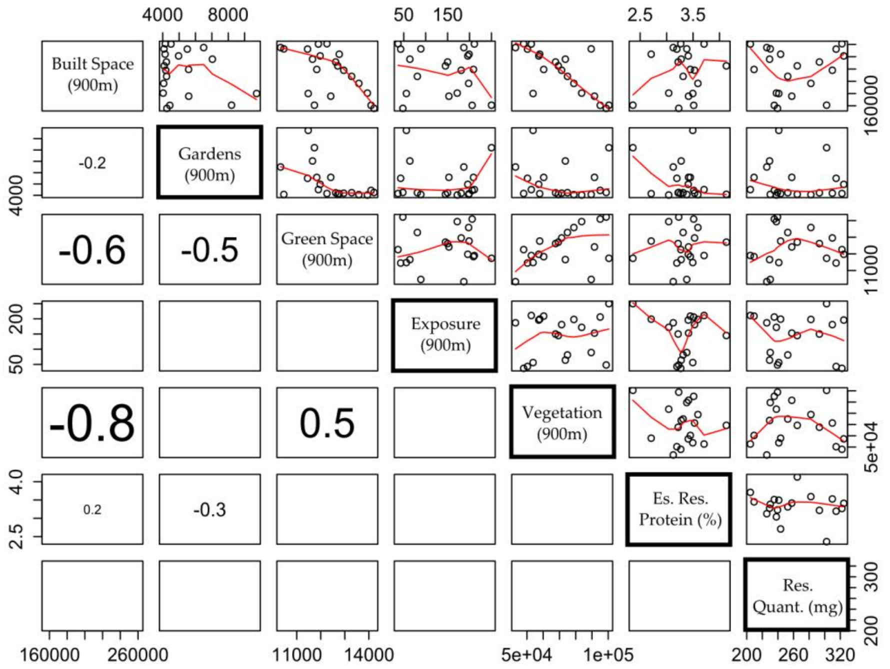


Figure 6.5: Correlation matrix of the explanatory variables uses in Generalised Linear Models (GLMs) to test the importance of resource quantity, quality and landscape context on the provisioning rate of *Osmia bicornis*. The correlation coefficient is given in the lower boxes and the relationship is presented in the boxes above. The variables chosen for the final model are highlighted in thick lines. Correlations with coefficients below 0.2 or -0.2 are not shown.

Table 6.2: The output from the provisioning success models for each landscape context and vegetation scale used to test the importance of resource quantity, quality and landscape context on the provisioning rate of *Osmia bicornis*. The IT approach highlights gardens and vegetation metrics recorded within a concentric circular area of 900 m from each site as the most parsimonious model scale. However, as there are no great differences in w^i it is observed that these landscape metrics did not have significant influence on the model output. The 900 m model is therefore used as the final model output. Δ_I = AICi – min(AIC). w^i = rounded Akaike weights.

Scale Ranking (m)	AIC	Δ_i	exp'-½Δi	w_i	Sum(exp'-½$\Delta_{j)}$
900	205.34	0.000	1.000	*0.180*	5.564
1,000	205.53	0.190	0.909	*0.163*	
800	205.69	0.350	0.839	*0.151*	
700	206.27	0.930	0.628	*0.113*	
600	206.894	1.554	0.460	*0.083*	
500	206.895	1.555	0.460	*0.083*	
200	206.92	1.580	0.454	*0.082*	
400	207.016	1.676	0.433	*0.078*	
300	207.27	1.930	0.381	*0.068*	
100	208.25	2.910	0.233	*0.042*	

Table 6.3: Generalized Linear Model (GLM) output from the final model of the 900 m scale provisioning success model used to test the importance of resource quantity, quality and landscape context on the provisioning rate of *Osmia bicornis*.

***Response*/Explanatory Variables**	*p*	R^2
Total Provisioned Brood Cells		*58.03*
Resource Quantity (mg)	0.155	
Resource Quality Estimate (mg)	<0.001	
Exposure	0.956	
Vegetation m^2 (900 m)	0.698	
Gardens m^2 (900 m)	0.062	

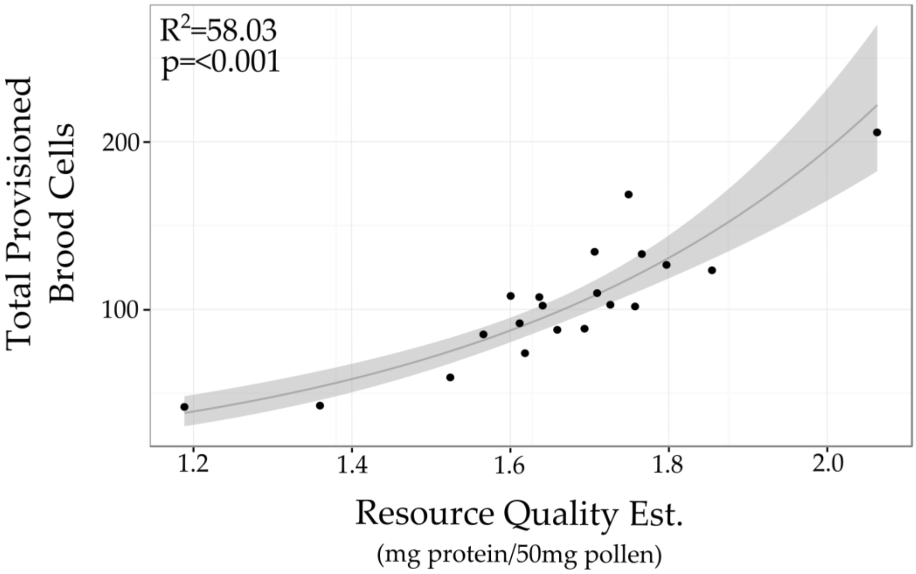


Figure 6.6: Scatter plot representing the significant relationship of total provisioned brood cells per site and the resource quality estimate taken from six provisioned brood cells at each site (Table 6.2). The grey line indicates the relationship and grey areas represent the 95% confidence interval. p = the significance of the relationship in the model, R^2 = the square of the correlation for each abundance metric model (also known as a proportion between 0-100% of model fit).

6.3.4 Reproductive Success

Of the 30 cocoons sub-sampled per site, the offspring weighed an average 130.3 ±9.2 mg per site, ranging from 117.8 to 151.9 mg. Per site mean offspring weight did not correlate between total provisioned brood cells (r=0.29, n=20, p=0.21) but it did increase with greater resource quality (r=0.49, n=20, p=0.026; Figure 6.7). Across all sites the mean percentage of females produced was 39±2.12 % with only four sites having >50% of females produced. The percentage of females appeared to be positively correlated with total brood cells provisioned (r=0.47, n=20, p=0.03; Figure 6.8).

Table 6.4: Correlation matrix showing the correlation coefficients (r) and significance for measures of provisioning success, resource quality, resource quantity and measures of reproductive success of *Osmia bicornis* for each site in an urban landscape.

	Total Provisioned Brood Cells	*Resource Quality Est.*	*Resource Quantity (mg)*	*Mean Offspring Weight (mg)*
Resource Quality Est. (mg protein/50mg pollen)	0.62**			
Resource Quantity(mg)	0.10	-0.15		
Mean Offspring Weight (mg)	0.29	0.49*	-0.37	
Proportion of Females	0.47*	0.29	-0.41	0.42

***p<0.05, **p<0.01**

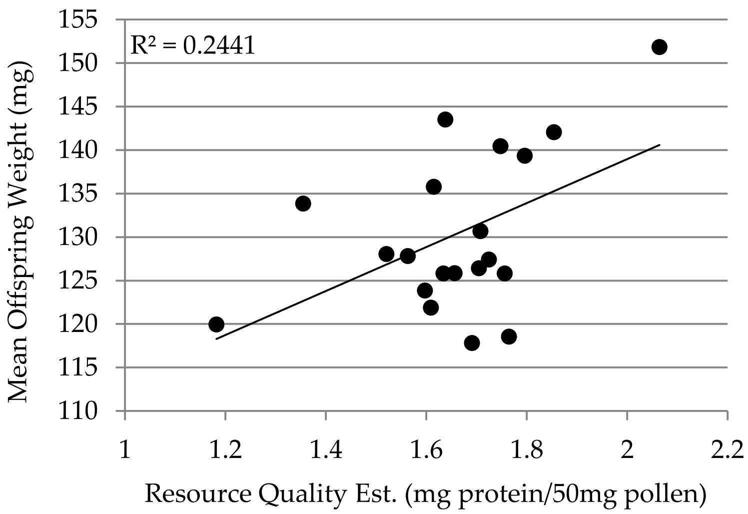


Figure 6.7: The resource quality of six brood cells was significantly correlated to the averaged offspring weight (mg) measured from 30 cocoons per site. r=correlation coefficient, *=p<0.05

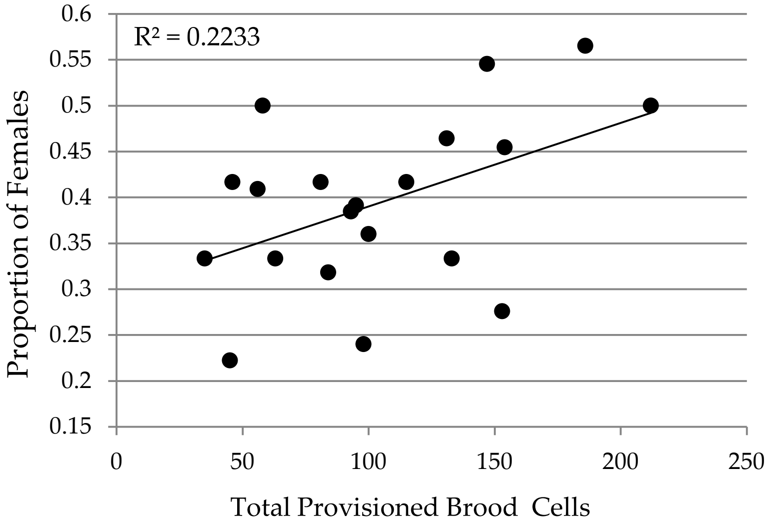


Figure 6.8: The proportion of females produced from a sub-sample of 30 cocoons was significantly correlated to the total number of provisioned brood cells per site. r=correlation coefficient, *=$p<0.05$

6.4 Discussion

The dietary generalist cavity-nesting bee *O. bicornis* is a species commonly found in urban and suburban areas in the UK (Bates et al., 2011; Chapter 2). *O. bicornis* populations are known to be predominantly determined by bottom-up pressures, most notably in nest-site limitation (Steffan-Dewenter & Schiele, 2008). This suggests nesting opportunities are available in urban areas for these species to provision brood cells and reproduce effectively.

With artificial nesting sites placed in the sample area, the quality of resources provisioned significantly increased the total provisioning success of *O. bicornis* (Figure 6.6). In contrast, landscape context and vegetation metrics showed no influence over the provisioning success of *O. bicornis* with different scales of these metrics having little impact on model performance. This suggests that the quality of available resources is more constraining of provisioning ability than other factors in an urbanised environment once nesting site availability is limited. Previous studies investigating *O. bicornis* nesting potential in urban environments show that micro-climatic variation can influence nest selection (Everaars et al., 2011). This chapter's analysis standardised nest site location on brick walls and showed no association with measures of exposure in statistical modelling. Therefore, with a lower possibility of nesting site location affecting provisioning rate, it is suggested that the quality of resources in the surrounding area of nesting site influences the provisioning success of *O. bicornis*.

In previous studies, the availability of a viable source of quality pollen is crucial in maintaining bee populations (Franzén & Nilsson, 2010). Using olfactory cues (Cook et al., 2003; Grüter et al., 2008), eusocial bees can regulate the quality of pollen collected and stored in the colony (Kitaoka & Nieh, 2009). This chapter did not accounted for pollen choice in the sample area by *Osmia bicornis*. The resulting increase in provisioning success is most likely an in-direct effect from a greater abundance of protein-rich resources in the local vicinity increasing the number of females choosing to use these particular nesting sites. Maternal site fidelity in *O. bicornis* is shown to be as high as 80% (Steffan-Dewenter & Schiele, 2004) and is thought to be higher if nesting and foraging resources are in abundance. This suggests that sites that did well in provisioning success may have already been in habitats of greater quality resources. Alternatively, there were natural populations of *O. bicornis* already present within these sites, thereby increasing the number of provisioning females in that site and the amount of provisioned cells. The dispersal rate and movement of bees between sites was not recorded in this study so it was not possible to corroborate this, although it is something that could be explored in future research following the methodology set out by Steffan-Dewenter & Scheile (2004).

Another explanation for this indirect effect would be that bees were able to select pollen of greater quality because within their foraging range there was increased availability of pollen sources on offer. Bees can provision more brood cells in a shorter period of time when suitable resources are in closer proximity to their nests (Zurbuchen et al., 2010). Therefore, as the surrounding area increased in floral availability and diversity, bees may have been able to provision cells at a faster rate and with greater selection of better quality resources. This is beyond this chapter however, but the implications of this should be taken into consideration in future research.

Previous literature has suggested that pollen quantity affects bee body size and subsequently important aspects of reproductive success more so than pollen quality (measured as the diversity of pollen species provisioned; Radmacher and Strohm, 2010). In this chapter's analysis however, resource quantity showed no relationship to provisioning success or any measures of reproductive success of *O. bicornis*. This may have been because the difference in provisioned resource quantity between brood cells was determined by its placement within the nesting tube (Figure 6.4). Previously, research has suggested that there is a link between the length of tubes and the proportion of females produced (Gruber et al., 2011). Bees exhibit haplodiploidy and are able to determine the sex of offspring they produce (Gerber & Klostermeyer, 1970; Bosch & Vicens, 2005; Seildelmann et al., 2010). From observations when opening overwintering cocoons of *O. bicornis*, it would appear males are usually laid towards the front of the cells whilst females are laid first at the back of the tube. Although the gender of bees was not recorded in relation to the quantity and quality of resources, it was apparent that the amount of pollen increased in the cells provisioned earliest. There could be a temporal influence with greater pollen resources available earlier in the season but is more likely associated with protecting female bees from parasitism and predation. Ultimately, this difference in resources provisioned along the nest tubes may have influenced the effect of quantity on the provisioning success models. Cell placement should therefore be taken into account when measuring the quantity of provisioned resources, and still may be an important factor in the provisioning success of bees.

It was apparent that alongside increased provisioning success in *O. bicornis*, there was an increase in the percentage of females produced (Figure 6.8). When foraging for resources becomes difficult, either through reduced or distant resources, sex allocation increases towards the sex which takes less effort to provision (i.e. males in *O. bicornis*). This is because male *O. bicornis* have on average smaller body weight and shorter lifespan meaning they require fewer resources to females (Ulbrich & Seildelmann, 2001; Ivanov, 2006). When potential foraging is closer and greater in abundance to nesting sites this is reversed and more females are produced (Ivanov, 2006). Therefore, the variation in females produced may have been due to larger female bees laying more female eggs in certain sites, whilst smaller females lay male eggs as they are easier to provision (Ivanov, 2006; Seildelmann et al., 2010). As the number and size of bees released was standardised throughout the sampling area, the differences in females produced were either as a result of potential foraging quality of the surrounding habitat allowing bees to provision sufficient resource for female production, or that the site quality attracted larger females to nest there and usurp smaller females (Kim, 1997; Steffan-Dewenter & Schiele, 2004). More females within a habitat could directly influence the population dynamics of bees with a greater number of potential nest founders in that habitat for next season. In addition, as females are more active in visiting flowers for provision collection they are considered greater in pollination effectiveness compared to males. Helping to promote increases in female numbers in bees is an important aspect for further conservation efforts to take into consideration. This chapter suggests promoting habitat quality alongside adequate nesting resources could promote greater percentages of females in urban habitats.

Another aspect of reproductive success in *O. bicornis* is the size of offspring, which was measured in this study as the weight of both bee's body and cocoon. It was found to increase alongside the estimate of resource quality (protein) for each site. It is clear from the literature that the survival and future reproductive success of bees is directly and indirectly based on the quality of provisioned resources is. In the early stages of life, larger offspring are more successful in overwintering and provisioning young the following season (Strohm et al., 2002; Bosch & Kemp, 2004) and less susceptible to parasitism and disease (Di Pasquale et al., 2013; Alaux et al., 2010). Once emerged, larger females can achieve more desirable nesting space (Kim, 1997; Steffan-Dewenter & Schiele, 2004), are more successful in egg laying (Kim, 1997; Strohm et al., 2002) and have increased foraging abilities (Kim, 1997; Gathmann & Tscharntke, 2002; Bosch & Vicens, 2006; Neff, 2008). Likewise, for male bees body size is linked to their ability to hold mating territory (Alcock, 1994). Pollen quantity and temperature have been shown to influence *O. bicornis* offspring development (Radmacher & Strohm, 2010), but pollen quality has not been shown to influence them. Controlled experiments in other species of bee show a larval diet with greater protein content increases offspring body size (Roulston & Cane, 2002; Quezada-Euán et al., 2011).

Therefore, not only does pollen quality indirectly affect provisioning success, but also the reproductive fitness of offspring produced. The next step towards increasing resource quality in terms of protein would be to identify what flowers bees are using in the urban environment, document their protein content and advise on planting schemes.

In previous studies where nesting resources have been supplemented in fruit orchards, *O. bicornis* populations display an increase of 2.8 (Gruber et al., 2011) and 2.4 (Steffan-Dewenter & Schiele, 2008) bees for each one in previous years. Over all sample sites, I recorded a population increase of 4.03 bees for each bee released. This is much higher than previous studies, but difficult to compare as this is for only one season and there was only 3.3% compared to other studies with 17.1% of nest tubes showing signs of parasitism and 13.6% found dead in their brood cells due to parasitism (Steffan-Dewenter & Schiele, 2008).In addition, this study design meant that unhealthy cocoons were excluded from the initial bees released, effectively removing the decrease in population due to fatalities. Therefore, although these results could suggest that urban areas are highly beneficial to *O. bicornis* population growth, this study represents a managed population more so than a naturally occurring one. Even so, this experiment's supplementation of nesting sites allowed a substantial number of *O. bicornis* offspring to be produced. Therefore, it seems likely that *O. bicornis* are nest-limited in the urban environment, supporting results obtained in managed orchards (Steffan-Dewenter & Schiele, 2008).

O. bicornis is a species of bee known for their success in urban and suburban environments specifically within this study region (Bates et al., 2011; Chapter 2), most likely due to their generalist foraging and cavity-nesting traits (Chapter 4). In terms of the conservation of bees, supplementing the nesting potential in urban sites could go far to increase the population of bees. Furthermore, once nesting sites are not limited, the quality of resources available is shown as an important determinant of both provisioning success and reproductive success. By providing artificial nesting sites in urban areas for other species, as long as adequate resources are available urban areas can become a viable habitat for bees and mitigate species' loss in light of increased urbanisation.

Chapter 7

Thesis Conclusions

7.1 Introduction

By 2030, it is estimated that over five billion people will reside within urban areas (United Nations, 2011). Increased levels of urbanisation to cater for population increase may lead to impervious surfaces limiting vegetation and suppressing potential habitat (McKinney, 2008) causing habitat reduction, isolation and fragmentation (Young & Jarvis, 2001). To ensure valuable ecosystem services continue to provide benefits to human populations, alleviating the loss of species diversity along urban-rural gradients is essential. Bees help to provide pollination services to 87.5% of all angiosperms (Ollerton et al., 2011) and to 35% of global food crops (Klein et al., 2007). Greater abundance, diversity and trait complementarity in bee assemblages can lead to increased pollination efficiency and effectiveness (Kremen et al., 2002; Hoehn et al., 2008; Bommarco et al., 2012; Garibaldi et al., 2013; Klatt et al., 2014). The impact of urbanisation on bees shows contrasting results depending on region and the type of site sampled. Consistent throughout the literature however, is how species-specific responses of bees along urban-rural gradients are evident across studies (Frankie et al., 2005; Cane et al., 2006; Kearns & Oliveras, 2009; Bates et al., 2011; Banaszak-Cibicka & Żmihorski, 2012).

This thesis set out to improve understanding of how bee assemblages are altered along the urban-rural gradient, to gauge the impact this may have on the ecosystem services of pollination, whilst also improving the documentation and understanding of bee traits important to function. To further understanding of bee assemblage change along an urban-rural gradient, this thesis increases regional and site type sampling replication within Birmingham, UK following on from Bates et al. (2011).

7.2 Main findings

7.2.1 Bee assemblage variation along an urban-rural gradient

In church yards and cemeteries, Bates et al. (2011) found greater bee abundance in rural areas compared to suburban areas. However, in Chapter 2 canal side and parkland sites showed contrasting results, with more bees found in urban and suburban areas. This implies that sample site type plays an important role in the bees recorded and reinforces the need for further site type replication along the urban-rural gradient. In Birmingham, gardens have not been sampled highlighting the need for further investigation in other habitat types.

McIntyre (2000) suggested that arthropods show patterns in their response between urban and rural areas with: (i) rural taxa showing little or no presence in urban sites; (ii) urban taxa showing greater abundance or only occur in urban sites; and (iii) some taxa showing no difference between the two. Chapter 2 and previous literature (e.g. Cane et al., 2006; Fetridge et al., 2008; Matterson et al., 2008; Banaszak-Cibicka & Żmihorski, 2011; Bates et al., 2011) show this is usually the case with bee assemblages. The species recorded in this thesis' urban and suburban areas included 64 species from the total 76 species recorded throughout the entire experiment. Previously, urban and suburban areas have been noted to represent a large proportion of the regional bee diversity (Frankie et al., 2009) helping to provide pollination services throughout urban landscapes (Tommasi et al., 2004). Hence, within Birmingham canal sides and parkland sites may be beneficial to bees along the urban-rural gradient.

The implications of species' variation on the ecosystem services of pollination are not clear. By further understanding the relative importance of species as individuals and as part of assemblages which facilitate pollination services, the effect of species-specific variation on pollination services will be assessed better.

7.2.2 Furthering knowledge of potential influential traits to function

The thesis set out to add to knowledge of the functional traits in bees that can add to or complement pollination efficiency in bees. It attempted to increase the understanding of the potential morphological differences in bees may affect pollen carryover by bees through an analysis of pilosity. This analysis found that body size and the type of pollen transport adaptation of bees explained a proportion of the variation found in bee pilosity. This could have implications for pollen carryover in bees as previous analysis of trait complementarity reported body size as an important component in providing pollination, with larger bees transferring more pollen between flowers (Hoehn et al., 2008; Albrecht et al., 2012). In terms of trait analysis, pollen transport adaptations have been used before (Moretti et al., 2009) and from our analysis, they explain hair length on areas adapted for pollen transport. Therefore, it is recommended that the pilosity of bees on areas of passive pollen collection and incidental pollen transport should be applied to future analysis, testing the variation and complementarity of pilosity metrics to pollination.

7.2.3 The importance of traits in defining bee assemblage variation along the urban-rural gradient and the potential this may have for pollination services

When traits were applied to the bee assemblage data collected as part of Chapter 2, there were clear associations between particular traits along the urban-rural gradient. Bees more commonly found in urban and suburban areas were social, foraging generalists, cavity nesters, that carried pollen on corbiculae, that were in general larger, had longer flight periods and were active in the spring and summer months. In contrast, bees in rural areas were more likely to be cleptoparasites, be ground-excavating nesters, have two broods per year, carry pollen on their legs and body with branched hairs and were active in the spring. In terms of trait diversity, urban and suburban sites had bee assemblages with a greater diversity of nesting strategies and durations of activity. On the other hand, bees of rural areas had increased trait diversity in foraging specialisation of assemblages and greater diversity in lecty, tongue types and seasonal presence.

This highlights the importance of traits in assessing the change in bee assemblages between land-use types. It could also indicate trait complementarity loss within urban areas, with traits relating to foraging specialisation (e.g. lecty and tongue type) significantly more abundant in rural sites. However, a greater abundance of larger generalist bees in urban and suburban areas could compensate and provide pollination services despite loss of certain species (Albrecht et al., 2012; but see Winfree & Kremen, 2009). Interestingly, foraging specialists were found to increase in urban areas suggesting that canal side and parkland sites in urban areas may provide potential foraging and nesting resources for specialist species (e.g. *Melitta* haemorrhoidalis). This was supported in Chapter 5 which recorded the oligolectic species *Chelostoma campanularum* visiting test plants *Campanula glomerata* in urban areas.

7.2.4 Testing the pollination services provided between urban and rural land-use types

Chapter 5 reported that seed-set of test species did not vary between the urban and rural sites of the study area. This suggests that across Birmingham, species-specific responses to the urban-rural gradient (Chapter 2) which led to trait diversity variation (Chapter 4) did not have significant influence on the pollination services bees provide. This was corroborated by similar levels of bee visits on test flowers between land-use types, suggesting equal pollen carryover in both land-use types. Before conclusions can be made regarding the implications of urbanisation on pollination services however, different species of plant (e.g. broad bean, vipers bugloss) will need to be tested in more site types (e.g. gardens and parkland) in different regions.

7.2.5 Resource provisioning potential and subsequent population dynamics of bees within an urban context

Once nesting sites were added to an urban environment, the main determinant of brood cell provisioning success was resource quality. This suggests that the quality of resources in the immediate area of nesting sites could play an important role in provisioning rate of their offspring. In addition to increased provisioning rate, sites showed larger offspring and greater percentages of females produced with protein content of resources provisioned. This suggests there were implications for the reproductive success for *O. bicornis* between sites based on the level of quality of resources bees were able to provision. This leads me to believe that both nesting and foraging resources are integral in promoting reproductive success of bees in urban environments. Therefore, although nesting sites are required to support a diversity of bees in the first place, along the urban-rural gradient quality foraging resources are needed to ensure population growth and reproductive success in bees.

7.2.6 General Conclusions

This thesis found that the canal sides and parkland of Birmingham are important habitats for bees along the urban-rural gradient and showed contrasting results to churchyards and cemeteries in the same area (Bates et al., 2011). Species-specific responses, most likely determined by their individual traits, were evident. However, this showed no deleterious impact on pollination service in *Campanula glomerata* between urban and rural areas. This highlights the importance of further analysis of functional traits and their individual and complementary importance towards the function of pollination. This would greatly benefit analysis of trait diversity along the urban-rural gradient and its impact on pollination services. One way this thesis suggests mitigating species change and potential trait diversity loss in urban areas is through providing a diversity of nesting and foraging resources throughout the urban-rural gradient. Chapter 4 shows how nesting and foraging traits dictate bee assemblage variation across this gradient, whilst Chapter 6 highlights the potential of urban bee populations as long as nesting and foraging resources are present and of sufficient quality.

7.3 Potential limitations of the study

- This thesis' investigation concentrates on one region only, namely Birmingham, UK and the wider area. Although this study builds upon the previous work of Bates et al. (2011) by increasing the level of regional and site type replication, there is still a limit to the comparability of findings to other cities. A study of multiple cities will allow for further interpretation of how bee assemblages are affected by different urban-rural gradients. However, for this thesis, it was decided that effort and resources were better suited to intensive survey and investigation within the same region to complement each another.

- In terms of methods, one limiting factor of this study is the division of land-use types. Urban, suburban and rural sites were grouped based on a more complex grouping system of 1 km^2 blocks of land by Owen et al. (2006). However, it could be argued that the ‘dense suburban’ group that Owen et al. (2006) used could be placed either in urban or suburban land-use type. Hernandez et al. (2009) stated that variety of ways the urban-rural gradient is measured one of the limiting factors in achieving a comprehensive conclusion in studies testing bee assemblages along urban-rural gradients. Therefore, it is important to future studies that the use of a methodological framework specifically associated with how urban, suburban and rural areas are defined.

- In addition to this, the vegetation data derived from photogrammetry may not be fully representative of all vegetation. This method involved detecting the colour green from aerial photography, rather than measuring vegetation itself. Although the vegetation layer was checked and corrected in ArcGIS v 10 before analysis, there is still a level of inaccuracy within this dataset.

- One main constraint of the analysis when comparing species and their responses to the urban-rural gradient is that presence does not necessarily mean nesting or survival at that particular site. The relative size of bees (Gathmann & Tscharntke, 2002) can define their foraging range and dispersal ability. Therefore, the greater abundance of social bumblebee species in urban areas could be a consequence of greater foraging range allowing them to use the resources available in isolated habitats. This is one potential consequence of analysing all species based on landscape scales as bees with greater foraging ranges may be surveyed but not necessarily be nesting in the site they are found. An experiment investigating the genetic diversity of the bees foraging in urban habitats (such as that of Chapman et al., 2003) could have provided clarity in how dense and related bumblebee populations were in this study area if genetic sampling was undertaken. Otherwise, estimating nesting sites through intensive sampling may have given an idea of the nesting potential each site provided.

- Bee assemblages are known to fluctuate between seasons and years (Oertli et al., 2005). Although bee assemblage data were collected over two years, there still may have been seasonal variation in the data. Likewise, for the artificial nesting experiment in Chapter 5 and 6, this was for only one season. Further research between seasons is required to support this chapter's findings and test that reproductive success is consistent with our findings between years.

7.4 Implications for conservation and planning policy

As nesting site limitation is noted as a main determinant of a species' population growth (Steffan-Dewenter & Schiele, 2008), this thesis suggests that creating more diverse nesting opportunities for a variety of different species of bee is an effective form of conservation for bees along urban-rural gradients. However, as foraging quality is associated with provisioning and reproductive success in urban areas (Chapter 6), a diversity of foraging resources is also essential in providing benefits to bees along urban-rural gradients. From the results of this thesis, it is suggested that increasing the diversity of nesting and foraging resources available throughout the urban-rural gradient is crucial in limiting the loss of species from different land-use types.

Through the experiments undertaken in this thesis, it is apparent that canal side and parkland sites used in the sampling of data for Chapters 2 and 4 provided beneficial resources to bees in urban areas. These sites could be used as focal points for such conservation efforts, to increase nesting and foraging opportunities for a diversity of bee species as they are already using these sites. However, the importance of other aspects of habitat should not be overlooked as a variety of green space and gardens could be potentially beneficial habitats for bees (Goulson et al., 2003; Cussans et al., 2010).

Through conservation efforts, evidence suggests that the application of management for wild bees has helped to increase a range of other biodiversity. The planting of meadows with nectar and pollen-rich seed mixtures intends to increase pollinator abundance and diversity. This has been seen to result in a cascade affect whereby ecosystem services such as soil protection and water quality have been increased in sites designated for bee conservation (Wratten et al., 2012). Hence, providing resources for bees along the urban-rural gradient may have a subsequent benefit to other taxa (e.g. hoverflies and wasps) and support a range of ecosystem services.

7.5 Recommendations for future research

Every city is unique in its development, form and geographical context, which limits the comparison of ecological analyses between cities. When assessing bee assemblages between cities, there are inherent differences in the species present, limiting comparison of species and their responses. The comparison of bee assemblages between cities is also restricted by the various methods and intensities of bee sampling between studies (Cane et al., 2006; Fetridge et al., 2008; Matteson et al., 2008; Bates et al., 2011; Banaszak-Cibicka & Żmihorski, 2012). Bates et al. (2011) suggested that a common framework for sampling bee assemblage variation along the urban-rural gradient could help to clarify understanding of how urbanisation impacts upon bees across cities and regions. This has been conducted with carabid assemblages using GLOBENET (Niemelä et al., 2000; Sadler et al., 2006; Niemelä & Kotze, 2009). By standardising the methodology, there could be greater interpretability in the results of studies investigating bee assemblage variation along the urban-rural gradient. Even if a common framework is not achieved, until regional and methodological replication is increased firm conclusions of the general response of bee assemblages to urbanisation may not be drawn.

This thesis underlines the importance of life-history traits in defining bee assemblage variation along the urban-rural gradient. Between regions, it becomes difficult to compare results of bee species' variation because regional species' distribution is different. Alongside a framework for sampling methodology, by using the traits associated with each species rather than the species as an individual unit of measurement itself, a greater level of comparability may be achieved. This could enable conservation and planning policy to plan for specific bee taxa in urban areas and use that information accordingly to reduce the variation in bee assemblages along the urban-rural gradient.

Wider than urban bee studies, I recommend further investigations into the importance of species variation along landscape gradients incorporate functional diversity into their analysis. Obviously, having trait data is essential in these analyses, but the potential benefits of applying ecological and behavioural traits to bee assemblage data are worthwhile. It is believed that with a constantly changing environment, the amount of functional redundancy that remains in bee assemblages is vital to continuing pollination for certain plant species (Albrecht et al., 2012; Fründ et al., 2013). More information regarding which functional traits are important for pollination services is required so it can be determined how pollination services may be affected alongside bee species' variation. Specifically, areas that require further investigation include testing the importance of specific traits and combinations of traits in influencing pollination efficiency and success. Pilosity and its relative importance as a complementarity trait in pollen carryover are highlighted as key aspects in the future for functional diversity analysis, and are recommended to be used in future analyses.

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Appendix I

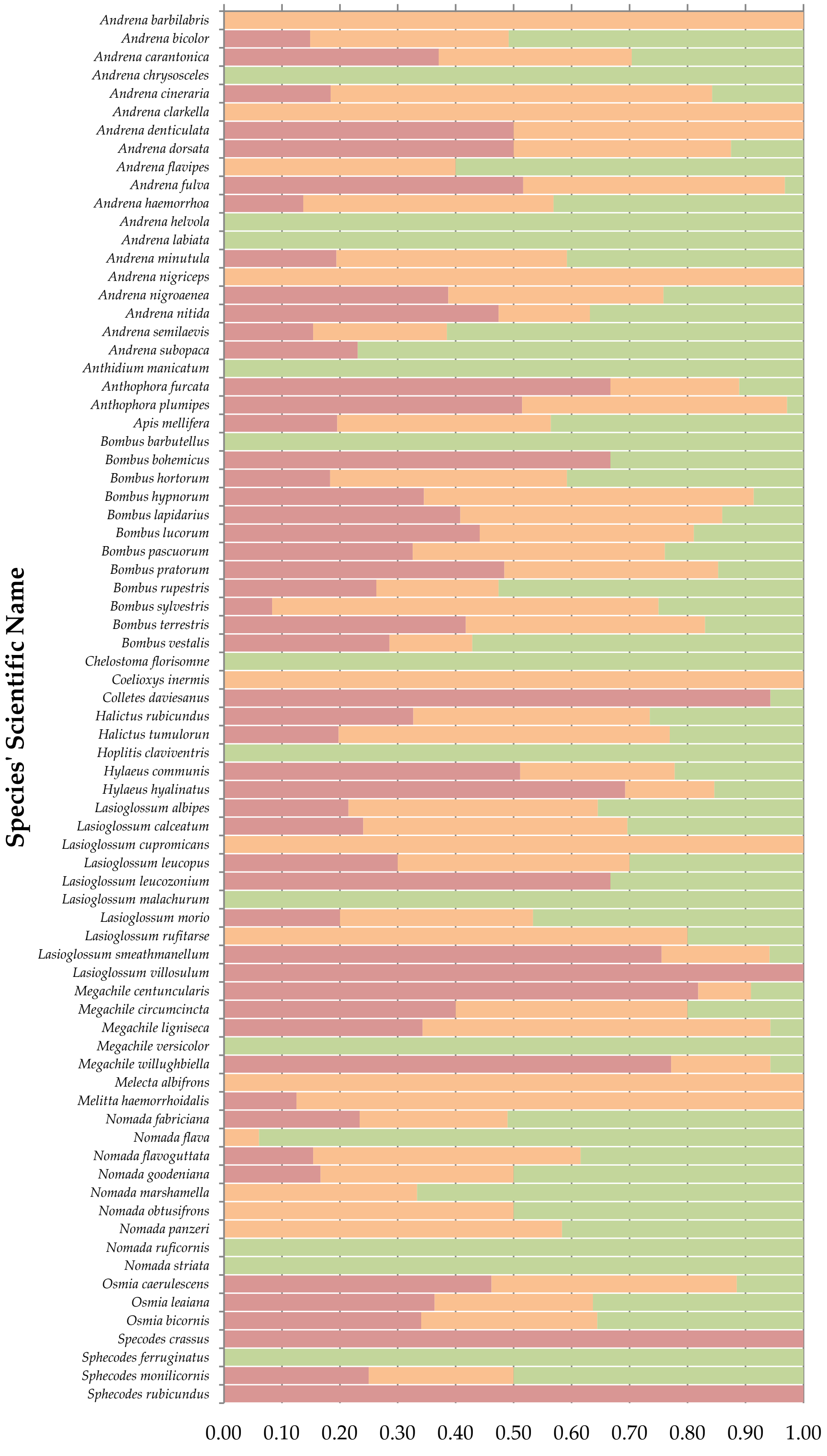
Supplementary information for Chapter 2

Table AI.1 Species table of recorded bees along the urban-rural gradient of Birmingham, UK from canal side and parkland sites using six pan trapping and two sweep netting surveys in 2011 and 2012

Species' Scientific Names	Urban Abundance	Suburban Abundance	Rural Abundance	Total Abundance
Andrena barbilabris	0	4	0	4
Andrena bicolor	43	99	147	289
Andrena carantonica	10	9	8	27
Andrena chrysosceles	0	0	2	2
Andrena cineraria	7	25	6	38
Andrena clarkella	0	1	0	1
Andrena denticulata	1	1	0	2
Andrena dorsata	4	3	1	8
Andrena flavipes	0	2	3	5
Andrena fulva	16	14	1	31
Andrena haemorrhoa	7	22	22	51
Andrena helvola	0	0	1	1
Andrena labiata	0	0	1	1
Andrena minutula	19	39	40	98
Andrena nigriceps	0	2	0	2
Andrena nigroaenea	24	23	15	62
Andrena nitida	9	3	7	19
Andrena semilaevis	2	3	8	13
Andrena subopaca	3	0	10	13
Anthidium manicatum	0	0	1	1
Anthophora furcata	6	2	1	9
Anthophora plumipes	18	16	1	35
Apis mellifera	26	49	58	133
Bombus barbutellus	0	0	1	1
Bombus bohemicus	2	0	1	3
Bombus hortorum	13	29	29	71
Bombus hypnorum	20	33	5	58
Bombus lapidarius	157	174	54	385
Bombus lucorum	98	82	42	222
Bombus pascuorum	83	111	61	255
Bombus pratorum	59	45	18	122
Bombus rupestris	5	4	10	19
Bombus sylvestris	1	8	3	12

Bombus terrestris	103	102	42	247
Bombus vestalis	2	1	4	7
Chelostoma florisomne	0	0	2	2
Coelioxys inermis	0	1	0	1
Colletes daviesanus	33	0	2	35
Halictus rubicundus	16	20	13	49
Halictus tumulorum	18	52	21	91
Hoplitis claviventris	0	0	3	3
Hylaeus communis	23	12	10	45
Hylaeus hyalinatus	18	4	4	26
Lasioglossum albipes	20	40	33	93
Lasioglossum calceatum	19	36	24	79
Lasioglossum cupromicans	0	1	0	1
Lasioglossum leucopus	12	16	12	40
Lasioglossum leucozonium	2	0	1	3
Lasioglossum malachurum	0	0	1	1
Lasioglossum morio	3	5	7	15
Lasioglossum rufitarse	0	4	1	5
Lasioglossum smeathmanellum	77	19	6	102
Lasioglossum villosulum	1	0	0	1
Megachile centuncularis	18	2	2	22
Megachile circumcincta	2	2	1	5
Megachile ligniseca	12	21	2	35
Megachile versicolor	0	0	1	1
Megachile willughbiella	27	6	2	35
Melecta albifrons	0	1	0	1
Melitta haemorrhoidalis	1	7	0	8
Nomada fabriciana	11	12	24	47
Nomada flava	0	2	31	33
Nomada flavoguttata	2	6	5	13
Nomada goodeniana	1	2	3	6
Nomada marshamella	0	1	2	3
Nomada obtusifrons	0	1	1	2
Nomada panzeri	0	7	5	12
Nomada ruficornis	0	0	6	6
Nomada striata	0	0	2	2
Osmia caerulescens	12	11	3	26
Osmia leaiana	4	3	4	11
Osmia bicornis	65	58	68	191
Sphecodes crassus	5	0	0	5
Sphecodes ferruginatus	0	0	1	1
All Species	**1142**	**1259**	**908**	**3309**

Figure AI.1: The proportion of bee species' abundance between Urban (Red), Suburban (Orange) and Rural (Green) land-use types in Birmingham, UK from canal side and parkland sites using six pan trapping and two sweep netting surveys in 2011 and 2012



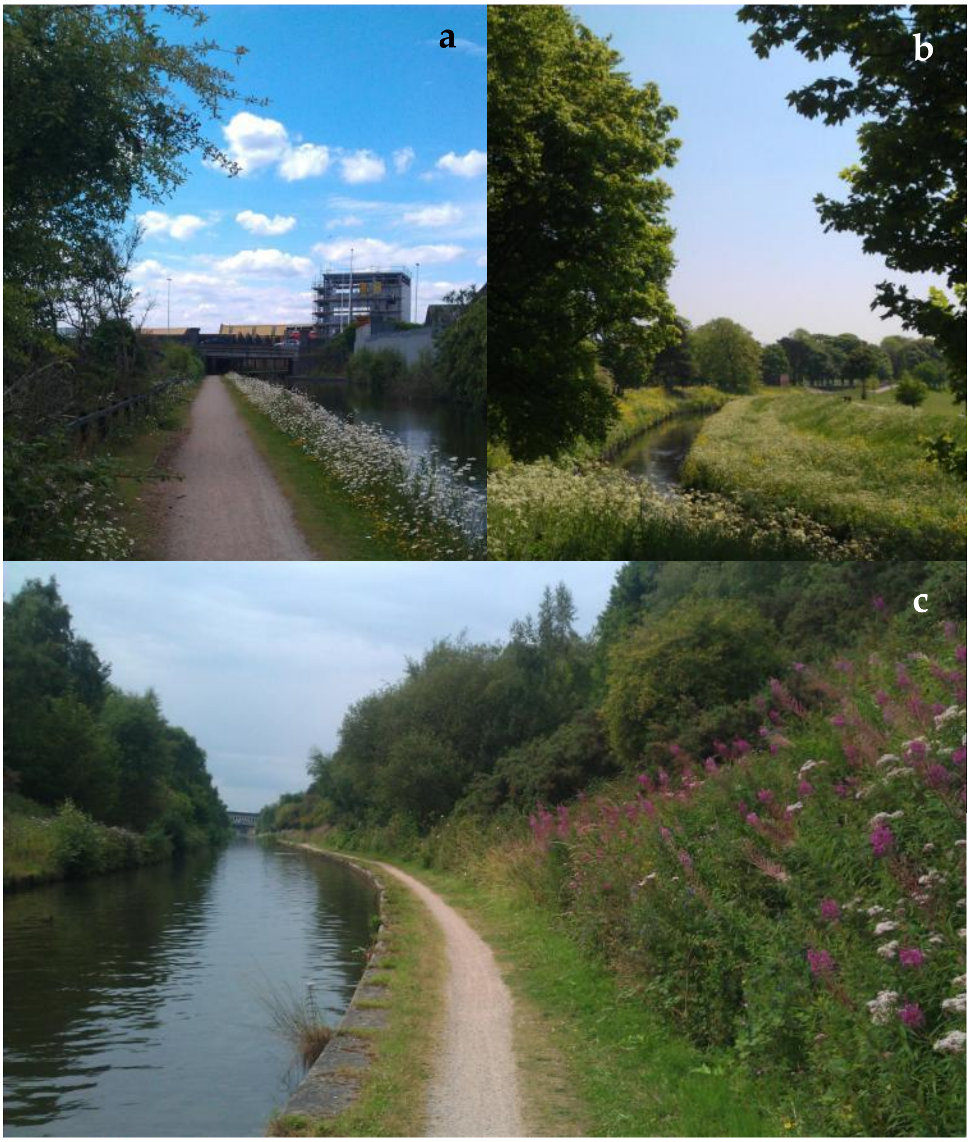


Figure AI.2: Photographs of typical sample sites along (a & c) canal sides and in (b) parkland. Photos: R Fowler.

Appendix II

Supplementary information for Chapter 3

Table AII.1: The species measured as part of Chapter 3's analysis of pilosity, including species' codes, and the mean (mode for hair type) values for each pilosity measurement, as well as their body size (ITD), the PTG they were assigned to and the cluster they were grouped into based on their pilosity values. [1] hair types consisted of 1=smooth, 2=scaled, 3=branched <1 μm, 4=branched 1-2 μm, 5=branched >2 μm. [2] ITD is the distance between the tegulae (wing bases) as a measure of body size. [3] Pollen Transport Groups (PTGs) were defined following Thorp (1979) (Table 3.1).

Species	Code	Hair Length (μm)	Hair Spacing (μm)	Hair Cover (%)	Hair Type[1]	ITD[2] (μm)	PTG[3]
Andrena angustior	A.ang	423	37.3	94	2	2384.4	1
Andrena barbilabris	A.bar	541.7	45.4	72	3	2318.6	1
Andrena bicolor	A.bic	476.2	33.5	100	4	1991.9	1
Andrena carantonica	A.car	549.9	41.0	100	4	2710.2	1
Andrena chrysosceles	A.chr	336.3	42.5	85.5	1	1811.4	1
Andrena cineraria	A.cin	584.4	47.5	78	2	2621.5	1
Andrena clarkella	A.cla	768.6	26.8	80	3	3298.2	1
Andrena coitana	A.coi	231.8	43.1	73	3	1511.4	1
Andrena denticulata	A.den	528.7	30.7	89	4	2153.6	1
Andrena dorsata	A.dor	373.5	49.8	78	3	2148.4	1
Andrena fucata	A.fuc	438.3	33.5	100	5	2127.1	1
Andrena fulva	A.ful	747.4	28.6	89.5	4	2880.2	1
Andrena fuscipes	A.fus	547.8	29.0	81	1	2425.7	1
Andrena haemorrhoa	A.hae	471.8	43.1	87	4	2615.3	1
Andrena helvola	A.hel	549.2	31.8	100	4	2200.2	1
Andrena labialis	A.lab	566.5	35.5	100	3	2599.3	1
Andrena lapponica	A.lap	598.4	39.7	100	3	2498.4	1
Andrena minutula	A.min	222.1	53.4	58.5	3	1377.6	1
Andrena nigroaenea	A.nig	673.4	28.4	100	4	2728.2	1
Andrena nitida	A.nit	592.4	34.7	100	4	2791.9	1
Andrena ovatula	A.ova	326.3	31.7	99	4	1982.8	1
Andrena praecox	A.pra	605.8	33.9	100	3	2157.3	1
Andrena semilaevis	A.sem	244.3	42.6	92	4	1323.6	1
Andrena similis	A.sim	369.3	50.7	84.5	1	2275.5	1
Andrena subopaca	A.sub	104.21	82.8	80	4	1350.6	1
Andrena synadelpha	A.syn	574.8	29.7	100	4	2133.9	1

Andrena tarsata	A.tar	276.6	36.8	57.9	3	1745.8	1
Andrena thoracica	A.tho	633.8	43.5	81	1	3091.8	1
Andrena wikella	A.wik	377.1	43.1	91.5	1	2340.9	1
Anthidium manicatum	An.man	453.4	47.3	100	1	2038.4	3
Anthophora furcata	A.fur	538.3	36.7	90	3	2792.2	5
Anthophora plumipes	A.plu	776	33.0	100	2	2613.1	5
Apis mellifera	Ap.mel	235.392	44.4	100	1	2917.9	6
Bombus barbutellus	B.bar	1448.8	36.9	100	4	1996.1	6
Bombus bohemicus	B.boh	1225.8	36.5	100	2	1405.2	6
Bombus campestris	B.cam	1555	37.3	100	3	1634.8	6
Bombus hortorum	B.hor	1416.9	42.0	90	3	1748.1	6
Bombus humilis	B.hum	1158.5	46.2	90	3	1261.4	6
Bombus hypnorum	B.hyp	1353.6	45.2	100	2	1453.9	6
Bombus jonellus	B.jon	936.8	39.6	100	3	1352.2	6
Bombus lapidarius	B.lap	1128	43.3	100	3	1554.4	6
Bombus lucorum	B.luc	1222	42.4	100	2	1113.2	6
Bombus monticola	B.mon	1277.6	108.7	86	2	1585.5	6
Bombus muscorum	B.mus	798.4	44.7	100	3	855.8	6
Bombus pascuorum	B.pas	1397.3	43.1	100	3	1113.4	6
Bombus pratorum	B.pra	1294	46.3	100	3	1242.0	6
Bombus ruderarius	B rudr	1293.2	45.3	100	2	1161.2	6
Bombus ruderatus	B.rudt	1203.5	34.3	90	3	1211.9	6
Bombus rupestris	B.rup	1005.8	40.8	90	2	1309.4	6
Bombus soroeensis	B.sor	1090.8	46.2	92	3	3230.5	6
Bombus sylvarum	B.sylm	1286.3	62.2	100	3	955.3	6
Bombus sylvestris	B.syls	1344.5	41.8	100	2	1728.2	6
Bombus terrestris	B.ter	1589.4	49.2	100	3	2738.2	6
Bombus vestalis	B.ves	1128.5	54.0	89	2	2655.4	6
Chelostoma campanularum	C.cam	117.9	36.9	77.5	1	3023.7	3
Chelostoma florisomne	C.flo	313.5	40.2	94	3	1756.3	3
Coelioxys elongata	C.elo	218.5	30.5	83	1	3228.9	3
Coelioxys inermis	C.ine	190.9	49.5	97	1	3549.7	3
Coelioxys rufescens	C.ruf	280	34.4	92	1	3932.5	3
Colletes daviesanus	C.dav	320.9	33.9	99.5	4	3757.2	1
Colletes fodiens	C.fod	382.2	37.6	100	4	3140.0	1
Colletes similis	C.sim	356.7	33.8	100	1	3690.1	1
colletes succinctus	C.suc	441.9	31.3	100	4	1895.5	1
Dasypoda hirtipes	D.hir	744.9	49.0	100	3	2374.3	4
Epeolus cruciger	E.cru	52	38.0	72	1	2449.8	7
Epeolus variegatus	E.var	77	37.8	92	3	3207.0	7
Eucera longicornis	E.lon	538.3	28.6	100	3	2778.8	6
Halictus rubicundus	H.rub	366.5	29.6	100	4	3330.6	2
Halictus tumulorun	H.tum	198.1	36.7	93	4	4726.9	2
Hoplitis claviventris	H.cla	272.2	47.3	88	1	2372.1	3

hylaeus brevicornis	H.bre	40.8	31.8	82	1	2686.6	7
Hylaeus communis	H.com	58.4	41.0	62	1	2176.2	7
Hylaeus confusus	H.con	66.5	67.0	100	1	1599.3	7
Hylaeus hyalinatus	H.hya	66.1	56.2	72	1	2713.8	7
Lasioglossum albipes	L.alb	271.4	38.2	100	4	4857.0	2
Lasioglossum calceatum	L.cal	246.3	41.5	100	4	5143.4	2
Lasioglossum cupromicans	L.cup	147.3	39.5	83	3	4596.6	2
Lasioglossum fratellum	L.fra	174.7	32.3	69	3	5517.8	2
Lasioglossum fulvicorne	L.ful	208.7	39.2	94	4	5761.9	2
Lasioglossum lativentre	L.lat	215.5	38.7	100	4	5599.2	2
Lasioglossum leucopus	L.leu	164.7	46.2	77	4	4502.1	2
Lasioglossum malacha	L.mal	132.408	40.5	75	4	4390.2	2
Lasioglossum minutissimum	L.min	132.8	39.9	81.5	4	5892.2	2
Lasioglossum morio	L.mor	151.3	42.3	81.5	4	5374.6	2
Lasioglossum nitidiusculum	L.nit	172.8	42.0	78	4	3663.3	2
Lasioglossum parvulum	L.par	158.8	55.6	89	4	6347.9	2
Lasioglossum smeathmanellum	L.sme	185.3	43.3	80	4	5293.3	2
Lasioglossum villosulum	L.vil	204.8	55.6	100	4	5859.6	2
Macropis europaea	M.eur	294.4	34.0	100	1	6250.2	5
Megachile centuncularis	M.cen	501.3	58.8	96	1	5677.4	3
Megachile circumcincta	M.cir	255.008	40.9	100	1	4922.2	3
Megachile ligniseca	M.lig	603.4	58.9	100	1	5810.8	3
Megachile maritima	M.mar	690.3	43.3	92	1	4798.4	3
Megachile versicolor	M.ver	484.1	36.8	90	1	6445.6	3
Megachile willughbiella	M.wil	613.7	47.8	100	1	5696.9	3
Melecta albifrons	M.alb	632.6	24.1	100	3	3471.5	6
Melitta leporina	M.lep	511.3	33.2	92	1	4124.0	5
Nomada fabriciana	N.fab	102.1	41.8	92	1	1906.9	7
Nomada flava	N.fla	140.2	34.9	100	1	1865.5	7
Nomada flavoguttata	N.flav	68.8	81.7	52	1	1055.3	7
Nomada goodeniana	N.goo	139.5	64.1	53	1	1321.0	7
Nomada lathburiana	N.lat	168.7	57.7	100	1	1308.8	7
Nomada leucophthalma	N.leu	161.9	67.8	72.5	1	1317.5	7
Nomada marshamella	N.mar	126.2	89.9	62	1	1579.8	7
Nomada panzeri	N.pan	110.7	63.5	76	1	1873.2	7
Nomada ruficornis	N.rufc	106	50.0	64	1	1088.5	7
Nomada rufipes	N.rufp	90.2	70.3	90	1	2050.6	7
Nomada striata	N.str	109.5	57.4	94	1	2171.7	7
Osmia aurulenta	O.aur	515.6	63.8	100	3	2015.9	3
Osmia caerulescens	O.bic	391.8	66.4	84	1	2519.2	3
Osmia leaiana	O.cae	457.3	92.7	89	1	1609.2	3
Osmia bicornis	O.lea	929.5	89.5	100	3	1820.0	3
Panurgus banksianus	P.ban	393.9	60.7	100	3	1524.3	5
Panurgus calcaratus	P.cal	274.1	78.8	100	1	1646.9	5

Sphecodes ephippius	S.eph	137.3	76.3	84	1	1350.4	7
Sphecodes geoffrellus	S.geo	132.8	92.2	62	1	1100.5	7
Sphecodes gibbus	S.gib	159.1	68.3	90	1	1629.2	7
Sphecodes hyalinatus	S.hya	122.8	63.1	100	1	1227.0	7
Sphecodes monilicornis	S.mon	215.5	77.9	55	1	1593.1	7
Sphecodes pellucidus	S.pel	182	46.4	84	1	1692.2	7
Sphecodes puncticeps	S.pun	120.3	58.2	86	1	1136.5	7
Sphecodes reticulatus	S.ret	180.7	112.8	91	1	1522.5	7

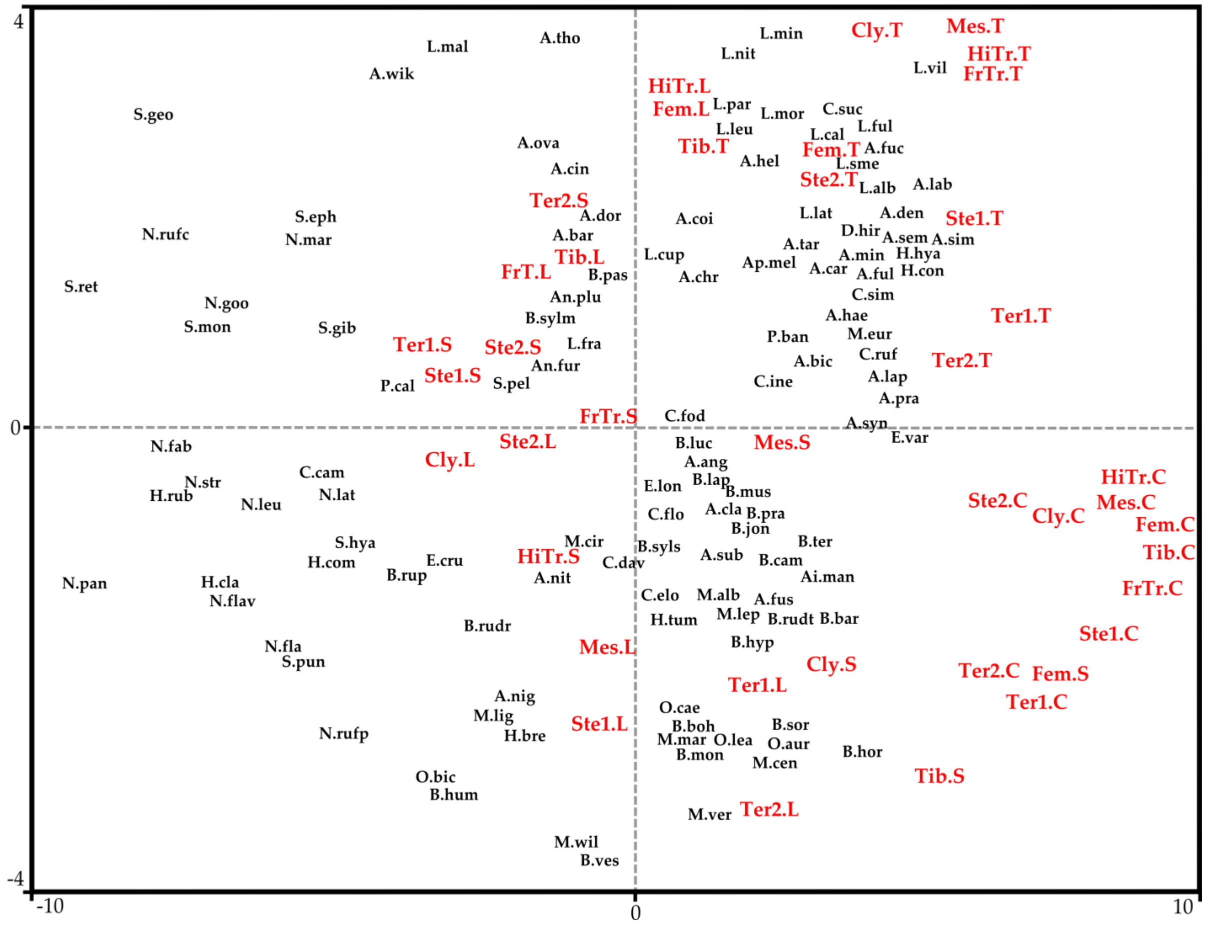


Figure AII.1: Principal Components Analysis biplot of hair length, spacing, cover and type measured from 122 species of bee. Variables in red indicate the area on the bee's body and type of measurement taken, those ending in '.L'=length, '.S'=spacing, '.C'=cover and '.T'=type. For species' code reference, see Table AII1.