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UNIVERSITY OF SUSSEX

THE EFFECT OF AGRI-ENVIRONMENT SCHEMES ON
FARMLAND BEE POPULATIONS

by

THOMAS JAMES WOOD

Thesis submitted for the degree of Doctor of Philosophy

School of Life Sciences,
Department of Evolution, Behaviour and Environment

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UNIVERSITY OF SUSSEX
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DEPARTMENT OF EVOLUTION, BEHAVIOUR AND ENVIRONMENT

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POPULATIONS

ABSTRACT

Over the past century there have been substantial declines in farmland biodiversity as a result of the intensification of agricultural practice. Concerns over these declines have led to the development of agri-environment schemes designed to mitigate the effects of intensive agriculture and to benefit biodiversity. Prior to commencing this thesis it was not clear if flower-rich, pollinator-focused agri-environment schemes had a population level impact on wild bees on farmland. Whilst previous work has shown that the creation of flower-rich habitat can provide suitable foraging resources for bumblebees, little was known about the impact of this management on bumblebee population sizes and even less on whether these resources were used by and benefited solitary bees. This thesis compares bee populations between farms with and without flower-rich, pollinator focused agri-environment schemes in Hampshire and West Sussex, UK. Using genetic techniques to estimate colony density, and hence population size, farms implementing targeted schemes had a significantly higher density of bumblebee nests for the four species studied (212 nests/km² against 112 nests/km²). However, there was no difference in the species richness of bees between these different farm types. When assessing pollen use by solitary bees, flowering plants sown as part of pollinator-focused agri-environment schemes were not widely used, representing 27% of pollen foraging observations and 23% of pollen collected by volume. Only 35% of solitary bee species were found to use sown plants for pollen to a meaningful extent, with most pollen collected from plants persisting in the wider environment. The creation of flower-rich habitat significantly increased resource availability, but did not increase resource diversity. These results indicate that if diverse bee populations are to be maintained on farmland then agri-environment schemes must be developed that effectively increase the number of flowering plant species present at the farm scale.

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DECLARATION OF AUTHORSHIP

I, Thomas Wood, hereby declare that the thesis entitled 'The effect of agri-environment schemes on farmland bee populations' and the work presented within it is my own. I confirm that:

- the thesis has not been, and will not be, submitted in whole or part to another University for the award of any other degree;
- where I have consulted the published work of others this is always clearly attributed;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others;

and parts of this work have been published as:

Wood, T.J., Holland, J.M. and Goulson, D. (2015) A comparison of techniques for comparing farmland bumblebee populations. *Oecologia*, 177, 1093-1102 (Chapter 2)

Wood, T.J., Holland, J.M., Hughes, W.O.H. and Goulson, D. (2015) Targeted agri-environment schemes significantly improve the population size of common farmland bee species. *Molecular Ecology*, 24, 1668-1680 (Chapter 3)

Wood, T.J., Holland, J.M. and Goulson, D. (2015) Pollinator friendly management does not increase the diversity of farmland bees and wasps. *Biological Conservation*, 187, 120-126 (Chapter 4)

Wood, T.J., Holland, J.M. and Goulson, D. (2016) Providing foraging resources for solitary bees on farmland: current schemes benefit a limited suite of species. *Journal of Applied Ecology*, currently online only (Chapter 5)

Wood, T.J., Holland, J.M. and Goulson, D. (2016) Diet characterisation of solitary bees on farmland: dietary specialisation predicts rarity. *Biodiversity and Conservation*, 25, 2655-2671 (Chapter 6)

Signature: Thomas Wood

Date: 21st December 2016

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‘Let us turn elsewhere, to the wasps and bees, who unquestionably come first in the laying up of a heritage for their offspring.’

J-H Fabre

Chapter 1 – General introduction

1.1 A changing agricultural landscape

The 20th century saw global change at an unprecedented scale, with great political, economic and social upheaval that affected all parts of the world. Agriculture was no exception and was strongly affected by the defining conflict of the 20th century, the Second World War. In Britain, low levels of domestic food production combined with the disruption to food imports caused by the war resulted in food shortages and mass rationing that persisted long after the cessation of hostilities. The combined desires to end rationing, to increase the general standard of living and to reduce food imports that were negatively affecting Britain's balance of payments led to the introduction of the 1947 Agriculture Act which sought to achieve self-sufficiency in food production (Blaxter and Robertson 1995; Robinson and Sutherland 2002). As stated by the Minister of Agriculture in cabinet briefings in 1945, the aim of the reform to agriculture would be "to promote a healthy and efficient agriculture capable of producing that part of the nation's food which is required from home sources at the lowest price consistent with the provision of adequate remuneration and decent living conditions for farmers and workers, with a reasonable return on capital invested" (The National Archives 1945).

The new Act brought in two major changes. Firstly, guaranteed prices were introduced for principal agricultural products, namely milk, fat livestock, eggs, cereals, potatoes and sugar beet. Secondly, a large programme of capital investment and subsidies was launched to encourage investment in agriculture. The major effect of this and subsequent policies was to increase agricultural output, principally through an increase in the area of arable land in the UK as farmers increased cereal production (Figure 1.1, Robinson and Sutherland 2002). Agricultural output grew faster between 1945 and 1965 than at any other period in history, before or after (Brassley 2000, Figure 1.2). Since the 1980s, UK agricultural output has been essentially stable, though productivity has increased as inputs have fallen (Figure 1.3, DEFRA 2016).

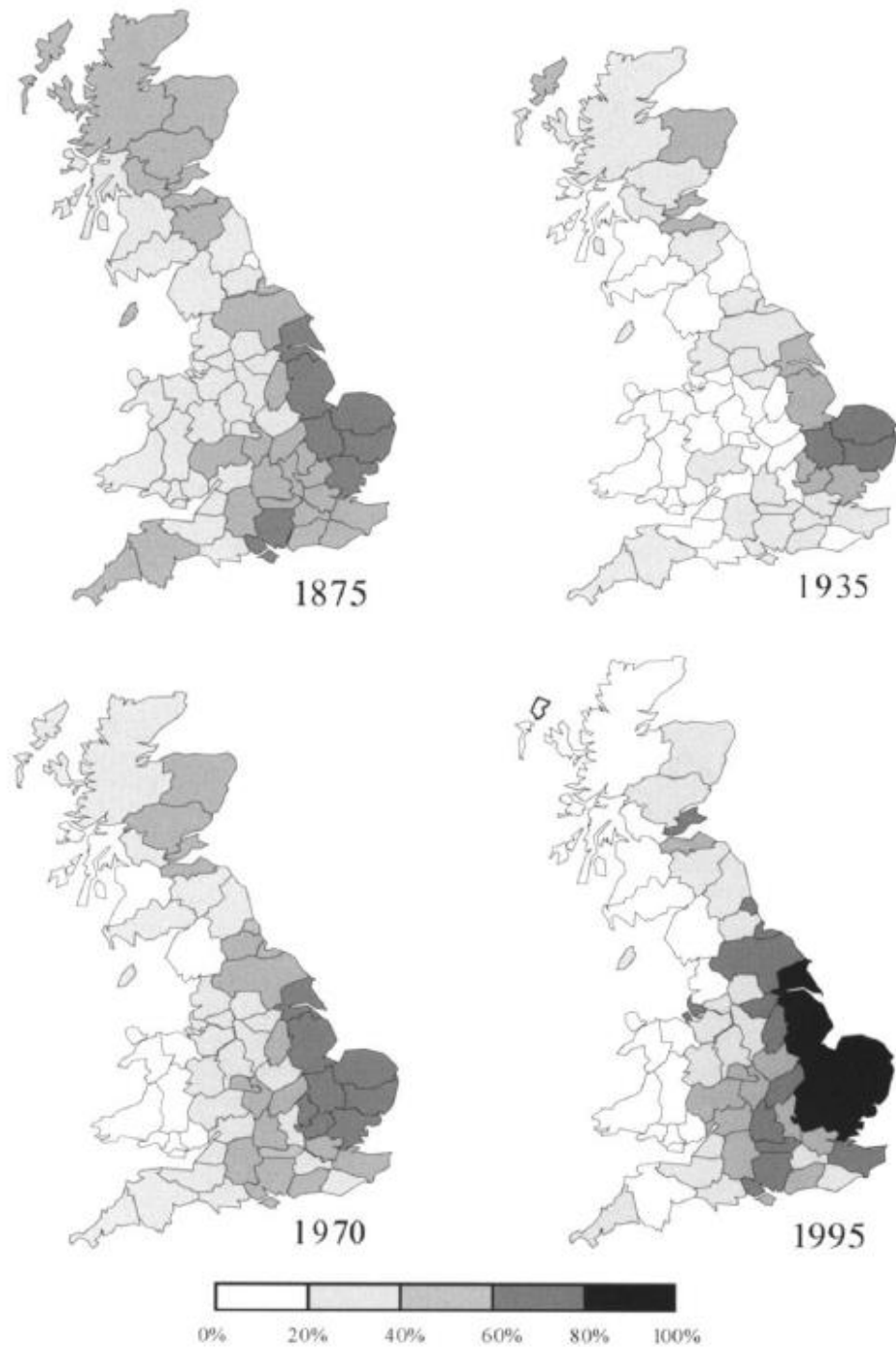


Figure 1.1. The amount of annually tilled land, as a percentage of total farmed area by county in 1875, 1935, 1970 and 1995. Based on DEFRA statistics. Reproduced from Robinson and Sutherland 2002.

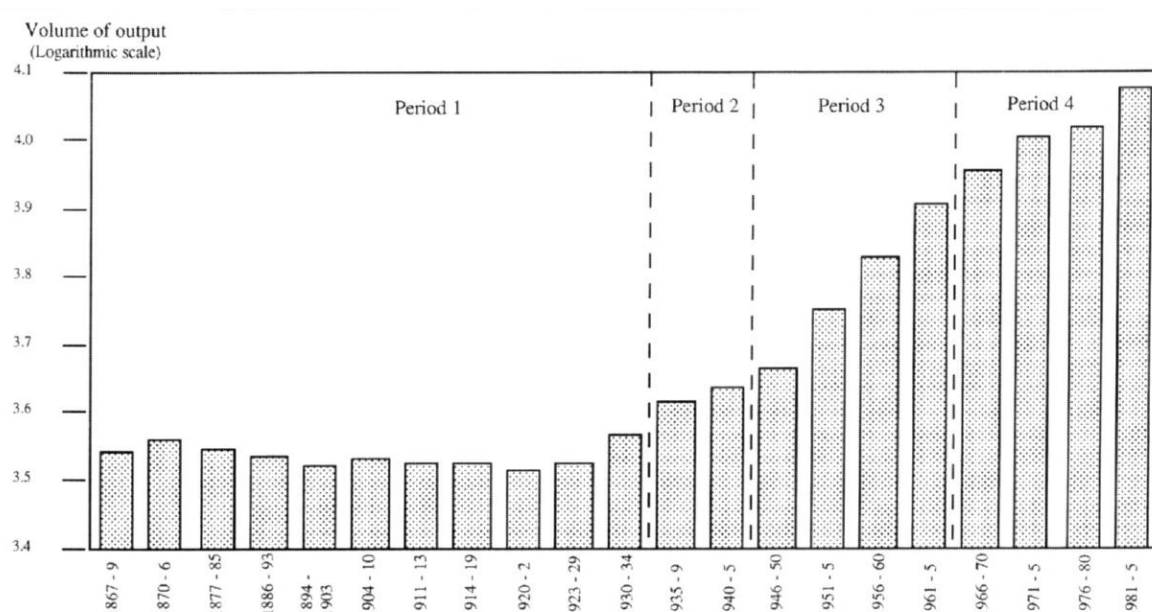


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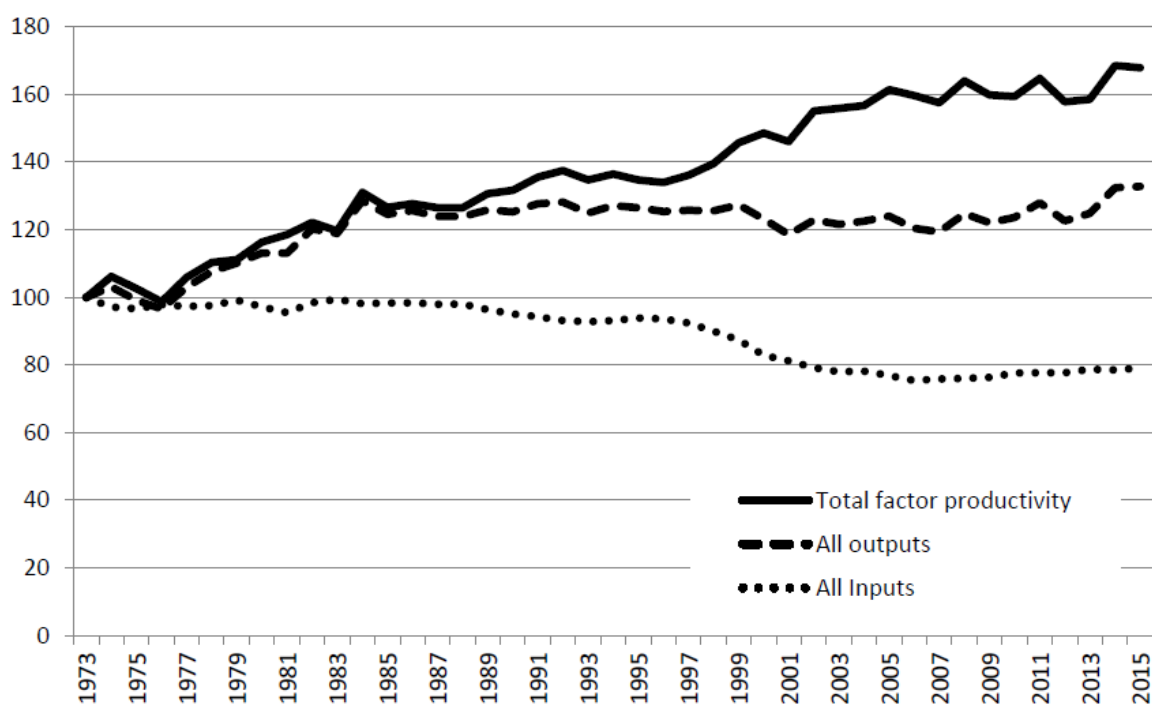


Figure 1.3. Productivity index for the UK agriculture industry (1973=100). Solid line = total factor productivity, dashed line = agricultural output, dotted line = all inputs. Reproduced from DEFRA 2016.

In addition to the increase in the area of land used for agriculture there were substantive changes to the manner in which the land itself was farmed. Farming became more dependent on widespread industrial scale input of inorganic chemical pesticides and fertilisers. The number of chemical compounds approved for agricultural use increased from 37 in 1955 to 344 in 1997 (Robinson and Sutherland 2002). The area sprayed with pesticides increased markedly over this period (Figure 1.4 a and b). The use of inorganic fertilisers applied to crops and grasslands also increased substantially during this time period (Figure 1.4 c). In 1962 nitrogen fertiliser was applied to 37% of permanent grassland across England and Wales. This rose to 77% of permanent grassland by 1982 with the application rate also rising from 20 to 96 kg ha⁻¹ in the same time period (Wells and Sheail 1988). The programme of capital investment grants saw many farmers expand their operations with the purchase of modern agricultural machinery such as tractors and combine harvesters (Figure 1.4 d).

The combined effects of these changes led to an increased proportion of specialised farms, focused on either arable or pastoral production (Figure 1.1). The ability to increase soil fertility with the use of chemical inputs reduced the need for rotational farming that would previously have been used to return nutrients to the soil, allowing continual cash cropping year after year. Inorganic fertilisers also reduced the requirement for animal manure, further driving a division between arable and pastoral farming. Due to the large capital overheads associated with machinery, the drive towards mechanisation favoured larger farms. Between 1949 and 1999 the number of farms declined by 35% and the proportion of farms over 500 ha in size increased from 1% to 6% (Robinson and Sutherland 2002). This increased use of machinery required fewer farm labourers, with a 77% decline in the same time period. The horse was replaced as a beast of burden, and the loss of horses from arable farms can be seen in the steady decline in oat cultivation from the 1940s onwards (Brassley 2000). In the 1960s hedgerows began to be removed on a large scale to increase field size (Figure 1.5) in order to make fields more accessible and amenable to larger machinery. This effect was most pronounced in more arable areas such as Cambridgeshire as there was no requirement for livestock, and therefore no need for stock-proof hedging, with an increase in average field size from 6.5 ha to 16.0 ha between 1945 and 1994. This compares with an increase of 5.5 ha to 9.5 ha in the more pastoral counties of Dorset and Somerset (Robinson and Sutherland 2002). The relative length of hedgerows in arable counties is now only around 20-30% of that found in pastoral counties (Barr *et al.* 1993; Westmacott and Worthington 1997).

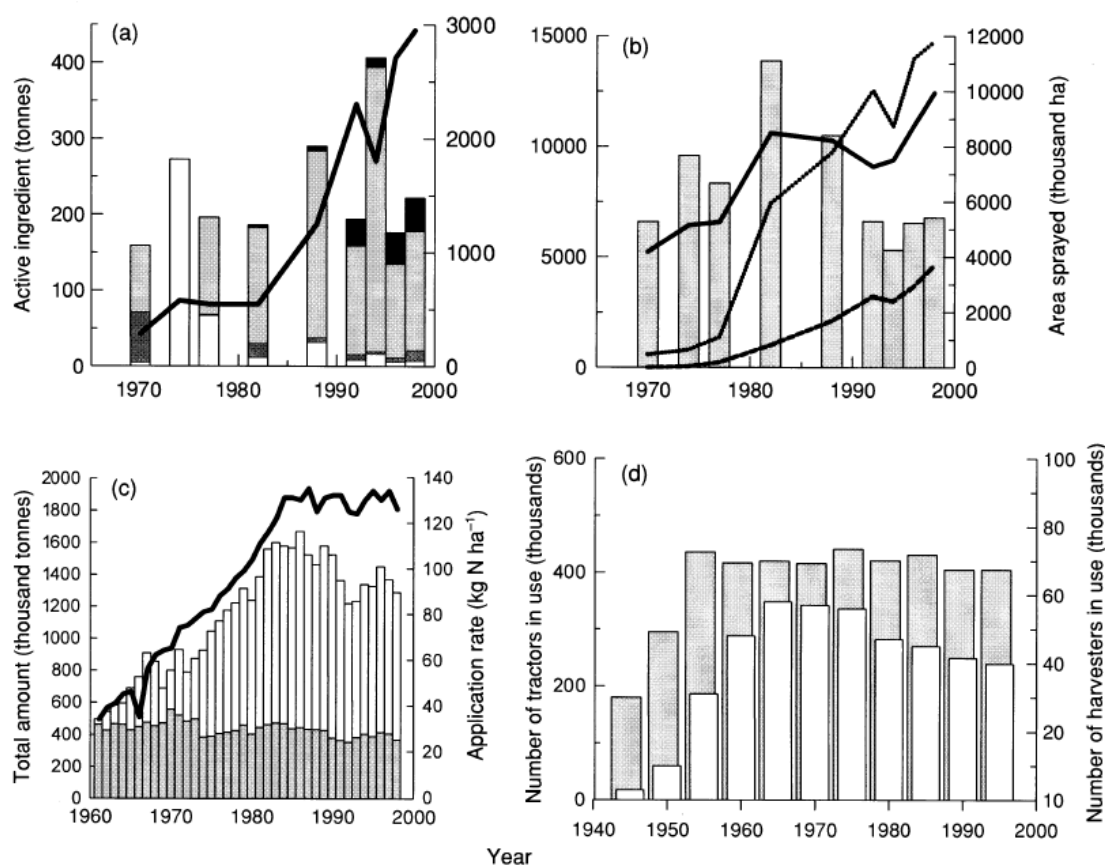


Figure 1.4. (a) Areas sprayed with insecticide in England and Wales (line and far right hand axis) and amount (active ingredient) of each type of insecticide used (bars and far left axis): carbamates (white), organochlorines (heavy stippling), organophosphates (light stippling) and pyrethroids (black), this breakdown not available for 1974. (b) Areas sprayed (right axis) with herbicides in England and Wales (at 1970, top line), fungicides (at 1970, middle line) and sown with chemically treated seeds (at 1970, bottom line). Bars (and left axis) indicate weight of herbicide (active ingredient) applied. (c) Total amount of fertiliser applied in Britain (bars and left axis): nitrogen (open) phosphate (filled) and average application rates of nitrogen to all crops (line and right axis). (d) Number of tractors (filled bars and left axis) and combine harvesters (open bars and right axis) in use in the UK. Reproduced from Robinson and Sutherland 2002.

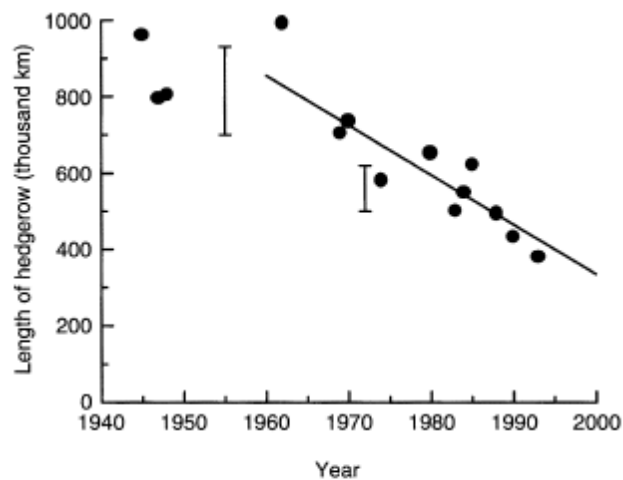


Figure 1.5. Published estimates of hedgerow length in England and Wales; dots indicate (mean) estimates, bars ranges. Slope of regression (1960-1998): -13,000 km year, $R^2=0.74$. Reproduced from Robinson and Sutherland 2002.

The magnitude of these post-war changes has led to this period being described as a second agricultural revolution, following on from the first British agricultural revolution in the late 17th to early 19th centuries (Blaxter and Robertson 1995). More broadly, many of the same desires drove reform of European agriculture and similar policies, namely guaranteed prices and efficiency investments, are outlined in the Common Agricultural Policy (CAP) of the European Union (EU) that was first adopted in 1962. Most of the trends seen in Britain are consequently reflected across much of Western Europe (Potter 1997; Donald *et al.* 2002). When measured in terms of food production, the post-war period of British and European agriculture was hugely successful, generating vast surpluses neutrally referred to as ‘intervention stocks’ by the EU but dubbed ‘wine lakes’ and ‘butter mountains’ by the Press (Krebs *et al.* 1999). However, this enormous increase in agricultural productivity has come at a substantial environmental cost.

1.2 Farmland biodiversity in decline across Europe

By the early 2000s concern was growing over the state of nature on British and European farmland. Due to the high level of public interest in bird watching and the historic strength of ornithology in the UK, robust long term datasets exist that chart the relative success of many

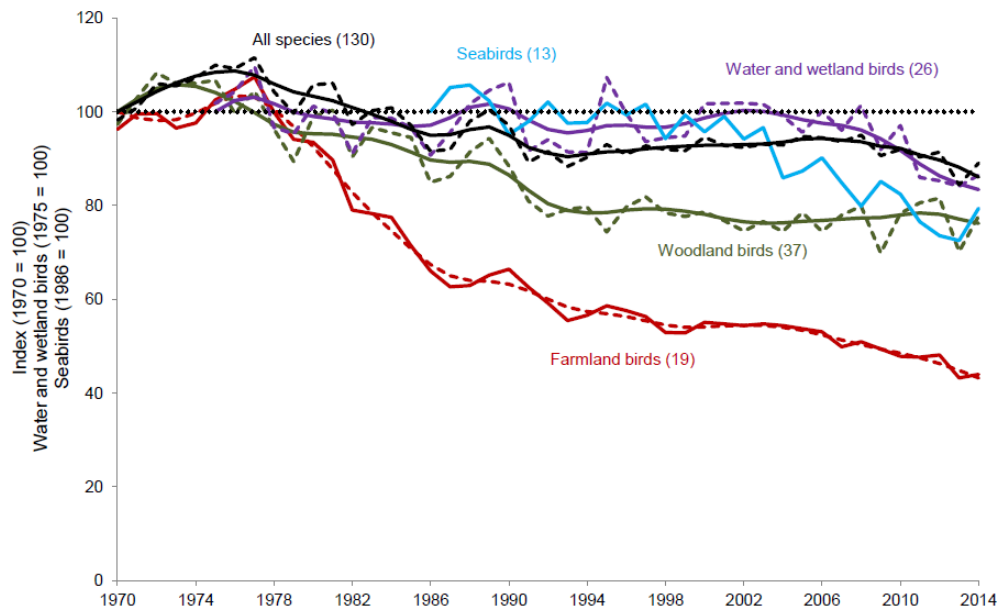


Figure 1.6. The relative population trends of different UK bird groups since 1970. Reproduced from DEFRA 2014.

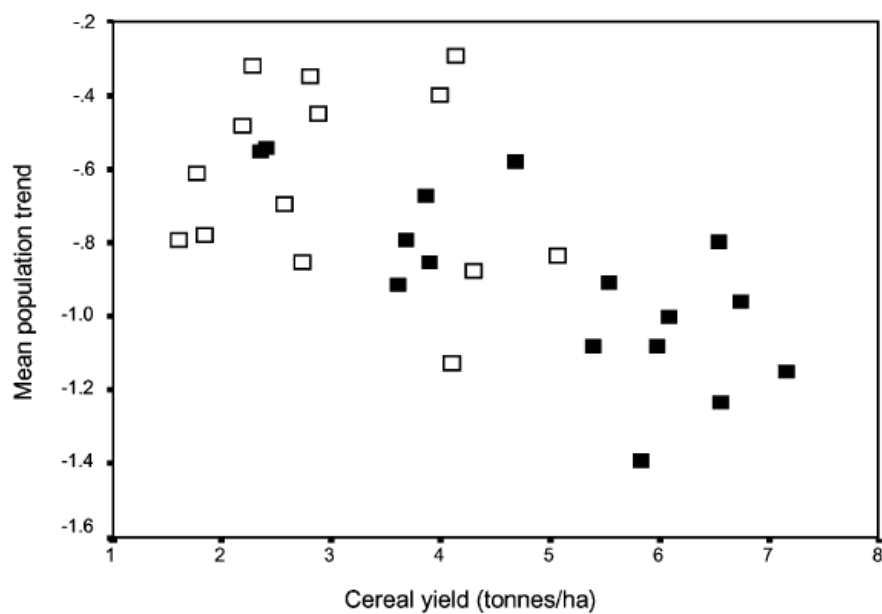


Figure 1.7. The relationship between mean farmland bird population trend and cereal yield across Europe ($r_{30} = -0.66$, $P < 0.001$). Open squares: Eastern Europe, filled squares: European Union member states. Reproduced from Donald *et al.* 2002. Note, this analysis was conducted before the 2004 enlargement of the European Union that brought in eight Eastern European states.

bird species over the past century. Whilst farmland birds recovered from earlier losses in the 1920s and 1930s to reach a high point in the 1970s (Gibbons *et al.* 1996), since that time they have declined substantially to less than half the population size (Figure 1.6, DEFRA 2014). During the period of most significant decline, between 1979 and 1999, an estimated 10 million breeding individuals of 10 species of farmland birds were lost from the British countryside (Krebs *et al.* 1999). Similar declines have been observed for European farmland birds over the same time period (Donald *et al.* 2001), with the greatest declines in the most intensively farmed areas.

Arable intensification has resulted in a simplification of the farmed environment and a consequent simplification of the plant and animal communities that inhabit it. In short, the more that farmland is focused on the goal of food production, the fewer resources remain for wild plants and animals. In assessing population trends in European farmland birds, average grain yield alone explains 30% of the variation in relative population decline (Figure 1.7, Donald *et al.* 2002). Most low intensity grassland has been lost from intensified agricultural systems, with over 90% of unimproved lowland grassland disappearing between 1930 and 1984 (Fuller 1987; Wilson 1992). In Central England, within agriculturally productive lowlands, most grasslands were directly replaced with arable fields (Hodgson *et al.* 2005). Within grasslands, increases in fertiliser application and more regular cutting for silage, rather than a less frequent cut for hay, has resulted in a decline in grassland diversity through favouring competitive species such as rye-grasses *Lolium* and meadow-grasses *Poa* (Peel *et al.* 1985; Wilson *et al.* 1999). Fertiliser drift into uncropped field margins and hedge bottoms has changed the nutrient balance in these areas, further reducing plant diversity as low-nutrient specialist species were outcompeted by more generalist plants (Marshall 1988; Boatmann *et al.* 1994). The level of direct fertiliser input is also important, with nitrogen input strongly negatively associated with plant diversity across both arable and grassland fields in multiple European countries (Kleijn *et al.* 2009).

As well as the effects on birds and plants, many invertebrate groups have also declined as a result of this intensification. Multiple studies link an increase in agricultural intensity to a decline in the abundance and diversity of farmland invertebrate populations (Woiwod and Harrington 1994; Wilson *et al.* 1999; Ewald and Aebischer 1999; Sotherton and Self 2000; Benton *et al.* 2002). Data is particularly good for butterflies and moths. Analyses have illustrated widespread declines in both common and rare species in Britain (Conrad *et al.* 2006; Fox *et al.* 2013) and elsewhere in Europe (Mattila *et al.* 2006; van Swaay *et al.* 2006; Franzen

and Johannesson 2007; Groenendijk and Ellis 2011). This decline has been most pronounced in agricultural areas such as Flanders in Belgium where 19 of the 64 indigenous butterfly species became extinct during the 20th century (Maes and van Dyck 2001), the most pronounced loss anywhere in Europe.

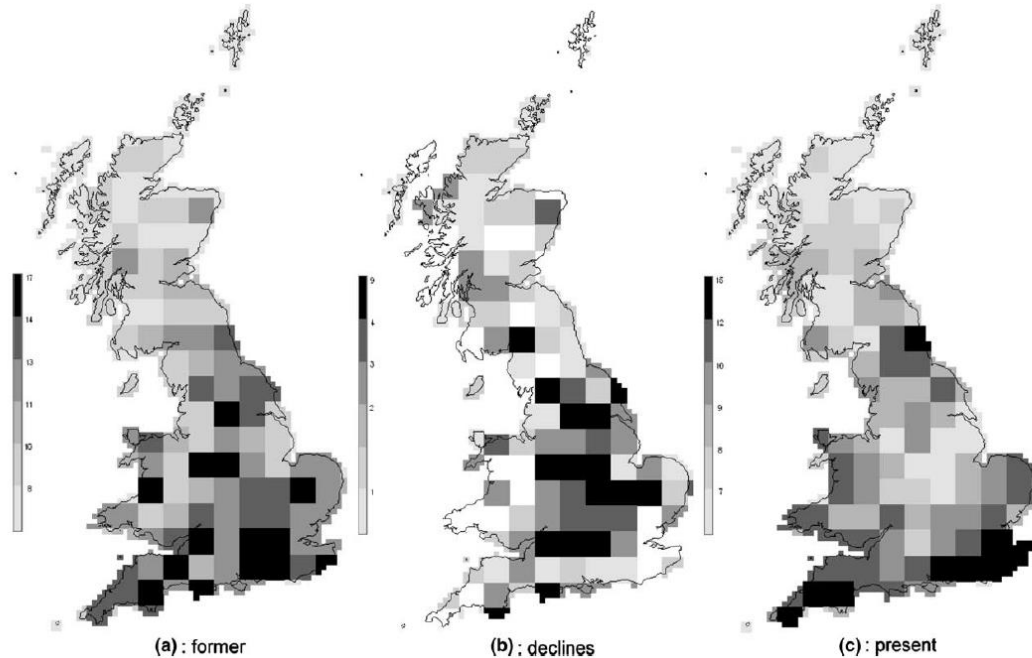


Figure 1.8. Maps of species richness for British bumblebees from the Bumblebee Distribution Maps Scheme data measured at the scale of 50 x 50 km grid cells for (a) former richness (pre 1960 and 1960 onwards records); (b) declines in richness (pre 1960s records); (c) present richness (1960 onwards records). Equal frequency grey-scale classes are used to maximise differentiation among regions. Reproduced from Williams 2005.

For bees, the data is most complete for European bumblebees (*Bombus*) which have also shown substantial post-war declines (Williams 1986; Williams 2005; Kosior *et al.* 2007; Goulson *et al.* 2008; Williams and Osborne 2009). In Britain, the most pronounced declines are in areas of greater agricultural intensification, such as Central and Eastern England (Figure 1.8, compare with Figure 1.1). Other bee species have also been affected with declines across Britain and Europe linked to agricultural intensification (Biesmeijer *et al.* 2006; Potts *et al.* 2010; Ollerton *et al.* 2014). However, there is a general lack of information about the status and population trends of most bee species due to the lack of national monitoring and recording schemes. In a recent assessment of European bee species, 1,101 out of a total of 1,942 species were listed as Data Deficient under IUCN criteria, and no population trend could be calculated for 79% of species (Nieto *et al.* 2014).

It is important to note that some authors have argued that the pre- and post-war analysis is oversimplified. In compiling a cumulative extinction curve of British bees and flower-visiting wasps, Ollerton *et al.* (2014) noted that the process of species extinction had been in progress long before the passing of the 1947 Agriculture Act (Figure 1.9). Whilst output itself did not increase as spectacularly as during the 1945-1965 period (Figure 1.2), there were extensive changes in agricultural practices during the 1874-1939 period, such as the large scale importation of South American guano as a grassland fertiliser (Brassley 2000). Nonetheless, the financial support provided by central government and the resulting widespread changes in agricultural practice marks a discrete change in societal attitude towards farming in Britain and makes the immediate post-war period an important cultural reference point from which the events of the 20th century can be viewed.

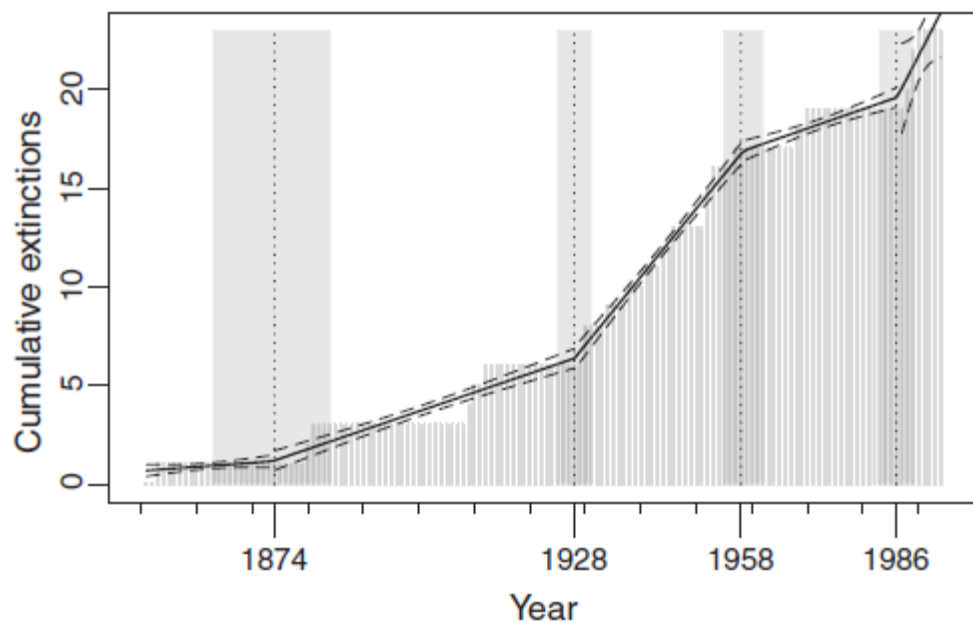


Figure 1.9. Cumulative British bee and flower-visiting wasps extinctions, 1851-1994. Data are plotted as cumulative number of extinctions per year (vertical grey bars). The four estimated break points are shown as vertical dotted black lines, with 99% confidence intervals depicted as transparent grey-shaded rectangles. The identified line segments are given by solid black lines, with 99% confidence intervals indicated by dashed lines. The smaller vertical tick marks on the x-axis show decades starting at 1850. Reproduced from Ollerton *et al.* 2014.

Whilst most groups of plants and animals living on farmland have been negatively affected by the intensification of agriculture, this thesis focuses on farmland bees. In recent years much research has focused on the global importance of bees as agricultural pollinators (Klein *et al.* 2007; Garibaldi *et al.* 2011; Garibaldi *et al.* 2013) and pollinators of wild plants (Ashman *et al.* 2004; Ollerton *et al.* 2011). As a result, the observed declines of wild bees are particularly concerning from both an environmental and an agricultural perspective. The domesticated honeybee (*Apis mellifera*) is used to pollinate crops all over the world and the physical importation of this species into fields remains the mainstream management approach to enhance agricultural pollination (Garibaldi *et al.* 2014). However, recent work has questioned the effectiveness of this approach. Studies have found that most agricultural pollination is carried out by wild bees, hoverflies and other insects (Winfree *et al.* 2008; Breeze *et al.* 2011; Woodcock *et al.* 2013). Honeybees are even less important when the pollination of wild plants is considered (Ollerton *et al.* 2012).

Not only do such wild pollinators provide a pollination service, the quality of this service increases with the diversity of the pollinator community (Klein *et al.* 2003). A diverse pollinator community can increase yield regardless of honeybee abundance (Garibaldi *et al.* 2013) and can actually make honeybee pollination more efficient through competition for resources (Brittain *et al.* 2013). Even with large numbers of imported honeybees, the stability of pollination services decreases with increasing isolation from natural areas (Garibaldi *et al.* 2011), highlighting that neglect of the wild pollinator community cannot be offset simply by bringing in more domesticated honeybees.

1.3 Reversing declines and benefiting bee populations on farmland

By the 1990s European food stockpiles were beginning to build up and the need for ever increasing agricultural production was being questioned (Marren 2002). The large cost of the CAP (peaking at some 73% of the EU budget in 1985, European Commission 2016) combined with the planned expansion of the EU led to concerns that its structure was not viable (Donald *et al.* 2002). Efforts were made to decouple subsidies from production, resulting in the publication of Agenda 2000 (Krebs *et al.* 1999). Though agri-environment schemes were initially introduced in the 1992 reform of the CAP, Agenda 2000 further supported their development (Stoate *et al.* 2001). Through financial incentives, these schemes encourage farmers to set aside land for nature and to farm in a less intensive manner. Continued reform to the CAP throughout the 2000s led to a further decoupling of subsidies and food production

and increased support for agri-environment schemes (Stoate *et al.* 2009). The stated goals of these schemes include reducing fertiliser and pesticide use, protecting biodiversity, restoring landscapes and preventing rural depopulation (Kleijn and Sutherland 2003).

The desire to improve European farming through the use of agri-environment schemes offers the opportunity to conserve and benefit bee populations on farmland. In England, agri-environment schemes are modular and optional, with the farmer selecting which schemes to implement. Much of current pollinator-focused scheme design is based around the foraging requirements of those bumblebees that have shown the greatest declines. When concerns over bumblebee decline were raised in earnest in the 1990s, five of the most negatively affected bumblebees were designated as Biodiversity Action Plan species. Research into their ecological requirements was carried out, highlighting the importance of Fabaceae and of widespread resources at a landscape scale (Edwards 1999). As a result, Fabaceae featured prominently in pollinator-focused agri-environment scheme design. Farms can implement three main scheme types. Pollen and nectar margins are short lived (4-5 year) rotational strips of flowers, predominantly made up of Fabaceae such as red clover *Trifolium pratense*. Florally-enhanced margins are non-rotational grassland alongside field edges containing a mixture of grasses and flowering plants such as bird's-foot trefoil *Lotus corniculatus* and common knapweed *Centaurea nigra*. The final scheme is the most extensive and comprises creation, restoration and maintenance of low-intensity species rich grassland. A reasonable number of studies have looked at the response of foraging bumblebees to these schemes, and other agri-environment scheme habitats, and found that they can have a positive impact, with targeted schemes providing appropriate forage for a wide range of bumblebee species (Edwards 2003; Pywell *et al.* 2006; 2011a; Carvell *et al.* 2006b; 2007).

However, there are a number of outstanding issues surrounding this research. Bumblebees are large, strong flying insects and have been shown to congregate in appropriate foraging habitat, with the strength of the response modulated by relative forage availability in the surrounding landscape (Heard *et al.* 2007). Whilst previous studies have demonstrated a response to these schemes, it is not clear to what extent these schemes are having a population level effect, rather than simply redistributing foragers in the landscape (Holland *et al.* 2015). As eusocial insects, bumblebees form colonies, and consequently, bumblebee effective population size is the number of colonies in an area. Nesting densities of wild bumblebees are poorly understood and direct surveys are infrequent and labour intensive (Fussell and Corbet 1992; Osborne *et al.* 2008a; O'Connor *et al.* 2012). However, the use of molecular genetic techniques

has enabled estimates of bumblebee nesting density in a given area to be made (Knight *et al.* 2005; Goulson *et al.* 2010; Carvell *et al.* 2012), allowing an assessment of the population size at the farm level to be made. If these agri-environment schemes are having a positive population level impact, then a molecular genetic comparison between resource-rich and resource-poor farms can be used to demonstrate this.

Another similar problem is the issue of measuring changes in diversity. Several European studies have found that, when compared to control areas, a greater diversity of bees can be found in pollinator-focused agri-environment schemes (Knop *et al.* 2006; Albrecht *et al.* 2007; Kohler *et al.* 2008). It has been argued that in order to demonstrate the efficacy of a scheme, a before and after control impact assessment should be made, requiring surveying of the same area before and after the intervention (Kleijn *et al.* 2006). When employed, this approach also demonstrates a positive effect of targeted schemes on bee diversity (Scheper *et al.* 2015). However, both approaches suffer from the same problem. Even small bees are surprisingly mobile and can travel hundreds of metres to forage (Beil *et al.* 2008; Zurbuchen *et al.* 2010). Whilst the presence of a bee in a scheme area would suggest that this area is suitable for foraging, this does not mean that the bee was not present on the wider farm, just that it was not previously recorded in either control or before intervention areas. Observation cannot prove a negative. For example, take a farm with 50 bee species present in a variety of habitats. A before intervention piece of grassland is surveyed, revealing 10 of the 50 species. After habitat creation the survey is repeated and now 30 of the 50 species are recorded. Has the diversity of bees on the farm increased? Demonstrating that agri-environment scheme habitat attracts more bee species and provides more resources for them than alternative habitat is an important aim. However, an assessment at the level of the farm, including surveys of non-scheme habitats, is necessary to make a wider assessment of whether or not schemes are having a real and positive impact on bee diversity.

1.4 Aims and objectives for this thesis

Despite the desire for a strong evidence base empirically demonstrating the impact of agri-environment schemes on farmland bee populations, the number of studies that have attempted to do this is remarkably low. Consequently, it is the aim of this project to determine:

a) If current pollinator-focused agri-environment schemes significantly increase the population size of widespread bumblebee species

and

b) If current pollinator-focused agri-environment schemes significantly increase the diversity of the bee community at the farm scale.

In addressing these questions, this thesis will provide a strong empirical base that will help answer the broader question of whether current measures are sufficient to effectively promote farmland bee populations. These stated aims will be addressed through an extensive programme of field data collection backed up with laboratory based identification and molecular work. The main objectives of this work are as follows:

1. Genetic samples were collected from a range of farmland bumblebee species in order to allow estimation of population size at the farm scale, enabling the comparison of population sizes on farms with and without agri-environment schemes targeted at bees

2. Farmland bee populations (all species) were extensively surveyed over multiple years using a combination of active and passive sampling techniques

and

3. Wild bee floral preferences and pollen diets were quantified in relation to flowers sown as part of agri-environment schemes and those persisting in the wider environment.

Chapter 2 – A comparison of techniques for comparing farmland bumblebee populations

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All authors commented on draft versions of the manuscript, and a slightly amended version of
the published paper is presented here.

2.1 Abstract

Agri-environment schemes have been implemented across the European Union in order to reverse declines in farmland biodiversity. To assess the impact of these schemes for bumblebees, accurate measures of their populations are required. Here we compared bumblebee population estimates on sixteen farms using three commonly used techniques: standardised line transects, coloured pan traps and molecular estimates of nest abundance. There was no significant correlation between the estimates obtained by the three techniques, suggesting that each technique captured a different aspect of local bumblebee population size and distribution in the landscape. Bumblebee abundance as observed on the transects was positively influenced by the number of flowers present on the transect. The number of bumblebees caught in pan traps was positively influenced by the density of flowers surrounding the trapping location, and negatively influenced by wider landscape heterogeneity. Molecular estimates of the number of nests of *Bombus terrestris* and *Bombus hortorum* were positively associated with the proportion of the landscape covered in oilseed rape and field beans. Both direct survey techniques are strongly affected by floral abundance immediately around the survey site, potentially leading to misleading results if attempting to infer overall abundance in an area or on a farm. In contrast, whilst the molecular method suffers from an inability to detect sister pairs at low sample sizes it appears to be unaffected by the abundance of forage and thus is the preferred survey technique.

2.2 Introduction

In addition to facilitating the reproduction of wild plants, pollinating animals provide a valuable ecosystem service to food producers with approximately 35% of the world's plant based food supply dependent to a greater or lesser extent on animal pollination (Klein *et al.* 2007). The importance of wild pollinators for agricultural pollination has received more recognition in recent years, with wild populations providing an important service independent of the size of honey bee populations (Garibaldi *et al.* 2011; Garibaldi *et al.* 2013). Given the well documented declines in honey bee stocks in both the US and Europe in recent years (NRC 2006; Potts *et al.* 2010), it is even more important to maintain wild bee populations to provide the continuation of pollination services (Winfree *et al.* 2007a) through their synergies with managed honey bees (Brittain *et al.* 2013), as well as for their contributions in areas where they provide the majority of crop pollination (Klein *et al.* 2003).

Bumblebees (*Bombus* spp.) are probably the most important wild pollinators in the UK for both crops and wild plants (Osborne and Williams 1996; Goulson 2010). Along with many other pollinating insects (Biesmeijer *et al.* 2006), the abundance and distribution of bumblebee species has declined substantially across Europe and North America since the Second World War (Kosior *et al.* 2007; Grixti *et al.* 2009; Williams and Osborne 2009). In common with many other taxa, declines in wild bumblebees have been particularly acute in intensively farmed areas and consequently the UK and the European Union have introduced agri-environment schemes to counteract and reverse declines in biodiversity. These schemes offer the opportunity to provide resources for wild bumblebee populations. Previous work has highlighted the importance of robust and accurate population measurements of target taxa in order to determine the impact of agri-environment schemes (Kleijn and Sutherland 2003; Kleijn *et al.* 2006). Whilst there have been comparisons of the relative ability of different sampling techniques to measure bee diversity (e.g. Roulston *et al.* 2007; Westphal *et al.* 2008), there has been little assessment of the techniques used to measure the size of bumblebee populations.

The most commonly used technique to assess bumblebee abundance has been direct observations on standardised transect walks, based on the method for surveying butterflies (Pollard and Yates 1993) and modified for bees (e.g. Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2006; Carvell *et al.* 2007). Transect walks are relatively time consuming and require trained surveyors and consequently low effort passive sampling techniques such as pan traps have been used to sample bee populations (e.g. Leong and Thorp 1999; Cane *et al.* 2000). Passive blue vane traps designed for sampling beetles have also been shown to capture a high proportion of bumblebees (Stephen and Rao 2005), but this technique is not in common usage. The use of pan traps in sampling bumblebee populations has been limited, in part because it is thought that they under-sample large bodied bees (Toler *et al.* 2005). Nevertheless, because other passive sampling techniques such as trap nests are limited to cavity nesting bees (Tscharntke *et al.* 1998), pan traps are one of the few passive sampling techniques that can be used to survey bumblebees.

Bumblebee population estimates have also been obtained through the use of microsatellite markers. As eusocial insects, bumblebees form colonies, and because the queen is the dominant reproductive unit the effective population size can be estimated from the number of colonies in an area. Nesting densities of wild bumblebees are poorly understood and direct surveys are infrequent and labour intensive (Osborne *et al.* 2008a; O'Connor *et al.* 2012).

Because bumblebee colonies are founded by a single queen, and most species are monoandrous (Estoup *et al.* 1995; Schmid-Hempel and Schmid-Hempel 2000), the resulting high relatedness between workers makes social bumblebees amenable to identification of sisters using molecular techniques, such as microsatellite sequencing, enabling an estimation of the number of nests present in an area (e.g. Knight *et al.* 2005; Goulson *et al.* 2010).

Bumblebees are known to be highly mobile insects, sometimes travelling up to several kilometres to forage (Walther-Hellwig and Frankl 2000; Knight *et al.* 2005). Where foraging resources are scarce, such as in intensively farmed landscapes, bumblebees may respond more strongly to the presence of sown forage patches (Heard *et al.* 2007; Scheper *et al.* 2013), and hence a direct survey of these areas may over-represent the total population present in the area. Here we compare bumblebee population estimates using all three techniques on 16 farms in Southern England. Using transects and pan traps we examine abundance of all bumblebee species present, and we also estimate nest density via genetic sampling for two species, *Bombus terrestris*, a short tongued bumblebee and *Bombus hortorum*, a long tongued bumblebee. We then investigate local and landscape factors that may affect the bumblebee population estimates provided by these survey techniques. Our results have clear implications for future effective assessment and interpretation of bumblebee survey data.

2.3 Materials and methods

2.3.1 Study area

Sixteen farms were selected in north Hampshire and West Sussex, UK, representing a range of farming intensities and landscape gradients. The minimum distance between a pair of farms was 5 km. Farms were predominantly arable or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

2.3.2 Sampling methods

A standardised 3 km line transect was designed for each farm passing through semi-natural habitats, primarily grass or floristically enhanced margins alongside agricultural fields, hedgerows and woodlands. Regularly grazed and mown grassland and cropped areas were not surveyed. Transects preferentially passed through areas of flower-rich habitat that were present. Moving at a standard pace, all bumblebees within 2 m of the surveyor were recorded

to species level. Transects were walked three times throughout the summer of 2013, between 25th May - 5th June, 26th June - 15th July and the 3rd - 11th August (henceforth described as rounds one, two and three). Transects were subdivided into habitat sections, and for each section, the number of flowering units of each plant species was recorded during each of the sampling periods. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a capitulum) counted as a single unit. All sampling was conducted between 0930-1700 hours and conformed to meteorological guidelines from the UK butterfly monitoring scheme (Pollard and Yates 1993).

Twelve pan traps were placed out on each farm. Pan traps consisted of 500 ml plastic bowls and were spray-painted fluorescent blue, yellow or white (Sparvar Leuchtfarbe, Spray-Color GmbH). One of each colour, adjacent to each other, were attached to a wooden post at an approximate height of 60 cm. On farms where flower-rich margins had been sown (8 farms), two such posts were placed in a flower-rich margin and two were placed in a general grass margin. On farms lacking any sown flower-rich margins (8 farms), two posts were placed in each of two separate grass margins. Twenty-seven of the 32 sampled margins were also surveyed as part of the transect, allowing for calculation of the floral density at the trapping location for each sampling round. Posts in the same margin were separated by a distance of 25 m. Sampled margins were not closer than 200 m. Traps were filled with approximately 400 ml of water with a few drops of odourless liquid detergent (Surcare Sensitive). Traps were left out for 96 h before being collected. All farms were sampled at the same time so they were all subject to the same weather conditions. Traps were set three times on the 10th-15th June, 8th-12th July and the 1st-5th of August (henceforth described as rounds one, two and three). Invertebrate samples were stored in 70% ethanol and pinned prior to identification.

Genetic samples of *B. hortorum* and *B. terrestris* workers were collected between 26th June and 15th July. Each farm was divided into four sections and each quarter was searched for approximately 90 min, giving a total collection period of six hours. This sampling area conformed to a circle with radius 800 m and covered the majority of each farm. Non-lethal tarsal samples (Holehouse *et al.* 2003) were taken from the mid-leg of collected workers. Tarsi were stored immediately in 95% ethanol for later DNA extraction.

2.3.3 Molecular methods

DNA was extracted from the tarsal sample using the HOTShot protocol (Truett *et al.* 2000) and amplified at nine polymorphic microsatellite loci (B100, B118, B132, B10, B11, B96, B119, B124, B126, Estoup *et al.* 1995, 1996). Polymerase chain reactions (PCR) were carried out on samples using the QIAGEN Multiplex PCR kit. Multiplex PCRs were run for combinations of the loci B100(VIC)-B118(NED)-B132(FAM), B10(VIC)-B11(NED)-B96(FAM) and B119(FAM)-B124(NED)-B126(PET) (fluorescent markers indicated in parenthesis). B119 amplified weakly in *B. hortorum* and was removed from further analysis. PCR reactions were 10 µl in volume and contained 1 µl of Q-solution, 5 µl of PCR MasterMix, 1.8 µl of RNase free water, 1.2 µl of primer solution (6 x 0.2 µl of each primer, forward primers labelled with VIC, NED, FAM and PET dyes, Applied Biosystems) and 1 µl of template DNA. Samples were denatured at 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 54°C annealing for 90 s and 72°C for 90 s. This was followed by a final step at 72°C for 10 min. PCR products were visualised on an ABI 3730 capillary DNA sequencer with a 1:125 dilution before the run and using a GeneScan LIZ 500 internal size standard. Fragments were sized using GeneMapper software (Applied Biosystems).

Colony v2.0.5.0 (Jones and Wang 2009) was used to assign workers to unique colonies within each farm. This program uses maximum likelihood methods to assign sibship or parent-offspring relationships, and has been found to be the most reliable method available for assigning sibship in bumblebees (Lepais *et al.* 2010). Genotyping error was set at 2% (allele dropout 0.5% and other errors 1.5%). DNA-based capture–recapture models allow for multiple sampling of an individual, and the frequency distribution of the number of times each individual is recaptured can be used to estimate the population size. Instead of trying to estimate the number of individuals we were interested in estimating the number of nests represented by our sample of workers. Following Goulson *et al.* (2010) the program Capwire (Miller *et al.* 2005) was used to estimate the number of colonies present on each farm using the Two Innate Rate Model.

2.3.4 Landscape analysis

A buffer 1 km in radius was drawn around the centre point of each transect covering the majority of each farm and some of the surrounding area. The buffer covered the entirety of the 3 km transect. Using satellite imagery, ordinance survey maps and extensive ground

truthing during the survey period, detailed land cover maps to a resolution of 2 m² were created in Google Earth (Google Inc). Habitat types were binned into nine discrete biotope classes: i) crops non-attractive to bumblebees (wheat, barley, maize); ii) crops attractive to bumblebees (oilseed rape, field beans); iii) permanent grassland (grassland that is continuously grazed through the year or regularly cut for silage); iv) general grass (agri-environment grass margins, road verges, grassed access tracks, flower-poor grassland which is not regularly cut or grazed); v) woodland (broadleaf, coniferous and mixed); vi) hedgerow (the woody centre, not including associated hedge bottom grass strips, maximum width 4 m); vii) flower-rich (florally enhanced margins, species-rich grassland and wild bird seed margins containing a high density of bumblebee attractive plants); viii) water (including drainage ditches, rivers and ponds) and ix) urban (farm buildings, paved roads, private houses and gardens). These habitat maps were exported to ArcGIS 10.1 (ESRI Inc 2013) where the total area covered by each biotope was calculated. Following Fuentes-Montemayor *et al.* (2012), a Shannon diversity index was calculated from the number of different habitat types and their proportional abundance as a measure of landscape heterogeneity. Each of these factors was selected for inclusion in the initial model on the basis that they are likely to impact the size and distribution of bumblebee populations across the landscape.

2.3.5 Data analysis

Bumblebee population results obtained by the different sampling techniques were summed across all sampling rounds and tested for significant correlation using Spearman's rank correlation test.

Generalised linear models (GLMs) were run to investigate the impact of landscape variables on the number of bumblebees recorded along transects, caught in the pan traps and the estimated number of bumblebee colonies present on each farm. Models were run for the abundance of all bumblebee species and the abundance of the four most common bumblebee species, *B. hortorum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris*, with the transect and pan trap data summed across all sampling rounds. Before performing the main analysis, explanatory factors were tested for collinearity revealing a group of significantly intercorrelated variables. This group represented farming intensity, with the cover of non-attractive crops and hedgerow positively correlated with each other and negatively correlated with the landscape Shannon diversity index, the cover of woodland and cover of permanent grassland. Following Fuentes-Montemayor *et al.* (2012) these explanatory factors were

correlated with the response variable for each model, and the most strongly correlated factor was selected for inclusion in the initial model. The following explanatory factors were not significantly correlated with any other factor and were always included in the initial model; cover of attractive crops, cover of general grass, cover of flower-rich grass, cover of water and cover of urban. Models were simplified using a backward-stepwise approach until only significant factors ($p=0.05$) remained. At each step models were assessed using an ANOVA for a significant increase in residual deviance. If removing a non-significant term led to a significant increase in residual deviance the factor was returned to the model.

Generalised linear mixed effect models (GLMMs) were run to investigate the impact of floral abundance on the number of bumblebees recorded on the transects and caught in pan traps. For the transect abundance model, floral abundance was measured by the number of floral units counted on each transect for each sampling round. Data were not summed across rounds; instead sampling round was included as a random factor to account for temporal variation. Floral abundance was included as a fixed factor. For the pan trap model, floral density measurements were available for 27 of the 32 margins in which pan traps were placed, so data without density measurements was excluded from this analysis. The number of bumblebees caught in the six traps placed out in each margin were summed, to give two measurements per farm per sampling round. The farm nested within sampling round were included as random factors to account for spatial and temporal variation. Floral density was included as a fixed factor.

The strength of the impact of floral density on both survey techniques was investigated further. The pan trap data was recalculated to provide a single bumblebee total and floral density measurement for each farm in each sampling round. A floral density score was calculated for each transect in each sampling round. A GLMM was run with floral density, survey type and their interaction as fixed factors, with sampling round as a random factor.

All GLMMs were compared by ANOVA with a null model to test for significance. GLMs and GLMMs were run with a Gaussian, negative binomial or Poisson/quasi-poisson error distribution for the response variable where appropriate and were tested for overdispersion. All data analysis was conducted in R version 3.0.2 using the lme4 package for GLMMs (R Development Core Team).

2.4 Results

2.4.1 Bumblebee abundance

A total of 6,014 bumblebees of 14 species were recorded on the transects. The 14 bumblebee species comprised the ubiquitous social species *B. terrestris*, *B. lucorum*, *B. pratorum*, *B. pascuorum*, *B. hortorum* and *B. lapidarius* as well as *B. jonellus*, *B. hypnorum* and *B. ruderatus*. Five cuckoo bumblebee species (*Psithyrus*) were also recorded comprising *B. vestalis*, *B. barbutellus*, *B. rupestris*, *B. sylvestris* and *B. campestris*. A total of 1,980 bumblebees of 12 species were recorded in the pan traps. The 12 bumblebee species were the same as those recorded on the transects, less *B. barbutellus* and *B. ruderatus*. A total of approximately 3.5 million flowering units of 209 species of insect pollinated flowering plants were recorded on the transects.

A total of 386 *B. hortorum* and 593 *B. terrestris* workers were genotyped for eight and nine microsatellite loci, respectively. Eighty-five *B. hortorum* sister pairs and 58 *B. terrestris* sister pairs were identified. No 'noncircular' nests were identified. Out of the 16 farms studied, no sister pairs were found for *B. hortorum* on four farms and no sister pairs for *B. terrestris* on four farms (i.e. all bees sampled were from unique nests). Consequently, no estimate of the number of undetected nests could be made for these farms, leaving a total of 12 farms for each species. Estimated numbers of colonies per farm from Capwire ranged from 107 to 412 for *B. hortorum* and from 92 to 1,000 for *B. terrestris*.

2.4.2 Relationship between different survey techniques

There were few similarities between the different sampling techniques. The abundance of bumblebees observed on the transects was not correlated with the number of bumblebees caught in the pan traps (Spearman's $\rho=0.18$, $n=16$, $P=0.50$). The Shannon diversity index of bumblebees observed on the transects was not correlated with the diversity of bumblebees caught in the pan traps (Spearman's $\rho=0.19$, $n=16$, $P=0.47$). The molecular estimate for the number of *B. terrestris* nests in an area was not correlated with either the abundance of *B. terrestris* seen on the transects (Spearman's $\rho=0.26$, $n=12$, $P=0.42$) or caught in the pan traps (Spearman's $\rho=0.16$, $n=12$, $P=0.63$). The molecular estimate of the number of *B. hortorum* nests in an area was not correlated with either the abundance of *B. hortorum* seen on the

transects (Spearman's $\rho=0.11$, $n=12$, $P=0.73$) or caught in the pan traps (Spearman's $\rho=0.06$, $n=12$, $P=0.85$).

2.4.3 Effects of the surrounding landscape

Sampled farms varied considerably in land use types (Table 2.1) with an average of $72.1 \pm 2.7\%$ of land covered in crop or permanent grassland and $21.8 \pm 2.8\%$ covered by semi-natural habitat. Few landscape variables were significantly associated with bumblebee abundance across the sampling techniques. Final model results are summarised in Table 2.2. There were no significant interactions between any of the final explanatory variables.

Table 2.1. Land use types for surveyed farms within a 1 km radius. Means \pm 1 SE are given for the 16 selected farms.

Land use type	Area (%)	Minimum (%)	Maximum (%)
Attractive crops	10.99 ± 1.81	3.13	32.58
Non-attractive crops	46.52 ± 4.23	16.68	70.59
Permanent grassland	14.62 ± 2.52	0.68	34.32
Woodland	11.49 ± 3.01	0.85	35.18
Hedgerow	2.10 ± 0.24	0.39	3.34
Flower-rich	0.99 ± 0.32	0.00	4.36
General grass	7.23 ± 0.99	2.88	16.17
Water	1.05 ± 0.84	0.00	13.44
Urban	5.00 ± 0.52	1.83	7.44

Table 2.2. Summary table showing significant effects of landscape variables on bumblebee population metrics.

	Variable	df	t	P	sig.
Transect abundance all species	<i>No significant factor</i>				
Pan trap abundance all species	Landscape heterogeneity	1,15	-2.382	0.032	*
<i>Bombus terrestris</i>					
Molecular nest estimate	Attractive crop cover	1,11	2.226	0.0502	.
Transect abundance	<i>No significant factor</i>				
Pan trap abundance	Landscape heterogeneity	1,15	-2.681	0.018	*
<i>Bombus hortorum</i>					
Molecular nest estimate	Attractive crop cover	1,11	2.948	0.016	*
	General grass cover	1,11	-2.316	0.046	*
Transect abundance	Flower-rich cover	1,15	3.259	0.0062	**
	General grass cover	1,15	-2.524	0.025	*
Pan trap abundance	General grass cover	1,15	-2.143	0.0502	.
<i>Bombus lapidarius</i>					
Transect abundance	<i>No significant factor</i>				
Pan trap abundance	<i>No significant factor</i>				
<i>Bombus pascuorum</i>					
Transect abundance	<i>No significant factors</i>				
Pan trap abundance	<i>No significant factors</i>				

Landscape heterogeneity was significantly negatively associated with both the total number of bumblebees caught in pan traps (GLM, $t_{1,15}=-2.382$, $P=0.032$) and the number of *B. terrestris* (GLM, $t_{1,15}=-2.681$, $P=0.018$), the most commonly caught bumblebee ($n=925$). The number of *B. hortorum* caught in pan traps was marginally non-significantly negatively associated with the cover of general grass habitats (GLM, $t_{1,15}=-2.143$, $P=0.0502$, Figure 2.1b). For the transect data, only the abundance of *B. hortorum* was associated with landscape variables, being positively associated with the area of flower-rich habitat (GLM, $t_{1,15}=3.259$, $P=0.0062$) and negatively associated with the cover of general grass habitats (GLM, $t_{1,15}=-2.524$, $P=0.025$, Figure 2.1a).

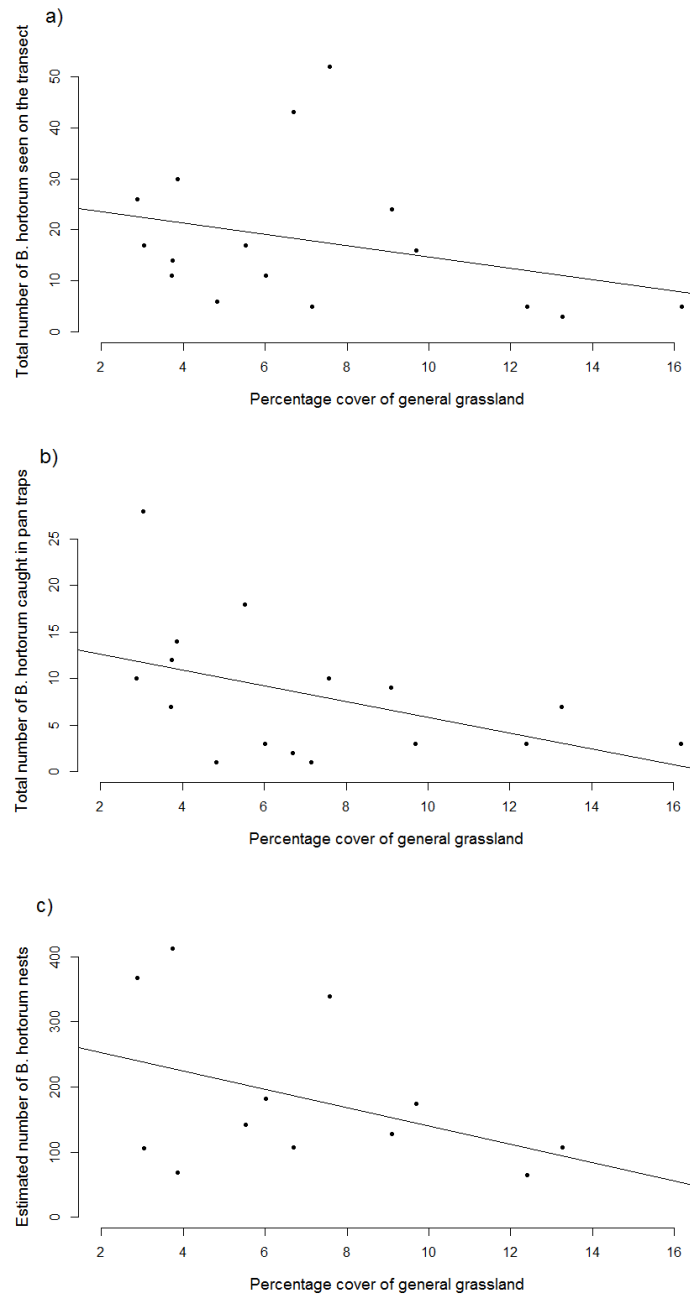


Figure 2.1. Effect of the proportion of general grass habitats on *Bombus hortorum* as measured by (a) the total number of individuals counted on the transect, GLM $t_{1,15}=-2.524$ $p=0.025$ (b) the total number of individuals captured in pan traps, GLM $t_{1,15}=-2.143$ $p=0.0502$ and (c) the molecular estimate of number of nests, GLM $t_{1,11}=-2.316$ $p=0.046$.

For the molecular estimates of the number of colonies present, *B. hortorum* was significantly (GLM, $t_{1,11}=2.948$, $P=0.016$) and *B. terrestris* marginally non-significantly ($t_{1,11}=2.226$, $P=0.0502$) associated with the cover of attractive crops. In addition, the abundance of *B. hortorum* colonies was negatively associated with the cover of general grass habitats (GLM, $t_{1,11}=-2.316$, $P=0.046$, Figure 2.1c).

2.4.4 Effect of local floral abundance

Bumblebee abundance on the transects was strongly associated with recorded floral abundance on the transects ($\chi^2=30.9$, $P<0.001$, Figure 2.2). The number of bumblebees caught in pan traps was also strongly associated with the density of flowers in the margin in which they were placed ($\chi^2=8.1$, $P=0.004$, Figure 2.3). This effect was similar for both methods with the inclusion of the interaction between floral density and survey technique not significantly improving the model ($\chi^2=0.8$, $P=0.364$).

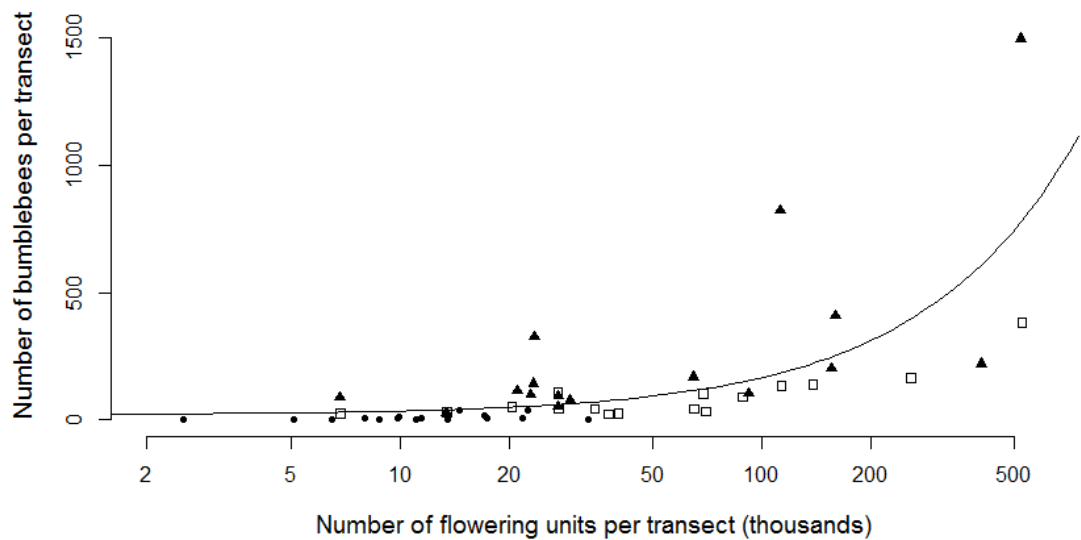


Figure 2.2. Relationship between floral abundance and number of observed foraging bumblebees on 3 km transects during the May-August sampling period. Sampling round one = circles, round two = open squares, round three = triangles. GLMM, $\chi^2=30.9$, $P<0.001$. Note log scale used on x-axis.

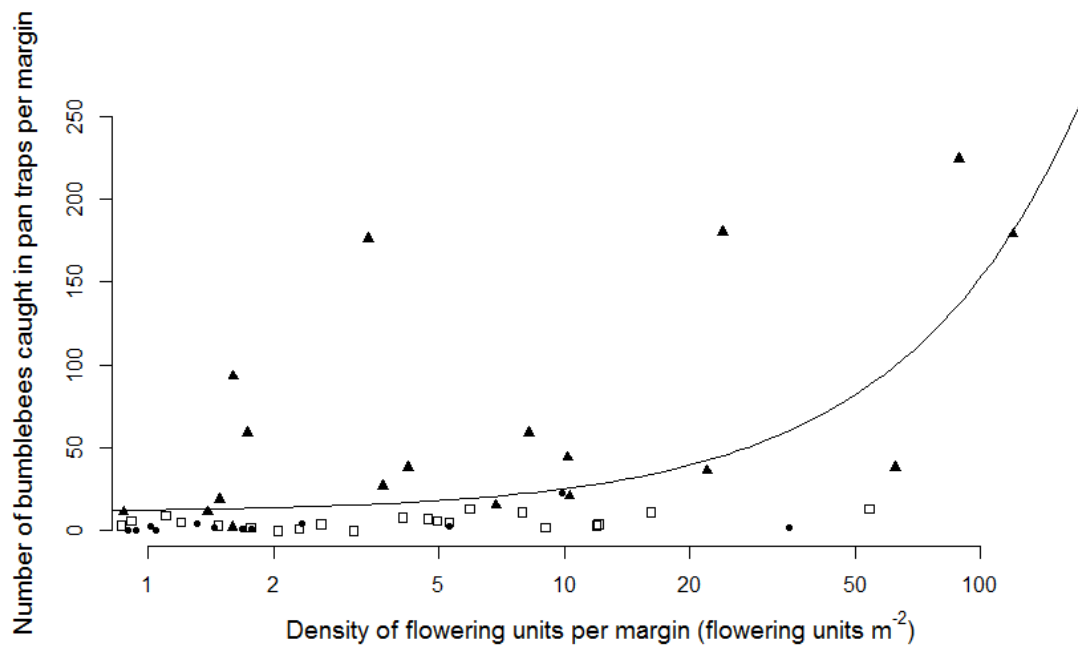


Figure 2.3. Relationship between floral density and the number of bumblebees caught in pan traps placed out in agricultural margins during the May-August sampling period. Sampling round one = circles, round two = open squares, round three = triangles. GLMM, $\chi^2=8.1$, $P=0.004$. Note log scale used on x-axis.

2.5 Discussion

If we are to devise sensible management strategies for bumblebees or other pollinators, to conserve rare species or maintain adequate populations of common species to deliver pollination services, then it is important that we have accurate means of measuring their populations. Without such measures we are unable to detect population change, compare populations in regions, or detect the effectiveness of different management techniques. It is thus of considerable concern that the three techniques here, all of which are in common use, appear to produce population measurements that do not correlate with one another. Rather than accurately measuring the bumblebee population at the farm level, each technique seems to capture a different aspect of local bumblebee abundance and distribution throughout the landscape. Such parallels can also be found with other invertebrate sampling techniques; for example pitfall traps have long been regarded as measuring invertebrate activity rather than just abundance, where activity is governed by factors such as the availability of food resources and vegetation density (Sunderland *et al.* 1995).

Semi-natural environments on agricultural land can provide suitable foraging resources and nesting sites for bumblebees and are expected to be beneficial to bumblebee populations (Kells and Goulson 2003; Pywell *et al.* 2006). However, in our study, only one positive association with semi-natural habitats or factors correlated with semi-natural habitats was found, that of the abundance of *B. hortorum* to the area covered in flower-rich habitat. Instead, in common with other studies, the number of bumblebees recorded on the transects was strongly associated with floral abundance on the transect itself (Pywell *et al.* 2006; Carvell *et al.* 2007).

Whilst previous pan trap studies have caught few large bodied bees (Toler *et al.* 2005; Westphal *et al.* 2008), pan traps in this study caught large numbers of bumblebees (1,980 individuals representing 70% of the total number of bees caught in pan traps). Whilst this represents a smaller proportion than the transects where the 6,014 bumblebees represented 93% of the total bees recorded, our data show that bumblebees can be attracted to pan traps in large numbers. The total number of bumblebees caught in pan traps over the survey period on each farm depends on the wider landscape structure, with a greater number caught in more intensively farmed, less heterogeneous environments. It has been argued that landscape heterogeneity is important for farmland biodiversity (Benton, Vickery and Wilson 2003) and it seems unlikely that less heterogeneous landscapes genuinely support more bumblebees, as the process of agricultural intensification that reduces landscape heterogeneity is the primary reason behind the decline in agricultural bumblebee populations (Goulson *et al.* 2005; Williams 2005; Carvell *et al.* 2006a). It is well known that bumblebees forage over large areas, in particular *B. terrestris* (Walther-Hellwig and Frankl 2000; Knight *et al.* 2005; Westphal *et al.* 2006), which was the most common bee caught in the pan traps. In landscapes with lower heterogeneity, bumblebees may have to travel greater distances to find suitable forage, and are more likely to encounter pan traps than bees in a more heterogeneous landscape where resources are more widely distributed. This may result in an overestimation of the total population with the largest effect found in those species with the greatest dispersal ability. Previous studies have found that pan traps catch more bees in flower-poor areas, arguing that the relative lack of resources may make the pan traps more attractive (Baum and Wallen 2011; Morandin and Kremen 2013). It is consequently surprising that, as with transect surveys, our results suggest that pan traps catch more bumblebees in areas with a greater density of flowers. Despite the differences in this relationship, pan traps are clearly strongly influenced by both landscape structure and floral availability and are not suitable for measuring bumblebee population sizes.

Previous studies have highlighted the lack of an apparent relationship between observed bumblebee abundance and the presence of varying areas of semi-natural habitat (e.g. Westphal *et al.* 2003; Hermann *et al.* 2007). It has been argued that as the proportion of arable land increases the abundance of semi-natural forage is reduced, resulting in sown forage patches being relatively more exploited because they represent a greater proportion of the total forage available in an area (Heard *et al.* 2007), making it difficult to assess the true benefit of semi-natural habitats to bumblebee populations. This is borne out by Carvell *et al.* (2011) who found that densities of *B. lapidarius*, *B. pascuorum*, *B. hortorum* and *B. terrestris* agg. on sown forage patches were significantly higher in areas with a greater proportion of arable land. Moreover, bumblebees also exhibit a degree of site constancy once lucrative locations have been found (Osborne *et al.* 1999), leading to a greater accumulation of workers where alternative forage is scarce. This 'ecological contrast' between sown floral resources and the resources present in the wider environment has been shown to affect how strongly pollinators respond to agri-environment schemes (Scheper *et al.* 2013). Given the strong aggregative effect of highly contrasting floral resources and the lack of similarity between different population measurements, it is not clear that a strong response to agri-environment schemes necessarily translates into a positive population level impact for bumblebees.

Whilst these aggregative effects may influence the distribution of foraging bumblebees throughout the landscape, they should not affect the distribution of workers from the same colony. Unlike honeybees, bumblebees lack a directional recruitment mechanism between foragers (Dornhaus and Chittka 1999), so sister workers should be distributed randomly throughout the environment. Consequently, even if samples are collected from an aggregation of foraging bees it should still be representative of the number of colonies present in an area. The molecular nest estimates for both *B. hortorum* and *B. terrestris* showed a positive association with the percentage of the landscape covered with oilseed rape and field beans. It has been argued that these mass flowering crops are good for providing resources for bumblebees in intensively farmed environments, and their presence has been associated with higher observed densities of bumblebees on sown forage patches (Westphal *et al.* 2003) and on transect walks (Hermann *et al.* 2007). Whilst this may also be as a result of bumblebee aggregation, our molecular estimates suggest that there may be a real beneficial effect of mass flowering crop on the number of colonies founded in an area.

However, the molecular population estimates obtained in this study show marked variation, particularly for *B. terrestris*. True nesting densities of wild bumblebees are poorly understood

so it is not possible to comment on whether these colony abundance estimates are reasonable, but the lack of detection of sister pairs from 4 sites suggests that sampling the same site over multiple days may be required to detect sufficient sister pairs to make more comprehensive population estimates. Additionally, the low similarity between the results obtained from the molecular estimates and those from the direct sampling techniques may be due to differences in average nest size between farms. Bumblebee colonies placed in resource-rich areas develop faster than those in resource-poor areas, though their reproductive output was unaffected (Goulson *et al.* 2002; Williams *et al.* 2012). Colony growth of wild bumblebee nests is poorly understood, but with variable emergence and colony establishment dates for individual queens it seems unlikely that consistent differences in nest size would exist between farms, but this is an issue that requires further study. Despite these shortcomings, the molecular technique does not appear to be biased by floral abundance in the study area and is likely to be a more accurate measure of bumblebee population size than the direct survey techniques.

The presence of general grass habitats was negatively associated with population estimates for the long tongued *B. hortorum* across all three sampling techniques. The majority of the general grass biotope comprised 2 to 6 m grass margins, usually located alongside field edges. This negative association may also be as a result of dilution, with *B. hortorum* preferring these habitats, leading to a reduced detection in other areas. However, this seems unlikely as from personal observation (TJW), during the transects and the collection of sampling techniques, few *B. hortorum* were seen foraging in general grass habitats because these areas did not provide suitable forage plants, in contrast to hedgerows and flower-rich grassland. General grass margins tend to be comprised of coarse grasses which may repress forage plants such as *Stachys sylvatica*. This is a typical hedgerow plant that is not included in conservation mixes and was the most popular forage plant for *B. hortorum* in this study, representing 62% of worker foraging visits during the collection of genetic samples and 38% of visits by all castes over the whole survey period. Given that the majority of the conservation priority bumblebee species in Britain are also long tongued and favour similar plant species to *B. hortorum* (Goulson *et al.* 2005), a potentially negative impact of general grass margins requires further investigation.

2.6 Conclusions

Distinguishing between factors that have a genuine population level effect and factors that simply cause a redistribution of individuals around the landscape is vitally important if the drivers of bumblebee population size at the farm scale are to be identified, understood and integrated into contemporary management schemes. Our results suggest that the bumblebee population size estimates from both transects and pan traps are highly affected by the availability of forage in both absolute terms and in relationship to the floral resources present in the wider environment, potentially leading to an overestimation of the population in intensively farmed areas and an underestimation of the population in less intensively farmed areas. Whilst the molecular technique does not appear to be affected by bumblebee aggregation it is substantially more expensive and time consuming and may not produce useful results if insufficient samples to detected sister pairs are collected. However, in the absence of a true population measurement it appears to be the best technique. Further detailed work is needed to determine a practical and cost effective way to accurately measure bumblebee populations in agricultural regions.

Chapter 3 – Targeted agri-environment schemes significantly improve the population size of common farmland bumblebee species

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W.O.H. Hughes assisted with the design of the molecular techniques. All authors commented on draft versions of the manuscript, and a slightly amended version of the published paper is presented here.

3.1 Abstract

Changes in agricultural practice across Europe and North America have been associated with range contractions and local extinction of bumblebees (*Bombus* spp.). A number of agri-environment schemes have been implemented to halt and reverse these declines, predominantly revolving around the provision of additional forage plants. Although it has been demonstrated that these schemes can attract substantial numbers of foraging bumblebees, it remains unclear to what extent they actually increase bumblebee populations. We used standardised transect walks and molecular techniques to compare the size of bumblebee populations between Higher Level Stewardship (HLS) farms implementing pollinator friendly schemes and Entry Level Stewardship (ELS) control farms. Bumblebee abundance on the transect walks was significantly higher on HLS farms than ELS farms. Molecular analysis suggested maximum foraging ranges of 566 m for *Bombus hortorum*, 714 m for *B. lapidarius*, 363 m for *B. pascuorum* and 799 m for *B. terrestris*. Substantial differences in maximum foraging range were found within bumblebee species between farm types. Accounting for foraging range differences, *B. hortorum* (47 vs 13 nests/km²) and *B. lapidarius* (45 vs 22 nests/km²) were found to nest at significantly greater densities on HLS farms than ELS farms. There were no significant differences between farm type for *B. terrestris* (88 vs 38 nests/km²) and *B. pascuorum* (32 vs 39 nests/km²). Across all bumblebee species, HLS management had a significantly positive effect on bumblebee nest density. These results show that targeted agri-environment schemes that increase the availability of suitable forage can significantly increase the size of wild bumblebee populations.

3.2 Introduction

Since the Second World War, many bumblebee species have suffered severe range contractions and local extinctions across Europe and North America (Kosior *et al.* 2007; Grixti *et al.* 2009; Williams and Osborne 2009). This decline has been particularly severe in arable areas, mirroring a wider decline in farmland biodiversity in this time period (Robinson and Sutherland 2002). The speed of this decline has been quite striking, with species considered widespread in Free and Butler (1959) described as rarities in Alford (1975).

The use of Fabaceae pollen by bumblebees and the decline in Fabaceae across the wider countryside has received a great deal of attention in recent years (Rasmont and Mersch 1988; Carvell *et al.* 2006a). Bumblebees that have declined most seriously tend to be medium to

long-tongued species that have more restricted diets and collect a greater proportion of their pollen from Fabaceae (Goulson *et al.* 2005). Analysis of pollen from museum specimens shows that before the 1950s, bumblebee species that subsequently declined collected pollen from almost one third fewer plant species than bumblebee species that have remained stable (Kleijn and Raemakers 2008). More generally, the loss of appropriate pollen host plants has been implicated as a key driver behind the loss of wild bee diversity (Biesmeijer *et al.* 2006; Scheper *et al.* 2014). In addition, it has been argued that certain species are inherently more susceptible to intensification as a result of their climatic and habitat specialisation. Aspects of their life history such as emergence period have also been implicated, with late emerging species more vulnerable to a shortage of summer forage compared to better established spring emerging species (Williams *et al.* 2009). Consequently, in order to counteract these declines the creation of Fabaceae-rich habitats has become an important part of the design and assessment of agri-environment schemes for bumblebees (Edwards 2003; Carvell *et al.* 2007).

Across the European Union, agri-environment schemes are now funded as part of the Common Agricultural Policy. In England, two tiers of environmental stewardship operated between 2005 and 2014. Entry Level Stewardship (ELS, DEFRA 2005a) was open to all farmers. Higher Level Stewardship (HLS, DEFRA 2005b) provided greater financial rewards for more substantial and rigorous agri-environmental schemes, often in targeted high priority areas. There are three main pollinator-focused HLS options. HF4 pollen and nectar mixes are rotational plots or strips sown with a mixture of predominantly leguminous plants such as *Trifolium pratense* L. and *Trifolium hybridum* L. which tend to be resown within a 5 year period. HE10 floristically enhanced grass buffer strips are non-rotational grassland alongside fields and are composed of a mixture of grasses and plants such as *Centaurea nigra* L. and *Lotus corniculatus* L. HK6/7/8 focus on the maintenance, restoration and creation of species rich grassland. These schemes are aimed at increasing the availability of forage in farmed environments to support populations of bumblebees and other pollinators. A number of studies have assessed how well flower-rich agri-environment schemes establish and provide resources throughout the year, specifically for bumblebees (Carvell *et al.* 2006b; Pywell *et al.* 2006; Carvell *et al.* 2007), but little work has been done to establish if these schemes are having a population level impact and are actually increasing the size of bumblebee populations, rather than simply redistributing existing foragers in the landscape (Holland *et al.* 2015).

As eusocial insects, bumblebees form colonies, and consequently bumblebee effective population size is the number of colonies in an area. Nesting densities of wild bumblebees are poorly understood and direct surveys are infrequent and labour intensive (Fussell and Corbet 1992; Osborne *et al.* 2008a; O'Connor *et al.* 2012). Moreover, previous studies that have assessed how agri-environment schemes have affected pollinators predominantly use counts of foraging workers when ideally we need to know how the schemes affect the abundance of bumblebee colonies. Bumblebee colonies are founded by a single queen, and most species are monoandrous (Estoup *et al.* 1995; Schmid-Hempel and Schmid-Hempel 2000; Hughes *et al.* 2008). The resulting high relatedness between workers makes social bumblebees amenable to identification of sisters using molecular techniques, such as microsatellite sequencing. These techniques have been used to quantify foraging distance and nesting density (Darvill *et al.* 2004; Knight *et al.* 2005; Carvell *et al.* 2012), conservation genetics and population structuring (Ellis *et al.* 2006; Charman *et al.* 2010) and dispersal distances of reproductive castes (Kraus *et al.* 2009; Lepais *et al.* 2010). Moreover, the development of molecular capture-recapture models (e.g. Miller *et al.* 2005) has enabled estimation of the number of bumblebee colonies present in an area, allowing assessment of nest mortality rates (Goulson *et al.* 2010). These studies have provided a novel understanding of bumblebee ecology that could not have been achieved with conventional techniques.

The aim of this study was to evaluate the effectiveness of pollinator-targeted HLS schemes in boosting bumblebee populations by comparing farms with and without such HLS schemes using a combination of molecular techniques and direct surveys. The molecular studies of nest density and foraging range focus on the four most common bumblebee species on farmland in Southern England: *Bombus hortorum*, *Bombus lapidarius*, *Bombus pascuorum* and *Bombus terrestris*. All species are members of the 'big six' bumblebee species that remain common in the UK, being found in almost every habitat (Goulson *et al.* 2006). They differ markedly in tongue length and hence the flowers that they visit. *B. lapidarius* and *B. terrestris* are ubiquitous short-tongued bumblebees and *B. pascuorum* has a medium length tongue. *B. hortorum* has a much longer tongue than the other members of this group, of around 13 mm compared to 5-8 mm (Goulson *et al.* 2008a). Both *B. pascuorum* and *B. hortorum* have remained relatively common whilst most long and medium-tongued bumblebees have declined markedly in western Europe in the last 60 years (Goulson *et al.* 2005; Kosior *et al.* 2007).

The relative success of these four contrasting bumblebees will therefore provide greater insight into the suitability of farm management for other bumblebee species and potentially other pollinators. In addition to the molecular studies of the nesting density of these four species, we investigate more broadly how the provision of additional forage for insect pollinators has affected populations of all bumblebee species using standard field surveys. Our results are examined in relationship to management type and floral availability and have clear implications for the management of pollinator populations on agricultural land.

3.3 Materials and Methods

3.3.1 Study area

Nine ELS and nine HLS farms were selected in north Hampshire and West Sussex, UK (Figure 3.1). On average, HLS farms covered 256.22 ± 37.80 ha, significantly more than ELS farms which covered 156.67 ± 22.07 ha (t-test, $t_{16}=2.565$, $p=0.021$). Selected HLS farms had been implementing an average of 5.56 ± 0.13 ha of pollinator focused flower-rich options representing $2.17 \pm 0.05\%$ of the farm area for a minimum of three years. Almost all farms in lowland England implement some form of agri-environment scheme and consequently basic ELS farms were selected as the control group. ELS farms were not implementing any pollinator focused schemes. The centre points of the two closest farms were 5.4 km apart. At this distance it is unlikely that bumblebees will be able to regularly travel between farms on foraging trips (see Knight *et al.* 2005; Osborne *et al.* 2008b). There was no pairing of farms. Farms were predominantly arable or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

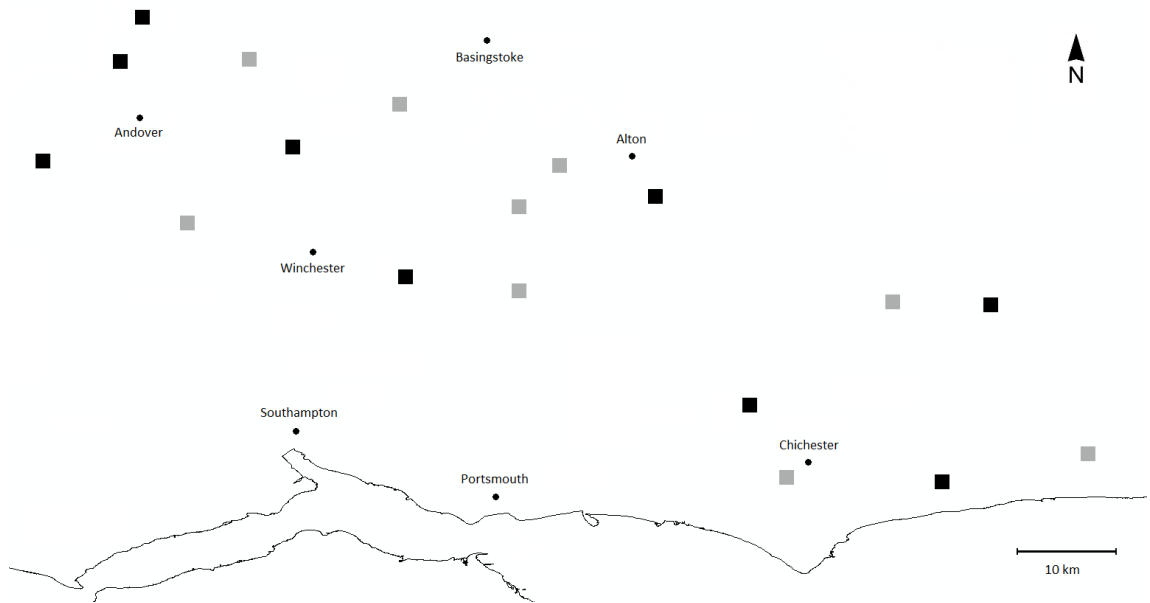


Figure 3.1. Map of the study area showing Higher Level Stewardship farms (black squares) and Entry Level Stewardship Farms (grey squares).

3.3.2 Line transect counts

A standardised 3 km line transect was designed for each farm. Transects on HLS farms were designed to pass through as many areas of flower-rich options as possible. Bumblebee activity was recorded along each transect following standard bee walk methodology (see Carvell *et al.* 2004). All bumblebees within 2 m of the recorder were recorded to species and caste level. The first flowering species visited and the purpose of the visit, for either pollen or nectar, was recorded. Bumblebees collect pollen by loading it onto specially adapted grooves on their hind legs. Many foraging bumblebees will visit a flower for nectar with pollen collected from different plant species, so only bees actively grooming pollen onto their hind legs were recorded as collecting pollen. For each transect, the number of flowering units of each plant species within 2 m of the recorder were estimated. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a head, a capitulum) counted as a single unit.

Sixteen farms (8 HLS, 8 ELS) were surveyed in 2013. Transects were walked three times throughout the year, between 25th May - 5th June, 26th June - 15th July and 3rd - 11th August. Seventeen farms (8 HLS, 9 ELS) were surveyed in 2014. Transects were walked three times throughout the year, between 17th - 27th May, 21st June - 9th July and 3rd - 15th August. Each of these six sampling periods are henceforth referred to as sampling rounds. This sampling followed the UK butterfly Monitoring Scheme guidelines (Pollard and Yates 1993) with all

surveys conducted between 0930-1700 hours when the temperature was above 13°C with at least 60% clear sky, or above 17°C with any sky conditions. Observation time was randomised between farms. No surveys were conducted when it was raining.

3.3.4 Genetic sample collection

Genetic samples were collected at the end of June and beginning of July as colony size should be approaching its peak at this time before the production of reproductive castes. Farms were sampled under the same climatic conditions as for the transects. *B. hortorum* and *B. terrestris* samples were collected from 8 HLS and 8 ELS farms between 26th June and 15th July 2013. HLS and ELS farms were visited on alternating days. *B. lapidarius* and *B. pascuorum* samples were collected from 8 HLS and 9 ELS farms between 21st June and 9th July 2014. From the centre of each farm an area of a circle with radius 800 m was searched for bumblebee workers on one day for six hours. The circle was divided into quarters and each part was searched for 90 min to maximise the detection of unique colonies present on each farm. The total area covered the majority of each farm and all flower-rich options present. Non-lethal tarsal samples (Holehouse *et al.* 2003) were taken from the mid-leg of collected workers. Tarsi were stored immediately in 95% ethanol for later DNA extraction. A GPS reading accurate to the nearest few metres was taken at the location of each sample. If the sampled bee had been visiting flowers, the first flowering species the bee had been seen to visit and the purpose of the visit, for either pollen or nectar, was recorded for *B. lapidarius* and *B. pascuorum*, but not for *B. hortorum* and *B. terrestris*.

3.3.5 Molecular methods

DNA was extracted from the tarsal sample using the HotSHOT protocol (Truett *et al.* 2000) and amplified at nine polymorphic microsatellite loci (Estoup *et al.* 1995, 1996). All species used the same nine markers (*B100*, *B118*, *B132*, *B10*, *B11*, *B96*, *B119*, *B124*, *B126*), with the exception of *B100* replaced with *B121* for *B. pascuorum* and *B119* replaced with *B131* in *B. pascuorum* and *B. lapidarius*. Polymerase chain reactions (PCR) were carried out on samples using the QIAGEN Multiplex PCR kit. Multiplex PCRs were run for combinations of the loci *B100*(VIC)/*B121*(VIC)-*B118*(NED)-*B132*(FAM), *B10*(VIC)-*B11*(NED)-*B96*(FAM) and *B119*(FAM)/*B131*(FAM)-*B124*(NED)-*B126*(PET) (fluorescent markers indicated in parenthesis). *B119* amplified weakly in *B. hortorum* and was removed from further analysis. PCR reactions were 10 µl in volume and contained 1 µl of Q-solution, 5 µl of PCR MasterMix, 1.8 µl of RNase

free water, 1.2 µl of primer solution (6 x 0.2 µl of each primer, forward primers labelled with VIC, NED, FAM and PET dyes, Applied Biosystems) and 1 µl of template DNA. Samples were denatured at 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 54°C annealing for 90 s and 72°C for 90 s. This was followed by a final step at 72°C for 10 min. PCR products were visualised on an ABI 3730 capillary DNA sequencer with a 1:125 dilution before the run and using a GeneScan LIZ 500 internal size standard. Fragments were sized using GeneMapper software (Applied Biosystems).

3.3.6 Identification of unique colonies

COLONY v2.0.5.0 (Jones and Wang 2009; Wang 2013) was used to assign workers to unique colonies within each farm. This program uses maximum likelihood methods to assign sibship or parent-offspring relationships, and has been found to be the most reliable method available for assigning sibship in bumblebees (Lepais *et al.* 2010) and has been used extensively for this purpose (Charman *et al.* 2010; Carvell *et al.* 2012). The analysis was run globally with all farms included. Genotyping error was accounted for in the analysis by setting the error rate at 2% (allele dropout 0.5% and other errors 1.5%), rates typical for bumblebee genotyping (Lepais *et al.* 2010; Goulson *et al.* 2010). This procedure may regroup a small fraction of unrelated individuals into a common colony (Type I errors, Lepais *et al.* 2010). Type I errors for this number of loci with an average of ten alleles per loci are expected to occur with a frequency of <0.005 (Wang 2013) which would lead to less than one worker from each species being falsely grouped. Global analysis resulted in a number of sister pairs identified between farms at distances of over 6 km (see results) and these are highly likely to be spurious. The proportion of false sister pairs was 0.0207 for *B. hortorum*, 0.0181 for *B. lapidarius*, 0.0334 for *B. pascuorum* and 0.0202 for *B. terrestris*. These false positive rates were used for the calculation of species specific foraging range estimates. Given that any falsely accepted sisterhoods within farms would be randomly distributed this was not considered to have a significant effect on the estimation of the number of unique colonies. Where markers departed from Hardy-Weinburg Equilibrium (HWE) or were in linkage disequilibrium they were individually removed and a separate analysis conducted to determine their impact on sister pair identification.

3.3.7 Genetic parameters

Due to high relatedness between sister pairs, only one worker per colony was randomly selected for the following analyses. Genepop v4.2 (Rousset 2008) was used to calculate F-

statistics, to test for genotypic linkage disequilibrium between pairs of loci, and the HWE of each locus on each farm. Bonferroni corrections for multiple tests were used to minimise type I errors (Rice 1989).

3.3.8 Estimation of number of unique colonies

DNA-based capture–recapture models allow for multiple sampling of an ‘individual’, and the frequency distribution of the number of times each ‘individual’ is sampled can be used to estimate the population size. In most instances, the individual will correspond to a single vertebrate organism. However, for bumblebees the colony represents the reproductive individual and capturing two sisters from the same colony is analogous to sampling the same individual twice. Instead of trying to estimate the number of individual workers we are interested in estimating the number of nests represented by our sample of workers. The program Capwire (Miller *et al.* 2005) can be used to obtain point estimates of the number of bumblebee colonies present at a farm from a subsample of workers (Goulson *et al.* 2010; Stanley *et al.* 2013). Capwire uses two different estimation methods, an Even Capture Model (ECM) that assumes equal chance of sampling workers from the same colony and a Two Innate Rate Model (TIRM) that assumes unequal sampling chance. Heterogeneity of capture probability is a characteristic of bumblebee nests so the TIRM model was chosen as it probably gives more accurate estimates of the number of nests present at a site (Goulson *et al.* 2010). Capwire models were run in 0.1 increments with capturability ratios of minimum 1, maximum 20; 95% confidence intervals for the estimate on population size based on 1000 bootstrap replicates and a largest population size of 2000 for dimensioning.

3.3.9 Estimation of foraging range and nesting density

A foraging range estimate was calculated for each species on each farm type from an analysis of the distribution of identified sisters. GPS marked sisters were plotted in Google Earth (Google Inc.) and the distance between them was measured. Sister pairs were ranked by distance of separation and plotted as a cumulative proportion of total sister pairs. A logarithmic line of best fit as measured by the highest adjusted R^2 was then plotted using the formula $y=a(\ln(x+b))+c$ and was used for foraging range calculations (Knight *et al.* 2005). The true separation distance was calculated for the point at which the proportion of sister pairs falls to the false positive rate as calculated for each species (see above). The resulting value was then halved to give an estimated maximum foraging range for each species, with the most

conservative assumption being that two detected sisters were both foraging equidistant from their nest but in opposite directions. It is highly likely that bumblebees will forage over greater distances than this estimate, but the chances of detecting sister pairs at great distances becomes remote and hence this estimate is best referred to as a minimum estimate of the maximum foraging distance (see Knight *et al.* 2005).

Whilst the area searched on each farm was a circle with radius 800 m, bumblebees are mobile organisms and the true area sampled is a function of the foraging range of each bumblebee species. A bumblebee sampled on the edge of the 800 m sampling area may have flown there from many hundreds of meters away. Consequently, estimates of nesting density were obtained by dividing the molecular estimate of the number of colonies present by the area of a circle of radius 800 m plus the estimated foraging range of the respective species on each farm type to more accurately reflect the size of the sampled area.

3.3.10 Data analysis

The effect of environmental stewardship on total bumblebee abundance and the abundance of the six most common bumblebee species was tested using Generalised Linear Mixed-Effect Models (GLMMs) with management type as a fixed factor. Each sampling round, nested within sampling year was included in the models as a random factor to account for the temporal data structure. Models were fitted with a negative binomial error structure. Differences in the number of genetic samples collected and the estimated nest density between farm types for each sampled bumblebee species were compared using Student t-tests. The effect of management type on estimated bumblebee nest density was tested in a GLMM as a fixed factor with bumblebee species included as a random factor. Models were fitted with a negative binomial error structure. The proportion of pollen visits to plants sown as part of HLS management and members of Fabaceae was calculated for *B. hortorum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris* for all farms over the two year sampling period. For each bumblebee species, proportions were only calculated for farms with a minimum of five recorded pollen visits. Differences were tested in a GLMM with bumblebee species included as a random factor. Models were fitted with a binomial error structure.

All GLMMs were tested for over dispersion to ensure appropriate error structure selection. No models were significantly over dispersed. Final models were compared by ANOVA with a null model containing the same random factors to test for significance. All data analysis was

conducted in R version 3.1.1 (R Development Core Team) using the lme4 package for the GLMMs (Bates *et al.* 2014).

3.4 Results

3.4.1 Transect counts

A total of 9,436 bumblebees of 15 species were recorded. The 15 bumblebee species comprised the ubiquitous social species *B. terrestris*, *B. lucorum*, *B. pratorum*, *B. pascuorum*, *B. hortorum* and *B. lapidarius* as well as *B. jonellus*, *B. hypnorum*, *B. ruderarius* and *B. ruderatus*. Five cuckoo bumblebee species (*Psithyrus*) were also recorded comprising *B. vestalis*, *B. barbutellus*, *B. rupestris*, *B. sylvestris* and *B. campestris*.

Significantly greater numbers of bumblebees were recorded on HLS farms over the two year sampling period ($\chi^2=8.5$, $p=0.004$). Of the six most abundant species, *B. lapidarius* ($\chi^2=11.5$, $p<0.001$), *B. pascuorum* ($\chi^2=8.4$, $p=0.004$) and *B. terrestris* ($\chi^2=8.3$, $p=0.004$) were significantly more abundant on HLS farms. *B. hortorum* ($\chi^2=0.3$, $p=0.589$), *B. lucorum* ($\chi^2=2.1$, $p=0.145$) and *B. pratorum* ($\chi^2=0.2$, $p=0.654$) showed no difference in abundance between farm types.

3.4.2 Genetic samples

A total of 386 *B. hortorum* (24.00 ± 3.69 samples per farm), 771 *B. lapidarius* (45.36 ± 7.02), 598 *B. pascuorum* (35.18 ± 4.44) and 593 *B. terrestris* workers (37.06 ± 4.45) were collected. There was no significant difference in the number of samples collected between farm types for *B. hortorum* (HLS 24.00 ± 4.83 against ELS 24.00 ± 5.93 , $t_{10}=0.118$, $p=0.908$), *B. pascuorum* (32.75 ± 4.77 against 37.33 ± 7.44 , $t_{15}=0.183$, $p=0.857$) and *B. terrestris* (36.00 ± 6.34 against 38.13 ± 6.65 , $t_{10}=0.057$, $p=0.956$). Significantly more *B. lapidarius* samples were collected on HLS farms than ELS farms (72.75 ± 3.80 against 21.00 ± 4.27 , $t_{8.071}=5.953$, $p<0.001$). Allelic diversity was high with an average of 18.88 ± 2.55 alleles per locus in *B. hortorum*, 9.56 ± 0.85 alleles per locus in *B. lapidarius*, 12.44 ± 2.29 alleles per locus in *B. pascuorum* and 14.67 ± 2.02 alleles per locus in *B. terrestris*.

At one farm only one individual *B. hortorum* sample was taken, which was excluded from the following assessments. For *B. hortorum* (364 unique colonies), departures from Hardy-Weinberg were significant after Bonferroni correction in a total of 16 out of 120 tests. Marker

B118 showed departure at 10 out of 15 farms. Significant linkage disequilibrium was found for B100 & B126, B118 & B132, B126 & B132 and B96 & B126. For *B. lapidarius* (664 unique colonies) departure from Hardy-Weinberg was significant after Bonferroni correction in a total of 3 out of 144 tests. No significant linkage disequilibrium was found. For *B. pascuorum* (488 unique colonies), departure from Hardy-Weinberg was significant after Bonferroni correction in a total of 6 out of 153 tests. Significant linkage disequilibrium was found for B96 & B126 and B96 & B132. For *B. terrestris* (580 unique colonies), departure from Hardy-Weinberg was significant after Bonferroni correction in a total of 7 out of 144 tests. Significant evidence of linkage disequilibrium was found for B124 & B126.

The inbreeding coefficient was low with $F_{is}=0.0969 \pm 0.0387$ for *B. hortorum*, $F_{is}=0.0091 \pm 0.0113$ for *B. lapidarius*, $F_{is}=0.0468 \pm 0.0171$ for *B. pascuorum* and $F_{is}=0.0948 \pm 0.0139$ for *B. terrestris*. Overall genetic differentiation between farms was very low with $F_{st}=0.001 \pm 0.001$ for *B. hortorum*, $F_{st}=0.001 \pm 0.0001$ for *B. lapidarius*, $F_{st}=0.001 \pm 0.001$ for *B. pascuorum* and $F_{st}=0.003 \pm 0.001$ for *B. terrestris*.

3.4.3 Identification of unique colonies

Eighty-five *B. hortorum* sister pairs ($n=386$, 8 loci), 185 *B. lapidarius* sister pairs ($n=771$, 9 loci), 193 *B. pascuorum* sister pairs ($n=598$, 9 loci) and 58 *B. terrestris* sister pairs ($n=593$, 9 loci) were identified. No 'noncircular' nests where sister A and sister B were both related to sister C, but not to each other, were identified. Global analysis identified a number of sister pairs between farms at distances over 6 km (8 pairs for *B. hortorum*, 14 pairs for *B. lapidarius*, 20 pairs for *B. pascuorum* and 12 pairs for *B. terrestris*). At these distances such sister pairs are highly likely to be spurious. Further analysis with the removal of markers that departed from HWE and were in linkage disequilibrium identified some additional sister pairs between farms at large distance (over 10 km), and these are also highly likely to be spurious. No existing sister pairs within farms were discarded. The use of these linked markers does not appear to be affecting the identification of sisters within farms. Out of the 16 farms studied in 2013, no sister pairs were found for *B. hortorum* on four farms and no sister pairs for *B. terrestris* on four farms (i.e. all bees sampled were from unique nests). Out of the 17 farms studied in 2014, no sister pairs were found for *B. lapidarius* on one farm. As at least one 'recapture' is required to estimate numbers of colonies present, no estimate of undetected nests could be made for these farms, leaving a total of 12 farms (6 HLS, 6 ELS) for *B. hortorum* and *B. terrestris* and 16 farms (8 HLS, 8 ELS) for *B. lapidarius* for which we can estimate nest density.

Point estimates from Capwire of the number of colonies present on each farm ranged from 64 to 412 for *B. hortorum*, 9 to 579 for *B. lapidarius*, 30 to 329 for *B. pascuorum* and 92 to 1,780 for *B. terrestris*. An average of 193 ± 63 nests of *B. hortorum*, 114 ± 37 nests of *B. lapidarius*, 152 ± 35 nests of *B. pascuorum* and 482 ± 185 nests of *B. terrestris* were detected on ELS farms. An average of 173 ± 36 nests of *B. hortorum*, 336 ± 55 nests of *B. lapidarius*, 140 ± 30 nests of *B. pascuorum* and 630 ± 236 nests of *B. terrestris* were detected on HLS farms.

3.4.4 Estimation of foraging range and nesting density

Foraging range estimates showed considerable differences between bumblebee species. *B. hortorum* was estimated to forage up to 566 m (Figure 3.2a), *B. lapidarius* up to 714 m (Figure 3.2b), *B. pascuorum* up to 363 m (Figure 3.2c) and *B. terrestris* up to 799 m (Figure 3.2d). Large differences in foraging range estimates were found between farm types, although the direction of change differed between species. On ELS farms *B. hortorum* was estimated to forage up to 1415 m (Figure 3.3a), *B. lapidarius* up to 484 m (Figure 3.3b), *B. pascuorum* up to 313 m (Figure 3.3c) and *B. terrestris* up to 1196 m (Figure 3.3d). On HLS farms, *B. hortorum* was estimated to forage up to 282 m, *B. lapidarius* up to 746 m, *B. pascuorum* up to 377 m and *B. terrestris* up to 709 m.

Using these foraging range estimates, significant differences in bumblebee nest density were found between farm types (Figure 3.4). Both *B. hortorum* and *B. lapidarius* were found at significantly greater nesting density on HLS farms than ELS farms ($t_{10}=4.014$, $p=0.002$ and $t_{13.983}=2.232$, $p=0.043$, respectively). There were no differences in the nesting density of *B. terrestris* or *B. pascuorum* between farm types ($t_{10}=1.907$, $p=0.0885$ and $t_{15}=0.323$, $p=0.751$, respectively). Overall, HLS farms had a significantly higher estimated bumblebee nesting density across all four species ($\chi^2=7.7$, $p=0.006$).

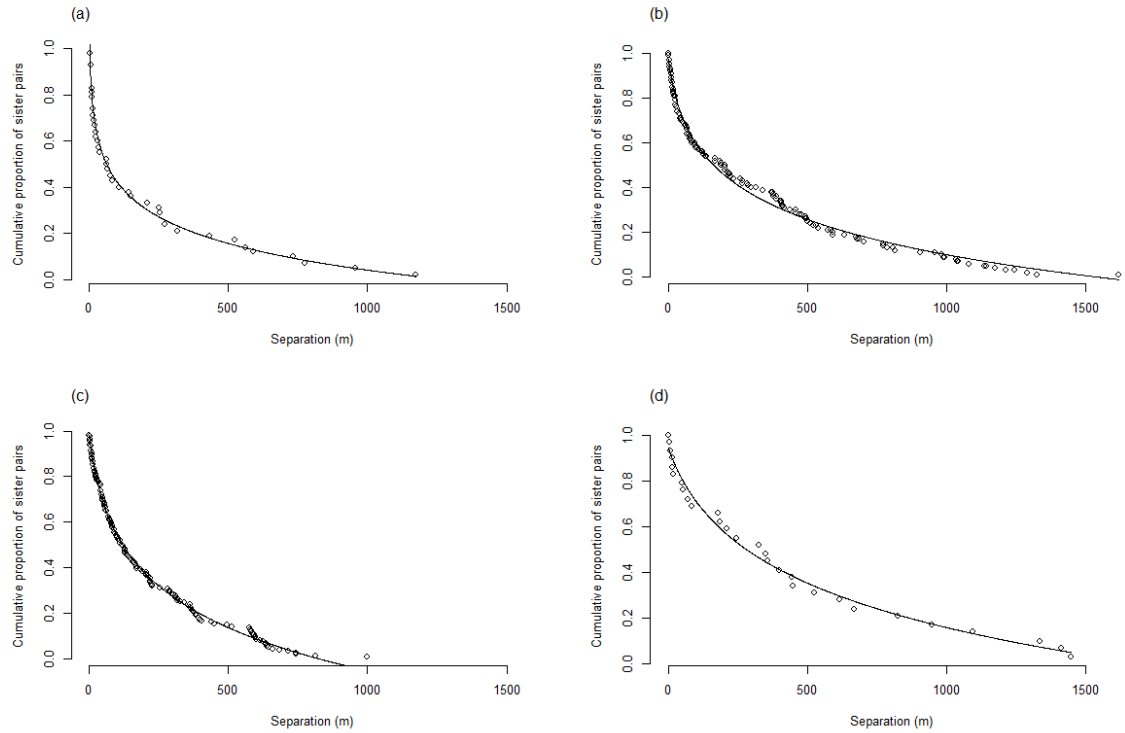


Figure 3.2. Separation distance of sister pairs for a) *Bombus hortorum*, b) *Bombus lapidarius*, c) *Bombus pascuorum* and d) *Bombus terrestris* across all farm types.

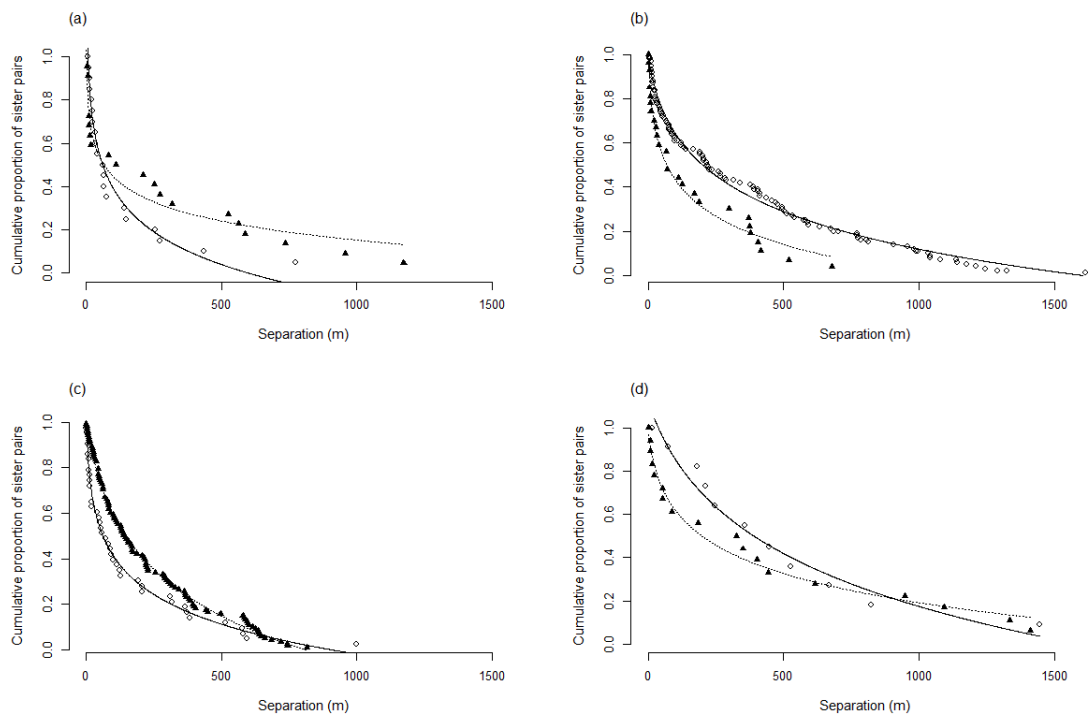


Figure 3.3. Separation distance of sister pairs for a) *Bombus hortorum*, b) *Bombus lapidarius*, c) *Bombus pascuorum* and d) *Bombus terrestris* for Higher Level Stewardship farms (white circles with line of best fit) and Entry Level Stewardship farms (black triangles with dotted line of best fit).

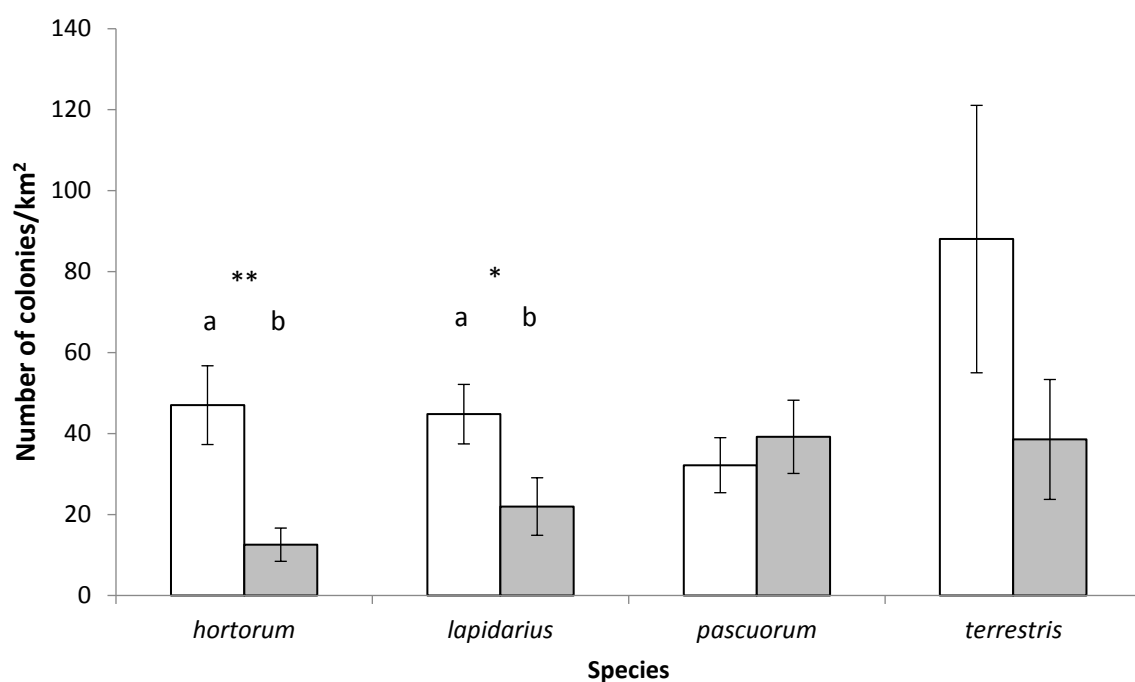


Figure 3.4. Differences in the nesting density of four bumblebee species between Higher Level Stewardship farms (white bars) and Entry Level Stewardship farms (grey bars). Errors bars are ± 1 standard error of the mean. Different letters above columns indicate farm types which differed significantly in a sampling round. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.4.5 Bumblebee foraging preferences

A total of approximately 9.69 million flowering units of 237 insect pollinated flowering plant species were recorded on the transects. A total of 9,288 foraging trips to 110 species were recorded by bumblebees with 102 species visited for nectar ($n=7,823$) and 47 species visited for pollen ($n=1,465$). *Centaurea nigra* was the most popular choice for nectar, accounting for 51% of visits. *Lotus corniculatus* was the most popular choice for pollen, accounting for 19% of visits. Nectar collection was dominated by visits to Asteraceae, whilst pollen collection was equally dominated by visits to Fabaceae (Table 3.1). Including pollen visitation data from the genetic sample collection during 2014 ($n=898$), substantial differences in pollen preferences were found between the four most abundant bumblebee species across farm types (Table 3.2). All four species collected a significantly higher proportion of their pollen from plants sown as part of HLS management on HLS farms compared to ELS farms ($\chi^2=11.8$, $p < 0.001$). The proportion of pollen collected from Fabaceae was also significantly higher on HLS farms, increasing by an average of 26.9 ± 2.31 percentage points ($\chi^2=12.2$, $p < 0.001$). Across all farm types, *B. lapidarius* visited Fabaceae most extensively, comprising 90% of total pollen visits.

Whilst still the most popular family, Fabaceae comprised between 26-49% of total pollen visits for the other three bumblebee species. *B. lapidarius* also visited plants sown as part of pollinator friendly HLS management most extensively, with these plants comprising 75% of total pollen visits compared to between 16-41% for the other three species.

Table 3.1. Percentage of visits for pollen and nectar recorded by bumblebees on the transects. The top ten most popular flowering plant families for each group over the sampling period are shown.

Family	Pollen (%)	Nectar (%)
<i>Apiaceae</i>	3.21	0.82
<i>Asteraceae</i>	2.12	66.33
<i>Boraginaceae</i>	0.68	3.13
<i>Brassicaceae</i>	0.89	-
<i>Dipsacaceae</i>	-	3.32
<i>Fabaceae</i>	60.27	12.58
<i>Lamiaceae</i>	15.09	7.27
<i>Rosaceae</i>	4.44	2.05
<i>Orobanchaceae</i>	11.13	1.05
<i>Scrophulariaceae</i>	-	1.60
<i>Ranunculaceae</i>	0.41	0.63
<i>Resedaceae</i>	1.09	-
Total	99.32	98.77

3.5 Discussion

A consistent problem in assessing the response of bumblebees to agri-environment schemes has been that it is unclear whether a high observed abundance of bumblebees was merely an attraction of workers to sown forage patches or a genuine population level increase. Here we show for the first time that the management of uncropped land under Higher Level Stewardship can significantly increase the size of bumblebee populations, and specifically those of *B. hortorum* and *B. lapidarius*.

Whilst the observed numbers of bumblebee species recorded over the study were high, comprising over half of the extant species in Britain, no sightings were made of *B. sylvarum*, *B. humilis* or *B. muscorum*, and *B. ruderarius* and *B. ruderatus* were only observed in very low numbers. These five medium to long-tongued bumblebees are the most threatened lowland England species and are most in need of assistance. All except *B. ruderatus* are members of the subgenus *Thoracobombus*.

Table 3.2. Percentage of pollen collected from different flowering plant species on different farm types by the four most abundant bumblebee species. Plant species highlighted in bold are commonly sown as part of pollinator friendly HLS management and are also found growing wild on all farm types.

<i>Entry Level Stewardship farms</i>							
Flowering plant species (n=8)	<i>Bombus hortorum</i> (visits n=106)	Flowering plant species (n=13)	<i>Bombus lapidarius</i> (visits n=150)	Flowering plant species (n=15)	<i>Bombus pascuorum</i> (visits n=393)	Flowering plant species (n=15)	<i>Bombus terrestris</i> (visits n=78)
<i>Lamium album</i>	39.62	<i>Trifolium repens</i>	48.00	<i>Stachys sylvatica</i>	38.42	<i>Rubus fruticosus agg.</i>	23.08
<i>Trifolium pratense</i>	30.19	<i>Lotus corniculatus</i>	11.33	<i>Odontites verna</i>	20.10	<i>Odontites verna</i>	16.67
<i>Stachys sylvatica</i>	25.47	<i>Rubus fruticosus agg.</i>	10.67	<i>Trifolium repens</i>	17.56	<i>Heracleum sphondylium</i>	10.26
Other (<2%)	4.72	<i>Reseda lutea</i>	6.00	<i>Trifolium pratense</i>	10.69	<i>Trifolium repens</i>	10.26
		<i>Trifolium hybridum</i>	5.33	<i>Lamium album</i>	3.05	<i>Rosa arvensis</i>	7.69
		<i>Brassica napus</i>	4.67	<i>Lotus corniculatus</i>	3.05	<i>Brassica napus</i>	6.41
		<i>Centaurea nigra</i>	4.00	<i>Ballota nigra</i>	2.04	<i>Lamium album</i>	5.13
		<i>Odontites verna</i>	4.00	<i>Rubus fruticosus agg.</i>	2.04	<i>Bryonia dioica</i>	3.85
		<i>Trifolium pratense</i>	3.33	Other (<2%)	3.05	<i>Plantago media</i>	3.85
		Other (<2%)	2.67			<i>Barbarea vulgaris</i>	2.56
						<i>Chaerophyllum temulum</i>	2.56
						<i>Pulicaria dysenterica</i>	2.56
						<i>Rhinanthus minor</i>	2.56
						Other (<2%)	2.56
Total sown	30.19		24.67		15.01		0.00
Total Fabaceae	30.19		68.00		33.59		10.26
<i>Higher Level Stewardship farms</i>							
Flowering plant species (n=9)	<i>Bombus hortorum</i> (visits n=93)	Flowering plant species (n=19)	<i>Bombus lapidarius</i> (visits n=936)	Flowering plant species (n=23)	<i>Bombus pascuorum</i> (visits n=438)	Flowering plant species (n=14)	<i>Bombus terrestris</i> (visits n=96)
<i>Trifolium pratense</i>	49.46	<i>Lotus corniculatus</i>	39.64	<i>Trifolium pratense</i>	36.30	<i>Heracleum sphondylium</i>	26.04
<i>Stachys sylvatica</i>	25.81	<i>Trifolium hybridum</i>	33.33	<i>Stachys sylvatica</i>	22.83	<i>Trifolium repens</i>	18.75
<i>Clinopodium vulgare</i>	7.53	<i>Trifolium repens</i>	11.43	<i>Lotus corniculatus</i>	9.59	<i>Trifolium hybridum</i>	11.46
<i>Vicia sepium</i>	6.45	<i>Melilotus officinalis</i>	5.13	<i>Odontites verna</i>	5.94	<i>Rubus fruticosus agg.</i>	10.42
<i>Rosa arvensis</i>	4.30	<i>Centaurea nigra</i>	2.35	<i>Trifolium hybridum</i>	3.88	<i>Lotus corniculatus</i>	7.29
<i>Lamium album</i>	2.15	<i>Odontites verna</i>	2.35	<i>Trifolium repens</i>	3.88	<i>Phacelia tanacetifolia</i>	7.29
<i>Vicia sativa</i>	2.15	<i>Trifolium pratense</i>	2.24	<i>Lamium album</i>	3.42	<i>Odontites verna</i>	6.25
Other (<2%)	2.15	Other (<2%)	3.53	<i>Vicia cracca</i>	2.51	<i>Reseda lutea</i>	4.17
				<i>Medicago sativa</i>	2.28	<i>Genista tinctoria</i>	3.13
				Other (<2%)	9.36	Other (<2%)	5.21
Total sown	51.61		83.33		58.68		27.08
Total Fabaceae	58.06		93.38		64.61		38.54
<i>All farm types</i>							
Total sown	41.21		75.32		36.58		16.09
Total Fabaceae	43.72		89.87		49.94		26.44

Bombus pascuorum is the final member of *Thoracobombus* in Britain and showed no difference in nesting density between farm types, despite using sown plants more extensively than *B. hortorum*. Plants sown as part of HLS provided good bumblebee forage with all four common species favouring them for pollen collection when they were present, and the provision of large quantities of Fabaceae has clearly benefited Fabaceae specialists like *B. lapidarius*. However, this management does not appear to have benefited rarer species which were formerly found more extensively in this part of England.

It has been argued that dietary specialisation alone is not sufficient to explain patterns of bumblebee decline. Species that have declined mostly emerge late from hibernation, and it has been suggested that such species may be more susceptible to a loss of food plants during mid to late colony development (Williams *et al.* 2009). Moreover, it has long been suspected that bumblebees with smaller colonies forage over shorter distances (Free and Butler 1959). Large colonies require more resources and, all else being equal, should have to travel further to collect sufficient food. *Bombus pascuorum*, along with other members of the *Thoracobombus*, tends to have small nests with around 20-100 workers, whilst *B. lapidarius* and *B. terrestris* nests are larger, with 100-400 workers (Sladen 1912; Alford 1975). *B. hortorum* nests tend to be more similar to *B. pascuorum*, with seldom more than 100 workers produced (Sladen 1912). Studies suggest that *Thoracobombus* have short foraging ranges, with *B. muscorum* only observed foraging up to 125 m (Walther-Hellwig and Frankl 2000). Species with shorter foraging ranges may be more susceptible to habitat fragmentation because resources are spread out over larger distances in such landscapes. Both *B. muscorum* and *B. sylvarum* show significant population structuring between their remaining habitat patches (Darvill *et al.* 2006; Ellis *et al.* 2006), and such small, isolated populations are unlikely to be viable in the long term, with metapopulation breakdown likely to be behind the extinction of *B. subterraneus* in Britain (Goulson, Lye and Darvill 2008b). Providing extra foraging resources may be sufficient to benefit those bumblebees with greater foraging ranges, but such options have had low uptake across England (Clothier and Pike 2013), and such forage patches may be too few and too scattered in the landscape to benefit those species unable to cover larger distances. Concentrated efforts on agricultural land surrounding existing populations in semi-natural habitats should therefore be prioritised for these species. Additionally, improving the quality of existing buffer strips and hedgerows, many of which have become dominated by nitrophilous species, would provide a more homogenous supply of suitable forage compared to isolated pollinator-friendly agri-environment schemes.

Numerous studies have measured bumblebee foraging range using direct observations such as marking workers (Walther-Hellwig and Frankl 2000; Wolf and Moritz 2008) and genetic markers (Knight *et al.* 2005; Carvell *et al.* 2012, Table 3.3). Our suggested foraging ranges for *B. pascuorum* (363 m) and *B. terrestris* (799 m) compare reasonably well with previous studies. The situation is less similar for our estimate for *B. lapidarius* (714 m), though the authors of the previous study note that their estimate of 450 m was likely an underestimate (Knight *et al.* 2005). To the best of our knowledge, these are the first data presented on the foraging range of *B. hortorum* (566 m). Despite considerable variation, probably due to inherent difference in the various techniques used, the same broad trends are found, with bumblebee species with larger colony sizes foraging over greater distances.

Table 3.3. Estimated foraging distances of worker bumblebee species included in this study.

Species	Maximum foraging distance (m)	Method	Reference
<i>B. hortorum</i>	566	Genetic markers	Present study
<i>B. lapidarius</i>	450	Genetic markers	Knight <i>et al.</i> (2005)
	714	Genetic markers	Present study
	1,032	Genetic markers	Carvell <i>et al.</i> (2012)
	1,500	Direct (marked workers)	Walther-Hellwig and Frankl (2000)
<i>B. pascuorum</i>	363	Genetic markers	Present study
	449	Genetic markers	Knight <i>et al.</i> (2005)
	990	Genetic markers	Carvell <i>et al.</i> (2012)
<i>B. terrestris</i>	631	Direct (radar tracking)	Osborne <i>et al.</i> (1999)
	758	Genetic markers	Knight <i>et al.</i> (2005)
	799	Genetic markers	Present study
	800	Direct (marked workers)	Wolf and Moritz (2008)
	1,500	Direct (marked workers)	Osborne <i>et al.</i> (2008b)
	1,750	Direct (marked workers)	Walther-Hellwig and Frankl (2000)

It is noteworthy that there were considerable differences in foraging range within species in different landscapes. As highly mobile foragers, bumblebees are capable of making profitable foraging trips over long distances and have the ability to respond to an increase in floral availability at the landscape scale. When seeking to maximise the amount of pollen and nectar brought back to the nest, the most important factor in the final profitability of the trip is the time spent foraging, as whilst travelling longer distances incurs a slightly higher energetic cost, the bigger cost is the lost time that could have been spent foraging for resources (Beutler 1951). Consequently, we would expect bumblebees to favour shorter foraging trips wherever possible (Heinrich 1979). Using molecular markers, Carvell *et al.* (2012) found that the foraging range of both *B. lapidarius* and *B. pascuorum* increased as the availability of semi-natural habitat decreased, increasing the average distance between bumblebee nests and the nearest forage patch and forcing workers to make longer foraging trips. However, in our study, whilst

B. hortorum and *B. terrestris* foraged over shorter distances on resource rich HLS farms, *B. lapidarius* actually foraged further on HLS farms than on ELS farms. If highly rewarding forage patches are added to a landscape, the quality of the resources they provide may offset the time cost of travelling to these patches, enabling profitable foraging trips to be made over larger distances. However, it is unclear why different species should respond in opposite ways to the provision of extra foraging resources. Whilst it is difficult to draw wider conclusions with so few studies having assessed this question directly, it may be the case that bumblebees will not always prefer a closer patch of forage to a further patch if the quality of the latter is sufficiently high.

3.6 Conclusions

It is not clear how we might define what a desirable bumblebee population density should be, either from a conservation or an ecosystem services provisioning perspective. Are conservation schemes for pollinators aimed at conserving rare species, providing high seed set in commercial crops or ensuring adequate pollination for wild flowers? In identifying key knowledge needs in the conservation of wild pollinators, Dicks *et al.* (2013) highlighted the importance of understanding the relationship between pollinator populations and the ecosystem services they provide. Our work suggests that current HLS conservation measures significantly increase populations of at least two of the bumblebee species studied, and it seems likely that this will boost pollination services for crops and wildflowers. On the other hand, our study farms do not appear to be supporting viable populations of any rare bumblebee species, many of which were formerly found in this region. These rarer species require more specific and geographically focused management that takes into account their shorter foraging range and other ecological requirements if they are to persist in agricultural landscapes.

Chapter 4 – Pollinator-friendly management does not increase the diversity of farmland bees and wasps

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4.1 Abstract

In order to reverse declines in pollinator populations, numerous agri-environment schemes have been implemented across Europe, predominantly focused on increasing the availability of floral resources. Whilst several studies have investigated how bees and wasps (aculeates) respond to management at the scale of the scheme (i.e. within the flower patch) there has been little assessment of how schemes affect diversity at the farm scale. In the current work we assessed whether farms implementing flower-rich schemes had richer aculeate communities than farms without such habitats. A total of 104 species of bee and 44 species of aculeate wasp were recorded. Farms providing flower-rich habitats had significantly greater floral abundance but there were no differences in the total number of aculeate or flowering plant species recorded compared to farms without these habitats. Using a rarefaction analysis to account for differences in sample size, farms without flower-rich habitats had more diverse aculeate communities, as the communities on farms providing flower-rich habitat were dominated by large numbers of bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*). These two groups foraged strongly from sown flowers, but the majority of bee species preferred wild plants that are not included in flower-rich schemes. Maintaining large numbers of bees is important for the delivery of ecosystem services, but the creation of pollinator-friendly habitats has not increased the diversity of flowering plants at the farm scale and consequently, such schemes will only benefit a limited suite of aculeate species. If diverse aculeate communities are to be retained and restored on farmland, agri-environment schemes that provide foraging and nesting resources for a wider range of pollinator species must be developed. A clearer understanding of the primary purpose of agri-environment schemes is necessary in order to evaluate their success in this regard.

4.2 Introduction

The process of agricultural intensification has resulted in a simplified and less heterogeneous landscape across Europe and North America and it is the primary driver behind long-term declines in farmland biodiversity (Benton, Vickery and Wilson 2003; Tscharntke *et al.* 2005). As with many taxa, wild bees have been negatively affected with serious declines across Europe and North America (Biesmeijer *et al.* 2006; Osborne and Williams 2009; Potts *et al.* 2010), though there is evidence that rates of decline are slowing (Carvalhiero *et al.* 2013). These declines are of serious concern as pollinators, particularly bees, provide an important pollination service which both supports wild plant communities (Ashman *et al.* 2004) and

affects the yield for approximately 70% of crop species worldwide, representing around 35% of total global food production (Klein *et al.* 2007). Up to 80% of this pollination service has been attributed to honeybees *Apis mellifera* L. (Carreck and Williams 1998), but more recent studies have highlighted the importance of the service provided by wild pollinators. The quality of this service depends on the diversity of the pollinator community, with richer assemblages improving yields in many crop species (Westerkamp and Gottsberger 2000; Klein *et al.* 2003; Hoehn *et al.* 2008). Wild bee pollinators can provide the majority of crop visitation, even in contemporary intensive farming systems (Winfree *et al.* 2008), and enhance fruit set regardless of honeybee abundance (Garibaldi *et al.* 2013), leading to suggestions that the role of honeybees as agricultural pollinators has been overstated (Breeze *et al.* 2011; Ollerton *et al.* 2012). Pollinator communities are sensitive to habitat loss (Kremen, Williams and Thorp 2002; Steffan-Dewenter *et al.* 2002), and consequently the service they provide can reduce as agricultural intensification degrades the semi-natural environments upon which they depend (Garibaldi *et al.* 2011).

In order to reverse the decline in farmland biodiversity, agri-environment schemes are now funded across the European Union as part of the Common Agricultural Policy. These schemes offer opportunities to create pollinator-friendly habitats with the objectives of increasing both pollinator abundance and diversity. In England, two tiers of environmental stewardship were established in 2005. Entry Level Stewardship (ELS, Natural England 2013a) was open to all farmers. Higher Level Stewardship (HLS, Natural England 2013b) which was targeted to high priority areas provided greater financial rewards for more substantial and rigorous agri-environmental schemes. Most agricultural land in England is under some form of environmental stewardship with 72% of land under at least ELS management and around 21% of this total under HLS management as of 2014. (JNCC 2014). There are three main pollinator-focused HLS options. HF4 pollen and nectar mixes are rotational plots or strips sown with a mixture of predominantly leguminous plants such as *Trifolium pratense* L. and *Trifolium hybridum* L. which tend to be resown within a 5 year period. HE10 floristically enhanced grass buffer strips are non-rotational grassland alongside fields and are composed of a mixture of grasses and plants such as *Centaurea nigra* L. and *Lotus corniculatus* L. HK6/7/8 focus on the maintenance, restoration and creation of species rich grassland.

The response of bumblebees to the creation of flower-rich schemes has received a great deal of attention. From the initial designation of five UK bumblebees as Biodiversity Action Plan species of conservation concern, and the resulting research into their ecological requirements,

it was argued that without landscape scale habitat restoration these species would continue to decline (Edwards 1999). Many bumblebee species that have suffered substantial declines tended to collect a large proportion of pollen from Fabaceae (Goulson *et al.* 2005), and consequently leguminous plants became an important part of agri-environment scheme design. A number of studies have focused on the response of foraging bumblebees to these schemes and other agri-environment scheme habitats (Edwards 2003; Pywell *et al.* 2006; Carvell *et al.* 2007; Pywell *et al.* 2011a; Holland *et al.* 2015), but the response of other bees to these options has received much less attention and is correspondingly less well characterised, both in the UK and abroad (Haaland *et al.* 2011). Studies suggest that the wider bee community can respond well to management at a local level, with higher species richness recorded in targeted areas, such as field margins or meadows, compared to unmanaged control areas (Knop *et al.* 2006; Albrecht *et al.* 2007). However, there has been little comparison of diversity between farms at a scale greater than that of the targeted area. It is not clear to what extent any positive benefits extend to the wider landscape, as species richness can drop off sharply outside target areas (Albrecht *et al.* 2007; Kohler *et al.* 2008). If schemes are effectively increasing species diversity we would expect farms implementing appropriate management to support significantly more diverse pollinator communities. Bees and wasps can be highly aggregative, congregating on appropriate habitat (Heard *et al.* 2007), but not all species will be attracted to agri-environment schemes, necessitating more extensive surveying within and between targeted areas to accurately capture the community present at any one site.

In a recent survey of insect pollination scientists and conservation practitioners, the top research priority identified was to understand how important the diversity of pollinator species was to the resilience and reliability of the pollination service (Dicks 2013). Other than bumblebees, there is little knowledge as to which bee and aculeate wasp species (henceforth collectively referred to as aculeates) persist on agricultural land and how they respond to agri-environment schemes. Without an appropriate evidence base, conservation interventions generally, and agri-environment schemes in particular are unlikely to be effective (Kleijn and Sutherland 2003; Sutherland *et al.* 2004). In this study we assess whether farms implementing pollinator-friendly management schemes support richer and more diverse aculeate communities at the farm level. Our results have clear implications for the future management of farmland aculeate populations.

4.3 Materials and Methods

Nine HLS and nine ELS farms were selected in Hampshire and West Sussex, UK. On average, HLS farms were significantly larger (256.22 ± 37.80 ha) than ELS farms (156.67 ± 22.07 ha, t-test, $t_{16,17}=2.565$, $p=0.021$). The selected HLS farms had been implementing an average of 5.56 ± 0.13 ha of pollinator focused flower-rich options representing $2.17 \pm 0.05\%$ of the farm area by ownership for a minimum of three years. As the majority of farms in England are in some form of environmental stewardship, ELS management was chosen as the control group for this study. Whilst pollinator-focused flower-rich options are available as part of ELS management, the selected ELS farms were not implementing any such schemes. Farms were predominantly arable or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

As the proportion of the landscape which is comprised of semi-natural habitat can affect the species richness of bees (Steffan-Dewenter *et al.* 2002; Holzschuh *et al.* 2010), the farms were mapped to ensure their overall similarity. A detailed land cover map to a resolution of 2 m^2 was constructed in Google Earth (Google Inc) based on detailed surveys during the field season and satellite imagery. A 1 km buffer was drawn around the centre point of each transect covering the majority of each farm and some of the surrounding area. Semi-natural habitats fell into four broad groups i) flower-rich agri-environment scheme grassland ii) flower-poor general grassland (not including permanent pasture and silage grassland, categorised as intensive grassland) iii) hedgerows and iv) woodland (Table 4.1). There was no significant difference in the proportion of the landscape covered by semi-natural habitats between farm types (GLM, $t_{16,17}=0.147$, $p=0.885$). The presence of water and urban structures can also affect the species richness of bees and wasps by creating moisture gradients that increase floral abundance and by providing floral resources and nesting areas (Goulson *et al.* 2002; Winfree *et al.* 2007b). There was no significant difference in the proportion of the landscape covered by either of these habitat types (GLM, $t_{16,17}=1.256$, $p=0.232$, $t_{16,17}=0.064$, $p=0.949$ respectively).

Table 4.1. Habitat composition within a 1 km radius for selected farms in Hampshire and West Sussex, UK. Means \pm 1 SE are given for nine study sites per farm type. Habitat types marked with a * were categorised as semi-natural.

Habitat type	Area (%)	Minimum (%)	Maximum (%)
(a) Higher Level Stewardship farms			
Arable land	59.73 \pm 5.13	28.18	72.99
Flower-rich grassland *	1.77 \pm 0.41	0.38	4.36
Flower-poor general grassland *	8.36 \pm 1.43	3.02	16.19
Intensive grassland	14.15 \pm 3.34	3.57	34.32
Water	0.34 \pm 0.22	0.00	2.01
Hedgerows *	1.96 \pm 0.24	0.77	3.12
Urban	4.89 \pm 0.79	1.83	8.60
Woodland *	8.81 \pm 3.06	0.84	24.96
(b) Entry Level Stewardship farms			
Arable land	55.76 \pm 6.06	25.08	78.23
Flower-rich grassland *	0.05 \pm 0.05	0.00	0.47
Flower-poor general grassland *	5.73 \pm 1.03	2.88	12.40
Intensive grassland	15.93 \pm 3.18	0.68	30.77
Water	1.52 \pm 1.49	0.00	13.44
Hedgerows *	2.26 \pm 0.36	0.39	3.40
Urban	4.82 \pm 0.58	2.68	7.44
Woodland *	13.92 \pm 4.41	1.52	35.17

A standardised 3 km transect was designed for each farm. Transects on HLS farms were designed to pass through as many pollinator-focused schemes as possible. HLS transects passed through an average of 1496 ± 148 m of flower-rich habitat in an average of 3.77 ± 0.24 discrete habitat patches. Aculeate activity was recorded along each transect following standard bee walk methodology (Carvell *et al.* 2007). On each 3 km transects all aculeates within 2 m of the recorder were identified to species level. Specimens which could not be immediately identified in the field were netted and later identified in the laboratory. Aculeate floral preferences were measured on the transects, with the first flowering plant species visited recorded for both observed and netted individuals. All surveys were conducted between 0930-1700 hours when the temperature was above 13°C with at least 60% clear sky, or above 17°C with any level of cloud. No surveys were conducted when it was raining. On each transect, the number of flowering units of each plant species was estimated. Grasses, sedges and rushes were not recorded. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a head, a capitulum) counted as a single unit. All aculeate and floristic surveys were conducted by the same individual (TJW) to minimise recorder bias.

Sixteen farms (8 HLS, 8 ELS) were surveyed in 2013. Transects were walked three times throughout the year, between 25th May - 5th June, 26th June - 15th July and 3rd - 11th August.

Seventeen farms (8 HLS, 9 ELS) were surveyed in 2014. Transects were walked three times throughout the year, between 17th - 27th May, 21st June - 9th July and 3rd - 15th August.

Twelve pan traps were placed out on each farm. Pan traps consisted of 500 ml plastic bowls and were spray-painted fluorescent blue, yellow or white (Sparvar Leuchtfarbe, Spray-Color GmbH, Germany). One of each colour was attached to a wooden post of approximate height 60 cm. On HLS farms two such posts were placed in a flower-rich margin and two were placed in a flower-poor general grass margin. On ELS farms two posts were placed in each of two separate flower-poor general grass margins. Posts in the same margin were separated by a distance of 25 m. Sampled margins were no closer than 200 m from each other. Traps were filled with approximately 400 ml of water with a few drops of unscented washing up liquid (Surcare Sensitive, UK). Traps were left out for 96 h before being collected.

Sixteen farms (8 HLS, 8 ELS) were surveyed in 2013. Traps were set three times between 10th-14th June, 8th-12th July and 1st-5th of August. Fourteen farms (7 HLS, 7 ELS; all of these farms were surveyed with transect walks) were surveyed in 2014. Trap placement was staggered over two days, with half of the farms (4 HLS, 3 ELS) trapped on day one and the other half of the farms (3 HLS, 4 ELS) trapped on day two. Traps were set three times between 29th/30th May - 2nd/3rd June, 10th/11th - 14th/15th July and 12th/13th - 16th/17th August. Invertebrate samples were stored in 70% ethanol and pinned prior to identification.

Differences in aculeate and floristic species richness between farm types were investigated using rarefaction and extrapolation curves. Both the transect and the pan trap techniques produce replicated sample-based abundance data, where it is the sampling unit rather than the individual species that is sampled randomly and independently (Gotelli and Colwell 2001). Following Colwell *et al.* (2012), this sample-based abundance data was converted to sample-based incidence data to calculate incidence-based extrapolation curves using a Bernoulli product model. Differences in the number of individuals per sampling unit is accounted for by rescaling the X-axis to individuals (Gotelli and Colwell 2001), allowing for a more meaningful comparison between assemblages of differing sample sizes. Rarefaction curves with 95% confidence intervals were produced using EstimateS 9.1.0 (Colwell 2013). Curves were calculated without replacement and were extrapolated to twice the number of collected samples. Similarity in community composition between different farm types was assessed using Simpson's similarity index (Simpson 1960). This index was chosen as it accounts for variable sampling effort between sites, as not every site was surveyed with transects or pan

traps in every year. Community similarity within and between farm management types was compared using a permutation ANOVA following Chase (2007).

The impact of the proportion of the landscape represented by semi-natural habitats (flower-rich grassland, flower-poor grassland, hedgerow, woodland and all habitats combined) on the number of aculeate species recorded on the transect and in the pan traps was investigated using Generalised Linear Models. No significant relationships were found (Appendix A).

Differences in total number of aculeate and flowering plant species between farm types were investigated using Generalised Linear Mixed-Effect Models (GLMMs). The total number of species recorded by each sampling technique was calculated for each farm in each year. Sampling year was included in the model as a random factor to account for temporal pseudoreplication. Differences in floral abundance between management types was tested with a GLMM with sampling round nested within sampling year included as random factors to account for the temporal data structure. The impact of floristic richness on aculeate richness was also tested using a GLMM. Sampling year was included in the model as a random factor. Models were fitted with a Gaussian error distribution unless found to be significantly non-normal. Where models were significantly non-normal they were fitted with Poisson error distributions though in all cases they were found to be significantly over dispersed and so were fitted with negative binomial error distributions instead. Final models were compared by ANOVA with a null model containing the same random factors to test for significance. Differences in the proportion of visits to plants sown as part of HLS management by different bee groups were tested using 2-sample tests for equality of proportions. All data analysis was conducted in R version 3.1.1 (R Development Core Team) using the package fossil to calculate Simpson's similarity indices, the package coin for the permutation ANOVA and the package lme4 for the GLMMs.

4.4 Results

A total of 16,821 aculeates of 148 species were recorded comprising 104 species of bee and 44 species of wasp, including 21 species of conservation concern (Table 4.2). This follows the rarity classifications in Falk (1991) but excludes *Lasioglossum malachurum* Kirby, *L. pauxillum* Schenck and *Bombus rupestris* Fabricius which have become much more widespread since this classification and no longer merit nationally scarce status (S. Falk, *pers. comm.*). The bumblebee *B. ruderarius* Müller is also included due its recent decline in the UK and

designation as a Biodiversity Action Plan species. Both *Nomada flava* Panzer and *N. panzeri* Lepeletier were recorded but as the males are indistinguishable these two species were merged for analysis purposes.

Table 4.2. Species of conservation concern recorded during the survey and the number of individuals recorded on HLS and ELS farms. Rarity status from Falk (1991), also includes Biodiversity Action Plan (BAP) species. RDB1 = Red Data Book 1 species in danger of extinction, RDB3 = Red Data Book 3 species estimated to occur in fewer than 15 10 km squares, Na = Nationally Scarce A species estimated to occur within 16 to 30 10 km squares and Nb = Nationally Scarce B species estimated to occur within 31 to 100 10 km squares.

Species	Rarity	Number of individuals recorded on HLS farms	Number of individuals recorded on ELS farms
<i>Andrena alfkenella</i> (Perkins 1914)	RDB3	2	1
<i>Andrena florea</i> (Fabricius 1793)	RDB3	1	7
<i>Andrena fulvago</i> (Christ 1791)	Na		1
<i>Andrena humilis</i> (Imhoff 1832)	Nb		2
<i>Andrena labiata</i> (Fabricius 1781)	Na		1
<i>Andrena minutuloides</i> (Perkins 1914)	Na	1	
<i>Bombus ruderarius</i> (Müller 1776)	BAP	4	
<i>Bombus ruderatus</i> (Fabricius 1775)	Nb/BAP	5	
<i>Hoplitis claviventris</i> (Thomson 1872)	Nb	1	
<i>Hylaeus cornutus</i> (Curtis 1831)	Na	15	
<i>Hylaeus signatus</i> (Panzer 1798)	Nb		1
<i>Lasioglossum puncticolle</i> (Morawitz 1872)	Nb	4	
<i>Lasioglossum xanthopus</i> (Kirby 1802)	Nb	1	
<i>Melitta tricincta</i> (Kirby 1802)	Nb	18	43
<i>Microdynerus exilis</i> (Herrich-Shäffer 1839)	Nb	1	1
<i>Mimumesa unicolor</i> (Vander Linden 1829)	Na		2
<i>Nomada flavopicta</i> (Kirby 1802)	Nb		1
<i>Nomada guttulata</i> (Schenck 1861)	RDB1	1	
<i>Nysson trimaculatus</i> (Rossi 1790)	Na	2	
<i>Osmia bicolor</i> (Schrank 1781)	Nb	2	1
<i>Sphecodes niger</i> (von Hagens 1874)	RDB3		3

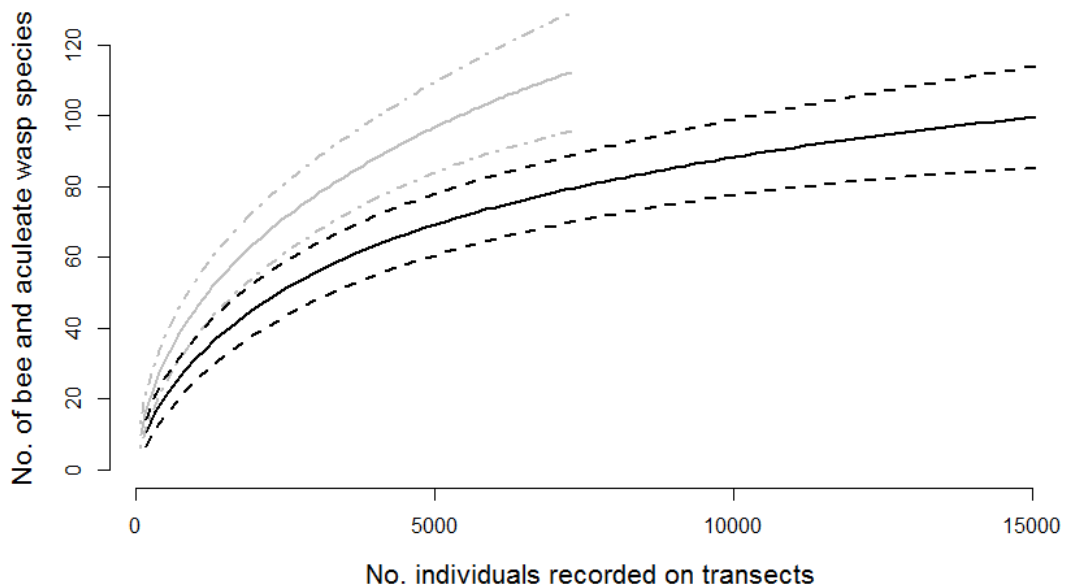


Figure 4.1. Rarefaction and extrapolation curves for aculeate richness recorded on the transects for ELS farms (upper grey line with dashed and dotted-line 95% confidence intervals) and HLS farms (lower black line with dashed-line 95% confidence intervals).

On the transects 12,136 aculeates of 112 species were recorded, with a total of 82 and 89 species found on HLS and ELS farms, respectively (Appendix B). There was no difference in the total number of species recorded between farm types ($\chi^2=0.1$, $p=0.767$). Rarefaction analysis showed that ELS farms had significantly more diverse aculeate communities than HLS farms after approximately 2000 sampled individuals (Figure 4.1). The pan traps caught 4,685 aculeates of 115 species, with a total of 89 and 98 species found on HLS and ELS farms, respectively (Appendix B). There was no difference in the total number of species recorded between farm types ($\chi^2=1.6$, $p=0.203$). Rarefaction analysis showed that there was no significant difference aculeate community diversity between farm types, though ELS farms tended to have more species (Figure 4.2). Aculeate community composition of farms did not more closely resemble farms of the same management type than farms of the other management type (permutation ANOVA, $Z=1.594$, $p=0.108$).

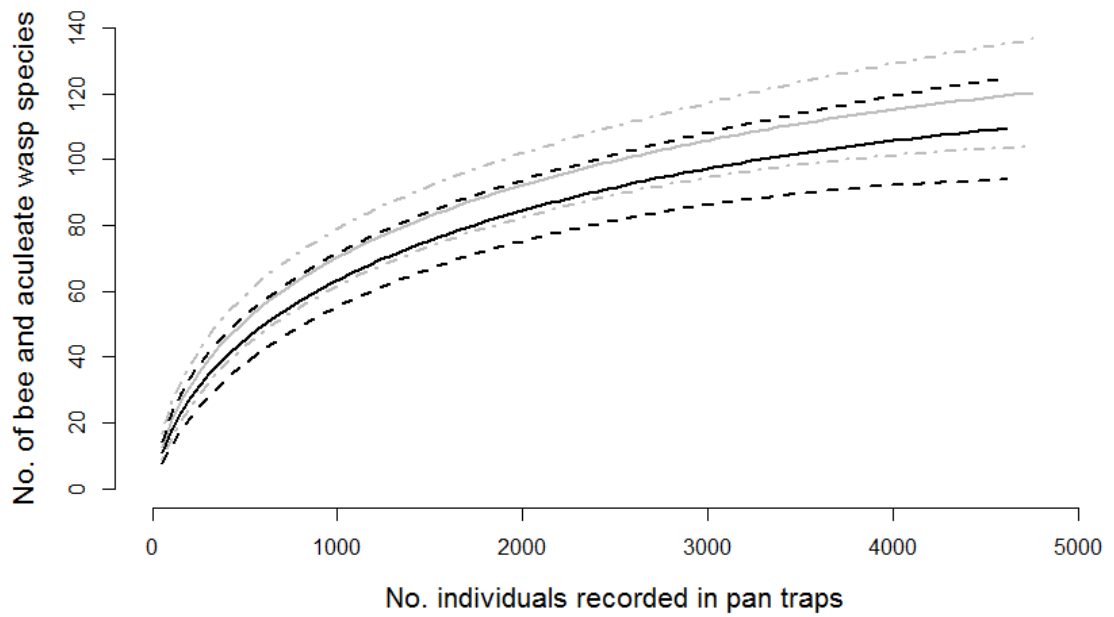


Figure 4.2. Rarefaction and extrapolation curves for aculeate richness recorded in the pan traps for ELS farms (upper grey line with dashed and dotted-line 95% confidence intervals) and HLS farms (lower black line with dashed-line 95% confidence intervals).

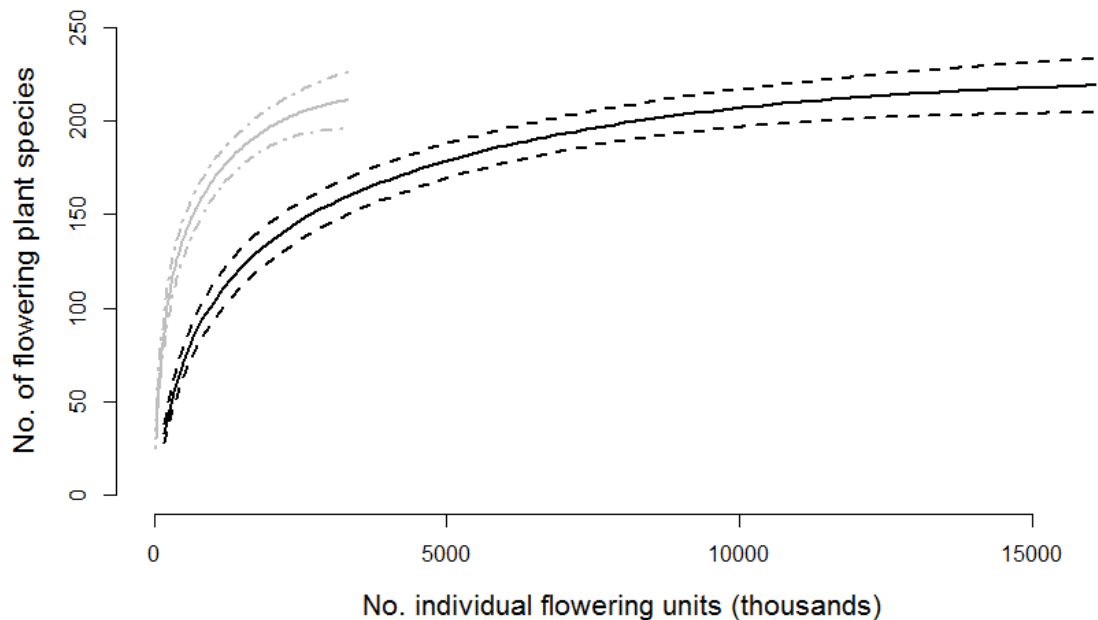


Figure 4.3. Rarefaction and extrapolation curves for floristic richness recorded on the transects for ELS farms (upper grey line with dashed and dotted-line 95% confidence intervals) and HLS farms (lower black line with dashed-line 95% confidence intervals).

On the transects 9.69 million flowering units of 237 species of flowering plant were recorded, with a total of 198 and 190 flowering plant species found on HLS and ELS farms respectively (Appendix C). Floral abundance was significantly greater on HLS farms than ELS farms ($502,523 \pm 74,324$ flowering units/year against $93,608 \pm 22,703$ flowering units/year, $\chi^2=49.0$, $p<0.001$). There was no difference in the number of flowering plant species between farm types ($\chi^2=1.1$, $p=0.293$), but rarefaction analysis showed that ELS farms had a significantly more diverse flowering plant community (Figure 4.3). Floristic richness had a significant effect on the richness of bees recorded on the transects, but not the richness of aculeate wasps ($\chi^2=12.6$, $p<0.001$, $\chi^2=0.6$, $p=0.797$, respectively). Floristic richness had no effect on the richness of bees recorded in the pan traps and a significantly negative effect on the richness of aculeate wasps ($\chi^2=0.0$, $p=0.948$, $\chi^2=6.6$, $p=0.010$, respectively).

On the transects 11,661 foraging trips to 124 species of flowering plant were recorded by 81 species of bee. Plants sown as part of HLS management were visited extensively by both bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*), with visits to these plants accounting for 69% ($n=6441/9288$) and 68% ($n=1147/1698$) of total visits, respectively, a similar overall proportion ($\chi^2=2.1$, $p=0.149$). However, only 32% ($n=23/72$) of the non-corbiculate bee species recorded on the transects were ever observed to visit sown flowers (including visits to the sown species growing wild on both farm types), these plants representing 33% ($n=224/675$) of total visits, a significantly lower proportion than for bumblebees ($\chi^2=370.0$, $p<0.001$) and honeybees ($\chi^2=232$, $p<0.001$). The sweat bee *Lasioglossum malachurum* accounted for 72% ($n=157/218$) of these visits to sown flowers. Only six species of non-corbiculate bees were observed visiting Fabaceae flowers (a dominant component of most sown flower mixes), representing 3% ($n=23/675$) of total visits. Excluding sown species, non-corbiculate bees foraged heavily from Asteraceae and Apiaceae, accounting for 73% ($n=261/457$ and $n=74/457$) of total visits, in particular *Heracleum sphondylium* L. ($n=69$), *Hypochaeris radicata* L. ($n=54$) and *Tripleurospermum inodorum* (L.) Schultz Bip ($n=44$).

Wasps rarely visit flowers, instead provisioning their offspring with invertebrates. A total of 91 visits to 13 species of flowering plant were recorded by 17 species of wasp. Plants sown as part of HLS management were seldom visited by wasps, accounting for 22% ($n=20/91$) of total visits. Wasps predominantly visited Apiaceae, accounting for 85% ($n=77/91$) of total visits, in particular *Heracleum sphondylium* ($n=44$), *Daucus carota* L. ($n=17$) and *Angelica sylvestris* L. ($n=10$). The most popular forage plants for different bee and wasp groups are shown in Table 4.3.

Table 4.3. The ten most popular forage plants for different aculeate groups. Plants sown as part of pollinator friendly management are highlighted in bold.

<i>Bombus</i> spp.	Visits (%)	<i>Apis mellifera</i>	Visits (%)	Other bees	Visits (%)	Wasps	Visits (%)
<i>Centaurea nigra</i>	43.21	<i>Centaurea nigra</i>	32.08	<i>Centaurea nigra</i>	18.52	<i>Heracleum sphondylium</i>	48.35
<i>Lotus corniculatus</i>	5.23	<i>Trifolium hybridum</i>	15.60	<i>Heracleum sphondylium</i>	10.22	<i>Daucus carota</i>	18.68
<i>Trifolium pratense</i>	5.23	<i>Heracleum sphondylium</i>	8.83	<i>Hypochaeris radicata</i>	8.00	<i>Angelica sylvestris</i>	10.98
<i>Trifolium hybridum</i>	4.73	<i>Melilotus officinalis</i>	6.24	<i>Leucanthemum vulgare</i>	7.11	<i>Cirsium arvense</i>	6.59
<i>Cirsium vulgare</i>	3.62	<i>Rubus fruticosus</i> agg.	5.06	<i>Tripleurospermum inodorum</i>	6.52	<i>Pastinaca sativa</i>	4.40
<i>Stachys sylvatica</i>	3.47	<i>Phacelia tanacetifolia</i>	3.65	<i>Crepis capillaris</i>	6.07	<i>Achillea millefolium</i>	2.20
<i>Arctium minus</i>	2.99	<i>Sonchus arvensis</i>	3.59	<i>Odontites verna</i>	4.89	<i>Scrophularia auriculata</i>	2.20
<i>Lamium album</i>	2.84	<i>Origanum vulgare</i>	2.77	<i>Pulicharia dysenterica</i>	4.59	<i>Anthriscus sylvestris</i>	1.10
<i>Dipsacus fullonum</i>	2.80	<i>Pulicharia dysenterica</i>	2.77	<i>Picris hieracioides</i>	3.26	<i>Euphorbia amygdaloides</i>	1.10
<i>Trifolium repens</i>	2.77	<i>Reseda lutea</i>	2.65	<i>Taraxacum</i> agg.	3.26	<i>Leucanthemum vulgare</i>	1.10

4.5 Discussion

The 104 bee species recorded in the current work represent approximately half of the bee species found in the south-east of England, with 198 and 199 species recorded since 1970 from the neighbouring counties of Surrey and Kent, respectively (Baldock 2008). Given that many habitat specialists are unlikely to be found on farmland this number is a substantial fraction of all possible species, with many European studies only recording around 40-70 bee species (e.g. Holzschuh *et al.* 2008; Kovács-Hostyánszki 2013; Le Féon 2013), despite surveying a more extensive European fauna, though see Batáry *et al.* (2010). Direct surveys for farmland wasps are uncommon, with most studies assessing trap nesting wasps only (e.g. Holzschuh *et al.* 2010). Bee faunas are known to be locally diverse, variable in space and time and rich in rare species with up to 50% of species represented by singletons, even in studies recording several thousand individuals (Williams *et al.* 2001). Our results show that reasonably high aculeate diversity, including species of national conservation concern, can persist in agricultural environments containing 21-22% semi-natural habitat. This proportion of semi-natural habitat is high, as areas where comparable studies have been carried out in continental Europe often have considerably less semi-natural habitat, typically 5-10% (e.g. Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2003; Herrmann *et al.* 2007). It has been argued that landscape complexity is more important than local management, with positive benefits of management seen only in simple landscapes (Tscharntke *et al.* 2005). The addition of 5.5 ha of flower-rich habitat to a landscape which already has around 20% semi-natural habitat may not be enough to make a significant difference to existing aculeate populations. It may be the case that current HLS prescriptions will significantly increase floristic and aculeate diversity in simpler, more intensively farmed landscapes comprising around 5-10% semi-natural habitat, but this remains to be tested.

The loss of meadows and leys rich in Fabaceae has been particularly associated with bumblebee declines (Carvell *et al.* 2006a; Williams and Osborne 2009), and many species that have declined substantially have likewise been shown to collect a larger proportion of their pollen from Fabaceae (Goulson *et al.* 2005; Kleijn and Raemakers 2008). It has also been argued that the abundance of the most rewarding forage plants is more important than the overall diversity of flowering plants for maintaining diverse bumblebee communities (Williams 1989). As a result, increasing floral abundance, particularly the abundance of leguminous forage, and observing the response of foraging bumblebees has been an important part of the design and assessment of pollinator-friendly agri-environment schemes (Edwards 2003; Pywell *et al.* 2006; Carvell *et al.* 2007). However, there has been relatively little assessment of the response of other wild bees to these options (Haaland *et al.* 2011), despite the fact that these other bees make up the large majority of species diversity. Where studies have been conducted, non-corbiculate bees show little preference for sown species. Carreck and Williams (2002) recorded only six species of non-corbiculate bees visiting annual wildflower mixes, representing just 1% (n=223/21841) of total visits by all bees. Our results demonstrate that whilst bumblebees and honeybees foraged strongly from sown flowers, the majority of non-corbiculate bee species were not observed visiting these plants, even when growing wild as part of an existing flora. Non-corbiculate species predominantly favoured Asteraceae and Apiaceae growing in wayside and hedgerow habitats. Whilst wasps visit flowers much less frequently, similar patterns were observed, with the majority of visits being to non-scheme plants, particularly Apiaceae.

Bee community richness has been frequently linked to floristic richness (Tscharntke *et al.* 1998; Potts *et al.* 2003; Batáry *et al.* 2010), and a loss of plant species has been associated with a decline in bee diversity in agricultural habitats (Banaszak 1992; Biesmeijer *et al.* 2006; Scheper *et al.* 2014). Options for establishing floristically enhanced habitats as part of HLS management typically involve sowing mixes containing around 20 native species of wildflowers (Carvell *et al.* 2007; Pywell *et al.* 2011a). However, the systematic addition of inorganic fertilisers over the past 60 years has negatively affected floral diversity in field margins and other non-cropped areas, primarily by increasing the competitiveness of coarse grasses (Kleijn and Verbeek 2000). High nutrient levels are not easily reduced and grasslands can suffer chronically low levels of biodiversity for decades after enrichment has ceased (Isbell *et al.* 2013). Consequently, establishing diverse floral communities on enriched soils faces a number of difficulties, with the most successful programmes requiring deep cultivation and sowing of a selection of ecologically appropriate species (Pywell *et al.* 2002).

In addition to foraging resources, aculeates require suitable nesting habitat to complete their lifecycles. Cavity nesting bees and wasps are generally thought to be limited by cavity availability (Holzschuh *et al.* 2010), and the provision of trap nests can significantly increase their abundance (Gathmann and Tschardt 1997). Whilst the majority of bees are ground nesters, the availability of nesting resources can play a small but important role in organising bee communities (Potts *et al.* 2005). There has been little assessment of how well agri-environment schemes can provide appropriate habitat for nesting bees other than for bumblebees (Lye *et al.* 2009). Deliberate habitat manipulation to create nesting habitat for aculeates on agricultural land has been advocated (Kremen *et al.* 2007), but there are currently no specific schemes under HLS management with this aim in mind.

Many farmers lack the requisite knowledge to restore grassland communities and the created habitats are often dominated by relatively few species, often *Centaurea nigra*, *Lotus corniculatus* and *Trifolium* spp. Whilst the creation of flower-rich habitats significantly increased floral abundance, in our study it did not significantly increase floral diversity at the farm level. Whilst the flower species that are currently sown and establish well in flower-rich margins provide attractive forage for bumblebees and honeybees, they are of limited use to the majority of bee species. Given that current schemes were designed around the foraging requirements of bumblebees, whilst they can significantly increase the population size of common bumblebee species (Wood *et al.* 2015a), it is perhaps not surprising that they are only visited by a restricted suite of aculeates. Because of the strong response of bumblebees and honeybees to sown plants and their consequent high abundance on HLS farms, the rarefaction analysis suggests that HLS farms are less diverse purely because they have large numbers of bees, even though there is no total difference in species richness. Whilst it is the case that a random sample of 10 bees from an HLS farm will probably contain fewer species than a sample from an ELS farm, and could consequently be considered less diverse, it is clear that HLS farms should not be described as less species rich, or supporting a smaller aculeate community.

Increasing the number of pollinators is important, as larger wild bee populations can significantly increase the quality of the pollination service provided (Blaauw and Isaacs 2014). Recent work has also highlighted the importance of a diverse pollinator community for providing a high quality pollination service (Westerkamp and Gottsberger 2000; Klein *et al.* 2003; Hoehn *et al.* 2008; Garibaldi *et al.* 2013). The extent to which agri-environment schemes should focus on ecosystem service delivery or biodiversity conservation is not clear, with most

schemes benefiting generalist pollinators that can respond rapidly to habitat creation (Scheper *et al.* 2013). Since both pollinator diversity and abundance are important for crop pollination (Garibaldi *et al.* 2014), such objectives may not be greatly opposed. Bumblebees and honeybees are important pollinators, and increasing the size of their populations is an appropriate goal, but a better appreciation that current schemes predominantly benefit corbiculate bees and are of limited use to the wider aculeate community is also desirable. Complementary ‘non-corbiculate-focused’ schemes should be developed and implemented, as in their current form pollinator-focused agri-environment schemes do not provide suitable resources to support a significantly more diverse aculeate community.

4.6 Conclusions

Increasing the size of pollinator populations is an important aim of environmental stewardship, but management that focuses purely on increasing floral abundance may fail to support a diverse aculeate community. Despite the lack of evidence that Fabaceae-rich options provide resources for non-corbiculate bees, they are the most highly appraised pollinator-focused agri-environment scheme (Breeze *et al.* 2014). Our results show that current management techniques described as pollinator-friendly appear to benefit only a limited suite of aculeate species, predominantly a small number of bumblebee species and honeybees. The majority of bee species foraged heavily from naturally regenerating Asteraceae and Apiaceae in non-scheme areas; it is these and other non-Fabaceae plant groups that also need to be encouraged to maintain a diverse bee community. Consequently, agri-environment schemes that enable land managers to create and maintain local floral diversity should be developed and investigated to more effectively retain and restore aculeate diversity on farmland.

Chapter 5 – Providing foraging resources for solitary bees on farmland: current schemes for pollinators benefit a limited suite of species

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5.1 Abstract

Changes in agricultural practice across Europe and North America have been associated with range contractions and a decline in the abundance of wild bees. Concerns at these declines has led to the development of flower-rich agri-environment schemes as a way to enhance bee diversity and abundance. Whilst the effect of these schemes on bumblebee species (*Bombus* spp.) has been well studied, their impact on the wider bee community is poorly understood. We used direct observations of foraging bees and pollen load analysis to quantify the relative contribution that sown flowers (i.e. those included in agri-environment scheme seed mixes) make to the pollen diets of wild solitary bees on Higher Level Stewardship farms (HLS) implementing pollinator-focused schemes and on Entry Level Stewardship farms (ELS) without such schemes in Southern England, UK. HLS management significantly increased floral abundance, and as the abundance of sown flowers increased these sown plants were utilised for pollen by a greater proportion of the solitary bee species present. However, the overall proportion of pollen collected from sown plants was low for both direct observations (27.0%) and pollen load analysis (23.3%). At most only 25 of the 72 observed species of solitary bee (34.7%) were recorded utilising sown plants to a meaningful degree. The majority of solitary bee species did not collect pollen from flower species sown for pollinators. Total bee species richness was significantly associated with plant species richness, but there was no difference in the total species richness of either bee or flowering plant species between HLS and ELS farms. Our results show that the majority of solitary bee species present on farmland in the south-east of England collect most of their pollen from plants that persist unaided in the wider environment, and not from those included in agri-environment schemes focused on pollinators. If diverse bee communities are to be maintained on farmland, existing schemes should contain an increased number of flowering plant species and additional schemes that increase the diversity of flowering plants in complementary habitats should be studied and trialled.

5.2 Introduction

Wild bees, in common with many other taxa, have experienced declines in richness and abundance across Europe and North America (Biesmeijer *et al.* 2006; Williams and Osborne 2009; Bartomeus *et al.* 2013; Goulson *et al.* 2015). This is of concern as pollinating insects, of which bees are the dominant group, provide a highly valuable pollination service to both crops and wild plants (Ollerton *et al.* 2011; Garibaldi *et al.* 2013). With the demand for increased

agricultural yields growing across the world, potential pollination deficits have increased the pressure to develop effective management techniques to conserve and maintain bee populations on agricultural land.

There is an increasing consensus behind the idea that food resources are the most important limiting factor for bee populations on farmland (Roulston and Goodell 2011), and that loss of flowering resources resulting from agricultural intensification is the major cause behind the declines in bee populations seen in the 20th century (Carvalho *et al.* 2013; Ollerton *et al.* 2014; Scheper *et al.* 2014). Moreover, a reduction in the diversity of pollen sources can have negative impacts on bee fitness through reduced development and immunocompetence (Alaux *et al.* 2010). As a result, the creation of flower-rich habitat through agri-environment schemes has been advocated and trialled as the primary means of conserving and enhancing bee abundance and diversity on farmland (Carvell *et al.* 2007; Winfree 2010). In the United Kingdom, much of the early work on agri-environment schemes focused on bumblebees (*Bombus* spp.), due in part to their particularly pronounced declines in agricultural areas (Goulson *et al.* 2005). Schemes were consequently designed with the foraging requirements of bumblebees in mind, specifically including a large Fabaceae component comprised of plants such as *Trifolium pratense*, *T. hybridum* and *Lotus corniculatus* (Edwards 2003; Carvell *et al.* 2007). Research has shown that these sown resources are attractive to a wide variety of common and threatened bumblebees (Carvell *et al.* 2006b; Carvell *et al.* 2007), and that when present in sufficient quantities they can significantly increase the population size of common bumblebee species (Wood *et al.* 2015a). However, much less work has been carried out on the impact of agri-environment schemes on the wider bee community.

In temperate areas such as Britain bumblebees make up only a small part of the overall bee community, representing around 10% of the total species list (25 out of c. 250 species), and in the larger continental faunas of Europe and North America they represent an even smaller proportion. The wider bee community consists of predominantly solitary species (and their associated kleptoparasites) that collect pollen to provision their own offspring. The fauna contains a number of species within the Halictidae that show variably developed and expressed eusocial behaviour (Plateaux-Quénu 2008). Whilst not technically correct, the term 'solitary bees' is generally used as an all-encompassing term to include the eusocial species of the Halictidae with all non-parasitic, non-corbiculate (non-*Apis* and non-*Bombus*) bees found in temperate regions, with this synthetic group the focus of this study.

In order to assess the benefit of an agri-environment scheme, field trials have often compared target areas with control areas and have recorded an increase in bee species richness and abundance (e.g. Knop *et al.* 2006 ; Kohler *et al.* 2007; Pywell *et al.* 2011a) or an increase in important behaviours, such as bumblebee queen nest site searching (Lye *et al.* 2009). Ideally, measures should be assessed by comparing bee trends on sown flower strips before and after implementation of the schemes (Kleijn *et al.* 2006), with such studies finding a positive impact on bee species richness and abundance (Holland *et al.* 2015; Scheper *et al.* 2015). However, whilst these studies show that enhanced areas provide resources for a greater variety of bee species than before, it is not clear that the overall bee community has become richer as a result of the intervention, as some bees that were already present in the landscape may simply have been attracted to enhanced areas. Moreover, the relative contribution of pollen from sown plants to the diet of different solitary bee species is poorly known, and whilst they may be attracted to sown flowers these resources may not make up a significant proportion of their overall diet. Data on *Bombus* species, other than presence, was not collected due to the extensive previous work conducted on this group on farmland and their response to agri-environment schemes (Carvell *et al.* 2006b; Carvell *et al.* 2007).

In this study we conducted extensive surveys across a range of farms in Southern England and quantified the pollen diets of wild solitary bees using direct observations and pollen load analysis to address the following objectives. (i) To compare the contribution that plants sown as part of agri-environment schemes make to the pollen diet of solitary bees relative to that provided by wild plants. We predict that as sown resources increase in relative abundance they will increase in relative utilisation by solitary bees. (ii) To quantify the proportion of solitary bee species using sown resources. We predict that as sown resources increase in relative abundance, they will be utilised by a relatively greater proportion of solitary bee species. (iii) To identify solitary bee species most likely to be benefiting from currently sown resources. (iv) To identify potential temporal resource gaps in current agri-environment scheme design, or key wild flowering plant species not currently included in seed mixes. This study will provide valuable information to scientists, governments and land managers in designing more effective measures to conserve the broader wild bee community on agricultural land.

5.3 Methods

5.3.1 Study area

Nine HLS and ten ELS farms were selected in Hampshire and West Sussex, UK. The selected HLS farms had been implementing an average of 5.56 ± 0.13 ha of pollinator-focused flower-rich schemes representing $2.17 \pm 0.05\%$ of the farm area by ownership for a minimum of three years. As 70% of farms in England were at the time in some form of environmental stewardship (Elliot *et al.* 2010), ELS farms were chosen as the control group for this study. Flower-rich schemes were available under ELS, but these schemes had a low uptake so only basic ELS farms without such management were selected for this comparison. Pollinator-focused flower-rich schemes were typically established with a seed mix containing c.15-30 flowering forb species (Carvell *et al.* 2007; Pywell *et al.* 2011a). Additional plant species such as *Hypochaeris radicata* and *Trifolium repens* are sometimes included in experimental mixes (i.e. Scheper *et al.* 2015), but this did not represent the situation in our study area and so these species were not characterised as sown. Whilst there were no such flower-rich areas on ELS farms, most of the species included in these seed mixes can be found growing in a wild state on these farms. Consequently, in order to allow a comparison of pollen choice preferences and relative rates of utilisation across farm types, plant species included in pollinator-friendly agri-environment schemes were characterised as 'sown' even when found growing wild as part of the wider plant community. For a full list of the plant species characterized as being sown as part of pollinator-focused management, see Appendix D. Farms were predominantly arable, or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

5.3.2 Bee and floristic surveys

In 2013 and 2014, a standardised 3 km transect was designed for each farm, passing through all major habitat types present. For HLS farms this included pollinator-focused flower-rich schemes (HE10 floristically enhanced grass margins, HK7 species-rich grassland restoration, HF4 pollen and nectar mixes), non-agricultural grass margins and hedgerow and woodland edge habitats. For ELS farms only non-agricultural grass margins and hedgerow and woodland edge habitats were surveyed, as no pollinator-focused schemes were present. Crops and areas of agricultural grassland were not surveyed. Each transect was subdivided into discrete sections, with each section covering a distinct habitat type. Transects on HLS farms were

designed to survey as many pollinator-focused schemes as possible whilst remaining contiguous and passed through an average of $1496 \pm 148\text{m}$ of flower-rich habitat in an average of 3.77 ± 0.24 discrete habitat patches per farm.

Bee activity was recorded along the transect following standard bee walk methodology (Carvell *et al.* 2007), with all bees within 2 m of the recorder identified to species level. Individuals that could not be identified in the field were netted for later identification. The first flowering plant species visited and the purpose of the visit, for either pollen or nectar, was recorded. *Hylaeus* species, which lack scopal hairs on their body, instead ingesting pollen and regurgitating it in the nest, cannot reliably be determined to be foraging for pollen and so all plant visits were recorded simply as visits. On each transect, the number of species of flowering plants and the number of flowering units of each plant species within 2 m of the recorder was estimated within each discrete transect section. Grasses, sedges and rushes were not recorded as these plant species are not attractive to bees in the study region. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a head, a capitulum) counted as a single unit. Sixteen farms (eight HLS, eight ELS) were surveyed in 2013. Transects were walked three times through the season, between 25th May–5th June, 26th June–15th July and 3rd–11th August. Seventeen farms (eight HLS, nine ELS) were surveyed in 2014. Transects were walked three times through the season, between 17th–27th May, 21st June–9th July and 3rd–15th August. These discrete sampling blocks are henceforth referred to as ‘sampling rounds’.

In 2015 farms were surveyed for a fixed period of time rather than using distance based transects. ELS farms were surveyed for 3 hrs with 1.5 hrs spent on non-agricultural grass habitats and 1.5 hrs on woody hedgerow/woodland edge habitats. HLS farms were surveyed for 3 hrs with 1 hr on pollinator-focused flower-rich schemes, 1 hr on non-agricultural grass habitats and 1 hr on woody hedgerow/woodland edge habitats. The survey followed standard bee walk methodology as described above, but at a reduced pace to ensure thorough sampling. All bees within 2 m of the recorder were identified to species level. The first flowering plant species visited and the purpose of the visit, for either pollen or nectar, was recorded. Solitary bees with clearly visible pollen on their body were collected, placed in individual Eppendorf tubes and frozen. The collection of pollen loads from foraging bees may overestimate pollen use of more easily observable flowers. Ideally, pollen would be sampled from bees as they return to their nest, but this method was not chosen for this study as is often time consuming and may lead to low sample sizes for species with difficult to locate nests. All flowering plant species present on the transects were recorded, but their abundance

was not quantified. Pollen samples from insect visited flowering plant species present were collected to form a pollen reference library. Pollen reference slides were prepared by transferring pollen-laden anthers to a drop of water on a microscope slide. The slide was gently heated to allow grains to absorb water and achieve their maximum size and to evaporate excess water. The remains of the anthers were removed, molten glycerine jelly stained with fuchsin was added and the slide was sealed with a coverslip. For a full list of sampled flowering plant species, see Appendix E. Fourteen farms (7 HLS, 7 ELS) were surveyed in 2015. Transects were walked four times throughout the season, between 22 April– 13th May, 26th May– 17th June, 25th June– 4th July and 29th July– 10th August. All bee surveys were conducted between 0930 and 1700 hrs when the temperature was above 13°C with at least 60% clear sky, or above 17°C with any level of cloud. No surveys were conducted when it was raining. All bee and floristic surveys were conducted by the same individual (TJW) to minimise recorder bias.

5.3.3 Pollen identification

The scopal pollen load of foraging solitary bees collected in 2015 was analysed by light microscopy using the method outlined by Westrich and Schmidt (1986). Before removing pollen from the scopae, the total load was estimated relative to a full load for that species, ranging from 8/8 (full load) to 1/8 (one eighth load). The pollen grains were removed from the scopae using an entomological pin and transferred to a drop of water on a microscope slide. Pollen that was not clearly held in the scopae was not sampled as this may have become attached to other parts of the body during nectar visits to non-host plant flowers. The slide was gently heated to allow grains to absorb water and achieve their maximum size and to evaporate excess water. Molten glycerine jelly stained with fuchsin was then added and the slide was sealed with a coverslip. The proportion of the load comprised of different plant species was estimated along three randomly selected lines across the cover slip at a magnification of x400. The proportion of the load by volume was estimated by the relative area of the slide occupied by each plant species, rather than the absolute number of grains, in order to better reflect the total volume of pollen collected, an important correction in mixed loads where pollen grains of different plant species often differ widely in size (Cane and Sipes, 2006). Species representing less than 1% of the load were excluded from further analysis as their presence may have arisen from contamination (Westrich and Schmidt 1986).

The proportions of pollen collected were corrected according to the overall size of each load to give a final weight, e.g. a full load (8/8) comprised of 50% *Centaurea nigra* and 50% *Leucanthemum vulgare* would receive a final *C. nigra* weight of 50 and a final *L. vulgare* weight of 50, whereas a quarter load (2/8) comprised of 100% *Hypochaeris radicata* would receive a final *H. radicata* weight of 25. The pollen grains were identified to species using Sawyer (1981) and the reference collection assembled during the project. The majority of samples were identified to species level, but where this was not possible pollen was identified to genus, for example in *Brassica*, *Plantago* and *Geranium*. For a full list of taxa and the level of identification, to either species or genus, see Appendix F.

5.3.4 Statistical analysis

Generalised Linear Mixed-Effect Models (GLMMs) were used to test for the impact of management type on bee and plant species abundance and diversity and the impact of plant species richness on bee species diversity and diet breadth. Models were fit using the maximum likelihood (Laplace Approximation) method. All data analyses were conducted in R version 3.1.1 (R Development Core Team) using the lme4 package for the GLMMs (Bates *et al.* 2014). All models were fitted with Poisson and negative binomial error distributions and were tested for overdispersion. In all cases negative binomial error structures were the most appropriate and final models were not overdispersed. Final models were compared by ANOVA with a null model containing the same random factor to test for significance.

Differences in the total number of bee and plant species and total floral abundance recorded between different farm types were analysed using GLMMs with management type as a fixed factor. Sampling year was included as a random factor to take account of the temporal data structure and differences in sampling methods. The abundance analysis used the 2013-2014 data and the species richness analysis used the 2013-2015 data. The impact of plant species richness on bee species richness (including *Apis*, *Bombus* and kleptoparasitic bee species) and oligolectic solitary bee species richness was analysed using GLMMs with plant species richness as a fixed factor and sampling year as a random factor. This analysis used the 2013-2015 species richness data.

The impact of plant species richness on the number of pollen species detected in bee pollen loads was analysed using a GLMM with plant species richness as a fixed factor and sampling round (April/May, May/June, June/July and July/August) as a random factor. The number of

pollen species detected in bee pollen loads was also calculated for the seven most common polylectic bee species for which a total of 30 pollen loads had been collected from each species, representing the majority of the pollen load data (759 of 1054 samples, *Andrena chrysosceles*, *A. flavipes*, *A. haemorrhoea*, *A. semilaevis*, *Lasioglossum calceatum*, *L. malachurum* and *L. pauxillum*). The number of species detected in pollen loads was summed over the year for each species to reduce temporal variation. Farms where no samples of a species were taken were excluded from that species' analysis, as the species may have been absent from the sample for reasons other than floristic composition, e.g. nesting site availability, low detection rate etc. The relationship between plant species richness and the number of pollen species collected by polylectic bee species was analysed using a GLMM with plant species richness as a fixed factor and bee species as a random factor. Both these analyses used the 2015 microscopic pollen load analysis data.

The proportion of sown flowers relative to total flowers was calculated for each farm over the 2013-2014 period. The proportion of observed solitary bee pollen visits to sown flowers and the proportion of solitary bee species visiting sown flowers for pollen was also calculated over the 2013-2014 period. The impact of the proportion of sown flowers on the proportion of observed solitary bee pollen visits and the proportion of solitary bee species visiting sown flowers was analysed using Spearman's rank correlation tests, as in each case the response variable could not be transformed to normality.

Differences in the proportion of pollen collected from different plant types were analysed using binomial tests. For the observational data, the proportion of pollen visits to sown and wild plants was calculated for each sampling round across all years for both farm types. For the pollen load data, a third category of crop plant data was included. A number of pollen loads contained Brassica type pollen, most of which is highly likely to have come from the crop plant oilseed rape *Brassica napus*. No wild Brassica species such as *B. nigra* were recorded during floristic surveys with the only other source being small areas of *B. rapa* that is sometimes sown as part of conservation management for birds. As a result, we are confident that the majority of the Brassica type pollen originated from crop plants and so this was excluded from the comparison between sown and wild plant pollen use. As the pollen load data is non-integer (with variably full pollen loads with mixed species composition), the proportion of each pollen type was used to calculate an appropriate value from the number of collected samples, i.e. where 173 bees were collected with pollen loads in total comprised of 9.7% pollen from sown plants and 90.3% pollen from wild plants by volume this was calculated as 17 samples from

sown plants and 156 samples from wild plants. These calculated values were used in the binomial tests.

5.4 Results

A total of 105 species of bee was recorded over the survey period. This comprised the honey bee *Apis mellifera*, 15 species of bumblebee *Bombus* (including five parasitic *Psithyrus* spp.), 72 species of solitary bee and 17 species of parasitic bee (Appendix G). There was no difference in the total number of bee species recorded on each farm type in each year (mean HLS 23.2 ± 2.4 , mean ELS 21.5 ± 2.1 , $\chi^2=0.6$, $p=0.418$). A total of 9.69 million flowering units was recorded on the transects in 2013-2014. Floral abundance was significantly greater on HLS farms than on ELS farms in each year (mean HLS $501,758 \pm 74,397$ flowering units, mean ELS $97,530 \pm 22,703$ flowering units, $\chi^2=32.6$, $p<0.001$). A total of 6.24 million flowering units of plant species characterised as sown was recorded on the transects in 2013-2014. Sown floral abundance was more than ten times higher on HLS farms than on ELS farms in each year (mean HLS $354,271 \pm 71,761$, mean ELS $33,579 \pm 12,739$, $\chi^2=23.0$, $p<0.001$). A total of 291 species of flowering plants was recorded over the survey period. There was no difference in the total number of plant species recorded on each farm type in each year (mean HLS 75.2 ± 4.2 , mean ELS 68.8 ± 3.1 , $\chi^2=1.9$, $p=0.171$).

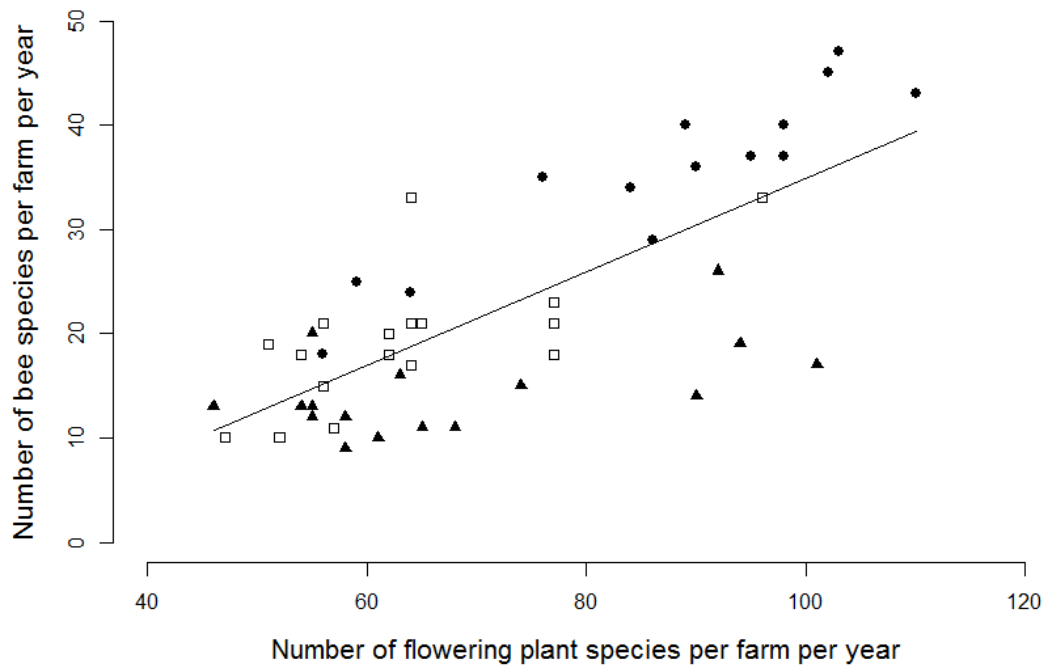


Figure 5.1. Relationship between plant species richness and bee species richness on surveyed farms. Squares = 2013, triangles = 2014, circles = 2015.

Over the whole survey period, bee species richness was significantly associated with plant species richness ($\chi^2=33.7$, $p<0.001$, Figure 5.1). A total of 16 oligolectic bee species was recorded following Westrich (1989), and oligolectic bee species richness was also significantly associated with plant species richness ($\chi^2=10.0$, $p=0.002$).

Fifty-six species of solitary bee were observed making 1,416 pollen foraging trips. One thousand and fifty-four individual bees with pollen loads from 47 bee species were collected for microscopic pollen analysis. The number of species of pollen detected in pollen loads on each farm was significantly associated with the number of flowering plants recorded on the transects, with this effect consistent over the survey period ($\chi^2=16.8$, $p<0.001$, Figure 5.2). However, for the seven most common polylectic bee species representing the bulk of the pollen load data (759 out of 1054 samples) there was no significant relationship between diet breadth and observed plant species richness ($\chi^2=0.7$, $p=0.416$), suggesting that the relationship is instead driven by the addition of more specialised bees to the community in floristically richer environments that collect pollen from a different suite of host plants.

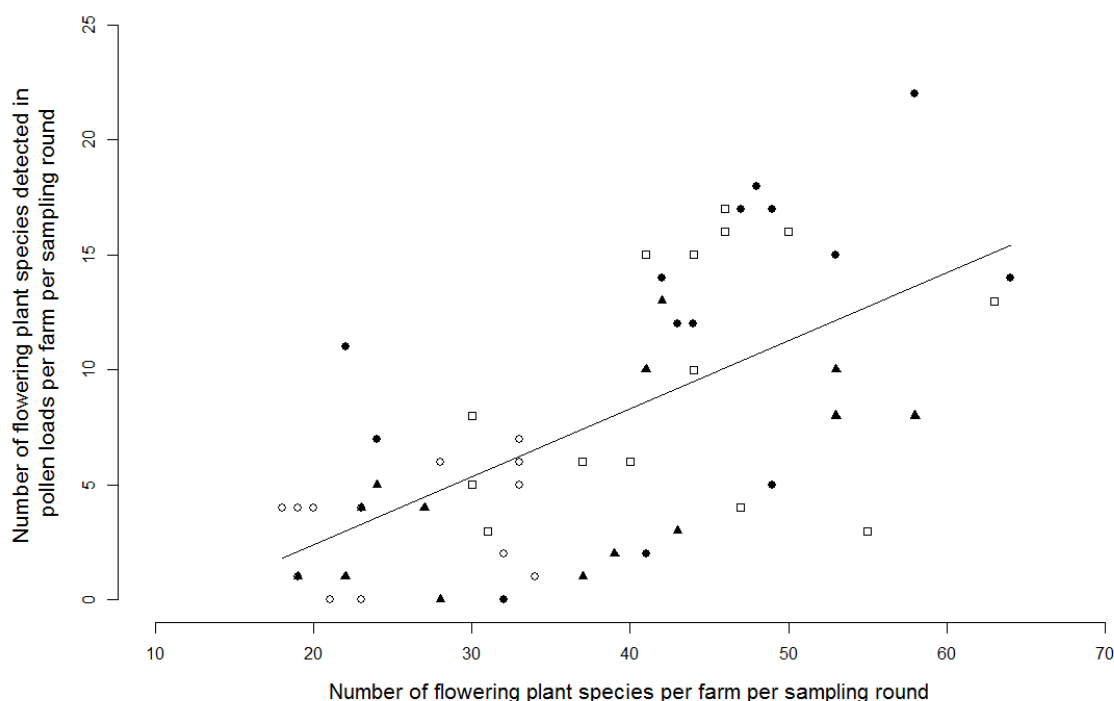


Figure 5.2. Relationship between plant species richness and the species richness of pollens detected in bee pollen loads on surveyed farms across the season. Open circles = late April/early May; open squares = late May/early June; closed circles = late June/early July; closed triangles = late July/early August.

Across all farms, as the proportion of sown plants increased, so the proportion of observed pollen foraging visits to sown plants increased as well (Spearman's $\rho=0.920$, $p<0.001$, Figure 5.3). However, the proportion of pollen collected by solitary bees from sown plants varied greatly throughout the year and between farm types. In late April/early May no pollen visits to sown plants were observed on either farm type (Figure 5.4 a, b). In late May/early June the proportion of observed pollen visits to sown plants was similarly low on both ELS and HLS farms (HLS 13.1%, ELS 9.4%, $\chi^2=0.83$, $p=0.364$, Figure 5.4 a, b). In late May/early June the proportion of observed pollen visits to sown plants was similarly low on both ELS and HLS farms (HLS 13.1%, ELS 9.4%, $\chi^2=0.83$, $p=0.364$, Figure 5.4 a, b). In late June/early July the proportion of observed pollen visits to sown plants increased to 60.1% of visits on HLS farms compared to 18.5% of visits on ELS farms, a significantly higher proportion ($\chi^2=113.92$, $p<0.001$, Figure 5.4 a, b). In late July/early August the proportion of pollen visits to sown plants decreased on both farm types, though it was still significantly greater on HLS farms (HLS 21.1%, ELS 10.1%, $\chi^2=4.5$, $p=0.033$, Figure 5.4 a, b).

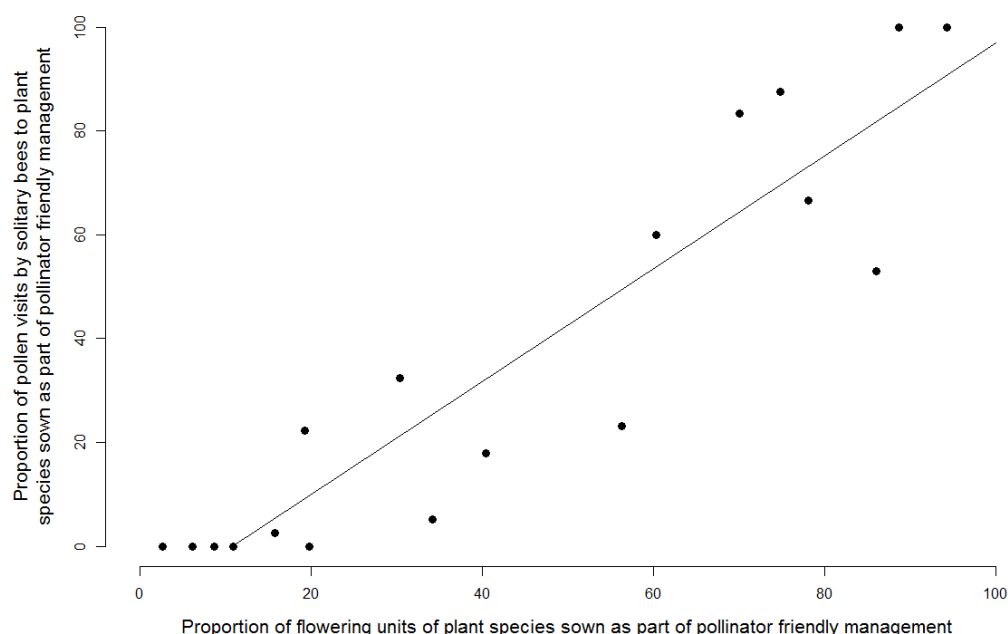


Figure 5.3. Relationship between availability of flowering units of plant species sown as part of pollinator friendly management and their observed utilisation for pollen by solitary bees on surveyed farms.

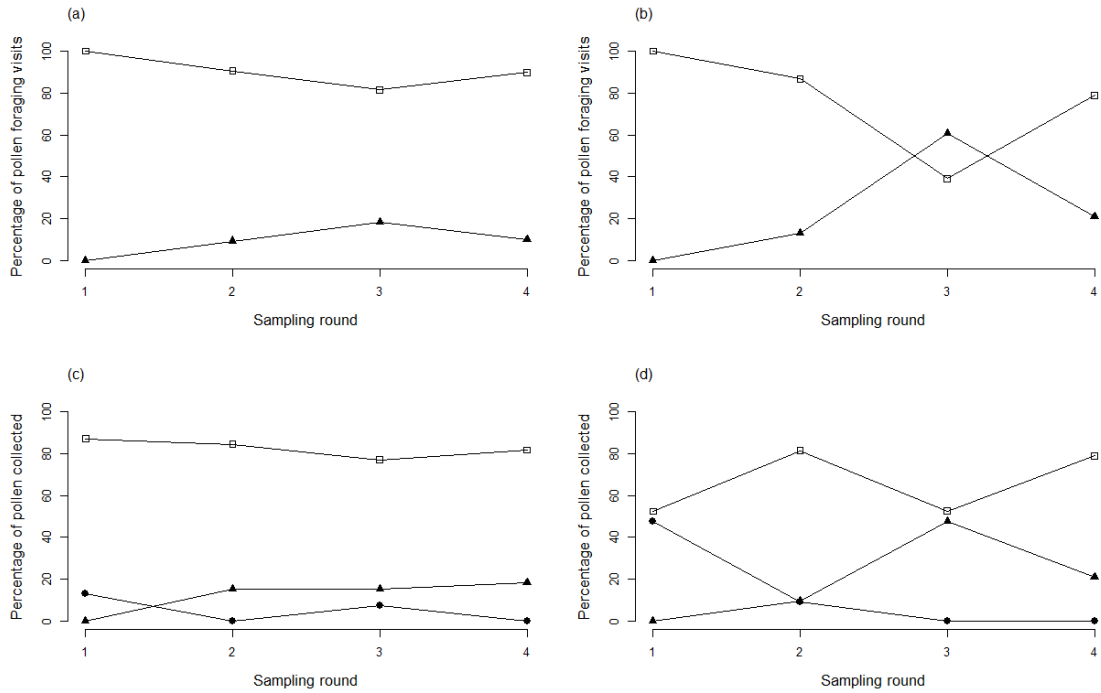


Figure 5.4. Proportion of pollen collected by solitary bees from a) observed pollen foraging trips on ELS farms, b) observed pollen foraging trips on HLS farms, c) pollen load analysis from ELS farms and d) pollen load analysis from HLS farms. Triangles = plant species sown as part of agri-environment management, squares = wild plant species and circles = crop plant species (*Brassica* spp). Sampling round one, late April/early May; sampling round two, late May/early June; sampling round three, late June/early July; sampling round four, late July/early August. A similar trend was observed in the analysis of collected pollen samples.

Excluding oilseed rape type (*Brassica* type) pollen from the analysis, no pollen was collected from sown plants in late April/early May. In late May/early June the proportion of pollen collected from sown plants was similarly low (ELS 15.4%, HLS 10.6%, $\chi^2=1.2$, $p=0.283$, Figure 5.4 c, d). In late June/early July the proportion of pollen collected from sown plants increased to 47.4% on HLS farms compared to 16.5% on ELS farms, a significantly higher proportion ($\chi^2=46.2$, $p<0.001$, Figure 5.4 c, d). In late July/early August the proportion of pollen collected from sown plants decreased to a similar level on both HLS and ELS farms (ELS 18.3, HLS, 21.1, $\chi^2=0.1$, $p=0.824$, Figure 5.4 c, d).

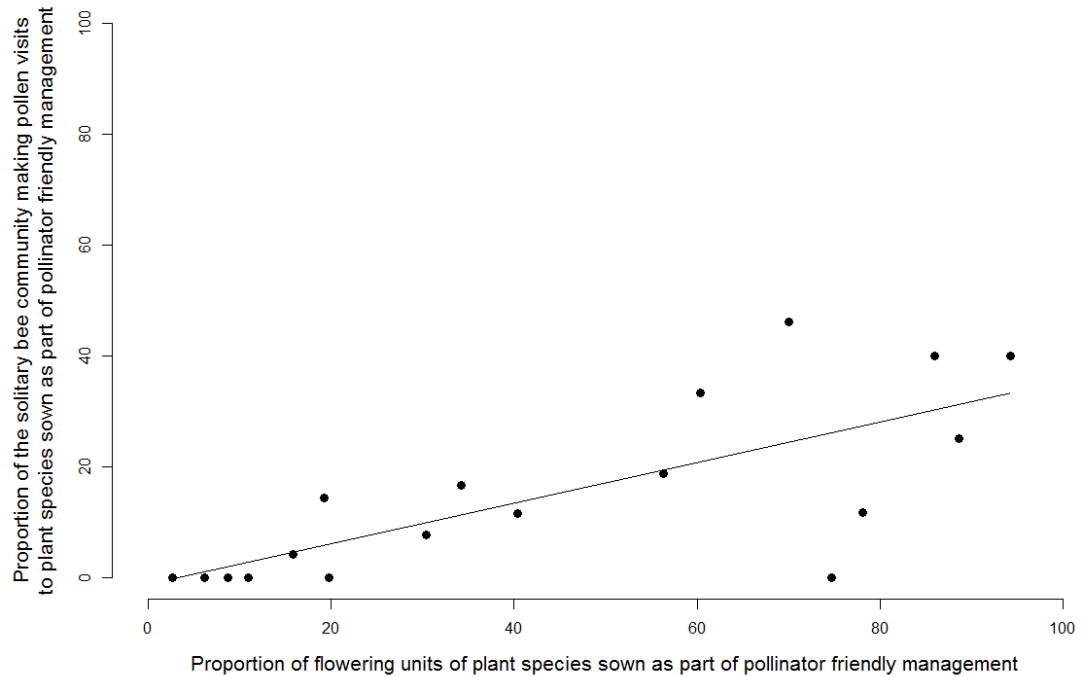


Figure 5.5. Relationship between availability of sown floral resources and the proportion of solitary bee species utilising them for pollen on surveyed farms.

Across all farms, as the proportion of sown plants increased the proportion of solitary bee species present observed making pollen foraging trips to sown plants increased as well (Spearman's $\rho=0.743$, $p<0.001$, Figure 5.5). However, at best only 46.2% of solitary bee species were observed visiting sown plants for pollen. Over the whole survey period, pollen collected from sown plants by solitary bees represented only 27.0% of pollen visitation observations and 23.3% of pollen collected by volume. The most popular sown plants were *Leucanthemum vulgare*, *Centaurea nigra* and *Daucus carota* (Table 5.1). Of the 72 species of solitary bee only 31 species had five analysed pollen loads or five observed pollen visits. Of these, 14 species collected at least 10% of their pollen from sown plants (Table 5.2) with 17 species collecting a lower proportion than this. Of the 41 species with fewer than five analysed loads or five observed visits, 11 were observed visiting, or their pollen loads contained pollen from, sown plants (*Andrena wilkella*, *Hylaeus brevicornis*, *H. cornutus*, *H. dilatatus*, *Lasioglossum albipes*, *L. smeathmenellum*, *Megachile centuncularis*, *M. versicolor*, *M. willughbiella*, *Osmia leaiana* and *O. spinulosa*). This list includes *Hylaeus* species which lack scopal hairs on their body, making accurate determination of pollen visits impossible in the field. However, all British species with the exception of *Hylaeus signatus* are known to be polylectic (Westrich 1989), and so any observed visits to sown plants were included in this list,

but not included in the main pollen visitation analysis. Five species were only recorded in the male sex and there was no evidence that females of the final 25 species visited sown plants for pollen. This most liberal estimate of 25 out of 72 solitary bee species (34.7%) likely to be using sown plants to a greater or lesser extent is similar to the overall observed proportion of pollen collected from sown plants.

5.5 Discussion

Our results show that, as expected, increasing resource availability through the creation of flower-rich habitat increased both the utilisation rates of sown plants for pollen by solitary bees and the proportion of solitary bee species utilising sown plants for pollen. These findings are in line with studies that have found that sown resources can provide improved resources for wild bees in enhanced areas (Knop *et al.* 2006; Kohler *et al.* 2007; Pywell *et al.* 2011a; Scheper *et al.* 2015). However, whilst utilisation rates of sown flowers were higher in areas in which they were abundant, the proportion of solitary bee species utilising sown plants for pollen was always a minority, even in areas where sown resources represented the vast majority of available forage. It is important to note that this study was conducted in a study region in which the landscape is composed of 21-22% semi-natural habitat (Wood *et al.* 2015b). This is considerably more than areas of conventional farmland in many developed European countries which often contain between 5-10% semi-natural habitat (e.g. Westphal *et al.* 2003; Herrmann *et al.* 2007). As such, the relatively high bee diversity and low sown resource utilisation may reflect the less intensified nature of this area, and more studies of a similar nature should be conducted in more intensified landscapes.

Table 5.1. Most important pollen forage plants for solitary bees at different times of the year from field observations and pollen analysis. Plant species sown as part of agri-environment management are marked in bold. Sampling round one, late April/early May; sampling round two, late May/early June; sampling round three, late June/early July; sampling round four, late July/early August.

Observations (2013-2015)							
Round one	% of visits	Round two	% of visits	Round three	% of visits	Round four	% of visits
<i>Taraxacum agg.</i>	66.89	<i>Chaerophyllum temulum</i>	19.37	<i>Tripleurospermum inodorum</i>	26.67	<i>Pulicaria dysenterica</i>	17.36
<i>Sinapis arvensis</i>	11.49	<i>Heracleum sphondylium</i>	14.25	<i>Centaurea nigra</i>	23.26	<i>Senecio jacobea</i>	12.81
<i>Brassica rapa</i>	10.14	<i>Crataegus monogyna</i>	9.69	<i>Leucanthemum vulgare</i>	16.15	<i>Odontites vernus</i>	11.98
<i>Crataegus monogyna</i>	4.05	<i>Leucanthemum vulgare</i>	7.69	<i>Heracleum sphondylium</i>	7.26	<i>Daucus carota</i>	11.16
<i>Anthriscus sylvestris</i>	2.70	<i>Tripleurospermum inodorum</i>	7.41	<i>Hypochaeris radicata</i>	5.19	<i>Heracleum sphondylium</i>	9.92
<i>Alliaria petiolata</i>	2.03	<i>Hypochaeris radicata</i>	7.12	<i>Bryonia dioica</i>	3.85	<i>Rubus fruticosus agg.</i>	9.09
<i>Bellis perennis</i>	0.68	<i>Sisymbrium officinale</i>	4.84	<i>Crepis capillaris</i>	3.70	<i>Tripleurospermum inodorum</i>	8.26
<i>Hyacinthoides non-scripta</i>	0.68	<i>Ranunculus repens</i>	4.56	<i>Chaerophyllum temulum</i>	1.63	<i>Picris hieracioides</i>	5.79
<i>Lamium album</i>	0.68	<i>Oenanthe crocata</i>	3.99	<i>Achillea millefolium</i>	1.48	<i>Hypochaeris radicata</i>	1.65
<i>Prunus spinosa</i>	0.68	<i>Crepis vesicaria</i>	2.85	<i>Trifolium repens</i>	1.04	<i>Centaurea nigra</i>	1.65
Pollen analysis (2015 only)							
Round one	% of pollen	Round two	% of pollen	Round three	% of pollen	Round four	% of pollen
<i>Brassica spp.</i>	31.22	<i>Heracleum sphondylium</i>	11.17	<i>Tripleurospermum inodorum</i>	27.52	<i>Senecio jacobea</i>	21.66
<i>Taraxacum agg.</i>	29.84	<i>Chaerophyllum temulum</i>	9.66	<i>Leucanthemum vulgare</i>	14.62	<i>Rubus fruticosus agg.</i>	17.52
<i>Alliaria petiolata</i>	13.41	<i>Sisymbrium officinale</i>	8.04	<i>Centaurea nigra</i>	12.53	<i>Daucus carota</i>	12.55
<i>Sinapis arvensis</i>	9.78	<i>Crataegus monogyna</i>	8.00	<i>Rubus fruticosus agg.</i>	4.50	<i>Pulicaria dysenterica</i>	11.58
<i>Bellis perennis</i>	3.48	<i>Tripleurospermum inodorum</i>	7.90	<i>Heracleum sphondylium</i>	4.43	<i>Heracleum sphondylium</i>	7.43
<i>Acer campestre</i>	3.07	<i>Leucanthemum vulgare</i>	6.06	<i>Sisymbrium officinale</i>	3.17	<i>Tripleurospermum inodorum</i>	6.03
<i>Hyacinthoides non-scripta</i>	1.79	<i>Hypochaeris radicata</i>	5.88	<i>Brassica spp.</i>	3.10	<i>Centaurea nigra</i>	4.11
<i>Lamium album</i>	1.36	<i>Brassica spp.</i>	5.19	<i>Sonchus arvensis</i>	3.06	<i>Odontites vernus</i>	3.74
<i>Glechoma hederacea</i>	1.28	<i>Oenanthe crocata</i>	4.35	<i>Bryonia dioica</i>	2.23	<i>Trifolium hybridum</i>	2.21
<i>Crataegus monogyna</i>	1.19	<i>Crepis vesicaria</i>	3.60	<i>Ranunculus repens</i>	2.18	<i>Trifolium repens</i>	2.07

Table 5.2. Solitary bee species potentially benefiting from plant species sown as part of agri-environment schemes. Selected species had a minimum of five analysed pollen loads or five observed pollen foraging visits and collected a minimum of 10% of their pollen from sown plant species in either category. *n p*, total number of pollen loads; *n obs*, total number of pollen foraging observations. * data only available for summer generation females.

Species	<i>n p</i>	<i>n obs</i>	Utilisation of sown plants		Important sown pollen sources (pollen analysis)	Important sown pollen sources (observations)
			% of pollen collected	% of observed pollen visits		
<i>Andrena alfkenella</i> *	6	10	78.6	90.0	<i>Daucus carota</i> (78.6)	<i>Daucus carota</i> (90.0)
<i>Andrena flavipes</i>	45	70	17.7	17.1	<i>Centaurea nigra</i> (8.9), <i>Trifolium hybridum</i> (5.2), <i>Leucanthemum vulgare</i> (3.4)	<i>Leucanthemum vulgare</i> (7.1), <i>Centaurea nigra</i> (4.3), <i>Trifolium hybridum</i> (2.9), <i>Trifolium pratense</i> (2.9)
<i>Andrena minutula</i>	15	30	19.8	26.7	<i>Daucus carota</i> (19.8)	<i>Daucus carota</i> (20.0), <i>Centaurea nigra</i> (3.3), <i>Sonchus arvensis</i> (3.3)
<i>Andrena minutuloides</i> *	8	10	85.0	70.0	<i>Daucus carota</i> (85.0)	<i>Daucus carota</i> (70.0)
<i>Colletes daviesanus</i>	2	5	100.0	40.0	<i>Achillea millefolium</i> (100.0)	<i>Achillea millefolium</i> (40.0)
<i>Halictus tumulorum</i>	21	23	46.5	34.8	<i>Ranunculus acris</i> (20.2), <i>Leucanthemum vulgare</i> (10.8), <i>Trifolium pratense</i> (6.2), <i>Medicago lupulina</i> (3.7), <i>Centaurea nigra</i> (3.7), <i>Silene dioica</i> (1.9)	<i>Leucanthemum vulgare</i> (17.4), <i>Ranunculus acris</i> (4.3), <i>Centaurea nigra</i> (4.3), <i>Medicago lupulina</i> (4.3), <i>Trifolium pratense</i> (4.3)
<i>Lasioglossum calceatum</i>	38	70	13.2	21.4	<i>Leucanthemum vulgare</i> (7.7), <i>Centaurea scabiosa</i> (3.0), <i>Phacelia tanacetifolia</i> (1.8), <i>Knautia arvensis</i> (0.6)	<i>Leucanthemum vulgare</i> (11.4), <i>Knautia arvensis</i> (2.9), <i>Leontodon hispidus</i> (2.9), <i>Centaurea nigra</i> (1.4), <i>Centaurea scabiosa</i> (1.4), <i>Phacelia tanacetifolia</i> (1.4)
<i>Lasioglossum leucozonium</i>	21	51	12.8	3.9	<i>Leontodon hispidus</i> (8.9), <i>Centaurea nigra</i> (4.0)	<i>Leontodon hispidus</i> (3.9)
<i>Lasioglossum malachurum</i>	437	553	32.1	47.2	<i>Leucanthemum vulgare</i> (14.8), <i>Centaurea nigra</i> (12.0), <i>Plantago</i> spp (1.5), <i>Phacelia tanacetifolia</i> (0.9), <i>Melilotus officinalis</i> (0.9), <i>Achillea millefolium</i> (0.7), others (1.2)	<i>Centaurea nigra</i> (26.8), <i>Leucanthemum vulgare</i> (17.7), <i>Achillea millefolium</i> (1.3), <i>Phacelia tanacetifolia</i> (0.5), others (0.9)
<i>Lasioglossum pauxillum</i>	70	93	19.5	15.1	<i>Leucanthemum vulgare</i> (14.1), <i>Centaurea nigra</i> (2.7), <i>Daucus carota</i> (1.1), <i>Plantago</i> spp (0.8), other (0.8)	<i>Leucanthemum vulgare</i> (11.8), <i>Centaurea nigra</i> (1.1), <i>Leontodon hispidus</i> (1.1), <i>Daucus carota</i> (1.1)
<i>Lasioglossum puncticolle</i>	2	8	100.0	37.5	<i>Centaurea nigra</i> (100.0)	<i>Centaurea nigra</i> (37.5)
<i>Lasioglossum villosulum</i>	25	32	34.8	21.9	<i>Leontodon hispidus</i> (34.8)	<i>Leontodon hispidus</i> (18.8), <i>Sonchus arvensis</i> (3.1)
<i>Lasioglossum xanthopus</i>	7	8	67.9	100.0	<i>Leucanthemum vulgare</i> (67.9)	<i>Leucanthemum vulgare</i> (100.0)
<i>Lasioglossum zonulum</i>	4	7	33.4	14.3	<i>Centaurea nigra</i> (20.6), <i>Silene dioica</i> (12.9)	<i>Centaurea nigra</i> (14.3)

The most important sown plants for solitary bees were *Leucanthemum vulgare*, *Centaurea nigra* and *Daucus carota*. The former two were widely used by polylectic *Lasioglossum* species with *D. carota* an important pollen source for scarce late summer species such as *Andrena alfkenella* and *A. minutuloides*. Plants from the family Fabaceae are often a major component of pollinator-focused agri-environment schemes, since many schemes were designed primarily to provide resources for threatened bumblebees that show close association with members of the Fabaceae (Edwards 2003; Goulson *et al.* 2005). These schemes can have significantly positive effects on the size of bumblebee populations on farmland (Carvell *et al.* 2015; Wood *et al.* 2015a), and whilst some threatened solitary bee species are dependent on Fabaceae (e.g. *Eucera longicornis*, Westrich 1989, not recorded in this study), overall use of Fabaceae as a pollen source by solitary bees was very low at 2.3% of pollen visitation observations and 3.0% of pollen collected by volume.

In contrast, an important pollen source for solitary bees in the spring was the genus *Brassica*. This predominantly comprises oilseed rape (*Brassica napus*), a major crop in England. *Brassica* species are also sometimes included in wild bird focused agri-environment schemes to provide winter seeds, but the area under this form of management is dwarfed by the area cropped with oilseed rape. *Brassica* type pollen was collected by 12 species of *Andrena*, in particular *A. scotica* (40.2% of total pollen collected by volume), *A. haemorrhoea* (23.1%), *A. minutula* (20.2%), *A. nitida* (19.1%), *A. cineraria* (15.7%) and *A. subopaca* (14.2%). Mass flowering crops, in particular oilseed rape, have been associated with higher densities of foraging bumblebees, larger bumblebee colonies and larger numbers of bumblebee colonies in agricultural environments, due to increased resources availability during early colony development (Westphal *et al.* 2003; Herrmann *et al.* 2007; Wood *et al.* 2015b). However, oilseed rape is widely treated with pesticides including neonicotinoids that have been linked to bee declines around the world (see Goulson *et al.* 2015). There are few field studies of impacts on solitary bees, but one study found reduced numbers of solitary bees foraging on neonicotinoid-treated oilseed rape and neighbouring field margins compared to controls, and also reduced occupancy of nest holes by *Osmia bicornis* (Rundlöf *et al.* 2015), so the net benefits of this mass flowering crop for solitary bees may depend upon its wider agricultural management.

The area of land cropped with oilseed rape in the UK has increased markedly in the past 30 years, from 269,000 ha in 1984 to a peak of 756,000 ha in 2012, driven mainly by rising commodity prices and demand for biofuels (DEFRA 2015). This increased availability of pollen and nectar resources during the period before agri-environment schemes begin to flower may

partly explain positive trends in some spring *Andrena* species in the UK. Widespread in the north and the west of the UK, *A. cineraria* had been scarce in the south of England until the 1990s when it markedly expanded its range (Baldock 2008). In addition, a number of scarce and threatened parasitic insects use spring flying mining bees such as *A. cineraria* as a host, specifically the oil beetle *Meloe proscarabaeus*, the bee fly *Bombylius discolor* (Nationally Scarce), the parasitic bee *Nomada lathburiana* (Red Data Book 3) and the conopid fly *Myopa pellucida* (Red Data Book 3). National recording efforts and county atlases show that these species have all increased in range and frequency since the 1990s, particularly in the south east of England (M. Edwards *pers. comm.*, Baldock 2008; Baldock and Early 2015), with the latter three species being recorded during this study.

Oilseed rape is an increasingly widely grown crop across much of the world and is the most important insect pollinated crop in arable areas of England. Whilst it can be wind pollinated, insect pollination increases seed yield, quality and market value (Bommarco *et al.* 2012). Solitary bees from the genera *Andrena*, *Osmia* and *Lasioglossum* are more efficient pollinators of oilseed rape than bumblebees and honeybees in Britain (Woodcock *et al.* 2013). However, current pollinator-focused schemes are dominated by herbaceous grassland plants which produce their peak flower abundance in July and August. These options provide low to non-existent resources in the spring, with none of the univoltine spring flying *Andrena* collecting more than 10% of their pollen from sown plants. Instead, visits to plants associated with woodland, hedgerow, grassland and arable weed habitats predominated. However, increasing floral abundance in the late summer is important, as pollen demand for larval rearing by social bumblebees is at its highest at this point (Dicks *et al.* 2015). Identifying bee species that are resource limited on farmland is an important part of targeted agri-environmental management, and it has been argued that current agricultural landscapes are particularly flower poor in the summer (Holland *et al.* 2015), with availability of suitable forage decreasing through the season (Scheper *et al.* 2014). The creation of 2% of predominantly summer flowering flower-rich habitat by farm area is sufficient to consistently supply a suite of common farmland bumblebee species with enough pollen to raise their larvae throughout the season (Dicks *et al.* 2015).

Current seed mixes biased towards summer flowering plants may consequently be an appropriate focus, but a change in composition to include more attractive species that flower in May and early June would support a greater variety of bee species, including important crop pollinators. Conservation management that takes land out of production can both benefit

biodiversity and increase agricultural yields, essentially making the intervention economically neutral (Pywell *et al.* 2015). However, as the majority of pollination is carried out by the most abundant bee species in any location, usually representing a small fraction of the total number of species present, a purely economic argument is insufficient to justify conserving bee diversity in general (Kleijn *et al.* 2015). In addition to bees being a valid conservation target in their own right, it has been argued that maintaining pollinator species diversity is crucial for providing ecosystem resilience in the face of future environmental change (Senapathi *et al.* 2015). Bee communities are strongly structured by, and associated with, plant species diversity (Tscharntke *et al.* 1998; Potts *et al.* 2003; Batáry *et al.* 2010), and so management that does not increase plant species diversity at the farm level is unlikely to increase bee diversity either, no matter the total increase in resource availability. Increasing the diversity of plants species included in wild seed mixes is likely to increase their efficacy (Scheper *et al.* 2015), and the inclusion of grassland species such as *Taraxacum* agg., *Hypochaeris radicata* and *Ranunculus repens* would provide resources for a wider variety of species. However, hedgerow plants such as *Heracleum sphondylium*, *Chaerophyllum temulum* and *Alliaria petiolata* and arable plants such as *Sinapis arvensis*, *Sisymbrium officinale* and *Tripleurospermum inodorum* are also popular, and their management relies on wider farmland management such as herbicide and hedge cutting regimes. Agri-environment schemes promoting improved hedgerow management and various uncropped or unharvested headlands for arable plants currently exist, but their ability to increase the abundance and diversity of pollen sources for solitary bees is poorly studied. These and similar schemes should be trialled as complementary methods to benefit solitary bee populations on farmland.

5.6 Conclusions

Current pollinator-focused agri-environment management can increase floral abundance and provide pollen resources for a limited suite of farmland bee species. However, in our study the majority of solitary bee species foraged from other plants persisting in the wider farm environment. As a taxon almost entirely dependent upon flowering plants for food, an association between bee species richness and flowering plant richness is to be expected. As the addition of flower-rich schemes as part of pollinator-friendly management did not significantly increase flowering plant richness at the farm scale, it should not be surprising that bee species richness was also not significantly different at this level. Management that increases resource availability is important, but our results suggest that techniques that

increase floristic richness at the farm scale are necessary if we wish to conserve a richer bee community on farmland.

Chapter 6 – Diet characterisation of solitary bees on farmland: dietary specialisation predicts rarity

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6.1 Abstract

Changes in agricultural practice across Europe and North America have been associated with declines in wild bee populations. Bee diet breadth has been associated with sensitivity to agricultural intensification, but much of this analysis has been conducted at the categorical level of generalist or specialist, and it is not clear to what extent the level of generalisation within generalist species is also associated with species persistence. We used pollen load analysis to quantify the pollen diets of wild solitary bees on 19 farms across Southern England, UK. A total of 72 species of solitary bees were recorded, but only 31 species were abundant enough to allow for formal diet characterisation. The results broadly conformed to existing literature with the majority of species polylectic and collecting pollen from a wide range of plants. Pollen load analysis consistently identified pollens from more plant species and families from each bee species than direct observation of their foraging behaviour. After rarefaction to standardise pollen load sample sizes, diet breadth was significantly associated with frequency of occurrence, with more generalist bees present on more farms than less generalist bees. Our results show that the majority of bee species present on farmland in reasonable numbers are widely variable in their pollen choices, but that those with the broadest diet were present on the greatest number of farms. Increasing the diversity of plants included in agri-environment schemes may be necessary to provide a wider range of pollen resources in order to support a diverse bee community on farmland.

6.2 Introduction

Since the Second World War, many wild bee species have undergone substantial range contractions and extinctions across Europe and North America (Biesmeijer *et al.* 2006; Kosior *et al.* 2007; Goulson *et al.* 2008b; Bartomeus *et al.* 2013). These declines have been linked to changes in agricultural practice which have reduced the abundance and diversity of flowering plants, reducing the amount and range of food resources available to foraging bees (Ollerton *et al.* 2014; Scheper *et al.* 2014; Goulson *et al.* 2015). A loss of bees from agricultural areas is of concern because of their important role as pollinators of both wild and crop plants (Ollerton *et al.* 2011; Garibaldi *et al.* 2013).

Partly to address these declines in wild bees, agri-environment schemes have been implemented across the European Union as part of the Common Agricultural Policy. Designed to deal with more general declines in agricultural biodiversity, they offer an opportunity to

provide important foraging resources for wild bees. However, there are still important knowledge gaps relating to the conservation of wild bees, in part relating to an incomplete understanding of their agro-ecology (Dicks *et al.* 2013). Previous authors have found that bee diet breadth is associated with sensitivity to habitat loss and agricultural intensification, with generalists faring better than specialists (Bommarco *et al.* 2010; De Palma *et al.* 2015). However, much of this analysis has been conducted at the categorical level of generalist or specialist, and it is not clear to what extent the level of generalisation within generalist species is also associated with species persistence. Providing appropriate foraging resources for wild bees on farmland is important if their populations are to be maintained, but the lack of data on their contemporary diets is detrimental as the assessment of the efficacy of current agri-environment schemes often has to work with an incomplete knowledge of bee species requirements (Dicks *et al.* 2015).

Whilst there has been reasonable assessment of the diet of bumblebees on and off agricultural land (Goulson *et al.* 2005; Carvell *et al.* 2006b; 2007; Kleijn and Raemakers 2008), less is known about the diet of the wider bee community (though see Scheper *et al.* 2014). In terms of the number of species, the bee fauna in temperate regions is dominated by ‘solitary’ bees that live independently and collect pollen to provision their own offspring. This term is used generically to contrast this group against the social bumblebees and the honeybee *Apis mellifera*. However, the ‘solitary’ bees include many species within the Halictidae that show variably expressed eusocial behaviour (Plateaux-Quénu 2008; Davison and Field 2016). Consequently, whilst not technically correct, the term solitary bees is used from here on to mean any non-parasitic (i.e. they forage for their own pollen), non-corbiculate (i.e. non-*Bombus*, non-*Apis*) bee species.

Some field studies have used direct field observations of pollen foraging behaviour to identify pollen preferences in wild bees (e.g. Minckley *et al.* 1999; Steffan-Dewenter and Tschantke 2001; Goulson *et al.* 2005). However, many wild bees are not flower constant and do not exclusively use the same flower species during a foraging trip, instead visiting and carrying pollen from many different flowering plants at once (Westrich 1989; Beil *et al.* 2008). Additionally, wild bees can forage over considerable distances (Beil *et al.* 2008; Zurbuchen *et al.* 2010) and may visit flowers inaccessible to or outside the areas visited by recorders. As a result, direct observations may not capture the full range of plant species visited for pollen, and may not accurately measure the relative contribution that different host plants make to the overall diet as the proportion of pollen collected from each particular plant species is

unknown. In establishing more definitive pollen preferences and diet breadth ranges, microscopic analysis of pollen loads has been favoured as the total volume of pollen collected from different sources can be quantified. The level of specialisation, as either oligolectic (collecting pollen from one plant family or genus) or polylectic (collecting pollen from many plant families) can then be made with greater confidence (Westrich 1989; Müller 1996; Müller and Kuhlmann 2008). The use of pollen load analysis is becoming more widely used in field studies to assess pollen utilisation of both semi-natural and agri-environment scheme plants (Carvell *et al.* 2006b; Kremen and Williams 2007; Beil *et al.* 2008) and to quantify historical bee diets through the analysis of remaining pollen loads present on museum specimens (Kleijn and Raemakers 2008; Scheper *et al.* 2014).

In this study we characterise the pollen foraging diets of wild solitary bees on farmland in Southern England, UK using pollen load analysis. Within a British context this is the most comprehensive assessment of solitary bee species since Chambers (1968). We examine the diet breadth results in the context of frequency of occurrence, with the prediction that those species with a wider diet will be present on a greater number of farms. This information will be of use to land managers and policy makers interested in maintaining pollinator populations on agricultural land.

6.3 Methods

6.3.1 Study area

In England, two tiers of environmental stewardship were established in 2005. Entry Level Stewardship (ELS, Natural England, 2013a) was open to all farmers. Higher Level Stewardship (HLS, Natural England, 2013b) which was targeted to high priority areas provided greater financial rewards for more substantial and rigorous agri-environmental schemes. At the time of the study, around 70% of farms in England were in some form of environmental stewardship (JNCC 2014). Nineteen farms were selected in Hampshire and West Sussex, UK. Nine farms were under HLS management and were implementing pollinator-friendly management. This consisted of an average of 5.6 ± 0.1 ha of flower-rich grassland per farm, typically established by sowing areas with seed mixes containing c.15-30 flowering forb species (Carvell *et al.* 2007). Ten farms were under ELS and were not implementing any pollinator-friendly management. ELS management can include schemes targeted at pollinators that can benefit wild bee

populations (Pywell *et al.* 2012), but the overall uptake of such schemes within ELS is low (Elliot *et al.* 2010). Consequently, general ELS farms not implementing any specifically pollinator-friendly management were selected as the control group for this study. The floral communities on the studied farms consequently consisted of sown plants in conservation areas and wild plants persisting in the wider farmed environment. Farms were predominantly arable, or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops representing the dominant agricultural practices in this region.

6.3.2 Bee surveys and sample collection

In 2013 and 2014, a standardised 3 km transect was designed for each farm, passing through all major habitat types present, excluding crops. These habitats types can be divided into flower-rich pollinator-focused schemes, non-agricultural grassland and hedgerows and woodland edges. Transects on HLS farms were designed to survey as many pollinator-focused schemes as possible and passed through an average of 1496 ± 148 m of flower-rich habitat in an average of 3.8 ± 0.2 discrete habitat patches. Solitary bee activity was recorded along the transect following standard bee walk methodology (Carvell *et al.* 2007), with all solitary bees within 2 m of the recorder identified to species level. Individuals that could not be named in the field were netted for later identification. The first flowering plant species visited and the purpose of the visit, for either pollen or nectar, was recorded. *Hylaeus* species, which lack scopal (pollen collecting) hairs on their body, instead ingesting pollen and regurgitating it in the nest, cannot be reliably determined to be foraging for pollen and so all plant visits were recorded simply as visits. Sixteen farms (eight HLS, eight ELS) were surveyed in 2013. Transects were walked three times through the season, between 25th May–5th June, 26th June–15th July and 3rd–11th August 2013. Seventeen farms (eight HLS, nine ELS) were surveyed in 2014. Transects were walked three times through the season, between 17th–27th May, 21st June–9th July and 3rd–15th August 2014.

In 2015 farms were surveyed on time based rather than distance based transects. ELS farms were surveyed for 3 hrs with 1.5 hrs on non-agricultural grass habitats and 1.5 hrs on woody hedgerow/woodland edge habitats. HLS farms were surveyed for 3 hrs with 1 hr on pollinator-focused flower-rich schemes, 1 hr on non-agricultural grass habitats and 1 hr on woody hedgerow/woodland edge habitats. The survey followed standard bee walk methodology as described above, but at a reduced pace to ensure thorough sampling. In addition, female bees with clearly visible pollen on their body were collected, placed in individual Eppendorf tubes

and frozen. Samples of all flowering plant species present were collected to form a pollen reference library. Fourteen farms (7 HLS, 7 ELS) were surveyed in 2015. Transects were walked four times throughout the season, between 22 April– 13th May, 26th May– 17th June, 25th June– 4th July and 29th July– 10th August 2015. All bee surveys were conducted between 0930 and 1700 h when the temperature was above 13°C with at least 60% clear sky, or above 17°C with any level of cloud. No surveys were conducted when it was raining. All bee surveys were conducted by the same individual (TJW) to minimise recorder bias.

6.3.4 Pollen identification

The scopal pollen load of foraging bees collected in 2015 was analysed by light microscopy using the method outlined by Westrich and Schmidt (1986). Before removing pollen from the scopae, the total load was estimated relative to a full load for that species, ranging from 8/8 (full load) to 1/8 (one eighth load). The pollen grains were removed from the scopae using an entomological pin and transferred to a drop of water on a microscope slide. Pollen that was not clearly held in the scopae was not sampled as this may have become attached to other parts of the body during nectar visits to non-host plant flowers. The slide was gently heated to allow grains to absorb water and achieve their maximum size and to evaporate excess water. Molten glycerine jelly stained with fuchsin was then added and the slide was sealed with a coverslip. The proportion of the load comprised of different plant species was estimated along three randomly selected lines across the cover slip at a magnification of x400. The proportion of the load by volume was estimated by the relative area of the slide occupied by each plant species, rather than the absolute number of grains, in order to better reflect the total volume of pollen collected, an important correction in mixed loads where pollen grains of different plant species often differ widely in size (Cane and Sipes, 2006). Species representing less than 1% of the load were excluded from further analysis as they may have arisen from contamination (Westrich and Schmidt 1986). As specimens were individually netted and stored in clean Eppendorf tubes such contamination was expected to be minimal.

The proportions of pollen collected were corrected according to the overall size of each load to give a final weight, e.g. a full load (8/8) comprised of 50% *Centaurea nigra* and 50% *Leucanthemum vulgare* would receive a final *C. nigra* weight of 50 and a final *L. vulgare* weight of 50, whereas a quarter load (2/8) comprised of 100% *Hypochaeris radicata* would receive a final *H. radicata* weight of 25. The pollen grains were identified to species using Sawyer (1981) and the reference collection assembled during the project. Where identification to species

level was not possible, pollen was identified to genus, for example in *Brassica*, *Plantago* and *Geranium*.

6.3.5 Diet characterisation

Characterisation of floral preferences in pollen host plants was carried out for species with a minimum of three pollen load samples following Müller and Kuhlmann (2008). A small sample size may result in mischaracterisation of bee diets as certain plant families may be under or overrepresented. However, in all cases for bees with sample sizes of less than five the results conformed closely to more extensive previous studies (see Results). Consequently, for species with small sample sizes the results presented here should be viewed within this broader context. In characterising diet we used the categories laid out by Müller and Kuhlmann (2008) who modified the work of Cane and Sipes (2006) to include additional subcategories of oligolecty and polylecty. This modification added the category 'polylectic with a strong preference' as this pattern of host use exists in many species (Müller 1996). Müller and Kuhlmann (2008) used two approaches to characterise oligolecty for a given bee species using microscopic analysis of pollen loads. The first averages use over all individuals so a bee species is deemed oligolectic if 95% of the pollen grains (or 95% of the pollen by volume) is from one plant family or genus (Müller 1996). The second approach looks at the proportion of pure and mixed pollen loads so a bee species is deemed oligolectic if 90% of females collect pure pollen loads of one plant family or genus (Sipes and Tepedino 2005). Both methods produce similar results (Müller and Kuhlmann 2008), but in the few cases where they produced different answers the category with the lower degree of specialisation was used in our analysis.

Flower visit observations from the 2013, 2014 and 2015 transects were pooled. Differences in the number of plant species and families utilised for pollen detected by the direct observation and pollen load analysis techniques were tested using Mann Whitney U tests. When comparing diet breadth between different bee species, rarefaction must be used to reduce the impact of differing samples sizes between species and the consequent effect on diet breadth calculations (Williams 2005). A rarefaction procedure was used to calculate the number of pollen types from different plant species (pollens) each different bee species would be expected to collect for a standardised number of pollen loads. Here we rarefied the diets of bees for i) species with a minimum of 10 pollen loads and ii) species with a minimum of three pollen loads. A subsample of i) 12 pollen loads (smallest sample size over 10, *Andrena subopaca* see Table 6.1) and ii) three pollen loads is made from the observed frequency of pollens collected, chosen at

random without replacement 1000 times. As this procedure is designed for use on integer data, the pollen load data was first transformed. For example, with a sample size of 14, the percentage of pollen collected from each plant species was multiplied by the sample size to give a whole pollen load equivalent, e.g. 40% becomes 5.6 pollen loads. These values were all multiplied by 10 and rounded to the nearest whole number to give an integer equivalent that was used in the rarefaction procedure. For the group with a minimum sample size of 10 pollen loads the relationship between diet breadth (the number of pollens collected) and frequency of occurrence (the number of farms at which the bee species was recorded at least once over the three year survey period) was tested using a linear model with a Gaussian distribution as the response variable conformed to a normal distribution. Bee family was included in the model as a fixed factor to control for the possible impact of bee phylogeny on the results. For the group with a minimum sample size of three pollen loads the response variable could not be transformed to normality, and for this dataset the relationship between diet breadth and frequency of occurrence was tested using Spearman's rank correlation.

Additionally, we also investigated the impact of farm type (HLS or ELS) on diet breadth and frequency of occurrence to ensure that the effect was consistent across different management types. Fourteen bee species had a minimum of 10 pollen loads from either HLS or ELS farms (9 species had a minimum of 10 pollen loads from both farm types, 5 species has a minimum of 10 pollen loads from only one farm type). Following the same protocol these data were rarefied to a sample size of 10 pollen loads and were tested in a linear model with a Gaussian distribution with diet breadth and farm management type included as fixed factors. All statistical analyses were conducted in R version 3.1.1 (R Development Core Team) using the package *vegan* (Oksanen *et al.* 2015) to calculate diet rarefaction scores.

6.4 Results

A total of 72 species of solitary bee was recorded over the sampling period. Fifty-six solitary bee species were observed making 1,416 pollen foraging trips to 62 flowering plant species from 19 families. One thousand and fifty-four bees with pollen loads from 47 solitary bee species were collected for microscopic pollen load analysis. Pollen analysis detected 93 pollen types from 32 plant families. Thirty-one solitary bee species were collected in sufficient quantities to allow diet breadth characterisation (Table 6.1).

Table 6.1. Host plant spectrum and inferred category of host use in sampled farmland solitary bee species. Only species with a minimum of three collected pollen loads are included. *n*, total number of pollen loads; *N*, number of pollen loads from different localities. s.s. = sensu strictu. Plant taxa: ACE, Aceraceae; API, Apiaceae; AST, Asteraceae; BER, Berberidaceae; BRA, Brassicaceae; CAM, Campanulaceae; CAP, Caprifoliaceae; CAR, Caryophyllaceae; CUC, Cucurbitaceae; FAB, Fabaceae; GER, Geraniaceae; LAM, Lamiaceae; LIL, Lilaceae; MAL, Malvaceae; OLE, Olaceae; ORO, Orobanchaceae; RES, Resedaceae; RAN, Ranunculaceae; RHA, Rhamnaceae; ROS, Rosaceae; RUB, Rubiaceae; SOL, Solanaceae; VER, Veronicaceae. * pollen data only available for summer generation females.

Bee species	<i>n</i>	<i>N</i>	Results of microscopic analysis of pollen grains (% pollen grains)	% pure loads of preferred host	% loads with preferred host	Host range	Host range in the literature
<i>Andrena alfkenella</i>	6	3	API 97.0, other 3.0	33.3	100.0	Broadly oligolectic (Apiaceae) *	Polylectic
<i>Andrena bicolor</i>	16	7	AST 29.6, BRA 21.4, CUC 13.7, API 13.7, CAM 10.5, LIL 7.8, other 2.0	37.5	37.5	Polylectic s.s.	Polylectic
<i>Andrena chrysosceles</i>	32	9	API 45.6, BRA 32.1, ROS 14.9, MAL 3.1, AST 2.2, other 2.1	43.8	78.1	Polylectic s.s.	Polylectic
<i>Andrena cineraria</i>	9	3	BRA 53.8, API 27.9, ROS 12.9, RHA 3.2, other 2.2	33.3	77.8	Polylectic s.s.	Polylectic
<i>Andrena dorsata</i>	22	9	ROS 66.6, API 11.8, BRA 9.7, AST 7.3, FAB 4.7	50.0	68.2	Polylectic s.s.	Polylectic
<i>Andrena flavipes</i>	45	10	AST 56.0, BRA 20.1, FAB 10.5, ROS 5.1, API 5.0, other 3.3	15.6	86.7	Polylectic s.s.	Polylectic
<i>Andrena florea</i>	3	2	CUC 100.0	100.0	100.0	Narrowly oligolectic (<i>Bryonia dioica</i>)	Narrowly oligolectic (<i>Bryonia</i>)
<i>Andrena haemorrhoa</i>	40	12	ROS 37.8, BRA 31.5, AST 14.7, RES 6.3, CAP 4.9, other 3.0	10.0	45.0	Polylectic s.s.	Polylectic
<i>Andrena labiata</i>	3	1	CAR 50.0, VER 16.7, RAN 12.5, BRA 10.8, AST 7.5, GER 2.5	0.0	66.7	Polylectic s.s.	Polylectic
<i>Andrena minutula</i>	15	8	API 56.4, BRA 23.6, ROS 11.6, AST 8.0, other 0.4	20.0	60.0	Polylectic s.s.	Polylectic
<i>Andrena minutuloides</i>	8	2	API 100.0	100.0	100.0	Broadly oligolectic (Apiaceae) *	Polylectic
<i>Andrena nigroaenea</i>	16	6	API 39.4, AST 34.5, BRA 14.2, RAN 6.3, RES 3.1, other 2.5	18.8	62.5	Polylectic s.s.	Polylectic
<i>Andrena nitida</i>	23	10	BRA 45.6, API 16.3, ACE 9.9, RHA 9.1, AST 4.7, SOL 3.8, LAM 3.8, ROS 2.4, other 4.5	21.7	69.6	Polylectic s.s.	Polylectic
<i>Andrena scotica</i>	18	7	BRA 65.7, ACE 19.6, API 5.4, ROS 4.2, other 5.1	55.6	77.8	Polylectic s.s.	Polylectic
<i>Andrena semilaevis</i>	97	9	API 91.3, BRA 5.3, VER 1.9, other 1.5	75.3	97.9	Polylectic with a strong preference (Apiaceae)	Polylectic
<i>Andrena subopaca</i>	12	4	API 48.4, BRA 19.3, ROS 18.2, VER 9.2, FAB 2.6, other 2.3	33.3	50.0	Polylectic s.s.	Polylectic
<i>Halictus tumulorum</i>	21	10	AST 24.2, RAN 22.2, FAB 16.9, ROS 13.9, BRA 12.4, RUB 6.82, LIL 3.0, other 2.62	38.1	71.4	Polylectic s.s.	Polylectic
<i>Lasioglossum albipes</i>	3	2	RAN 65.3, ROS 33.3, other 1.3	33.3	66.7	Polylectic s.s.	Polylectic
<i>Lasioglossum calceatum</i>	38	11	AST 46.9, BRA 17.5, ROS 11.3, API 6.0, LAM 3.9, BER 3.7, OLE 3.5, RAN 2.6, other 4.6	42.1	68.4	Polylectic s.s.	Polylectic
<i>Lasioglossum fulvicorne</i>	7	2	BRA 67.2, ROS 26.9, API 5.0, other 0.8	57.1	71.4	Polylectic s.s.	Polylectic
<i>Lasioglossum lativentre</i>	9	3	FAB 95.3, RAN 2.6, AST 2.1	77.8	88.9	Polylectic with a strong preference (Fabaceae)	Polylectic
<i>Lasioglossum leucopus</i>	7	6	BRA 68.1, API 22.9, RAN 6.3, AST 2.5, other 0.3	0.0	57.1	Polylectic s.s.	Polylectic
<i>Lasioglossum leucozonium</i>	21	6	AST 95.3, RAN 4.7	71.4	100.0	Polylectic with a strong preference (Asteraceae)	Polylectic
<i>Lasioglossum malachurum</i>	437	12	AST 73.1, BRA 9.9, ROS 3.3, other 13.7	53.3	83.3	Polylectic with a strong preference (Asteraceae)	Polylectic
<i>Lasioglossum morio</i>	7	5	BRA 51.2, AST 15.1, API 12.1, CAP 11.2, ROS 8.4, other 2.1	28.6	42.9	Polylectic s.s.	Polylectic
<i>Lasioglossum parvulum</i>	7	5	ROS 28.5, LAM 23.5, RAN 20.2, API 11.2, ACE 9.4, AST 5.9, other 1.3	0.0	42.9	Polylectic s.s.	Polylectic
<i>Lasioglossum pauxillum</i>	70	10	AST 62.1, API 9.8, RAN 9.4, ROS 8.7, BRA 3.5, VER 2.3, FAB 2.2, other 2.0	45.7	68.6	Polylectic s.s.	Polylectic
<i>Lasioglossum villosulum</i>	25	5	AST 100.0	100.0	100.0	Broadly oligolectic (Asteraceae)	Polylectic
<i>Lasioglossum xanthopus</i>	7	3	AST 67.8, BRA 32.0, other 0.2	42.9	85.7	Mesolectic	Polylectic
<i>Lasioglossum zonulum</i>	4	1	ROS 53.6, AST 21.6, CAR 12.9, RAN 6.1, CAP 4.3, other 1.4	0.0	100.0	Polylectic s.s.	Polylectic
<i>Melitta tricincta</i>	3	1	ORO 100.0	100.0	100.0	Narrowly oligolectic (<i>Odontites vernus</i>)	Narrowly oligolectic (<i>Odontites</i>)

The host plant use results broadly conformed to existing literature (Chambers 1968; Westrich 1989; Amiet *et al.* 2010), with the majority of species characterised as polylectic or polylectic with some preferences. Five species (*Andrena alfkenella*, *A. florea*, *A. minutuloides*, *Lasioglossum villosulum* and *Melitta tricincta*) were characterised as oligolectic. However, *A. alfkenella*, *A. minutuloides* and *L. villosulum* are not considered oligolectic by other authors (see Discussion). Excluding these three doubtful species a total of 15 solitary bee species well known to be oligolectic was recorded (Table 6.2). Only *A. florea* and *M. tricincta* were collected in sufficient numbers to allow formal diet characterisation. For the other 13 oligolectic species, observed pollen visits were in line with their expected host preferences. Important pollen sources in the study region are summarised in Table 6.2, with likely pollen host plants based on observed nectar visits to suitable plants present at the localities where they were recorded.

Table 6.2. Oligolectic solitary bee species recorded during the survey and their observed pollen host plants in the study area. Likely pollen sources are based on nectar visits to suitable host plants present at the locality. Plants sown as part of pollinator-friendly management are highlighted in bold.

Bee species	Pollen sources in the study area	Host range in the literature
<i>Andrena florea</i>	<i>Bryonia dioica</i>	Narrowly oligolectic (<i>Bryonia</i>)
<i>Andrena fulvago</i>	none recorded (likely <i>Hypochaeris radicata</i>)	Broadly oligolectic (Asteraceae)
<i>Andrena humilis</i>	<i>Hypochaeris radicata</i>	Broadly oligolectic (Asteraceae)
<i>Andrena nitidiuscula</i>	none recorded (likely <i>Heracleum sphondylium</i> , <i>Daucus carota</i>)	Broadly oligolectic (Apiaceae)
<i>Andrena wilkella</i>	<i>Trifolium repens</i> , <i>Trifolium hybridum</i> , <i>Lotus corniculatus</i>	Broadly oligolectic (Fabaceae)
<i>Anthophora furcata</i>	<i>Stachys sylvatica</i>	Broadly oligolectic (Lamiaceae)
<i>Chelostoma campanularum</i>	none recorded (likely <i>Campanula trachelium</i>)	Narrowly oligolectic (<i>Campanula</i>)
<i>Chelostoma florisomne</i>	<i>Ranunculus repens</i>	Narrowly oligolectic (<i>Ranunculus</i>)
<i>Colletes daviesanus</i>	<i>Achillea millefolium</i> , <i>Tripleurospermum inodorum</i> , <i>Leucanthemum vulgare</i>	Broadly oligolectic (Asteraceae)
<i>Hylaeus signatus</i>	<i>Reseda lutea</i>	Narrowly oligolectic (<i>Reseda</i>)
<i>Melitta leporina</i>	<i>Trifolium repens</i>	Broadly oligolectic (Fabaceae)
<i>Melitta tricincta</i>	<i>Odontites vernus</i>	Narrowly oligolectic (<i>Odontites</i>)
<i>Osmia leaiana</i>	<i>Centaurea nigra</i> , <i>Crepis capillaris</i>	Broadly oligolectic (Asteraceae)
<i>Osmia spinulosa</i>	<i>Achillea millefolium</i> , <i>Helminthotheca echioides</i>	Broadly oligolectic (Asteraceae)
<i>Panurgus calcaratus</i>	none recorded (likely <i>Hypochaeris radicata</i>)	Broadly oligolectic (Asteraceae)

Excluding the narrowly oligolectic *A. florea* and *M. tricincta* (that each only collect pollen from one plant species in Britain), direct observation recorded bees collecting pollen from an average of 6.9 ± 1.0 plant species from an average of 3.4 ± 0.3 plant families per bee species (Table 6.3). Microscopic pollen analysis detected significantly more pollens from an average of 13.6 ± 1.9 plant species from an average of 7.6 ± 0.9 plant families per bee species ($W=211.5$, $p=0.001$; $W=151.5$, $p<0.001$ respectively). Of the 13 additional plant families detected in pollen load analysis, seven were represented by woody genera in the study area, specifically Aceraceae (*Acer*), Aquifoliaceae (*Ilex*), Berberidaceae (*Berberis*), Cornaceae (*Cornus*), Fagaceae (*Castanea*, *Fagus*), Malvaceae (*Tilia*) and Rhamnaceae (*Rhamnus*). For the 17 species with a minimum sample size of 10 analysed pollen loads, after rarefaction there was a significant

relationship between diet breadth and frequency of occurrence (Figure 6.1, $t_{14,16}=3.411$, $p=0.004$, adjusted $R^2=0.413$). There was no impact of bee family on this relationship ($t_{14,16}=0.186$, $p=0.855$). After removing bee family from the model diet breadth was still a significant predictor of frequency of occurrence ($t_{15,16}=3.756$, $p=0.002$, adjusted $R^2=0.450$).

Table 6.3. Number of pollens from different flowering plant species and flowering plant families collected by solitary bee species (excluding narrowly oligolectic species), by direct observation and pollen load analysis. Data was rarefied for species with a minimum of 10 pollen loads. *obs*, number of observations; *n*, number of pollen loads.

Bee species	<i>obs</i>	<i>n</i>	Number of plant species			Number of plant families	
			Observations	Pollen loads	Rarefied	Observations	Pollen loads
<i>Andrena alfenella</i>	10	6	2	7		1	5
<i>Andrena bicolor</i>	17	16	5	11	9.61	4	9
<i>Andrena chrysosceles</i>	38	32	7	16	13.40	4	8
<i>Andrena cineraria</i>	16	9	6	9		3	6
<i>Andrena dorsata</i>	34	22	9	15	10.30	5	5
<i>Andrena flavipes</i>	70	45	14	28	18.06	4	12
<i>Andrena haemorrhoa</i>	43	40	6	18	11.03	5	11
<i>Andrena labiata</i>	2	3	1	6		1	6
<i>Andrena minutula</i>	30	15	9	11	9.78	3	5
<i>Andrena minutuloides</i>	10	8	2	2		1	1
<i>Andrena nigroaenea</i>	13	16	5	16	11.07	4	10
<i>Andrena nitida</i>	24	23	7	16	14.14	5	12
<i>Andrena scotica</i>	13	18	4	12	11.24	3	9
<i>Andrena semilaevis</i>	127	97	7	18	10.33	3	9
<i>Andrena subopaca</i>	7	12	3	13	11.00	3	8
<i>Halictus tumulorum</i>	23	21	14	19	16.14	5	11
<i>Lasioglossum albipes</i>	3	3	2	4		2	4
<i>Lasioglossum calceatum</i>	70	38	17	31	19.76	7	16
<i>Lasioglossum fulvicorne</i>	6	7	3	4		3	4
<i>Lasioglossum lativentre</i>	8	9	3	5		2	3
<i>Lasioglossum leucopus</i>	5	7	2	8		2	5
<i>Lasioglossum leucozonium</i>	51	21	9	8	7.56	2	2
<i>Lasioglossum malachurum</i>	553	437	22	50	22.98	8	22
<i>Lasioglossum morio</i>	6	7	6	11		5	7
<i>Lasioglossum parvulum</i>	6	7	4	9		4	7
<i>Lasioglossum pauxillum</i>	93	70	17	28	18.09	4	12
<i>Lasioglossum villosulum</i>	32	25	7	5	3.93	1	1
<i>Lasioglossum xanthopus</i>	8	7	1	5		1	4
<i>Lasioglossum zonulum</i>	7	4	5	9		4	6
Average			6.9 ± 1.0	13.6 ± 1.9	12.9 ± 1.2	3.4 ± 0.3	7.6 ± 0.9

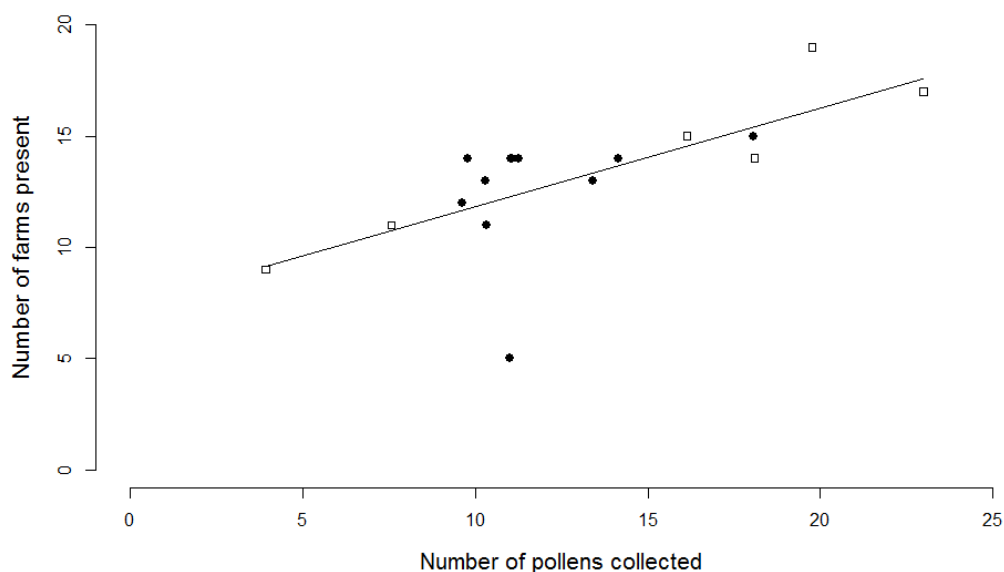


Figure 6.1. The relationship between diet breadth of solitary bee species ($n=17$) after rarefaction (to a standardised sample size of 12 pollen loads) and the number of farms each bee species was recorded on. Circles = bee species from the family Andrenidae, Squares = bee species from the family Halictidae.

The same relationship was found if the analysis is repeated for all 31 species with a minimum of 3 analysed pollen loads (Spearman's $\rho=0.794$, $p<0.001$). There was no impact of farm management type on the relationship between diet breadth and frequency of occurrence ($t_{20,22}=0.616$, $p=0.545$) with diet breadth remaining significant with ($t_{20,22}=2.384$, $p=0.027$, adjusted $R^2=0.150$) and without ($t_{21,22}=2.379$, $p=0.027$, $R^2=0.175$) the inclusion of this term in the model.

6.5 Discussion

Amongst the solitary bee species found on contemporary farmland in Southern England, the majority of common species are polylectic and forage from a wide range of flowering plants. Whilst overall bee diversity was high, representing almost half the regional total (Baldock 2008), most of the generalist solitary bee species and almost all the specialised oligolectic solitary bee species were too scarce to allow formal diet characterisation. Within characterised bees there was a strong relationship between diet breadth and frequency of occurrence, with more generalist species found on a greater number of farms. Whilst earlier studies have shown that generalist bees are less sensitive to agricultural intensification (Bommarco *et al.* 2010; De

Palma *et al.* 2015), the level of generalisation has not previously been shown to be a good predictor of frequency of occurrence. As more specialised bees are *de facto* less flexible in their dietary choices, the loss of floristic diversity resulting from agricultural intensification is likely to be the main driver behind their range declines over the past century (Scheper *et al.* 2014; Ollerton *et al.* 2014). Since bee species richness is strongly associated with plant species richness (Potts *et al.* 2003; Wood *et al.* 2015c), the effectiveness of agri-environment schemes in providing resources for a wider variety of bee species is likely to be enhanced by increasing the number of flowering plant species in seed mixes (Scheper *et al.* 2015). For oligolectic species, only five out of 14 species were recorded collecting pollen from plant species currently sown as part of agri-environment schemes (Table 6.2). The addition of a wider range of species such as *H. radicata* to these mixes would provide resources for a wider variety of specialised bee species. However, increasing floristic diversity is not a straightforward process as sown species do not always develop or persist depending on the local soil type, the plant establishment method, competition between sown and unsown plants and subsequent management (Pywell *et al.* 2011b). Many of these important plant species are associated with low intensity grassland and hedgerow habitats, so complementary techniques that maintain and improve floristic richness in long term habitats may also be effective.

Pollen load analysis provided a more complete description of solitary bee pollen diets than direct observation, consistently detecting pollens from a greater number of plant families across all bee species. In particular, this analysis identified plant families represented by woody plant genera whose flowers are often located well above the height of the surveyor. Due to this spatial structuring, these woody genera are consequently under-recorded as pollen sources by direct observations, and as a result their importance to bees may be widely underestimated. Beil *et al.* (2008) produced similar findings with a small number of bees collected on low growing herbaceous plants found to be carrying pollen from woody plant genera. In some cases in this study the nearest trees of this type were located over 1000 metres away from the collection point. There is a positive relationship between bee body size and foraging distance, with small bees predicted to have a maximum foraging distance of only a few hundred metres (Greenleaf *et al.* 2007). However, detailed study of experimental solitary bee populations confirms that whilst the majority of individuals do only forage over distances of a few hundred metres, a small proportion are able to successfully forage over 1000 metres from their nest, even in small bees such as *Hylaeus* (Zurbuchen *et al.* 2010). Since at least a proportion of solitary bee individuals are capable of making long pollen foraging trips

of over 1000 metres, the importance of flowering plants that may not be immediately apparent in the sampling location and their contribution to the diet of farmland bees should be considered, further emphasising the importance of pollen load analysis for building a more complete picture of solitary bee diets.

Whilst host plant use for studied species broadly conformed to the literature, three polylectic species were characterised here as oligolectic. All analysed pollen loads of *Lasioglossum villosulum* were comprised of Asteraceae pollen, 98.2% from the 'hawkish' Asteraceae genera *Hypochaeris* (49.3%), *Leontodon* (34.8%) and *Crepis* (14.0%). This would clearly suggest broad oligolecty under the conditions laid out by Müller and Kuhlmann (2008), but *L. villosulum* is known to be at least occasionally polylectic, for example in the Mediterranean collecting pollen from *Ecballium elaterium*, a member of the Cucurbitaceae (Rust *et al.* 2003). At least in Britain, *L. villosulum* should probably be considered an oligolectic species, or a polylectic species with an extremely strong preference for Asteraceae. Both *A. alfkenella* and *A. minutuloides* were characterised as oligolectic on Apiaceae, though only summer generation females were collected. Both species are bivoltine in mainland Europe (Amiet *et al.* 2010), but in Britain it has been noted for a long time that both species are markedly less common in the spring generation (Perkins 1919; Baldock 2008). In the present study no spring generation males or females were recorded. Both species are considered polylectic as the spring generation forages from various plant families but the summer generation shows a strong preference for Apiaceae (Perkins 1919; Westrich 1989; Amiet *et al.* 2010). Because of the lack of spring females, neither species was considered to be oligolectic in this study.

The basal clades of most bee families include a high proportion of oligolects (Westrich 1989; Wcislo and Cane 1996) and it has been argued that oligolecty is the basal state in bees with polylecty being a derived state with multiple origins (Müller 1996; Danforth *et al.* 2013). One of the suggested mechanisms by which oligolecty is maintained is that plants may chemically protect their pollen to prevent overexploitation, thus necessitating specialisation to process difficult metabolites (Praz *et al.* 2008). Asteraceae pollen is known to have a low protein content (Roulston *et al.* 2000; Hanley *et al.* 2008), is lacking in essential amino acids (Wille *et al.* 1985) and may possess a toxic pollenkitt, the oily liquid found on the surface of the pollen grain (Williams 2003). Consequently Asteraceae pollen is difficult to utilize by non-specialised bees, even in widely polylectic species such as the honey bee (Herbert *et al.* 1970) and solitary bees such as *Osmia lignaria* (Williams 2003). Even bees specialised on Asteraceae pollen may

incur other costs such as extended development time, as in a comparison of specialised bees the Asteraceae oligolec *Heriades truncorum* had the longest development time on its preferred pollen despite being the smallest bee in the comparison (Praz *et al.* 2008). In reviewing host plant use and diet breadth in 60 species of Western Palearctic *Colletes*, Müller and Kuhlmann (2008) found that 12 species collected pollen exclusively from Asteraceae with a further two showing a strong preference. However, amongst widely polylectic species Asteraceae pollen played a very marginal role, with pollen loads from 27 species not containing Asteraceae pollen at all. This striking difference, suggesting a high degree of specialisation or almost total avoidance, is referred to as the Asteraceae paradox.

Interestingly, in the present study we found that four clearly polylectic solitary bee species collected a substantial proportion of their pollen from Asteraceae in the study area, these species being *Andrena flavipes* (56.0%), *Lasioglossum calceatum* (46.9%), *L. malachurum* (73.1%) and *L. pauxillum* (62.1%). The three *Lasioglossum* species also collected small amounts of *Ranunculus* pollen, a genus known to have pollen toxic to insects (Jürgen and Dötterl 2004). Additionally, after rarefaction, these four species had the widest diet of any of the characterised species and were each present on at least three-quarters of all surveyed farms. That these broad polylects have developed the physiological mechanisms to digest a range of difficult pollens may be related to their long flight periods. *A. flavipes* is bivoltine, flying in the spring and again in the summer in discrete generations. *L. malachurum* and *L. pauxillum* are obligately primitively eusocial (Plateaux-Quénu 2008) and *L. calceatum* is facultatively eusocial with the eusocial phenotype dominating in the south of England (Davison and Field 2016). Producing two generations in a season, or in the case of social *Lasioglossum*, a worker and a reproductive generation, necessitates the ability to collect and digest pollen collected over a long flowering season from April to September. In contrast to these effectively bivoltine species, the Western Palearctic *Colletes* are almost always univoltine and the resultant shorter flight season and temporally limited resource competition may favour an all or nothing investment in the physiological capacity to process Asteraceae pollen. Indeed, xeric environments with short flowering seasons tend to have bee faunas with a greater degree of pollen specialisation (Minckley and Roulston 2006; Michener 2007). A more thorough understanding of the physiological mechanisms used by both specialised and generalist bees to detoxify and digest chemically protected pollen is necessary to better explain the different strategies pursued by foraging bees (Praz *et al.* 2008).

6.6 Conclusions

The majority of solitary bees persisting on farmland in reasonable numbers are polylectic and make use of a wide variety of flowering plants. However, the level of generalisation is important, with the species with the widest diet breadth being found on the greatest number of farms. The ability to digest pollens from a large number of plant species is one reason that these more generalised bees are better able to deal with a wider variety of agricultural landscapes than bees with a narrower diet. Given that current agri-environment schemes targeted at pollinators do not result in an increase in either floristic or bee species richness at the farm scale (Wood et al. 2015c), if the aim of agri-environment schemes is to support a diverse community of farmland bees this will require a change in design to provide more appropriate foraging resources for more specialised bee species. This may rely on increasing the number of flowering plant species than are currently included in agri-environment schemes for pollinators.

Chapter 7 – General discussion and synthesis

7.1 Main findings

At the start of this thesis in January 2013, although much work had been conducted on the observable response of foraging bumblebees to agri-environment schemes, there was a lack of evidence as to whether or not these schemes significantly increased bumblebee population size. Because of the aggregative foraging behaviour seen by worker bumblebees, whereby large numbers of bees are attracted to patches of high density rewards, assessing whether high observed abundance was reflective of a genuinely larger population size or simply redistribution in the landscape was not straightforward. The development of bumblebee genetic profiling techniques over the past 20 years has enabled this question to be answered empirically and in the affirmative for three of the four common bumblebee species studied.

Even though there had not been a definitive demonstration of the effect of agri-environment schemes on bumblebee population size, bumblebees had generally received a lot of attention and study from the 1990s onwards as concern over the extent of their declines grew. As a result, their foraging ecology both on farmland and in the wider environment is generally well understood for most species. In contrast, whilst general pollen preferences have been established for many Central European solitary bee species with publications such as Westrich (1989), Müller (1996), Müller and Kuhlmann (2008) and Sedivy *et al.* (2013), there was a lack of data on solitary bee pollen use on agricultural land, their use of agri-environment scheme plants for pollen and whether or not the schemes significantly increase the diversity of the overall bee community. Moreover, there was a lack of knowledge of the British species in particular, with the last published work on pollen use by British solitary bees published by Chambers (1968), from data collected in the 1940s. The data presented here demonstrate that current schemes only provide appropriate pollen resources for a minority of solitary bee species, with most species collecting most of their pollen from plants persisting unaided in the wider environment. In addition, the diversity of the bee community is no different between farms with and without pollinator-friendly schemes, suggesting that the schemes are not effective at increasing bee diversity.

7.2 The response of bumblebees to agri-environment schemes – are all species equal?

The causes of decline of British bumblebees specifically and worldwide bumblebees more generally has been extensively discussed and debated by previous authors. Relative diet breadth and loss of key forage plants (Goulson *et al.* 2005; Goulson *et al.* 2008a; Kleijn and Raemakers 2008), time of initiation of the colony cycle and climatic niche breadth (Williams 2005; Williams and Osborne 2009) have all been implicated in their differing fortunes over the 20th and early 21st centuries. The work presented here has shown that by increasing food resources, current agri-environment schemes help three of the common farmland bumblebees, *B. lapidarius*, *B. hortorum*, and *B. terrestris*, but not *B. pascuorum*, despite the suitability of sown resources for this latter species. The relative response of bumblebee species can be explained by their differing ecology and life history traits as well as their diet breadth.

The most declined bumblebees of lowland England belonged to the following subgenera: *B. humilis*, *B. sylvarum*, *B. muscorum*, *B. ruderarius* (*Thoracobombus*), *B. soroeensis* (*Kallobombus*), *B. subterraneus*, *B. distinguendus* (*Subterraneobombus*, both extinct) and *B. ruderatus* (*Megabombus*) (Williams 1986; 2005; Goulson *et al.* 2008). The example of *B. ruderatus* is particularly interesting and illustrative of the variable trials and tribulations of British bumblebees over the past 70 years. After declining in the post-war period, by the 1990s it was largely restricted to river valleys in the Midlands and East Anglia where it predominantly foraged on yellow iris *Iris pseudacorus*, marsh woundwort *Stachys palustris*, comfrees *Symphytum* spp. and other deep flowered plants of damp habitats (Edwards and Jenner 2005). Its fortunes contrast greatly with *B. hortorum*, the only other member of *Megabombus* in Britain which has remained widespread during this period of decline. In common with the declined bumblebees, *B. ruderatus* favours Fabaceae and has a narrow diet, narrower than that of *B. hortorum* (Kleijn and Raemakers 2008). However, since the 1990s, a period that coincides with the expansion of Fabaceae-rich agri-environment schemes, *B. ruderatus* has become much more common in Central and Eastern England (Falk 2011; BWARS data, M. Edwards, *pers. comm.*). It can now be commonly found visiting agri-environment scheme sown red clover *Trifolium pratense* in these areas and is now frequent enough to be included in genetic studies of farmland bumblebees in certain regions (e.g. Buckinghamshire, Dreier *et al.* 2014). Whilst *B. ruderatus* is the most improved of the six scarce lowland species it still remains uncommon in Southern England, with only two males collected from two different Hampshire farms during data collection for this thesis. Why has *B. ruderatus* responded so

much more positively to these schemes than the members of the *Thoracobombus*? The answer may lie in their differing ecology.

B. ruderatus is one of Britain's largest bumblebees and forages over longer distances than *B. hortorum*. Using a different metric of foraging range than the one employed in this thesis, mean worker foraging distances rather than maximum foraging range estimates, Redhead *et al.* (2016) estimated *B. terrestris*, *B. lapidarius* and *B. ruderatus* were to have mean foraging distances of 551 m, 536 m and 501 m respectively compared to distances of 336 m and 272 m for *B. hortorum* and *B. pascuorum*. As previously discussed in Chapter 3, members of the *Thoracobombus* tend to have small nests with under 100 workers. *B. ruderatus* has much larger colonies of up to 400 workers, much larger than its close relative *B. hortorum* (Sladen 1912). The longer foraging range of *B. ruderatus* would agree with the hypothesis that foraging range at the species level is driven by colony size, with the larger colonies requiring more resources and hence requiring the ability to travel further to find sufficient forage. Most published estimates of bumblebee foraging range generally agree with this trend, though given the various differing techniques and interpretations there is a wide degree of variability (Table 7.1). One surprising result is that of *B. distinguendus* which in Britain has declined precipitously across the whole British Isles and is now found only in northernmost Scotland. *B. distinguendus* has small nests with very seldom more than 100 workers (Löken 1973), so the foraging ranges estimated by Charman *et al.* (2010) are surprisingly high compared to those obtained for *B. pascuorum* and *B. hortorum* which have colonies of similar sizes (Chapter 3; Redhead *et al.* 2015).

Two other important life-history strategies relate to emergence date and nesting ecology. *B. ruderatus* emerges in April, which puts it much closer to the March-April emergence time of the big six (big seven with the addition of *B. hypnorum*) than to the late May-June emergence time of the declined species of *Thoracobombus*. Late emergence date is a good predictor for declining bumblebees across the world as it makes them more susceptible to changes in agricultural practice that reduce floral availability in the summer (Williams and Osborne 2009; Williams *et al.* 2009). This is mirrored in the wider bee community as bees associated with summer flowering plants have declined more than those associated with spring flowering plants (Scheper *et al.* 2014). Secondly, *B. ruderatus* is a cavity nester like *B. hortorum*, not a surface nester like most of the *Thoracobombus*. The loss of flower-rich grassland has not only deprived summer flying bumblebees of their forage, but also their nesting habitat. Both *B.*

distinguendus and *B. subterraneus*, as the name suggests, nest underground but have late emerging queens, like the *Thoracobombus*. Interestingly, *B. pascuorum* is the most catholic in its nest choice amongst the *Thoracobombus*, another factor that may help explain its success relative to its sister species.

The difference in the strong response of *B. ruderatus* at a country level and the neutral response for *B. pascuorum* presented here (and absence of a response from the rest of the *Thoracobombus*) suggests that whilst the foraging resources created by agri-environment schemes are appropriate in composition, if they are not provided at a small enough spatial scale and with appropriate nearby nesting habitat then surface nesting bumblebee species with small foraging ranges will not be able to take advantage of this. Short-term rotational pollen and nectar mixes do not provide appropriate nesting habitat for surface nesters. Long-term species rich grassland should be favoured for the conservation of *Thoracobombus* in conjunction with rotational pollen and nectar mixes. Though anecdotal in nature, where concerted efforts have been made to increase the abundance of appropriate forage as well as suitable undisturbed grassland as part of the *B. subterraneus* reintroduction project at Dungeness and Romney Marsh in Kent, there have been substantial increases in abundance of scarce species such as *B. humilis*, *B. ruderatus*, *B. muscorum* and even a return of previously absent species such as *B. ruderarius*, *B. sylvarum* and *B. soroeensis* (N. Gammans, *pers. comm.*), probably because all aspects of their life-cycle are being catered for, not just their dietary requirements.

The differing response of bumblebees to intensification depending on their individual ecologies can equally be seen in the response of the wider bee community. The traits of a narrower diet and smaller foraging range are also important predictors for the response of solitary bees to agricultural intensification (Bommarco *et al.* 2010; De Palma *et al.* 2015). However, it bears repeating that these traits are not always perfect predictors in and of themselves. Some highly specialised bees remain common where their host plants have remained common, for example the spring flying mining bee *Andrena praecox* collects pollen from willows (*Salix* spp.), and both have remained common and relatively unaffected by agricultural intensification. In contrast, the summer flying *Andrena hattorfiana* and *Andrena marginata* have been negatively affected as they collect pollen only from scabiouses (*Knautia*, *Succisa* and *Scabiosa* species), plants that are dependent on the maintenance of low-intensity

grassland and consequently both bee species have fared poorly in recent years. It is the relative fortune of the host plants that determines the success of the species that feed on them (Scheper *et al.* 2014). However, it should not be surprising that bee diet breadth alone is a generally good predictor of their persistence, given the far-reaching negative effects of intensification on most agricultural plant communities. Few British bee species are specialised on the kind of woody plants that have remained relatively common in the farmed landscape, and so the reduction in herbaceous plant diversity has affected the majority of bee species present on farmland.

Table 7.1. Estimated foraging ranges of worker bumblebees (*Bombus*).

Species	Foraging distance (m)		Method	Reference
	Average	Maximum		
<i>B. distinguendus</i>	391	955	Genetic markers	Charman <i>et al.</i> 2010
<i>B. hortorum</i>	-	556	Genetic markers	Wood <i>et al.</i> 2015a
	336	-	Genetic markers	Redhead <i>et al.</i> 2015
<i>B. lapidarius</i>	-	450	Genetic markers	Knight <i>et al.</i> 2005
	536	-	Genetic markers	Redhead <i>et al.</i> 2015
	-	714	Genetic markers	Wood <i>et al.</i> 2015a
	260	1,500	Direct (marked workers)	Walther-Hellwig and Frankl 2000
<i>B. muscorum</i>	55	125	Direct (marked workers)	Walther-Hellwig and Frankl 2000
<i>B. pascuorum</i>	272	-	Genetic markers	Redhead <i>et al.</i> 2015
	-	363	Genetic markers	Wood <i>et al.</i> 2015a
	-	449	Genetic markers	Knight <i>et al.</i> 2005
<i>B. pratorum</i>	-	674	Genetic markers	Knight <i>et al.</i> 2005
<i>B. ruderatus</i>	501	-	Genetic markers	Redhead <i>et al.</i> 2015
<i>B. terrestris</i>	275	631	Direct (radar tracking)	Osborne <i>et al.</i> 1999
	-	758	Genetic markers	Knight <i>et al.</i> 2005
	-	799	Genetic markers	Wood <i>et al.</i> 2015a
	267	800	Direct (marked workers)	Wolf and Moritz 2008
	-	1,500	Direct (marked workers)	Osborne <i>et al.</i> 2008b
	551	-	Genetic markers	Redhead <i>et al.</i> 2015
	663	1,750	Direct (marked workers)	Walther-Hellwig and Frankl 2000

7.3 What should the countryside look like?

Humans have been present across Europe for thousands of years, and the habitats and species assemblages present are predominantly as a result of human activities. Habitats such as open arable farmland, deforested heathlands, coppiced woodlands and grazed moorlands would not exist without the influence of man. Arable 'weeds' such as corn marigold *Chrysanthemum segetum* and corn buttercup *Ranunculus arvensis* are archeophytes, introduced to Britain many hundreds of years ago through the trade of crop seeds (Stace 2010). These species are essentially Mediterranean in distribution and can only persist in Britain in the pseudo-Mediterranean conditions resulting from the annual cultivation typical of arable farming. Now modern herbicides, improved seed sorting and winter sowing of cereal crops have changed this environment drastically, and these introduced species have become exceedingly scarce (Wilson 1992; Stewart *et al.* 1994).

The nature of agricultural change is important to bear in mind when considering post-war declines in bumblebee species in the UK. The species that have suffered the greatest declines mostly show a preference for Fabaceae, the abundance of which has declined as low-intensity agricultural grassland was replaced with arable or high-input grassland. It has been argued that, as farming practices have changed this flower-rich habitat has been lost, and the bumblebees that depend on it have consequently disappeared from agricultural habitats, unable to cope with this changed environment (Rasmont and Mersch 1988; Goulson *et al.* 2005; Carvell *et al.* 2006a). However, much of this flower-rich grassland was a relatively recent part of the British landscape. Prior the 17th century most agriculture occurred in large open field systems with common grazing rights and minimal crop rotation, with a large proportion of the land standing fallow to recover fertility (Overton 1996). It was not until the 17th and 18th centuries that the land began to be enclosed in a meaningful way. Enclosure was the transfer of common land to private ownership and private grazing and agricultural practice, with associated demarcation of boundaries. Much of the hedging in England was created as a result of the 18th century Enclosure Acts (Pollard, Hooper and Moore 1974). This system of private ownership allowed the extensive uptake of the more effective Norfolk four-course crop rotation including the import and widespread planting of legumes (Fabaceae) to increase nitrogen levels in the soil. Fallow consequently decreased from around 20% of the arable land area in England in 1700 to around 4% by 1900 (Overton 1996). Moreover, growing international trade in food imports in the 1870s subjected British arable farmers to intense

competition, especially from North American cereals. By the 1930s grain acreage had fallen by 33% (3,402,000 ha to 2,291,000 ha) as farmers shifted from arable to pastoral production, with a 51% increase in the acreage of permanent grassland (4,890,000 ha to 7,389,000 ha, Brassley 2002; see General Introduction Figure 1.1).

All in all, the widespread shift to a patchwork of smaller fields including a large legume-rich permanent grassland component was a radical departure from the previous open field system. As a result of these major changes, the landscape seen in the early part of the 20th century may have been supporting an ‘unnaturally’ high density of bumblebees that favour Fabaceae pollen, or at least a density that may only have been representative of a few hundred years of farming practice at most. A study of bumblebee pollen diet based on specimens collected between 1878 and 1949 in Britain, Belgium and the Netherlands, countries with similar changing agricultural practices, found that bumblebee species that subsequently declined in the post-war period had a narrower diet than bumblebee species that did not decline over the same period (Kleijn and Raemakers 2008). Additionally, a similar analysis looking at the genetic diversity of bumblebee species collected in the Netherlands between 1918-1926 showed that the species that subsequently declined had a lower genetic diversity than bumblebees that have remained stable (Maebe *et al.* 2015).

All this suggests that the decline of Fabaceae favouring bumblebees may be a regression towards a historic mean. Is there a moral difference between the agricultural intensification that led to a mass expansion of Fabaceae-rich grassland, and the agricultural intensification that led to its subsequent destruction? Both were changes freely made with the aim of increasing agricultural productivity and human wellbeing. What of the forgotten species characteristic of fallow land that would have dwindled to obscurity in the landscape of 1900? When considering what changes to make to the countryside, we have to ask what kind of a world do we want to see? What world do we want to promote and conserve?

7.4 Concluding remarks

In his 1917 lecture ‘Science as a Vocation’ (Gerth and Wright Mills 1946) the sociologist Max Weber argues that science can provide an explanation of natural phenomena and provide support and justification for holding a position, but it cannot explain why it is worth holding that position in the first place. The question – ‘do agri-environment schemes benefit farmland

bee populations' can be answered empirically. The question 'should farmland bees be conserved' cannot. The role of bees as agricultural pollinators is clearly of great importance, but justifying their conservation on this basis relies on the supposition that we value our own continued survival and quality of life, and in providing a pollination service bees contribute towards our desired outcomes. In other words, we value them for what they provide us with, not for what they are.

When considering this pollination service, the diversity of the pollinator community is an important factor. Wild bees can provide an important pollination service independent of honeybee abundance (Garibaldi *et al.* 2013), with more diverse pollinator communities directly increasing crop yields (Westerkamp and Gottsberger 2000; Klein *et al.* 2003; Hoehn *et al.* 2008). The presence of different bee species can even increase the efficiency of honey bee pollination (Brittain *et al.* 2013). However, whilst diversity is important, this is not a sufficient argument to conserve the overwhelming majority of bee species, as most agricultural pollination is carried out by a small minority of the bee community present in any one area that can be enhanced through relatively simple conservation measures (Kleijn *et al.* 2015). For example, approximately 20-25% of named bee species are obligate parasites of other bees and provide a very limited pollination service due to their reduced need to visit flowers and greatly reduced body hair (Michener 2007).

However, as it stands, people do not value bees as purely for the pollination service they provided. Britain is a wealthy nation, and the factors that motivated the post-war intensification of agriculture are less pressing than they were. The strength of feeling in the general public, as seen in the membership of organisations such as the National Trust, the Royal Society for the Protection of Birds and many others show that there is a desire to protect nature and for the negative effects of intensified agriculture to be mitigated and diminished. Bees, along with other wild organisms are valued for their aesthetic and cultural benefits, and people do not want to see a countryside denuded of variation and diversity. Moreover, as well as concern over short term extinction of species, maintaining diversity within agroecosystems is desirable over the medium to long term as well. Bee and plant communities are highly interdependent and declines in one are closely linked to declines in the other (Biesmeijer *et al.* 2006; Scheper *et al.* 2014). The loss of species and simplification of plant-pollinator communities suggests that these networks will be less resilient to future change (Burkle *et al.* 2013). Given the ongoing challenge of climate change that has the potential to seriously affect

global agriculture, it is likely that only sufficiently diverse agroecosystems in complex landscapes will have the capacity to adapt to changing conditions (Tcharntke *et al.* 2005). In short, whilst most agriculture can probably get by with low levels of biodiversity for now, it is prudent to conserve biodiversity because of the strong possibility of future change.

This thesis demonstrates that agri-environment schemes in their current form can, at the farm scale, significantly increase the population size of common bumblebees but do not increase the diversity of the bee community or the plants that they feed on. Whilst providing important resources for common species they fail to meet the needs of species with more particular requirements and preferences. As it stands, current schemes provide a good baseline measure for what conservation intervention can achieve, but given the importance of maintaining diverse bee communities on farmland it would be reasonable to develop additional techniques and schemes that effectively increase the diversity of flowering plants present on farmland, rather than just increasing their abundance. Attention needs to be paid to the ecology of different bee species in order to cater to their specific requirements which can be markedly different even within genus level groups, and a more detailed knowledge of the autoecology of individual species will further inform our understanding of why species thrive or dwindle. Agri-environment schemes will never be able to provide all resources to all species, and there will have to be separate measures that look after the rarest parts of the fauna, but a more in depth knowledge of the bee community is key to benefiting the majority of species and to the maintenance of a more biodiverse countryside.

Chapter 8 - References

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Appendices

Appendix A - Impact of the proportion of semi-natural habitats on aculeate diversity

<i>Number of aculeate species</i>	Variable	<i>df</i>	<i>t</i>	<i>P</i>
Recorded on transects	Flower-rich agri-environment grassland	16,17	-0.636	0.534
	Flower-poor general grassland	16,17	-0.614	0.548
	Hedgerow	16,17	-1.524	0.147
	Woodland	16,17	1.063	0.304
	Total semi-natural	16,17	0.744	0.467
Recorded in pan traps	Flower-rich agri-environment grassland	15,16	-0.636	0.534
	Flower-poor general grassland	15,16	-0.745	0.468
	Hedgerow	15,16	-0.624	0.542
	Woodland	15,16	1.704	0.109
	Total semi-natural	15,16	1.393	0.184

Appendix B - Abundance of aculeate species recorded on transects and in pan traps

	Transects		Pan traps	
	Entry Level Stewardship	Higher Level Stewardship	Entry Level Stewardship	Higher Level Stewardship
<i>Ancistrocerus gazella</i>	0	2	1	3
<i>Ancistrocerus parientinus</i>			0	1
<i>Ancistrocerus trifasciatus</i>	1	0	2	2
<i>Andrena alfkenella</i>	1	2		
<i>Andrena barbilabris</i>	0	1		
<i>Andrena bicolor</i>	3	3	43	18
<i>Andrena carantonica</i>	2	4	7	6
<i>Andrena chrysosceles</i>	2	1	0	5
<i>Andrena cineraria</i>	3	7	28	23
<i>Andrena dorsata</i>	10	12	0	1
<i>Andrena flavipes</i>	6	8	43	13
<i>Andrena florea</i>	7	1	10	27
<i>Andrena fulva</i>	1	0	1	0
<i>Andrena fulvago</i>	1	0	3	6
<i>Andrena haemorrhoa</i>	7	10	43	73
<i>Andrena helvola</i>			15	0
<i>Andrena humilis</i>	1	0	1	0
<i>Andrena labialis</i>	0	4	2	2
<i>Andrena labiata</i>			1	0
<i>Andrena minutula</i>	15	8	42	23
<i>Andrena minutuloides</i>	0	1		
<i>Andrena nigroaenea</i>	9	3	32	32
<i>Andrena nitida</i>	1	9	8	5
<i>Andrena semilaevis</i>	4	5	1	0
<i>Andrena subopaca</i>	0	2	3	2
<i>Andrena synadelpha</i>			1	0
<i>Andrena wilkella</i>	0	3		
<i>Anoplius nigerrimus</i>			5	4
<i>Anthidium manicatum</i>	0	1		
<i>Anthophora furcata</i>	1	4	1	0
<i>Anthophora plumipes</i>	1	0		
<i>Apis mellifera</i>	377	1338	289	446
<i>Arachnospila anceps</i>			2	0
<i>Arachnospila spissa</i>	0	1		
<i>Arachnospila trivialis</i>			2	2
<i>Bombus barbutellus</i>	3	5		
<i>Bombus campestris</i>	3	1	1	2
<i>Bombus hortorum</i>	313	343	112	71
<i>Bombus hypnorum</i>	27	19	13	8
<i>Bombus jonellus</i>	2	0	2	1
<i>Bombus lapidarius</i>	1710	3566	190	264
<i>Bombus lucorum</i>	82	98	184	130

<i>Bombus pascuorum</i>	590	1054	108	141
<i>Bombus pratorum</i>	75	82	78	45
<i>Bombus ruderarius</i>	0	5		
<i>Bombus ruderatus</i>	0	7		
<i>Bombus rupestris</i>	46	10	6	8
<i>Bombus sylvestris</i>	3	1	503	478
<i>Bombus terrestris</i>	396	905	10	3
<i>Bombus vestalis</i>	54	36	32	20
<i>Caliadurgus fasciatellus</i>			1	1
<i>Cerceris rybyensis</i>	5	0	1	2
<i>Chelostoma campanularum</i>	0	1	50	1
<i>Chelostoma florissomne</i>	2	0	1	0
<i>Chrysis ignita</i>			3	1
<i>Chrysis impressa</i>			4	1
<i>Chrysis impressa</i>	1	0		
<i>Chrysis mediata</i>			1	0
<i>Colletes davesianus</i>	5	2		
<i>Crabro cribrarius</i>			0	1
<i>Crossocerus cetratus</i>	1	0		
<i>Crossocerus nigrinus</i>	1	0		
<i>Crossocerus podagricus</i>	7	2	4	3
<i>Crossocerus megacephalus</i>			1	0
<i>Crossocerus nigrinus</i>			2	0
<i>Crossocerus quadrimaculatus</i>	1	0		
<i>Dolichovespula saxonica</i>	2	0		
<i>Dolichovespula sylvestris</i>	7	9	5	0
<i>Ectemnius continuus</i>	2	0	3	2
<i>Ectemnius lituratus</i>	7	22	11	2
<i>Ectemnius rubicola</i>	1	0		
<i>Gorytes quadrifasciatus</i>	0	1		
<i>Halictus rubicundus</i>	1	2	17	3
<i>Halictus tumulorum</i>	13	8	24	33
<i>Hoplitis claviventris</i>	0	1		
<i>Hylaeus brevicornis</i>			1	1
<i>Hylaeus communis</i>	7	1	25	9
<i>Hylaeus confusus</i>			4	3
<i>Hylaeus cornutus</i>	0	14	0	1
<i>Hylaeus dilatatus</i>			0	1
<i>Hylaeus signatus</i>	1	0		
<i>Hylaeus pectoralis</i>			1	0
<i>Lasioglossum albipes</i>	1	0	8	3
<i>Lasioglossum calceatum</i>	28	24	116	133
<i>Lasioglossum fulvicorne</i>	0	1	4	6
<i>Lasioglossum laevigatum</i>			1	0
<i>Lasioglossum lativentris</i>	2	0		
<i>Lasioglossum leucopus</i>	5	1	21	2

<i>Lasioglossum leucozonium</i>	29	8	3	3
<i>Lasioglossum malachurum</i>	47	191	52	65
<i>Lasioglossum minutissimum</i>	1	0		
<i>Lasioglossum morio</i>	17	29	45	39
<i>Lasioglossum parvulum</i>	1	0	3	0
<i>Lasioglossum pauxillum</i>	14	29	25	17
<i>Lasioglossum puncticolle</i>	0	4		
<i>Lasioglossum smeathmanellum</i>	1	0		
<i>Lasioglossum villosulum</i>	5	7	3	3
<i>Lasioglossum xanthopus</i>	0	1		
<i>Lasioglossum zonulum</i>	1	4	0	4
<i>Lindenius albilabris</i>	1	0	1	5
<i>Megachile centuncularis</i>	3	3	1	1
<i>Megachile ligniseca</i>	1	2	0	2
<i>Megachile versicolor</i>	0	3		
<i>Megachile willughbiella</i>	1	3	1	1
<i>Megachile versicolor</i>			1	2
<i>Melitta haemorrhoidalis</i>			2	7
<i>Melitta leporina</i>	0	1		
<i>Melitta tricineta</i>	40	16	3	2
<i>Microdynerus exilis</i>	1	0	0	2
<i>Mimumesa unicolor</i>	1	0	1	0
<i>Myrmosa atra</i>			0	1
<i>Nomada fabriciana</i>	1	1	5	2
<i>Nomada flava/panzeri</i>	31	20	9	6
<i>Nomada flavoguttata</i>	1	1	1	2
<i>Nomada flavopicta</i>	1	0	3	1
<i>Nomada goodeniana</i>	12	7		
<i>Nomada guttulata</i>			0	1
<i>Nomada marshamella</i>	4	0	2	1
<i>Nomada ruficornis</i>	1	3	3	6
<i>Nomada sheppardana</i>	2	0		
<i>Nomada striata</i>			0	1
<i>Nysson trimaculatus</i>			0	2
<i>Osmia bicolor</i>	1	2		
<i>Osmia bicornis</i>	0	2	1	3
<i>Osmia caerulea</i>	0	1		
<i>Osmia leaiana</i>	2	2	1	1
<i>Osmia spinulosa</i>			0	1
<i>Panurgus calcaratus</i>	3	0	1	0
<i>Passaloecus corniger</i>			1	0
<i>Passaloecus singularis</i>	1	0	2	0
<i>Pemphredon inornata</i>			2	0
<i>Pemphredon lethifer</i>	1	2	0	1
<i>Prionemis exaltata</i>	1	0	1	1
<i>Psenulus pallipes</i>			0	1

<i>Sphecodes crassus</i>	0	1	3	0
<i>Sphecodes ephippius</i>	2	1	3	1
<i>Sphecodes geoffrellus</i>			1	1
<i>Sphecodes monilicornis</i>	0	3		
<i>Sphecodes niger</i>			3	0
<i>Spilomena beata</i>			1	0
<i>Stigmus solskyi</i>			1	1
<i>Tiphia femorata</i>	6	6	7	4
<i>Trypoxylon attenuatum</i>			0	11
<i>Trypoxylon clavicerum</i>			4	0
<i>Vespa crabro</i>	1	1	2	0
<i>Vespula germanica</i>	5	3	16	8
<i>Vespula vulgaris</i>	23	8	30	23

Appendix D List of flowering plant species recorded during surveys

Species marked with * were sown as part of HLS management

Species recorded on ELS farms

Achillea millefolium
Aesculus hippocastanum
Agrimonia eupatorium
Ajuga reptans
Alliaria petiolata
Allium ursinum
Anagallis arvensis
Anchusa arvensis
Anthriscus sylvestris
Anthyllis vulneraria
Aquilegia vulgaris
Arctium minus
Artemisia vulgaris
Ballota nigra
Barbarea vulgaris
Bellis perennis
Brassica napus
Bryonia dioica
Calystegia sepium
Calystegia silvatica
Campanula trachelium
Capsella bursa-pastoris
Cardamine pratensis
Carduus crispus
Carduus nutans
Centaurea nigra
Centaurea scabiosa
Centaurium erythraea
Cerastium fontanum
Chaerophyllum temulum
Chamerion angustifolium
Chelidonium majus
Cichorium intybus
Circaea lutetiana
Cirsium arvense
Cirsium palustre
Cirsium vulgare
Clematis vitalba
Clinopodium vulgare
Conium maculatum
Convolvulus arvensis
Conyza canadensis
Cornus sanguinea
Crataegus monogyna
Crepis capillaris
Crepis vesicaria
Cruciata laevipes
Cytisus scoparius
Dactylorhiza fuchsii
Daucus carota
Digitalis purpurea
Dipsacus fullonum
Epilobium hirsutum
Eupatorium cannabinum
Euphorbia helioscopia
Euphorbia lathyris
Euphrasia spp.
Fragaria vesca

Species recorded on HLS farms

* *Achillea millefolium*
Aegopodium podagraria
Aethusa cynapium
Agrimonia eupatorium
Ajuga reptans
Alliaria petiolata
Anacamptis pyramidalis
Anagallis arvensis
Angelica sylvestris
* *Anthemis austriaca*
Anthriscus sylvestris
* *Anthyllis vulneraria*
Arctium minus
Artemisia vulgaris
Ballota nigra
Barbarea vulgaris
Bellis perennis
Betonica officinalis
* *Borago officinalis*
* *Brassica rapa*
Bryonia dioica
Calystegia sepium
Calystegia silvatica
Campanula rotundifolia
Campanula trachelium
Capsella bursa-pastoris
Cardamine pratensis
Carduus crispus
Carduus nutans
* *Centaurea cyanea*
* *Centaurea nigra*
Centaurea scabiosa
Centaurium erythraea
Cephalanthera longifolia
Cerastium fontanum
Chaerophyllum temulum
Chamerion angustifolium
Chelidonium majus
* *Cichorium intybus*
Cirsium arvense
Cirsium eriophorum
Cirsium palustre
Cirsium vulgare
Clematis vitalba
Clinopodium acinos
* *Clinopodium vulgare*
Conium maculatum
Convolvulus arvensis
Cornus sanguinea
Crepis capillaris
Crepis vesicaria
Cruciata laevipes
Dactylorhiza fuchsii
* *Daucus carota*
* *Digitalis purpurea*
* *Dipsacus fullonum*
Epilobium hirsutum
Epilobium montanum

Fumaria officinalis
Galeopsis tetrahit
Galium aparine
Galium mollugo
Galium odoratum
Galium verum
Geranium columbinum
Geranium dissectum
Geranium molle
Geranium pratense
Geranium pyrenaicum
Geranium robertianum
Geum urbanum
Glechoma hederacea
Helminthotheca echinoides
Heracleum sphondylium
Hieracium agg.
Hyacinthoides non-scripta
Hyacinthoides x massartiana
Hypericum perforatum
Hypochaeris radicata
Kickxia elatine
Knautia arvensis
Lamium album
Lamium purpureum
Lapsana communis
Lathyrus pratensis
Leontodon hispidus
Leucanthemum vulgare
Ligustrum vulgare
Linaria vulgaris
Linum usitatissimum
Lithospermum officinale
Lonicera periclymenum
Lotus corniculatus
Lotus pedunculatus
Lychnis flos-cuculi
Malva moschata
Malva sylvestris
Matricaria discoidea
Medicago arabica
Medicago lupulina
Melilotus officinalis
Mentha spicata
Myosotis arvensis
Myosotis sylvestris
Odontites verna
Oenanthe crocata
Onobrychis viciifolia
Origanum vulgare
Orobancha elatior
Papaver dubium
Papaver rhoeas
Pastinaca sativa
Persicaria lapathifolia
Persicaria maculosa
Phacelia tanacetifolia
Picris echioides
Pilosella officinarum
Pimpinella saxifraga
Plantago lanceolata
Plantago media
Potentilla anserina
Potentilla reptans
Primula veris

Galeopsis tetrahit
Galium aparine
Galium mollugo
Galium palustre
Galium verum
Genista tinctoria
Euphorbia amygdaloides
Euphorbia helioscopia
Euphrasia spp.
** Fagopyrum esculentum*
Fragaria vesca
Fumaria officinalis
Geranium columbinum
Geranium dissectum
Geranium molle
Geranium pratense
Geranium pyrenaicum
Geranium robertianum
Geum urbanum
Glechoma hederacea
Helianthemum nummularium
** Helianthus annuus*
Helminthotheca echinoides
Heracleum sphondylium
Hesperis matronalis
Hieracium agg.
Hyacinthoides x massartiana
Hypericum perforatum
Hypericum pulchrum
Hypericum tetrapterum
Hypochaeris radicata
Inula conyzia
Kickxia elatine
** Knautia arvensis*
Lamium album
Lamium purpureum
Lapsana communis
** Lathyrus pratensis*
Lathyrus nissolia
Leontodon hispidus
** Leucanthemum vulgare*
Ligustrum vulgare
Linaria vulgaris
Linum catharticum
Linum usitatissimum
Lithospermum officinale
** Lotus corniculatus*
Lotus pedunculatus
** Lychnis flos-cuculi*
Lythrum salicaria
Malva moschata
Malva sylvestris
Matricaria discoidea
** Medicago lupulina*
** Medicago sativa*
** Melilotus alba*
** Melilotus officinalis*
Mentha arvensis
Myosotis arvensis
Myosotis ramosissima
Odontites verna
Oenanthe crocata
** Onobrychis viciifolia*
** Origanum vulgare*
Ornithogalum angustifolium

Primula vulgaris
Prunella vulgaris
Pulicaria dysenterica
Ranunculus acris
Ranunculus repens
Reseda lutea
Rhinanthus minor
Rosa arvensis
Rosa canina agg.
Rosa rubiginosa
Rubus fruticosus agg.
Rumex acetosa
Sambucus nigra
Sanguisorba minor muricata
Sanicula europaea
Scabiosa columbaria
Scrophularia auriculata
Scrophularia nodosa
Senecio erucifolius
Senecio jacobea
Senecio vulgaris
Sherardia arvensis
Silene dioica
Silene latifolia
Silene vulgaris
Silene x hampeana
Sinapis arvensis
Sison amomum
Sisymbrium officinale
Solanum dulcamara
Solanum nigrum
Sonchus arvensis
Sonchus asper
Sonchus oleraceus
Stachys sylvatica
Stellaria graminea
Stellaria holostea
Symphytum grandiflorum
Symphytum orientale
Tamus communis
Tanacetum vulgare
Taraxacum agg.
Torilis japonica
Tragopogon pratensis
Trifolium campestre
Trifolium dubium
Trifolium hybridum
Trifolium pratense
Trifolium repens
Tripleurospermum inodorum
Ulex europaeus
Valerianella locusta
Verbascum nigrum
Verbascum thapsus
Verbena officinalis
Veronica chamaedrys
Veronica montana
Veronica persica
Viburnum lantana
Vicia cracca
Vicia faba
Vicia hirsuta
Vicia sativa agg.
Vicia sepium
Vicia tetrasperma
Viola arvensis

Orobanche minor
Papaver rhoeas
Pastinaca sativa
** Persicaria maculosa*
** Phacelia tanacetifolia*
Picris hieracioides
Pimpinella saxifraga
Plantago lanceolata
** Plantago media*
Polygonatum multiflorum
Potentilla anserina
Potentilla reptans
Primula veris
** Prunella vulgaris*
Pulicaria dysenterica
** Ranunculus acris*
Ranunculus bulbosus
Ranunculus repens
Raphanus raphanistrum
Reseda lutea
** Rhinanthus minor*
Rosa arvensis
Rosa canina agg.
Rubus caesius
Rubus fruticosus agg.
** Rumex acetosa*
Sambucus nigra
** Sanguisorba minor minor*
** Sanguisorba minor muricata*
Sanicula europaea
Senecio erucifolius
Senecio jacobea
Senecio vulgaris
Sherardia arvensis
** Silene dioica*
Silene latifolia
Silene vulgaris
Silene x hampeana
** Sinapis alba*
Sinapis arvensis
Sisymbrium officinale
Solanum dulcamara
** Sonchus arvensis*
Sonchus asper
Spergularia arvensis
Stachys palustris
Stachys sylvatica
Stellaria graminea
Stellaria holostea
Taraxacum agg.
Torilis japonica
Tragopogon pratensis
Trifolium campestre
Trifolium dubium
** Trifolium hybridum*
** Trifolium incarnatum*
** Trifolium pratense*
Trifolium repens
Tripleurospermum inodorum
Verbascum thapsus
Verbena officinalis
Veronica arvensis
Veronica chamaedrys
Veronica montana
Veronica officinalis
Veronica persica

Veronica serpyllifolia

Viburnum lantana

* *Vicia cracca*

Vicia hirsuta

* *Vicia sativa* agg.

Vicia sepium

Vicia tetrasperma

Viola arvensis

Appendix D - Flowering plants sown as part of pollinator-focused agri-environment schemes and their average abundance per farm 2013-2014

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Plant species	Average abundance 2013-2014		Relative proportion of ELS to HLS sown flowers (%)
	Entry Level Stewardship	Higher Level Stewardship	
<i>Achillea millefolium</i>	127.88±59.35	1519.00±706.11	8.42
<i>Centaurea cyanus</i>	0.0±0.0	2.13±2.06	0.0
<i>Centaurea nigra</i>	12996.18±6591.33	56632±17910.19	22.95
<i>Centaurea scabiosa</i>	19.47±10.88	88.12±57.22	22.09
<i>Daucus carota</i>	2000.65±1320.72	8450.63±4188.34	23.67
<i>Galium verum</i>	1172.94±855.57	545.88±181.96	214.87
<i>Geranium pratense</i>	1.76±1.34	17.88±12.92	9.87
<i>Knautia arvensis</i>	236.53±192.43	425.44±160.67	55.59
<i>Lathyrus pratensis</i>	106.59±31.43	608.19±306.90	17.52
<i>Leontodon hispidus</i>	164.18±161.75	829.69±579.29	19.78
<i>Leucanthemum vulgare</i>	3428.59±2200.02	13355.88±4763.56	25.67
<i>Lotus corniculatus</i>	1416.12±588.41	84387.44±24117.45	1.68
<i>Lotus pedunculatus</i>	17.65±17.64	1282.94±880.30	1.38
<i>Lychnis flos-cuculi</i>	1.0±0.79	5.0±3.02	20.00
<i>Malva moschata</i>	2.65±1.80	48.13±37.94	5.50
<i>Medicago lupulina</i>	803.89±456.77	20192.06±13736.21	3.98
<i>Medicago sativa</i>	0.0±0.0	4603.81±2441.53	0.0
<i>Melilotus officinalis</i>	5.59±5.59	20872.50±20.789.15	0.0
<i>Onobrychis viciifolia</i>	17.12±8.24	121.43±75.99	14.09
<i>Origanum vulgare</i>	19.70±10.80	13418.56±6655.28	0.14
<i>Phacelia tanacetifolia</i>	400.18±400.18	11237.50±4655.08	3.56
<i>Plantago lanceolata</i>	533.65±302.01	533.69±166.25	99.99
<i>Plantago media</i>	142.23±110.29	0.81±0.75	17505.88
<i>Primula veris</i>	16.76±9.33	2.13±1.88	650.52
<i>Prunella vulgaris</i>	336.88±180.26	1653.06±729.86	20.56
<i>Ranunculus acris</i>	1646.88±1013.00	1018.25±372.06	161.73
<i>Rhinanthus minor</i>	2473.77±1657.92	85.93±82.66	2878.56
<i>Rumex acetosella</i>	10.18±8.58	49.69±27.72	20.48
<i>Sanguisorba minor</i>	11.12±7.40	5.875±4.02	189.24
<i>Silene dioica</i>	144.00±57.13	1658.94±1173.54	8.68
<i>Sonchus arvensis</i>	73.12±31.22	1553.25±915.34	4.71
<i>Trifolium hybridum</i>	168.59±63.83	53810.75±25635.92	0.31
<i>Trifolium pratense</i>	5083.23±4828.95	55254.06±20521.24	9.20
Total	33579.06±12739.26	354270.88±71760.77	9.47

Appendix E - Plant species included in the pollen reference library

<i>Acer campestre</i>	<i>Lamium purpureum</i>
<i>Acer pseudoplatanus</i>	<i>Lapsana communis</i>
<i>Achillea millefolium</i>	<i>Lathyrus pratensis</i>
<i>Aegopodium podagraca</i>	<i>Leontodon hispidus</i>
<i>Alliaria petiolata</i>	<i>Leucanthemum vulgare</i>
<i>Anthriscus sylvestris</i>	<i>Ligustrum vulgare</i>
<i>Arctium minus</i>	<i>Lonicera periclymenum</i>
<i>Bellis perennis</i>	<i>Lotus corniculatus</i>
<i>Berberis vulgaris</i>	<i>Lotus pedunculatus</i>
<i>Brassica napus</i>	<i>Malva sylvestris</i>
<i>Brassica rapa campestris</i>	<i>Medicago lupulina</i>
<i>Bryonia dioica</i>	<i>Medicago sativa</i>
<i>Campanula trachelium</i>	<i>Melilotus officinalis</i>
<i>Cardamine hirsuta</i>	<i>Mentha arvensis</i>
<i>Carduus acanthoides</i>	<i>Myosotis arvensis</i>
<i>Centaurea nigra</i>	<i>Odontites verna</i>
<i>Centaurea scabiosa</i>	<i>Oenanthe crocata</i>
<i>Cerastium fontanum</i>	<i>Origanum vulgare</i>
<i>Chaerophyllum temulum</i>	<i>Pastinaca sativa</i>
<i>Chamerion angustifolium</i>	<i>Persicaria lapathifolia</i>
<i>Cirsium arvense</i>	<i>Phacelia tanacetifolia</i>
<i>Cirsium vulgare</i>	<i>Picris hieracioides</i>
<i>Clematis vitalba</i>	<i>Plantago lanceolata</i>
<i>Clinopodium vulgare</i>	<i>Plantago media</i>
<i>Convolvulus arvensis</i>	<i>Potentilla anserina</i>
<i>Cornus sanguinea</i>	<i>Potentilla reptans</i>
<i>Crataegus monogyna</i>	<i>Primula veris</i>
<i>Crepis capillaris</i>	<i>Prunella vulgaris</i>
<i>Crepis vesicaria</i>	<i>Prunus spinosa</i>
<i>Daucus carota</i>	<i>Pulicaria dysenterica</i>
<i>Dipsacus fullonum</i>	<i>Ranunculus acris</i>
<i>Euphorbia amygdaloides</i>	<i>Ranunculus bulbosus</i>
<i>Fagopyrum esculentum</i>	<i>Ranunculus ficaria</i>
<i>Galium aparine</i>	<i>Ranunculus repens</i>
<i>Genista tinctoria</i>	<i>Raphanus raphanistrum</i>
<i>Geranium columbinum</i>	<i>Reseda lutea</i>
<i>Geranium pyrenaicum</i>	<i>Rhamnus cathartica</i>
<i>Glechoma hederacea</i>	<i>Rosa arvensis</i>
<i>Helminthotheca echioides</i>	<i>Rosa canina agg.</i>
<i>Heracleum sphondylium</i>	<i>Rubus fruticosus agg.</i>
<i>Hyacinthoides non-scripta</i>	<i>Sambucus nigra</i>
<i>Hypericum perforatum</i>	<i>Senecio erucifolius</i>
<i>Hypericum pulchrum</i>	<i>Senecio jacobea</i>
<i>Hypochaeris radicata</i>	<i>Senecio vulgaris</i>
<i>Knautia arvensis</i>	<i>Silene dioica</i>
<i>Lamium album</i>	<i>Sinapis arvensis</i>

Sisymbrium officinale
Sonchus arvensis
Sonchus asper
Stachys sylvatica
Stellaria graminea
Stellaria media
Taraxacum agg.
Torilis japonica
Trifolium campestre
Trifolium dubium

Trifolium hybridum
Trifolium pratense
Trifolium repens
Tripleurospermum inodorum
Veronica chamaedrys
Veronica persica
Viburnum lantana
Viburnum opulus
Vicia sepium

Appendix F - Plant species identified in pollen analysis and level of taxonomic detail

Species identified to species level

Acer campestre
Acer pseudoplatanus
Achillea millefolium
Aegopodium podagraria
Alliaria petiolata
Anthriscus sylvestris
Bellis perennis
Berberis vulgaris
Bryonia dioica
Campanula trachelium
Carduus acanthoides
Castanea sativa
Centaurea nigra
Centaurea scabiosa
Cerastium fontanum
Chaerophyllum temulum
Chamerion angustifolium
Cirsium arvense
Cirsium vulgare
Clinopodium vulgare
Convolvulus arvensis
Cornus sanguinea
Crataegus monogyna
Crepis capillaris
Crepis vesicaria
Daucus carota
Euphorbia amygdaloides
Fagus sylvatica
Galium aparine
Glechoma hederacea
Helminthotheca echioides
Heracleum sphondylium
Hyacinthoides non-scripta
Hypochaeris radicata
Ilex aquifolium
Knautia arvensis
Lamium album
Leontodon hispidus
Leucanthemum vulgare
Ligustrum vulgare
Lonicera periclymenum
Lychnis flos-cuculi
Medicago lupulina
Medicago sativa

Melilotus officinalis
Odontites verna
Oenanthe crocata
Origanum vulgare
Phacelia tanacetifolia
Potentilla reptans
Prunella vulgaris
Prunus spinosa
Pulicharia dysenterica
Ranunculus acris
Ranunculus bulbosus
Ranunculus repens
Raphanus raphanistrum
Reseda lutea
Rhamnus cathartica
Rosa arvensis
Rosa canina agg.
Rubus fruticosus agg.
Sambucus nigra
Senecio jacobea
Senecio vulgaris
Sherardia arvensis
Silene dioica
Sinapis arvensis
Sisymbrium officinale
Solanum dulcamara
Sonchus arvensis
Sonchus asper
Stachys sylvatica
Stellaria graminea
Taraxacum agg.
Torilis japonica
Trifolium campestre
Trifolium dubium
Trifolium hybridum
Trifolium pratense
Trifolium repens
Tripleurospermum inodorum
Veronica chamaedrys
Veronica persica
Viburnum lantana
Viburnum opulus

Species identified to genus level

Brassica

Geranium
Phlox
Plantago

Tilia
Vicia

Appendix G - Full list of bee species recorded during the study with species authorities

<i>Andrena angustior</i> Kirby 1802	<i>Chelostoma campanularum</i> 1802
<i>Andrena alfenella</i> Perkins 1914	<i>Chelostoma florisomne</i> Linnaeus 1758
<i>Andrena bicolor</i> Fabricius 1775	<i>Colletes daviesanus</i> Smith 1846
<i>Andrena bucephala</i> Stephens 1846	<i>Halictus rubicundus</i> Christ 1791
<i>Andrena chrysosceles</i> Kirby 1802	<i>Halictus tumulorum</i> Linnaeus 1758
<i>Andrena cineraria</i> Linnaeus 1758	<i>Hoplitis claviventris</i> Thomson 1872
<i>Andrena dorsata</i> Kirby 1802	<i>Hylaeus brevicornis</i> Nylander 1852
<i>Andrena flavipes</i> Panzer 1799	<i>Hylaeus communis</i> Nylander 1852
<i>Andrena florea</i> Fabricius 1793	<i>Hylaeus confusus</i> Nylander 1852
<i>Andrena fulva</i> Müller 1766	<i>Hylaeus cornutus</i> Curtis 1831
<i>Andrena fulvago</i> Christ 1791	<i>Hylaeus dilatatus</i> Kirby 1802
<i>Andrena haemorrhoa</i> Fabricius 1781	<i>Hylaeus hyalinatus</i> Smith 1842
<i>Andrena helvola</i> Linnaeus 1758	<i>Hylaeus signatus</i> Panzer 1798
<i>Andrena humilis</i> Imhoff 1832	<i>Lasioglossum albipes</i> Fabricius 1781
<i>Andrena labialis</i> Kirby 1802	<i>Lasioglossum calceatum</i> Scopoli, 1763
<i>Andrena labiata</i> Fabricius 1781	<i>Lasioglossum fulvicorne</i> Kirby 1802
<i>Andrena minutula</i> Kirby 1802	<i>Lasioglossum laevigatum</i> Kirby 1802
<i>Andrena minutuloides</i> Perkins 1914	<i>Lasioglossum lativentre</i> Schenck 1853
<i>Andrena nigroaenea</i> Kirby 1802	<i>Lasioglossum leucopus</i> Kirby 1802
<i>Andrena nitida</i> Müller 1776	<i>Lasioglossum leucozonium</i> Schrank 1781
<i>Andrena nitidiuscula</i> Schenck 1853	<i>Lasioglossum malachurum</i> Kirby 1802
<i>Andrena scotica</i> Perkins 1916	<i>Lasioglossum minutissimum</i> Kirby 1802
<i>Andrena semilaevis</i> Perez 1903	<i>Lasioglossum morio</i> Fabricius 1783
<i>Andrena subopaca</i> Nylander 1848	<i>Lasioglossum parvulum</i> Schenck 1853
<i>Andrena trimmerana</i> Kirby 1802	<i>Lasioglossum pauperatum</i> Brule 1832
<i>Andrena wilkella</i> Kirby 1802	<i>Lasioglossum pauxillum</i> Schenck 1853
<i>Anthidium manicatum</i> Linnaeus 1758	<i>Lasioglossum puncticolle</i> Morawitz 1872
<i>Anthophora furcata</i> Panzer 1798	<i>Lasioglossum smeathmanellum</i> Kirby 1802
<i>Anthophora plumipes</i> Pallas 1772	<i>Lasioglossum villosulum</i> Kirby 1802
<i>Apis mellifera</i> Linnaeus 1758	<i>Lasioglossum xanthopus</i> Kirby 1802
<i>Bombus barbutellus</i> Kirby 1802	<i>Lasioglossum zonulum</i> Smith 1848
<i>Bombus campestris</i> Panzer 1801	<i>Megachile centuncularis</i> Linnaeus 1758
<i>Bombus hortorum</i> Linnaeus 1761	<i>Megachile ligniseca</i> Kirby 1802
<i>Bombus hypnorum</i> Linnaeus 1758	<i>Megachile versicolor</i> Smith 1844
<i>Bombus jonellus</i> Kirby 1802	<i>Megachile willughbiella</i> Kirby 1802
<i>Bombus lapidarius</i> Linnaeus 1758	<i>Melitta leporina</i> Panzer 1799
<i>Bombus lucorum</i> Linnaeus 1761	<i>Melitta tricineta</i> Kirby 1802
<i>Bombus pascuorum</i> Panzer 1801	<i>Nomada fabriciana</i> Linnaeus 1767
<i>Bombus pratorum</i> Linnaeus 1761	<i>Nomada flava</i> Panzer 1798
<i>Bombus ruderarius</i> Müller 1776	<i>Nomada flavoguttata</i> Kirby 1802
<i>Bombus ruderatus</i> Fabricius 1775	<i>Nomada fucata</i> Panzer 1798
<i>Bombus rupestris</i> Fabricius 1793	<i>Nomada goodeniana</i> Kirby 1802
<i>Bombus sylvestris</i> Lepeletier 1832	<i>Nomada hirtipes</i> Perez 1844
<i>Bombus terrestris</i> Linnaeus 1758	<i>Nomada lathburiana</i> Kirby 1802
<i>Bombus vestalis</i> Geoffroy 1785	<i>Nomada marshamella</i> Kirby 1802

Nomada ruficornis Linnaeus 1758
Nomada sheppardana Kirby 1802
Osmia bicolor Schrank 1781
Osmia bicornis Linnaeus 1758
Osmia caerulea Linnaeus 1758
Osmia leaiana Kirby 1802
Osmia spinulosa Kirby 1802
Panurgus calcaratus Scopoli 1763
Sphecodes crassus Thomson 1870
Sphecodes ephippius Linnaeus 1767
Sphecodes geoffrellus Kirby 1802
Sphecodes monilicornis Kirby 1802

Sphecodes niger von Hagens 1874
Sphecodes puncticeps Thomson 1870
Sphecodes spinulosus von Hagens 1875